Exxon Valdez Oil Spill
Restoration Project Annual Report

Sound Ecosystem Assessment (SEA): An Integrated Science Plan for the Restoration of Injured Species in Prince William Sound, Alaska

Restoration Project 95320
Annual Report

This annual report has been prepared for peer review as part of the *Exxon Valdez* Oil Spill Trustee Council restoration program for the purpose of assessing project progress. Peer review comments have not been addressed in this annual report.

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Study History: The SEA program was initiated in April 1994 as an integrated, interdisciplinary, multi-project study of processes constraining the recovery of damaged pink salmon and herring populations in Prince William Sound. An April 1995 annual report by R.T. Cooney entitled SEA94, Sound Ecosystem Assessment (SEA) - An Integrated Science Plan for the Restoration of Injured Species in Prince William Sound was comprised of eleven chapters detailing the results of each individual project, and a final synthesis chapter. The 1995 annual report submitted as this document reports progress toward SEA goals and objectives during the second of five anticipated years of funding. FY 98 will be the last field year for most elements of SEA. As presently envisioned, a final report of the five year study will be prepared as a group synthesis task in FY 99.

Abstract: This study describes mechanisms responsible for recruitment of juvenile pink salmon and herring in Prince William Sound. Regional oceanographic cruises examined circulation patterns, hydrography and plankton populations. Products include a 3-dimensional numerical model of circulation to measure differences in plankton biomass. Stable isotope tracers suggest that, at times, shelf zooplankton may augment local populations. Observations demonstrated that lake and river conditions prevail as part of the annual pattern of currents. Juvenile pink salmon and herring rear in shallow "nursery zones" where they grow and become food for predators including walleye pollock, kittiwakes and tomcod. Transient glut feeding probably takes most hatchery fry within weeks after ocean entry and abrupt, massive early fry mortality may be easily overlooked. Juvenile herring reinvade the nearshore zone in late summer as survivors of larval drift. Older juveniles were distributed in the region and along the eastern Kenai Peninsula. Herring work is guided by the supposition that juveniles may starve during winter if energy reserves are insufficient to survive periods of low plankton. Laboratory studies established threshold levels of somatic energy to judge pre and post-winter measures of energy to evaluate overwintering survival. Pre-winter condition is variable within and between populations from different locations.

Key Words: Avian predation, biophysical models, circulation, energetics, experimental fry release, food-web, herring spawn, marine acoustics, modeling, numerical models, nutrients, oceanography, Pacific herring, phytoplankton, pink salmon, stable isotopes, zooplankton.

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Preface

Each of the SEA project individual annual reports compiled here as separate chapters has been written as a stand-alone document except for the opening Synthesis Chapter. Bundling the reports together under one cover with synthesis has been done to address the integrative nature of the overall SEA approach, and to provide the Trustee Council and its agencies access to FY 95 results in a convenient form. It must be understood that SEA is a work in progress, and that results presented here represent the status of the study after only 18 months of field and modelling work, not an exhaustive analysis.

SEA was designed in the fall of 1993 to test hypotheses describing the mechanisms believed at that time to control survival in juvenile populations of pink salmon and Pacific herring in Prince William Sound. Refinements to these conjectures continue to guide the integrated research efforts:

a. The survival of juvenile pink salmon and post-larval herring populations in Prince William Sound and adjacent waters is determined primarily by losses to bird, fish and mammal predators.

b. For juvenile herring, the energetic condition prior to winter, and the winter environment (temperature and food) also establish the survival potential during periods of food deprivation. Under certain conditions, starvation may account for significant brood-year losses during this critical period.

c. Predation losses are related, in part, to the energetic condition and size of juvenile pink salmon and herring. Condition and size are established by growth rates that vary within and between years.

d. Predation losses are modified by the numbers and kinds of predators, and by the numbers, kinds and time/space distributions of alternative prey for these predators. Macrozooplankton serves as alternative prey during some seasons and years.

e. Macrozooplankton populations are established by local reproduction, and modified by phytoplankton productivity and timing and by currents that both flush the region and seed the Sound from adjacent shelf and ocean populations.

The SEA approach incorporates close coupling between field studies of processes and ecological mechanisms, and numerical modelling activities. The results of work completed in 1994 and 1995 - Phase I of SEA - have led to the creation of three field/model focus groups that have been designed to expedite the creation of management tools for pink salmon and herring in Prince William Sound. These numerical tools are presently envisioned as major contributions of the SEA program. Because of the continuous nature of the field and modelling work, the results presented here do not represent an exhaustive examination of the growing SEA data base, but rather a snap-shot of progress to date.

Chapter 1

SEA 1995 Synthesis

A Sound Ecosystem Assessment (SEA) Synthesis with Emphasis on Results Reported for the FY 95 Funding Period

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As the result of work competed in the first phase of SEA - FY 94 and FY 95 - the program has undertaken an internal reorganization designed to optimize the development, testing and eventual validation of resource models for pink salmon and herring populations in Prince William Sound. This focusing of the overall study addresses the original goals of SEA: 1) develop an ecosystem-level understanding of factors and mechanisms constraining the production of pink salmon and herring; 2) use this information to create a series of numerical tools and a nominal monitoring program to assist with increasingly informed management, enhancement and restoration of these injured species; and 3) establish a comprehensive data base of SEA (and possibly other) observations for management and continuing scientific activities in the region. An ocean state and plankton dynamics group (OP), a pink salmon recruitment dynamics group (PS), and a Pacific herring recruitment dynamics group (HR) provide critical exchanges between the field observations and modeling activities that address these goals. Most SEA principal investigators participate in at least two of these working groups; many participate in all three.

Even though the overall study was not organized in this way for phase I, I find it convenient to provide a synthesis of FY 95 information under these major working categories. Some may see this grouping and its functioning as divisive of the effort, but we do not. Certainly, the factors influencing the survival of juvenile pink salmon and herring in the region are likely similar at some level - oceanographically-modified predation and perhaps starvation (for herring) - but differences in the life history of herring and salmon, and local amounts of information available from previous work on these species suggested to us that the science might be most efficiently addressed by considering herring, salmon and ocean process separately. An integration of the modelling efforts has been established as a key task for Phase II of SEA, planned for completion in FY 98.

After two years in the field and laboratory, SEA has gone from data "poor" to data "rich". Now our growing list of results is able to effectively inform the continuing investigation. In addition, most investigators are now actively pursuing a SEA synthesis as the basis for developing collaborative manuscripts, and as a means to more carefully craft the remaining field activities in relation to stated modelling goals.

Ocean State and Plankton Dynamics

This sub-set of SEA projects is attempting a numerical translation of external forcing factors (winds, freshwater, tides) to account for observed seasonal and spatial patterns in hydrography, deep and shallow currents, and lower trophic level production in Prince William Sound. In the vernacular of the original study plan, the OP group is describing "lake/river" attributes of the system, reflected in both the physics and the biology. Our approach is similar to that being supported by the U.S. GLOBEC study of cod production on Georges Bank, and NOAA FOCI studies of walleye pollock in Shelikov Strait and the eastern Bering Sea (Kendall, et al., 1996). These other studies emphasize process queries designed to elucidate major mechanisms of mortality in the larval and juvenile stages. These mechanisms are then captured in numerical simulations that have robust predictive properties. Once created, the models also provide a means to examine a variety of management "what if" scenarios from which appropriate management actions can be selected. In the case of the recently completed Shelikov study, stochastic mathematical formulations and coupled bio-physical models have demonstrated strong applied predictive capabilities (Megrey, et al., 1996; Hermann et al., 1966).

The development and initial testing in SEA of a 1.2 km, 3-dimensional, eddy resolving model of the circulation of Prince William Sound represents a huge accomplishment for FY 95. Although yet to be tuned to the specifics of local winds, freshwater and tidal forcing, the model presently simulates many of the features observed in direct measures of current flow in the region. From reasonable northward transport values impressed at Hinchinbrook Entrance, the model reproduces the major current regimes in the Sound, including a lake-like northern region and river-like southern extension of the Alaska Coastal Current (ACC). The addition of a hydrological model of seasonal freshwater input in FY 96 and incorporation of wind and density data are expected to improve the simulation, and provide some of the first experimental opportunities for SEA hypothesis testing.

Regional-scale physical oceanographic studies continue to focus on understanding seasonality in upper-layer density structure and transport processes, variables that influence the timing, distribution and seasonal production schedules of plankton communities. Some of the first continuous records of flow fields extending from the seabed to 50 m were obtained during the summer and early fall from an upward-looking acoustic doppler current profiler (ADCP) located on the seabed north of Hinchinbrook Entrance. Preliminary analyses of this data portray a generally weak and variable flow regime, punctuated regularly by events that may represent spring/neap tidal forcing. There is no record of sustained deep inflow in the latter part of the record, indicating that the annual deep-water renewal cycle had not as yet begun before the ADCP was retrieved. It is believed that deep-water renewal occurs each year (Neibauer et al., 1994).

Seasonal changes in temperature, salinity and density agree well with historical records from the Sound (Schmidt, 1977). A deeply mixed cold winter layer was present to about 200 m for a central Sound location in March, the month generally accepted as the middle of oceanographic winter in Subarctic waters. Slight warming and freshening occurred in April and May,

apparently sufficient to promote the annual plankton blooms during those months. By late spring and early summer, the upper 50 m began to exhibit strong seasonal stability that carries forward into September. Continuing analyses of upper layer structure will include mapping the mixed-layer depth in March and April to help assess regional-scale differences in the timing of the phytoplankton blooms. Napp, et al., (1996) compare and contrast the timing of phytoplankton blooms occurring over deep and shallow water in Shelikof Strait and conclude that phytoplankton production is initiated annually in the more stable deeper water in the central portion of the Strait. We have evidence that in 1995, the spring phytoplankton bloom was likewise initiated over deep water in the central basin, quiet possibly the result of upper-layer stratification in that part of the Sound.

Upper-layer currents (20-100 m) obtained from a towed ADCP support a growing contention (and numerical model result) that flow fields in the eastern, northern and western Sound regions are generally weak and variable relative to south Sound flow - the so-called river region. Plots of potential density also reveal physical structure that in some cases is persistent for weeks and months. A cyclonic gyre appeared in the record north of Hinchinbrook Entrance during May, June and September, 1995. This structure is not apparent in the preliminary numerical simulations of upper-layer currents in the region, perhaps because the model has yet to be tuned to seasonal changes in density.

SEA biophysical modeling draws on simulations that track field observations of the timing and magnitude of phyto and zooplankton blooms each spring. That "timing is everything" is recognized by SEA as potentially establishing the matches and mismatches between predators and their prey. Since zooplankton require food to fuel their growth and reproductive cycles, peaks in the biomass of these two components are generally phased sequentially. Continuous monitoring of chlorophyll since 1993 (UAF C-LAB buoy) demonstrates interannual differences of up to three weeks in the timing of the plant bloom. We also find that the springtime zooplankton bloom generally follows the plants by 2-3 weeks. How these differences are related to problems with juvenile fish survival are unknown at present, but we do suspect they could be substantial. Census of wild fry outmigrations from index streams in southwestern Prince William Sound in 1990 and 1991 demonstrated that entry into the nearshore zone from adjacent natal areas began in early April each year despite a 2 degree C difference in temperature between years (Cooney et al., 1995). If juvenile salmon begin entering the marine system at about the same time each year (regardless of environmental cues), shifts in the timing of the zooplankton bloom might afford more food (for fry), and better sheltering from predators some years, but not in others.

Unlike the oceanic Gulf of Alaska, an early spring large diatom bloom is characteristic of Sound waters in late March and April. The timing of this event is modelled accurately using the Sverdrup critical depth theory which identifies light levels (relative to photosynthesis) and the mixed layer depth relationships as principal criteria for triggering" the bloom. April and May are transition months for the Gulf of Alaska, separating the fall/winter downwelling regime from the spring/summer period of relaxation or weak upwelling. Our observations indicate the bloom quickly becomes nutrient limited (nitrogen or silicon), falling to low standing stocks by early

May. The C-LAB records often depict one or more weak secondary blooms during the summer. In 1995, we have evidence that most of the bloom was associated with the "lake" portion of the region rather than the southern "river". Just why this was so remains to be investigated. However, since nutrient levels in the river water were elevated relative to the lake region, a light/mixing complication is suggested. We are presently plotting the basin-scale distribution of mixed-layer depths to address that possibility.

Zooplankton populations, measured in the Plankton Watch at the AFK hatchery in the southern region, were the highest since 1989, the last real "lake"year. Also in 1995, the peak in zooplankton biomass occurred a week or so earlier than historical averages (10 May). This difference was driven primarily by the timing and extent of the large calanoids - principally *Neocalanus plumchrus* and *N. flemingeri.* The first and second copepodite stages of this composite were present at most stations in and outside the Sound in March, and not restricted to regions over the deepest water. By April, all copepodite stages were occurring regionally and dominated by CIII. By May, the early stages were disappearing, and the biomass was mostly accounted for by the massive CV stage. Using intermolt periods similar to those in the literature (Miller, 1993) for these species, we note that to contribute to the May peak in biomass, *Neocalanus* arising from deepwater populations must remain in the upper layers of the Sound for at least 50-60 days. This may be possible for the more northern lake-like region, but probably not in the south where flow from the Alaska Coastal Current traverses the area in a week or so. Thus, it seems likely that south-Sound large calanoid populations are derived from open shelf stocks via the inflow, but that northern stocks may have more local origins. If this is true, lake/river differences are associated with transport and seeding from Gulf of Alaska stocks (Cooney, 1986), not by washout events as originally proposed. Work with the ocean state circulation model in 1996 should resolve these issues.

SEA is actively pursuing an independent test of this possibility using differences in the stable isotopes of carbon to identify different source populations. Our work to date on a congeneric, *Neocalanus cristatus*, supports the notion that shelf populations of large calanoids do invade the region with the coastal water inflow at Hinchinbrook Entrance. SEA is also examining an archive of zooplankton samples acquired in 1989, the last "lake" year, for evidence that *N. flemingeri* and *N. plumchrus* occured in different proportions that spring than are presently observed. *Neocalanus plumchrus* is the more oceanic form, and thus a natural marker for the inflow population (Miller and Clemons, 1988).

Pink Salmon Recruitment Dynamics

Wild juvenile pink salmon enter the coastal zone in the Gulf of Alaska from April to early June each year (Taylor, 1988). Cooney et al., (1995) note that this timing corresponds closely with the macrozooplankton bloom in Prince William Sound. We now believe that this outmigration timing maximizes the potential for survival by matching the fry with their food at a time of increasing water temperature, and also by providing a refuge from predation by adult fishes that are drawn to plankton rather than fry at this time.

Much was known about juvenile pink salmon during early marine residence from studies conducted prior to, and following the EVOS (Willette, 1996). Some of this work pointed to the importance of nearshore fry nurseries where juveniles feed and grow before leaving for open shelf and open ocean feeding grounds. The juvenile salmon literature has long suggested that losses of juveniles to predation was growth-rate dependent, the slowest growing fry being exposed in the smallest, presumably weakest stages for longer periods of time. Since both temperature and food influence growth rate, it seems reasonable to assume that the fry will always attempt to optimize their growth by exploiting gradients in food and temperature. Pink fry feed immediately on pelagic resources in the deep waters of Prince William Sound (Urquhart, 1979). During the period of early marine residence (April and May) waters of the region are generally warming and stabilizing. However, in the nearshore tidally-mixed zone, temperatures may actually be cooler than in adjacent stratified waters, and food diluted by mixing (see Juvenile Salmon Growth report). Under these conditions, it seems odd the fry would remain in water that is less than optimal (food and temperature) for growth unless there are other factors dictating these distributions. Given the results of the SEA research on juvenile pink salmon predators, it now seems reasonable to assume the nearshore "nurseries" are actually only marginal predation refuges, where fry are pinned between birds feeding from above and larger fish from below. In response to these threats, fry probably remain in the shallows until they have achieved a swimming ability (size) that tips the balance away from predation loss toward more optimal foraging opportunities (offshore). Another factor, the genetic clock driving an obligatory migration from coastal to open ocean feeding areas, must also play a role in dictating when fry are obligated to leave the shelter of the nearshore. In colder-than-average years, fry may be smaller when they begin migrating south to open coastal waters. Temperature-modulated size-dependent mortality could be contributing to the general relationship between fry-spring water temperatures and regional adult returns (Cooney et al., 1995).

While most researches believe that predation losses are highest during early marine residence, there is by no means agreement as to when these losses actually occur. Results from an analysis of coded-wire tag group survivals for fry released from the WN Hatchery in 1994, coupled with collections of these fish after release, suggests that the majority of losses of hatchery-reared fry may have occurred prior to the middle of June, or roughly within the first month of ocean residence (see Salmon Growth and Mortality report). Experimental releases of large fry (50 to 55-mm) in mid June survived at rates about an order of magnitude greater than fry released earlier under general hatchery protocols. If surviving and similar-sized fry from earlier hatchery releases can be assumed to have survived at this same higher rate after mid June, the poor overall survivals must be attributable to huge local mortalities in late April and May. These losses were apparently also absorbed by wild stocks in the Sound in 1994 since the wild pink salmon return was poor in 1995 in the west and southern districts.

Early SEA studies of salmon fry predators focused on offshore midwater collections of fishes and squids, and hatchery-based observations of losses to birds. An assessment of bird predation following the release of fry from pens implicates black-legged kittiwakes and marbled murrelets as species targeting fry. Energy models of bird food demands provided a means to evaluate the

impact of observed bird numbers. For observations in 1995 near the WN Hatchery at Esther Island, modeled forage demands suggest that up to 2.4% of the overall numbers of fry released or roughly 6.0 million fry could have been taken by birds. There was also evidence that bird predation increases during large releases because small numbers of birds attract larger numbers. Bird counts were highest near hatcheries in April and May, but declined in June (after most releases had been made). An association between gull-like (white birds) and herring spawning events suggests that predation at hatcheries may be influenced each year by the timing, location and duration of the herring spawning cycle.

Studies of fry predators in 1995 added tomcod and 1+ and older juvenile pollock to the list of fish that prey on fry in April, May and June. Unlike the previous year, a nearshore assemblage of fishes including herring, cod, pollock (juvenile), other juvenile salmon , dolly varden and an assortment of benthic species was present from early May on through mid June. We speculate that warmer water temperatures earlier in the season permitted this "assemblege" to invade the nearshore sooner than had been the case the year before. Small pollock and cod seemed to particularly target fry populations.

A surprise in 1995 was to find that distribution, feeding rate and diet composition of adult walleye pollock was statistically related to tidal range in May and early June. Catches were much reduced, and a diet shift was apparent during the spring-tide portion of the tidal cycle. Also, marine survival rates for tag groups of fry released over the same period in 1994 and returning as adults in 1995 were also statistically related to tidal range. Marine survivals were higher for fry entering during the spring part of the cycle. It is not clear what these relationships mean, except perhaps that predation by fish (adult pollock and/or other) may be reduced during the highest tidal flow rates as the result of reduced feeding rates or distributional changes in predators. We also speculate that under the highest flow rates, the nearshore refuge environment will increase in volume due to a deepening of the tidal mixed layer. Increasing the volume of this zone should reduce the encounter rates between fry and their predators resulting in lower mortalities. In contrast, fry released during neap tides enter predation refuges that have been reduced in volume. Substantial hatchery releases or wild fry entries at this time could "flood" the nearshore zone (in some places), forcing some fry to move out over deeper water at a time when predators were not only present, but actively feeding. These, and other possibilities provide guidance for continuing studies of fry and their predators.

SEA studies of fry predation losses are supported by acoustic surveys of nekton and plankton in the region. This methodology is providing estimates of fry predators in relatively shallow water near some hatcheries (AFK), and of off-shore adult pollock and herring stocks (spawning and feeding populations). Problems associated with separating pollock and plankton acoustic signals have been solved using a counting rather than echo integration technique to estimate fish abundance. This "problem" confirmed the ecological co-occurrence of macrozooplankton and pollock, the latter probably filter-feeding in layers of high plankton biomass (see Role of Zooplankton report). Studies to interpret the acoustic information relative to plankton species composition, size and densities in 1995 will be pursued again in 1996 using a MOCNESS system to provide discrete-depth samples of layers and zooplankton swarms. Also, work started

in 1995 to describe spatial scales in distributions of plankton and fish populations will continue. We hope to determine minimal copepod densities required by filter-feeding adult pollock as one "trigger mechanism" forcing a switch to other prey like small fishes.

Questions about wild and hatchery-released fry interactions explore density-dependent competition and will be greatly enhanced by the thermal otolith marking of all hatchery fry released in 1996 in the Sound. Somatic energy for three collections of pink salmon fry in 1995 demonstrated differences that appear to be associated with school size; the condition being poorer for fry sampled from the largest schools (see Energetics of Herring, Pollock and Pink Salmon report). Since the measurement of whole body energy represents an integration of feeding success, this method can be used to rank the condition of wild and hatchery fry rearing in the region as one means of evaluating possible adverse interactions. In a more general sense, overall differences in fry condition (wild and hatchery) could describe interannual differences in the rearing capacity of the entire region each year, and perhaps represents a predictor of adult run strength. As mentioned previously, growth rate (and presumably condition) likely mediates size-dependent losses to predators during early marine residence.

Modelling prey predator relationships in the "salmon fry ecosystem" is one of the most ambitious undertakings of the entire SEA program. The multi-dimensional approach is centered on the bioenergetics of the fry and their predators - the behaviors of both being driven by gradients in food and predation which in turn are modified by the oceanography - currents, mixing and temperature. This effort focuses SEA field investigations on "mechanisms of loss" for juvenile pink salmon and herring as the principal means for creating representative numerical simulations of these processes. The approach also draws from the literature to create bioenergetic models for the principal environmental components of the system. In 1995, an onshore-offshore model of fry, adult pollock, large copepods and euphausiids was completed and used to test our notions about prey switching and the time/space overlaps of fry, their food and their predators. New information about the effect of tidal range on fry survival will be used to modify future simulations.

A present, we are still uncertain about the actual "mechanisms" that couple fry and their predators during early marine residence. Our preliminary attempts to account for numbers of fry consumed on the basis of direct measures of predator gut content, their modeled energetic demands, and numbers of predators in the area fell well short of losses determined from adult returns. However, these preliminary results did not take into account the possibility of glut feeding or new information about the role that juvenile pollock may play as predators on fry. We now believe that intense and episodic feeding events probably account for most losses of fry near natal areas. These events have actually been witnessed at some hatcheries. In mid-May, 1994, a large school of juvenile pollock (and possibly other species) were observed in a feeding frenzy at Sawmill Bay adjacent to the AFK Hatchery following seasonal fry releases. Hook and line samples acquired fortuitously at this time revealed most small pollock were glutted with salmon fry. These events are well known to hatchery personnel who have devised release strategies to protect juveniles immediately after release. Obviously, these strategies do not always work.

Field studies in 1996 of pink salmon recruitment dynamics take advantage of what we have learned during Phase I of the program to narrow the remaining field effort on times and places that represent the best candidates to observe and describe the mechanisms of loss . We are aware that conditions vary each year, but expect to "capture" the most dominant interactions and possibly their causes.

Herring Recruitment Dynamics

SEA sponsored herring studies began in 1994 with an investigation of the influence of avian predation on herring embryo mortalities in intertidal spawning areas. That research demonstrated that glaucous-wing gulls, mew gulls, surf scoters, surfbirds and black turnstones obtained all or a very large percentage of their daily rations from herring eggs while eggs were present in the natal areas. The application of a bioenergetic model predicted that in 1994, this assemblage of birds could account for an average loss of 2.5 % of the eggs per day for a spawning location on Montague Island. Depending on the numbers of birds, and their arrival times, bird predation can account for measurable losses of eggs each year (see Avian Predation on Herring Spawn executive summary).

In 1995, an internal reorganization of SEA budgets was undertaken to accommodate the inclusion of studies designed to describe juvenile herring growth and habitats, and their energetic condition as a means to investigate how overwintering survivals might determine cohort recruitment strength to adult populations each year. Laboratory studies indicate that fasting herring use substantial energy, enough to result in starvation under some conditions (see Energetics of Herring, Pollock and Pink Salmon report). Herring store energy during the growth cycle each year, apparently drawing on that energy reserve to bridge seasonal reductions in plankton and other forage. If these reserves are exceeded during the winter, the fish may starve. Unusually warm winter temperatures might raise the metabolism if the juveniles enough to substantially increase their energy demands at times when plankton populations are at annual lows. Approaching starvation or weakening the fish could also increase their vulnerability to predation in these conditions.

SEA investigators entered the juvenile herring picture with little previous local information available about local growth or habitat preferences. Some observations indicated that larvae begin drifting seaward from natal areas in mid May. Depending on water temperatures, the larval forms could drift from 30-60 days before metamorphosis to post-larvae and eventual re-invasion of the nearshore zones. It was reasoned that during the drift, some unknown percentage of larvae could be swept from the Sound via the Alaska Coastal Current and thus possibly be lost to the system as recruits.

In 1995, emphasis was placed on describing the growth and habitats of post-larval forms in Prince William Sound and along the eastern Kenai Peninsula (see Juvenile Herring Growth and Habitats report). Aerial surveys coupled with some direct sampling found the juveniles to be widely distributed in shallow water environments from April through October; 0-age juveniles

began appearing in the nearshore zones in late summer. The pre-winter condition of 0-age fish was variable within and between sampling locations indicating that summer growth conditions are also highly variable. On the basis of laboratory experiments, it appears that many individuals collected in the fall of 1994 were at risk to overwintering starvation. This observation strengthens our conjecture that winter starvation losses may establish (in large part) eventual levels of recruitment to adult populations in the region.

Collections of herring and other fishes were obtained in October, 1995 during an intensive survey of herring distributions inside and outside Prince William Sound. Some of these observations are expected to provide some of the first local information on juvenile herring predators during a period when the trophic status of consumers may be shifting from planktivory to piscivory. Those results will enter the reporting schedule in 1997.

Herring research in 1996 will exploit the ocean state circulation model to examine retention, washout, and time-dependent distributions of "larvae" injected at natal areas and subject to upper and mid-level flow fields. The results of this work are expected to provide insight on the washout mechanism and its effects each year. Modeled larval trajectories will also provide some clues on where the nearshore post-larvae might be found on the basis of drift alone. It is hypothesized that some locations will provide better growth than others on the basis of measured oceanographic variables (temperature and food). Information on the characteristics of good and poor juvenile growth habitats determined from whole-body energy content, will then be used to evaluate the variables influencing the summer growth stanza. As mechanisms of growth and loss are identified, a modelling procedure similar to that applied to juvenile salmon will be developed to simulate the processes defining recruitment. Since herring presumably reside in the region for at least 2 years before entering adult populations, their local life-history is extended and more complex than that of pink salmon. We expect this complexity to complicate the development of suitable simulations for management purposes. However, SEA has allocated a significant portion of its remaining funds to assure at least partial success with the modelling by the end of 1998. Since herring is probably the most important forage species in the region, it seems reasonable that enough continuing support can be found within the EVOS Trustee Council to carry the work forward to completion sometime after FY98.

Summary

After 2 years of intensive field work, and hundreds of hours of analysis and discussion among the principal investigators in a variety of public and private professional forums, the skeleton of processes influencing the survival of juvenile pink salmon and herring in Prince William Sound is beginning to emerge. True to the adage "timing is everything", we believe that good and poor survival years are somehow established by differences in the phenological development of the pelagic marine production cycle each year which in turn influences when and where energy is transferred from producers to consumers in complex exchanges dictated by overlapping time space distributions of predators and their prey.

In a very fundamental sense, our preliminary results suggest significant "deviations" in the onset, magnitude and duration of the pelagic plant bloom. This variability is apparently driven by interannual noise in ocean state and meteorological forcing. The consequences of an early or late bloom seem most apparent in the subsequent timing of the zooplankton response through growth and reproduction. Variations in plant stocks are thus transferred forward to plankton forage populations for fishes, birds and marine mammals. Our interpretations of "Mother Nature's" past experiments have begun to provide insight about mechanisms setting limits on juvenile salmon and herring stocks each year. For salmon (the more-studied species), we now have evidence that a very critical time may actually be early marine residence - perhaps the first few days following ocean entry. The degree to which this is true is related to coincident predator and alternative prey fields. In most simulations of prey/predator interactions, we have found that zooplankton must occur in extremely high abundance to draw "pollock" away from fry. However, pollock can obtain suitable rations by filter feeding on zooplankton occurring in layers near the surface during the spring. In the absence of fry (and they seem relatively dilute over deep water) adult pollock seem content to utilize passive layers of large calanoid copepods.

If however, adult pollock stray into the nearshore zone, or fry are forced into deeper water (foraging needs or school size), a prey-switch to fry can occur and glut feeding may ensue. As we have discovered, adult herring, tomcod, several demersal fishes, squids, and juvenile pollock also feed in salmon fry and may be subject to similar relationships with alternative prey. Again, the phenology of events will be a key issue determining how energy is passed from juvenile salmon to consumer populations each year. Phenology plays an additional role in terms of controlling physical factors. The relationship between hatchery marine survivals and tidal range demonstrates an interaction we believe may be a modification of consumer feeding and distributions during monthly periods of high and low tides. Apparently during spring tides, some fish redistribute and apparently reduce or cease feeding. These periods of 3-6 days which may occur a couple of times during massive hatchery releases in April and May could " shelter" tens of millions of fry from immediate predation, allowing time for individuals to adjust to life outside the protection of net pens and to establish natural feeding patterns on local plankton. In 1993, a seven-day period of spring tides in early May may have contributed to better-than-average survival of fry returning as adults returning 1994. In contrast, a much weaker spring-tide series preceded by a neap in early May might have left much of the 1994 releases at high risk to predation losses. The return of adults in 1995 was one of the poorest on record. In 1995, the period 4-12 May was neap condition and several tens of millions of fry were released during this time. This "experiment" awaits evaluation during the coming summer as both wild and hatchery adults return to the region.

The picture is more opaque for herring, both for lack of data and the more complex life-history of this species in the Sound. Fisheries oceanographic theory counsels that discrete spawning locations for Prince William Sound stocks are probably sited in ways that maximize the potential for subsequent larval and post larval survivals. Spawning in April places emerging larvae in a mid-May pelagic system that is rapidly warming, is transitioning from downwelling to weak upwelling, and is seeing an increase in populations of small copepods like *Pseudocalanus*, *Acartia* and *Centropages* and their reproductive products (eggs and nauplii). These conditions

may provide enhanced local retention, and promote an optimal growth environment for the larvae. We know nothing about predation losses at this time, but surmise they will be high.

A re-invasion of the nearshore waters by surviving juveniles in the late summer may be in response to better growth environments, or for reasons of predator avoidance, or both. Without a better understanding of what constitutes growth habitat for juveniles and the predators that feed on them, we are unable to account for this behavior.

Herring have apparently adopted a "energy storage" strategy for coping with reduced forage availability during the winter. Evidence from the laboratory indicates that juveniles enter a fast at low temperature, presumably conserving body energy. We predict that variable summer growth conditions will prepare groups of juveniles for winter differently each year, and that perhaps some years will provide for better growth than others. If this is true, and our measurements of juvenile whole body energy content demonstrate this variability, some proportion of young herring will be at risk to weakening and starvation during the winter each year. The numbers lost will probably reflect balances between metabolic demand and predation.

Studies by Rogers et al. (1987) of nearshore fish communities in Prince William Sound document significant seasonal differences; most species move offshore or deeper in the water during the winter. It appears that juvenile herring may have adopted a means to take advantage of these seasonal shifts in fish abundance by opting to overwinter in habitats avoided by most other fish. If the fast is temperature dependent (a metabolic cue), the coldest water temperatures will occur in the upper mixed layer during the winter. Juveniles sheltering in local embayments (an observed strategy), would thus be subject to cold temperatures and a general separation from predators that move into warmer, deeper water during this time. Surprisingly, the greatest risk to starvation and loss might occur during years of warmer-than-average winter mixed-layer temperatures. An elevated metabolic demand could accelerate the utilization of energy stores at a time when local forage (zooplankton) was at seasonal lows. Warmer temperatures might also allow some larger fishes to remain in the shallow water environments, increasing the risk of predation. These and other factors will be examined in detail as SEA herring studies focus more on overwintering conditions and their relationship to recruitment.

The special technologies being utilized by SEA have matured in 1995 and are now contributing strongly to the data base. This includes digital transducer acoustic support, optical plankton counting, underway continuous CTD and florescence measurements, real-time buoy and weather-station observations, a moored ADCP, and computerized data base and internet services. In this sense, Phase I developmental exercises have been largely completed and SEA Phase II is in a standardized and ongoing data collection, analysis and synthesis mode.

I judge that only unexpected budget reductions could impair the ability of SEA to meet most of its program objectives by the end of 1998. After several noteworthy discoveries, the study has now arrived at the routine, difficult work of data interpretation and application. This stage represents the "old fashioned way" of grinding out defensible scientific results upon which much of the applied use of the work must ultimately rest. I trust the sponsors of SEA research

recognize the importance of these tasks as proposed and reviewed, both in terms of the recovery of pink salmon and herring resources in the region, and from the vantage of damaged services that also depend on the health of these stocks. The SEA consortium is dedicated to completing its remaining tasks in a professional and expedient manner.

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Chapter 2

95320A Salmon Growth and Mortality

Exxon Valdez Oil Spill
Restoration Project Annual Report

Sound Ecosystem Assessment: Salmon Growth and Mortality

Restoration Project 95320A
Annual Report

This annual report has been prepared for peer review as part of the *Exxon Valdez* Oil spill Trustee Council Restoration program for the purpose of assessing project progress. Peer review comments have not been addressed in this annual report.

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March 22, 1996

Study History: This project was initiated under Restoration project 94320A. An annual report was issued in 1994 by Willette, M., G. Carpenter, E. Debevec, under the title Sound Ecosystem Assessment: Salmon Growth and Mortality. The project effort was continued under Restoration Project 95320A, the subject of this annual report. In 1996, this project will be merged with project 96320E. A final report will be prepared for both projects in FY98.

Abstract: This project is a component of the Sound Ecosystem Assessment program. The project collected data needed to test several hypotheses related to predator-prey interactions affecting the mortality of pink salmon (*Oncorhynchus gorbuscha*) in Prince William Sound. Diel studies were conducted at four sites during three sampling periods in both May and June. Total zooplankton biomass and the abundance of large calanoid copepods was significantly greater in offshore than nearshore habitats. This result indicates that the growth potential for juvenile pink salmon may be greater offshore when growth is limited by low food abundance nearshore. When this condition occurs juvenile salmon may face a trade off between growth and predation risk, because the abundance of predatory fish is at times much greater in offshore habitats. Several lines of evidence indicate that significant mortality may have occurred among an early group of relatively small juvenile salmon released from the Wally H. Noerenberg Hatchery prior to the middle of June, 1994. Fry-to-adult survival was an order of magnitude greater for a late (mid-June) release of relatively large fry compared to an early release of much smaller fish. Recoveries of coded-wire tagged fry indicated that these two groups of fish were mixed together in lower Knight Island Passage after the middle of June. Several biological characteristics were not significantly different between the two groups after they were mixed together. Finally, relative abundances of the early-release group in purse seine catches declined exponentially, consistent with an instantaneous mortality rate assuming equal mortality between the early- and late-release groups after they were mixed together. High mortality among the early-release group largely caused the failure of the 1995 pink salmon return to the Wally H. Noerenberg Hatchery. High mortality among juvenile pink salmon prior to the middle of June may have been the cause of weak returns of both wild and hatchery-reared pink salmon in western Prince William Sound in 1995.

Key Words: *Exxon Valdez*, pink salmon, *Oncorhynchus gorbuscha*, migration, food consumption, diet composition, growth, mortality, coded-wire tagging.

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Executive Summary

This project is a component of the Sound Ecosystem Assessment (SEA) program. SEA is a multi-disciplinary effort to acquire an ecosystem-level understanding of the marine and freshwater processes that interact to constrain levels of pink salmon and herring production in Prince William Sound (PWS). Pink salmon runs to PWS failed in 1992 and 1993, and herring biomass dropped sharply in 1993. These run failures have drastically affected the economy of the PWS region which is largely based on the salmon and herring resources. This project collected data needed to test several hypotheses related to predator-prey interactions affecting the mortality of pink salmon (*Oncorhynchus gorbuscha*) in PWS. Several other projects within SEA also contribute to this hypothesis testing effort. These hypotheses include the following concepts (1) predation on juvenile salmon and other age 0 fish is inversely related to the abundance of large calanoid copepods, (2) predation risk is related to the daily foraging times of juvenile salmon, and (3) spatial patterns of adult pink salmon production are related to the distribution of large calanoid copepods and walleye pollock during the early marine period. This project was designed to achieve the following objectives (1) estimate the daily foraging times of juvenile pink salmon, (2) estimate prey abundance and composition in nearshore nursery habitats utilized by juvenile pink salmon, (3) estimate the diet composition of juvenile pink salmon, and (4) estimate the size composition and mean growth rate of juvenile pink salmon. Diel feeding studies were conducted at 4 sites during 3 time periods in May and June. Juvenile salmon were sampled with a purse seine and tow net every three hours during a 24-hour period in nearshore and offshore habitats at each site. Four thousand and six hundred juvenile pink and chum salmon were collected to estimate size composition, condition, daily foraging times and the proportion of the diet comprised of large calanoid copepods. Preliminary estimates of foraging time and daily food consumption have been made for four sites. Daily foraging time and food consumption increased by a factor of 2 and 6 between May and June, respectively. An additional 1,800 samples of fish (<150mm FL) from seven species were collected to estimate species/size composition of nearshore fish assemblages. Stomach contents analysis will be conducted on a portion of these samples to examine diet overlap among species under project 95163 Forage Fish Influence on Recovery of Injured Species'. Coded-wire tagged juvenile salmon released from the Wally H. Noerenberg (WHN) Hatchery were sampled in June to estimate growth rate. Mean growth rate was significantly different (P=.010) among three treatment groups, but not different from the mean growth of juvenile salmon in 1994. Zooplankton samples were collected every three hours at each site to test for differences in plankton abundance between nearshore and offshore habitats. Total zooplankton biomass and the abundance of large calanoid copepods was significantly greater (P<.05) in offshore than in nearshore habitats. Laboratory studies have shown that the feeding rate of juvenile pink salmon is about 3 times greater when feeding on large rather than small calanoid copepods. In the present study, large calanoid copepods were 3 times more abundant offshore than nearshore in June. This result indicates that the growth potential for juvenile pink salmon may be greater offshore when growth is

limited by low food abundance nearshore. When this condition occurs juvenile salmon may face a trade off between growth and predation risk, because the abundance of predatory fish is at times much greater in offshore habitats.

Several lines of evidence indicate that significant mortality may have occurred among an early group of relatively small juvenile salmon released from the WHN hatchery prior to the middle of June in 1994. High mortality among this early-release group largely caused the failure of the 1995 pink salmon return to the WHN Hatchery. Fry-to-adult survival was an order of magnitude greater for a late release of relatively large fry (1.5 g) compared to the early release of much smaller fish (.30 g). Recoveries of CWT fry indicated that these two groups of fish were mixed together in lower Knight Island Passage after the middle of June. Several biological characteristics were not significantly different between the two groups after they were mixed together. Finally, relative abundances of the early-release group in purse seine catches declined exponentially, consistent with an instantaneous mortality rate assuming equal mortality between the early and late groups after they were mixed together. Several alternative hypotheses were examined to evaluate the possible causes of these observations. High mortality among juvenile pink salmon prior to the middle of June, 1994 may have also been the cause of weak returns of wild pink salmon in western PWS in 1995.

Introduction:

This project is a component of the Sound Ecosystem Assessment (SEA) program. SEA is a multi-disciplinary effort to acquire an ecosystem-level understanding of the marine and freshwater processes that interact to constrain levels of pink salmon and herring production in Prince William Sound (PWS). Pink salmon runs to PWS failed in 1992 and 1993, and herring biomass dropped sharply in 1993. These run failures have drastically affected the economy of the PWS region which is largely based on the salmon and herring resources.

Recruitment to adult salmon populations is strongly affected by mortality during the early marine period, because mortality at this time is typically very high (Parker 1968; Ricker 1976; Hartt 1980; Bax 1983). During this period, slow-growing individuals sustain a higher mortality, because they are vulnerable to predators for a longer time than fast-growing individuals (Parker 1971; Healey 1982; West and Larkin 1987). Low returns of hatchery-produced salmon in 1992 and 1993 indicates that the run failures were likely caused by processes occurring during the early marine period. Damage assessment studies on juvenile pink salmon in PWS have demonstrated that growth during the juvenile lifestage is related to survival to adult (Willette et al. 1994). Growth rates of juvenile salmon were estimated in 1991 and 1992 after the fish were released from hatcheries. Juvenile growth and ocean temperatures were low in PWS during the early marine period in 1991. However, in 1992 juvenile growth and ocean temperatures were near average; although, zooplankton abundance was very low. The relationship between juvenile growth and mortality changed dramatically for pink salmon released in 1992 suggesting a change in predation rate.

In FY95 this project focused on the relationship between the daily foraging time of juvenile pink salmon and predation risk (Walters and Juanes 1993). Juvenile salmon spend much of their time in nearshore nursery habitats (Cooney et al. 1978) that likely provide a refuge from predation. This behavior is common among juvenile fish, and often juveniles must move out of the predation refuge to feed in areas where food abundance is greater (Helfman 1994). The amount of time spent feeding is likely related to food abundance and particularly the abundance of large calanoid copepods (Parsons and LeBrasseur 1973, Willette et al. 1994). High abundances of juvenile salmon or other age-0 fish may also lead to increased foraging time and predation risk.

Coded-wire tagged fish have played a major role in tracking the migration, growth and mortality of juvenile salmon in this study. During the past decade, five salmon hatcheries have been established within PWS. These facilities, operated by private non-profit corporations, released approximately 500 million juvenile pink salmon in 1995. Approximately one million of these fish were marked with a coded-wire tag (CWT). Several aspects of this project would not have been feasible without the CWT program. Comparison of survival rates of hatchery-reared salmon and return per spawner of wild salmon suggests that similar mortality processes may be affecting both groups (Figure 1).

This project will provide data needed to test several of the following predator\prey hypotheses posed by SEA investigators.

I. Walleye pollock and seabirds are the principal predators on juvenile salmon during the first 30 days of marine residence. After the initial 30 days, walleye pollock, herring, and adult salmon are the principal predators.

Sub-hypotheses:

I-1. *The juvenile salmon consumption rate of seabirds and age 1+ walleye pollock is greater than for all other predators combined during the initial 30 days of marine residence.*

I-2. *The juvenile salmon consumption rate of age 1+ walleye pollock, herring, and salmon is greater than for all other predators combined after the initial 30 days of marine residence.*

II. The rate of predation on juvenile salmon and other age 0 fish is strongly affected by the timing and duration of the macrozooplankton bloom modulated by ocean temperatures during the early marine period. During the macrozooplankton bloom, predators largely consume large calanoid copepods and predation on age-0 fish is low. As the abundance of macrozooplankton declines, predators switch to age 0 fish. Predation on age 0 fish is also size dependent; predation risk being substantially less for fish greater than approximately 60 mm FL. The survival of juvenile pink salmon and other age-0 fish therefore depends largely on their growth rate prior to reaching a size of approximately 60 mm FL and the coincident timing of the decline of the macrozooplankton bloom. Ocean temperature during this period is also critical, because the growth of juvenile pink salmon is largely temperature dependent.

Sub-hypotheses:

II-1. *The proportion of predator diets comprised of juvenile salmon and other age-0 fish is inversely related to the proportion of the diet comprised of large calanoid copepods.*

II-2. *The juvenile salmon consumption rate is greater when the abundance of large calanoid copepods is low.*

II-3. *Greater than 75% of the juvenile salmon consumed by predators are less than 60 mm FL.*

II-4. *Growth rates of juvenile salmon are positively related to ocean*

temperature and the proportion of the diet comprised of large calanoid copepods.

II-5. *The survival of pink salmon to the adult stage is positively related to growth rate before the fish reach 60 mm FL.*

III. The carrying capacity of PWS for juvenile salmon is determined by the availability of predation refuges that are both temporally and spatially limited. Temporal limitation of the predation refuge for juvenile salmon results from seasonal inshore movements of predators. Foraging time of juvenile salmon and thus predation risk is inversely related to interannual and seasonal changes in prey density and composition. Increased juvenile salmon density leads to longer juvenile salmon foraging times and increased predation risk.

Sub-hypotheses:

III-1. *The abundance of fish predators increases substantially from May to June in nearshore nursery habitats occupied by juvenile salmon.*

III-2. *The seasonal increase in fish predator abundance in nearshore nursery habitats is related to ocean temperature structure.*

III-3 *Zooplankton abundance and the abundance of large calanoid copepods is greater offshore (outside of predation refuge) than nearshore (within predation refuge).*

III-4. *The daily foraging time of juvenile salmon is inversely related to total prey density and the proportion of large calanoid copepods in the diet.*

III-5. *The daily foraging time of juvenile salmon is positively related to juvenile salmon abundance.*

III-6. *The juvenile salmon consumption rate of predators is positively related to the daily foraging time of juvenile salmon.*

IV. Predation on wild salmon fry is greater when wild fry are mixed with larger hatchery-reared fry in nearshore nursery habitats. Behavioral responses of predators lead to predator aggregations and greater predation rates in areas of high juvenile salmon abundance. Predators select smaller wild fry in mixed schools of wild and hatchery salmon.

Sub-hypotheses:

IV-1. *Predator abundance is positively related to juvenile salmon abundance.*

IV-2. *Wild salmon fry are smaller than hatchery-reared fry in nearshore nursery habitats.*

IV-3. *The ratio of wild to hatchery salmon in predator stomachs is greater than the ratio of wild to hatchery salmon in nearby nearshore nursery areas.*

V. Spatial patterns of adult pink salmon production in Prince William Sound are determined by the distribution of age 1+ walleye pollock and macrozooplankton during the early marine period.

Objectives:

Original objectives in detailed project description for 95320A:

1. Estimate the daily foraging time of juvenile pink salmon at 16 study sites in PWS.

2. Estimate the relative abundance of juvenile pink salmon at 16 study sites in PWS.

3. Estimate prey abundance and composition in nearshore nursery habitats utilized by juvenile pink salmon and in adjacent offshore areas at 16 study sites in PWS.

4. Estimate diet composition of juvenile pink salmon at 16 study sites in PWS and collect juvenile fish stomach samples for project 95163 (Forage Fish).

5. Estimate the size composition and condition of untagged juvenile pink salmon at 16 study sites in PWS.

6. Estimate the growth rate of juvenile CWT salmon recovered at 16 study sites in PWS.

7. Conduct preliminary tests of predator\prey hypotheses.

Objective added as result of new information obtained in FY95:

8. Evaluate differences in fry-to-adult survival, biological characteristics and relative

abundance of small early-fed and large late-fed juvenile salmon released in 1994 from the Wally H. Noerenberg Hatchery.

Methods:

The study design in FY95 focused on diel patterns of feeding and movements of juvenile salmon and their predators. The large releases of juvenile salmon from the Wally H. Noerenberg (WHN) Hatchery provide an opportunity to investigate processes regulating the growth and mortality of juvenile pink salmon. Results from the first year of this project indicate that similar process-oriented studies may not be feasible in areas of much lower juvenile salmon abundance. The study examined the relationship between the daily foraging time of juvenile pink salmon and predation risk modulated by changes in prey abundance and juvenile salmon abundance. This approach required sampling at a number of sites exhibiting a range of both prey abundance and juvenile salmon abundance. Four nearshore study sites were sampled in western PWS during each of four time periods (Figure 2).

Objective 1:

The daily foraging time of juvenile pink salmon was estimated from diel feeding periodicity studies conducted at each of 16 nearshore study sites. Each site consisted of an approximately 3000 m long segment of shoreline. Juvenile salmon sampling was conducted at 3-hour intervals throughout a 24-hour period at each site. A small-mesh purse seine (70 m length) was used to sample juvenile pink salmon in nearshore nursery habitats where the fish were aggregated. In offshore habitats, juvenile pink salmon were sampled with a tow net (3m x 4m) deployed from a 6 m long aluminum skiff. Samples of untagged juvenile pink salmon (n=100) were preserved in 10% buffered formaldehyde solution.

Stomach contents analysis was conducted later in the laboratory on a random subsample of 15 individuals. Fish showing signs of regurgitation were not included in the subsample. Whole body wet weight was measured to an accuracy of 0.01 g. Total stomach contents weight was measured to an accuracy of 0.1 mg. The proportion of the diet comprised of large calanoid copepods (>2.5 mm), small calanoid copepods (<2.5 mm), and 'other prey' was visually estimated. Large calanoid copepods in each stomach were enumerated. Stomach fullness was expressed as the ratio of stomach contents weight and whole body weight.

The daily foraging time of juvenile pink salmon at each site was estimated by examining changes in stomach fullness by time of day. Temperature-specific gastric evacuation rate was estimated from data provided by Bailey et al. (1975). Expected stomach fullness assuming no feeding was estimated for each sampling time from stomach fullness during the previous sampling time reduced by gastric evacuation. If measured stomach fullness was greater than expected it was assumed that the fish were feeding during the interval between sampling times. Food consumption during the interval between sampling times was estimated from the

difference between expected stomach fullness and measured stomach fullness. Daily food consumption was estimated from the sum of food consumption during the intervals between sampling times. In cases where data was not available for all 7 sampling times within a day, daily food consumption and foraging time was estimated by extrapolating the results obtained during the period sampled to the entire 24-hour period. Stomach data from all stations sampled during each time period were pooled in the analysis.

Objective 2:

The relative abundance of juvenile pink salmon was estimated at each study site from visual surveys of nearshore nursery habitats. Relative abundance from visual surveys was described as low (=1), medium (=2) or high (=3). Relative abundance in offshore areas adjacent to nearshore nursery habitats was estimated by catch per net set in purse seines (1 cm stretch mesh; 20m x 240m) and catch per hour in tow nets (3m x 4m). Purse seines were fished by holding a hook into the direction of the prevailing current for 20 min. Tow nets were fished for approximately one-half hour along a transect 100m from shore.

Objective 3:

Zooplankton samples were collected every three hours during daylight with a 0.5 m ring net (243 um mesh) at two stations nearshore and two stations offshore at each of the 16 study sites. Nearshore stations were located in areas inhabited by juvenile salmon. Offshore stations were approximately two kilometers from the shore in water generally exceeding 200 m depth. Of the 228 samples collected, 179 were 20 m vertical tows and 48 were 10 m vertical tows. Ten meter vertical tows were only taken at nearshore stations when the bottom depth was less than 20 m. All samples were preserved in 10% buffered formaldehyde solution for later laboratory analysis.

In the laboratory, a vacumn pump was used to remove excess water from each sample. The sample was placed on a 100 um filter and the pump operated for 1 minute. Total sample wet weight was measured to an accuracy of 0.1 mg. The sample was then washed into a graduated beaker and subsampled with a Stimple pipette. Large calanoid copepods (>2.5 mm), small calanoid copepods (<2.5 mm), and 'other zooplankters' were enumerated in each subsample. A sufficient volume was subsampled to obtain a minimum count of 100 large calanoid copepods. The total wet weight of animals in each category was estimated from the product of abundance and average wet weight (Coyle et al. 1990). Biomass (g m^{-3}) and abundance (no.m^{-3}) were estimated for each taxonomic group in both nearshore and offshore habitats at each site.

Objective 4:

The field sampling design and laboratory procedures described in objective 1 were used to estimate diet composition of juvenile pink salmon at each study site. Whenever possible, samples of other juvenile fishes (forage fish) were collected along with samples of juvenile

salmon. These juvenile salmon and forage fish samples were provided to project 95163 (Forage Fish) for a later more detailed diet analysis. A paired comparison of diet overlap among various species of juvenile fishes occupying nearshore habitats will be conducted under project 95163.

Objective 5:

The size composition and condition of juvenile pink salmon at each study site was estimated from samples of untagged juvenile pink salmon (n=60) collected as described in objective 1. Fork length was measured to the nearest 0.5 mm and total body wet weight to the nearest 0.01 g. Each fish was blotted dry before weighing. Condition of juvenile pink salmon was examined to evaluate feeding and growth conditions at each site. The relationship between body weight (W) and length (L) was described by

$$W = a L^b \quad (1)$$

where a is the condition factor and b is the slope of the linear-transformed model (Ricker 1975). All juvenile pink salmon collected at each site were pooled to estimate the parameters of the model.

Objective 6:

In 1995, approximately one million CWT juvenile pink salmon were released from four hatcheries in PWS. Juvenile salmon were sampled as described in objective 1 to obtain CWT juvenile salmon for estimation of growth rate. A portable tube CWT detector was used to isolate CWT juvenile salmon from untagged fish in purse seine catches. Only about one fish in a thousand were coded-wire tagged on average, so a large number of juvenile salmon were captured to obtain an adequate sample of CWT salmon. When a large number of fish were caught, the total number of fish in the catch was estimated volumetrically. Live fish were placed in a volumetric beaker with a known volume of water. The displacement volume of the fish was calculated by subtraction. The number of beakers of live fish in the total catch was recorded. Total number of fish in the catch was estimated from the number of beakers and the number of fish per displacement volume.

Individual CWT juvenile salmon were placed in pre-weighed vials and frozen. The vials were weighed later on shore when accuracies of 0.01 g were obtained. Fork length was measured to the nearest 0.05 mm. Methods developed by the ADF&G CWT Laboratory for extracting and interrogating CWTs were employed. An exponential model was used to estimate growth rates (G_i) of individual CWT juvenile salmon, i.e.

$$G_I = \frac{\ln(W_c) - \ln(W_r)}{t_c - t_r} \quad (2)$$

where W_c is the weight of the fish at capture, W_r is the mean weight at release of the fish in a specific tag-code group, t_c is the date at capture, and t_r is the mean date at release.

Objective 7:

Sub-hypotheses I-1 & I-2.

See DPD for project 95320E.

Sub-hypotheses II-1 through II-3.

See DPD for project 95320E.

Sub-hypothesis II-4.

A bioenergetics model will be used to examine the effects of ocean temperature and diet composition on the growth of juvenile pink salmon (Willette et al. 1994). Holling (1966) developed a model to estimate the feeding rate of invertebrates in relation to prey density, i.e.,

$$I_f = \frac{\gamma\ p\ U}{1 + \gamma\ p\ U\ h} \quad (3)$$

where I_f is the feeding rate (g sec^{-1}), γ is the cross-sectional area of the reactive field (cm^2), p is the prey density (g cm^{-3}), U is the swimming speed (cm sec^{-1}), and h is the prey handling time (sec g^{-1}). This model was successfully used by Ware (1975, 1978) to estimate the feeding rate of fish. To account for prey that are attacked but not captured, equation (3) will be multiplied by the prey capture success rate. A prey capture success rate of 85% is typical for juvenile fishes (Ware 1972). The distance from which a fish will approach prey is called the reactive distance (Ware 1972). This distance is a function of fish size (Ware 1978) and prey size (Ware 1972). Data provided by Ware (1972) was used to estimate a regression equation relating reactive distance to fish length and prey length, i.e., $d_r = 0.29\ L_f^{1.1} + 3.3\ L_p$ ($r=.98$, $P=.005$), where d_r is the reactive distance (cm), L_f is total fish length (cm) and L_p is prey length (mm) (Willette et al. 1994). Given d_r, the cross-sectional area of the reactive field (γ) is πd_r^2. Bailey et al. (1975) estimated that pink salmon swim at 11 to 20 cm sec^{-1} when feeding in currents. In the present study, an average swimming speed of 15 cm sec^{-1} will be assumed, because juvenile pink salmon are often observed feeding while swimming in currents. For a 1 g pink salmon, this is approximately the critical swimming speed, i.e. 3.0 body lengths per second. Parsons and LeBrasseur (1973) estimated the feeding rates of juvenile pink salmon in tanks at different prey densities. Their data have not be used to

estimate feeding rates directly, because the prey densities used in their experiment were an order of magnitude greater than those measured in PWS. Their data were used to estimate handling times for fish feeding on *Pseudocalanus spp.* and *Neocalanus plumchrus* assuming an experimental duration of two hours.

Estimates of daily food consumption will be obtained from equation 3 using prey composition data obtained from stomach contents analyses of juvenile pink salmon in PWS (Willette et al. 1994). It will be assumed that light levels in PWS are adequate for feeding for twenty hours each day. The daily food consumption will be estimated using handling times for large and small copepods (Parsons and LeBrasseur 1973). Handling times for small copepods will be used for all prey items other than large copepods. The analysis will be conducted using data collected in 1989, 1990, 1991, 1993, 1994, and 1995, because estimates of prey density by taxonomic group are available for these years (Wertheimer et al. 1993, Willette et al. 1994).

Daily growth will be estimated by a simple mass balance equation, i.e.

$$G = a I - R \qquad (4)$$

where G = growth rate (cal day^{-1}), I = food consumption (cal day^{-1}), R = total metabolism (cal day^{-1}), and a = assimilation coefficient. An assimilation coefficient (a) of 0.86 will be used (Ware 1975). Total metabolism (R) is composed of feeding metabolism, standard metabolism, active metabolism, and migration metabolism (Brett and Groves 1979). Brett and Glass (1973) estimated the active metabolism (including standard metabolism) of sockeye salmon at the critical swimming speed. The critical swimming speed is the maximum speed that can be sustained without incurring an oxygen debt. The critical swimming speed is typically 2.5 to 3.0 body lengths per second. Juvenile pink salmon appear to swim at this speed while feeding along steep rocky shorelines (Bailey et al. 1975). Data provided by Brett and Glass (1973) will be used to estimate temperature-specific active metabolic rates for a 1 g pink salmon. Feeding metabolism is a function of the rate of food consumption, i.e. $R_f = sI$, where s is the weighted mean of the specific dynamic action factors associated with protein, lipid, and carbohydrate catabolism (i.e. ~0.16, Ware 1975). Feeding metabolism will be added to active metabolism after an initial estimate of food consumption. Migration metabolism will not be included in total metabolism, because active metabolism has been estimated while the fish were swimming at the critical speed. The model will be validated by comparing predicted growth to measured growth of coded-wire tagged juvenile pink salmon in PWS.

Sub-hypothesis II-5.

The relationship between juvenile growth rate and fry-to-adult survival was evaluated from recoveries of CWT juveniles and adults. Restoration project 95320B (Pink Salmon Coded-wire Tag Recovery) provided data on survival rates of CWT pink salmon released in 1994. Analysis of covariance was used to test for differences in the intercept and slope of the regression model between years (1989-1994). Mean growth and survival for each tag code

group were used in the analysis. The independent variable was release year with mean growth rate of juvenile salmon as a covariate. Only juvenile salmon less than 60 mm FL at capture were included in the analysis .

Sub-hypotheses III-1 & III-2.

See DPD for project 95320E.

Sub-hypothesis III-3.

Analysis of variance was used to test for differences in total zooplankton biomass (g m^{-3}), and the abundance of large calanoid copepods (no. m^{-3}), small calanoid copepods, and 'other' zooplankters between nearshore and offshore habitats. Zooplankton biomass and composition was estimated as described in objective 3. The analysis was conducted using data from both 10 and 20m vertical net tows, and only data from 20 m vertical net tows. Analysis of variance was also used to test for differences the abundance of large copepods in nearshore habitats at three levels of current speed (<.10 m sec^{-1}, .10 m sec^{-1} > and < .20 m sec^{-1}, and >.20 m sec^{-1}). This analysis was conducted to determine if periodic tidal mixing causes large calanoid copepods to be pulsed into nearshore habitats. Current speed estimates were obtained from tide programs provided by NOAA.

Sub-hypotheses III-4 & III-5.

Multiple linear regression analysis will be used to examine the relationship between the daily foraging time of juvenile salmon and prey density, the proportion of large calanoid copepods in the diet, ocean temperature, and the abundance of juvenile salmon. The daily foraging time of juvenile salmon, prey density, diet composition, juvenile salmon abundances will be estimated as described in objectives 1, 2, 3, and 4. Data from each diel study conducted at nearshore sampling sites will be used as the sample unit in the analysis.

Sub-hypothesis III-6.

Regression analysis will be employed to examine the relationship between juvenile salmon consumption rate and the daily foraging time of juvenile salmon. The juvenile salmon consumption rate will be obtained from project 95320E. The daily foraging time of juvenile salmon will be estimated as described in objective 1. Data from each diel study conducted at nearshore sampling sites will be used as the sample unit in the analysis.

Sub-hypothesis IV-1.

Analysis of variance was used to test for a relationship between the abundance of fish predators and the abundance of juvenile salmon. Catch per unit effort in trawl, purse seine and fixed gear was used as an index of predator abundance in offshore, nearshore pelagic, and nearshore benthic habitats at each site. The abundance of juvenile salmon was estimated as

described in objective 2.

Sub-hypotheses IV-2 and IV-3.

Deferred until FY96 when otolith thermal marked juvenile pink salmon will be released from all PWS hatcheries.

Objective 8:

In 1995, estimates of survival to adult became available for pink salmon released in 1994. A striking difference in survival of early and late groups released from the Armin F. Koernig and WHN hatcheries prompted an examination of possible causes. The distribution of the two groups of fish were visually compared on maps indicating the location of recovery of CWT juveniles. An analysis of variance was conducted to test for differences in growth, length-adjusted body weight (condition), and length between the two groups. Only data from CWT juveniles were used in this analysis. Growth rate of the early release group was estimated for the period when the two groups of fish were mixed together (after the middle of June). The body weight of the early release group in mid-June was used as an initial weight to calculate growth after that time. The relative abundance of each group was estimated from the number of CWT juveniles recovered in each net set expanded by the appropriate tagged-to-untagged ratio. An analysis of variance was conducted to test for differences in the relative abundance of the two groups after the middle of June. The dependent variable in the analysis was the natural logarithm of catch per net set. Two instantaneous mortality rates were calculated for the early release group for the initial 45 days of marine residence (i.e. prior to the release of the late group) assuming (1) equal mortality for the early and late release groups after the middle of June, and (2) constant mortality for the early release group throughout the marine lifestage and differential mortality between the early and late release groups after the middle of June. The instantaneous mortality of the early release group under assumption #1 was estimated by

$$\frac{z_{ij} = z_i t_i + z_j t_j}{t_{ij}} \qquad (5)$$

$$z_i = x\, z_j$$

where z_{ij} is the instantaneous mortality for the entire fry-to-adult lifestage, z_i is the instantaneous mortality during the initial 45 days of marine residence, t_i is the duration of the initial period (45 days), z_j is the instantaneous mortality after the initial 45 day period, t_j is the duration of the period after the initial 45 days, t_{ij} is the duration of the entire fry-to-adult stage, and x is a constant. Changes in relative abundance of the early release group were modelled using these two assumed instantaneous mortality rates, i.e

$$N_t = N_o e^{-zt} \quad (6)$$

where N_t is the population size at time t, N_o is the initial population size, and z is the instantaneous mortality rate. Estimated and simulated changes in the relative abundance of the early release group were visually compared.

Results:

The 1995 release of juvenile salmon from the WHN Hatchery began on April 29 and continued until June 14. During this time period, approximately 168 million juvenile pink salmon averaging .24-.35 g were released (Figure 3). About 300,000 of these fish carried a CWT. The last release occurred on June 14 when 7 million juvenile pink salmon averaging 1.0 g swam out of two net pens at the WHN Hatchery. This group of relatively large juveniles was released to test the hypothesis that mortality is size dependent.

Objective 1:

Four thousand and six hundred juvenile pink and chum salmon were collected to estimate daily foraging times at sixteen sites in western PWS. Stomach sample processing was initiated in December, 1995. Approximately 40% of these samples have been laboratory analyzed to date. Preliminary estimates of daily foraging time were developed for 4 sites from which sufficient data was available. Stomach fullness differed significantly by time of day ($P<.001$) at each of the 4 sites (Figure 4). Daily food consumption was relatively low in early May and increased by a factor of 6 by early June (Table 1). Daily foraging times increased by a factor of two between early May and early June (Table 1). A more comprehensive analysis will be conducted when all data is available.

Objective 2:

The relative abundance of juvenile salmon varied considerably among sixteen sites sampled in western PWS (Table 2). There was no apparent relationship between the relative abundance of juvenile salmon in nearshore and offshore habitats. In offshore habitats, catch per net set in purse seines and tow nets did not appear to be related. Further analyses will be conducted when acoustic data from project 95320N becomes available.

Objective 3:

Mean total zooplankton biomass ranged from 0.3 to 1.8 g m^{-3} and varied significantly ($P<.001$) among sixteen sites sampled in western PWS (Table 3). The mean abundance of large calanoid copepods (no. m^{-3}), small calanoid copepods, and 'other' zooplankters also varied significantly ($P<.001$) among the sixteen sites sampled in 1995 (Table 3).

Objective 4:

Four thousand and six hundred juvenile pink and chum salmon were collected at sixteen sites in western PWS to estimate diet composition. Stomach sample processing was initiated in December, 1995. Approximately 40% of these samples have been laboratory analyzed to date. Analyses of these data will be conducted when all data becomes available. An additional 1,513 samples of various species of forage fish were collected for later stomach contents analysis by the NMFS, Auke Bay Laboratory under project 96163 (Table 4).

Objective 5:

Juvenile pink salmon increased in length from 32 mm in early May to 59 mm in the middle of June (Table 5). Analysis of covariance indicated significant differences (P<.001) in condition among the six sites for which data is presently available.

Objective 6:

Two hundred and sixty four juvenile CWT pink salmon were recovered during June in western PWS. Growth rates of juvenile pink salmon differed significantly among three treatment groups (P=.011) and were similar to growth rates observed in 1994 (Table 6).

Objective 7:

Sub-hypothesis II-4.

An analysis has not yet been conducted to determine if the growth of juvenile pink salmon was likely limited by low food abundance. Diet composition data for juvenile salmon collected in 1995 is not yet fully from the laboratories. Work on this and other components of data analysis will continue in July after the 1996 field season.

Sub-hypothesis II-5.

Analysis of covariance indicated significant differences (P<.0001) among years in the slope of the relationship between juvenile growth and fry-to-adult survival (Willette et al. 1995). The slope of the growth-survival relationship was significantly different (P<.0001) from zero in all years, except 1991 (P=.1470) and 1992 (P=.1986). For fry released from the WHN Hatchery in 1994, the slope of the growth-survival relationship was again not significantly different (P=.1904) from zero (Figure 5).

Sub-hypothesis III-3.

A total of 230 vertical ring net samples were collected to test for differences in zooplankton biomass and species composition between nearshore and offshore habitats. Forty nine of these samples were 10 m vertical net tows taken in nearshore habitats where bottom depth was less

than 20 m. The remainder were 20 m vertical net tows. When all samples were included in the analysis, the total zooplankton biomass was significantly greater offshore than nearshore in both May (P=.003) and June (P=.001). When all samples were included in the analysis, the abundance of large copepods was significantly greater offshore than nearshore in both May (P=.005) and June (P=.004). When only 20 m vertical net tows were included in the analysis, the total zooplankton biomass was still significantly greater offshore than nearshore in May (P=.024) and June (P=.007). When only 20 m vertical net tows were included in the analysis, the abundance of large copepods was still significantly greater offshore than nearshore in May (P=.063) and June (P=.006). The abundance of large copepods was approximately 50% greater in offshore habitats in May and 3 times greater in June (Tables 7 & 8). There was generally no relationship between tidal current speed and zooplankton biomass or abundance. However, the abundance of 'other' zooplankters was significantly greater (P=.038) at intermediate current speeds relative to both low and high current speeds in May (Table 9). It is not clear what mechanism if any may have caused this difference.

Sub-hypothesis III-4 & III-5.

An analysis has not yet been conducted to examine the relationship between the daily foraging time of juvenile pink salmon and environmental conditions. Diet composition data for juvenile salmon collected in 1995 is not yet fully from the laboratories. Work on this and other components of data analysis will continue in July after the 1996 field season.

Sub-hypothesis III-6.

An analysis has not yet been conducted to examine the relationship between the daily foraging time of juvenile pink salmon and juvenile salmon consumption rates. Work on this and other components of data analysis will continue after the 1996 field season.

Sub-hypothesis IV-1.

The relative abundance of juvenile salmon was not significantly related to catch of predatory fish per unit effort in trawl gear (P=.3148), seine gear (P=.9238), or fixed gear (P=.8646, Table 10) Trawl catch per unit effort was marginally significantly related to sampling site (P=.0974), but no site effect was detected for the other gear types. Further analyses will be conducted when data from project 95320N becomes available.

Objective 8:

Survival to adult in 1995 was an order of magnitude greater for a late release of large fry compared to an early release of small fry at the WHN Hatchery (Table 11). Recoveries of coded-wire tagged juvenile salmon indicated that these two groups of fish were mixed together in southwest PWS after the middle of June (Figure 6). Comparison of several biological characteristics between these two groups indicated no difference in growth (P=.247) or length-adjusted body weight (P=.351) after the fish were mixed together (Table 12);

although, length was slightly less for the late release group compared to the early release group (Table 12). After the middle of June, catch per unit effort of coded-wire tagged fry was roughly equal for these two groups despite the fact that 235,000 tags had been applied to the early-release group and only 20,000 tags to the late-release group (Figure 7). These results suggest that significant mortality may have occurred among the early release group prior to the middle of June, 1994. Assuming equal mortality for the early and late release groups after the middle of June, the instantaneous mortality rate (z) of the early release group prior to the middle of June was estimated to be -.0846. The instantaneous mortality rate for the early release group (assuming constant mortality throughout the marine lifestage) was estimated to be -.0133. Comparison of simulated and estimated changes in relative abundance (catch per unit effort) support the conclusion that significant mortality may have occurred among the early release group prior to the middle of June, 1994 (Figure 8).

Discussion:

Greater zooplankton biomass and abundances of large calanoid copepods in offshore habitats (Tables 7 & 8) indicates that the growth potential for juvenile pink salmon may be greater offshore when growth is limited by low food abundance nearshore. Willette et al. (1994) found that growth rates of juvenile pink salmon may be limited by low food abundance when zooplankton biomass is less than 0.10 g m^{-3} and water temperature exceeds 10° C. When growth is limited by low food abundance in nearshore habitats, juvenile salmon may face a trade off between growth and predation risk, because the abundance of predatory fish is at times much greater in offshore habitats (Willette et al. 1995b, Walters and Juanes 1993). The abundance of large calanoid copepods likely has a strong effect in determining whether juvenile salmon are food limited, because the feeding rate of juvenile pink salmon is approximately 3 times greater when the fish consume large rather than small calanoid copepods (Parsons and LeBrasseur 1973). Preliminary estimates of daily food consumption and foraging times suggest that juvenile pink salmon may not have been food limited in nearshore habitats in May when foraging times were approximately 12 hours per day (Table 1). This is consistent with earlier estimates of daily foraging times in May which were based on estimated feeding rates and maximum daily ration (Willette et al. 1994). Further analyses will be conducted to evaluate when low food abundance may have limited the growth of juvenile pink salmon in nearshore habitats. We will also examine how the timing of food limitation in nearshore habitats may coincide with apparent offshore movements of fry and seasonal changes in predator abundances.

Several lines of evidence indicate that significant mortality may have occurred among the early group released from the WHN hatchery prior to the middle of June, 1994. Fry-to-adult survival was an order of magnitude greater for the late release of relatively large fry compared to the early release of much smaller fish (Table 11). Recoveries of CWT fry indicated that these two groups of fish were mixed together in lower Knight Island Passage after the middle of June (Figure 6). Several biological characteristics were not significantly different between the two groups after they were mixed together (Table 12). Finally, relative

abundances of the early-release group in purse seine catches declined exponentially, consistent with an instantaneous mortality rate assuming equal mortality between the early and late groups after they were mixed together. Several alternative hypotheses must be examined to evaluate the possible causes of these observations: (1) the mortality of the early-release group was high in some area within their overall distribution after the middle of June, (2) high condition (energetic content) among the late-release group caused lower mortality compared to the early-release group after the middle of June, and (3) the apparent exponential decline in relative abundance of the early-release group was due to sampling error.

It seems unlikely that the mortality of the early-release group was high in some area within their overall distribution after the middle of June. In 1994, shoreline surveys were conducted in Knight Island Passage to collect samples of CWT juvenile salmon. Each survey typically began in the lower reaches of the passage below the area where fry from the WHN Hatchery had last been observed. The sampling effort then moved northward toward WHN Hatchery recovering CWT fry as they were encountered. The overall sampling effort moved southward in the passage as the main body of fry from WHN Hatchery moved south. After the middle of June, sampling effort was focused in southern PWS. Thus, it is possible that the bulk of the late-release group was not mixed with the early-release group and was distributed more in northern PWS. If so, higher mortality among the early-release group could be due to higher predation in southern PWS. However, mid-water trawl sampling indicated very low abundances of walleye pollock and squid in both northern and southern PWS in early July (Willette et al. 1995b). Also, catch per net set for the early-release group abruptly declined several weeks prior to the middle of June (Figure 8).

High condition (energetic content) among the late-release group may have caused lower mortality compared to the early-release group after the middle of June. Sequential measurements of carbon-nitrogen ratios of juvenile pink salmon indicated that condition declined rapidly after release (See chapter 95320I, Confirming food web dependencies). These measurements were made on fish from the early-release group. The initial high condition of fish upon release was likely due to a period of feeding in net pens. Measurements of energy content were not made for the late release of large fry from the WHN Hatchery. However, it is likely that the condition of these fish was very high after more than 45 days of net pen feeding. In the present study, the condition of the early and late release groups was estimated from measurements of whole body wet weight (Table 11). This method of estimating condition may not be adequate for describing the energy content of fish due to differences in water content of tissues (Brett et al. 1969). Several studies have shown that predation rate is related to the condition of juvenile salmon (Bams 1967, Hatfield and Anderson 1972, Sylvester 1972, Ginetz & Larkin 1976, Olla and Davis 1989, Olla et al. 1992, Gadomski and Hall-Griswold 1992). Future studies should focus on condition-dependent predation on juvenile pink salmon.

It does not appear that the exponential decline in catch per net set of juvenile pink salmon from the early-release group was due to sampling error. In the present study, juvenile salmon were sampled with a small-mesh purse seine operated from a skiff. As juvenile pink salmon

approach 60-70 mm in length, they tend to move offshore and deeper in the water column (Hoar 1976, Cooney et al. 1978). In the present study, the relative abundance of the early-release group declined abruptly between early and late May, 1994 (Figure 8). Over this same time interval, the mean length of the early-release group increased from 38 to 42 mm (Willette et al. 1995a). It seems unlikely that this relatively small increase in mean length could account for the observed magnitude of the decline in catch per net set for the early-release group. It is possible that a significant number of fish from the early-release group moved out of our study area. However, previous juvenile CWT recovery surveys (1989-1991) conducted over a broader area documented that the majority of juvenile pink salmon released from WHN Hatchery typically move southward into Knight Island Passage (Willette et al. 1994).

High mortality among the early-release group largely caused the failure of the 1995 pink salmon return to the WHN Hatchery. Low survival of early-release groups and high survival of late-release groups was also observed at the Armin F. Koernig (AFK) Hatchery in southwest PWS (Table 11). The return of pink salmon to the AFK Hatchery also failed in 1995. Comparison of percent deviation of district-specific wild pink salmon escapement from the sound-wide mean indicates that wild pink salmon returns may have also been weak in western PWS in 1995 (Figure 9). These results suggest that high mortality among juvenile pink salmon prior to the middle of June may have been the cause of weak returns of both wild and hatchery-reared pink salmon in western PWS in 1995.

Conclusions:

1. Greater zooplankton biomass and abundances of large calanoid copepods in offshore habitats indicates that the growth potential for juvenile pink salmon may be greater offshore when growth is limited by low food abundance nearshore.

2. Several lines of evidence indicate that significant mortality may have occurred among an early group of juvenile pink salmon released from the WHN hatchery prior to the middle of June, 1994.

3. High mortality among early-release groups of juvenile pink salmon largely caused the failure of the 1995 pink salmon return to the WHN Hatchery. Low production of wild and hatchery-reared pink salmon throughout western PWS in 1995 may have been caused by high mortality of juveniles prior to the middle of June, 1994.

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Table 1: Estimated daily food consumption and foraging time for juvenile pink salmon at four sites in western Prince William Sound, 1995.

Date	Description	Temp. (deg. C)	Daily Food Consumption (%BW)	Foraging Time (hours)
5/8-5/9	W. Esther	6.0	4.8	9
5/9-5/10	S. Esther	6.0	4.5	12
5/30-5/31	Esther Pt.	7.0	28.0	24
6/1-6/2	Tipping Pt.	7.0	31.5	24

Table 2: Relative abundance of juvenile pink salmon in nearshore and offshore habitats at sixteen study sites in western Prince William Sound, 1995. Catch per net set in purse seines (PS) and tow nets (TN) was used as a measure of relative abundance in offshore habitats.

Date	Site	Description	Relative Abundance		
			Nearshore	Offshore (PS)	Offshore (TN)
5/3-5/4	501	Esther Pt.	high	157.2	-
5/5-5/6	506	Tipping Pt.	low	1.5	-
5/8-5/9	525	W. Esther Is.	moderate	1430.8	-
5/9-5/10	526	S. Esther Is.	high	1.9	-
5/11-5/12	501	Esther Pt.	high	0.9	-
5/13-5/14	525	W. Esther Is.	moderate	0.6	-
5/15-5/16	506	Tipping Pt.	low	0.2	-
5/17-5/18	502	Hodgkin Pt.	high	0.5	-
5/30-5/31	501	Esther Pt.	high	0.3	5.8
6/1-6/2	506	Tipping Pt.	moderate	3.6	5.5
6/3-6/4	525	W. Esther Is.	moderate	39.7	-
6/5-6/6	502	Hodgkin Pt.	moderate	575.3	4.0
6/8-6/9	506	Tipping Pt.	low	0.4	3.1
6/9-6/10	504	NE Culross	high	27.3	3.2
6/11-6/12	505	SE Culross	high	79.4	3.9
6/13-6/14	509	Herring Pt.	low	178.9	2.8

Table 3: Mean total zooplankton biomass (g m^{-3}) and mean abundance of large calanoid copepods (g m^{-3}), small calanoid copepods, and 'other' zooplankters at sixteen study sites in western Prince William Sound, 1995.

Date	Site	Description	n	Total	Lg. Cop.	Sm. Cop.	Other
5/3-5/4	501	Esther Pt.	20	.41	122	676	133
5/5-5/6	506	Tipping Pt.	8	.58	88	3255	157
5/8-5/9	525	W. Esther Is.	8	.33	78	1446	161
5/9-5/10	526	S. Esther Is.	11	.98	272	4340	386
5/11-5/12	501	Esther Pt.	15	.47	89	2381	209
5/13-5/14	525	W. Esther Is.	8	.47	103	1922	162
5/15-5/16	506	Tipping Pt.	6	.48	106	2873	397
5/17-5/18	502	Hodgkin Pt.	11	.44	48	3493	402
5/30-5/31	501	Esther Pt.	11	.69	123	5148	541
6/1-6/2	506	Tipping Pt.	1	1.79	115	12116	1077
6/3-6/4	525	W. Esther Is.	12	.50	49	3495	586
6/5-6/6	502	Hodgkin Pt.	3	.54	23	3598	366
6/8-6/9	506	Tipping Pt.	4	.38	37	1855	459
6/9-6/10	504	NE Culross	16	.72	24	3726	580
6/11-6/12	505	SE Culross	14	.28	21	2618	370
6/13-6/14	509	Herring Pt.	11	.53	29	1601	330

Table 4: Number of samples collected for later stomach contents analysis under project 96163 'Forage Fish Influence on Recovery of Injured Species.

Site	Date	Greenling	Wolfish	Herring	Tom Cod	Pollock	Capelin	Sandlance	Stickleback
521	5/2			21			48		
501	5/3						56		
506	5/5			36		12	12		
525	5/8	5					12		
526	5/9								23
501	5/12								93
525	5/13						35		12
506	5/16	20					24		10
502	5/17	24		12					51
517	5/26							12	
528	5/27	12		48				12	
501	5/30	36					24		96
506	6/1	9	13	12			24	30	
525	6/3					47			24
502	6/5	17					12		
506	6/8	2						13	
504	6/9	14	15			71			12
505	6/12				12	151	36		
509	6/13	54	2	11		171			
521	6/14	12							
Total		205	30	140	12	452	283	67	321

Table 5: Mean length, body weight, and condition of juvenile pink salmon collected at sixteen study sites in western Prince William Sound, 1995.

Date	Site	Description	Length (mm)	Weight (g)	Condition
5/3-5/4	501	Esther Pt.	32.0	.20	3.22
5/5-5/6	506	Tipping Pt.	32.7	.21	2.47
5/8-5/9	525	W. Esther Is.	32.5	.20	1.81
5/9-5/10	526	S. Esther Is.	34.6	.26	2.85
5/11-5/12	501	Esther Pt.	33.5	-	-
5/13-5/14	525	W. Esther Is.	36.1	-	-
5/15-5/16	506	Tipping Pt.	36.1	-	-
5/17-5/18	502	Hodgkin Pt.	39.5	-	-
5/30-5/31	501	Esther Pt.	40.6	.50	3.18
6/1-6/2	506	Tipping Pt.	48.9	.90	1.95
6/3-6/4	525	W. Esther Is.	43.3	-	-
6/5-6/6	502	Hodgkin Pt.	49.2	-	-
6/8-6/9	506	Tipping Pt.	53.1	-	-
6/9-6/10	504	NE Culross	52.0	-	-
6/11-6/12	505	SE Culross	54.4	-	-
6/13-6/14	509	Herring Pt.	58.9	-	-

Table 6: Mean growth (%BW day^{-1}) of coded-wire tagged juvenile pink salmon released from four hatcheries in Prince William Sound, 1989-1994.

Hatchery	Treatment	1989	1990	1991	1992	1993	1994	1995
AFK	Early Fed	3.77 (39)	4.30 (133)	3.12 (146)	4.13 (159)	4.41 (12)	4.59 (7)	-
	Direct Release	2.56 (22)	3.81 (50)	3.08 (13)	4.14 (32)	4.11 (5)	4.74 (7)	-
	Late Fed	2.16 (36)	3.35 (20)	3.12 (18)	5.14 (14)	6.11 (37)	4.62 (24)	-
WHN	Early Fed	4.12 (24)	3.96 (80)	3.38 (148)	4.43 (3)	4.57 (2)	4.26 (162)	4.21 (181)
	Direct Release	3.89 (9)	3.65 (12)	2.80 (19)	4.04 (1)	4.49 (1)	-	4.37 (27)
	Late Fed	9.19 (5)	7.09 (11)	2.09 (15)	-	6.84 (10)	-	3.37 (56)
CCH	Early Fed	5.64 (6)	4.76 (17)	3.08 (18)	-	-	5.25 (9)	4.21 (1)
	Direct Release	4.00 (8)	4.61 (8)	-	-	4.12 (1)	-	-
	Late Fed	3.87 (10)	5.29 (54)	2.80 (4)	4.48 (3)	6.84 (4)	5.92 (56)	-
SGH	Early Fed	5.60 (6)	6.53 (1)	-	-	2.22 (2)	4.32 (30)	-
	Direct Release	-	-	-	-	-	4.61 (24)	-
	Late Fed	5.10 (1)	2.50 (5)	-	-	-	-	-

AFK - Armin F. Koernig Hatchery
WHN - Wally H. Noerenberg Hatchery
CCH - Cannery Creek Hatchery
SGH - Solomon Gulch Hatchery

Table 7: Tests for differences in mean total zooplankton biomass and the abundance of large copepods, small copepods, and other zooplankters between nearshore and offshore stations during May and June. All stations used in analysis.

Month	Variable	Mean Nearshore	Mean Offshore	P-value
May	total biomass	.46	.65	.003
	abdn. lg. copepods	95	159	.005
	abdn. sm. copepods	1967	2020	.908
	abdn. other zoops.	219	223	.933
June	total biomass	.48	.74	.001
	abdn. lg. copepods	31	80	.004
	abdn. sm. copepods	3687	4291	.309
	abdn. other zoops.	529	605	.306

Table 8: Tests for differences in mean total zooplankton biomass and the abundance of large copepods, small copepods, and other zooplankters between nearshore and offshore stations during May and June. Only 20 m vertical net tows.

Month	Variable	Mean Nearshore	Mean Offshore	P-value
May	total biomass	.49	.65	.024
	abdn. lg. copepods	106	159	.063
	abdn. sm. copepods	1599	2020	.303
	abdn. other zoops.	194	223	.481
June	total biomass	.45	.69	.007
	abdn. lg. copepods	22	74	.006
	abdn. sm. copepods	2993	3868	.117
	abdn. other zoops.	480	602	.126

Table 9: Tests for differences in mean total zooplankton biomass and the abundance of large copepods, small copepods, and other zooplankters among 3 levels of current speed (low: <10 m sec-1, medium: >.10 m sec-1 and < .20 m sec-1, and high: >.20 m sec-1) during May and June. Only 20 m vertical net tows.

Month	Variable	Low	Medium	High	P-value
May	total biomass	.34	.60	.48	.196
	abdn. lg. copepods	103	118	86	.496
	abdn. sm. copepods	1151	2261	1994	.420
	abdn. other zoops.	136	293	193	.038
June	total biomass	.43	.45	.45	.989
	abdn. lg. copepods	45	19	17	.163
	abdn. sm. copepods	2301	3498	2908	.497
	abdn. other zoops.	337	484	446	.817

Table 10: Mean catch per unit effort of potential predatory fish at sixteen study sites in western Prince William Sound, 1995.

Site	Relative Fry Abund.	n	Gear Type		
			Trawl	Seine	Fixed
Tipping Pt.	low	3	24.0	7.7	1.6
Herring Pt.	low	1	6.7	176.3	1.6
Hodgkin Pt.	moderate	1	42.7	42.9	2.8
Tipping Pt.	moderate	1	58.0	7.7	2.0
W. Esther Is.	moderate	1	46.7	147.4	2.3
Esther Pt.	high	3	47.9	73.3	2.2
Hodgkin Pt.	high	1	3.2	125.7	2.2
NE Culross	high	1	33.7	24.0	2.6
SE Culross	high	1	146.9	225.2	2.0
S. Esther Is.	high	1	32.8	47.6	1.4
Statistical Significance (P)			.315	.923	.865

Table 11: Survival to adult of the early-fed and late-fed groups released from the Wally H. Noerenberg Hatchery in 1994.

Hatchery	Release Date	Length (mm) at Release	Number Released (millions)	Survival to Adult (%)
AFK	early May	30	84.8	.36
AFK	early June	50	7.0	7.21
WHN	early May	33	154.7	.38
WHN	early June	55	7.7	22.12

Table 12: Biological characteristics of the early-fed and late-fed groups released from the Wally H. Noerenberg Hatchery and recovered after mid-June, 1994.

Characteristic	Treatment Group		P-value
	Early-fed	Late-fed	
Growth Rate (% BWday^{-1})	4.86	5.19	.247
Length-adj. Body Weight (g)	1.60	1.57	.351
Length (mm)	84.6	78.8	.002

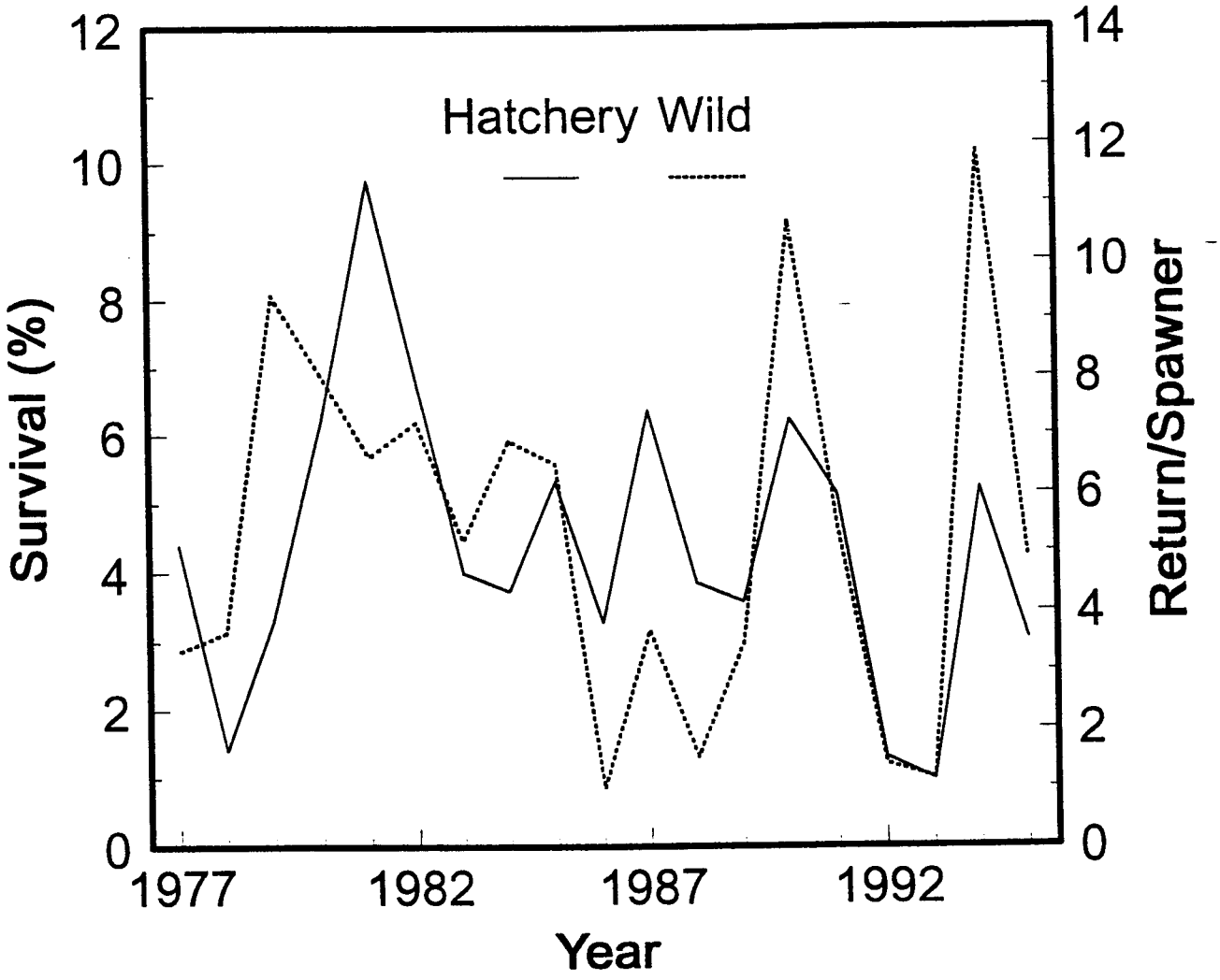


Figure 1: Survival of hatchery-reared pink salmon and return per spawner of wild pink salmon in Prince William Sound, 1977-1995..

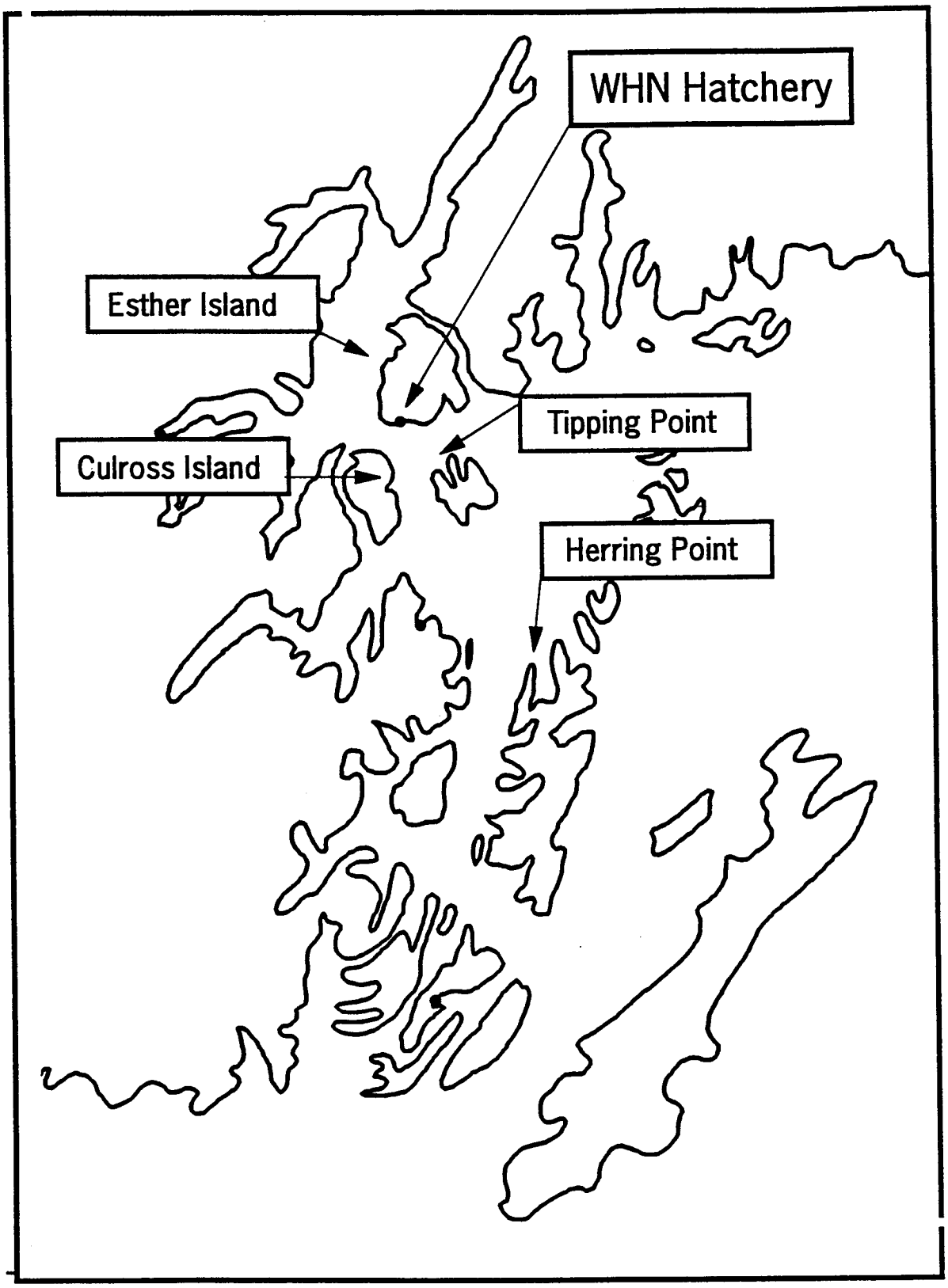


Figure 2: Study sites in western Prince William Sound.

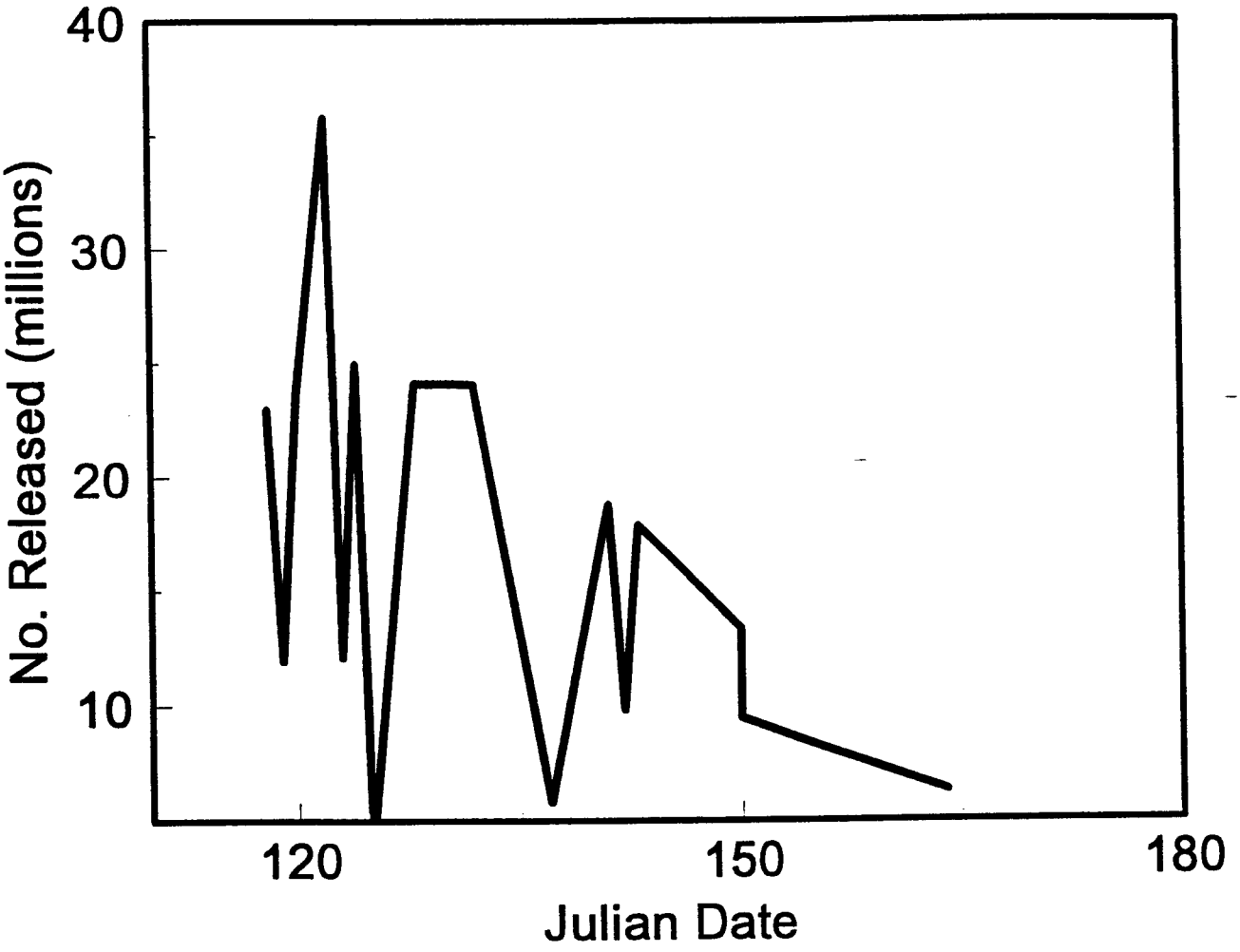


Figure 3: Timing of releases of juvenile pink salmon from the Wally H. Noerenberg Hatchery in 1995.

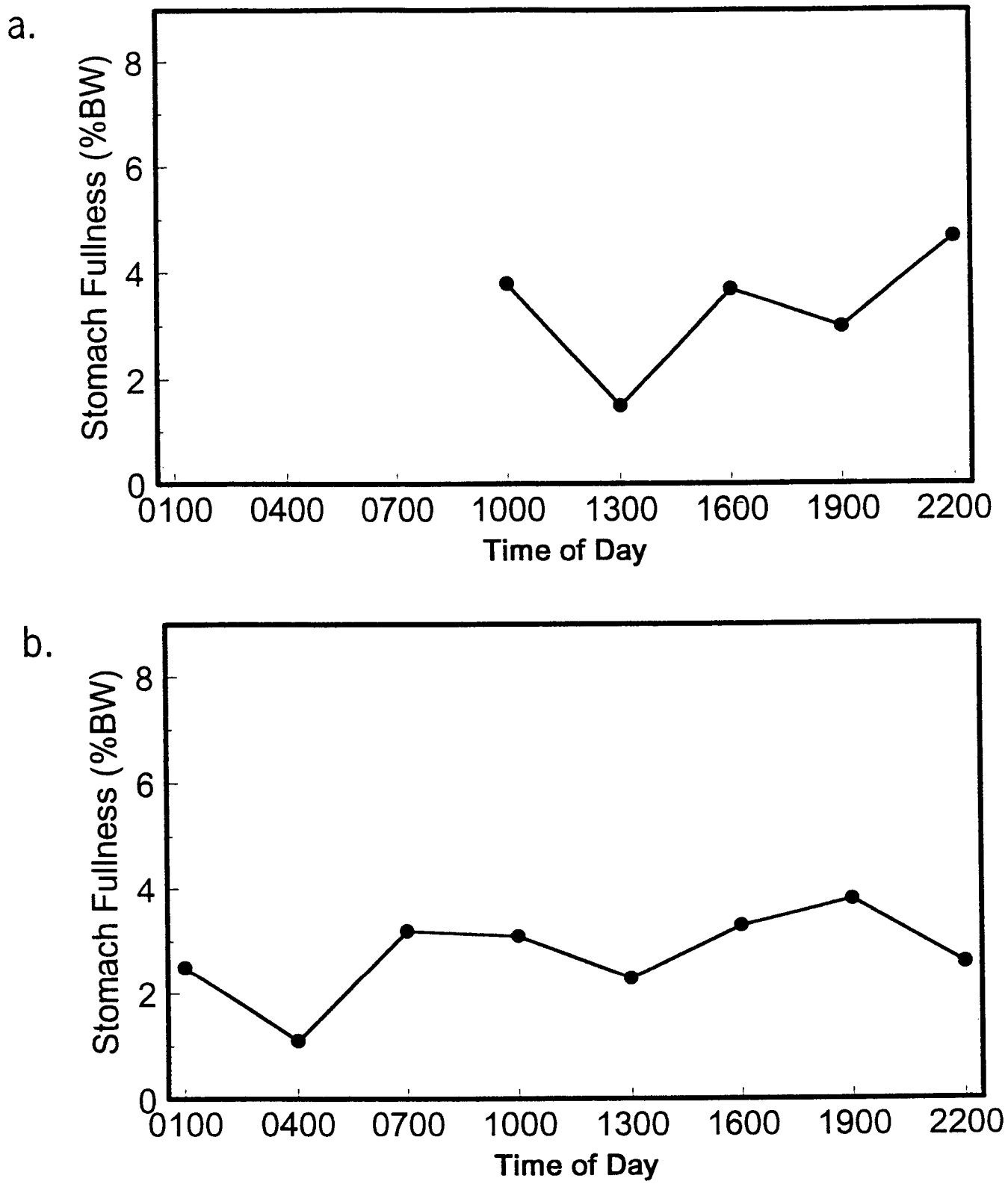
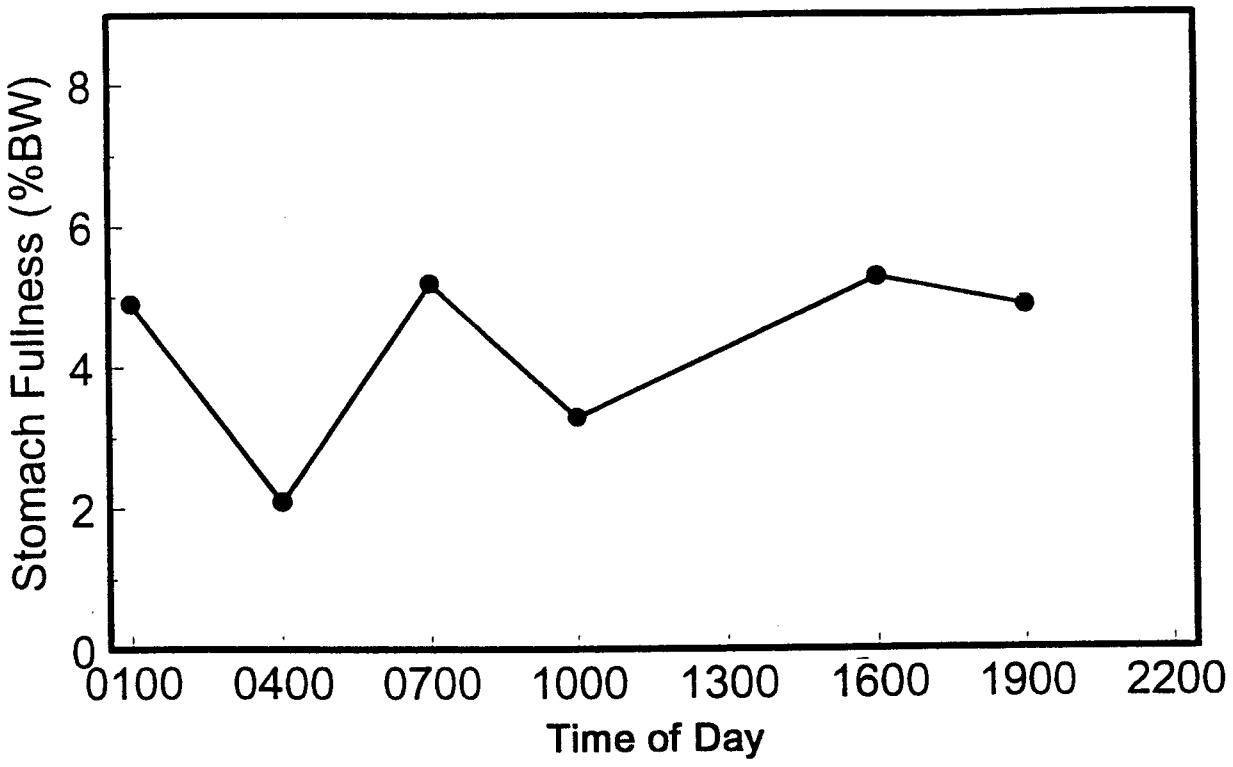


Figure 4: Stomach fullness of juvenile pink salmon sampled along the (a) west coast of Esther Island (May 8-9) and (b) south coast of Esther Island (May 9-10).

c.



d.

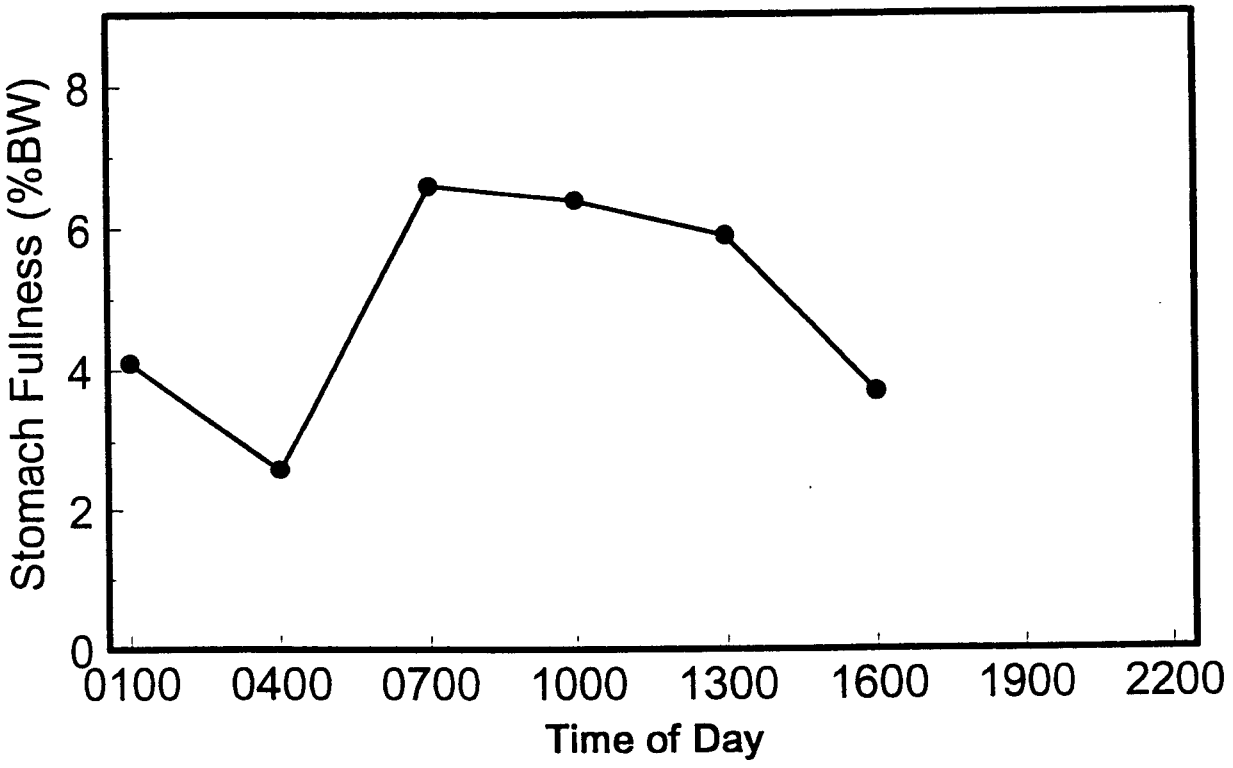


Figure 4: Stomach fullness of juvenile pink salmon sampled (c) near Esther Point (May 30-31)and (d) near Tipping Point (June 1-2).

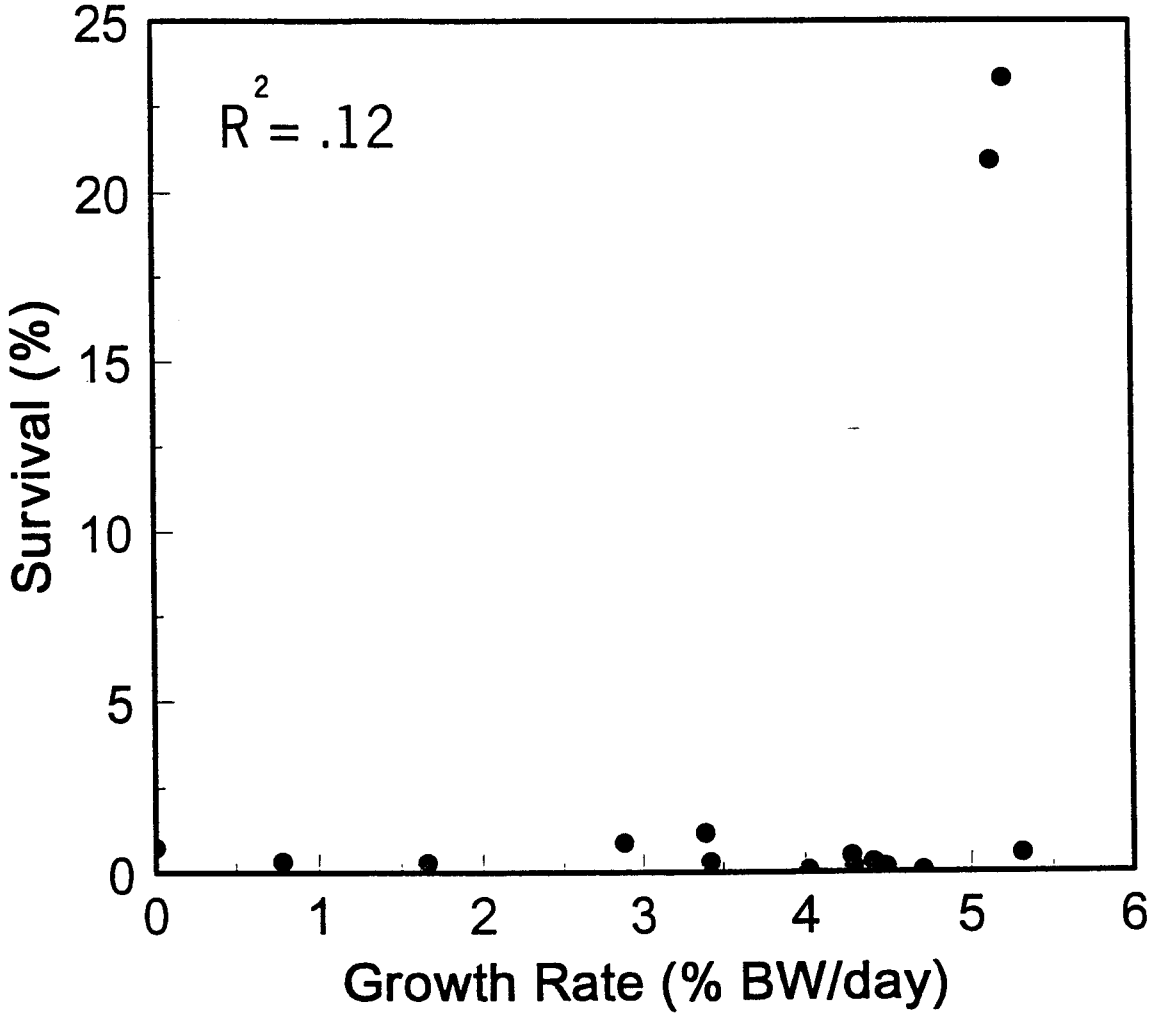


Figure 5: Relationship between growth rate of juvenile pink salmon and survival to adult for fish released from the Wally H. Noerenberg Hatchery in 1994.

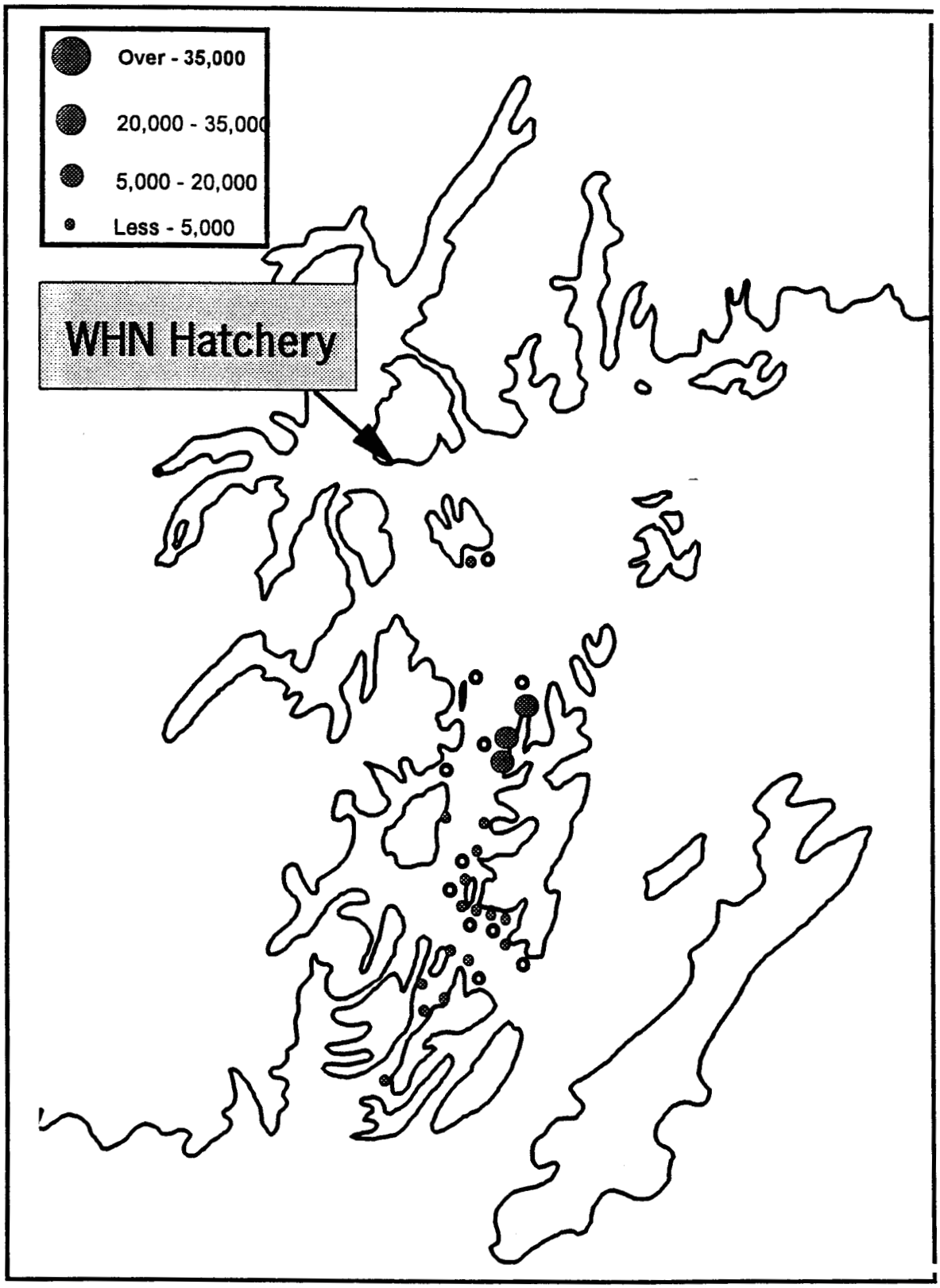


Figure 6: Distribution of the early and late groups released from the Wally H. Noerenberg (WHN) Hatchery and recovered in late June and early July, 1994. Solid circles indicate the early release group and open circles indicate the late release group.

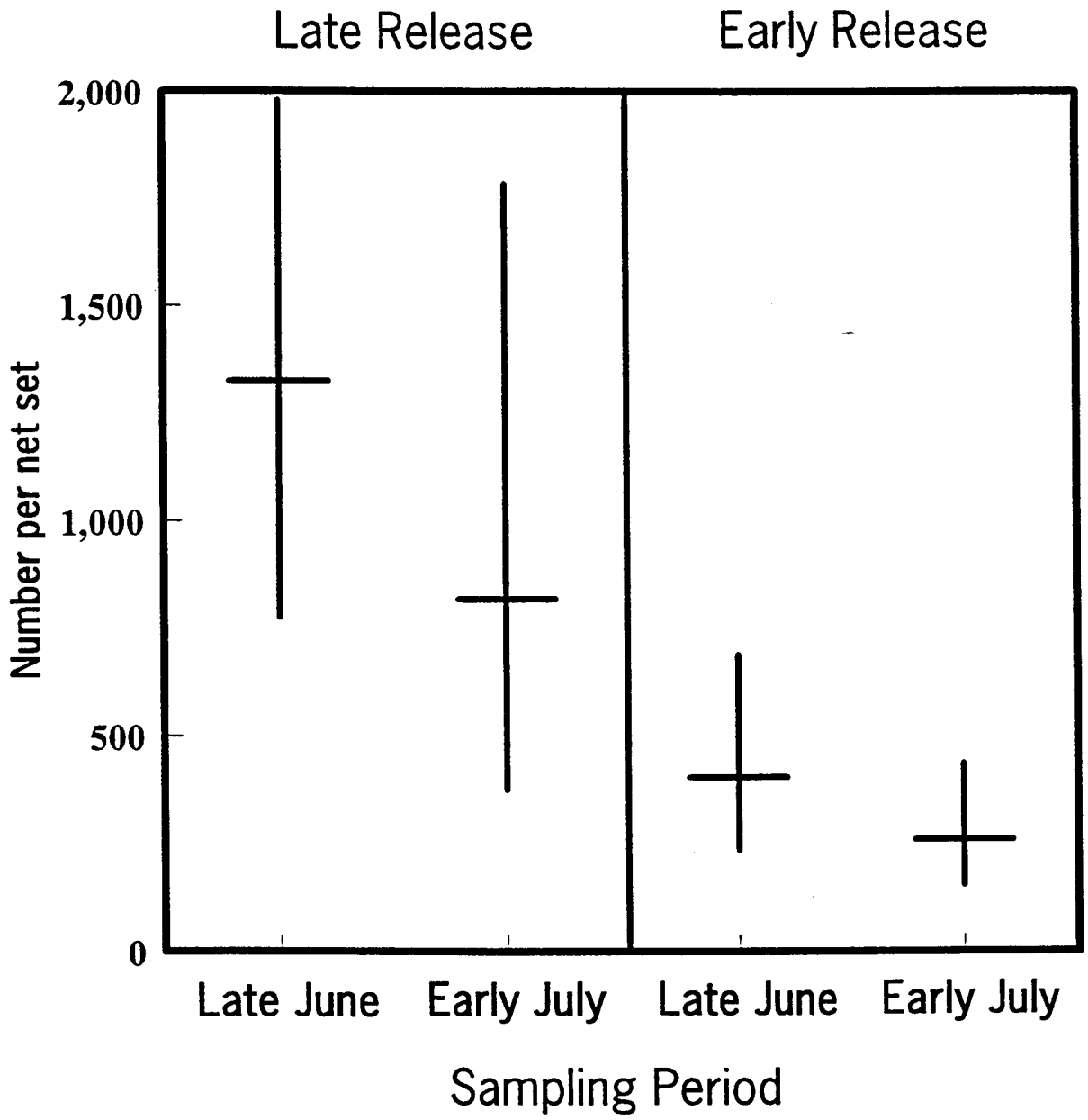


Figure 7: Comparison of catch per net set of juvenile pink salmon for the early and late groups released from the Wally H. Noerenberg Hatchery in 1994.

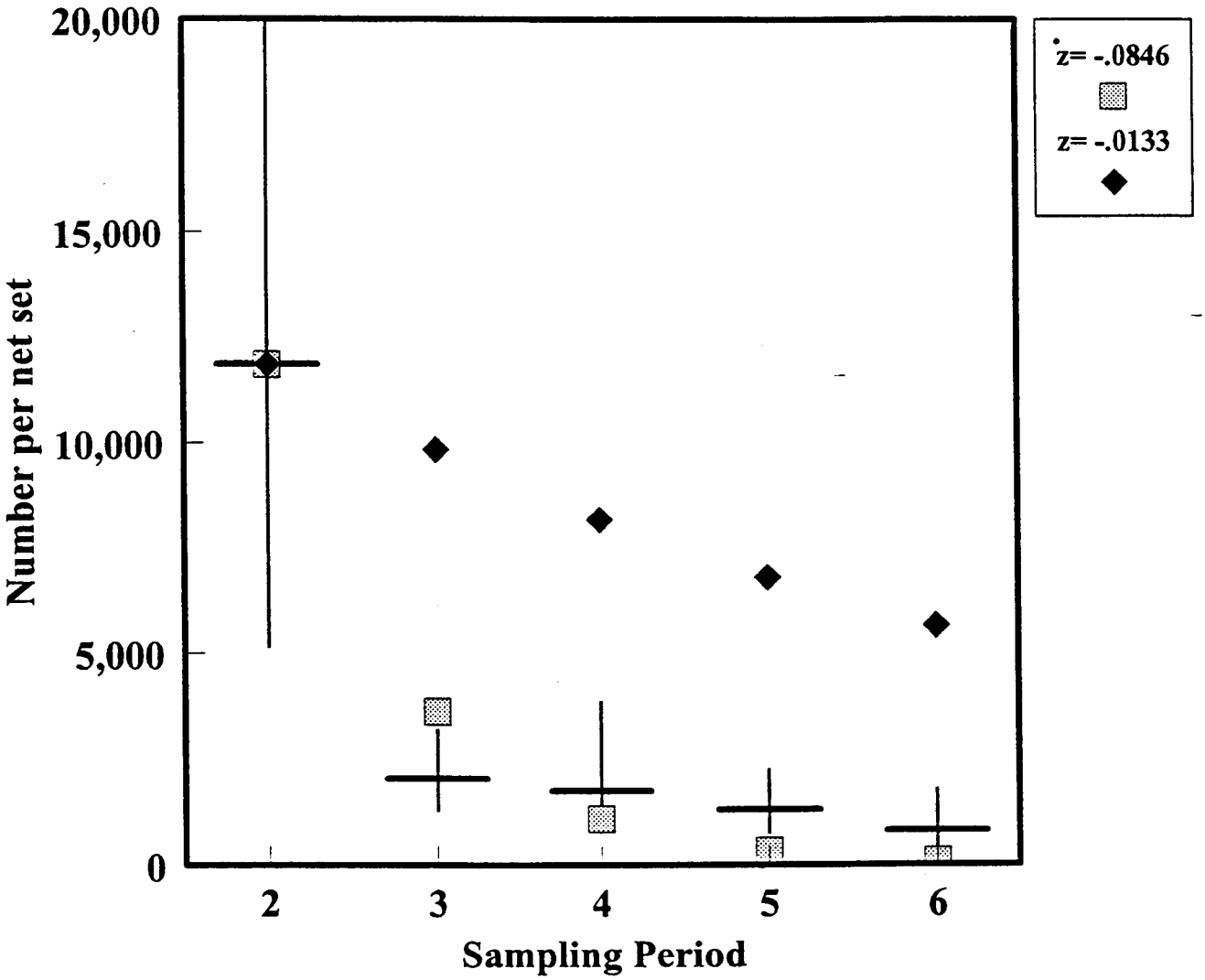


Figure 8: Estimated catch per net set of juvenile pink salmon from the early group released from the Wally H. Noerenberg Hatchery in 1994 and simulated relative abundance at two assumed levels of instantaneous mortality.

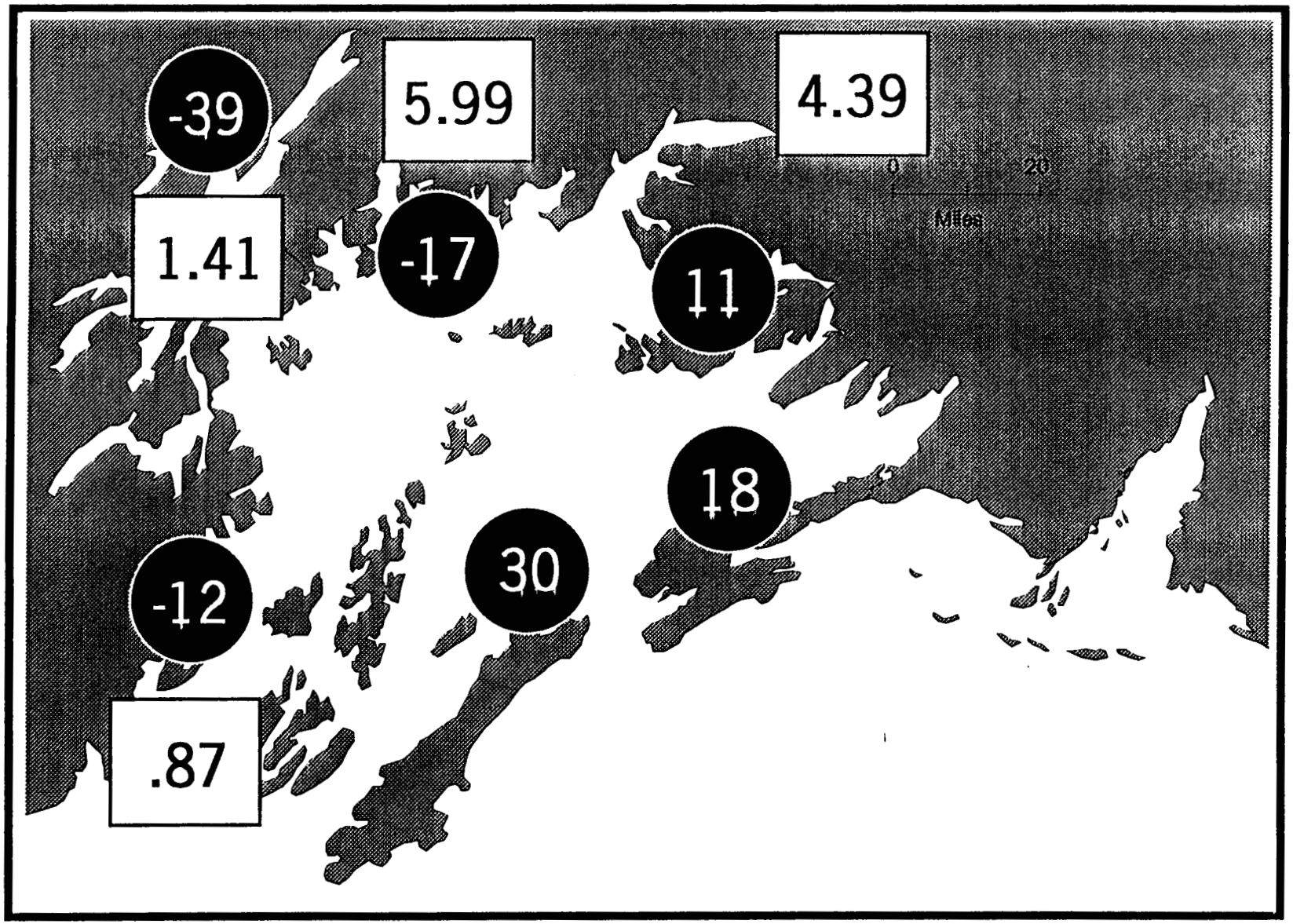


Figure 9: District-specific pink salmon escapement deviations from the sound-wide mean (circles) and survival of pink salmon returning to four hatcheries (squares) in Prince William Sound, 1995.

Exxon Valdez Oil Spill
Restoration Project Annual Report

Coded Wire Tag Recoveries from Pink Salmon in Prince William Sound Salmon Fisheries, 1995

Restoration Project 95320B
Annual Report

This annual report has been prepared for peer review as part of the *Exxon Valdez* Oil Spill Trustee Council restoration program for the purpose of assessing project progress. Peer review comments have not been addressed in this annual report.

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Seawan Gehlbach
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Restoration Project 95320B
Annual Report

Study History: The pink salmon coded wire tag program in Prince William Sound was initiated in 1986 to partition returns of pink salmon into wild and hatchery stocks, and to determine the size of the hatchery return. After the *Exxon Valdez* oil spill, the program was incorporated into Natural Resource Damage Assessment Fish/Shellfish Study Number 3, to document effects of the spill on wild pink salmon by comparing returns to oiled and unoiled streams, as well as to estimate the size of hatchery and wild stock returns. The project continued under Restoration Study Number 60A (Coded wire tag studies on Prince William Sound pink salmon), Restoration Project 93067 (Coded wire tag recoveries from pink salmon in Prince William Sound salmon fisheries, 1993), and Restoration Project 94320B (Coded wire tag recoveries from pink salmon in Prince William Sound salmon fisheries, 1994).

Abstract: During 1994, approximately half billion pink salmon fry were released into Prince William Sound from the A.F. Koernig, W.H. Noerenberg, Cannery Creek, and Solomon Gulch hatcheries. About one million were tagged with half-length coded wire tags. Tags from these releases were recovered in the 1995 commercial catch. Estimates of hatchery contributions based upon detected tags, a historical W.H. Noerenberg adjustment factor (1989-1994) to account for tag loss and differential mortality, and an overall expansion factor were given to management biologists. These estimates did not agree with postseason estimates due to the extraordinary survival of experimental releases that were tagged at a higher rate. Postseason analysis using tag-specific expansion factors and an updated historical W.H. Noerenberg adjustment factor (1989-1995) revealed that of 17.16 million pink salmon caught commercially in 1995, the A.F. Koernig, W.H. Noerenberg, Cannery Creek and Solomon Gulch hatcheries contributed 0.78 million, 2.37 million, 3.17 million, and 6.76 million pink salmon, respectively. The wild contribution was 4.08 million. The 1995 Cannery Creek hatchery contribution may have been underestimated due to tag shedding problems. The overall survival rates for pink salmon from A.F. Koernig, W.H. Noerenberg, Cannery Creek, and Solomon Gulch hatcheries were 0.83%, 1.42%, 3.75% and 4.52%, respectively.

Key Words: Coded wire tag, commercial harvest, hatcheries, *Oncorhynchus gorbuscha*, pink salmon, Prince William Sound, wild stock.

Citation:

Riffe, R.R., S. Gehlbach, D.G. Evans, and B.G. Bue. 1996. Coded wire tag recoveries from pink salmon in Prince William Sound salmon fisheries, 1995, *Exxon Valdez* Oil Spill Restoration Project Annual Report (Restoration Project 95320B), Alaska Department of Fish and Game, Commercial Fisheries Management and Development Division, Cordova, Alaska.

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EXECUTIVE SUMMARY

This report documents Restoration Study 95320B, one of the projects designed to restore the pink salmon *Oncorhynchus gorbuscha* resource of Prince William Sound to its pre-spill status. Coded wire tags applied in 1994 at four hatcheries in Prince William Sound, the W.H. Noerenberg, Cannery Creek, A. F. Koernig and Solomon Gulch facilities, were recovered in the commercial catch of 1995 and used to provide inseason and postseason estimates of hatchery contributions. Inseason estimates were used by fishery managers to target the numerically superior hatchery returns, and thus to reduce the pressure placed upon oil-damaged wild stocks. Inseason estimates were made in two stages. Preliminary estimates were based solely on detected tags (not extracted) and were made available to managers upon completion of sampling. These estimates were then updated approximately three days later with code-specific information.

The postseason analysis revealed that out of a commercial catch of 17.16 million pink salmon, 4.08 million fish were estimated to be of wild origin. Of the hatchery component (estimated at 13.08 million pink salmon), 0.78 million, 2.37 million, 3.17 million, and 6.76 million originated from the A.F. Koernig, W.H. Noerenberg, Cannery Creek and the Solomon Gulch hatcheries, respectively. Overall adult survival rates of hatchery reared pink salmon were 0.83%, 1.42%, 3.75%, and 4.52%, for the A.F. Koernig, W.H. Noerenberg, Cannery Creek, and Solomon Gulch facilities, respectively.

INTRODUCTION

Between 1961 and 1976, when hatcheries were absent from Prince William Sound, the commercial seine harvest of wild pink salmon *Oncorhynchus gorbuscha* averaged about 3.4 million fish. In the early 1970's, run failures led to an aggressive enhancement program which included construction of hatcheries. By 1986 five hatcheries were operating in Prince William Sound (Figure 1): the Solomon Gulch hatchery, producing pink salmon, and later, chum *O. keta*, and coho salmon *O. kisutch*, the A. F. Koernig hatchery, producing pink salmon, the W.H. Noerenberg hatchery, producing pink salmon, and later, chum, coho and chinook salmon *O. tschawytscha*, the Cannery Creek hatchery, producing pink salmon, and the Main Bay hatchery which produced chum and presently raises sockeye salmon *O. nerka*.

To protect wild stocks in a hatchery-dominated fishery, managers needed information pertaining to the temporal and spatial distributions of hatchery and wild fish. To meet this requirement, a coded wire tagging program was initiated in 1986 for hatchery releases of pink salmon with recovery of tagged returning adults in commercial and cost-recovery fisheries beginning in 1987. Tag recovery data enabled managers to estimate hatchery and wild contributions to catches from temporal and spatial strata within the fishery.

The March 24, 1989, *Exxon Valdez* oil spill exacerbated the problems faced by the fishery manager. The spill contaminated intertidal portions of streams where the majority of wild salmon stocks in western Prince William Sound spawn as well as the marine waters traversed by juvenile salmon on their migration seaward through the Sound. The decisions made by fishery managers suddenly became more complicated in so far as they affected wild populations injured by the oil spill. The coded wire tagging program was expanded under the Natural Resource Damage Assessment Fish/Shellfish # 3 study (Sharr et al, 1995a), and Restoration Studies 60A, 93067 and 94320B (Sharr et al, 1995b,c and e) to include tagging of wild fish, which allowed comparison of the survival rates of wild salmon in oiled versus unoiled streams. In recent years, the emphasis of the program has been to provide management biologists with timely data on the relative abundances of wild and hatchery stocks, and has allowed direction of fishing effort towards the hatchery returns. For 1995, the program was supported by Restoration study 95320B, along with matching funds from the Prince William Sound Aquaculture Corporation, the Valdez Fisheries Development Association, and the Alaska Department of Fish and Game.

This report documents the activities and results of the coded wire tag program for the 1995 recovery year. It focuses primarily upon hatchery contributions to the different fisheries, survival rates of different hatchery release groups, and inseason estimation of contributions. Unaggregated data is presented in appendices .

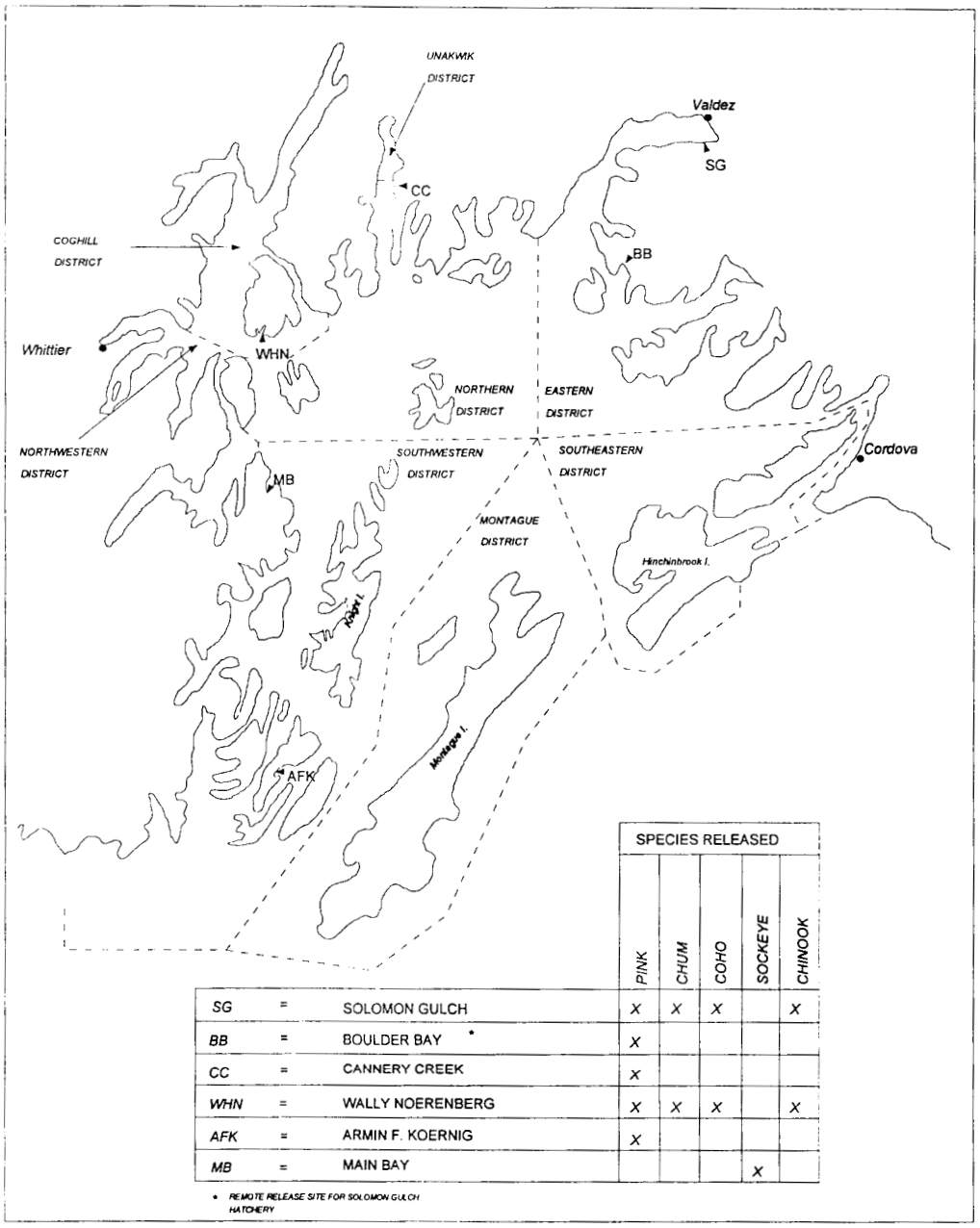


Figure 1 Fishing districts and hatcheries of Prince William Sound, Alaska

OBJECTIVES

1. To make determinations of wild and hatchery components of the pink salmon commercial fisheries of 1995 and to make these available to fishery managers on an inseason basis, so that fishing effort may be directed towards hatchery stocks.

2. To estimate marine survival rates for each uniquely coded hatchery release group returning in 1995.

3. To evaluate the method selected in 1993 for inseason analysis of coded wire tag data, whereby an historical adjustment factor and numbers of detected (undecoded) tags are used to estimate the hatchery and wild contributions.

METHODS

Tagging

Tagging of pink salmon fry occurred at the three Prince William Sound Aquaculture Corporation facilities (W.H. Noerenberg, Cannery Creek, and A. F. Koernig hatcheries) and at the Valdez Fisheries Development Association facility (Solomon Gulch hatchery). Tagging rates and recovery efforts should yield contribution estimates which are sufficiently precise to allow fishery managers to make meaningful inseason decisions. Assuming a potential sampling rate of approximately 20% of all commercial and cost-recovery harvests and following an analysis of the performance of previous tagging studies (Peltz and Miller 1990; Peltz and Geiger 1990; Geiger and Sharr 1990), an overall tagging rate of 0.00167 (1 coded wire tag per 600 fish) was chosen. A different tag code was given to each release group, a release group representing a batch of fish subjected to a certain feeding regimen (early feeding, late feeding or no feeding) and release timing. During 1994, some fish were tagged at a rate of 0.005 (1 coded wire tag per 200 fish), or three times the normal overall tagging rate. These lots were part of a Sound Ecosystem Assessment experiment to ascertain whether juvenile salmon above 60 mm in length had higher survival rates than those less than 60 mm in length.. The A.F. Koernig and W.H. Noerenberg facilities both had 2 lots of the fish tagged at the higher rate. In addition, at the W.H. Noerenberg facility, about 7,000 tagged fish meant to represent a "normal" release group were inadvertently dumped into a pen containing fish in the Sound Ecosystem Assessment predator-prey experiment. The tag code associated with these fish was voided. Fish marked with this tag were treated as a separate release group, and a subsample of fry were marked with a different code to represent the fish in the original release group.

Pink salmon fry to be tagged were randomly selected as they emerged from incubators. Fry were anesthetized in a 1 ppm solution of MS-222 prior to removal of adipose fins and application of tags. Half-length coded wire tags were applied with a Northwest Marine Technology tag injector (model MKIV). Adipose fin-clipped and tagged fish were passed through an electronic quality control device to test for tag retention. Rejected fish were held and retested later. If rejected a second time, they were killed to minimize the number of untagged clipped fish in the release. Fry which retained tags were held overnight at the Prince William Sound Aquaculture Corporation hatcheries and for 72 hours at the Solomon Gulch facility to determine short-term mortality and tag-loss. Mortality was determined by counting the number of fish floating on the surface after the holding period. The tag loss rate was estimated by randomly selecting 200 fish and testing them with the quality control device before release into saltwater rearing pens. Tag placement was checked periodically, but not quantified.

At the Prince William Sound Aquaculture Corporation hatcheries, after the overnight holding period and prior to release, all tagged fry were introduced into saltwater pens within the larger pens holding their unmarked cohorts. This allowed determination of short-term saltwater mortalities through enumeration of floating mortalities. At the Solomon Gulch hatchery, tagged fry were transferred to the saltwater net pen holding their unmarked cohorts following the 72 hour mortality check in freshwater; no saltwater mortality estimate was made on the tagged fish. The number of fry released with tags of tag code *t*, Tr_t, was estimated for each release group by deducting both the short-term tagging and saltwater rearing mortalities (for the Prince William Sound Aquaculture Corporation facilities) from the number of fry initially tagged, and accounting for tag loss :

$$\hat{Tr}_t = (T_t - Mo_t - Msw_t)(1 - \hat{Lo}_t)\,, \qquad (1)$$

where,

T_t	=	total number of tagged (*t*) fish
Mo_t	=	number of deaths during holding period among tagged (*t*) fish
Msw_t	=	number of deaths during saltwater rearing period among tagged (*t*) fish (Prince William Sound Aquaculture Corporation only) and,
Lo_t	=	proportion of tagged (*t*) fish which lost their tags during the holding period.

At all Prince William Sound Aquaculture Corporation facilities, unmarked fry entering the large saltwater rearing pens were enumerated with electronic fry counters, while at the Solomon Gulch hatchery, numbers of unmarked fry released were estimated from an inventory of embryos and subsequent mortality. Pink salmon fry mortalities were estimated visually immediately prior to release. These mortality estimates were applied equally to tagged and untagged fish to obtain

final release estimates. With the exception of experimental release groups, fry releases were timed to coincide with peak plankton abundances near the hatcheries.

Tag Recovery

Commercial and Cost-Recovery Harvests

Recoveries were stratified by district, week, and processor. This stratification was chosen as a result of the findings of Peltz and Geiger (1990) who detected significant differences between the proportions of some tag codes among such strata. The differences indicate that processors tend to receive catches from only certain parts of a district and is believed to be the result of traditional tendering patterns.

Recoveries of pink salmon tags from commercial and cost-recovery harvests were made after each opening as the fish were pumped from tenders onto conveyor belts at land-based processors located in Cordova, Valdez, Seward, Anchorage, Whittier, Kodiak, Kenai, Uganik Bay and aboard two floating processors in PWS. Technicians sampled fish that were moving down the conveyer belt, and subjected each sampled fish to a visual and tactile examination for a missing adipose fin.

Data recorded for each tender included harvest type (i.e., commercial or cost-recovery catch), fishing district(s) from which the catch was taken, catch date, processor, and the number of fish examined. Catch data were later verified from fish tickets.

Heads of adipose-fin clipped fish were excised, identified with a uniquely numbered cinch strap and bagged. Once sampling was finished, individual heads were passed through a Northwest Marine Technology field sampling tag detector. The detector produced an audible signal upon detection of a metal tag in the head. This procedure yielded the numbers of tags in the sample.

All heads were then frozen, and together with sample data, were shipped twice weekly from each site to the Alaska Department of Fish and Game Coded Wire Tag Processing Laboratory in Juneau. Laboratory staff located and removed tags from heads, decoded extracted tags, and entered tag code and sample data into a database accessible to biologists in Cordova.

Brood Stock Harvests

Tag shedding from release to return and differential mortality between tagged and untagged fish lead to discrepancies between marking rates at release and recovery. Hatchery brood stocks were scanned for tags in order to estimate adjustment factors which could be used to account for the loss of tags from the population. Three assumptions inherent in the use of the brood stock

for this purpose are: a) the brood stock consists only of fish reared at the hatchery, b) the tendency for a tagged fish to lose a tag or to die is similar for all fish marked at the same hatchery, and c) for a specific tag code, the marking rate in the commercial fishery is the same as that in the brood stock. It is believed that the first of these assumptions is violated at all facilities except the W.H. Noerenberg hatchery (Sharr et al. 1995c). Consequently, only the adjustment factor calculated from the brood stock from the W.H. Noerenberg hatchery was considered an appropriate quantity with which to adjust for tag loss and differential mortality. Historical average W.H. Noerenberg adjustment factors were used for both inseason (1989-1994) and postseason (1989-1995) estimations.

The adjustment factor for the W.H. Noerenberg hatchery for a given year was estimated as the ratio of sampled fish in the brood stock to the expanded number of fish based on tags found in the sample :

$$\hat{a} = \frac{s}{\sum_{i}^{T} \frac{x_i}{p_i}} \qquad (2)$$

where

T	=	number of tag codes released from the W.H. Noerenberg hatchery *in 1994*,
p_i	=	tagging rate at release for the *i*th tag code (defined as number of tagged fish released with the *i*th code divided by the total number of fish in release group *i*),
x_i	=	number of tags of the *i*th code found in *s* and,
s	=	number of brood stock fish examined at the *W.H.* Noerenberg facility in 1995

The W.H. Noerenberg historical average adjustment factor was then used to adjust contribution estimates (Equation 3) if it could be shown that it was significantly greater than 1.0 at the 90% level. An appropriate test of the hypothesis : $Ho : a \leq 1.0$ is given in Sharr *et al.* (1995a).

While only the adjustment factor associated with the W.H. Noerenberg facility was used in contribution estimations, brood stock samples were taken during hatchery egg-take operations at all four Prince William Sound pink salmon hatcheries, and adjustment factors calculated. Technicians stationed at each hatchery examined approximately 99% of the fish by visual and tactile means for missing adipose fins. The number of fish sampled was recorded daily. When adipose-clipped fish were found, the heads were excised and shipped on a weekly basis along with sample data to the Tag Lab.

Estimation of Contributions and Survival Rates

Postseason Hatchery Contributions and Survival Rates

The contribution of release group t to the sampled common property, cost-recovery, brood stock and special harvests, and escapement, C_t , was estimated as:

$$\hat{C}_t = \sum_{i=1}^{L} x_{it}\left(\frac{N_i \hat{a}}{s_i p_t}\right) \qquad (3)$$

where

x_{it}	=	number of group t tags recovered in the ith stratum,
N_i	=	total number of fish in the ith stratum,
s_i	=	number of fish sampled from the ith stratum,
p_t	=	proportion of group t tagged,
a	=	historical adjustment factor associated with W.H. Noerenberg facility and,
L	=	number of recovery strata associated with common property, cost-recovery, brood stock, special harvests and escapement in which tag code t was found.

The contribution of release group t to unsampled strata, Cu_t , was estimated from contribution rates associated with strata which were sampled from the same district-week openings as the unsampled strata:

$$\hat{C}u_t = \sum_{i=1}^{U}\left[N_i * \left(\frac{\sum_{j=1}^{S} \hat{C}_{tj}}{\sum_{j=1}^{S} N_j} \right) \right] \qquad (4)$$

where

U	=	number of unsampled strata,
N_i	=	number of fish in ith unsampled stratum
S	=	number of strata sampled in the period in which the unsampled stratum resides,
C_{tj}	=	contribution of release coded with tag t to the sampled stratum j, and
N_j	=	number of fish in jth sampled stratum.

When a district-week opening was not sampled at all (an infrequent occurrence), the catch from that opening was treated as unsampled catch of th subsequent opening in the same district.

An estimate of the contribution of tag group t to the total Prince William Sound return for 1995 was obtained through summation of contribution estimates for sampled and unsampled strata. An estimate of the total hatchery contribution to the Prince William Sound return was calculated through summation of contributions over all release groups.

A variance approximation for $\hat{C}_t$, derived by Clark and Bernard (1987) and simplified by Geiger (1990) was used:

$$\hat{V}(\hat{C}_t) = \sum_{i=1}^{L} x_{it} * \left[\frac{N_i \hat{a}}{s_i p_t}\right]\left[\frac{N_i \hat{a}}{s_i p_t} - 1\right] \qquad (5)$$

Assuming that covariances between contributions of different release groups to a stratum could be ignored, summation of variance components over all tag codes provided an estimate of the variance of the total hatchery contribution. Inspection of the formula given by Clark and Bernard (1987) for the aforementioned covariances shows them to be negligible for large N and s, and to be consistently negative, so that when ignored, conservative estimates of variance are obtained. Variances associated with unsampled strata are believed to be small (Sharr et al,1995b).

The survival rate of the release group coded with tag t (S_t), was estimated as:

$$\hat{S}_t = \frac{\hat{C}_t + \hat{C}u_t}{R_t} \qquad (6)$$

where,

C_t	=	contribution of release group coded with tag t to sampled strata,
Cu_t	=	contribution of release group coded with tag t to unsampled strata,
R_t	=	total number of fish in release group coded with tag t released from hatchery.

Assuming the total release of fish associated with a tag code is known with negligible error, and that the cumulative variance contributions associated with the unsampled strata are small, a suitable variance estimate for S_t is given by:

$$\hat{V}(\hat{S}_t) = \frac{\sum_{i=1}^{L} x_{it} * \left[\frac{N_i \hat{a}}{s_i p_t}\right]\left[\frac{N_i \hat{a}}{s_i p_t} - 1\right]}{R_t^2} \qquad (7)$$

Inseason Hatchery Contributions

Two inseason estimates of hatchery contributions of pink salmon were generated for each opening. The first and more timely estimate was made using the method suggested by Sharr et al. (1995b). This method depended on the number of (undecoded) tags detected in heads of adipose-clipped fish by a Northwest Marine Technology tag scanner. Estimates using undecoded detected tags required that assumptions be made about adjustment (a) and expansion ($1/p_t$) factors (see Equation 3). For all inseason estimation, an adjustment factor of 1.71 was used, which is the historical average adjustment factor (1989-1994) associated with the W.H. Noerenberg facility. Fishery openings in the western and northern portions of Prince William Sound were assumed to harvest only late run hatchery returns to the Prince William Sound Aquaculture Corporation facilities. For openings in the Southwestern district, an expansion factor of 517 was used; this is a weighted average of all expansion factors associated with tags released at the A.F. Koernig (517), W.H. Noerenberg (514) and Cannery Creek (600) hatcheries in 1994. The weighting scheme depended upon historical contributions of hatcheries to the Southwestern district. Using a similar weighting scheme for the Coghill and Northern districts, expansion factors of 534 and 582 were calculated. Openings in the Eastern district were assumed to harvest only the early run hatchery returns to Solomon Gulch, and an expansion factor of 489 was used. This number is the average of all expansion factors associated with releases from the Solomon Gulch facility in 1994. The second method, which used fully decoded data, was invoked less frequently. Fully decoded data were usually available about one week after the heads were collected, and the results were consequently not as useful to managers. Calculations of inseason contributions were consistent with those used to generate postseason results (Equation 3). Postseason estimation was a more thorough, but less timely method which used data from extracted and fully decoded tags, and which allowed use of tag-specific expansion factors and an updated W.H. Noerenberg adjustment factor.

RESULTS

Tagging

Pink salmon fry were released from the A.F. Koernig, W.H. Noerenberg, Cannery Creek, and Solomon Gulch hatcheries in 1994 (Table 1). Pink salmon were by far the most abundant salmon species cultivated and released from Prince William Sound hatcheries. Numbers of pink salmon fry released ranged from 85 million for the Cannery Creek hatchery to 162 million for the W.H. Noerenberg hatchery. Excluding experimental releases, tagging rates were in the region of 0.0017. Experimental release groups were tagged at a rate of 0.005. The numbers of codes applied were 6, 9, 17 and 16 by the Solomon Gulch, Cannery Creek, W.H. Noerenberg, and A.F. Koernig hatcheries, respectively. Approximately 7.0 and 7.7 million fry were released in the experimental groups from the A.F. Koernig and W.H. Noerenberg hatcheries, respectively.

Table 1 Pink salmon tagging data for fish released into Prince William Sound in 1994, returning in 1995.

Hatchery	No. Fish Released (millions)	No. Tag Codes	No. Fish Tagged	Range of tagging rates
Armin F. Koernig	92.08	16	178,900	0.00167-0.005[a]
W.H. Noerenberg	162.4	17	316,100	0.00165
Cannery Creek	84.6	9	141,100	0.00166-0.00170
Solomon Gulch	149.5	6	305,700	0.00169-0.00233
Totals	488.58	48	941,800	

a Including experimental release group

Tag Recoveries

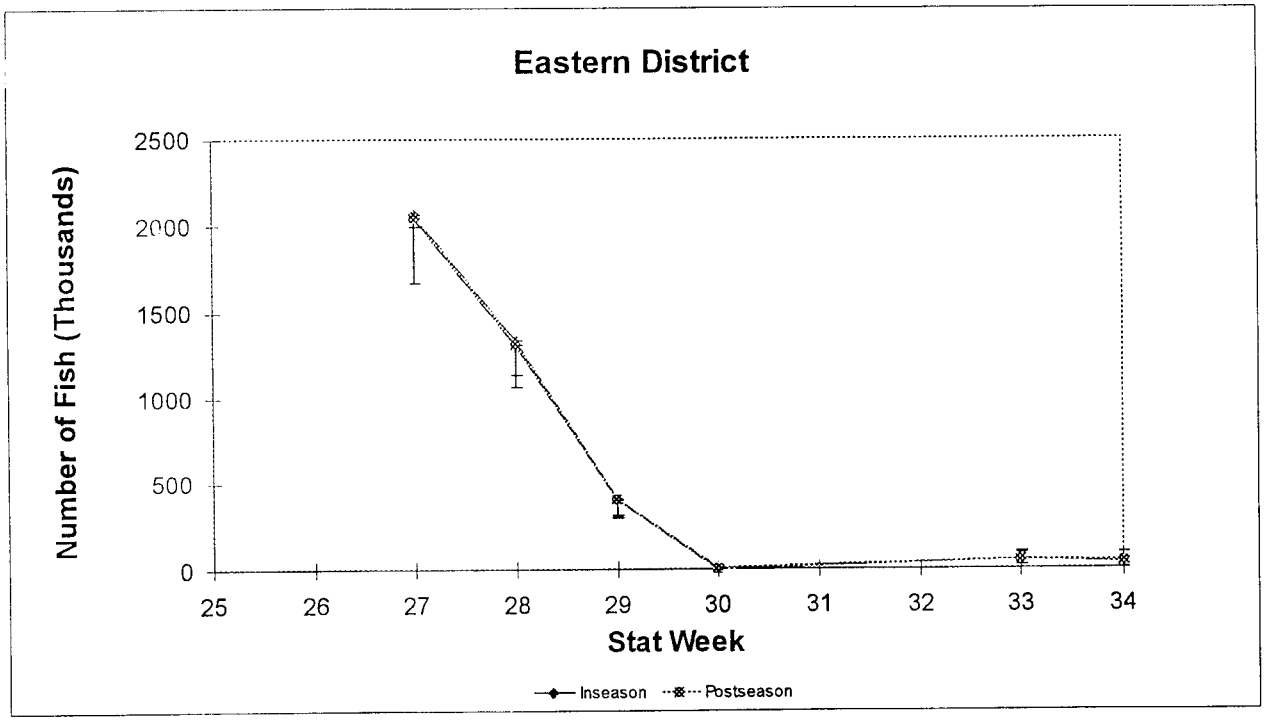
Sampling Rates

Approximately 23% of the pink salmon captured in the common property and 24% of those captured in the cost-recovery harvests were sampled during 1995. These sampling rates were functions of the magnitudes of the catch, the number of samplers and the time period the fish were accessible to the samplers. The proportion of the pink salmon brood stock sampled was 99%.

Estimates of Contributions

Tags from hatchery-produced pink salmon were recovered in the common property, cost-recovery, test fishery and brood stock harvests. Hatcheries contributed 13.08 million pink salmon (76%) to the total Prince William Sound catch of 17.16 million (Table 2). The Solomon Gulch hatchery contributed the largest number (6.76 million fish:39% of total catch), while the A.F. Koernig facility contributed the smallest number(0.78 million: 5% of total catch). The common property fisheries harvested 10.8 million pink salmon, of which 7.82 and 2.98 million were estimated to be of hatchery and wild origin, respectively (Table 2). The cost recovery fisheries harvested 5.10 million pink salmon of which 4.26 and 0.84 million were estimated to be of hatchery and wild origin, respectively.

The agreement between inseason estimates based upon detected tags and postseason estimates of hatchery contributions was district-dependent. Inseason and postseason estimates of hatchery contributions to the Eastern district common property fishery agreed very closely (Figure 2). Some of the differences between the estimates were attributable to changes in the catch data over the season. The inseason estimates for the Southwestern district were significantly higher than the postseason estimates, however. The reason for the discrepancies was an unusually high survival rate of the experimental release groups that had been tagged at a rate three times that of the other release groups. Since the detected but undecoded tags cannot be differentiated into separate tagging groups, all tags were expanded equally. This led to an overestimation of contributions for tagged fish originating from the experimental release groups by a factor of about three. Inseason estimates based on decoded tags agreed closely with postseason estimates, but were not generally used in management decisions because the data from which they were generated were not available for several days after an opening had occurred. Overestimation of hatchery contributions also occurred with openings in the Coghill district, again because of high survival rates of the differentially tagged experimental release groups. In contrast, the 1994 inseason estimates for all districts compared very favorably to postseason estimates (Sharr *et al.*, 1995e). Northern district inseason estimates were not used in management decisions, because of concerns over the possibility of excessive tag shedding in fish tagged at the Cannery Creek facility



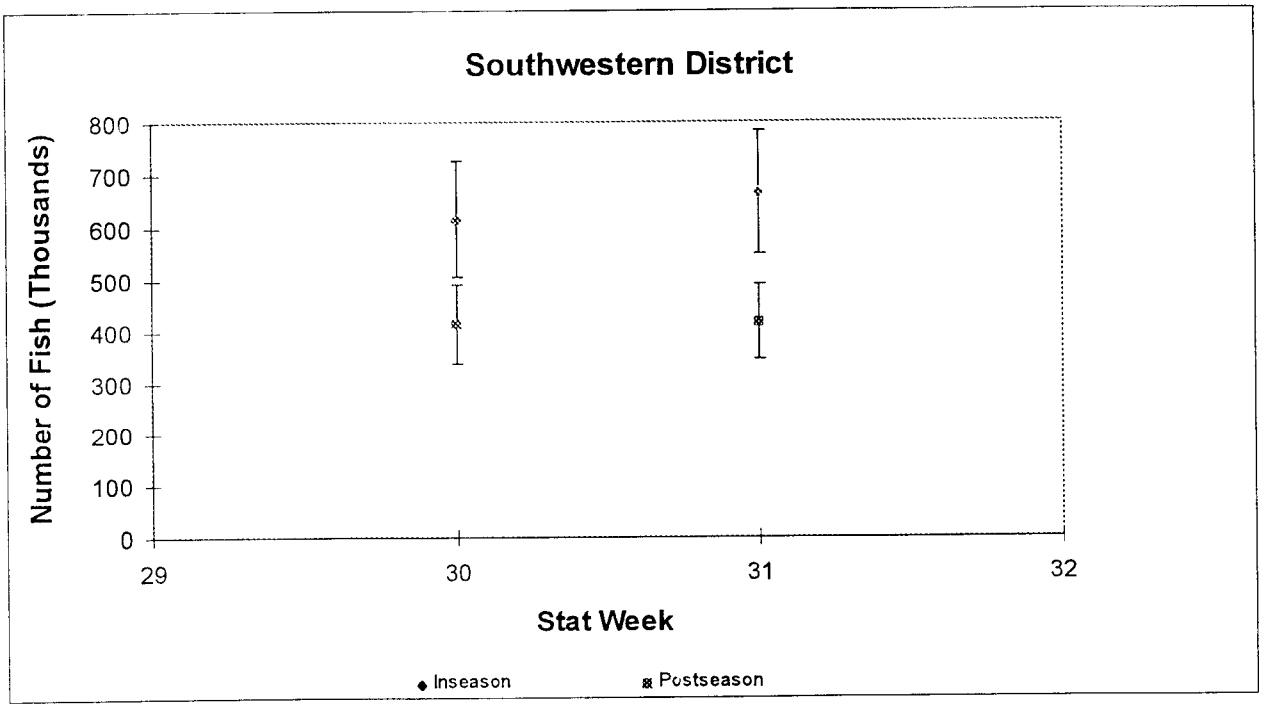


Figure 2 Inseason and postseason estimates of hatchery contributions to Eastern and Southwestern district common property fisheries in Prince William Sound in 1995.

Table 2 Postseason estimates of hatchery and wild stock contributions to the Prince William Sound catch of 1995 (millions of fish).

Contributor	Common Property	Cost Recovery	Test Fishery	Brood Stock[a]	Total Contribution	95% Bounds	Percent of Total Catch
Armin F. Koernig	0.20	0.45	0.003	0.13	0.78	0.66 - 0.90	4.6
Wally Noerenberg	1.19	0.86	0.01	0.31	2.37	2.21 - 2.53	13.8
Cannery Creek	2.62	0.41	0.02	0.12	3.17	2.94 - 3.40	18.5
Solomon Gulch	3.81	2.54	0.005	0.41	6.76	6.25 - 7.27	39.4
Hatchery Total	7.82	4.26	0.04	0.97	13.08	12.26-13.9	76.2
Wild Stocks	2.98	0.84	0.11	0.15	4.08		23.7
Grand Total	10.80	5.10	0.15	1.12	17.16		100.00

a Brood stock numbers include fish used for roe stripping (chiefly at Solomon Gulch hatchery)

Test Fishery Catches

Catches during the first weeks of the Eastern district fisheries are comprised almost exclusively of hatchery fish, and the Southwestern district test fishery is the first opportunity during the season for coded wire tag information to affect management decisions. The Alaska Department of Fish and Game has conducted the Southwestern district test fishery since 1993 in order to determine when the number and percentage of hatchery fish moving through the district is high enough to warrant opening the district to commercial fishing. Approximately 12 sampling stations are scattered throughout the district, of which 9 are used regularly. Boats assigned to a specific sampling station make three sets. All catches for a given fishing period are loaded onto one tender, and the load is sampled intensively for coded wire tagged fish upon arrival at a processing plant. The 1995 test fishery began on July 25 and ended on August 2, and was divided into 5 periods. A total of 147,895 pink salmon were caught, of which 33,825 were estimated to be hatchery fish (Appendix A.1). When the catches are stratified by period, the peak catch and peak wild stock contribution occurred during the 4th period (Appendix A.1, Figure 3). The wild stock estimate from the fourth period was biased upwards by a seiner at the Chenega Wall sampling station who made ten sets before Alaska Department of Fish and Game personnel could stop the fishing. Since Chenega Wall is a very productive fishing site, oversampling at that site strongly affected the overall catch. Chenega Wall is located in the northern part of the Southwestern District, and the large quantity of wild fish caught there reflected the high catches of wild fish caught at more southerly sampling stations earlier in the test fishery.

Common Property Catches

In the common property fishery, 10.8 million pink salmon were harvested, of which 3.81, 2.62, 1.19, 0.2 and 2.98 million were estimated to be of Solomon Gulch, Cannery Creek, W.H. Noerenberg, A.F. Koernig and wild origin, respectively (Table 2).

The Eastern district common property harvest accounted for 39.2% of the total common property harvest, due to large returns of Solomon Gulch-reared fish. The Eastern district harvest was 4.23 million fish, of which 3.78 million were estimated to originate from Solomon Gulch. The majority of the fish were harvested during the weeks of July 2 and July 9 (Statistical weeks 27 and 28; Figure 4, Appendix B2). Between July 16 and September 9, the southern portion of the district was opened to fishing as a result of high aerial survey counts. Between July 16 and September 9, 0.87 million fish were harvested, of which 43% were estimated to be wild fish.

The Northern district common property harvest was the second largest after that of the Eastern district, with 3.66 million fish being harvested (Appendix B.2). The peak weekly harvest occurred from August 13 to 19 (Stat Week 33), with 1.99 million fish harvested (Figure 5, Appendix B2). The Northern district hatchery contribution may be biased downward. In the 1995 Cannery Creek brood stock, the percentage of marked fish that did not have tags was 56%, which is considerably higher than the 37% found by Sharr et al. (1995b, Appendix A) in a multi-year study of the tag to clip ratio in the commercial fishery. Use of the W.H. Noerenberg adjustment factor of 1.77 may have underestimated the contributions from this facility.

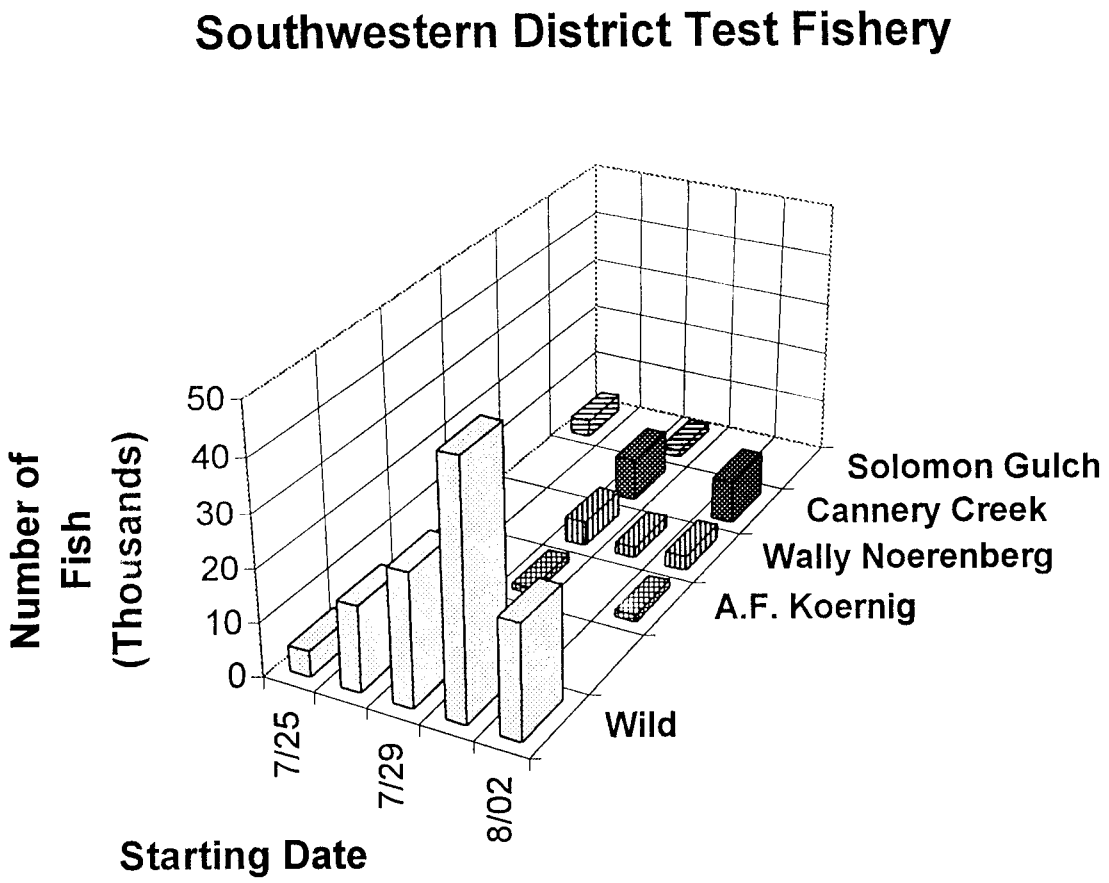


Figure 3 Hatchery and wild stock contributions to Southwestern district test fishery catches in Prince William Sound by fishing period in 1995.

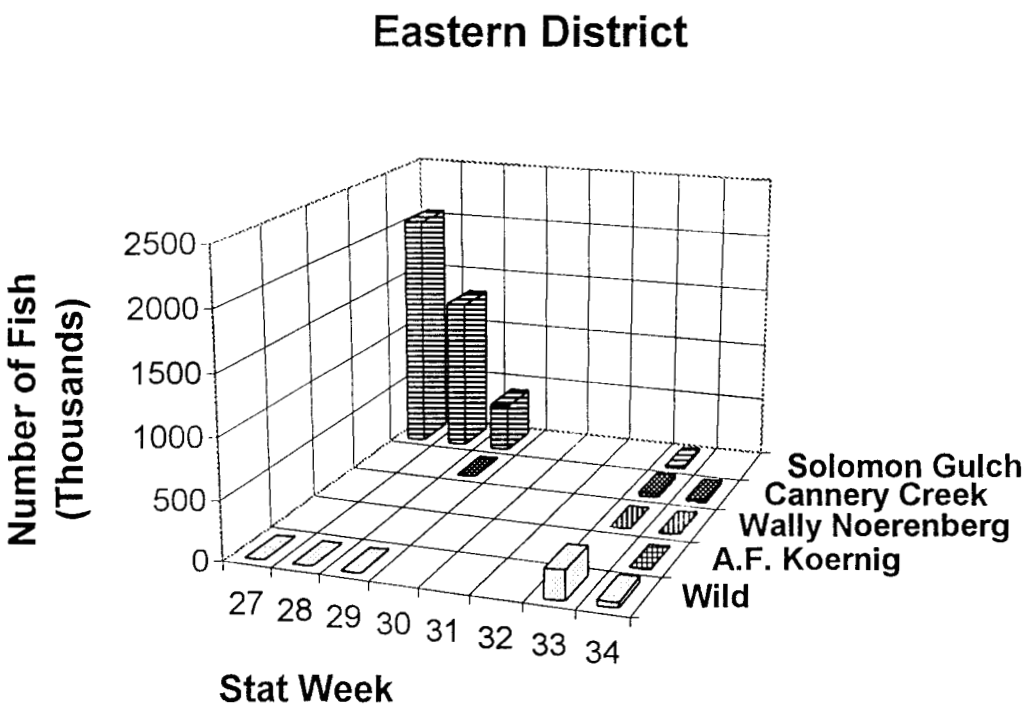


Figure 4 Hatchery and wild stock contributions to Eastern district common property fishery catches by district and week in Prince William Sound in 1995.

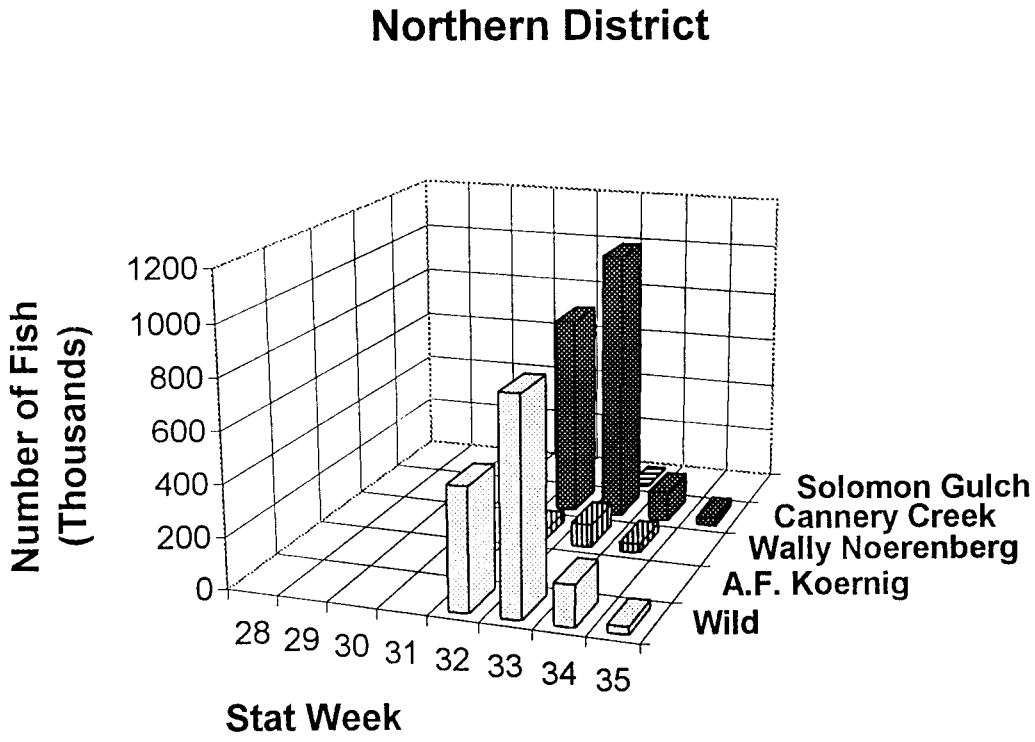


Figure 5 Hatchery and wild stock contributions to the Northern district common property fishery catches by district and week in Prince William Sound in 1995.

The 1995 Coghill district common property pink salmon harvest was 1.08 million fish (Appendix B2). The Coghill district purse seine fishery began the week of July 30 to August 5 (Stat Week 31), with 5,619 fish being caught, all of which were estimated to be of wild origin (Figure 6, Appendix B2). Peak harvest weeks were those ending on August 12 and August 19 (Stat Weeks 32 and 33), with 0.326 and 0.584 million fish being caught, respectively. Prior to July 31, the pink salmon harvested were incidental to the gillnet fishery targeting sockeye salmon. The W.H. Noerenberg hatchery was the largest contributor of fish in the Coghill district, with Cannery Creek and wild fish being the second and third largest contributors, respectively. The majority of the W.H. Noerenberg fish originated from the Sound Ecosystem Assessment project release groups.

The Eshamy district had one of the smallest common property pink salmon harvests, with 88,830 pink salmon caught. The only sampling conducted in the Eshamy district occurred during the last week of the fishery. Wild fish were the largest contributor to the common property harvest, with the W.H. Noerenberg and Cannery Creek hatcheries being the second and third largest contributors, respectively.

The Southwestern District was fished for only 2 weeks (Figure 7). It is likely that the common property fishery would have been further restricted had the true hatchery contribution not been obscured by the differential tagging of the experimental release groups. The inseason estimate based upon detected tags indicated a hatchery contribution of 82%, while the decoded tag estimate was 50%. In total, 1.71 million fish were caught in the district with wild fish being the largest contributor, and the Cannery Creek, W.H. Noerenberg and A.F. Koernig facilities being the second, third and fourth largest contributors, respectively.

Cost Recovery Catches

The total 1995 cost recovery harvest was 5.10 million fish (Table 2). Almost 50% of the harvest was taken in the Eastern district (Appendix B.3). The high percentage reflects both the comparatively high survival rates of Solomon Gulch pink salmon, and the Valdez Fisheries Development Association's policy of harvesting fish for a specific revenue goal instead of for a fixed percentage of the catch, as is required of the Prince William Sound Aquaculture Corporation. Solomon Gulch pink salmon comprised the largest portion of the total cost recovery catch (2.54 million), followed by W.H. Noernberg fish (0.86 million), wild stocks (0.84 million), A.F. Koernig fish (0.45 million), and Cannery Creek fish (0.41 million).

The 1995 cost recovery fishery for the Eastern district began during the week of June 18 (Stat Week 25), and peaked during the week of July 2 (Stat Week 27) with 1.05 million fish caught (Figure 8, Appendix B3). Tag recoveries indicated that the cost recovery harvest was exclusively comprised of Solomon Gulch hatchery pink salmon. Cost recovery was completed the week ending July 22 (Stat Week 29).

For the cost recovery harvest in the Northern District, over 60% was estimated to be of wild origin, with Cannery Creek hatchery pink salmon comprising the rest (Figure 9, Appendix B3).

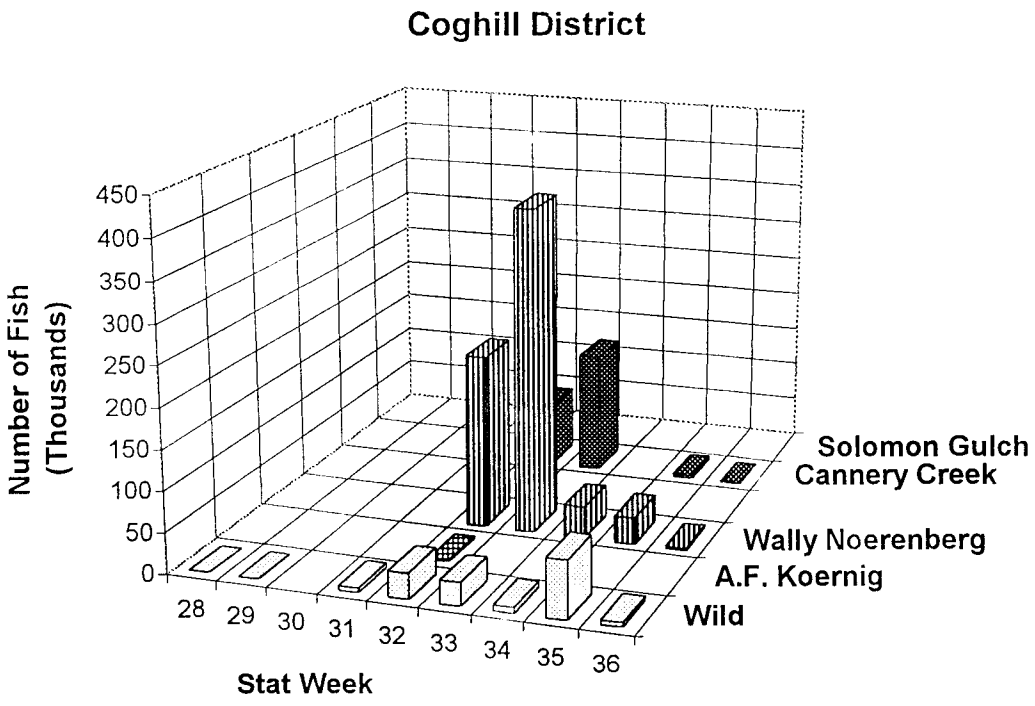


Figure 6 Hatchery and wild stock contributions to Coghill district common property fishery catches by district and week in Prince William Sound in 1995.

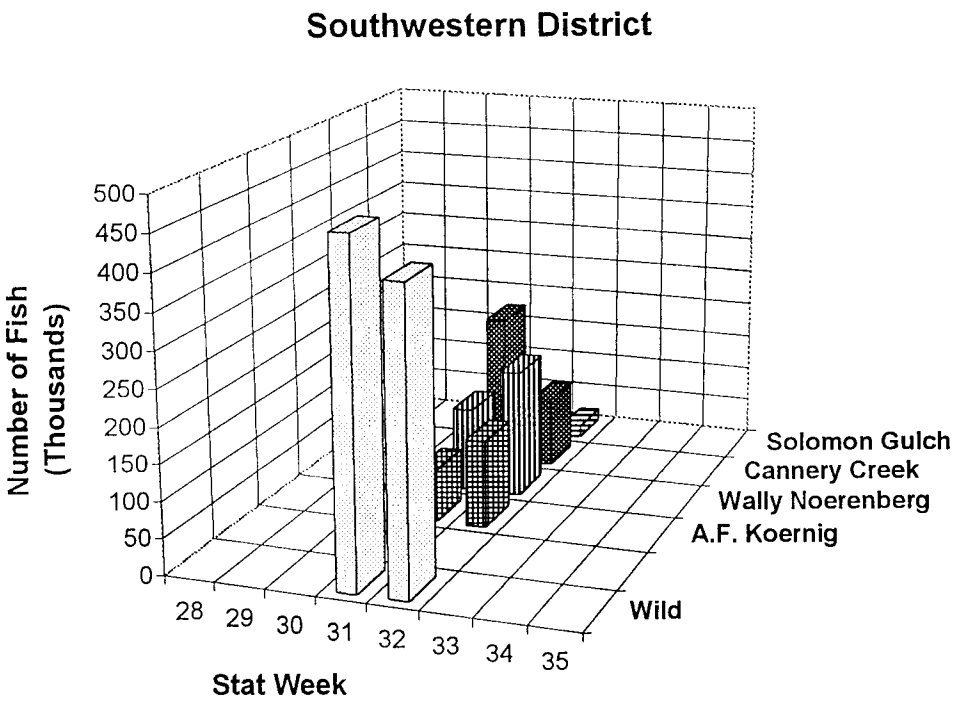


Figure 7 Hatchery and wild stock contributions to Southwestern district common property fishery catches by district and week in Prince William Sound in 1995.

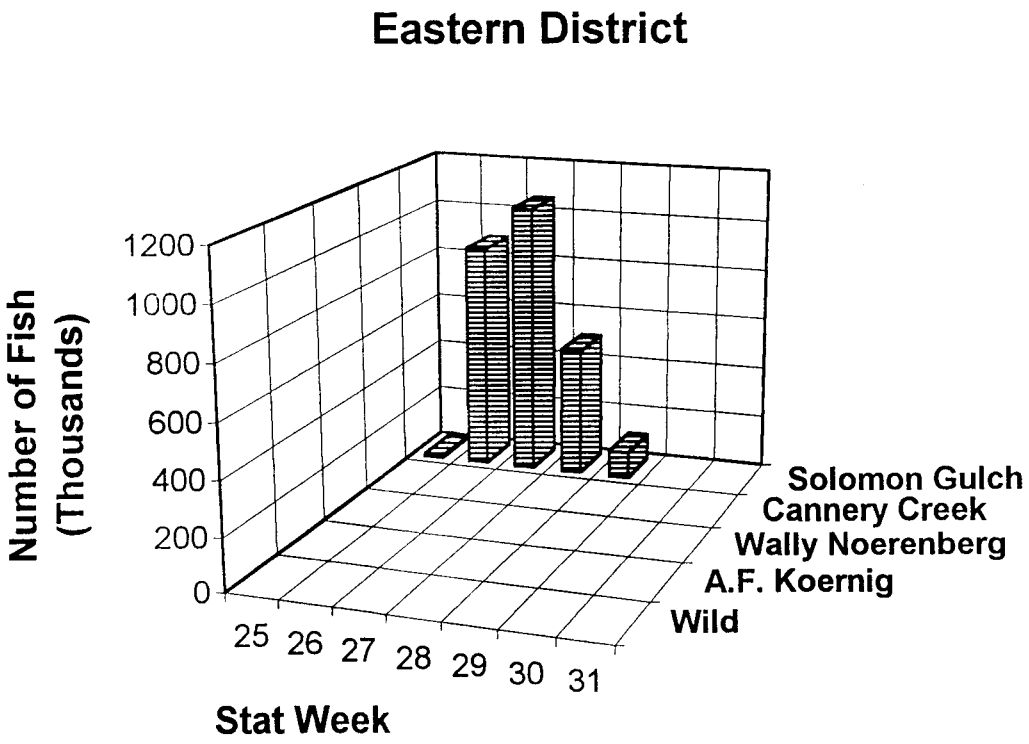


Figure 8 Hatchery and wild stock contributions to Eastern district cost recovery fishery catches by district and week in Prince William Sound in 1995.

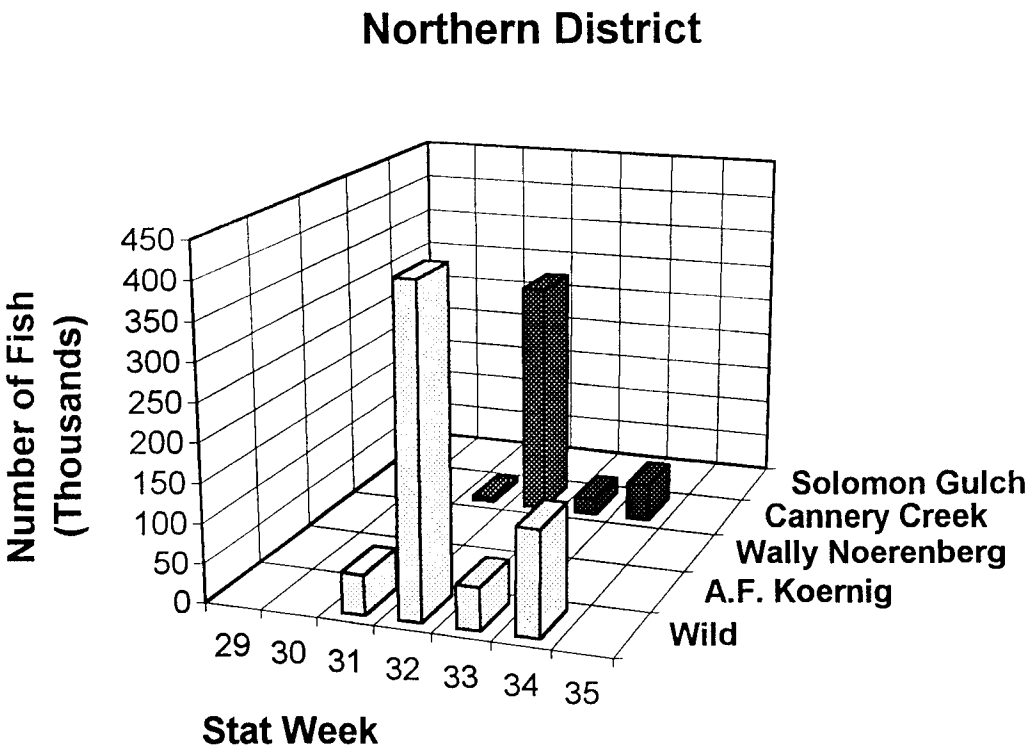


Figure 9 Hatchery and wild stock contributions to Northern district cost recovery fishery catches by district and week in Prince William Sound in 1995.

The hatchery contribution estimates are somewhat suspect, since 72% of the marked fish retrieved in sampling did not have tags, and aerial surveys in the district indicated mediocre escapements for wild stocks. Cost recovery began during the week of July 30 (Stat Week 31), and ended the week of August 20 (Stat Week 34). A total of 1.04 million fish were harvested, with a peak of 0.722 million fish harvested during the week of August 6 (Stat Week 32).

In the Coghill district, the first harvests of pink salmon occurred during the week ending July 22 (Stat Week 29) in the chum salmon cost recovery harvest (Figure 10, Appendix B3). A total of 0.93 million pink salmon were caught, of which 0.84 million were estimated to originate from the W.H. Noerenberg hatchery. Large harvests of pink salmon began during the week of August 5 (Stat Week 31), and peaked during the week ending August 19 (Stat Week 33).

A total of 0.04 million pink salmon were caught in the Eshamy district cost recovery fishery which included nearly 0.03 million believed to be of wild origin. No fish from the Eshamy district cost recovery were scanned, and the estimates of hatchery contributions were based upon samples taken from the Eshamy district common property fishery during the week ending September 2.

The Southwestern district pink salmon cost recovery harvest totaled 0.55 million fish, of which 0.45 million were estimated to have originated from the A.F. Koernig hatchery (Figure 11, Appendix B3). The second and third largest contributors were wild fish and the Cannery Creek hatchery with 0.078 and 0.014 million fish, respectively. The harvest peaked during the week ending August 19 (Stat Week 33).

Survival Rates

Survival rates (over all tag codes) of adult hatchery pink salmon were 4.52% for Solomon Gulch, 3.75% for Cannery Creek, 1.42% for W.H. Noerenberg, and 0.83% for A.F. Koernig (Table 3). Significant differences ($\alpha=0.05$) in survival rates of hatchery-reared fish were detected between all hatcheries. Since evidence exists that Cannery Creek hatchery returns may be underestimated, comparisons of survival rates between Cannery Creek and other hatcheries (most especially Solomon Gulch) should be treated with caution. The overall survival rates of the W.H. Noerenberg and A.F. Koernig facilities were considerably affected by returns of fish associated with the experimental release groups (Figure 12, Appendix C). Ten out of sixteen tag codes from the A.F. Koernig and ten out of seventeen tag codes from the W.H. Noerenberg hatchery were associated with survival rates below 0.5%. The Sound Ecosystem Assessment project release groups had extraordinarily high survival rates, especially those from the W.H. Noerenberg hatchery. The estimated survival rates of these experimental release groups were 7.46% and 6.29% for the two groups released from the A.F. Koernig facility and 23.5% and 21.1% for the groups released from the W.H. Noerenberg facility. The survival rates of the non-experimental release groups released from the A.F. Koernig hatchery continue to decline (see Sharr et al., 1995c and e).

Adjustment Factors

Adjustment factors were estimated from pink salmon brood stocks and are presented in Table 4. The smallest brood stock adjustment factor was for Solomon Gulch at 1.45. Cost recovery adjustment factors for the Solomon Gulch and Cannery Creek hatcheries were 1.34 and 5.55 respectively. The brood adjustment factor for W.H. Noerenberg was 1.96, which was the second smallest of the brood adjustment factors. The W.H. Noerenberg historical (1989-1995) adjustment factor estimate of 1.77 was found to be significantly greater than 1.0, and was used for all postseason contribution estimates. Adjustment factors for 1989 through 1995 are presented in Table 5.

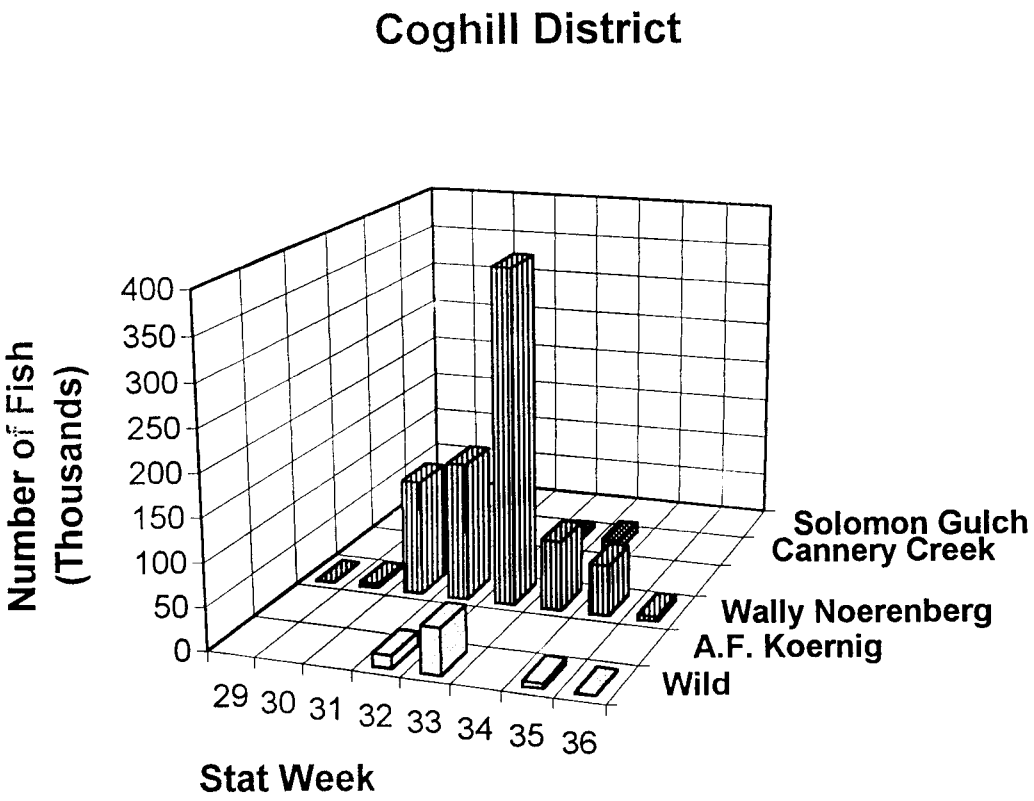


Figure 10 Hatchery and wild stock contributions to Coghill district cost recovery fishery catches by district and week in Prince William Sound in 1995.

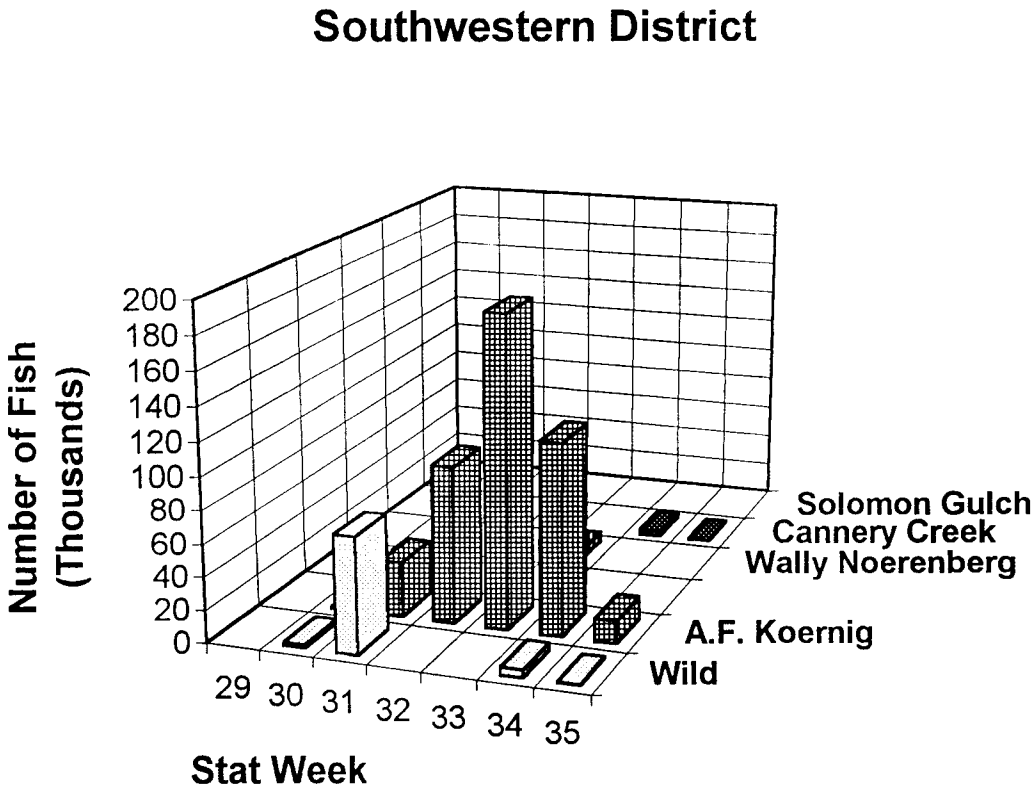


Figure 11 Hatchery and wild stock contributions to Southwestern district cost recovery fishery catches by district and week in Prince William Sound in 1995.

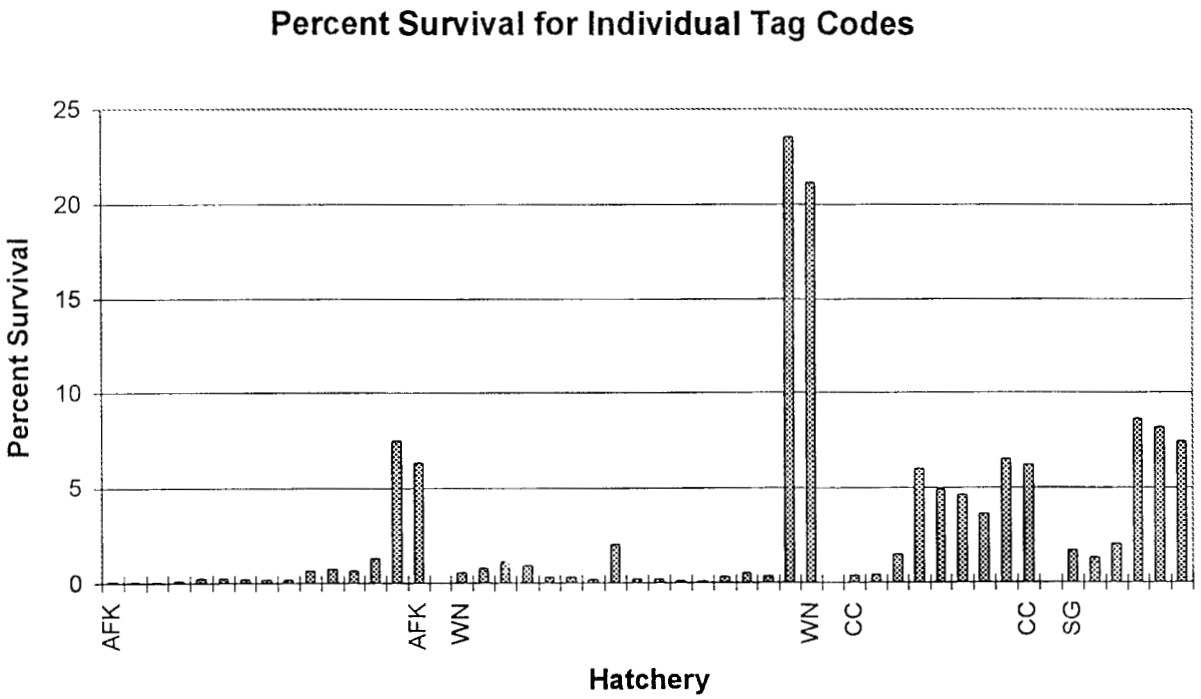


Figure 12 Percent survival rates for individual tag codes, delineated by hatchery, for tagged pink salmon returning to Prince William Sound in 1995.

Table 3 Overall survival rates by hatchery of tagged pink salmon returning to Prince William Sound in 1995.

Hatchery	Survival Rate (%)	95% Bounds
A.F. Koernig	0.83	0.71-0.96
W.H. Noerenberg	1.42	1.33-1.52
Cannery Creek	3.75	3.48-4.02
Solomon Gulch	4.52	4.00-5.04

Table 4 Adjustment factors by hatchery, estimated from the 1995 brood stock harvests.

Hatchery	Adjustment Factor		P Value for Ho: A. Factor < = 1.0
	Estimate	SE	
A.F. Koernig	2.13	0.067	0
W.H. Noerenberg	1.96	0.030	0
Cannery Creek	3.21	0.30	0
Solomon Gulch	1.45	0.053	0
Historical Average	1.77	0.127	0

Table 5 Adjustment Factors estimated from brood and cost-recovery harvests by facility for pink salmon from 1989 through 1995.

	Brood				Cost-Recovery	
Year	WHN[a]	AFK[b]	SG[c]	CC[d]	SG[c]	CC[d]
1989	1.73	1.56	1.13	2.12	1.11	1.81
1990	1.28	1.58	1.82	1.96	1.23	1.71
1991	1.82	1.45	1.94	2.28	1.55	1.97
1992	1.63	1.43	2.55	2.74	1.25	1.58
1993	1.78	2.06	3.82	2.91	2.41	2.36
1994	2.05	1.75	3.15	2.38	1.89	2.64
1995	1.96	2.13	1.45	3.21	1.34	5.55

[a] W.H. Noerenberg
[b] A.F. Koernig
[c] Solomon Gulch
[d] Cannery Creek

DISCUSSION

Contributions of Hatchery Fish to the Commercial Catch

Hatchery production of salmon in Prince William Sound has complicated management of the commercial salmon fisheries. While wild pink salmon production is overshadowed by total hatchery production, wild stocks produce a significant portion of the harvest. Wild salmon stocks were one of the three largest contributors (out of five) to the common property harvest from 1989 to 1995. During this time, a strong wild stock return in conjunction with weak hatchery returns has not taken place. For the most part, the peak returns of hatchery fish have coincided with peak returns of wild stocks in all districts, and management biologists have used spatial restriction of harvest areas to protect the weak elements with varying degrees of success.

As in the previous year, the most important task of the coded wire tag program in 1995 was to provide accurate and timely inseason estimates of hatchery contributions to fishery managers. This was attempted through the method recommended by Sharr et al. (1995c), i.e. preliminary estimation of hatchery contributions was based solely upon numbers of detected but undecoded tags. Inseason estimates of contribution rates were made available to fishery managers within 24-48 hours of the termination of the fishing period. The program was seriously compromised in 1995, however, by the extraordinary survival rates of some intensively tagged experimental release groups. Significant overestimation of hatchery contributions in the preliminary estimates for some of the openings resulted. Upon receipt of tag-specific information, the estimates were revised downwards. They were, however, of limited value to managers because of the time which had elapsed. In addition, it is believed that tagging problems at the Cannery Creek hatchery and the use of the W.H. Noerenberg adjustment factor of 1.71 may have resulted in underestimation of contributions from this facility. The agreement between preliminary and postseason estimates for the Eastern district (Figure 2) underscores, however, the utility of the coded wire tag program as a management tool when differential tagging and tag-retention problems are not factors.

No intensively tagged experimental groups were released in 1995, and thus the overestimation problems encountered in this study should not be a factor in 1996. Tagging practices at the Cannery Creek facility did not change from 1994 to 1995, and so if there was an underestimation of hatchery contributions in 1995 because of a tagging problem in 1994, there is no reason to suspect that it will not reoccur in 1996, i.e. the problem may be chronic in nature. Although tag to clip ratios can be misleading in as far as they are subject to sampler bias with regards to variable definitions of a clipped adipose fin, inspection of the ratio in Cannery Creek broodstocks from 1989 to the present (around 50-60)%) suggests that tag shedding may be problematic for pink salmon tagged at Cannery Creek. There is some anecdotal evidence to support this contention. Hatchery staff have noted that the radio will interfere with the quality control device responsible for determining whether the injected tags remain in place. When interference occurs, the quality control device randomly accepts fish without tags and rejects fish with tags. The response of the

tagging crew has sometimes been to turn off the quality control device when this occurs, which means that they are tagging without feedback on tagging success. While there is evidence of tag application problems at Cannery Creek, it is considered prudent to wait for data from the otolith-marking program to make more definitive statements regarding the matter. As mentioned, tag-clip ratios can be misleading when sampler bias exists, and the large jump in adjustment factors in 1995 in the brood harvests without a concomitant decrease in the brood stock tag-clip ratios warrants caution in use of these ratios in the formulation of conclusive statements regarding problems at Cannery Creek. Otolith data will allow us, for example, to assess the degree of straying of wild fish into the brood pond, and will indicate the extent to which it influences the adjustment factor. Absence of wild fish in the brood would be evidence in favour of the tag-shedding hypothesis.

Survival Rates of Hatchery Fish

The overall marine survival rates dropped for all hatcheries by about 50% from the previous year, but were nevertheless average for the Cannery Creek and Solomon Gulch hatcheries. If tagging problems exist at the Cannery Creek facility, the survival rates for that hatchery are being underestimated. The overall marine survival was poor for the W.N. Noerenberg and A.F. Koernig hatcheries, and would have been much poorer were it not for the experimental release groups. Roughly 63% of the return to the A.F. Koernig hatchery originated from these groups, which constituted about 7.5% of the pink salmon released, while about 74% of the pink salmon return to W.H. Noerenberg hatchery originated from experimental release group (5% of release). It is possible that predators are preying heavily on pink salmon juveniles released from these hatcheries, and also perhaps on wild pink salmon stocks on the western side of Prince William Sound.

Adjustment Factors

Adjustment factors were developed to address violations of underlying assumptions in the analysis; namely that fish do not lose tags, and that mortality rates are the same for tagged and untagged fish. Appropriate use of this concept relies on a further set of assumptions, however, such as that regarding the absence of wild fish in the brood pond from which the adjustment factor is calculated. It has been believed that the latter assumption is violated at all facilities except the W.H. Noerenberg hatchery, and a historical W.H. Noerenberg adjustment factor has been used in all inseason and postseason estimates of hatchery returns from 1993 through 1995. In light of the hypothesized tagging problems at the Cannery Creek hatchery, it is possible that the original adjustment factor calculated for that facility may not be as seriously inflated as previously thought. In the absence of data quantifying either wild stock contribution to the brood or tag loss, however, reversion to the use of a separate adjustment factor for Cannery Creek hatchery is thought premature.

The adjustment factors calculated for the Solomon Gulch cost recovery and brood were the smallest of all of the hatcheries (Table 5). This is interesting, since the large Solomon Gulch adjustment factors of 1992-1994 were instrumental in the conversion to the W.H. Noerenberg historical factors. Inspection of the standard deviation (σ) of the brood stock adjustment factors over the years at each facility shows that the variation of the adjustment factor associated with the W.H. Noerenberg facility to be the smallest (σ=0.25) and that σ associated with the A.F. Koernig, Cannery Creek and Solomon Gulch facilities to be 0.31, 0.45 and 0.96, respectively. It could be speculated that the variability for the latter two facilities is due to natural oscillations in wild populations local to the facility, with corresponding variations in the degree of immigration of wild fish into the brood pond. It is clear from the above discussion that further data are needed to answer the questions regarding the correct use of adjustment factors at all facilities.

Proper investigation of adjustment factors requires additional information, which we hope will be furnished by the otolith marking program (R95320C). In that study, all pink salmon released from Prince William Sound hatcheries in 1996 will be thermally marked, with fish from each facility receiving a different mark. Every fish lacking the otolith mark in the 1997 brood stocks will be considered wild, and an estimate of the proportion of wild fish present will be available. Comparisons between actual brood stock composition based on otolith marking and calculations of adjustment factors should allow us to evaluate the very contentious issue of the effect of wild fish in the brood on adjustment factors. In addition, otolith marking combined with coded wire tags should allow a better investigation of tag retention rates, and possibly, rates of naturally missing adipose fins in Prince William Sound pink salmon.

CONCLUSIONS

The major objective of this study was to provide fishery managers with time and location-specific data relating to the occurrence of wild stocks in the commercial fishery, and to do this in real-time with a technique based upon detected (undecoded) tags. Some of the preliminary estimates of hatchery contributions based upon detected but undecoded tags were biased upwards because of high survival rates of intensively tagged experimental groups. It is possible that management decisions would have restricted Southwestern district fishing more had the preliminary estimates been unbiased. Following discovery of the bias in the preliminary estimates, coded wire tag information was used with caution in making management decisions. In postseason analysis, reasonably precise estimates of hatchery contributions were obtained, as were estimates of hatchery survival rates. Possible tag retention problems at Cannery Creek hatchery were uncovered, which may have caused underestimation of Cannery Creek pink salmon. Further information is required to assess this problem and may be available from the thermal otolith marking program.

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Appendix A Pink salmon hatchery and wild stock contributions to Prince William Sound test fisheries by period and week for 1995.

Appendix A.1 Pink salmon hatchery and wild stock contributions to Prince William Sound test fisheries by district and fishing period for 1995.

Southwestern District

Date	Period	AFK Hatchery		WN Hatchery		CC Hatchery		SG Hatchery		Total Hatchery		Total Wild	Total Catch	Number of Tags
		Contrib.	Variance	Contrib.	Variance	Contrib.	Variance	Contrib.	Variance	Contrib.	Variance			
7/25 - 7/26	1							3261	10633532	3261	10633532	4742	8003	1
7/27 - 7/28	2									0		15857	15857	0
7/29 - 7/30	3	1375	946375	4825	8996407	8265	17078178	1476	2177183	15941	29198143	24786	40727	12
7/31 - 8/01	4			2095	4387100					2095	4387100	47485	49580	1
8/02	5	1390	1933053	2786	3880072	8352	34878853			12528	40691978	21200	33728	5
Subtotals		2766	2879428	9706	17263579	16617	51957031	4737	12810715	33825	84910753	114070	147895	19

Appendix B Pink salmon hatchery and wild stock contributions in test common property and cost recovery fisheries, and hatchery brood stock in Prince William Sound by district and week for 1995.

Appendix B.1 Pink salmon hatchery and wild stock contributions to Prince William Sound test fisheries by district and week during 1995.

Southwestern District

Week Ending	Stat Week	AFK Hatchery		WN Hatchery		CC Hatchery		SG Hatchery		Total Hatchery		Total Wild	Total Catch	Number of Tags
		Contrib.	Variance	Contrib.	Variance	Contrib.	Variance	Contrib.	Variance	Contrib.	Variance			
7/29/95	30	707	500065	4958	9515908	2127	4522549	4896	13690000	12685	28228522	27644	40329	8
8/05/95	31	1966	1485671	4545	6655512	14354	14354			20865	8155537	86701	107566	11
Grand Totals		2673	1985736	9503	16171420	16481	4536903	4896	13690000	33550	36384059	114345	147895	19

Appendix B.2 Pink salmon hatchery and wild stock contributions to Prince William Sound common property fisheries by district and week during 1995.

Eastern District

Week Ending	Stat Week	AFK Hatchery		WN Hatchery		CC Hatchery		SG Hatchery		Total Hatchery		Total Wild	Total Catch	Number of Tags
		Contrib.	Variance	Contrib.	Variance	Contrib.	Variance	Contrib.	Variance	Contrib.	Variance			
7/08/95	27							2048084	3.76E+10	2048084	3.76E+10	0	2048084	884
7/15/95	28							1312439	1.68E+10	1312439	1.68E+10	0	1312439	648
7/22/95	29					1228	347953	401677	2.29E+09	402905	2.29E+09	1160	404065	334
7/29/95	30									0		96105	96105	0
8/05/95	31													
8/12/95	32													
8/19/95	33			2344	3421171	35681	2.23E+08	16353	94940000	54378	3.21E+08	241176	295554	10
8/26/95	34	1091	464666	8867	1.45E+07	24960	1.05E+09			34918	1.20E+08	44161	79079	9
9/02/95	35													
9/09/95	36									0		312	312	0
Subtotals		1091	464666	11211	1.79E+07	61869	3.28E+08	3778553	5.69E+10	3852724	5.72E+10	382914	4235638	1885

Northern District

Week Ending	Stat Week	AFK Hatchery		WN Hatchery		CC Hatchery		SG Hatchery		Total Hatchery		Total Wild	Total Catch	Number of Tags
		Contrib.	Variance	Contrib.	Variance	Contrib.	Variance	Contrib.	Variance	Contrib.	Variance			
8/12/95	32			36318	1.57E+08	793870	3.63E+09			830188	3.78E+09	469885	1300073	194
8/19/95	33			91223	3.89E+08	1069141	5.20E+09	4204	14750000	1164568	5.60E+09	825853	1990421	229
8/26/95	34			36041	48660000	114392	3.29E+08			150433	3.78E+08	156929	307362	28
9/02/95	35					28913	21630000			28913	21630000	29349	58262	11
Subtotals		0		163582	5.95E+08	2006316	9.18E+09	4204	14750000	2174102	9.79E+09	1482016	3656118	462

Coghill District

Week Ending	Stat Week	AFK Hatchery		WN Hatchery		CC Hatchery		SG Hatchery		Total Hatchery		Total Wild	Total Catch	Number of Tags
		Contrib.	Variance	Contrib.	Variance	Contrib.	Variance	Contrib.	Variance	Contrib.	Variance			
6/17/95	24											1	1	0
6/24/95	25											27	27	0
7/01/95	26											11	11	0
7/08/95	27											60	60	0
7/15/95	28											103	103	0
7/22/95	29											304	304	0
7/29/95	30													
8/05/95	31											5619	5619	0
8/12/95	32	1680	2349728	213997	7.69E+08	78772	1.39E+08			294449	9.1E+08	31729	326178	183
8/19/95	33			403334	2.21E+09	151969	3.13E+08			555303	2.53E+09	28730	584033	281
8/26/95	34			38691	17290000					38691	17290000	7496	46187	15
9/02/95	35 1/			32599	91520000	4253	15570000			36852	1.07E+08	70844	107696	15
9/09/95	36			2558	563684	334	95898			2892	659582	5560	8452	0
9/16/95	37											22	22	0
Subtotals		1680	2349728	691179	3.09E+09	235328	4.67E+08	0		928187	3.57E+09	150506	1078693	494

1/ Proportions from week 34 were used to allocate the catch.

Eshamy District

Week Ending	Stat Week	AFK Hatchery		WN Hatchery		CC Hatchery		SG Hatchery		Total Hatchery		Total Wild	Total Catch	Number of Tags
		Contrib.	Variance	Contrib.	Variance	Contrib.	Variance	Contrib.	Variance	Contrib.	Variance			
7/08/95	27									0		271	271	0
7/15/95	28									0		1656	1656	0
7/22/95	29 2/	34	484	1292	303582	327	10713			1653	314779	2900	4553	0
8/29/95	30 2/	18	148	713	9263	180	3269			911	12680	1604	2515	0
8/05/95	31													
8/12/95	32	594	148866	22649	93340000	5736	3293728			28979	68782594	50856	79835	15
Subtotals		646	149498			6243	3307710	0		31543	97110053	57287	88830	15

2/ Proportions from week 32 were used to allocate the catch.

Southwestern District

Week Ending	Stat Week	AFK Hatchery		WN Hatchery		CC Hatchery		SG Hatchery		Total Hatchery		Total Wild	Total Catch	Number of Tags
		Contrib.	Variance	Contrib.	Variance	Contrib.	Variance	Contrib.	Variance	Contrib.	Variance			
8/05/95	31	73269	1.68E+08	118077	2.75E+08	212231	1.04E+09	10051	48070000	413628	1.53E+09	465052	878680	149
8/12/95	32	122602	3.56E+08	179440	4.23E+08	99770	4.59E+08	16442	95460000	418254	1.33E+09	410811	829065	188
Subtotals		195871	5.23E+08	297517	6.98E+08	312001	1.50E+09	26494	1.44E+08	831882	2.86E+09	875863	1707745	337

Montague District

Week Ending	Stat Week	AFK Hatchery		WN Hatchery		CC Hatchery		SG Hatchery		Total Hatchery		Total Wild	Total Catch	Number of Tags
		Contrib.	Variance	Contrib.	Variance	Contrib.	Variance	Contrib.	Variance	Contrib.	Variance			
8/26/95	34									0		12292	12292	0
9/02/95	35									0		5947	5947	0
Subtotals		0		0		0		0		0		18239	18239	0

Southeastern District

Week Ending	Stat Week	AFK Hatchery		WN Hatchery		CC Hatchery		SG Hatchery		Total Hatchery		Total Wild	Total Catch	Number of Tags
		Contrib.	Variance	Contrib.	Variance	Contrib.	Variance	Contrib.	Variance	Contrib.	Variance			
8/19/95	33													
Subtotals		0		0		0		0		0		11418	11418	0
Grand Totals		199288	5.26E+08	1188143	4.49E+09	2621757	1.15E+10	3809250	5.7E+10	7818438	7.4E+10	2978243	10796681	3193

Appendix B.3 Pink salmon hatchery and wild stock contributions to Prince William Sound cost recovery fisheries by district and week during 1995.

Eastern District

Week Ending	Stat Week	AFK Hatchery		WN Hatchery		CC Hatchery		SG Hatchery		Total Hatchery		Total Wild	Total Catch	Number of Tags
		Contrib	Variance	Contrib.	Variance	Contrib.	Variance	Contrib.	Variance	Contrib.	Variance			
6/24/95	25							8282	11440000	8282	11440000	0	8282	6
7/01/95	26							878823	4.04E+10	878823	4.04E+10	0	878823	497
7/08/95	27							1054189	4.29E+10	1054189	4.29E+10	0	1054189	416
7/15/95	28							494839	1.59E+10	494839	1.59E+10	0	494839	212
7/22/95	29							99445	6.86E+08	99445	6.86E+08	0	99445	39
Subtotals		0		0		0		2535578	9.99E+10	2535578	9.99E+10	0	2535578	1170

Northern District

Week Ending	Stat Week	AFK Hatchery		WN Hatchery		CC Hatchery		SG Hatchery		Total Hatchery		Total Wild	Total Catch	Number of Tags
		Contrib.	Variance	Contrib.	Variance	Contrib.	Variance	Contrib.	Variance	Contrib.	Variance			
8/05/95	31					5380	9645212			5380	9645212	51249	56629	3
8/12/95	32					305680	2.14E+09			305680	2.14E+09	417212	722892	57
8/19/95	33					20003	44440000			20003	44440000	54650	74653	9
8/26/95	34					47524	1.79E+08			47524	1.79E+08	134913	182437	12
Subtotals		0		0		378587	2.38E+09	0		378587	2.38E+09	658024	1036611	81

Coghill District

Week Ending	Stat Week	AFK Hatchery		WN Hatchery		CC Hatchery		SG Hatchery		Total Hatchery		Total Wild	Total Catch	Number of Tags
		Contrib.	Variance	Contrib.	Variance	Contrib.	Variance	Contrib.	Variance	Contrib.	Variance			
7/22/95	29 1/			444	11091					444	11091	0	444	0
7/29/95	30 1/			4788	1289734					4788	1289734	0	4788	0
8/05/95	31			134385	1.02E+09					134385	1.02E+09	0	134385	21
8/12/95	32			160433	2.29E+08					160433	2.29E+08	14504	174937	15
8/19/95	33			392949	5.04E+08	5784	1631351			398733	5.06E+08	52232	450965	170
8/26/95	34			81176	1.12E+08	10611	6027524			91788	1.18E+08	0	91788	21
9/02/95	35			59036	29650000					59036	29650000	6256	65292	36
9/09/95	36 2/			5732	279477					5732	2479477	607	6339	0
Subtotals		0		838943	1.89E+09	16396	7658875	0		855339	1.9E+09	7359	928938	263

1/ Proportions from week 31 were used to allocate the catch.

2/ Proportions from week 35 were used to allocate the catch.

Eshamy District

Week Ending	Stat Week	AFK Hatchery		WN Hatchery		CC Hatchery		SG Hatchery		Total Hatchery		Total Wild	Total Catch	Number of Tags
		Contrib.	Variance	Contrib.	Variance	Contrib.	Variance	Contrib.	Variance	Contrib.	Variance			
8/05/95	31									0		619	619	0
8/12/95	32									0		1946	1946	0
8/19/95	33													
8/26/95	34 3/	152	9782	5806	6133447	1470	216134			7428	6359663	13037	20465	0
9/02/95	35 3/	152	9782	5779	6077232	1464	214450			7395	6301464	12976	20371	0
Subtotals		304	19564	11585	12210679	2934	430884	0		14823	12661127	28578	43401	0

3/ Proportions from week 35 of Eshamy district common property fishery were used to allocate the catch.

Southwestern District

Week Ending	Stat Week	AFK Hatchery		WN Hatchery		CC Hatchery		SG Hatchery		Total Hatchery		Total Wild	Total Catch	Number of Tags
		Contrib.	Variance	Contrib.	Variance	Contrib.	Variance	Contrib.	Variance	Contrib.	Variance			
7/29/95	30	708	250042							708	250042	1841	2549	2
8/05/95	31	34075	1.38E+08							34075	1.38E+08	70918	104993	8
8/12/95	32	95739	5.83E+08			10170	25960000			105910	6.09E+08	0	105910	21
8/19/95	33	187585	1.97E+09	5644	4980080					193503	1.97E+09	0	193503	103
8/26/95	34	116543	2.01E+08			2938	8632293			119481	2.10E+08	4507	123988	90
9/02/95	35 4/	13799	2819448			348	46178			14147	2865626	534	14681	0
Subtotals		448728	2.89E+09	5644	4980080	13456	34538471	0		467824	2.93E+09	77800	545624	224
Grand Totals		449028	2.89E+09	856172	1.91E+09	411373	2.42E+09	2535578	9.99E+10	4252151	1.01E+11	838001	5090152	1738

4/ Proportions from week 34 were used to allocate the catch

Appendix B.4 Pink salmon hatchery and wild stock contributions to Prince William Sound hatchery brood stock by district and week during 1995.

Eastern District

Week Ending	Stat Week	AFK Hatchery		WN Hatchery		CC Hatchery		SG Hatchery		Total Hatchery		Total Wild	Total Catch	Number of Tags
		Contrib.	Variance	Contrib.	Variance	Contrib.	Variance	Contrib.	Variance	Contrib.	Variance			
7/29/95	30							38936	32720000	38936	32720000	4145	43801	47
8/05/95	31							89593	5.15E+08	89593	5.15E+08	0	89593	138
8/12/95	32							106101	7.70E+08	106101	7.70E+08	0	106101	162
8/19/95	33							63287	3.28E+08	63287	3.28E+08	0	63287	134
8/26/95	34							71543	66460000	71543	66460000	15853	87396	77
9/02/95	35							37589	1.25E+08	37589	1.25E+08	0	37589	75
9/09/95	36							551	75900	551	75900	0	551	1
9/16/95	37											16	16	0
Subtotals								407600	1.84E+09	407600	1.84E+09	20014	427614	634

Northern District

Week Ending	Stat Week	AFK Hatchery		WN Hatchery		CC Hatchery		SG Hatchery		Total Hatchery		Total Wild	Total Catch	Number of Tags
		Contrib.	Variance	Contrib.	Variance	Contrib.	Variance	Contrib.	Variance	Contrib.	Variance			
8/26/95	34					1008	254016			1008	254016	0	1008	1
9/02/95	35					11702	12440000			11702	12440000	4459	16161	11
9/09/95	36					49947	53030000			49947	53030000	40128	90075	47
9/16/95	37					56372	59900000			56372	59900000	50646	107018	53
9/23/95	38					4254	4520766			4254	4520766	5392	9646	4
Subtotals						123283	1.30E+08			123283	1.30E+08	100625	223908	116

Coghill District

Week Ending	Stat Week	AFK Hatchery		WN Hatchery		CC Hatchery		SG Hatchery		Total Hatchery		Total Wild	Total Catch	Number of Tags
		Contrib.	Variance	Contrib.	Variance	Contrib.	Variance	Contrib.	Variance	Contrib.	Variance			
7/15/95	28			1						1		0	1	0
7/22/95	29			7						7		0	7	0
7/29/95	30			3						3		0	3	0
8/05/95	31			21						21		0	21	0
8/12/95	32			4						4		0	4	0
8/19/95	33													
8/26/95	34			18920						18920		0	18920	38
9/02/95	35			58645						58645		0	58645	137
9/09/95	36			102929						102929		0	102929	258
9/16/95	37			117913						117913		0	117913	282
9/23/95	38			15576						15576		0	15576	34
Subtotals		0		314019		0		0		314019		0	314019	749

Southwestern District

Week Ending	Stat Week	AFK Hatchery		WN Hatchery		CC Hatchery		SG Hatchery		Total Hatchery		Total Wild	Total Catch	Number of Tags
		Contrib.	Variance	Contrib.	Variance	Contrib.	Variance	Contrib.	Variance	Contrib.	Variance			
8/26/95	34	1819	1483994							1819	1483994	1083	2902	3
9/02/95	35	41128	23690000							41128	23690000	4965	46093	92
9/09/95	36	74185	48230000							74185	48230000	11527	85712	152
9/16/95	37	13857	9493414							13857	9493414	8793	22650	27
Subtotals		130989	82897408	0		0		0		130989	82897408	26368	157357	274
Grand Totals		130989	82897408	314019		123283	1.30E+08	407600	1.84E+09	975891	2.05E+09	147007	1122898	1773

Appendix C **Percent survival by tag code of pink salmon returning to Prince William Sound in 1995.**

Appendix C.1 Percent survival by tag code of pink salmon returning to Prince William Sound in 1995.

Origin	Tag Code	# Tagged	# Released	Estimated Percent Survival	Standard Error	Lower 95% Conf. Interval	Upper 95% Conf. Interval
A. F. Koernig	1301030108	13,427	6,618,697	0.049596	0.36406	0.	0.120954
	1301030109	10,541	60324,498	0.016791	0		
	1301030110	9,213	5,527,509	0.019212	0		
	1301030111	9,741	5,844,629				
	1301030113	9,179	5,507,274	0.095969	0.038343	0.20816	0.171122
	1301030114	10,208	6,125,031	0.231033	0.095736	0.043389	0.418677
	1301030115	8,570	5,142,018	0.226232	0.082136	0.065245	0.387219
	1301030201	8,243	4,946,477	0.197874	0.11882	0.	0.430762
	1301030202	10,577	6,345,996	0.16298	0.05695	0.051356	0.274603
	1301030203	10,794	6,476,718	0.146823	0.065212	0.019006	0.274640
	1301030204	11,143	6,685,569	0.650078	0.205886	0.246541	1.053616
	1301030205	10,450	6,270,226	0.747400	0.15818	0.437366	1.057433
	1301030206	11,368	6,821,127	0.644830	0.200624	0.251606	1.038054
	1301030207	10,191	6,398,894	1.303599	0.251904	0.809865	1.797333
	1301030303	17,732	3,547,896	7.461411	1.119573	5.26705	9.655774
	1301030304	17,481	3,496,392	6.291389	0.893904	4.539336	8.043441
Wally Noerenberg	1301020401	15,977	9,371,637	0.534149	0.099969	0.33821	0.730089
	1301021214	2,229	1,300,230	0.793112	0.183494	0.433464	1.152761
	1301021312	18,674	11,211,336	1.113184	0.153949	0.811441	1.414925
	1301021313	19,208	11,540,914	0.906253	0.137294	0.637156	1.175351
	1301021314	19,917	12,040,148	0.294916	0.068438	0.160777	0.429056
	1301021315	19,744	11,872,060	0.324124	0.084269	0.158955	0.489293
	1301021401	20,181	12,163,694	0.156990	0.0875	0.	0.328491
	1301021402	19,977	19,977	1.996349	0.133013	1.735642	2.257056
	1301021403	20,324	12,055,003	0.206729	0.073913	0.061859	0.351599
	1301021404	20,706	12,328,148	0.179387	0.043744	0.093648	0.265126
	1310121405	20,214	12,126,815	0.094160	0.046921	0.002193	0.186127
	1301021406	20,098	12,106,415	0.065842	0.039793	0.	0.143838
	1301021407	20,113	12,214,122	0.315546	0.078754	0.161187	0.469905
	1301021408	20,385	12,336,261	0.495401	0.10529	0.289032	0.701770
	1301021409	19,965	12,010,977	0.332658	0.069442	0.196550	0.468766
	1301030305	18,990	3,803,426	23.53498	1.343817	20.9011	26.16886
	1301030306	19,469	3,905,582	21.15258	1.247775	18.70694	23.59822

Appendix C.1 Page 2 of 2.

Origin	Tag Code	# Tagged	# Released	Estimated Percent Survival	Standard Error	Lower 95% Conf. Interval	Upper 95% Conf. Interval
Cannery Creek	1301021513	16,084	9,485,711	0.364058	0.119929	0.128997	0.599122
	1301021514	15,523	9,329,671	0.425376	0.139545	0.151867	0.698886
	1301021515	15,793	9,492,115	1.464074	0.273948	0.927134	2.001013
	1310130101	15,691	9,429,516	5.993971	0.525123	4.964729	7.023213
	1301030102	45,797	9,494,035	4.886346	0.482306	3.941026	5.831666
	1301030103	16,252	9,767,701	4.605903	0.460585	3.703154	5.508651
	1301030104	16,434	9,876,333	3.60213	0.402492	2.813245	4.391015
	1301030105	15,961	9,580,712	6.481187	0.548845	5.405451	7.556923
	1301030106	13,569	8,160,820	6.165203	0.551855	5.083567	7.24684
Solomon Gulch	1301030209	49,718	28,140,000	1.67472	0.303499	1.079862	2.269578
	1301030210	49,513	29,370,000	1.309377	0.223177	0.871948	1.746805
	1301030211	50,381	24,170,000	2.017086	0.269197	1.489458	2.544713
	1301030212	53,421	23,740,000	8.600124	1.023131	6.594788	10.60546
	1301030213	68,860	29,553,648	8.166784	0.899825	6.403126	9.930443
	1301030214	33,785	14,500,000	7.397067	0.735555	5.955378	8.838755

Exxon Valdez Oil Spill
Restoration Project Annual Report

Otolith Marking of Pink Salmon in Prince William Sound Salmon Hatcheries, 1995

Restoration Project 95320C
Annual Report

This annual report has been prepared for peer review as part of the *Exxon Valdez* Oil Spill Trustee Council restoration program for the purpose of assessing project progress. Peer review comments have not been addressed in this annual report.

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Renate R. Riffe

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February 1996

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Otolith Marking of Pink Salmon in Prince William Sound Salmon Hatcheries, 1995

Restoration Project 95320C
Annual Report

Study History: After the *Exxon Valdez* oil spill, separation of hatchery and wild stocks was accomplished through coded wire tag recoveries in the salmon fisheries of Prince William Sound (PWS). Therma' otolith marking is a relatively new technology capable of providing more precise and accurate estimates at lower costs than those afforded by the CWT program. In 1995, the first otoliths of hatchery pink salmon were marked with the vision that the technique would eventually replace the CWT program as a tool for stock separation of PWS pink salmon.

Abstract: Otolith mass marking of hatchery pink salmon *Oncorhynchus gorbuscha* is introduced as a potential replacement for coded wire tag technology in an effort to provide more precise and accurate information to the fishery personnel charged with managing the mixed stock fisheries in PWS. All four pink salmon hatcheries in PWS installed water heating systems at a cost of $573,600 to the EVOS Trustee Council to allow rapid and sustained temperature changes to the incubation water. Thermal marks were applied on the otoliths of approximately 684.7 million pink salmon embryos in PWS in 1995. Each hatchery was assigned a distinct thermal mark to allow separation between hatchery stocks and wild stocks in returning adults. A preliminary study was undertaken to determine the amount of mixing that occurs on tenders from the time salmon are taken aboard to the time they are unloaded at the processor. The results of this study will be used in determining the complexity of the otolith sampling program required to provide managers with information on the hatchery and wild stock composition of the common property fishery.

Key Words: Commercial harvest, hatchery, *Oncorhynchus gorbuscha*, otolith, pink salmon, Prince William Sound, thermal mark, wild stock.

Citation

Joyce, T.L., D.G. Evans, and R.R. Riffe. 1996. Otolith marking of pink salmon in Prince William Sound hatcheries, 1995, *Exxon Valdez* Oil Spill Restoration Project Annual Report (Restoration Project 95320C), Alaska Department of Fish and Game, Commercial Fisheries Management and Development Division, Cordova, Alaska.

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EXECUTIVE SUMMARY

This report documents Restoration Study 95320C, one of the projects designed to restore the pink salmon *Oncorhynchus gorbuscha* resource of Prince William Sound (PWS) to its pre-spill status. Water heaters were installed at four hatcheries in PWS; A.F. Koernig, W. H. Noerenberg, Cannery Creek, and Solomon Gulch. Approximately 684.7 million pink salmon embryos had marks applied to their otoliths by a sustained 4°C change in the water temperature for a 24 hour period. Application of hatchery-specific marks will allow separation between hatcheries and between wild stocks when adults return in 1997. To aid in the development of an efficient otolith sampling strategy, a study was undertaken to determine the amount of mixing that occurs in salmon tenders.

INTRODUCTION

Between 1961 and 1976, when hatcheries were absent from Prince William Sound (PWS), the commercial seine harvest of wild pink salmon *Oncorhynchus gorbuscha* averaged about 3.4 million fish. In the early 1970's, run failures led to an aggressive enhancement program which included construction of hatcheries. By 1986 five hatcheries were operating: the Solomon Gulch hatchery, producing pink salmon, and later, chum *O. keta,* coho *O. kisutch* and chinook salmon *O. tschawytscha*, the A. F. Koernig hatchery, producing pink salmon, the W. H. Noerenberg hatchery, producing pink salmon, and later, chum, coho and chinook salmon, the Cannery Creek hatchery, producing pink salmon, and the Main Bay hatchery which produced chum and presently raises sockeye salmon *O. nerka.* From the late 1980's to the present, returns to these facilities have contributed approximately 20 million fish to the annual pink salmon run. Significant numbers of sockeye, coho, chum and chinook salmon have also been produced.

Parent stocks for PWS hatchery production were selected from native populations in the Sound with the consequence that the migratory timings of adult hatchery and wild returns coincided. Furthermore, virtually all these salmon stocks migrate to their natal streams or hatcheries through corridors in the southwestern and western areas of the Sound. The coincident timing and location of the large hatchery return and the considerably smaller wild returns lead to the danger of over-exploitation of the latter by the commercial fishery. Indeed, shortfalls in wild escapements were observed in more than half of the 15 years prior to hatchery production, when the average exploitation rate was 42%, a figure considerably lower than the 60% considered appropriate today for returning hatchery fish.

To protect wild stocks in a hatchery-dominated fishery, managers needed information pertaining to the temporal and spatial distributions of hatchery and wild fish. To meet this requirement, a coded wire tagging (CWT) program was initiated in 1986 for hatchery releases of pink salmon with recovery of tagged returning adults in commercial and cost-recovery fisheries beginning in 1987. Tag recovery data enabled managers to estimate hatchery and wild contributions to catches from temporal and spatial strata within the fishery.

The March 24, 1989, *Exxon Valdez* oil spill exacerbated the problems faced by the fishery manager. The spill contaminated intertidal portions of streams where the majority of wild salmon stocks in western PWS spawn as well as the marine waters traversed by juvenile salmon on their migration seaward through the Sound. Natural Resource Damage Assessment Fish/Shellfish (F/S) studies 2 and 4, demonstrated significant detrimental effects of oil contamination upon pink salmon embryos, pre-emergent fry, and juvenile salmon from wild populations in the Sound. The

decisions made by fishery managers suddenly became more complicated in as far as they affected wild populations injured by the oil spill.

The CWT program was continued through the years following the spill, and was funded under the Natural Resource Damage Assessment study F/S 3 through 1991 (Sharr et al, 1995a). During this period, the program continued to provide information pertaining to the nature of the commercial salmon catch. In 1992, the pink salmon tagging program was supported through Restoration Study R60A (Sharr et al, 1995b) and in 1993 and 1994 through Restoration Study 93067 (Sharr et al, 1995c) along with matching funds from the Prince William Sound Aquaculture Corporation (PWSAC), Valdez Fisheries Development Association (VFDA) and the State of Alaska. In 1995, the program was supported by R95320B, along with matching funds from PWSAC, VFDA, and the State of Alaska.

Hatchery contribution estimates provided by the CWT program are based on several assumptions including the premise that a tagged fish does not behave any differently from its untagged cohorts. In reality, tagged fish likely experience higher mortality rates, and an adjustment factor, based upon tag recoveries from the brood stock, has been used to compensate for this effect and the fact that tags are inevitably shed after implantation. The validity of the adjustment factor corrections are dependent upon the assumption that brood stocks contain only fish reared at the hatchery in question, and that for a given cohort, the tag rate in the brood is equal to that in the commercial fishery. It is thought that these assumptions may be flawed. Immigration of wild fish into the brood stocks is suspected (Sharr et al. 1995c) and Habicht (1996) presents evidence that tags may induce straying, so that marks present in the commercial fishery may not be fully represented in the brood stocks. Identifiable thermal marks have been produced on otoliths of chinook salmon , coho salmon, sockeye salmon, chum salmon, pink salmon and Atlantic salmon (*Salmo salar*) (Munk et al, 1993). It is thought that use of such a non-intrusive mark will eliminate the need for adjustment factors and will result in more accurate and precise estimates of hatchery contributions to the commercial fishery. It is also anticipated that the cost of applying and recovering marks will be less than that of the current CWT program. Otolith marking of all the hatchery pink salmon production was initiated in 1995 with support from R95320C. Two years of marking PWS pink salmon with both thermal marks and CWT's will allow direct comparisons between the two methods and provide insight on the drawbacks and benefits of each type of mark

This report documents the activities and results of the otolith marking program in 1995 as it pertains to hatchery pink salmon. It focuses primarily upon hatchery equipment installation and mark application.

OBJECTIVES

1. To install and operate a water heating system capable of sustaining a 3.5°C change in incubation water temperature for a minimum of 24 hours.

2. To apply unique and distinct thermal marks to the otoliths of developing pink salmon embryos at all four pink salmon hatcheries in PWS.

3. To determine the amount of mixing of fish that occurs from loading to unloading in salmon tenders.

METHODS

Equipment Purchase and Installation

The water heating system used at all four pink salmon hatcheries in PWS was designed by KCM. Inc. of Seattle, Washington. The design packet was let for bid and was awarded to Ramset Mechanical of Renton, Washington. The equipment was installed in a containerized van by Ramsett Mechanical. One van was then shipped to each hatchery in the Sound. Hatchery personnel located the van and made all the water, fuel and electrical connections. Once installed, Ramsett Mechanical supplied one inspector to perform a final inspection and test the system. Each van contained two boilers designed to heat process water with flow rates from 50 to 200gpm at temperatures ranging from 1.7 to 9.4°C to a *delta* T of 11.7°C at the maximum process water temperature and at 200 gpm. Additional technical specifications can be found in the Technical Specifications for the Design and Construction of a Containerized Process Water Heating System (Appendix A).

Application of Thermal Marks

Otoliths are composed of three pairs of bones in salmon, the sagittae, lapillae and asteriscae. The sagittae is the bone used for applying thermal marks because of its early development, size and hardiness. Thermal marks were applied to hatchery produced pink salmon embryos in PWS after otolith development proceeded beyond the primordial stage, approximately 275 temperature units (1 temperature unit is 1°C above 0°C for 24 h). This development also coincides with what is commonly referred to as the "eyed" stage in salmon egg development. The thermal marking was to be completed prior to egg hatch to eliminate the masking of the mark at hatch and to prevent gas supersaturation problems in the alevins from the heated water.

Thermal marks on otoliths are classified using a "Region, Band, and ring" (RBr) code. The Region (R) of the mark is broken down into 3 parts. Region 1 occurs in that area after the primordial stage and before the hatch mark. Region 2 occurs after the hatch mark and Region 3 describes a thermal mark that occurs in both Region 1 and Region 2. The Band (B) of the mark is composed of one or more rings (r). Generally speaking Bands will have a minimum of three rings as fewer rings often may be overlooked as a normal growth sequence. The ring number describes the number of dark colored rings in a band. The RBr code is written numerically R:B.r and is described schematically (Table 1) as a series of "I's" (Munk, in process).

Thermal marks were applied to all pink salmon embryos incubated in PWS hatcheries in the fall of 1995. Marks were chosen to distinguish all four hatcheries and to be applicable within the available marking window (Table 1). A suitable thermal mark schedule was devised to provide the chosen marks. Thermal marks were induced by causing rapid temperature declines of 4°C in the incubation water. This decline was accomplished by raising the ambient water temperature for 24 hours and then returning it back to its ambient temperature in repeated cycles until the desired number of rings were applied to the otolith of the fish. Rings are laid down during the temperature decline as the amount and rate of materials being deposited on the otolith are changed (Munk, et al, 1993). Early in the marking schedule when ambient water temperatures were high, the schedule used 24 hour alternating cycles. Later in the season as the ambient temperatures dropped the schedule was modified to 36 hour alternating cycles at Cannery Creek and W.H. Noerenberg hatcheries to insure proper spacing between rings. Solomon Gulch and A.F. Koernig hatcheries had completed their marking prior to the lower ambient temperatures.

Table 1. Banding paterns and associated thermal schedules.

Hatchery	Schedule	R:B.r Ring pattern	
A.F.KOERNIG	(4X)24H:24C	1:1.4	IIII
CANNERY CREEK	(3X)24H:24C,(1X)72H:36C,2(X)24H:24C	1:1.3,2.3	III III
W.H. NOERENBERG	(8X)24H:24C	1:1.8	IIIIIII
SOLOMON GULCH	(6X)24H:24C	1:1.6	IIIII

The marking schedules were set up so that the oil-fired boilers ran continuously after a second row of incubators reached the minimum developmental stage needed for marking. The alternating hot and cold cycles were timed to run one row of incubators hot while the second row was running cold. The sequence was reversed for the next 24 hours. This marking schedule allowed for the maximum number of embryos to be marked in the shortest time period.

Determination of the Travel-induced Redistribution of a Localized Collection of Marks within a Tender

Pectoral fin-clipped fish were added to two tenders at the A.F. Koernig hatchery during cost recovery harvests and to one tender at Payday Point in the Unakwik district during a commercial fishery opening. When a suitable tender was identified, a group of three to four technicians flew out to the tender, and removed pectoral fins from approximately 2,000 pink salmon using garden pruning shears. The clipped fish were added *en masse* to the fish hold. Stratified random

samples of the tender loads were subsequently taken from the processing belt at the North Pacific processing plant in Cordova. A lap-top computer using a random-sampling algorithm, was used to indicate when a fish should be selected from the belt and examined for a missing pectoral fin. Data were analyzed using a χ^2 -test of independence, a logit and linear logit analysis and a runs test.

RESULTS

Equipment Operation

All the hatcheries were able to apply their assigned thermal mark to all lots of pink salmon embryos prior to hatching. None of the hatcheries experienced any difficulties with the water heating equipment that would have compromised their assigned mark. All of the hatcheries were able to maintain temperatures from 3.8° to 4.0°C above the ambient temperature when the marking process required it.

Thermal Marks

High quality marks were found in samples taken from each of the four hatcheries three weeks after completion of the marking process (Figure 1). Voucher samples will be taken at the time of emergence from each lot at each hatchery so that any confounding marks laid down during the remaining incubation period will be apparent and documented.

Assessment of Degree of Mixing within a Tender

Sampling Statistics

The following stipulations/assumptions were made regarding the sampling scenario:

-Five strata were to be examined at the processor
-Ten marks were to be found per interval, in the event that marks were randomly distributed
-The number of signals per minute that a sampler could deal with was 10 (Event 1) and 15 (Events 2 and 3)
-The rate of unloading at the processor was 500 (Event 1) and 650 (Events 2 and 3) fish per minute

The above led to a required addition of 2,500 marks to the tender load for Event 1 and 2,166 marks for Events 2 and 3. Circumstances conspired to yield three strata for events 1 and 2 (the unloading rate was underestimated).
Descriptions of the three marking and sampling events are given in Table 2, and results pertaining to found marks in Table 3.

Figure 1. Thermal Marks on Pink Salmon Otoliths from Four Different Hatcheries in Prince William Sound

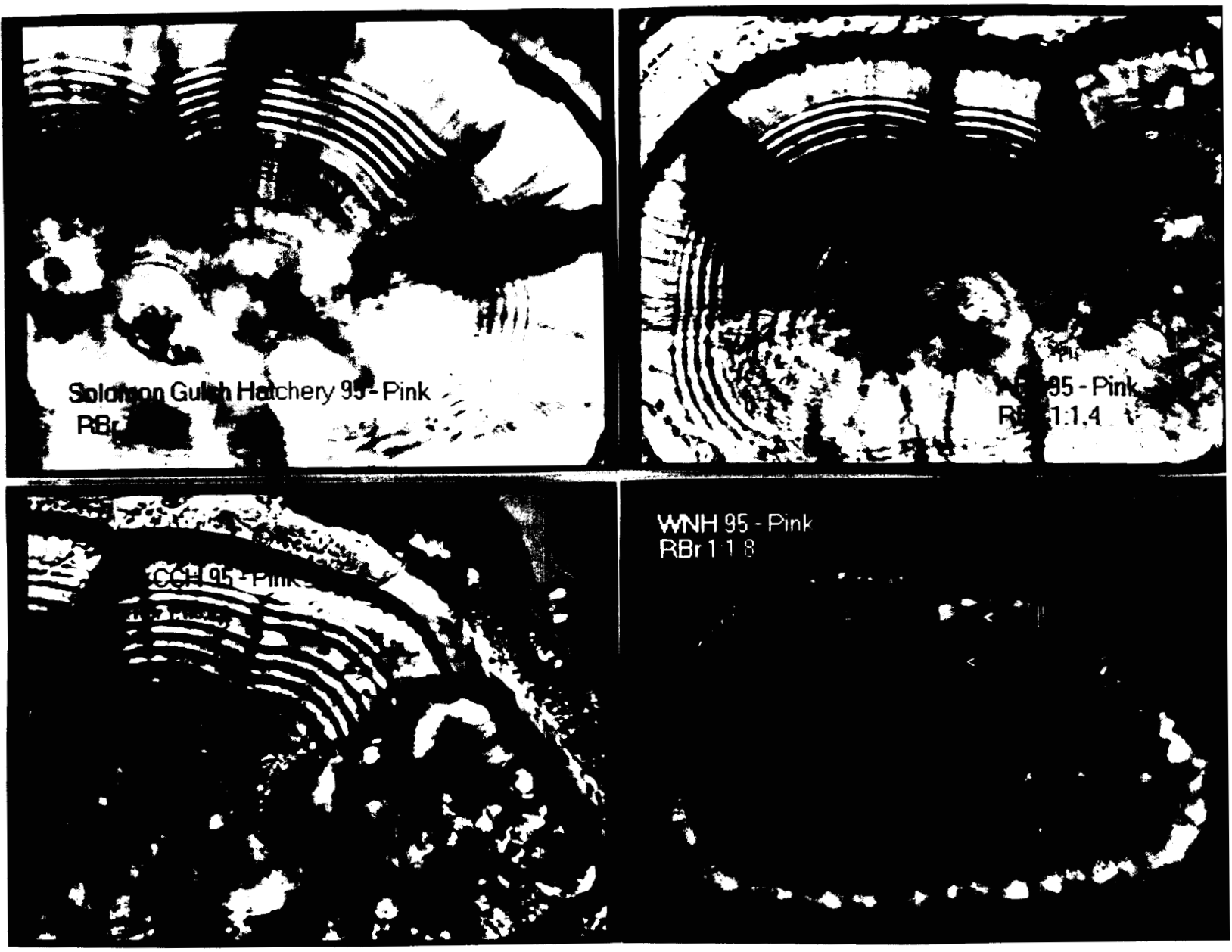

Table 2. Marking and sampling scenarios for Events 1 through 3.

	EVENT		
	1	2	3
Tender Name	Spartan	James A.	Spartan
Load (# fish)	31,805*	43,000	25,820
Loading Site	A.F. Koernig	Payday Point (Unakwik)	A.F. Koernig
# Marks Added	1,948	2,300	2,242
Marks Added after :	27% loading complete	0% loading complete	100% loading complete
Distance to Processor (miles)	85	60	85
Processor	North Pacific	North Pacific	North Pacific
Date of Sampling	08/15/95	08/18/95	08/27/95

* Total load was 45,850 fish, but marked fish only added to one of the fish holds, which contained 31,805 fish.

Table 3. Found marks for Events 1 through 3.

	STRATUM					
	I	II	III	IV	V	Total
Event 1						
Marked	7	12	3			22
Unmarked	168	150	87			405
Total Sampled	175	162	90			427
Event 2						
Marked	4	8	18			30
Unmarked	116	110	136			362
Total Sampled	120	118	154			392
Event 3						
Marked	15	9	1	4	1	30
Unmarked	78	74	109	91	109	461
Total Sampled	93	83	110	95	110	491

Analyses

χ^2 test of independence

A χ^2 -test of independence between the mark variable (two levels, marked and unmarked) and the stratum variable was conducted.. The question addressed was "Is the ratio of marked to unmarked fish independent of the stratum number?". P values for events 1, 2 and 3 were 0.25, 0.033 and 0, respectively. More detailed results are given in Table 4.

Table 4. χ^2 and P values for test of independence.

	EVENT		
	1	2	3
χ^2	2.8	6.84	30.5
df	2	2	4
P value	0.25	0.033	0

Logit analysis

Two logit models were fitted, with marked/unmarked being the binary response. The first model followed the form:

$$\log\left[\frac{\#Marked_i}{\#Unmarked_i}\right] = \alpha + \beta_i \qquad \text{EQ 1}$$

where i indexes stratum number. A full (saturated) and reduced ($\beta i=0$) model were fitted, and a likelihood ratio test used to determine whether the β_i's explained a significant amount of variation in the data. The interpretation of, for example, β_{III} for any of Events 1, 2, or 3 under the restrictions ($\beta_I=0$) imposed by GLIM software (Numerical Algorithms Group) is :

$$\beta_{III} = Log\left[\frac{\frac{\Pi_{m,III}}{\Pi_{u,III}}}{\frac{\Pi_{m,I}}{\Pi_{u,I}}}\right] \qquad \text{EQ 2}$$

where $\pi_{m,III}$ is the probability of marked fish in stratum III, and $\pi_{u,III}$ is the probability of unmarked fish in stratum III (similarly for stratum I). The parameter β_{III} is thus the log odds ratio for stratum I vs III. A similar interpretation may be made for β_{II} The model is a general one, in that it allows the existence of two different log-odds ratios. Results are given in Table 5.

Table 5. Parameter estimates and *P* values for logit model.

		EVENT		
		1	2	3
Parameter Estimates:				
	α	-3.178	-3.367	-1.649
	β_{II}	0.652	0.746	-0.458
	β_{III}	-0.189	1.345	-3.04
	β_{IV}	-	-	-1.48
	β_{V}	-	-	-3.04
P value for test $H_o: \beta_{II}=\beta_{III}=0$		0.259	0.028	0

The second model follows the form:

$$\log\left[\frac{\#Marked_i}{\#Unmarked_i}\right] = \alpha + \beta X_i \qquad \text{EQ 3}$$

where X_i is an ordinal explanatory variable indicating stratum number.? The model now fits one parameter to describe differences between strata, and assumes that the log-odds ratio between adjacent strata is constant:

$$\beta = Log\left[\frac{\frac{\Pi_{m,i+1}}{\Pi_{u,i+1}}}{\frac{\Pi_{m,i}}{\Pi_{u,i}}}\right] \qquad \text{EQ 4}$$

The model is a more parsimonious one than the one described in Equation 1), since it has only two parameters. A full and reduced (β=0) model were fitted, and a likelihood ratio test used to determine whether the linear component explained a significant amount of variation in the data. Results are given in Table 6.

Table 6. Parameter estimates and *P* values for linear logit model..

	EVENT		
	1	2	3
Parameter Estimates:			
α	-2.969	-3.98	-0.909
β	0.031	0.656	-0.732
P value for test H_o:β=0	0.91	0.007	0

Comparisons of proportions of marks between strata

The sample size in each stratum was considered large enough, and the ratio of sample to total catch small enough that the calculated proportions could be assumed to be normally distributed. Z-tests of differences (number of strata-1 orthogonal comparisons) in proportions between strata were therefore considered appropriate. The results are given in Table 7.

Table 7. Estimated proportions of marks and *P* values for Z-tests.

	STRATUM				
	I	II	III	IV	V
Event 1 Estimated proportions	0.04	0.074	0.033	-	-
	P value for Ho:Proportion in Stratum I =Proportion in Stratum j				
	Stratum j				
	I	II	III	IV	V
	-	0.179	0.780	-	-
Event 2 Estimated proportions	0.033	0.068	0.117	-	-
	P value for Ho:Proportion in Stratum I =Proportion in Stratum j				
	Stratum j				
	I	II	III	IV	V
	-	0.223	0.0063	-	-
Event 3 Estimated proportions	0.161	0.108	0.009	0.042	0.009
	P value for Ho:Proportion in Stratum I =Proportion in Stratum j				
	Stratum j				
	I	II	III	IV	V
	-	0.299	<0.0061	0.0061	<0.0061

Runs test

Here the number of runs (in the sequence marked, marked, unmarked, unmarked, marked, marked, there are three runs) in the sample is compared to the number expected in a random sequence of marked and unmarked fish. While large-sample Z-tests are available for such a comparison, simulations revealed that distributions of the number of runs under the null hypothesis of random sequences were fairly non-normal for the combinations of marked and unmarked fish present in this study. *P* values were obtained using simulated empirical distributions of the number of runs in random sequences. The results are presented in Table 8.

Table 8. Observed number of runs and *P* values for H_o: sequence is random.

	EVENT		
	1	2	3
Observed number of runs	43	55	55
P value for test H_o:Sequence is random	0.71	0.39	0.28

DISCUSSION

Equipment

All equipment purchased and installed proved to be adequate to thermally mark all the pink salmon embryos at all four PWS hatcheries, thus meeting Objective 1. The heated water was able to maintain close to a 4°C rise in temperature over ambient for the prescribed amount of time. The systems required little maintenance or repair in this first year of operation with the exception of some minor initial problems encountered at the Cannery Creek and Solomon Gulch hatcheries.

Thermal Marks

High quality marks are one of the prerequisites to the successful implementation of an otolith marking program intended to separate hatchery and wild stocks. Preliminary sampling of otoliths indicates that such marks were indeed placed on the otoliths of all pink salmon embryos produced by PWS hatcheries. While further voucher samples will yield a more detailed view of the nature and success of the marking process, it currently appears that the goals outlined in Objective 2 have been met.

Mixing of Fish within a Tender

For the events where marked fish were added at either the beginning or the end of the load (events 2 and 3, respectively), strong evidence was found to indicate that added marks were clustered within the hold of the tenders upon unloading at the processor. The χ^2, logit, linear logit and z-test analyses all led to strong rejection of the hypothesis that marks were evenly distributed throughout the load. The runs test did not reject the hypothesis. Non-parametric tests are notoriously weak, and it is suggested that this is the reason for the inability of the runs tests to identify any effect. The linear logit β parameter explained a significant amount of variation for both events. This is consistent with the notion that there was an incomplete mixing, with the result that the number of marks appearing on the processing belt decreased as fish were pumped from sections of the tender distant from the location to which the marks were added. The signs of the estimates of the linear logit parameters are also consistent with the mark-loading scenario. For event 2, where marks were added at the beginning of the load, the estimate of β is +0.656, which makes sense in that it estimates that the odds for marked fish versus unmarked fish for stratum III is almost twice that of stratum II, which is twice that of stratum I. For event 3, where

marks were added at the end of the loading process, the estimate of β is -0.732. Also of interest is that these estimates are of similar magnitude, suggesting that the degree of mixing is similar for both tenders. Fish appear to mix equally in both directions, bottom to top and top to bottom.

No evidence was found from event 1 to indicate that marks were clustered in the hold of the tender. It is suggested that this is because marks were added to the middle of the load, and some mixing occurred in both directions. The load therefore appeared mixed at the processor.

The relevance of the above to the design of otolith-sampling schemes is that it appears that we cannot rely on tender travel to efficiently mix loads. Tenders themselves may be heavily stratified if they receive, for example, predominantly wild fish at the beginning of the loading process, and hatchery fish towards the end. It seems that systematic samples taken from one section of the load may be biased, and that to achieve a representative sample, some sort of stratified sampling will be required from each tender.

CONCLUSIONS

The major objective of this project was to thermally mark all hatchery produced pink salmon in a manner that would allow the separation of hatchery produced fish between hatcheries and between wild stocks. The samples taken three weeks postmark indicate that unique high quality marks were applied using the equipment purchased and installed in 1995 at the four PWS pink salmon hatcheries. With respect to catch-sampling strategies, it appears that a tender cannot be relied upon to efficiently mix a load to the extent that systematic samples can be assumed to mimic, even distantly, random samples. It is apparent that any sampling strategy should sample otoliths throughout the unloading process.

LITERATURE CITED

Habicht, C., S. Sharr, and J.E. Seeb. 1996. Coded wire tag placement affects homing precision of pink salmon. *In press.*

Munk, K.M., W.W. Smoker, D.R.Beard, and R.W. Mattson. 1993. A hatchery water-heating system and its application to 100% thermal marking of incubating salmon. The Progressive Fish-Culturist. 55:284-288.

Munk, K.M., (*in process*), Thermal Marking Manual: A guideline to the induction of thermal marks in otoliths for the purpose of mass-marking hatchery stocks. Alaska Department of Fish and Game, Special Publication, Juneau.

Sharr, S., T.M. Willette, C.J. Peckham, D.G. Sharp, J.L. Smith, D.G. Evans, and B.G. Bue. 1995a. Coded wire tag studies on Prince William Sound salmon. Natural Resource Damage Assessment Fish/Shellfish Study Number 3, Alaska Department of Fish and Game, Cordova.

Sharr, S., C.J. Peckham, D.G. Sharp, J.L. Smith, D.G. Evans, and B.G. Bue. 1995b. Coded wire tag studies on Prince William Sound salmon. Natural Resource Damage Assessment Restoration Study Number R60A, Alaska Department of Fish and Game, Cordova.

Sharr, S., C.J. Peckham, D.G. Sharp, J.L. Smith, D.G. Evans, and B.G. Bue. 1995c. Stock identification of chum, sockeye, coho and chinook salmon in Prince William Sound. Natural Resource Damage Assessment Restoration Project 93068, Alaska Department of Fish and Game, Cordova.

APPENDICES

Appendix A: Technical Specifications for the Design and Construction of Containerized Process Water Heating System

PRINCE WILLIAM SOUND AQUACULTURE CORPORATION

PWSAC

TECHNICAL SPECIFICATIONS FOR THE DESIGN AND CONSTRUCTION OF A CONTAINERIZED PROCESS WATER HEATING SYSTEM

February 1995

by

KCM

KCM, Inc.
230 South Franklin
Suite 204
Juneau, AK 99801-1364

Project: PWSAC Containerized Process Water Heating Systems

Bid Submitted to: Prince William Sound Aquaculture Corporation

c/o KCM, Inc.
1917 First Avenue
Seattle, Washington 98101
Attention: Barry Scott
Telephone: (206)443-5377
Fax: (206)443-5372

Bids to Submitted on or before: February 23, 1995 at 2:00 PM Pacific Standard Time

1. The undersigned Bidder proposes and agrees, if this Bid is accepted, to enter into an Agreement with the Owner in a form to be agreed upon to complete all work as specified or indicated in the contract documents for the contract price and within the contract time indicated in this Bid and in accordance with the contract documents. A Performance and Payment Bond (in a form to be agreed upon) will be required.

2. The Bidder shall insert unit bid price or a lump sum price in figures and in words opposite each pay item for which an estimated quantity appears in the Bid Form. A unit bid price or lump sum bid price is not to be entered or tendered for any pay item for which no estimated quantity appears in the Bid Form. The estimated quantity of Work for payment on a lump sum basis will be "all required".

Notice: Contract award will be made on the basis of the Total Amount of Basic Bid, or the Total Amount of Basic Bid plus the Total Amount of Bid Alternative No. 1. Owner reserves the right to award the Basic Bid, or the Basic Bid plus Bid Alternative No. 1, or to reject all bids.

BASIC BID

Pay Item No.	Pay Item Name Lump Sum Bid Price Written in Words	Amount Bid
001 Lump Sum All Required	One (1) Complete Containerized Process Water Heating System, for Solomon Gulch Hatchery (SGH) Delivered to Valdez, Alaska on or before June 1, 1995 at _____ _____ (amount in words)	$_____
002 Lump Sum All Required	One (1) Complete Containerized Process Water Heating System, for W. Noerenberg Hatchery (WNH) Delivered to Whittier, Alaska on or before June 15, 1995 at _____ _____ (amount in words)	$_____
003 Lump Sum All Required	One (1) Complete Containerized Process Water Heating System, for A. F. Koernig Hatchery (AFK) Delivered to Whittier, Alaska on or before June 15, 1995 at _____ _____ (amount in words)	$_____
004 Lump Sum All Required	One (1) Complete Containerized Process Water Heating System, for Cannery Creek hatchery (CCH) Delivered to Whittier, Alaska on or before June 15, 1995 at _____ _____ (amount in words)	$_____
	TOTAL AMOUNT OF BASIC BID:	$_____

BID ALTERNATIVE NO. 1

Pay Item No.	Pay Item Name Lump Sum Bid Price Written in Words	Amount Bid
005 Lump Sum All Required	One 91) Complete Process Water Heating System, for Solomon Gulch Hatchery (SGH) Delivered to Valdez, Alaska on or before June 1, 1995, skid-mounted as specified herein in lieu of the containerized unit provided for in the Basic Bid (Check Deduct or Add below) _____ DEDUCT FROM ITEM 001 FROM THE BASIC BID _____ ADD TO ITEM 001 FROM THE BASIC BID at _____ _____ (amount in words)	($_____) Deduct $_____ Add
TOTAL AMOUNT OF BID ALTERNATIVE NO. 1:		($_____) Deduct $_____ Add

3. Unless otherwise stated in the Bid Form, it will be assumed by the Owner that the bidder has accepted without reservation or amendment the whole of the contract documents. At a bidder's discretion, a Bid may be conditioned upon such qualifications of or amendments to the contract documents as do not materially change the requirments contained therein. Such qualifications or amendments shall be fully described on the form provided herewith. The Owner reserves the right to accept or reject any other qualifications or amendments and to consider price variations thereof (if any) in detemining the lowest responsible bid.

4. When the SGH heating system is delivered to Valdez, Alaska the ownership of the system will transfer from PWSAC to the Valdez Fisheries Development Association (VFDA). All maintenance and guarantee work requests for the SGH system will come from VFDA. The remaining three containerized heating systems will remain under the ownership of PWSAC.

5. In the event that Bid Alternate No. 1 is awarded, the contractor shall be fully responsible for shipment of the skid-mounted system to SGH such that the system arrives complete, intact, undamaged and suitable for installation in a room provided by others. VFDA will be responsible for provision of an appropriate and adequate room for the system at SGH.

Submitted on: _____ Day of _____, 1995

Name of Bidding Firm: _____

If Bidder is an Individual:

Submitted by : _____
(Signature)

Business Address: _____

_____

_____

Telephone No: _____

Business License No: _____

If Bidder is a Partnership:

Submitted by : _____
(Signature)

Name: _____

Title: General Partner

Business Address: _____

_____

_____

Telephone No: _____

Business License No: _____

If Bidder is a Corporation:

Submitted by : _____
(Authorized Signature)

Name: _____

Title: _____

State of Incorporation: _____

Business Address: _____

_____

_____

Telephone No: _____

Business License No: _____

PWSAC
CONTAINERIZED PROCESS WATER HEATING SYSTEM(S)

PROPOSED QUALIFICATIONS AND AMENDMENTS TO BID

The following is a full and complete statement and description of the Bidder's suggested qualifications of and amendments to the specifications, drawings and other contract documents. Additional supporting data outlining this portion of the bid shall be numbered, titled PWSAC Containerized Process Water Heating System(s) Proposed Qualifications and Amendments to Bid, and shall be signed by the bidder.

_____

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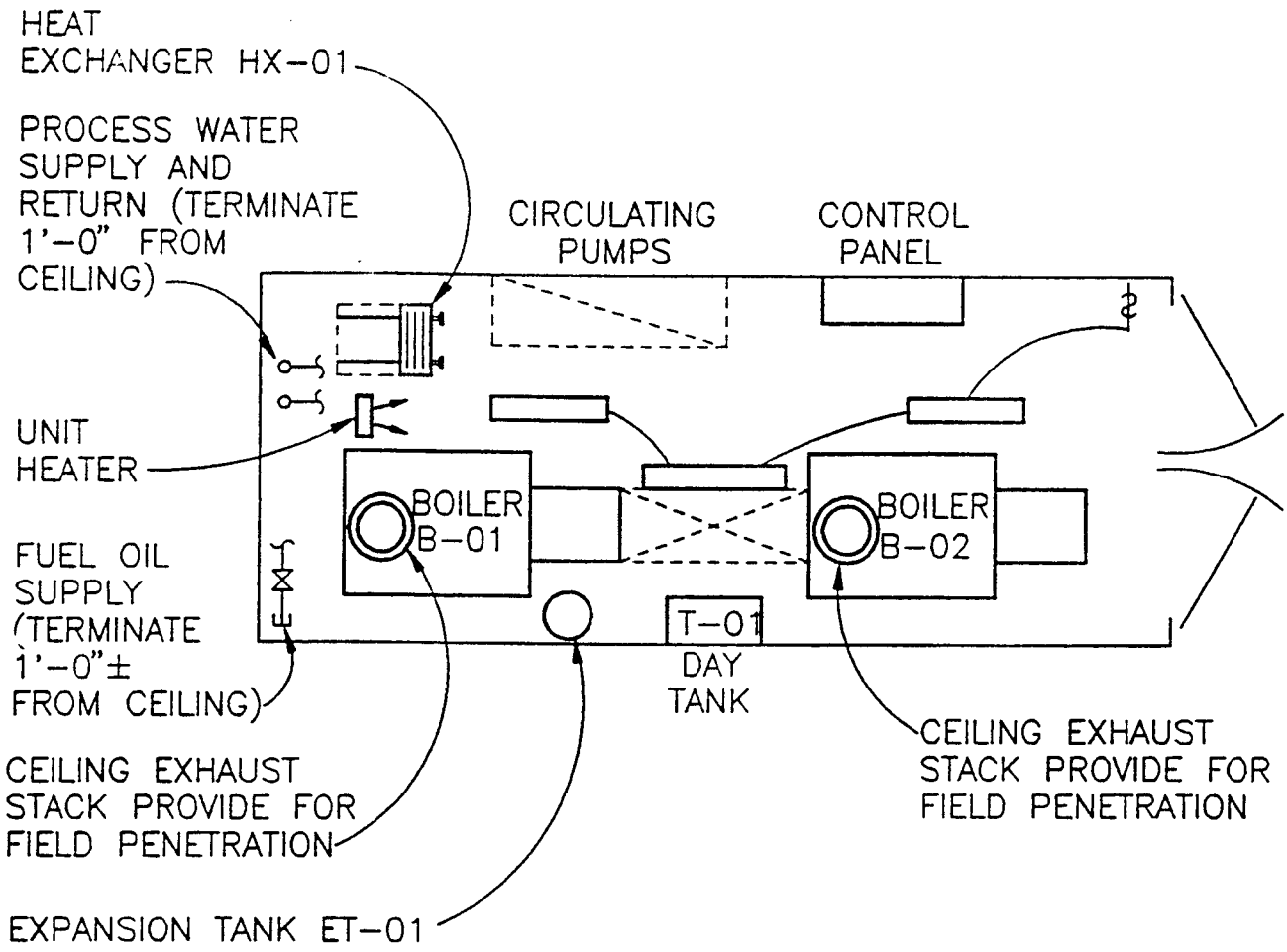
By: _____
(Authorized Signature)



SGH PLAN (BID ITEM 001) 1/1

1/4"=1'-0"

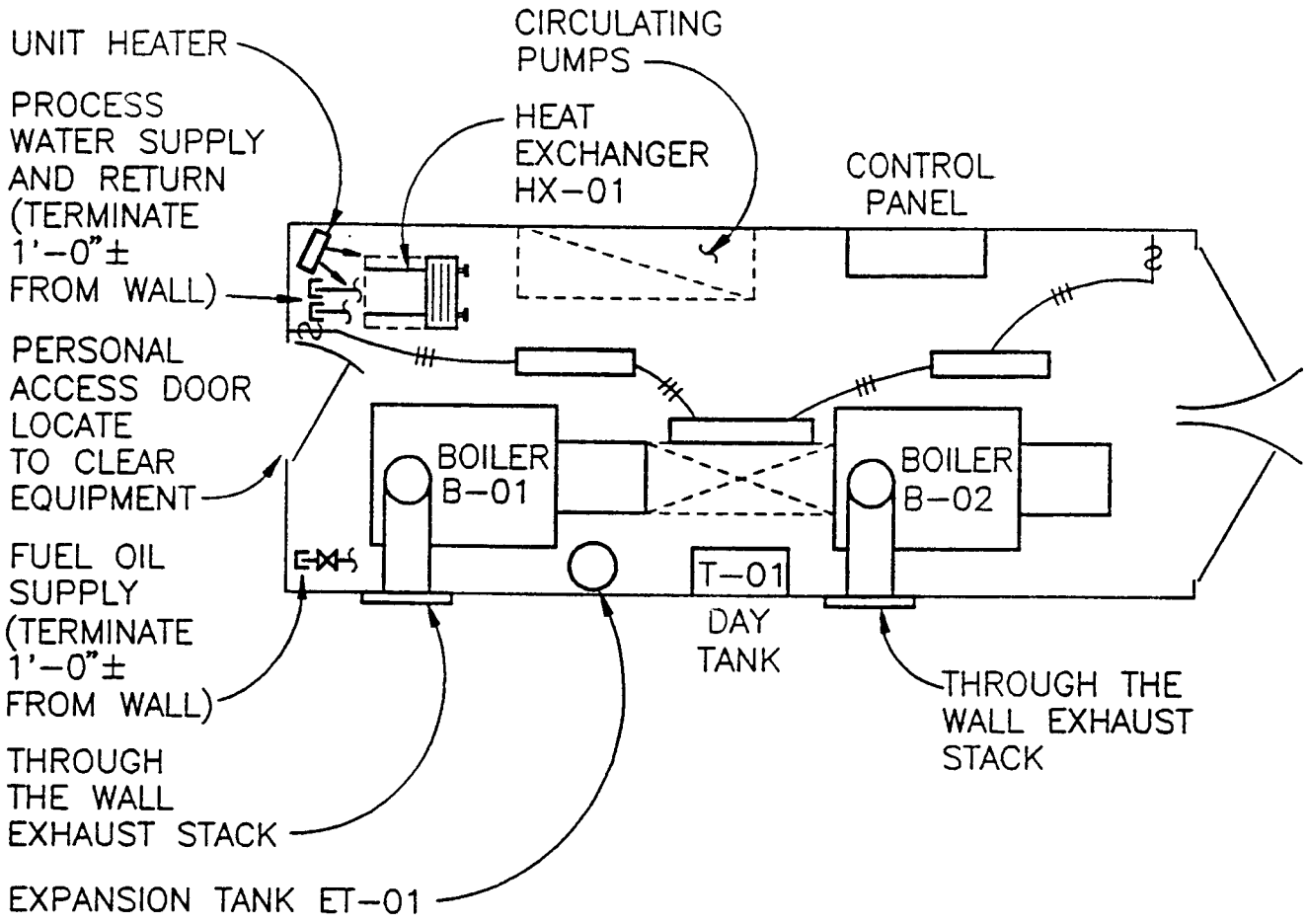
NOTES:

1. PLAN SHOWN FOR CONCEPT ONLY. CONTRACTOR TO MODIFY PLAN TO ACCOMODATE THEIR PROPOSED EQUIPMENT. LAYOUT TO COMPLY WITH ALL APPLICABLE CODES.
2. CONTAINER SHALL BE CLEARLY MARKED WITH IDENTIFICATION CODE SGH.

50011F01 1=48 2450011 1/20F

KCM

PRINCE WILLIAM SOUND AQUA CORP, PWSAC	CONTAINERIZED HEATING SYSTEM



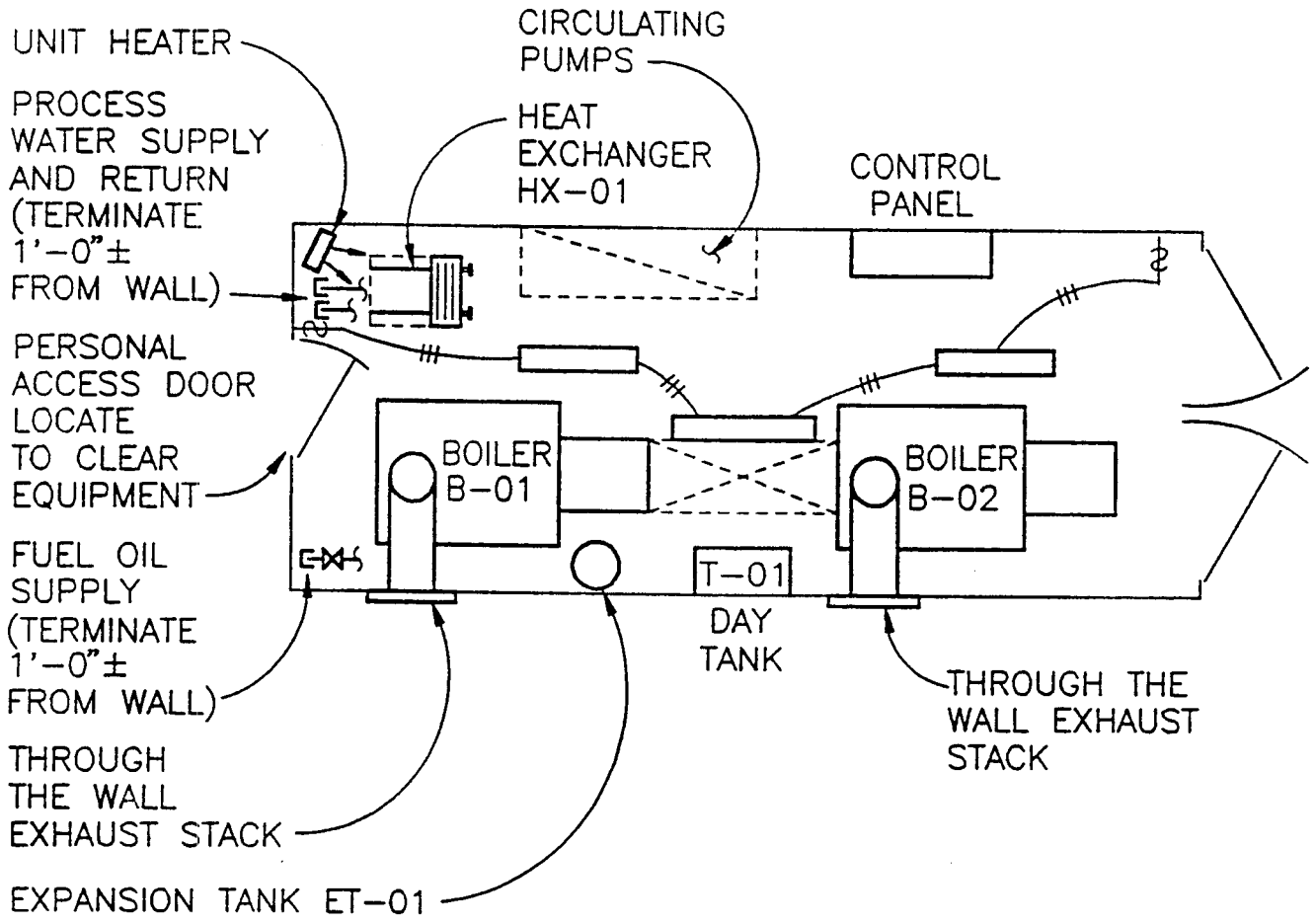
WNH PLAN (BID ITEM 002) 2/–

1/4"=1'-0"

NOTES:

1. PLAN SHOWN FOR CONCEPT ONLY. CONTRACTOR TO MODIFY PLAN TO ACCOMODATE THEIR PROPOSED EQUIPMENT. LAYOUT TO COMPLY WITH ALL APPLICABLE CODES.
2. CONTAINER SHALL BE CLEARLY MARKED WITH IDENTIFICATION CODE WNH.

50011F03 1=48 2450011 1/20



AFK PLAN (BID ITEM 003)

3/—

1/4"=1'-0"

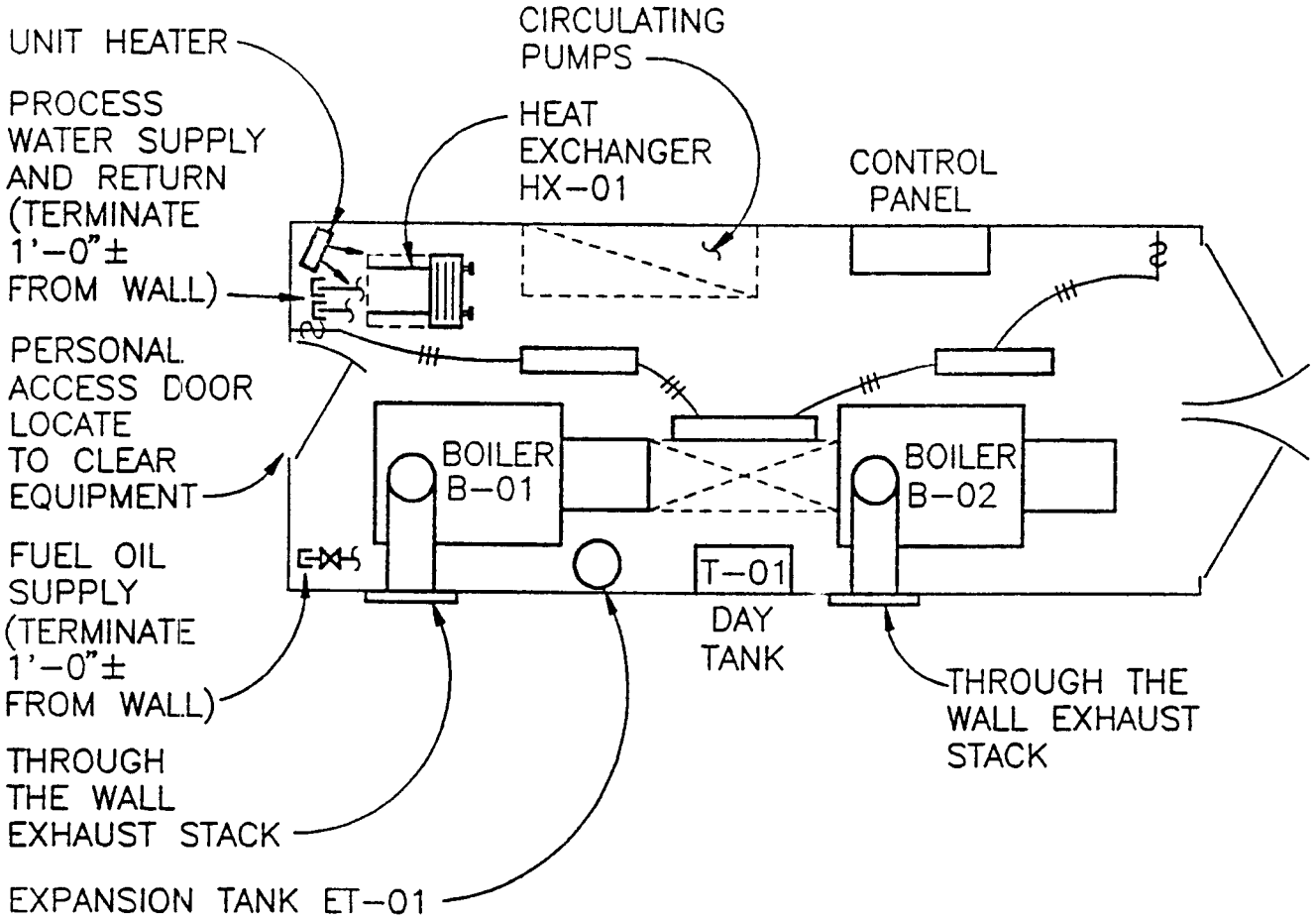
NOTES:

1. PLAN SHOWN FOR CONCEPT ONLY. CONTRACTOR TO MODIFY PLAN TO ACCOMODATE THEIR PROPOSED EQUIPMENT. LAYOUT TO COMPLY WITH ALL APPLICABLE CODES.
2. CONTAINER SHALL BE CLEARLY MARKED WITH IDENTIFICATION CODE AFK.

50011F04 1-48 2450011 1/20F

KCM

PRINCE WILLIAM SOUND AQUA CORP, PWSAC	CONTAINERIZED HEATING SYSTEM



CCH PLAN (BID ITEM 004) 4

1/4" = 1'-0"

NOTES:

1. PLAN SHOWN FOR CONCEPT ONLY. CONTRACTOR TO MODIFY PLAN TO ACCOMODATE THEIR PROPOSED EQUIPMENT. LAYOUT TO COMPLY WITH ALL APPLICABLE CODES.
2. CONTAINER SHALL BE CLEARLY MARKED WITH IDENTIFICATION CODE CCH.

50011F4A 1=48 2450011 2/7S

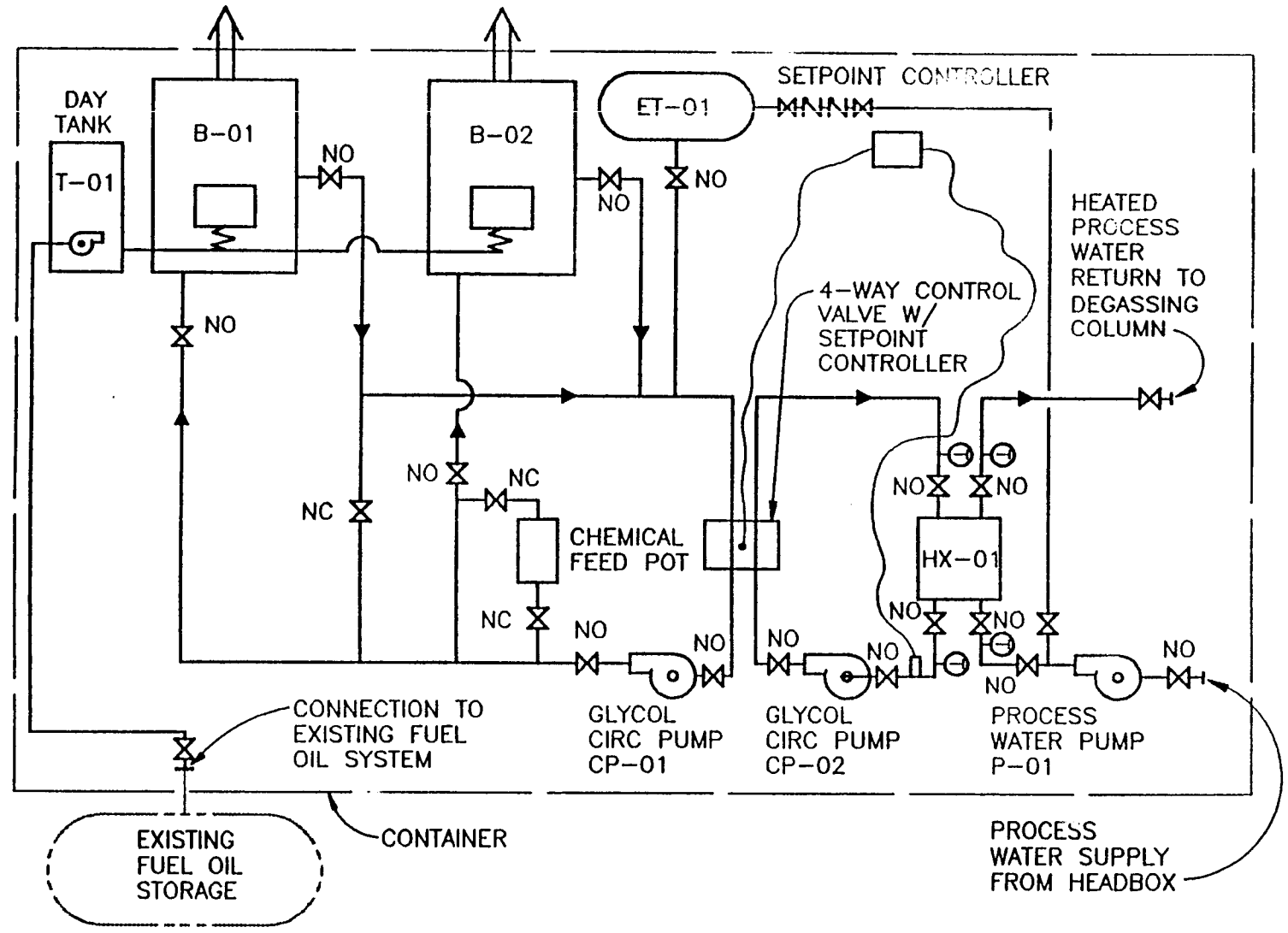
KCM

PRINCE WILLIAM SOUND AQUA CORP, PWSAC	CONTAINERIZED HEATING SYSTEM

KCM

PRINCE WILLIAM SOUND AQUA CORP, PWSAC

CONTAINER HEATING SYSTEM



PROCESS FLOW SCHEMATIC

NO SCALE

5
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1. GENERAL

A. LIST OF ARTICLE TITLES

1.01 SUBMITTAL REQUIREMENTS
1.02 SCHEDULE
1.03 DETAILED BREAKDOWN OF LUMP SUM PRICES

1.01 SUBMITTAL REQUIREMENTS

A. General: All submittals shall be identified by project title, hatchery name, and number and shall include Contractor's name, date and revision date. In addition, shop drawings, product data and samples shall include names of subcontractor and supplier, applicable specification section number and Contractor's stamp, initialed or signed, certifying to review of submittal, verification of field measurements and compliance with contract documents.

1. All submittals shall be accompanied by a submittal transmittal form. Equipment numbers shall be listed for items being submitted. A separate form shall be used for each specific item, class of material, equipment, and items specified in separate, discrete sections, for which the submittal is required. Submittals for various items shall be made with a single form when the items taken together constitute a manufacturer's package or are so functionally related that expediency indicates checking or review of the group or package as a whole.

2. A unique number, sequentially assigned, shall be noted on the transmittal form accompanying each item submitted. Original submittal numbers shall have the following format: "XXX"; where "XXX" is the sequential number assigned by the Contractor. Resubmittal shall have the following format: "XXX-Y"; where "XXX" is the originally assigned submittal number and "Y" is a sequential letter assigned for resubmittals; i.e., A, B, or C being the 1st, 2nd, and 3rd resubmittals, respectively. Submittal 25B, for example, is the second resubmittal of Submittal 25.

3. Submittal Completeness: Submittals which do not have all the information required to be submitted are not acceptable and will be returned without review.

B. Shop drawings: Submit 5 copies of each shop drawing required by the specifications. Show the information, dimensions, connections and other details necessary to ensure that

the shop drawings accurately interpret the contract documents. show adjoining work in such detail as required to indicate proper connections. Where adjoining connected work requires shop drawings or product data, submit such information for review at the same time so that connections can be accurately checked.

C. Product data: Submit 5 copies of each item of product data required by the specifications. Modify product data by deleting information which is not applicable to the project or by marking each copy to identify pertinent products. Supplement standard information, if necessary, to provide additional information applicable to project.

D. Review Procedure: Unless otherwise specified, the Engineer will review the submittal. The returned submittal will indicate one of the following actions:

1. If the review indicates that the material, equipment, or work method is in general conformance with the design concept and complies with the Drawings and specifications, submittal copies will be marked "NO EXCEPTION TAKEN" and given review action 1. In this event the Contractor may begin to implement the work method or incorporate the material or equipment covered by the submittal.

2. If the review indicates that limited corrections are required, copies will be marked 'NOTE MARKINGS" and given review action 2. The Contractor may begin implementing the work method or incorporating the material and equipment covered by the submittal in accordance with the noted corrections. Where submittal information will be incorporated in O&M data, a corrected copy shall be provided, otherwise no further action is required.

3. If the review reveals that the submittal is insufficient or contains incorrect data, copies will be marked "COMMENTS ATTACHED". If the comments are of a nature that can be confirmed without a resubmittal, copies will be further marked "CONFIRM" and given review action 3. If the comments require a revision and resubmittal, copies will be further marked "RESUBMIT" and given review action 4. Except at its own risk, the Contractor shall not undertake work covered by this submittal until the attached comments have been either confirmed by a separate written communication or the submittal has been revised, resubmitted and returned marked with "NO EXCEPTIONS TAKEN" or "NOTE MARKINGS".

4. If the review indicates that the material, equipment, or work method is not in general conformance with the

design concept or in compliance with the Drawings and specifications, copies of the submittal will be marked "REJECTED" and given review action 5. Except at it own risk, the Contractor shall not undertake work covered by such submittals until a new submittal is made and returned marked either "NO EXCEPTIONS TAKEN" or "NOTE MARKINGS".

E. Effects of Review of Contractor's Submittals: Review of Drawings, method of work, or information regarding materials or equipment the Contractor proposes to provide, shall not relieve the Contractor of its responsibility for errors therein and shall not be regarded as an assumption of risks or liability by the Engineer on behalf of the Owner, and the Contractor shall have not claim under the Contract on account of the failure, or partial failure, of the method of work, material, or equipment so reviewed. A mark of "NO EXCEPTIONS TAKEN" or "NOTE MARKINGS" shall mean that the Engineer has no objection to the Contractor, upon the Contractor's own responsibility, using the plan or method of work proposed, or providing the materials or equipment proposed.

1.02 SCHEDULE

A. The Contractor shall provide the following schedule and submit them not later than 15 days after notice to proceed.

1. Contractor's construction schedule:

a. The Contractor will be required to prepare and submit to the Engineer for review an overall construction schedule covering all work to be performed.

b. The construction schedule shall include, but not be limited to the following item:

1) Shop drawing receipt from Contractor, submitted to Engineer, review and return to Contractor.

2) Material and equipment order, delivery and installation and check-out.

3) Piping installation.

4) Final cleanup.

5) Testing activities.

6) Start-up at site.

c. The Contractor will be required to accept the risk of any delays caused by the rate of progress of the work to be performed under the above contract, and that in the event the Contractor is delayed in the prosecution and completion of his work because of such conditions, he shall have no claim for damages or contract adjustment.

1.03 DETAILED BREAKDOWN OF LUMP SUM PRICES

A. The Contractor shall, within 15 days of receipt of the notice to proceed, submit a breakdown of the lump sum price.

END OF SECTION

1. GENERAL

A. LIST OF ARTICLE TITLES

1.01 WRITTEN GUARANTEES
1.02 FIELD TESTS AND ADJUSTMENTS
1.03 PROJECT RECORD DRAWINGS (AS-BUILT)

1.01 WRITTEN GUARANTEES

A. Written guarantees, in duplicate, addressed to each Owner, but submitted to the Engineer.

1. Guarantee by Contractor: Guarantee by Contractor covering the entire work for the 1-year period from date of certificate of substantial completion as specified hereinbefore. Letter to be substantially as follows:

(Re: Project)

(Owner) (Address)

Gentlemen:

"I (We) the undersigned do hereby guarantee for a period of one year(s) from date of certificate of substantial completion all work performed under the terms of the contract documents. I (We) will remedy at my (our) expenses any defects appearing during that period due to poor materials or workmanship and will pay for any damage to other work resulting from occurrence of said defects or the correction of same.

This guarantee shall not be interpreted as holding the Contractor responsible for any deterioration of the work due to normal use or the abuse of the work by the Owner.

Very truly yours,

_____ Contractor

2. A guarantee bond for the face value of the contract shall be provided to the owner for the one-year guaranteed period."

1.02 FIELD TESTS AND ADJUSTMENTS

A. All mechanical and electrical equipment shall be tested by the Contractor to the satisfaction of the Engineer before unit

is shipped. Tests shall be made to determine whether the equipment has been properly assembled, aligned and connected. Any changes, adjustments or replacements required to make the equipment operate as specified shall be carried out by the Contractor as part of the work.

B. During the testing of the mechanical, instrumentation, and electrical equipment, the Contractor shall make available as necessary representatives of the manufacturers of all the various pieces of equipment or other qualified persons who shall instruct the Owner's personnel in the operation and care thereof. Instructions shall include written step-by-step operation and troubleshooting procedures with a complete description of all necessary test equipment and all protective device settings.

1.03 PROJECT RECORD DRAWINGS (AS-BUILT)

A. Maintenance: Maintain, at the jobsite, one set of the contract drawings for recording as-built conditions. Mark (in red) changes made during the course of construction.

B. Completion of work: Upon completion of the work, turn over the one marked-up set of prints to the Engineer.

C. Partial payment: Requests for partial payment will not be approved if the marked-up prints are not kept current and request for final payment will not be approved until the marked-up prints are delivered to the Engineer.

END OF SECTION

1. GENERAL

A. LIST OF ARTICLE TITLES

1.01 DESCRIPTION OF WORK
1.02 QUALITY ASSURANCE
1.03 FORM OF SUBMITTALS
1.04 CONTENT OF MANUAL
1.05 MANUAL FOR EQUIPMENT AND SYSTEMS
1.06 SUBMITTAL SCHEDULE

1.01 DESCRIPTION OF WORK

A. For each containerized system compile product data and related information appropriate for Owner's maintenance and operation of products furnished under the contract.

1. Prepare operating and maintenance data as specified in this section and as referenced in other pertinent sections of specifications.

B. Instruct Owner's personnel in the maintenance of products and in the operation of equipment and systems.

1.02 QUALITY ASSURANCE

A. Preparation of data shall be done by personnel:

1. Trained and experienced in maintenance and operation of the described products.

2. Completely familiar with requirements of this section.

3. Skilled as a technical writer to the extent required to communicate essential data.

4. Skilled as a draftsman competent to prepare required drawings.

1.03 FORM OF SUBMITTALS

A. Prepare data in the form of an instructional manual for use by Owner's personnel.

B. Format

1. Size: 8-1/2 inches by 11 inches

2. Paper: 20-pound minimum, white, for typed pages.

3. Text: Manufacturer's printed data, or neatly type-written.

4. Drawings:

   a. Provide reinforced punched binder tab, bind in with text.

   b. Fold larger drawings to the size of the text pages.

5. Provide fly-leaf for each separate product, or each piece of operating equipment.

   a. Provide typed description of product, and major component parts of equipment.

   b. Provide indexed tabs.

6. Cover: Identify each volume with typed or printed title "Operating and Maintenance Instruction." List:

   a. Title of project.

   b. Identity of separate structure as applicable.

   c. Identity of general subject matter covered in the manual.

C. Binders

1. Binders shall be similar and equal to National 98-381.

1.04 CONTENT OF MANUAL

A. Neatly typewritten table of contents for each volume, arranged in a systematic order.

1. Contractor, name of responsible principal, address and telephone number.

2. A list of each product required to be included, indexed to the content of the volume.

3. List with each product, the name, address and telephone number of:

   a. Subcontractor or installer.

   b. Maintenance contractor, as appropriate.

   c. Identify the area of responsibility of each.

   d. Local source of supply for parts and replacement.

4. Identify each product by product name and other identifying symbols as set forth in contract documents.

B. Product data

1. Include only those sheets which are pertinent to the specific product.

2. Annotate each sheet to:

a. Clearly identify the specific product or part installed.

b. Clearly identify the data applicable to the installation.

c. Delete references to inapplicable information.

C. Drawings

1. Supplement product data with drawings as necessary to clearly illustrate:

a. Relations of component parts of equipment and systems.

b. Control and flow diagrams.

2. Coordinate drawings with information in project record documents to assure correct illustration of completed installation.

3. Do not use project record documents as maintenance drawings.

D. Written text, as required to supplement product data for the particular installation.

1. Organize in a consistent format under separate headings for different procedures.

2. Provide a logical sequence of instruction for each procedure.

E. Copy of each warranty, bond and service contract issued.

1. Provide information sheet for Owner's personnel, give:

a. Proper procedures in the event of failure.

b. Instances which might affect the validity of warranties or bonds.

1.05 MANUAL FOR EQUIPMENT AND SYSTEMS

A. Submit 3 copies of complete manual in final form for each containerized system (12 total).

B. Content, for each unit of equipment and system, as appropriate:

1. Description of unit and component parts.

a. Function, normal operating characteristics, and limiting conditions.

b. Performance curves, engineering data and tests.

c. Complete nomenclature and commercial number of all replaceable parts.

2. Operating procedures:

a. Start-up, break-in, routine and normal operating instructions.

b. Regulation, control, stopping, shut-down and emergency instructions.

c. Summer and winter operating instructions.

d. Special operating instructions.

3. Maintenance procedures:

a. Routine operations.

b. Guide to "trouble-shooting."

c. Disassembly, repair and reassembly.

d. Alignment, adjusting and checking.

4. Servicing and lubrication schedule:

a. List of lubricants required.

5. Manufacturer's printed operating and maintenance instructions.

6. Description of sequence of operation by control manufacturer.

7. Original manufacturer's parts list, illustrations, assembly drawings and diagrams required for maintenance.

a. Predicted life of parts subject to wear.

b. Items recommended to be stocked as spare parts.

8. As-installed control diagrams by controls manufacturer.

9. List of original manufacturer's spare parts, manufacturer's current prices, and recommended quantities to be maintained in storage.

C. Content, for each electric and control system, as appropriate.

1. Description of system and component parts.

a. Function, normal operating characteristics, and limiting conditions.

b. Performance curves, engineering data and tests.

c. Complete nomenclature and commercial number of replaceable parts.

2. Circuit directories of panelboards.

a. Electrical service.

b. Controls.

c. Communications.

3. As-installed color coded wiring diagrams.

4. Operating procedures:

a. Routine and normal operating instructions.

b. Sequences required.

c. Special operating instructions.

5. Maintenance procedures:

a. Routine operations.

b. Guide to "trouble-shooting."

c. Disassembly, repair and reassembly.

d. Adjustment and checking.

6. Manufacturer's printed operating and maintenance instructions.

7. List of original manufacturer's spare parts, manufacturer's current prices, and recommended quantities to be maintained in storage.

8. Other data as required under pertinent sections of specifications.

D. Prepare and include additional data when the need for such data becomes apparent during instruction of Owner's personnel.

E. Additional requirements for operating and maintenance data: The respective sections of specifications.

1.06 SUBMITTAL SCHEDULE

A. Submit 2 copies of preliminary draft of proposed formats and outlines of contents for each containerized system prior to delivery of the units to the docks in Seattle.

1. Engineer will review draft and return 1 copy with comments.

B. Submit specified number of copies of approved data in final form prior to the scheduled date for receiving payment for 75% of the work.

END OF SECTION

1. GENERAL

A. LIST OF ARTICLE TITLES

1.01 DESCRIPTION OF WORK

A. The work covered in this section consists of furnishing all labor, materials, and equipment necessary to provide a fully operational and functional containerized process water heating system as a base bid or a skid mounted unit for SGH in Valdez, Alaska as Bid Alternate No. 1. The system shall be similar to that shown on the layout and flow Schematic Drawings. Overall layout, construction, acquisition of products however shall be finalized by the Contractor.

B. The containerized process water heating system shall consist of two oil fire boilers, one process water to boiler system heat

exchanger, process water pump, glycol system pumps, expansion tank, chemical pot feeder, day tank, piping, temperature control valve with a set point controller, isolation valves, unit heater, lighting, convenience outlets, insulated and gypsum board lined used seaworthy container with outside air louvers, painting, controls and power entrance for a single 480 volt, 3 phase connection. The containerized system is to be fully functional ready for shipment to Whittier or Valdez, Alaska.

C. Bid Alternate No. 1: In place of a containerized system for SGH in Valdez provide as Bid Alternate No. 1 a fully functional skid mounted heating system. The requirements for this system are the same as the containerized systems except the equipment would be skid mounted. In addition to the container, the container improvements such as louvers, lighting, unit heaters, insulation, fire protection, convenience outlets and man door will not be provided. The skid shall include forklift pockets for transporting and the outside plan dimensions shall not exceed 10 feet by 17 feet and the height shall not exceed 8 feet.

1.02 QUALITY ASSURANCE

A. Contractor shall have fabrication capabilities for the construction of the containerized boiler system. Contractor shall have a minimum of 10 years of experience in the design and installation of hot water boiler systems.

B. Service engineer: After delivery of the boiler system to the job site and installation by the site contractor, the fabrication contractor shall provide the services of the manufacturer's field representative for starting the units and training the operator for a minimum of 2 days excluding travel time for each containerized system.

1.03 SUBMITTALS

A. Product data: All equipment.

B. Shop drawings: In accordance with Section 01300. Include dimensioned layout drawings showing piping, electrical control requirements, boiler control schematics, temperature control value requirements, lighting, etc.

C. Operation and maintenance manuals: Include parts lists and control schematics for each specific model of equipment. Furnish in accordance with Section 01730, Operating and Maintenance Data.

1.04 GUARANTEE

A. Containerized boiler system shall be warranteed for 1-year following acceptance of the system, Section 01700, Project Closeout.

B. Contractor shall ensure containerized system for its replacement value to the point of delivery specified in Bid Form.

2. PRODUCTS

2.01 GENERAL DESCRIPTION

A. For the base bid the Contractor shall obtain a used shipping container, insulate and surface the interior walls, ends and ceiling with 5/8-inch gypsum board. The container shall be modified to include wall louvers for boiler combustion air, boiler stacks (through the wall preferred at WNH, AFK, and CCH; ceiling at SGH), lighting, a 2-foot 6-inch steel man door on three of the containers, convenience outlets and an electric unit heater for freeze protection. The exterior of the container shall be painted, the interior of the container shall be painted on the floor, walls, ends, and ceiling. Wiring for lights, switches and outlets shall be concealed by the gypsum board liner, or in exposed conduit. Locks shall be included for all doors.

B. As Bid Alternate No. 1, the Valdez Heating System (SGH) shall be delivered as a skid mounted unit. The skid shall be no larger than 10 feet by 17 feet and shall be designed to be placed into an existing building upon delivery to Valdez, Alaska. The system shall be "layed out" similar to the containerized system to match the building requirements. The skid shall be fabricated of steel, rigid enough to allow safe transportation of the heating system and include forklift pockets to allow for transport in Valdez. The skid shall be painted as specified for the containerized system. Except for the container, container improvements, unit heater, and fire supression system, the skid mounted system shall be the same as the containerized systems.

C. Inside of the container the Contractor shall develop a complete boiler/heat exchanger system to heat process water in the volume and temperature specified in Paragraph 2.02. The boiler system shall be fully automated such that the desired process water temperature can be set through a set point controller and automatically maintained with varying process water flow rates and incoming temperature.

D. The control panels shall be fully designed and fabricated by the Contractor to include all necessary relays, motor starters, temperature relays, etc. to allow a single 480 volt, 3 phase power connection. Metering of this power will not be required.

E. Utilities including fuel oil, supply and return process water connections, domestic water, and power will be supplied at each hatchery location. It is required that the connections be made inside the container to prevent damage to any potentially protruding element of the system. All boiler exhausts should be designed to leave the container through the side or ceiling to match the site requirements. All necessary penetrations through the container wall or ceiling for connection to on site piping shall be made by the Contractor and covered and sealed to be waterproof during shipping.

F. An acceptable layout is provided for design suggestions. The Contractor may suggest an alternative layout as long as it complies with all applicable codes and allows for proper maintenance of equipment and retains the same man and piping access shown on the Drawings. The flow schematic is provided showing schematically the desired piping and control arrangement.

G. Piping inside of the container shall be copper for the two propylene glycol heating loops, PVC for the process water loop with at least 2 feet of steel pipe where it attaches to the heat exchanger to prevent "heat drift"" to the PVC system, copper or black steel for the fuel oil piping. In no instance may any yellow metal piping or equipment come into contact with the process water.

H. The contents of the container shall be layed out so that the load is evenly distributed and the container can be handled by a forklift.

2.02 DESIGN CRITERIA

A. The containerized two boiler system shall be designed to meet the following process water conditions:

1. Minimum flow rate: 50 gpm.

2. Maximum flow rate: 200 gpm.

3. Minimum incoming temperature: 35 degrees F.

4. Maximum incoming temperature: 49 degrees F.

5. Required Δ T in the heated side stream is 21 degrees F at maximum process water temperature and 200 gpm flow rate. Contractor shall account for all inefficiencies in heat transfer.

B. The process is to use two boilers. The boilers shall be sized so that each boiler can supply 60 percent of the total heat required. The process shall use a single plate and frame heat exchanger sized to meet the above design criteria. The temperature controller and mixing valve must maintain the set water temperature to ±0.5 degrees F.

C. The process water circulation pump shall be sized to allow for fluctuations in flows. The process water flows are expected to range between 50 and 200 gpm depending on which valves are open downstream of the heating system. The two glycol circulating pumps shall be sized by the Contractor to meet the heating and control requirements stated above using 50% propylene glycol.

2.03 HOT WATER BOILERS (B-01, B-02)

A. Boilers shall be constructed of cast-iron sections manufactured in accordance with ASME requirements for low pressure boilers. The boiler-burner unit shall not require a refractory combustion chamber and the sections shall be of wet base construction and suitable for installation on combustible floors.

1. Boiler sections shall be assembled with precision machined cast-iron push nipples, pressed into mating machined nipple port in the section eliminating the need for any material such as gaskets.

2. Boiler shall have individual clean-out openings between sections covered with insulated cast-iron covers with rope gaskets to ensure a gas-tight seal.

3. The boiler jacket shall be constructed of heavy gage steel with 1/2-inch insulation and have a rust resistant baked enamel finish. Boilers shall be rated for installation on combustible floor.

4. Water boiler sections shall be hydrostatically tested for 50 psi water working pressure in accordance with ASME Code Section IV.

5. Water boiler trim shall include a 3-1/2-inch pressure temperature gage with separate scales for pressure and water temperature. In addition, an ASME approved water

relief valve shall be factory furnished and sized to exceed the boiler output capacity and shall be factory set to relieve pressure at 50 psi.

6. Each boiler shall have automatic reset high limit, automatic reset low water cutoff.

B. Burner shall be forced draft. Burner shall be complete with integral motor driven blower, fuel oil electric ignition assembly, combustion safeguard, motor starters, complete fuel train including pressure regulator and all valves and all necessary controls for safe and efficient operation in accordance with IRI requirements. Mode of operation shall be full modulation with proven low fire start. Boiler shall have a net hot water output of sufficient BTU to meet the heating water requirement identified in Paragraph 2.02 when including boiler and heat exchanger efficiencies and pipeline losses when fired on fuel oil.

C. The boilers shall operate in a lead-lag system integral with the burner control panel(s) and shall allow for manual selection of the lead boiler. Lag boiler shall automatically fire when the lead boiler is unable to satisfy the system load. If the process water circulating pump is shut down (zero flow) the remainder of the boiler system shall remain on and the boiler system shall operate to maintain a fixed boiler temperature without damage.

D. Control cabinet shall be mounted on front of burner and shall include:

1. Manual-automatic selector switch and manual firing rate potentiometer shall permit either automatic firing in accordance with load demands, or manual control of firing rate at any desired point between low fire and maximum rating.

2. Burner package shall consist of:

a. A series of annunciation lights mounted to allow operators a view of the operational status.

b. A prewired and factory tested wiring harness and quick disconnect device that enables quick removal of door from cabinet.

c. Functions to be displayed for full modulation:

1) Power on

2) Limit circuit closed

3) Modulation mode

4) Flame failure

E. All control circuits shall be 120 volts, 60 Hz, 1 phase, with all switches in the underground leg. Fuse protection for the control circuit shall be provided. Electrical supply to the boiler(s) will be 120 volt single phase.

F. Boiler stack may either be Metalbestos double wall self insulating or insulated welded steel. Stack shall be stainless and sized to meet the backpressure requirements of the boiler. Boiler stack shall be directed out through the side of the container for 3 units and through the roof on one. For the SGH unit for Valdez the container shall supply enough boiler stack to extend a minimum of 12 feet above the container and 13 feet of breeching for a total of 24 feet of stack outside of the container.

G. Accessories: Provide all necessary accessories to make the system a fully operational and functional system meeting all applicable boiler and building codes and Fire Marshal requirements.

H. Boiler shall be installed on a drip pan to contain any oil spill at the boiler.

2.04 UNIT HEATER

A. Unit heater shall be Trane, 5 kW, or equal single stage, 480 volt, 3 phase units with heavy gage steel casing with baked enamel finish, factory horizontal wall/ceiling mounting bracket, adjustable louvers, finned tubular steel elements, totally enclosed motor, manual reset thermal overload protection for motor and elements, integral factory installed thermostat and disconnect. Units shall be UL rated and conform to all applicable local and national codes. Integral thermostat shall activate unit on a drop in temperature to 45 degrees F, and shut off unit at 55 degrees F.

2.05 CIRCULATION PUMPS (CP-01, CP-02)

A. Close coupled glycol circulating pumps shall have capacities required to meet the heating load and headloss requirements of the piping, boiler heat exchanger, isolation valves and temperature control valve system. Close coupled pumps shall be of compact style, single stage, vertical split case design of bronze fitted construction. The pump internals shall be

capable of being serviced without disturbing the piping connections. The working pressure shall be 175 psi and the pump so nameplated.

1. The impeller shall be of the enclosed type, dynamically and hydraulically balanced and keyed to the shaft and secured with a suitable locknut.

2. The pumps shall utilize a mechanical seal with a carbon seal ring and ceramic (or equal) seat.

3. The bearing frame assembly shall be furnished with oil-lubricated sleeve bearings with readily accessible lubrication fittings.

4. The pump shall be coupled to the motor by means of a spring type coupling to ensure quiet operation.

5. The motors shall have sleeve bearings for 1,750 rpm selections and sleeve/ball bearings for 3,500 rpm selections.

6. The pump shall be factory tested at the operating conditions specified, thoroughly cleaned, and painted with one coat of machinery enamel. A set of installation-and-operation instructions shall be packed with each pump.

7. The motor shall be resilient mounted, equipped with oil lubricated journal bearings.

8. The pump shall be factory tested, thoroughly cleaned, and painted with one coat of machinery enamel prior to shipment. A set of installation instructions shall be included with the pump at the time of shipment.

B. Operating voltages and controls integral horsepower drive motors shall be 460 volt, 3-phase, 60 hertz. Provide combination starter and start/stop controls. Fractional horsepower motors shall be 120 volt, single phase.

2.06 PROCESS WATER PUMP (P-01)

A. Process water pump shall be similar to the circulating pumps except as stated below. The capacity to pump 50 to 200 gpm with a residual pressure following the heat exchanger HX-01 of 30 psi. The full head of the pump is to be determined by the Contractor following his pipe routing, boiler and heat exchanger selection. Close coupled pumps shall be of compact style, single stage, vertical split case design of all iron

construction. The pump internals shall be capable of being serviced without disturbing the piping connections. The working pressure shall be 175 psi and the pump so nameplated. The pump shall contain no yellow metals that can come into contact with the process flow.

2.07 CHEMICAL POT FEEDER

A. Feeder capacity shall be at least 1/1000 times the system volume. A label shall be affixed to the feeder stating the approximate system volume in gallons.

2.08 HEAT EXCHANGERS (HX-01)

A. Heat exchanger shall be of the plate and frame type for use with heating water. The exchanger shall be of the counterflow type and contain no yellow metals. Frames shall be of carbon steel with baked epoxy enamel finish and chrome plated carbon-steel guide bars, zinc-plated carbon-steel tie bolts, and zinc-treated painted protection shroud. Plates shall be of 304 stainless steel with gaskets of NBR. Gasket and plate design shall be such that there is no possibility of cross contamination of flows. Nozzles shall be 150-pound ASA rated hose flange type. Head loss shall be included in process water pump (P-01) requirements. Heat exchanger shall be sized to be used with 50% propylene glycol.

2.09 EXPANSION TANKS

A. Expansion tanks shall be Bell and Gossett, or equal. Tanks shall be sized by the Contractor.

2.10 THERMOMETERS

A. Thermometers for determining water temperatures shall be industrial grade, adjustable angle constructed of die cast aluminum with a vee shape. The case shall have a chrome plated finish. Scale ranges shall be selected to provide "normal" readings at approximately midscale for the particular use. The bulb chamber shall be machined to match the tapered separable socket to assure metal to metal contact. Thermometer shall be adjustable for a full 180 degree rotation. Thermometers shall have a 9 inch scale.

B. Thermometers shall be provided at the following locations and where shown on the drawings:

1. Heating water inlets and outlets of both boilers.

2. Inlets and outlets of heat exchanger (all four ports of all HX's).

2.11 DAY TANK

A. A 10 gallon day tank shall be provided to supply fuel oil to the boilers. The fuel system supplying fuel to the day tank is single pressurized line and the day tank must be able to "shut-off" the flow when full with either a normally closed solenoid valve or float valve. Provide a "catch tray" under the day tank to capture any overflows. The day tank shall include an audible high level alarm in case the tank fill valve sticks open. Day tank shall be Simplex, or equal.

2.12 CONTAINER

A. For the base bid the Contractor shall obtain a good used steel seaworthy watertight container to house each packaged heating system. The container shall have florklift pockets and be in structurally sound condition. The container shall be cleaned and include 5/8-inch thick Type X gypsum board on the walls, ends, and ceiling attached with galvanized screws to 2-inch by 2-inch studs which are glued to the wall and ceiling and secured with gasketed galvanized screws from the outside of the container or equivalent to maintain a watertight container. The container shall be insulated with 1-1/2 inches of extruded polystyrene insulation. The floor shall be prepared and painted with 2 coats of floor paint following the manufacturer's requirements for preparatives and application. The exterior shall be prepared and painted with 2 coats of high gloss enamel paint applied per the paint manufacturer requirements.

B. The container shall have a minimum of three 2 tube 4-foot long fluorescent lights operated by a light switch located adjacent to the doors. The container shall include 4-duplex convenience outlets for power tools. Wiring for the lights shall be concealed beneath the gypsum board or in surface mounted conduit.

C. The container and all exposed components on the container shall be designed for a minimum of 125 psf snow load.

D. A man-door shall be installed on 3 of the 4 containers as identified on the Drawings.

2.13 SKID

A. For Bid Alternate No. 1 the skid shall be made of fabricated steel and include forklift pockets. The skid shall be rigid

enough to allow transport without damaging the equipment or interconnecting piping or electrical conduits. The maximum outside dimensions of the skid shall be no larger than 10 foot by 17 foot to fit into an existing building.

B. The skid shall be painted with a rust prohibitive primer and finished with 2 coats of floor paint.

2.14 GATE VALVES

A. Gate valves for the glycol circulating system shall be iron body, bronze-mounted, double disc, nonrising stem, 200-pound working pressure, threaded or flanged. Flange ends shall be Class 125 in accordance with ANSI B16.1.

B. Gate valves for the process water shall be similar to above except they shall contain no yellow metals.

C. PVC valves for the process water shall be butterfly valves or ball valves. Butterfly valves shall be ASAHI or equal. Ball valves shall be ASAHI, or equal.

2.15 PIPE

A. PVC pipe shall conform to ASTM D 1785 and shall be Schedule 80 with solvent weld joints or flanged. Connections to all valves shall be flanged.

B. Copper pipe shall be Type K or L with soldered fittings. Solder shall contain no lead.

2.16 TEMPERATURE CONTROL SYSTEM

A. The temperature control valve shall be a Tekmar 4-way mixing valve with electric operator, or equal.

B. Motor for temperature control valve shall be sized for continuous service.

C. The set point controller shall be Tekmar Type 153 or equal. The unit shall have a control accuracy of ± 0.5 degrees F and shall include a digital display for setpoint and measured temperature. The controller shall modulate the 4-way valve to control the flow of hot water to maintain the setpoint temperature of the process water.

D. The temperature sensor shall be installed in a stainless steel thermo-well. A strap on sensor will not be acceptable.

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C. The set point controller shall be Tekmar Type 153 or equal. The unit shall have a control accuracy of $\pm$ 0.5 degrees F and shall include a digital display for setpoint and measured temperature. The controller shall modulate the 4-way valve to control the flow of hot water to maintain the setpoint temperature of the process water.

D. The temperature sensor shall be installed in a stainless steel thermo-well. A strap on sensor will not be acceptable.

2.17 CONTROL PANEL

A. The control panel shall be complete with all required motor starters, relays, transformers, pushbuttons, running lights, hour meters, etc. to provide a complete operating boiler and temperature control system in the heated and lighted shipping container. The panel enclosure shall be NEMA 1 and have all necessary start/stop and monitoring features on the front panel. To operate the water heating system it should not be necessary to open the control panel.

B. The controls shall be set up to manually start each system and then automatically maintain the setpoint temperature of the process water. The control panel shall be designed so that any failure will not overheat the water causing harm to the fish. The control panel shall include an audible alarm to indicate a drop in temperature (adjustable) below setpoint.

C. The control panel shall be designed so that a single 480 volt, 3 phase connection can be made at the hatchery and all systems are operational including the 120 volt and lower voltage systems.

D. All alarms shall be audible outside of the container, and provided a spare set of normally open and normally closed contacts for future connection to the existing alarm system at each hatchery.

2.18 FIRE ALARM

A. A fire alarm sensor shall be provided inside of the container. The alarm shall provide an audible alarm outside of the container, and provided a spare set of normally open and normally closed contacts for future connection to the existing alarm system at each hatchery.

2.19 FIRE SUPPRESSION

A. Container shall include a packaged automatic dry chemical fire suppression system suitable for use in closed containers specified. The system shall include a high temperature switch and smoke detector and the system shall be activated by either activation of the system shall sound an external alarm and include a normally open and normally closed contacts for future connection.

3. EXECUTION

3.01 INSTALLATION

A. All drains shall be piped to outside of the container.

B. Boiler: The boilers shall be installed in strict accordance with the manufacturer's instructions in the mechanical rooms so that the service requirements are met. Start-up shall be supervised by manufacturer's qualified and authorized representative. Manufacturer's representative shall provide demonstration and instruction to owner's operating personnel. All boilers shall be installed on a noncombustible pad with a drip pan.

C. Specialties:

1. Balancing and temperature control valves shall be installed and provided so that 1) they may be adjusted, 2) so that the measuring lines may be attached, and 3) so that the insulation covers may be installed and taped in place.

2. Exhaust stacks shall be coordinated with the equipment supplied assuring that pressure developed in the stack is acceptable for piece of equipment.

3. Stacks shall be completed during fabrication of the unit, removed for shipping, the stack penetration made waterproof, and reinstalled for final installation.

D. Note the drawings show design configurations based on particular manufacturer's equipment. If selected manufacturer's equipment configuration is different from that which is shown, the Contractor shall provide all necessary modifications to support the boiler system, fuel supply, electrical requirements, and piping systems at no additional cost to the owner.

E. Piping: Piping shall be run in straight horizontal and vertical runs. Pipes shall be supported from the floor wherever possible. Pipes and equipment shall be supported to allow transport of the container without damaging the piping or equipment. All low spots in the piping shall include a drain valve and high spots an air release valve. Connections to equipment shall include isolation valves so the equipment can be removed for service without draining the entire system. The system shall include a bypass line so that a single boiler can be removed from service without impacting the second boiler.

Valves shall be installed for isolation at each hatchery connection point.

F. All water piping and valves shall be insulated with a minimum of 2 inches of fiberglass preformed insulation with an exterior vapor barrier.

3.02 CLEANING

A. Prior to shipping the Contractor shall fill the hot water piping system with a solution of water and tri-sodium phosphate mixed at a concentration of 1 pound tri-sodium phosphate per 50 gallons of water. Vent system to assure complete fill and circulate the cleaning solution for a 3-hour duration. Flush system completely as many times as necessary to obtain a flushed water pH reading of 7.0. Remove startup strainer element after flushing entire system and prepare for shipment to Alaska.

3.03 TESTING

A. After the Owner has installed the unit the Contractor shall fill the system with 50% propylene glycol solution and perform functional tests and start-up of all equipment specified herein for a minimum period of 2 days for each containerized system with various thermostat settings to assure proper operation over the full design range. It is anticipated that this will occur during July or August 1995.

B. All equipment shall be tested for proper operation, bearing integrity and performance by the Contractor once the installation is complete.

C. The testing and start-up of all equipment specified herein shall be done by authorized manufacturer's representative or direct employee. Written reports of this testing and start-up shall be supplied to the Owner.

D. Specialties shall be tested as part of the circulating system except that air vents shall not be installed until system has been thoroughly flushed and dirt removed.

E. The Contractor shall furnish all equipment, material and labor to perform the testing at the installation site including travel costs to and from Cordova and Valdez. PWSAC will provide transportation between Cordova and each remote site, and all living accommodations at the sites and in Cordova, AK. Valdez Fisheries Development Association will provide transportation and living accommodations in the City of Valdez and at Solomon Gulch Hatchery.

END OF SECTION

Exxon Valdez Oil Spill
Restoration Project Annual Report

Genetics of Populations of Pink Salmon Inhabiting Prince William Sound

Restoration Projects 94320D and 95320D
Annual Report

This annual report has been prepared for peer review as part of the *Exxon Valdez* Oil Spill Trustee Council restoration program for the purpose of assessing project progress. Peer review comments have not been addressed in this annual report.

James E. Seeb
Christopher Habicht
William B. Templin
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August 1997

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Restoration Project Annual Report

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Genetics of Populations of Pink Salmon
Inhabiting Prince William Sound

Restoration Projects 94320D and 95320D
Annual Report

Study History: This study was submitted as a preproposal in FY 1991; it was deferred until funding was approved in FY 1994 as Restoration Project 94320D. The project continued in FY 1995 as 95320D, and in subsequent years as Restoration Project 9x196.

Abstract: Allozyme and mtDNA data were collected from 27 putative populations of pink salmon spawning throughout Prince William Sound (PWS) in 1994. Sampling included two hatchery, five upstream, and 20 tidal locations distributed among five management regions (Southeast, East, North, Southwest, and Montague). Seventy-seven allozyme loci were screened in up to 100 fish per population. Thirty-eight loci had frequencies for alternate alleles ≥ 0.01 in one or more populations and were used for population analyses. Forty fish per collection were screened for haplotype variation at the ND5/ND6 region using six restriction enzymes; eight haplotypes were detected. Significant differences between upstream and tidal collections were detected within Lagoon Creek (allozymes) and within Koppen Creek (mtDNA). Significant regional heterogeneity was detected within upstream (allozymes and mtDNA) and tidal (allozymes) collections. In pair-wise tests between management regions, only the test between two best represented regions (Southwest and East) was significant for tidal populations. Armin F. Koernig Hatchery was indistinct from all regions, while there was indication that Solomon Gulch Hatchery was distinct from all regions but East. These results support managing native populations of pink salmon in PWS at the regional level, considering local subpopulation structure, rather than as a single panmictic population.

Key Words: Allozymes, *Exxon Valdez* oil spill, mtDNA, *Oncorhynchus gorbuscha*, pink salmon, Prince William Sound, stock identification.

Project Data: (will be addressed in the final report)

Citation:

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EXECUTIVE SUMMARY

- Allozyme and mtDNA data were collected from 27 aggregates of pink salmon spawning in 1994 from Prince William Sound (PWS). These collections were distributed throughout PWS and included locations within each of the five major management regions (Southeast, East, North, Southwest, and Montague). Samples were collected from spawners from two hatchery, five upstream, and 20 tidal locations.

- We screened 77 allozyme loci from 92 to 100 fish per population for a total of 2686 fish. Of these loci, 38 had frequencies for alternate alleles ≥ 0.01 in at least one population and were retained for analysis.

- Haplotype data were collected from the ND5/ND6 region of mtDNA using six restriction enzymes on 40 fish per population for a total of 1080 fish. Four of these enzymes yielded a total of eight haplotypes.

- We analyzed the data for genetic structure in three steps. First, the wild collections were organized hierarchically to test for homogeneity: a) among collections within regions within elevation, b) among regions within elevation, and c) among wild collections from different elevations (tidal and upstream). The highest level of the hierarchy was a test between all the wild collections and the hatchery collections. Second, we performed pairwise log-likelihood tests within streams where we had both tidal and upstream collections. Third, we applied similar tests between collections pooled within regions to test regions against each other and to examine hatchery relationships to these regions.

- Significant differences between overall upstream and tidal collections were detected. Further examination with paired tests revealed that both Lagoon Creek (allozymes) and Koppen Creek (mtDNA) tidal and upstream collections were different. Significant regional heterogeneity was detected within upstream (allozymes and mtDNA) and tidal (allozymes) collections. In pair-wise tests between management regions after statistically accounting for multiple tests, only the test between the two best represented regions (Southwest and East) was significant for tidal populations. However, before accounting for multiple testing, 8 of the 21 tests made were significant, suggesting that we may have lacked statistical power to detect differences present among less sampled regions.

- In the region-by-region analysis of allozyme data for tidal collections, Armin F. Koernig Hatchery was indistinct from all regions, while there was indication that Solomon Gulch Hatchery was different from all regions but East. These hatchery results follow expectations based on the hatchery locations, original broodstock sources, and annual broodstock acquisition methods.

- These data support managing native populations of pink salmon in PWS at the regional level, considering local subpopulation structure, rather than as a single panmictic population.

INTRODUCTION

On March 24, 1989, the supertanker *Exxon Valdez* ran aground on Bligh Reef in Prince William Sound (PWS), Alaska, spilling 41 million liters of crude oil. The oil slick, pushed by winds and currents, moved through western PWS and the western Gulf of Alaska, contaminating approximately 2000 km of coastal habitat (see overview in Wells et al. 1995), killing thousands of sea otters *Enhydra lutris* (Garrott et al. 1993; Bodkin and Udevitz 1993) and hundreds of thousands of seabirds (Ford et al. 1993), and adversely affecting many other taxa (e.g., Barber et al. 1995; Bowman et al. 1995; Bowyer et al. 1995; Duffy et al. 1994). Sublethal effects, including reproductive impairment (Ford et al. 1993) and chromosome damage (Hose 1994), were documented. Subsurface oil remains in some of the beaches in spite of the multi-billion dollar clean-up and restoration effort (Wolfe et al. 1994). Populations of some species including pink salmon *Oncorhynchus gorbuscha* may not be fully recovered (Bue et al. 1996).

Pink salmon is the most abundant North American species of the Pacific salmon (Neave 1967; Heard 1991), making it an ecological cornerstone in biological communities of the Pacific Rim and an economic mainstay for many coastal communities. Pink salmon are both anadromous and semelparous: in their natural range, they make long oceanic migrations, home to their natal streams to spawn, and die at age two. Annual catches of pink salmon ranged from 46 to 128 million fish in Alaska alone during the period from 1985-1995.

Pink salmon, of both wild and hatchery origin, was also one of the most abundant vertebrate species inhabiting the spill area. Historically, wild populations produced approximately five hundred million pink salmon fry which emerged from streams throughout PWS each year to migrate seaward. Adult returns from these juvenile migrations averaged over 10 million fish annually. These returning wild-stock adults play a critical role in the total PWS ecosystem: they convey essential nutrients and minerals from the marine ecosystem to estuaries, freshwater streams, and terrestrial ecosystems. Both juveniles and adults are important sources of food for many fishes, birds, and mammals. Wild pink salmon also play a major role in the economy of PWS because of their contribution to commercial, sport, and subsistence fisheries in the area.

Up to 75% of wild pink salmon spawning within PWS occurs in intertidal areas (Helle et al. 1964; Roys 1971). This extensive use of intertidal areas made pink salmon susceptible to adverse effects from the oil spill. Pink salmon embryos and alevins suffered increased mortality, diminished growth, and a high incidence of somatic cellular abnormalities as a result of spawning ground contamination and rearing in oiled areas. Elevated mortality of embryos in the oiled streams continued through 1993, three generations after the oiling, implicating genetic damage (Bue et al. 1996). Also in 1989, the commercial harvest of pink salmon was shifted away from the hatchery and wild stocks in the oiled areas to target the wild stocks in eastern PWS (Geiger and Savikko 1990). This resulted in over-harvest and depletion of these stocks evidenced by general run failures of eastern PWS populations of non-hatchery origin in 1991 (Geiger and Savikko 1992).

An array of conservation and restoration alternatives have been proposed for "species" impacted by the *Exxon Valdez* oil spill. But, species-based proposals often do not provide the resolution needed to sustain the conservation of genetically diverse aggregates of salmon

populations; it is essential to manage and restore these damaged pink salmon resources on a population basis in order to conserve between-population diversity (e.g., Cuenco et al. 1993; Waples 1995). Between-population diversity provides optimal production for species inhabiting diverse ecosystems such as PWS; highly diverse population mixes also provide a biological buffer to environmental change (droughts, floods, major earthquakes, major shifts in oceanic conditions, and other routine catastrophic events that occur in Pacific Rim ecosystems). Our goal was to examine naturally occurring genetic markers to delineate the population structure of PWS pink salmon and to provide a genetic basis for fish management.

Two categories of molecular markers have been used extensively to define population structure of salmonids: allozymes and mitochondrial DNA (mtDNA). Allozyme analysis remains the preferred approach for study of population genetics of salmonids because of its power to resolve populations of many species in the tetraploid-derived family by assaying many nuclear loci rapidly and at low cost (Allendorf 1994). Additional advantages of allozymes for this study include the existence of a pre-spill allozyme data set for comparison and that many laboratories cooperate on inter-institutional examinations of pink salmon using allozymes, providing a support structure including a wealth of compatible data for comparison among Pacific Rim populations (e.g., Seeb and Wishard 1977; Utter et al. 1980; Beacham et al. 1985, 1988; Gharrett et al. 1988; Shaklee et al. 1991; White and Shaklee 1991; Shaklee and Varnavskaya 1994).

The utility of mtDNA approaches to study genetic diversity of salmonid populations is controversial for reasons such as relatively high cost and slow throughput (Allendorf 1994). Additionally, sometimes mtDNA data reveal less diversity than that detected through allozymes because mtDNA cannot recombine and is maternally inherited as a single locus so that the variation is absolutely linked (Smouse et al. 1994; contrast the lack of geographic resolution observed for mtDNA data for populations of chum salmon in Park et al. [1993] with the geographic resolution apparent for allozyme data for similar populations in Winans et al. [1994]). However, haplotype data from a pilot examination (Fetzner et al. in prep.; Appendix A) indicate a potential east-west-island and upstream-intertidal separation of populations within PWS. We believed that the complementary use of the two techniques should provide optimal resolution of the population structure for this study.

Our objective was to test for both temporal and geographical structuring among even- and odd-year classes by examining genetic differences between early- and late-season spawners, upstream and intertidal spawners, and stream-of-spawning. Additionally, genetic positioning of the local hatchery stocks within this structure was of interest because the extensive releases of pink salmon fry in PWS in recent decades may have affected the partitioning of naturally occurring genetic diversity. Some fear that hatchery production may pose a threat to native populations as or more substantial to that posed by the oil spill (see discussion in Gharrett and Smoker 1993).

Also important to this study was the fact that even- and odd-year classes have independent population structures because of the rigid two-year life cycle of pink salmon. For example, climactic, tectonic or other such events (such as the 1964 earthquake [Roys 1971] or the 1989 oil spill) may affect the population structure of one year class, cycle through subsequent generations, and leave the alternate cycle of year-classes relatively unchanged (see data in Fetzner et al. in prep; Appendix A). Therefore, population structure

and conservation strategies must be independently assessed for the even- and odd-year classes.

In this paper we report the genetic structure of even-year populations of wild pink salmon inhabiting PWS. After the assay of 2686 individuals from 27 collections for variation at 77 allozyme loci and assay of a subset of 1080 individuals from each collection for variation at the ND5/ND6 region of mtDNA, we found genetic structuring within PWS in comparisons between elevation of spawning and among regions.

OBJECTIVES

Our objective is to define the genetic structure of pink salmon stocks in the EVOS-affected area of PWS. In this multi-year project we will test for:

1. genetic differences between spawners from the five primary management regions within PWS (Southeast, East, North, Southwest, Montague).

2. genetic differences between spawners from different streams within PWS.

3. genetic differences between upstream and intertidal spawners within the same streams.

4. genetic relationships between hatcheries and native populations.

5. genetic differences between temporally isolated spawners within the same streams.

6. genetic differences between odd- and even-year pink lineages.

7. inheritance of newly detected isozyme variants and loci.

In this report, we review the results for the 1994 collections and address objectives 1, 2, 3, and 4. The study is ongoing, and objectives 5, 6, and 7 will be addressed in future years.

METHODS

Field Sampling

Tissues were collected from 92 - 100 individuals from each of 25 spawning aggregations from wild-stock streams and two hatcheries during 1994 (Table 1). Sampling incorporated a broad geographical distribution of locations within PWS; primary consideration was given to the sampling of tributaries that routinely support large runs of fish on both even and odd years.

We also distributed sampling effort among the current harvest management zones. The Sound was historically divided into subdivisions for management and conservation

purposes according to biological, geographical, and geological factors (Anonymous 1960; Randall et al. 1983; Rugolo 1984). Sampling was done to include at least one collection from each of the five major subdivisions (Southeast, East, North, Southwest, Montague; Figure 1).

Consideration was also given to the physiography of PWS. Sampling included both areas uplifted by the major 1964 earthquake, where even-year populations were reduced by up to 98%, as well as areas where populations were relatively unaffected (Roys 1971; Figure 2).

Finally, although a majority of pink salmon spawning in PWS occurs in areas of tidal influence, some larger tributaries also possess somewhat discrete aggregations that spawn in upstream areas, above the influence of tides. Samples were collected from both tidal and upstream sites from five of these creeks (Table 1; Figure 1).

Tissue samples from heart, liver, muscle, and vitreous humor from each individual were immediately frozen on liquid nitrogen and returned to Anchorage for storage at -80° C. Subsamples were shipped to the Washington Department of Fisheries and Wildlife, Olympia, Washington, on dry ice where they were also stored at -80° C prior to allozyme analysis.

Allozyme Analysis

Genetic data were collected using the techniques of allozyme electrophoresis on all samples (Aebersold et al. 1987). An extensive screening for resolution of allozyme phenotypes on 45 individuals from two collections, Erb Creek and Humpback Creek, detected 77 putative loci (Table 2). These 77 loci were screened for genetic variation in all remaining individuals. Our nomenclature followed the American Fisheries Society standard (Shaklee et al. 1990).

Alleles present at frequencies above 0.01 in one or more collections were retained for data analysis. Allele observations from alleles that did not meet this criterion were excluded to reduce statistical noise associated with low frequency alleles, thereby increasing our power to detect genetic structuring (see Shaklee et al. 1994). This criteria reduced the number of loci further analyzed to 38: *sAAT-1,2*; sAAT-3*; sAAT-4*; mAAT-1*; ADA-1*; ADA-2*; sAH*; mAH-3*; mAH-4*; CK-A2*; FDHG*; bGALA*, G3PDH-1*; G3PDH-2*; G3PDH-3*; GDA-1*; GPI-B1,2*; IDDH-1*; mIDHP-1*; sIDHP-2*; LDH-A2*; LDH-B2*; sMDH-A1,2*; sMDHB-1,2*; mMEP-1*; NTP*; PEPB-1*; PEPD-2*; PEPLT*; PGDH*; PGM-2*; mSOD*; sSOD-1*; TPI-2**. Loci dropped from the population analyses included: *mAAT-2*; mAH-1*; mAH-2*; AK*; ALAT*; CK-A1*; CK-B*; CK-C1*; CK-C2*; ESTD*; FH*; GAPDH-1*; GAPDH-2*; GAPDH-3*; GAPDH-4*; GAPDH-5*; bGLUA*; GPI-A*; GR; mIDHP-2*; sIDHP-1*; LDH-A1*; LDH-B1*; LDH-C*; aMAN*; mMDH-1*; mMDH-2,3*; mMEP-2*; MPI*; PEPA*; PEPB-2*; PEPD-1*; PGK-1*; PGK-2*; sSOD-1*; sSOD-2*; TPI-1*; TPI-3*; TPI-4**.

Individual genotypic data were summarized into allelic frequencies, and tests for departure from Hardy-Weinberg were made using log-likelihood tests, α=0.05 (modified from Weir 1990) with the experimentwise significance level set at 0.05 and adjusted for multiple tests (Rice 1989). For isoloci (*sAAT-1,2*; GPI-B1,2*; sMDH-A1,2*; sMDH-B1,2**), allele frequencies were calculated using a multinomial model, assuming independence of alleles at both loci. Observed and expected heterozygosities were computed using the reduced set of loci. Paired *t*-tests were made to determine if observed heterozygosities in upstream samples

were significantly greater than tidal samples from the same system and to test for differences in heterozygosities between hatchery and wild collections.

We performed hierarchical analyses using log-likelihood ratios to test for homogeneity within and among groups of pink salmon collections (modified from Weir 1990). The wild collections were organized hierarchically to test for homogeneity: 1) among collections within regions within elevation, 2) among regions within elevation, and 3) among wild collections from different elevations (tidal and upstream). The highest level of the hierarchy was a test between all the wild collections and the hatchery collections. The log-likelihood ratio statistic is distributed approximately chi-squared with $(n - 1)(m - 1)$ degrees of freedom, where n is the number of alleles and m is number of collections in the test. If an allele was observed in a collection, we assumed that it existed within all collections, potentially at an infinitely small frequency. Therefore, the degrees of freedom and log-likelihood statistics are summable, and differences among and within collection subdivisions can be examined.

For the hierarchical analysis, comparisonwise significance levels were adjusted for multiple tests using a sequential Bonferonni adjustment (modified from Miliken and Johnson 1984 and Rice 1989) with the overall experimentwise significance level set at 0.05. The first step in the analysis was a sequentially adjusted test for differences at the first hierarchical level, i.e., between sources (hatchery and wild) and within sources. If a significant difference was found within sources, then a sequentially adjusted test was applied at the next level. Testing proceeded in this way through the hierarchy. If a test was not significant, then all remaining lower levels were combined, and a final sequentially adjusted multiple test of significance was performed.

A gene diversity analysis (Nei 1973) was performed among the wild collections to partition variation into hierarchical levels. As before, this analysis was partitioned by wild/hatchery, then by elevation, and then by region. Isoloci were excluded.

Separate hierarchical groupings were used to test for differences among paired collections within streams and to test for differences among regions. Comparison-wise significant levels were adjusted for all tests within each hierarchical grouping using a sequential Bonferonni adjustment (modified from Miliken and Johnson 1984 and Rice 1989) with the overall experimentwise significance level set at 0.05. To test for differences between tidal and upstream collections within streams, we performed log-likelihood tests between the paired collections within the five streams from which we had both. To test for differences between individual regions, we performed two groupings of pairwise log-likelihood tests after pooling tidal collections within regions and after pooling upstream collections within regions. Within the same groupings we also tested these pooled collections with individual hatchery collections.

Cavalli-Sforza and Edwards (1967) chord distances were calculated to evaluate genetic relationships and examined with classical multidimensional scaling analysis (MDS; Lessa 1990) and with a tree constructed using unweighted pair-group method (UPGMA; Sneath and Sokal 1973). The MDS ordination technique plots genetic relationships in two dimensions so that the plotted distances between collections closely match the observed distances in multidimensional space. This technique provides a means to confirm expected structure and uncover unexpected structure by providing insight into structural demarcations. All calculations were performed using functions in *S-Plus* (Mathsoft, Inc., Seattle, WA).

Mitochondrial DNA Analysis

A subset of 40 individuals from each of the 27 collections analyzed for allozyme variation was assayed for variation at sites previously identified in the ND5/ND6 region (Fetzner et al. in prep.; Appendix A). Genomic DNA was extracted using Puregene DNA isolation kits for animal tissues (Gentra Systems, Inc. P.O. Box 13159, Research Triangle, NC 27709-13159). This process included: (1) a cell lysis solution to break down cell and nuclear membranes; (2) a Proteinase K digest to denature proteins; (3) an RNase treatment to digest RNA; (4) protein precipitation to remove Proteinase K, RNase, and denatured proteins; (5) isopropanol to precipitate DNA; (6) 70% ethanol to wash DNA; and finally (7) a hydration solution to rehydrate DNA.

After extraction, DNA was amplified using the polymerase chain reaction (PCR; Saiki et al. 1988; Kocher et al. 1989). Amplified DNA was cut with the six restriction enzymes found to detect haplotype polymorphisms (of the 30 screened in Fetzner et al. [in prep.]; *Apa I, BstU I, EcoR V, Hinf I, Rsa I, Xba I*) and electrophoresed on agarose gels. Fragments were visualized under UV light, and a photographic record was made of each gel. The restriction sites detected for each enzyme were pooled as composite haplotypes for the statistical analyses.

Nucleotide (π) and haplotype (h) diversity measures (Nei 1987) were calculated for all collections using the restriction enzyme analysis package (*REAP*; McElroy et al. 1992). These measures estimate the number of nucleotide substitutions per site between DNA sequences (i.e., sequence divergence) and the amount of DNA polymorphism within collections, respectively.

To test for heterogeneity among populations, Monte Carlo simulations with 10,000 replicates were performed (Roff and Bentzen 1989) using the REAP analysis program. Independent tests were performed to test for heterogeneity in a hierarchical manner following the levels identified in the log-likelihood analysis. However, unlike the log-likelihood analysis, the χ^2 values for individual tests are not summable. Monte Carlo tests were also performed between the paired upstream and tidal collections, and among-region tests were conducted by pooling collections within region. All significance levels were adjusted using sequential Bonferroni techniques (Rice 1989).

An analysis of the distribution of molecular variance was made using AMOVA (Excoffier et al. 1992) and utilizing a matrix of Euclidean distances between haplotypes. Pairwise Euclidean distances were calculated as the total number of site changes between haplotypes. The AMOVA analysis incorporates distance between haplotypes in the calculation of haplotypic diversity at different hierarchical levels. Haplotype correlation measures are expressed as Φ-statistics (Excoffier et al. 1992). Among regions, Φ_{CT} is defined as the correlation of random haplotypes within a group of collections relative to that of random pairs of haplotypes drawn from the entire set of collections. For the analysis among collections within regions, Φ_{SC} is the correlation of random haplotypes within collections relative to that of random pairs of haplotypes from the regions. Finally for the within-collection analysis, Φ_{ST} is the correlation of random haplotypes within collections relative to that of random pairs of haplotypes drawn from the entire set of collections. The AMOVA

analysis allows for only a two-level hierarchy, so we were unable to partition regions within elevations as in the preceding analyses. Rather, we performed two separate analyses, one based on elevation and one based on geographic regions. The significance of the observed variance components and Φ-statistics were tested using a random permutation procedure in AMOVA. The permutation approach to significance testing avoids the parametric assumptions of normality and independence that are not met by molecular distance measures (Excoffier et al. 1992). The number of permutations was set at 1000 for each analysis. Φ_{ST} between pairs of populations, a modified coancestry coefficient, were also calculated as a genetic distance and examined with MDS.

RESULTS

Allozymes

Variation was detected at 56% of the allozyme loci (43/77), although five polymorphic loci were dropped as alleles were present at a frequencies below 0.01 in all collections (Appendix B). The screening also yielded 28 rare alleles (<0.01 in each collection) which were excluded from analyses.

Observed heterozygosities based on 38 loci varied over a relatively narrow range (mean 0.142, range 0.132 to 0.163; Table 3). No significant difference in heterozygosities was observed between tidal and upstream collections within the same streams (mean tidal =0.149, mean upstream = 0.147, t = 0.693, df = 8, P = 0.741). Heterozygosities of hatcheries were not different from wild collections (mean hatcheries = 0.138, mean wild = 0.147, t = 1.63, P = 0.116). No differences between hatchery and wild collections were apparent with respect to rare allele frequencies, average number of alleles, or proportion of polymorphic loci.

All polymorphic alleles were tested for departures from Hardy-Weinberg (H-W) expectations. No collection had an overall deviation from H-W. We made 743 tests, of which 15 were significant at the 0.05 level before adjusting for multiple tests, well within the range of positive results expected, and none were significant after adjusting for multiple tests. The most significant deviations were spread over nine loci, and no locus deviated in more than three collections.

Heterogeneity among wild populations

We tested for heterogeneity within regions for each elevation. For the tidal collections, Montague and Southeast regions were represented by only a single site each , Rocky and Constantine Creeks, respectively and therefore were not tested. The other three regions were represented by tidal collections from a minimum of four steams each. Heterogeneity was detected only within the Southwest region (eight collections; Table 4). No differences were detected within either the North (four collections) or East regions (seven collections).

Significant heterogeneity was detected overall among all five regions for the tidal

collections (Table 4). A closer examination of the pattern of heterogeneity was conducted through pairwise comparison of the regions (Table 5a). After statistically accounting for multiple tests, only the test between the two best represented regions (Southwest and East) was significant.

Heterogeneity tests were also made for the upstream collections. Three of the upstream collections originated from the East region, while one each originated from the North and Southeast regions (Table 1). Significant heterogeneity was detected within the East region and also in the test among all regions (Table 4).

Pairwise comparisons between pooled upstream collections within regions (Table 5a) indicated that every region was significantly different from every other region. The upstream collection from Lagoon Creek was especially divergent from the rest. This collection had the most divergent allele frequencies for: *sAAT-4*-10; ADA-2*90; mAH-4*81; G3PDH-1* -52; G3PDH-2*120; GDA-1*108,*113; sIDHP-2*125; PEPB-1*138; PEPD-2*120; PEPLT*108;* and *PGDH*86.*

The test for overall heterogeneity between the upstream and tidal collections was also highly significant (Table 4). We conducted tests between the paired upstream and tidal collections originating from Mink, Olsen, Constantine, Koppen, and Lagoon Creeks. The test between the two Lagoon Creek samples was highly significant ($P < 0.01$; Table 6).

Total diversity

A hierarchical gene diversity analysis was performed using 30 loci (isoloci were excluded). The hierarchical analysis was stratified by collection, region, and elevation. By far the majority of the variation (99.29%) occurred within collections (Table 7) and was heavily weighted by variation at *sAAT-4**, *GDA-1**, *sIDHP-2**, and *PEPD-2**. The remaining heterogeneity was divided among collections within regions (0.45%), among regions within elevation (0.19%), and between elevations (0.07%).

The UPGMA tree and the MDS including all collections confirm the uniqueness of the upstream Lagoon Creek collection (Figures 3 and 4). The tree constructed using UPGMA does not show any genetic structuring based on region or elevation (Figure 3). To better visualize the relationships among the other collections, a second MDS was generated excluding the Lagoon Creek upstream collection (Figure 5). Some regional structuring is apparent from the plot. The Southwest collections tend to occupy the left and upper portions of the plot, while the East collections occupy a lower area that extends to the extreme right of the plot. Some overlap between the Southwest and East regions occurs. The North collections tend to occupy space across both the Southwest and East regions. The hatchery collections both occur in the central positions of their respective regions, and Armin F. Koernig (AFK) Hatchery is located near the area of overlap between the Southwest and East collections.

The position of the upstream collections is particularly interesting. Upstream collections from Olsen and Koppen Creeks occupy space within the area bounded by East collections. However, upstream collections from both Mink Creek and Constantine Creek are outliers. Interestingly, the tidal collection from Mink Creek is also an outlier and shows affinity to the upstream Mink Creek collection rather than to other tidal collections from the

North region. As mentioned earlier, Lagoon Creek upstream was not included in this plot because of its highly distant position.

Hatchery collections

No significant difference was detected in the heterogeneity test between the two hatchery collections (Table 5a). The log-likelihood test for homogeneity between the wild and hatchery groups at the highest level of the hierarchy was also not significant (Table 4). However paired log-likelihood tests between each hatchery and pooled collections within regions the test between Solomon Gulch Hatchery and upstream Southeast collections was significant. AFK Hatchery was not different from any of the regions for tidal collections.

In the MDS analysis, although both hatcheries clustered into their respective regions, AFK Hatchery clustered near the area overlapped by the East region collections (Figures 4-5). AFK Hatchery also clustered closely with Duck River, an eastern PWS site from which gametes were collected to found its even-year hatchery stock in 1976. Again the tree constructed using UPGMA does not show any apparent genetic relationship between the hatcheries and streams within regions (Figure 3).

Mitochondrial DNA

Forty individuals from each of the 27 collections were examined for variation at ND5/ND6 using six restriction enzymes previously identified to reveal polymorphisms in pink salmon (Fetzner et al. in prep.; Table 8). Eight unique haplotypes were defined from 1080 individuals detected with four of the six restriction enzymes tested (Table 9). No polymorphic sites were detected with two enzymes, *Rsa I* or *Xba I* . Four of the haplotypes (V, VI, VII, XV) were rare with seven or fewer individuals observed and frequencies less than 0.01. The two rarest haplotypes, VII and XV, were observed only once each.

Haplotype and nucleotide diversity

Haplotype diversity (h) ranged from 0.144 in Hartney Creek to 0.543 in Cathead Creek and averaged 0.381 (Table 9). Corresponding nucleotide diversity values (π) ranged from 0.0012 in Hartney Creek to 0.0050 in Cathead Creek and averaged 0.0039. No regional or elevational patterns in diversities were observed. Nucleotide and haplotype diversities were high for both hatchery collections, Solomon Gulch Hatchery (h = 0.504, π = 0.0047) and AFK Hatchery (h = 0.470, π = 0.0042), although neither of the hatchery values were the largest observed. No significant difference in the nucleotide diversities between the paired upstream and tidal collections were detected (paired t-test; $P > 0.80$).

Heterogeneity detected by Monte Carlo tests

A Monte Carlo test of all collections (hatchery, upstream, and tidal) yielded a significant test statistic (Table 10). Tidal collections were tested for homogeneity within each region, among regions, and among all tidal collections. No test was significant indicating

overall homogeneity among tidal collections. The upstream collections were evaluated in a similar manner; however unlike the tidal tests, all upstream tests were significant ($P < 0.01$, Table 10). Hatchery collections were tested and were not significantly different from each other (Table 10). We also performed an analysis on a region-by-region basis with hatcheries, pooled tidal, and pooled upstream collections similar to that performed with allozymes (Table 5a). For the comparisons between tidal regions, none were significantly different after adjusting for multiple tests (Table 5a). However, in the upstream comparisons, North and East were significantly different from each other after adjusting for multiple tests. For comparisons with hatcheries, no differences between Solomon Gulch Hatchery and tidal collections within any regions were found after adjusting for multiple tests (Table 5a). AFK Hatchery was significantly different from the North upstream region after adjusting for multiple tests. All other tests were not significant.

We also performed a series of Monte Carlo simulations between paired tidal and upstream collections. Only the test for Koppen Creek was significant after adjusting for multiple tests (Table 6). This within-stream difference was quite apparent in the haplotype counts and distribution of haplotypes. For example, haplotype II occurred at a frequency of 0.200 in the Koppen Creek tidal collection, but was absent from the upstream collection.

AMOVA analyses

An AMOVA analysis that partitioned the molecular variation by elevation was also performed. The majority of the variation (98.4%) was within collections ($\Phi_{ST} = 0.016$, Table 11). Most of the variation among collections was within elevation ($\Phi_{SC} = 0.011$). Both Φ_{ST} and Φ_{SC} were significant based on the permutation analysis (Table 11). The between-elevation component, Φ_{CT} , was not significant. A second AMOVA analysis was performed with the partitioning by region (Table 11). The results were quite similar to that obtained for the elevation analysis, indicating that much of the among-collection variation was among collections within regions.

An MDS plot was generated using distances computed from Φ-statistics (Figure 7). The plot resembles that of the allozyme data with Lagoon Creek as the most divergent collection. Other divergent collections include Koppen Creek upstream, Swanson Creek, Hartney Creek, and Constantine Creek upstream.

DISCUSSION

Understanding genetic structure of Pacific salmon populations is critical to their management and conservation. For example, managing on too fine a scale may adversely affect the fishing industry and waste management resources, while managing on too large a scale may result in loss of genetic adaptations and diversity (see Mundy et al. 1993). Here we report our initial findings in an examination of the even-year lineage of commercially important populations of pink salmon that inhabit PWS, Alaska.

Inferences from studies showing genetic homogeneity for allozymes over vast geographic distances (e.g., Shaklee and Varnavskaya 1994) lead some to suggest that pink salmon populations within PWS, spanning only 100 kilometers, should be genetically

homogenous. In contrast, implications from other allozyme studies (Lane 1990) suggest that pink salmon populations in PWS might be substantially heterogenous. Our objective was to generate molecular genetic data to support or reject these alternatives.

Three recent and major factors have impacted these populations. The *Exxon Valdez* oil spill of 1989 adversely affected pink salmon through a combination of direct lethal effects, sublethal effects, and alterations in fishing pressure (Bue et al. 1996); study of effects of the oil spill instigated our study. Further, the major tectonic upheaval of 1964 produced bottlenecks in some populations. However, arguably one of the most serious factors influencing population structure may be deleterious effects of hatchery/wild-stock interactions and the potential erosion of locally adapted genotypes (Gharrett and Smoker 1993). Prince William Sound is the center of one of the world's largest aquacultural industries. Six-hundred million pink salmon fry of hatchery origin are released annually. Alaska Department of Fish and Game has been grappling with management of the wild populations in face of intractable hatchery/wild-stock interactions for nearly a decade. The *Exxon Valdez* oil spill-related damages to wild populations, coupled with full-scale hatchery egg takes, exacerbated wild-stock conservation concerns.

Our analysis of the 1994 collections showed significant substructuring of pink salmon in PWS based upon both allozyme and mtDNA data sets. The heterogeneity analysis, a conservative analysis because all alleles observed are assumed to exist in all collections thereby inflating the degrees of freedom, showed significant allele frequency differences occurring between stream elevations and among and within regions. In the allozyme data, pairwise homogeneity tests among regions indicate that, for tidally spawning aggregates, the Southwest and East regions are distinct from each other after adjusting for multiple tests. However, these were also the two most heavily sampled regions. Other regions may have also been different from eachother had there been more sampling. Evidence of this is found in the number of tests that were significant before accounting for multiple tests. Before adjusting critical values for multiple tests, 4 of the 10 regional tests were significant (more than would be expected by chance if no heterogeneity among regions existed), suggesting that we may have lacked statistical power to detect differences present among less sampled regions (Table 5b). For upstream spawners, pairwise comparisons show genetic differences occurring among all regions where upstream spawners were sampled.

These data provided insight not only into the structure of the wild fish within PWS, but also into the genetic relationships between hatchery fish and these wild fish. Allozyme data did not distinguish AFK Hatchery from any of the regions when tidal fish within region were pooled. The even-year lineage for AFK Hatchery was founded originally with gametes from Duck River, a site across PWS in the East region. Annual propagation at the hatchery comes from broodstock seined from fish milling in front of the hatchery, and evidence from coded-wire-tag recoveries suggests that these milling fish include some wild fish headed for other areas (Sharr et al. 1995). AFK Hatchery is located adjacent to the strait through which most pink salmon enter PWS on their way to their spawning streams (Templin et al. 1996); it is possible that wild fish included in the hatchery broodstock may come from anywhere throughout PWS. Therefore the inability to distinguish AFK Hatchery fish from other regions is not surprising. Conversely, Solomon Gulch Hatchery is located at the end on the Valdez Arm in eastern PWS. Few pink salmon bound for other regions of PWS are likely to be

milling near this hatchery when broodstock are seined for Solomon Gulch Hatchery. In addition, founding broodstock for this hatchery was locally obtained. Although the Solomon Gulch Hatchery collection was not different from any other region after accounting for multiple tests, there is again evidence that this inability to detect differences may be due to a lack of statistical power. Before adjusting the critical values for multiple tests, significant differences were found between Solomon Gulch Hatchery and all regions except the East tidal region (Table 5b). These differences disappear after multiple test adjustments are made, indicating that statistical power may not be adequate to test the hypothesis until regions are better represented.

The fact that the mtDNA data and allozyme data provided generally concordant results strengthens our interpretations. Concordance is not always observed (c., Ward et al. 1989; Adams et al. 1994), but in this study both approaches demonstrate similar heterogeneity among the spawning aggregates. Interestingly, in contrast to expectations generated by mtDNA differences observed in the pilot study (Fetzner et al. in prep), the allozyme data tend to provide comparatively better resolution of regional population structure within PWS. However, both show significant differences between tidal and upstream spawning aggregates and substantial structuring among upstream-spawning populations. Multidimensional scaling analyses for both data sets (Figures 4, 5, and 7) indicated Lagoon Creek upstream to be genetically distinct from all other spawning aggregates. The differences observed within Koppen and Lagoon Creeks are particularly interesting and somewhat surprising given the relatively close geographic proximity of the upstream and tidal spawning areas.

When there were discrepancies in results between allozyme and mtDNA data, in all but two cases, mtDNA data were less able to detect differences than were allozyme data. Allozyme data detected various differences among tidal collections (Table 4), many differences between upstream regions (Table 5a), and differences between upstream and tidal collections at Lagoon Creek (Table 6) which were not detected with mtDNA data (Tables 10, 5 and 6). Three hypotheses might explain this discrepancy: higher straying rates in females than in males, bottlenecks or extinctions and recolonizations, or lack of statistical power. Higher straying rates in females could homogenize mtDNA allele frequencies because of strict maternal inheritance, while allozyme heterogeneity might be maintained if males stray little (Allendorf 1994). However, evidence from coded wire tag data indicates that straying rates of pink salmon in PWS are similar for males and females (Habicht, unpublished data). Other studies have observed low mtDNA variation in populations with high allozyme variation and have attributed these results to historical bottlenecks or extinction and subsequent recolonizations (reviewed in Allendorf 1994). However, mtDNA data in this study were variable; we found eight haplotypes of which three had frequencies greater than 5% (Table 9). The last hypothesis for the lack of significant tests in the mtDNA data analysis may have been a lack of statistical power resulting from the lower allele counts observed per population using this single-locus method. We analyzed 40 fish for mtDNA data which translates to 40 haplotypes per population; conversely, we analyzed 100 fish using allozymes which translates to 200 alleles per locus, and we analyzed 40 loci per population.

The two cases where mtDNA data detected differences where allozyme data did not were between Koppen upstream and tidal collections (Table 6) and between AFK Hatchery and the North collections (Table 5a). One hypothesis that would explain this difference is

that mtDNA data were gathered from the first 40 fish collected in each collection while allozyme data were collected from all 100 fish collected. These first 40 fish, especially in the upstream or tidal collections at Koppen Creek, could have been distinct from the second 60 fish which would have resulted in more heterogeneous allozyme data, but more homogeneous mtDNA data within elevations. To test this hypothesis, we analyzed the allozyme data for the first 40 individuals from all these collections. No differences were detected in either comparison, nor did this result appear to be caused by the decrease in statistical power of only analyzing 40 individuals. Alternatively, in these locations males may stray more than females, however, no data exists to test this hypothesis at these locations. Finally, these discrepancies might be the result of sampling error (Type II error). Data from additional year(s) will allow us to determine if these differences hold between years within the same year-classes.

We recognize that the data show the even-year lineage to have a shallow genetic structure (in contrast to the structure of sockeye salmon populations from a similar geographic range in Cook Inlet, Alaska, for example; Seeb et al. 1995a). In both MDS analyses in this study, collections within each of the *a priori* management regions did not cluster into tight, regional groups. Shallow structure is usually an indication of highly dispersive taxa with limited barriers to gene flow (Avise 1994).

Yet population structure and barriers to gene flow do exist for these fish in the face of oil spills, tectonic upheavals, and potential hatchery straying (Habicht et al. in prep.; available in Seeb et al. 1995b). Our goal is to provide the basis for key management decisions by defining the genetic structure of populations from throughout PWS. The commercial harvest of pink salmon fluctuated dramatically between six and 44 million fish during the years since the oil spill because of ecological instability. Maintenance of genetic diversity will play a key role in ameliorating the affects of this instability. Our data confirm that harvest- and hatchery-management decisions made for conservation purposes should best be made on a population-specific rather than species-specific basis. Expansion of this study to include additional even-year collections as well as comparable odd-year collections is continuing; the analysis of data from multiple year classes will allow us to better test the appropriateness of current management regions.

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Table 1. Pink salmon collected from Prince William Sound in 1994. Map numbers refer to Figure 1. All fish were screened for allozyme variation. Forty fish from each collection were screened for mtDNA variation.

Sample #	Map #	Location name	Elevation	Region	Sample Date	N
1	1	Rocky Creek	tidal	Montague	8/29	100
2	2	Armin F. Koernig Hatchery	-	Southwest	9/08	100
3	3	Cathead Creek	tidal	Southwest	8/22	99
4	4	Herring Creek	tidal	Southwest	8/22	100
5	5	Halverson Creek	tidal	Southwest	8/23	100
6	6	Countess Creek	tidal	Southwest	8/23	100
7	7	Chenega Creek	tidal	Southwest	8/22	100
8	8	Totemoff Creek	tidal	Southwest	8/22	100
9	9	Erb Creek	tidal	Southwest	8/24	100
10	10	Mink Creek	tidal	North	8/24	100
11	10	Mink Creek	upstream	North	8/24	100
12	11	Swanson Creek	tidal	North	8/06	100
13	12	Coghill River	tidal	North	8/24	100
14	13	Jonah Creek	tidal	North	8/23	96
15	14	Solomon Gulch Hatchery	-	East	8/12	100
16	15	Duck River	tidal	East	8/16	100
17	16	Millard Creek	tidal	East	8/16	100
18	17	Lagoon Creek	tidal	East	8/14	100
19	17	Lagoon Creek	upstream	East	8/14	99
20	18	Olsen Creek	tidal	East	8/17	100
21	18	Olsen Creek	upstream	East	8/17	100
22	19	Koppen Creek	tidal	East	8/15	100
23	19	Koppen Creek	upstream	East	8/13	100
24	20	Humpback Creek	tidal	East	8/13	100
25	21	Hartney Creek	tidal	East	8/12	100
26	22	Constantine Creek	tidal	Southeast	8/18	92
27	22	Constantine Creek	upstream	Southeast	8/18	100

Table 2. Enzymes, loci, and primary tissue-buffer combinations used to screen for allozyme variation. Enzyme nomenclature follows Shaklee et al. (1990), and locus abbreviations are given.

Enzyme	Enzyme Number	Locus	Tissue	Buffer[1]
Aspartate aminotransferase	2.6.1.1	*sAAT-1,2**	Heart	ACEN 6.8
		*sAAT-3**	Eye	TG
		*sAAT-4**	Liver	TG
		*mAAT-1**	Heart	ACEN 6.8
		*mAAT-2**	Muscle	ACE 6.5
Adenosine deaminase	3.5.4.4	*ADA-1**	Muscle	AC 6.1
		*ADA-2**	Muscle	AC 6.1
Aconitate hydratase	4.2.1.3	*mAH-1**	Heart	ACEN 6.8
		*mAH-2**	Heart	ACEN 6.8
		*mAH-3**	Muscle	ACE 6.8
		*mAH-4**	Muscle	ACE 6.8
		*sAH**	Liver	ACEN 6.8
Adenylate kinase	2.7.4.3	*AK**	Muscle	TG
Alanine aminotransferase	2.6.1.2	*ALAT**	Muscle	TG
Creatine kinase	2.7.3.2	*CK-A1**	Muscle	TG
		*CK-A2**	Muscle	TG
		*CK-B**	Eye	TG
		*CK-C1**	Eye	TG
		*CK-C2**	Eye	TG
Esterase-D	3.1.1.-	*ESTD**	Muscle	ACE 6.5
Formalin dehydrogenase	1.2.1.1	*FDHG**	Heart	ACEN 6.8
Fumarate hydratase	4.2.1.2	*FH**	Muscle	ACE 6.8

Table 2. Continue.

Enzyme	Enzyme Number	Locus	Tissue	Buffer[1]
β-N-Acetylgalactosaminidase	3.2.1.53	*βGALA* *	Muscle	TG
Glyceraldehyde-3-phosphate dehydrogenase	1.2.1.12	*GAPDH-1**	Muscle	AC 6.1
		*GAPDH-2**	Heart	ACEN 6.8
		*GAPDH-3**	Heart	ACEN 6.8
		*GAPDH-4**	Eye	TG
		*GAPDH-5**	Eye	TG
Guanine deaminase	3.5.4.3	*GDA-1**	Liver	TG
N-Acetyl-β-glucosaminidase	3.2.1.53	*βGLUA* *	Liver	ACE 6.8
Glycerol-3-phosphate dehydrogenase	1.1.1.8	*G3PDH-1**	Muscle	TG
		*G3PDH-2**	Heart	ACEN 6.8
		*G3PDH-3**	Heart	ACEN 6.8
Glucose-6-phosphate isomerase	5.3.19	*GPI-B1,2**	Muscle	TG
		*GPI-B2**	Heart	TG
		GPI-A *	Muscle	TG
Glutathione reductase	1.6.4.2	*GR**	Heart	TC4
L-Iditol dehydrogenase	1.1.1.14	*IDDH-1**	Liver	TBCL
Isocitrate dehydrogenase (NADP+)	1.1.1.42	*mIDHP-1**	Muscle	ACE 6.5
		*mIDHP-2**	Heart	ACEN 6.8
		*sIDHP-1**	Liver	ACE 6.8
		*sIDHP-2**	Liver	ACE 6.8

Table 2. Continue.

Enzyme	Enzyme Number	Locus	Tissue	Buffer[1]
L-Lactate dehydrogenase	1.1.1.27	*LDH-A 1**	Muscle	TG
		*LDH-A 2**	Muscle	TG
		*LDH-B1**	Heart	TG
		*LDH-B2**	Heart	TG
		*LDH-C**	Eye	TG
αMannosidase	3.2.1.24	*αMAN**	Heart	TG
Malate dehydrogenase	1.1.1.37	*sMDH-A 1,2**	Heart	ACEN 6.5
		*sMDH-B1,2**	Heart	ACEN 6.5
		*mMDH-1**	Heart	ACEN 6.5
		*mMDH-2,3**	Heart	ACEN 6.5
Malic enzyme (NADP+)	1.1.1.40	*mMEP-1**	Muscle	ACE 6.8
		*mMEP-2**	Muscle	ACE 6.8
Mannose-6-phosphate isomerase	5.3.1.8	*MPI**	Heart	TG
Nucleoside-triphosphate pyrophosphatase	3.6.1.19	*NTP**	Muscle	ACE 6.5
Dipeptidase	3.4.-.-	*PEPA**	Muscle	TG
Tripeptide aminopeptidase	3.4.-.-	*PEPB-1**	Heart	TG
		*PEPB- 2**	Heart	TG
Proline dipeptidase	3.4.13.9	*PEPD- 1**	Heart	ACEN 6.5
		*PEPD- 2**	Heart	ACEN 6.5
Peptidase-LT	3.4.-.-	*PEPLT**	Muscle	TG
Phosphogluconate dehydrogenase	1.1.1.44	*PGDH**	Muscle	ACE 6.5

Table 2. Continue.

Enzyme	Enzyme Number	Locus	Tissue	Buffer[1]
Phosphoglycerate kinase	2.7.2.3	*PGK-1**	Muscle	ACE 6.8
		*PGK-2**	Muscle	ACE 6.8
Phosphoglucomutase	5.4.2.2	*PGM-2**	Heart	TG
Superoxide dismutase	1.15.1.1	*sSOD-1**	Heart	ACEN 6.8
		*sSOD-2**	Heart	ACEN 6.8
		*mSOD**	Heart	ACEN 6.8
Triose-phosphate isomerase	5.3.1.1	*TPI-1**	Muscle	TG
		*TPI-2**	Muscle	TG
		*TPI-3**	Muscle	TG
		*TPI-4**	Muscle	TG

Buffers: AC: amine-citric acid buffer, pH 6.8 (Clayton and Tretiak 1972) modified with EDTA (E), NAD (N), or both (Harris and Hopkinson 1976); TBCL: Tris-citric acid gel, pH 8.7 and lithium hydroxide-boric acid electrode buffer, pH 8.0 (Ridgway et al. 1970); TC4: Tris-citric acid buffer pH 5.8 (Schaal and Anderson 1974); TG: Tris-glycine buffer, pH 8.5 (Holmes and Masters 1970).

Table 3. Observed and expected heterozygosities calculated from 38 polymorphic loci.

	Observed Heterozygosity		Expected Heterozygosity	
	H	Std. Dev.	*H*	Std. Dev.
Rocky Ck. T	0.144	0.054	0.150	0.000
A. F. Koernig Hatchery	0.138	0.049	0.144	0.000
Cathead Ck. T	0.132	0.048	0.137	0.000
Herring Ck. T	0.147	0.058	0.148	0.001
Halverson Ck. T	0.147	0.057	0.148	0.000
Countess Ck. T	0.142	0.054	0.144	0.000
Chenega Ck. T	0.145	0.053	0.151	0.001
Totemoff Ck. T	0.159	0.064	0.157	0.000
Erb Ck. T	0.162	0.069	0.152	0.000
Mink Ck. T	0.150	0.061	0.144	0.000
Mink Ck. U	0.140	0.054	0.142	0.000
Swanson Ck. T	0.145	0.054	0.149	0.000
Coghill R. T	0.152	0.059	0.152	0.000
Jonah Ck. T	0.151	0.057	0.158	0.000
Solomon Gulch Hatchery	0.138	0.050	0.143	0.001
Duck R. T	0.158	0.062	0.153	0.001
Millard Ck. T	0.155	0.060	0.154	0.001
Lagoon Ck. T	0.140	0.052	0.144	0.000
Lagoon Ck. U	0.136	0.054	0.139	0.000
Olsen Ck. T	0.138	0.052	0.138	0.000
Olsen Ck. U	0.148	0.056	0.149	0.001
Koppen Ck. T	0.153	0.060	0.152	0.001
Koppen Ck. U	0.146	0.056	0.145	0.001
Humpback Ck. T	0.163	0.070	0.155	0.000
Hartney Ck. T	0.149	0.059	0.151	0.000
Constantine Ck. T	0.153	0.060	0.153	0.001
Constantine Ck. U	0.154	0.060	0.158	0.000

Table 4. Hierarchical analysis of 1994 pink salmon collections in PWS using log-likelihood ratios. Comparisonwise significance levels (α_c) were adjusted for multiple tests done within the same test groups (Test) using sequential Bonferonni adjustments (modified from Miliken and Johnson 1984 and Rice 1989). Experimentwise significance level was set to 0.05. Complete allozyme table with all loci is in Appendix B.

Source of Variaton	DF	Overall	*P*-value	α_c	Test
Between Sources	56	55.13	0.508	0.050	1
Within Sources	1400	1750.02 *	0.000	0.025	1
Wild	1344	1691.30 *	0.000	0.025	2
Between elevations	56	126.50 *	0.000	0.050	3
Within elevations	1288	1564.80 *	0.000	0.025	3
Upstream	224	356.90 *	0.000	0.025	4
Among Regions	112	184.10 *	0.000	0.050	5
Within East Region	112	172.80 *	0.000	0.025	5
Tidal	1064	1207.90 *	0.001	0.050	4
Among Regions	224	289.50 *	0.002	0.025	6
Within Regions	840	918.40 *	0.031	0.050	6
Southwest	336	402.80 *	0.007	0.017	7
North	168	175.10	0.338	0.025	7
East	336	340.50	0.421	0.050	7
Hatchery	56	58.72	0.376	0.050	2

* Significant at experimentwise $\alpha = 0.05$.

Table 5a. Pairwise homogeneity tests, within stream elevation, between regions and hatcheries. Log-likelihood ratios and degrees of freedom (in parentheses) are given below diagonal for allozyme data; χ^2 values from Monte Carlo simulations are given above the diagonal for mtDNA data.

Tidal

	Montague	Southwest	North	East	Southeast	AFK	Solomon Gulch
Montague	-	3.23	5.30	1.84	1.73	1.42	4.39
Southwest	70.6 (54)	-	6.77	9.96	2.43	4.74	5.63
North	67.4 (49)	79.1 (56)	-	14.33	1.62	12.12	10.26
East	65.8 (51)	93.6 (55)*	73.8 (54)	-	4.78	2.60	6.82
Southeast	53.0 (45)	60.4 (54)	66.4 (53)	57.4 (52)	-	4.10	5.10
AFK	43.7 (44)	52.6 (55)	42.4 (48)	45.9 (50)	58.2 (49)	-	4.31
Solomon Gulch	63.3 (42)	73.4 (54)	85.0 (52)	61.8 (51)	65.8 (46)	58.72 (46)	-

Upstream

	North	East	Southeast	AFK	Solomon Gulch
North	-	16.98*	11.15	14.44*	9.86
East	86.0 (47)*	-	7.82	8.39	2.38
Southeast	87.4 (45)*	94.6 (46)*	-	7.11	8.14
AFK	47.4 (44)	67.3 (45)	62.6(45)	-	5.10
Solomon Gulch	68.4 (45)	62.4 (45)	72.8 (44)*	58.72 (46)	-

* Significant at experimentwise $\alpha = 0.05$ (Rice 1989)

Table 5b. *P*-values for pairwise homogeneity tests from Table 5a, within stream elevation, between regions and hatcheries. *P*-values for the log-likelihood ratios are given below diagonal for allozyme data; *P*-values for the χ^2 values from Monte Carlo simulations are given above the diagonal for mtDNA data.

Tidal

	Montague	Southwest	North	East	Southeast	AFK	Solomon Gulch
Montague	-		0.264	0.759	0.582	0.717	0.198
Southwest	0.064	-	0.338	0.104	0.828	0.486	0.400
North	0.042	0.023	-	0.008	0.792	0.031	0.031
East	0.080	0.001*	0.038	-	0.369	0.596	0.245
Southeast	0.193	0.256	0.102	0.282	-	0.316	0.154
AFK	0.484	0.567	0.701	0.639	0.173	-	0.317
Solomon Gulch	0.018	0.041	0.003	0.143	0.029	0.099	-

Upstream

	North	East	Southeast	AFK	Solomon Gulch
North	-	0.004*	0.016	0.005*	0.049
East	0.000*	-	0.152	0.123	0.784
Southeast	0.000*	0.000*	-	0.050	0.029
AFK	0.336	0.017	0.042	-	0.317
Solomon Gulch	0.014	0.044	0.004*	0.099	-

* Significant at experimentwise $\alpha = 0.05$ (Rice 1989)

Table 6. Heterogeneity between paired tidal and upstream collections for allozyme and haplotype frequencies. Log-likelihood tests were performed to test homogeneity of allozyme frequencies. Homogeneity of mtDNA was tested using Monte Carlo simulations; probabilities of exceeding the original χ^2 by chance alone are given.

	Allozyme			mtDNA	
Stream	Log-likelihood	df	*P*	χ^2	*P*
Olsen Ck.	51.80	47	0.2920	2.20	0.9033
Mink Ck.	58.73	47	0.1172	3.94	0.5990
Lagoon Ck.	115.73	43	0.0000*	6.90	0.0223
Koppen Ck.	56.97	46	0.1288	13.56	0.0016*
Constantine Ck.	63.07	51	0.1196	1.17	0.7382

* Significant at experimentwise $\alpha = 0.05$ (Rice 1989)

Table 7. Gene diversity analysis (Nei 1973) by locus between stream elevations, among regions within elevations, among collections within regions, and within collections.

	Absolute Gene Diversity		Relative Gene Diversity			
	Total	Within Collection	Within Collection	Collections Within Region	Region Within Elevation	Between Elevation
*sAAT-3**	0.3528	0.3504	0.9931	0.0061	0.0008	0.0001
*sAAT-4**	0.5229	0.5184	0.9914	0.0067	0.0018	0.0001
*mAAT-1**	0.0167	0.0166	0.9940	0.0040	0.0020	0.0001
*ADA-1**	0.0028	0.0028	0.9935	0.0035	0.0017	0.0012
*ADA-2**	0.1510	0.1499	0.9929	0.0046	0.0024	0.0001
*sAH**	0.0065	0.0064	0.9943	0.0036	0.0018	0.0004
*mAH-3**	0.0044	0.0044	0.9944	0.0046	0.0008	0.0002
*mAH-4**	0.0734	0.0728	0.9916	0.0058	0.0017	0.0008
*CK-A2**	0.0048	0.0048	0.9949	0.0041	0.0010	0.0000
*FDHG**	0.0151	0.0150	0.9945	0.0041	0.0012	0.0002
*bGALA**	0.2067	0.2051	0.9922	0.0049	0.0027	0.0002
*G3PDH-1**	0.3031	0.3000	0.9898	0.0066	0.0035	0.0000
*G3PDH-2**	0.2494	0.2476	0.9930	0.0038	0.0029	0.0003
*G3PDH-3**	0.0157	0.0156	0.9939	0.0044	0.0012	0.0005
*GDA-1**	0.5150	0.5120	0.9941	0.0043	0.0003	0.0013
*IDDH-1**	0.0060	0.0060	0.9947	0.0036	0.0016	0.0001
*mIDHP-1**	0.0089	0.0088	0.9957	0.0031	0.0011	0.0000
*sIDHP-2**	0.4528	0.4485	0.9906	0.0059	0.0026	0.0008
*LDH-A2**	0.0032	0.0032	0.9878	0.0092	0.0025	0.0004
*LDH-B2**	0.0204	0.0203	0.9945	0.0045	0.0009	0.0001
*mMEP-1**	0.3869	0.3844	0.9935	0.0017	0.0030	0.0018
*NTP**	0.0020	0.0020	0.9920	0.0073	0.0005	0.0003
*PEPB-1**	0.2230	0.2217	0.9939	0.0045	0.0004	0.0012
*PEPD-2**	0.6089	0.6058	0.9951	0.0025	0.0022	0.0003
*PEP-LT**	0.2580	0.2561	0.9928	0.0037	0.0026	0.0008
*PGDH**	0.4386	0.4360	0.9941	0.0033	0.0013	0.0013
*PGM-2**	0.0040	0.0040	0.9950	0.0032	0.0017	0.0001
*mSOD**	0.0204	0.0202	0.9927	0.0059	0.0012	0.0002
*sSOD-1**	0.0160	0.0159	0.9945	0.0047	0.0004	0.0004
*TPI-2**	0.0301	0.0299	0.9934	0.0048	0.0018	0.0000
Overall	0.1640	0.1628	0.9929	0.0045	0.0019	0.0007

Table 8. Restriction enzymes, length of recognition sequence (r), and fragment sizes detected in ND5/ND6 haplotypes.

Restriction Enzyme	r	Haplotype	Fragment sizes (bp)
Apa I	6	A	1300, 1100
		B	1300, 650, 450
BstU I	4	A	1650,750
		B	1200, 750, 450
		C	1150, 750, 500
EcoR V I	6	A	2400
		B	1500, 900
Hinf I	4	A	800, 500, 350, 300, 250[a]
		B	1050, 500, 350, 300, 250
		C	500, 450, 350[a], 300, 250[a]
Rsa I	4	A	1605, 265[b]
Xba I	6	A	2400

[a] There are two fragments of the indicated size in these patterns.
[b] There are three fragments of the indicated size in these patterns.

Table 9. Haplotype counts for 1994 collections from Prince William Sound (T = tidally spawning, U = upstream spawning, H = hatchery). Haplotype designations after Fetzner et al. (in prep.): I = AAAAAA, II = ACAAAA, III = AAABAA, IV = ABAAAA, V = AABAAA, VI = BAAAAA, VII = AAACAA, XV = ACBAAA. Order of restriction enzymes is *Apa I, BstU I, EcoR V, Hinf I, Rsa I, Xba I.* Haplotype diversity (h) and nucleotide diversity (π) are given.

			ND5/ND6 Haplotypes									
	Sampling Site		I	II	III	IV	V	VI	VII	XV	h	π
1	Rocky Creek	T	31	7	2	0	0	0	0	0	0.3709	0.0032
2	Armin F. Koernig	H	28	8	3	0	1	0	0	0	0.4696	0.0042
3	Cathead Creek	T	26	7	2	3	0	2	0	0	0.5430	0.0050
4	Herring Creek	T	34	2	1	2	0	0	0	0	0.3083	0.0026
5	Halverson Creek	T	32	5	3	0	0	0	0	0	0.3430	0.0030
6	Countess Creek	T	26	8	3	2	1	0	0	0	0.5354	0.0049
7	Chenega Creek	T	32	3	3	2	0	0	0	0	0.3506	0.0031
8	Totemoff Creek	T	27	5	5	1	0	1	1	0	0.5177	0.0049
9	Erb Creek	T	31	5	3	0	1	0	0	0	0.3823	0.0034
10	Mink Creek	T	33	2	2	3	0	0	0	0	0.3127	0.0027
11	Mink Creek	U	28	1	3	6	0	1	0	1	0.4861	0.0047
12	Swanson Creek	T	36	4	0	0	0	0	0	0	0.1823	0.0015
13	Coghill River	T	30	4	3	2	0	1	0	0	0.4241	0.0038
14	Jonah Creek	T	35	2	2	1	0	0	0	0	0.2316	0.0020
15	Solomon Gulch	H	27	5	7	1	0	0	0	0	0.5038	0.0047
16	Duck River	T	31	4	4	0	1	0	0	0	0.3835	0.0034
17	Millard Creek	T	29	7	3	0	1	0	0	0	0.4430	0.0039
18	Lagoon Creek	T	33	4	3	0	0	0	0	0	0.3076	0.0027
19	Lagoon Creek	U	26	2	12	0	0	0	0	0	0.4911	0.0045
20	Olsen Creek	T	29	5	4	1	0	1	0	0	0.4532	0.0041
21	Olsen Creek	U	29	6	5	0	0	0	0	0	0.4418	0.0040
22	Koppen Creek	T	29	8	3	0	0	0	0	0	0.4342	0.0038
23	Koppen Creek	U	35	0	1	2	1	1	0	0	0.2329	0.0020
24	Humpback Creek	T	29	6	5	0	0	0	0	0	0.4418	0.0040
25	Hartney Creek	T	37	1	1	0	1	0	0	0	0.1443	0.0012
26	Constantine Creek	T	33	5	1	1	0	0	0	0	0.3063	0.0026
27	Constantine Creek	U	35	4	0	1	0	0	0	0	0.2266	0.0018

Table 10. Analysis of geographic patterns of heterogeneity in mtDNA haplotypes. A total of 10,000 Monte Carlo simulations were performed to compute the probabilities of exceeding the original χ^2 by chance alone.

Region		Test	χ^2	*P*
WILD				
TIDAL				
Within Region				
	Southwest	5	38.10	0.367
	North	5	11.01	0.560
	East	5	27.08	0.680
Among Regions		5	23.88	0.420
All tidal		3	127.23	0.166
UPSTREAM				
Within Region				
	East	4	26.73	<0.001*
Among Region		4	27.79	0.004*
All Upstream		3	57.58	<0.001*
ALL WILD		2	233.70	<0.001*
HATCHERY		2	4.31	0.317
TOTAL PWS		1	251.25	<0.001*

* Significant at experimentwise $\alpha = 0.05$ (Rice 1989)

Table 11. Hierarchical analysis of molecular variation (AMOVA) observed in Prince William Sound pink salmon collections from 1994.

a. Elevation

Variance Component	Observed Partition		P^a	Φ-statistic
	Variance	% Total		
Among elevation	0.001	0.55	0.119	$\Phi_{CT} = 0.006$
Among collections within elevation	0.002	1.07	0.008	$\Phi_{SC} = 0.011$
Within collections	0.209	98.38	0.007	$\Phi_{ST} = 0.016$

b. Region

Variance Component	Observed Partition		P^a	Φ-statistic
	Variance	% Total		
Among regions	0.001	0.55	0.084	$\Phi_{CT} = 0.005$
Among collections within regions	0.002	0.90	0.044	$\Phi_{SC} = 0.009$
Within collections	0.209	98.55	0.003	$\Phi_{ST} = 0.014$

[a] Probability of having a more extreme variance component than the observed value by chance alone.

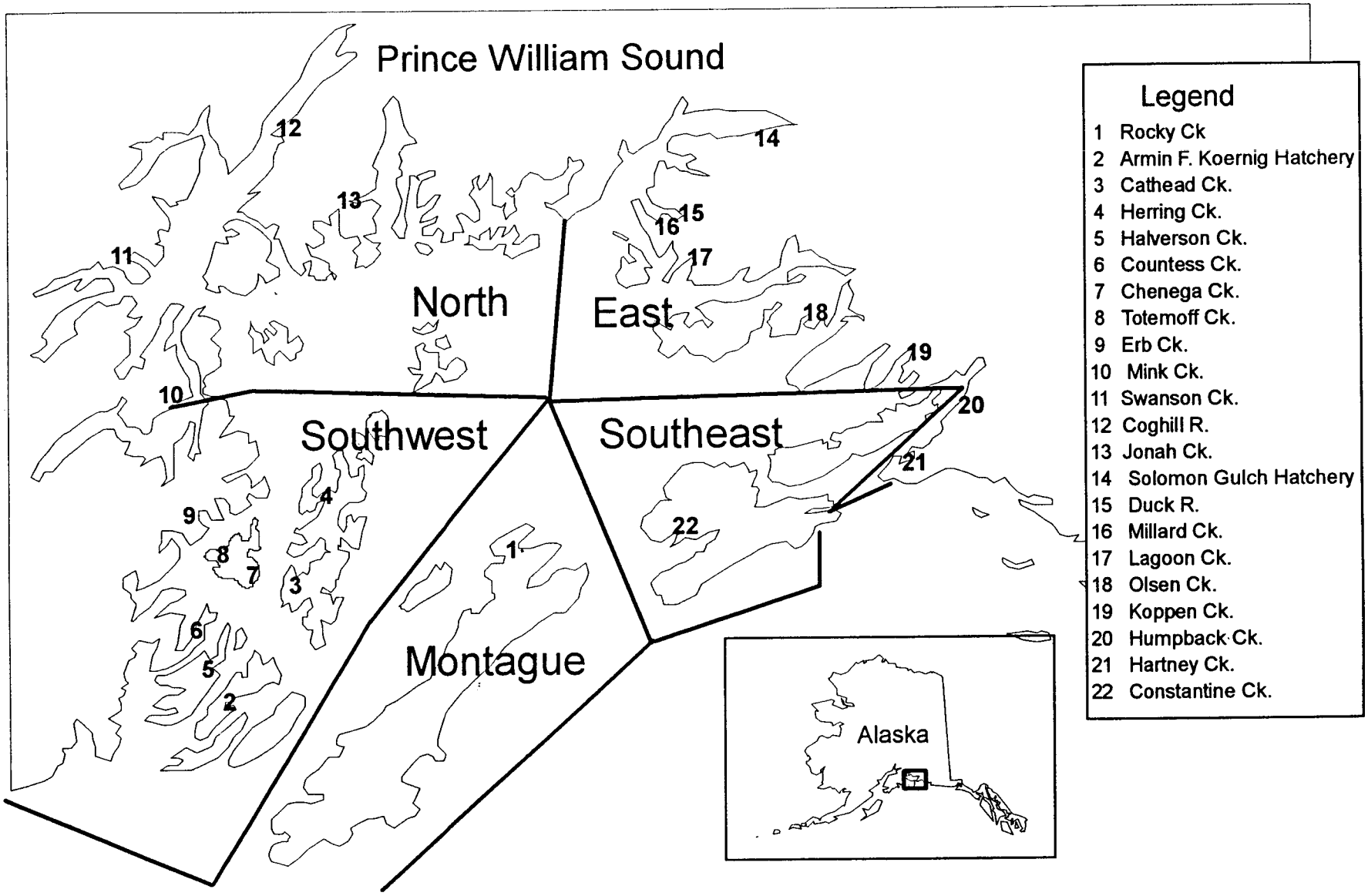

Figure 1. Location of sample collection sites within the major managment regions of Prince William Sound.

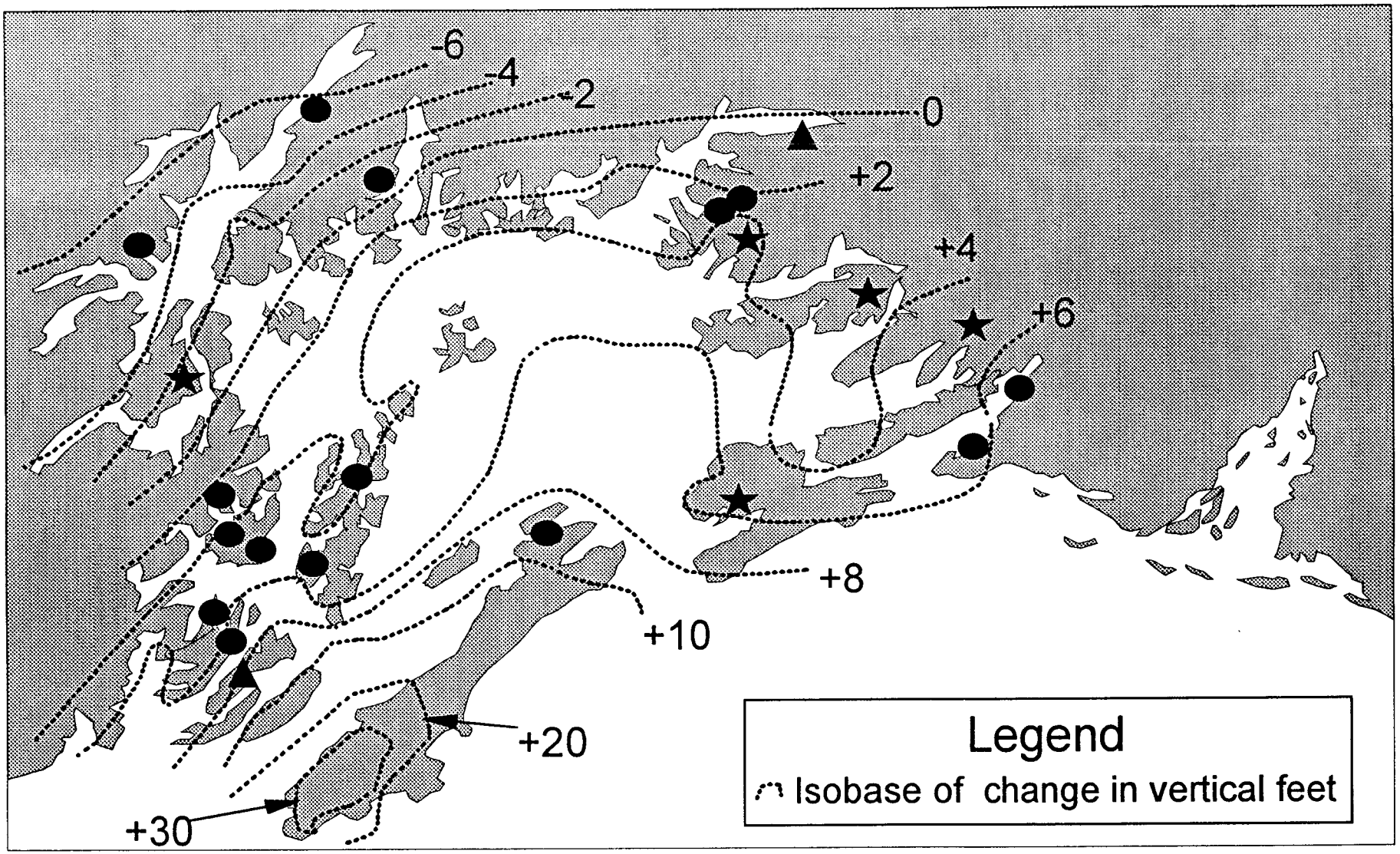

Figure 2. Shift in elevation following the 1964 earthquake in Prince William Sound. Collections with both upstream and tidal samples are indicated with stars, tidal collections are indicated with circles, and hatchery collections are indicated with triangles. Adapted from Plafker and Mayo (1965).

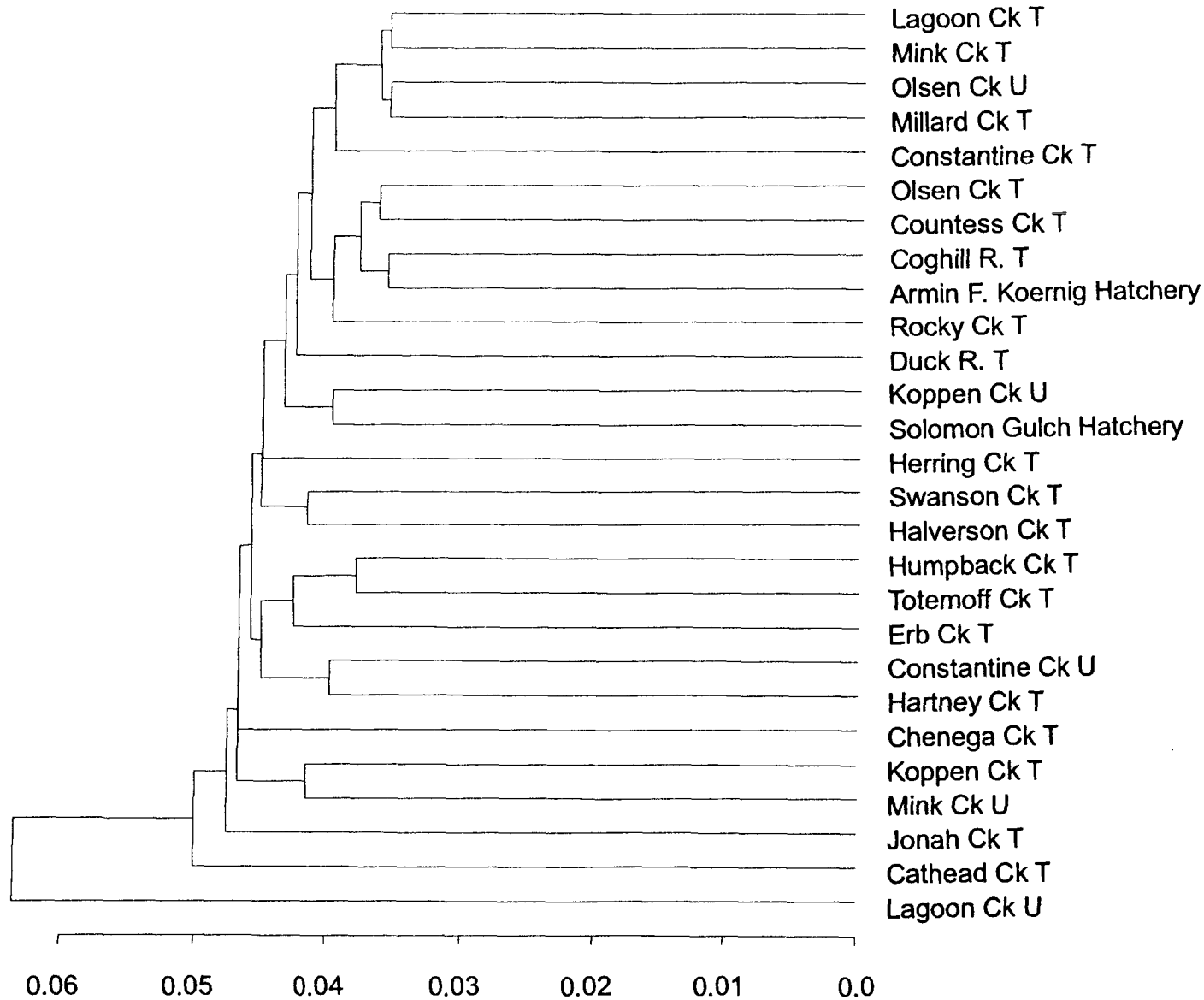

Figure 3. Tree constructed using unweighted pair-group method (UPGMA; Sneath and Sokal 1973) with Cavalli-Sforza and Edwards (1967) chord distances derrived from allozyme variation in pink salmon collections made in Prince William Sound, AK in 1994.

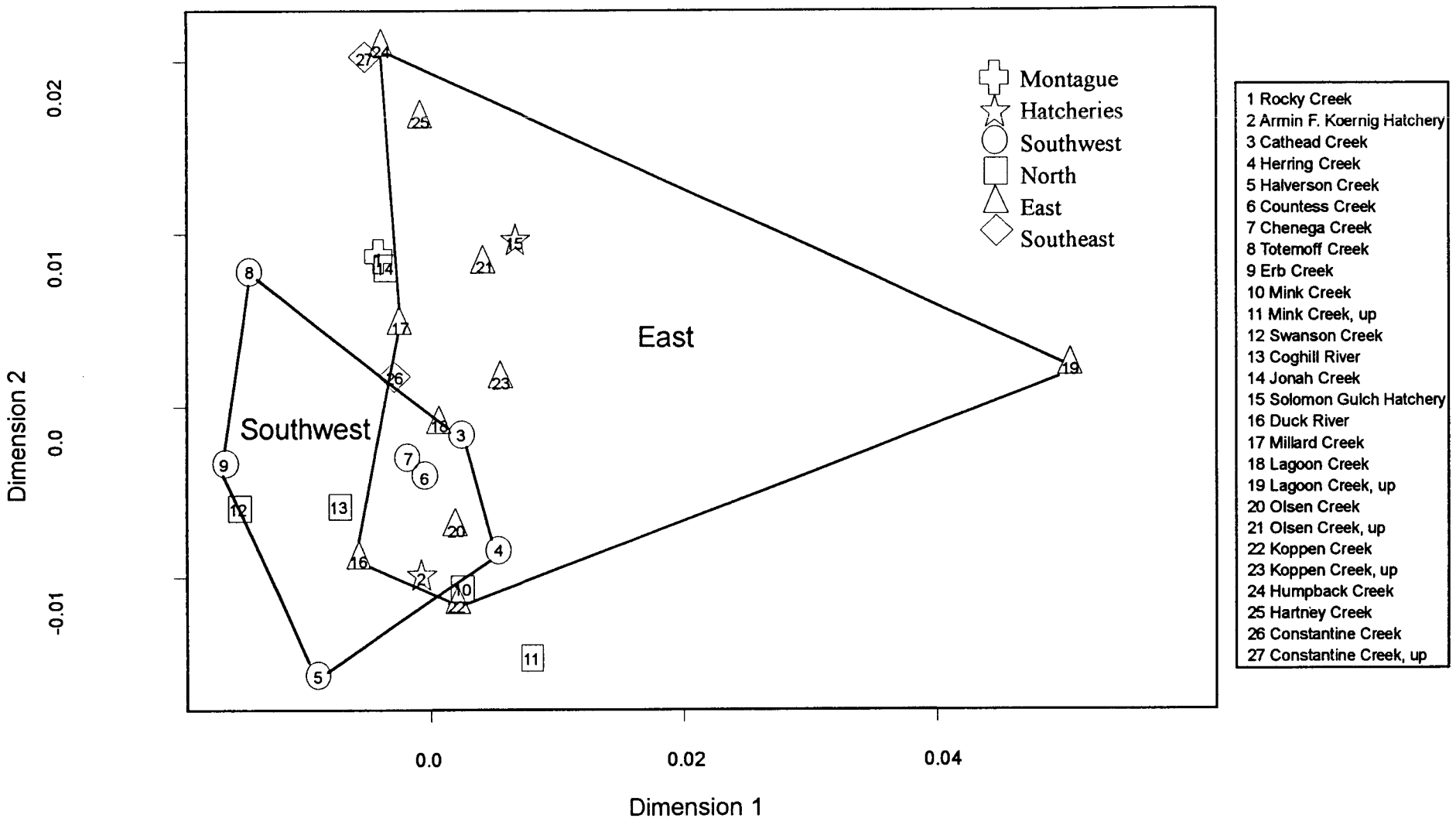

Figure 4. Multidimensional scaling analysis. Cavalli-Sforza and Edwards chord distances, calculated from 38 allozyme loci, were used. Polygons including all Southwest and East collections are superimposed.

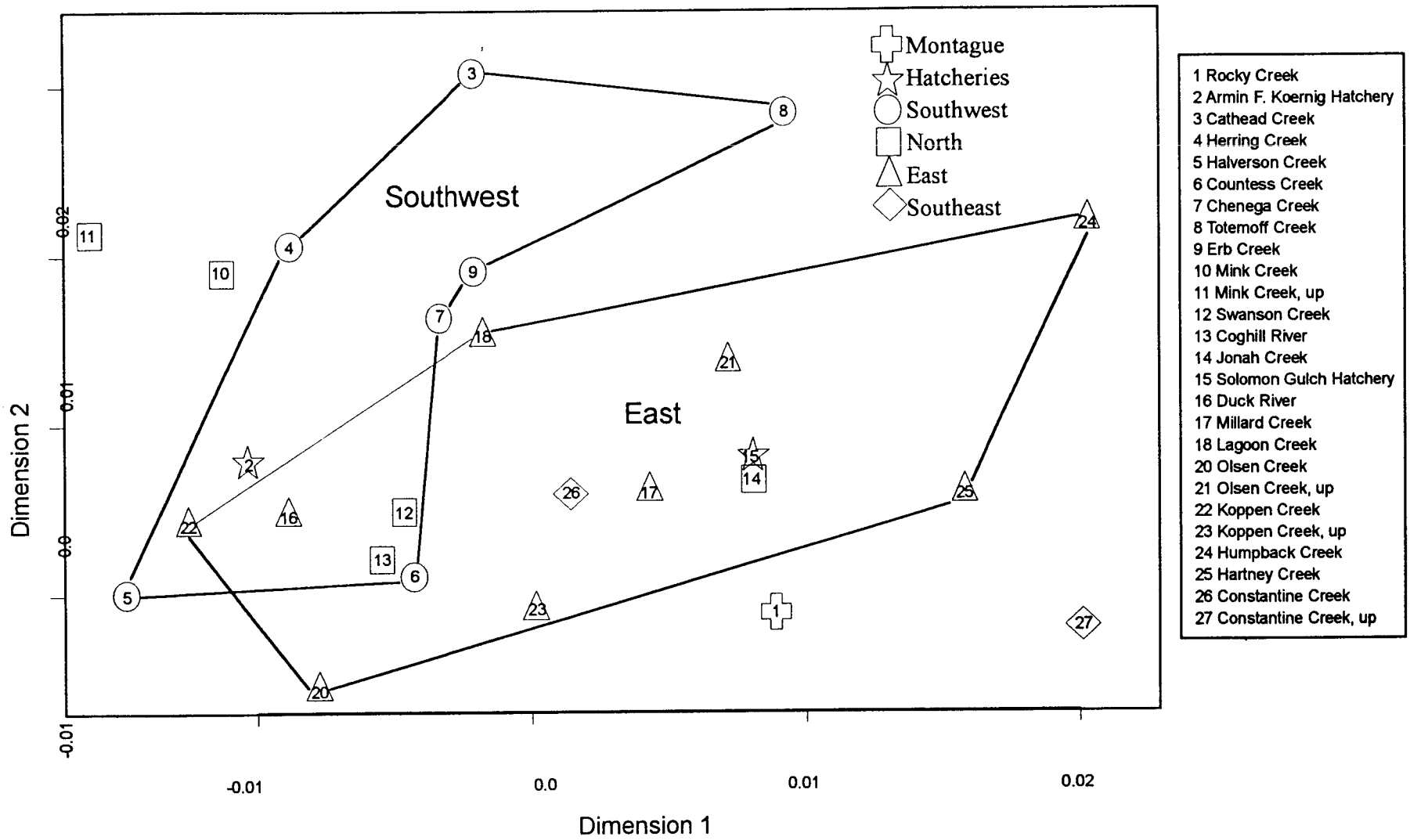

Figure 5. Multidimensional scaling analysis. The upstream collection from Lagoon Creek was excluded to clarify relationships among remaining collections. Cavalli-Sforza and Edwards chord distances, calculated from 38 allozyme loci, were used. Polygons including all Southwest and East collections are superimposed.

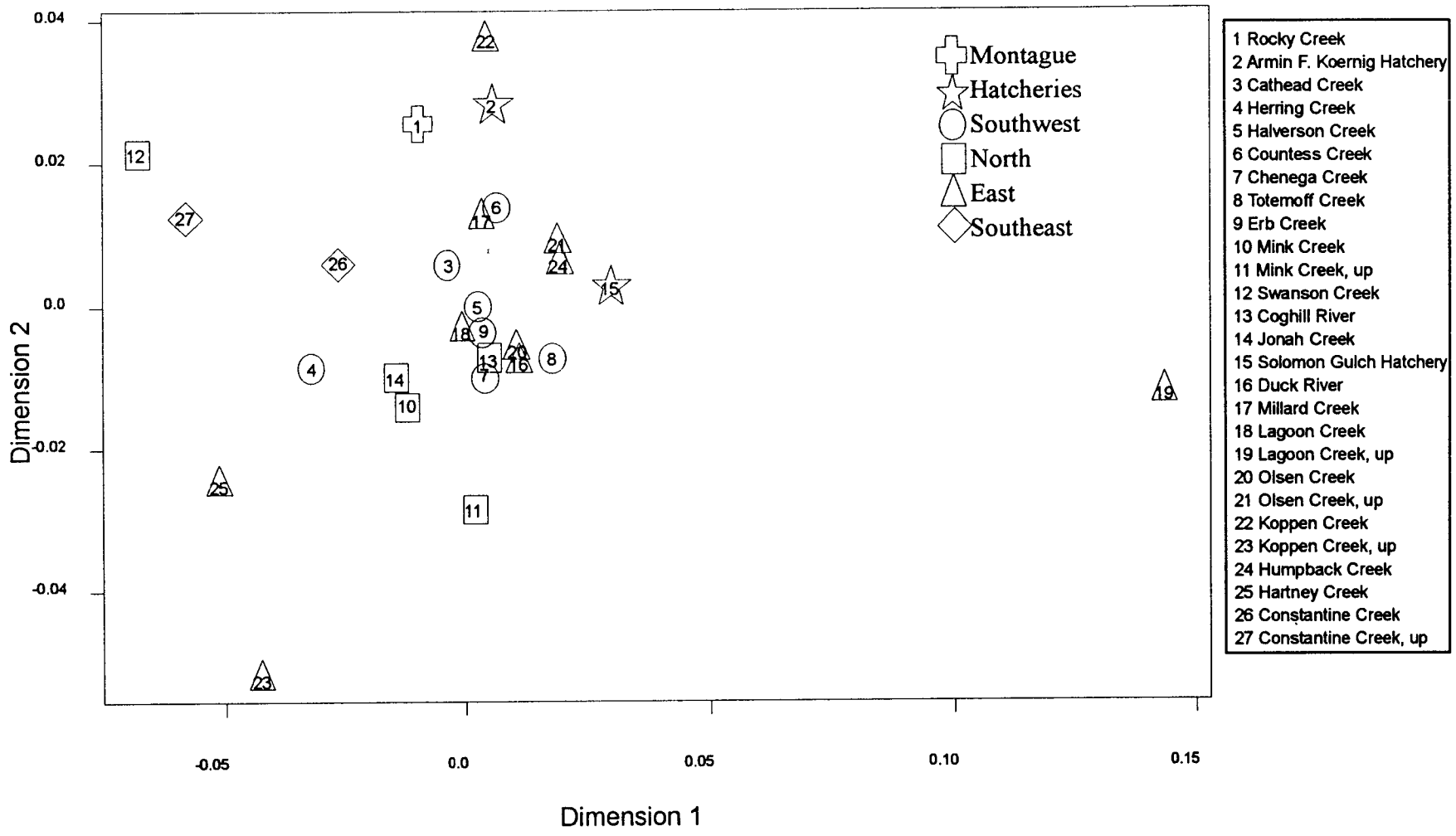

Figure 6. Multidimensional scaling analysis generataed from Φst distances calculated from mtDNA data.

Appendix A. Variation at ND5/ND6 discriminates even- and odd-year pink salmon (*Oncorhynchus gorbuscha*) populations from Alaska.

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J. W. Fetzner Jr., L. W. Seeb, and J. E. Seeb[‡]

To be submitted to Molecular Ecology

Running Title: mtDNA variation in pink salmon

Keywords: Mitochondrial DNA, *Oncorhynchus gorbuscha*, PCR-RFLPs, pink salmon, *Exxon Valdez*.

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Abstract--Mitochondrial NADH dehydrogenase subunits 5 and 6 regions were amplified from 160 odd-year and 204 even-year pink salmon (*Oncorhynchus gorbuscha*) from Alaska using the polymerase chain reaction (PCR). Samples included in the analyses were from Norton Sound, Kachemak Bay, Prince William Sound, and Southeast Alaska. Restriction fragment length polymorphism (RFLP) analyses revealed this region of mtDNA to be variable in pink salmon. Haplotype diversity values within populations were relatively high and ranged from 0.097 to 0.636 (mean = 0.466). The most common haplotype (I) was found in all populations examined but was present at a higher frequency in the even-year samples than in the odd-year samples. Overall, haplotype I was detected in 69% of sampled individuals. The frequency of the twelve composite haplotypes were found to be geographically informative across regions, and the frequencies also varied greatly among populations within regions as well as between the even- and odd-year collections.

Introduction

The pink salmon (*Oncorhynchus gorbuscha*) is the most abundant North American species of Pacific salmon (Neave 1967; Heard 1991), thus making it an economic and ecological cornerstone in biological communities of the Pacific Rim. Pink salmon are both anadromous and semelparous: in their natural range, they make long oceanic migrations, home to their natal stream to spawn, and die at age two. Commercial catches of pink salmon exceeded 100 million fish annually in Alaska during this decade. Pink salmon are an important food source for many marine and terrestrial species, and their spawning migration provides a pathway for transferring nutrients from marine ecosystems to nearshore and terrestrial ecosystems.

Pink salmon are unique in the family Salmonidae, having the fixed two-year life span which produces two reproductively isolated lineages in the non-overlapping even- and odd-year classes (Davidson 1934). Their range extends from Puget Sound, Washington, north to the Mackenzie River, which flows into the Beaufort Sea (modified from Heard 1991). Southern populations are primarily limited to odd-years, while even-year populations are common in more northerly rivers where odd-year populations are absent or in low numbers. Rivers in the center of the range are characterized by abundant populations in both even- and odd-years. Previous studies show that differences occur in allozyme frequencies between the even- and odd-year lineages inhabiting the same river (Aspinwall 1974; Utter et al. 1980; Beacham et al. 1988) as well as in morphological and life history characters (Beacham et al. 1988).

Prince William Sound, Alaska, site of the 1989 *Exxon Valdez* oil spill, is approximately the center of the North American range of the pink salmon. These populations were impacted by the *Exxon Valdez* oil spill of 1989, and the effects appear to be rippling through both the even- and odd-year lineages (Bue et al. 1996). In addition to effects of the oil spill, the Prince William Sound populations are subjected to intense pressures from both harvest and adverse interactions with hatchery fish (an average of over 600 million hatchery fry have been released annually from Prince William Sound hatcheries since 1985). For these and other reasons the aggregate census fluctuated an order of magnitude from 1.8 million to

21.0 million annually during recent decades. However, we know little about the genetic structure of these native populations, and a better understanding of the genetic structure of wild stocks inhabiting Prince William Sound is critical to their long-term management and conservation.

Previous studies of the genetic diversity of pink salmon populations rely almost solely on data collected from allozyme electrophoresis (e.g., Aspinwall 1974; Beacham et al. 1988; Gharrett et al. 1988; Shaklee et al. 1991; Varnavskaya and Beacham 1992; Shaklee and Varnavskaya 1994). Results show that pink salmon possess comparatively high allozyme diversity (proportion of polymorphic loci often $\geq$ 33%, heterozygosity $\geq$ 10%), but diversity is not always apparent among geographically adjacent collections (e.g., Gharrett et al. 1988). If heterogeneity is apparent among populations, it may not be partitioned along obvious geographical boundaries (Shaklee and Varnavskaya 1994). Therefore, Zhivotovsky et al. (1994) suggest that the population structure of pink salmon would best be studied by using complementary data sets gathered from more than one technique.

Variation in mitochondrial DNA (mtDNA) haplotypes may offer an additional opportunity for characterizing the structure of salmonid populations. The analysis of restriction fragment length polymorphisms (RFLPs) in mtDNA has sometimes shown resolving power complementary to that of allozyme electrophoresis for salmonids (e.g., Gyllensten and Wilson 1987; Birmingham 1990; Park et al. 1993; Adams et al. 1994; Bickham et al. 1995).

Haplotype diversity has yet to be examined among pink salmon populations. Our goal in this study is to document the levels of variability and diversity in mtDNA in Alaska pink salmon to provide a baseline for future comparative studies both within Alaska and throughout the species range. We hope to utilize markers identified in this study to help resolve the population structure of pink salmon from Prince William Sound affected by the *Exxon Valdez* oil spill.

We chose to examine the mitochondrial NADH dehydrogenase subunits 5 and 6 (ND5/ND6) in pink salmon using the polymerase chain reaction (PCR, Saiki et al. 1988) and utilizing restriction fragment length polymorphisms (RFLP). Previous examinations of many areas of the mitochondrial genome have shown the ND5/ND6 region to be variable in salmonids (Cronin et al. 1993; Park et al. 1993). We examined 14 even- and odd-year populations from across the species range in Alaska, including paired even- and odd- year collections from three streams in Prince William Sound. This allowed for comparisons between years, as well as comparisons within years across Alaska and within Prince William Sound.

Materials and Methods

Samples

We sampled three paired even- and odd-year populations in Prince William Sound and seven additional populations ranging from the northern Bering Sea to the eastern Gulf of Alaska (Figure 1). All tissues were collected on liquid nitrogen or dry ice and stored at -80°C

until analysis. In total, 364 pink salmon specimens were analyzed representing both odd- and even-year populations from: Norton Sound, Kachemak Bay, Prince William Sound, and Southeast Alaska (Table 1).

Total genomic DNA was extracted from 100 mg of liver or muscle tissue. Standard protocols of Proteinase-K and RNase-A digestion, phenol-chloroform extraction, and ethanol precipitation were used (Sambrook et al. 1989). After isolation, the DNA was resuspended in TE buffer (10mM Tris, 0.1 mM EDTA), quantitated by spectrophotometry, and diluted to 50 ng/μl for use in the polymerase chain reaction (PCR, Saiki et al. 1988).

Polymerase Chain Reaction

The entire *ND5/ND6* region of the mitochondrial genome was amplified using PCR and was conducted in 100μl volumes which contained 4mM $MgCl_2$, 1.0μM of each primer, 200μM each dNTP, 2.5U of *Taq* DNA polymerase (Perkin-Elmer Cetus) and 20-50*ng* of sample DNA. The reactions were initially denatured at 94°C for 2.5 minutes followed by amplification using 40 cycles consisting of strand denaturation (94°C, 40 seconds), primer annealing (55°C, 1 minute) and polymerase mediated primer extension (72°C, 3.5 minutes). A final extension of 7 minutes at 72°C was included to minimize partial extension products. Primers used in the reaction were those of Cronin et al. (1993; Table 2). The L and H designations were added and refer to the light and heavy strands of the mtDNA molecule, respectively. These primers are located in the tRNA genes flanking the *ND5/ND6* region. The LND5/ND6 primer is in the $tRNA^{LEU}$ gene and the 3' base is 12896 relative to the rainbow trout (*O. mykiss*) sequence (Zardoya et al. 1994, unpublished). The HND5/ND6 primer is in the $tRNA^{GLU}$ gene and the 3' base is 15337 relative to the rainbow trout sequence.

Restriction Digests and Analyses

An initial screen for variation in the *ND5/ND6* region of mtDNA was conducted on twenty individuals from 11 pink salmon collections using 16 restriction endonucleases. Following amplification, the DNA samples were digested directly with the restriction endonucleases which recognized both four (*BstU I, Dpn II, Hae III, Hinf I*, and *Rsa I*) and six (*Apa I, BamH I, Bcl I, BstE II, EcoR I, EcoR V, Hind III, Kpn I, Pst I, Stu I*, and *Xba I*) base sequences. Each digest was conducted in a total volume of 20μl with 2.5 units of enzyme and then incubated according to the manufacturer's specifications. After incubation, samples were loaded into a 0.8% agarose gel and run for approximately 1.5 hours. A pGEM molecular weight standard (Promega, #G-1741) was run on each gel in order to estimate fragment lengths. Following electrophoresis, gels were stained with ethidium bromide, photographed under ultraviolet light (312*nm*), and scored based on the fragment patterns detected for each individual. Haplotypes were assigned an alphabetic character according to their order of occurrence, with "A" being the most common haplotype detected.

Unbiased estimates of haplotype diversity (h, Nei and Tajima 1981) and nucleotide diversity (π) were estimated to examine the amount of mtDNA variation within populations of pink salmon by using the restriction enzyme analysis package (REAP) of McElroy et al.

(1992). Where the collections allowed, we tested for population subdivision at several hierarchical levels by using Monte Carlo simulations (Rolf and Bentzen 1989). The hierarchical levels we examined were 1) among populations within regions, 2) among regions (Norton Sound, Kachemak Bay, Prince William Sound, Southeast Alaska), and 3) among the even- and odd-year classes. We also performed an analysis of molecular variance (AMOVA) with Φ-statistics (Excoffier et al 1992). Transformed Φ-distances between pairs of populations were used to generated a UPGMA phenogram depicting relationships among populations with the PHYLIP package (Felsenstein 1993). A network connecting the different haplotypes was constructed using site data and the maximum-likelihood RESTML program of PHYLIP.

Sequence Analysis

We sequenced the entire *ND5/ND6* region from a single pink salmon to verify restriction sites detected in the RFLP segment of this study. The amplified *ND5/ND6* PCR products were separated by agarose gel electrophoresis, and the band of interest was excised from the gel. The product was purified from the agarose gel slices using the QIAquick gel extraction kit (Qiagen; Chatsworth, CA) and then further purified and concentrated with Microcon-100 spin columns (Amicon; Beverly, MA). Final concentrations of samples were 30 ng/μl. Sequencing reactions were conducted in 20μl volumes using the Applied Biosystems (ABI) Prism Dye-deoxy terminator kit with AmpliTaq FS. Sequencing reactions contained 3.2 pmol of primer and 225ng of purified template DNA. Primers for sequencing included those listed in Table 1. Samples were run for 14 hours on an ABI 373A automated DNA sequencer using 6% polyacrylamide (8.3M urea) gels. Samples were aligned to the known rainbow trout sequence obtained from Genbank (accession L29771) using the Sequence Navigator program supplied with the ABI sequencer.

Results

Restriction Enzyme Variation

Of the 16 enzymes examined in the initial survey, six (*Apa I, BstU I, EcoR V, Hinf I, Rsa I,* and *Xba I*) were found to be polymorphic (Table 3), three (*BamH I, Kpn I, and Pst I*) failed to recognize any sites within the region, and the remaining seven (*Bcl I, BstE II, Dpn II, EcoR I, Hae III, Hind III, and Stu I*) produced identical fragment patterns in all populations. A total of twelve different composite haplotypes were detected in the *ND5/ND6* region of pink salmon (Table 4). After the initial survey, all remaining populations (Table 2, Figure 1) were examined with the polymorphic enzymes detected in the screening process. The number of sites recognized by any one enzyme ranged from zero to seven. Of the 12 composite haplotypes detected in this study, four were produced by variants at *Hinf I* sites, with all but one of these being detected at low frequencies (≤ 0.05) within populations.

Odd-year Populations

The haplotype diversity (h) values within the eight odd-year populations ranged from 0.385 to 0.636 (mean = 0.524), and nucleotide diversity (π) ranged from 0.015 to 0.022 (mean = 0.019; Table 4).

For the odd-year populations, haplotypes I and II were the most prevalent, with overall frequencies of 0.544 and 0.400, respectively. In half of the odd-year populations examined the frequency of haplotype II surpassed that of haplotype I. Seven additional haplotypes (IV, V, VIII, IX, XI, XII, and XIII) were also detected in the odd-year populations but were at very low frequencies ($\leq$0.025, Table 4). For odd-year populations, haplotypes VIII and IX were found only in Prince William Sound and Kachemak Bay while haplotypes V, XI, XII, and XIII were detected only in populations from Southeast Alaska. However, samples sizes for all these collections were small (N=20), so the possibility that these haplotypes occur throughout Alaska cannot be excluded.

Overall, the odd-year populations had rather heterogeneous composite haplotype frequencies. We were able to detect geographic heterogeneity among the regions examined based on Monte Carlo simulations (χ^2=89.34, P<0.001; Table 5). Within regions, a significant difference was detected between the Norton Sound samples, Nome and Snake Rivers (χ^2=3.75, P=0.023), but no significant differences were detected among populations of Prince William Sound or Southeast Alaska (Table 5).

Even-year Populations

Haplotype diversity values for the even-year populations ranged from 0.097 to 0.442 (mean = 0.312) and nucleotide diversity ranged from 0.003 to 0.013 (mean = 0.010; Table 4). The even-year populations overall contained a higher frequency (0.799) of haplotype I while haplotype II was detected at a much lower frequency (0.088) when compared to odd-years (0.400). Frequency of haplotype II did not exceed 0.150 in any even-year population examined. Five additional haplotypes (III, IV, V, X, and XIV) were detected among the even-year populations but most were found at relatively low frequencies (see Table 4). Three of these (III, X and XIV) were unique to the even-year populations. Among all populations (odd- and even-years) examined, haplotype III was the only haplotype besides I and II which was detected at a relatively high frequency (>0.05) within any one population.

Significant heterogeneity in haplotype frequencies was detected among regions (χ^2= 26.18, P=0.0002; Table 5) for the even-year populations based on Monte Carlo simulations. Tests within Norton Sound and Prince William Sound were possible; no differences in haplotype frequencies were detected among populations within either region. Frequencies of composite haplotypes were also found to be temporally stable among our two even-year Duck River samples (χ^2= 1.69, P=0.6653; Table 5).

Comparison between even- and odd-years

Values for both haplotype and nucleotide diversity were significantly smaller (Wilcoxon rank-sum test [Wilcoxon 1945], P=0.0027 and P=0.0007, respectively) in even-year populations than in odd-year populations, indicating the even-year populations are less diverse in mtDNA variation. Significant heterogeneity in haplotype frequencies was detected in pooled even- versus pooled odd-year comparisons (χ^2=72.52, P=0.0000). We found that 22.4% of the variance (Φ_{ST}=0.224) detected was attributable to between year class differences based on AMOVA analyses. The phenogram (Figure 2) shows that the even-year and odd-year populations form distinct groups. In addition, some geographic clustering is seen among the even-year populations, with branches separating Prince William Sound from the Norton Sound populations. However, this type of pattern was not seen for the odd-year populations which formed two relatively divergent groups, neither of which showed geographic affinities. We constructed a phylogenetic network connecting the different haplotypes based on the restriction site differences detected. All haplotypes detected in this study could be unambiguously assigned to the network, and the differences between haplotypes could be inferred by single restriction site differences (Figure 3a). Differences in haplotype distribution between the even- and odd-year collections are apparent (Figures 3b and 3c). The even-year collections had a very high frequency of haplotype I (0.799), and a low frequency of haplotype II (0.088). It is interesting to note that all the other haplotypes detected among even-year collections are derivatives from haplotype I (Figure 3b). In contrast, we found that haplotypes from the odd-year collections, which had a higher frequency of haplotype II (0.400) and a lower frequency of haplotype I (0.543), were predominantly derived from haplotype II (Figure 3c).

Sequence Analysis

The entire sequence from a single individual exhibiting haplotype VII was obtained (Figure 4) and compared to the known rainbow trout sequence. The sequence divergence between the pink salmon and rainbow trout was 9.8% for *ND5* and 14.8% for *ND6*. The higher value of sequence divergence for the *ND6* gene was due to an 18 bp deletion in pink salmon (starting at position 2105) when compared to the rainbow trout sequence.

Discussion

Intraspecific mtDNA Variability

At the population level (within regions) composite haplotype frequencies varied among the streams examined but was only significantly different in the Norton Sound odd-year populations. Odd-year runs in this region are relatively small, especially when compared to even-year runs, and can fluctuate widely from year to year. In this type of situation, bottlenecks that increase genetic drift can cause rapid population differentiation. This heterogeneity among odd-year Norton Sound streams would seem to suggest that straying is limited among these pink salmon populations or at least below the level necessary to counteract the effects of drift. If this were not the case, one would expect to find

homogeneous haplotype frequencies among streams, especially in intertidal areas, where most straying is thought to occur. It would be interesting to examine if even-year populations from areas where their runs sizes are low (i.e., Washington and southern British Columbia) show this high level of differentiation in mtDNA haplotype frequencies.

Both the odd- and even-year populations showed some level of convergence in composite haplotype frequencies, but significant differences were still detected among regions for both year classes. Haplotype composition also differed among regions suggesting that several haplotypes are unique to a particular region or a particular year class. However, larger sample sizes are necessary to verify with a high level of certainty that the haplotypes are indeed absent.

We were able to detect significant differences between even- and odd-year populations in both haplotype and nucleotide diversity, with values for odd-year populations almost double those estimated for even-year populations. This result is consistent with previous allozyme studies, which found odd-year populations to be more diverse than those in even-years (Gharrett et al. 1990; Shaklee et al. 1991). In addition, we found that the haplotype relationships between even- and odd-year pink salmon to be divergent, with even-year populations having haplotypes derived exclusively from haplotype I while for odd-year populations the majority of haplotypes were derived from haplotype II (Figure 3).

The AMOVA analysis indicated that 22.4% of the variation detected could be attributed to between year-class differences. Values of G_{ST} between year classes, though not directly comparable to Φ_{ST}, have been reported for allozyme data and are quite variable, ranging from estimates of 2.7% - 14.9% (Zhivotovsky et al. 1994; McGregor 1983). The differentiation among year classes is thought to be caused by their temporal separation into independent glacial refugia during the Pleistocene (Aspinwall 1974) and subsequent reproductive isolation. The even-year class is thought to have survived in the Bering Refugia, while the odd-year class may have survived in a southern refuge, possibly in the Columbia River drainage (Withler and Morley 1982). The differences we detected in haplotype affinities would seem to support this hypothesis (Figure 3) and suggest that some lineage sorting has occurred between the even- and odd-year classes.

We were also able to compare sequence obtained from haplotype IX to that of haplotype VII and rainbow trout. Although this second pink salmon haplotype was not observed in any individuals from this study, it did originate from Prince William Sound (Seeb et al. in prep.). We calculated percent sequence divergence between the two haplotypes (*Hinf I* variants); the sequence divergence was estimated to be 1.5%. This comparison suggests that additional variation is present in the ND5/ND6 region of pink salmon and could be helpful in identifying additional restriction enzymes for future RFLP studies conducted on pink salmon. The value obtained for the comparison between pink salmon and rainbow trout sequences was 9.8% for *ND5* and 14.8% for *ND6*, which is akin to results obtained by other researchers that have examined mtDNA diversity in salmonids (Domanico and Phillips 1995; McVeigh and Davidson 1991; Shedlock et al. 1992).

Interspecific mtDNA Variability

Previous studies of mtDNA variation in other salmonid species (Parks et al. 1993; Cronin et al. 1993) have found the *ND5/ND6* region to possess somewhat limited amounts of variation, but more so than other areas of the mtDNA molecule. The current study, however, has detected a considerable amount of sequence divergence (p) in the *ND5/ND6* region in pink salmon (p=0.015 between two distinct haplotypes). Cronin et al. (1993) found sequence divergence to be low among chinook (*O. tshawytscha*) and chum (*O. keta*) salmon populations (p=0.0003-0.0044 and 0.0006-0.0062, respectively), and Parks et al. (1993) suggested a recent bottleneck event as a most likely reason for less variability in mtDNA of chum salmon.

The apparent higher levels of genetic variability observed in pink salmon may be linked to their unique life history. Generational effects (i.e., the length of time between generations) have been suggested as a possible reason for genetic variability among taxa (Sibley et al. 1988; Li et al. 1987). It has been noted that organisms with shorter generation times accumulate mutations at a faster rate and usually have larger population sizes than taxa with longer generation times (Wilson et al. 1987). In comparing Pacific salmon species, pink salmon have the shortest generation time and are the most abundant of the five species (Heard 1991). While there is considerable variability in the ages of returning spawners (anywhere from two to seven years old in chinook salmon), the majority of spawners are three to five years old. This difference may explain the higher levels of diversity detected in pink salmon mtDNA.

mtDNA Variation as a Reflection of Population Structure

One of the objectives of this study was to develop mitochondrial markers to delineate population structure of pink salmon in Prince William Sound and potentially throughout the range of the species. Mitochondrial DNA, as compared to nuclear DNA, has the unique properties of being haploid and transmitted through maternal lines. As a result, the effective population size for mtDNA may be only one fourth that of nuclear genes (Birky et al. 1989), so that the effects of bottlenecks and genetic drift are more pronounced for mtDNA. Further, we may expect additional differentiation in mtDNA if male migration rates are greater than female rates.

Allozyme electrophoresis, reflecting variation in nuclear genes, has been used extensively to distinguish among Pacific salmonid populations (Allendorf et al., 1987). Allozymes sometimes do not discriminate among geographically proximal populations of pink salmon, but they routinely exhibit significant heterogeneity on a broader scale, thus allowing the discrimination of lineages important for management and conservation (Shaklee and Varnavskaya 1994).

Mitochondrial DNA also appears capable of distinguishing pink salmon populations from different regions and in some cases among populations within regions. This study provides only a framework for delineating population structure of pink salmon; additional data are needed before a comprehensive understanding of geographic variability in pink salmon can be obtained from mtDNA. Our data suggest that mtDNA techniques will compliment allozyme and other nuclear markers in discriminating among pink salmon populations, and the smaller effective population sizes in mtDNA as compared to nuclear markers may make it a

more sensitive to technique to reflect historical events of genetic drift.

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Table 1. Primers used in PCR amplifications of the *ND5/ND6* region for RFLP and sequencing analyses (as in text). Numbering system for primers designed in this study are according to the rainbow trout sequence.

Primer Name	Sequence	Reference
LND5/ND6	5'-AATAGTTTATCCRTTGGTCTTAGG-3'	Cronin et al. (1993)
HND5/ND6	5'-TTACAACGATGGTTTTTCATRTCA-3'	Cronin et al. (1993)
L13331	5'-CCTCCTCCTCTTCCTGATTGCCATAA-3'	this study
L13734	5'-GTGGCGGGCATCTTCCTATTAATTCG-3'	this study
L14130	5'-TAGCTGGGTTCTTCTCCAAAGACTC-3'	this study
L14532	5'-TACATAACTTCTCCAACATACTGGG-3'	this study
L14878	5'-AATTAACATTCCCCCTCCATGAGAG-3'	this study

Table 2. Populations, collection year, sample sizes, and regional location of specimens examined in this study.

Map #	Population	Year	N	Region
	Odd-Year			
1	Nome River	1991	20	Norton Sound
2	Snake River	1991	20	Norton Sound
4	Tutka Bay Hatchery	1993	20	Kachemak Bay
5	Swanson Creek	1991	20	Prince William Sound
6	Duck River	1991	20	Prince William Sound
7	Humpback Creek	1991	20	Prince William Sound
8	Gastineau Hatchery	1993	20	Southeast Alaska
9	Little Port Walter Hatchery[a]	1993	20	Southeast Alaska
	Even-Year			
1	Nome River	1994	20	Norton Sound
3	Solomon River	1994	20	Norton Sound
5	Swanson Creek	1994	40	Prince William Sound
6	Duck River	1992	44	Prince William Sound
6	Duck River	1994	40	Prince William Sound
7	Humpback Creek	1994	40	Prince William Sound

[a] Specimens were F1 progeny from 50 wild caught parents used in hatchery experiments.

Table 3. Pink salmon mtDNA restriction fragment sizes and haplotype patterns for polymorphic enzymes examined in this study. Relative frequencies of composite haplotypes within and among populations are given in Table 4.

Restriction Enzyme	Fragment r	size (bp)	Haplotype Pattern
Apa I	6	1240	A B - - -
		1117	A - - - -
		651	- B - - -
		466	- B - - -
BstU I	4	1576	A - - - -
		1200	- B - - -
		1150	- - C - -
		781	A B C - -
		500	- - C - -
		450	- B - - -
EcoR V	6	2375	A - - - -
		1466	- B - - -
		909	- B - - -
Hinf I	4	1057	- B D - -
		850	- - - E -
		803	A - - E -
		550	- - - - F
		500	A B - - F
		362	- - D - -
		351	A B D - F
		258	A B D E F
		239	A[a] B D E[a] F[b]
		90	- - D - -
Rsa I	4	1610	A - - - -
		1100	- B - - -
		405	- B - - -
		298	A B - - -
		274	A B - - -
		187	A B - - -
Xba I	6	2375	A - - - -
		1706	- B - - -
		669	- B - - -

[a]There are two fragments of the indicated size in these patterns.
[b]There are three fragments of the indicated size in these patterns.

Table 4. Distribution of composite haplotypes, haplotype diversity, and nucleotide diversity for the ND5/ND6 region of mtDNA for fourteen populations of Alaskan pink salmon (*Oncorhynchus gorbuscha*) examined in this study.

				Composite Haplotypes[a]											Diversity Values	
Population	Year	N	I	II	III	IV	V	VIII	IX	X	XI	XII	XIII	XIV	Haplotype	Nucleotide
Duck River	1991	20	8	11	-	-	-	1	-	-	-	-	-	-	0.5487	0.0200
Humpback Creek	1991	20	14	4	-	-	-	2	-	-	-	-	-	-	0.4718	0.0173
Swanson Creek	1991	20	9	10	-	1	-	-	-	-	-	-	-	-	0.5590	0.0194
Nome River	1991	20	15	5	-	-	-	-	-	-	-	-	-	-	0.3846	0.0146
Snake River	1991	20	9	11	-	-	-	-	-	-	-	-	-	-	0.5077	0.0193
Gastineau Hatchery	1993	20	7	10	-	-	1	-	-	-	1	-	1	-	0.6359	0.0224
Little Port Walter Hat.	1993	20	13	6	-	-	-	-	-	-	-	1	-	-	0.4974	0.0180
Tutka Bay Hatchery	1993	20	11	7	-	-	-	1	1	-	-	-	-	-	0.5846	0.0209
Odd Year Totals		**160**	**87**	**64**	**0**	**1**	**1**	**4**	**1**	**0**	**1**	**1**	**1**	**0**	**0.5237**	**0.0190**
Duck River	1992	44	33	4	7	-	-	-	-	-	-	-	-	-	0.4086	0.0117
Duck River	1994	40	31	4	4	-	1	-	-	-	-	-	-	-	0.3835	0.0120
Humpback Creek	1994	40	29	6	5	-	-	-	-	-	-	-	-	-	0.4418	0.0134
Swanson Creek	1994	40	36	4	-	-	-	-	-	-	-	-	-	-	0.1823	0.0069
Nome River	1994	20	19	-	-	-	-	-	-	1	-	-	-	-	0.0974	0.0025
Solomon River	1994	20	15	-	-	2	1	-	-	1	-	-	-	1	0.3590	0.0106
Even Year Totals		**204**	**163**	**18**	**16**	**2**	**2**	**0**	**0**	**2**	**0**	**0**	**0**	**1**	**0.3121**	**0.0095**
TOTALS		**364**	**250**	**82**	**16**	**3**	**3**	**4**	**1**	**2**	**1**	**1**	**1**	**1**		

[a]Haplotypes were determined from polymorphic enzymes (*Apa I, BstU I, EcoR V, Hinf I, Rsa I* and Xba I, respectively) and are as follows: I=AAAAAA, II=ACAAAA, III=AAABAA, IV=ABAAAA, V=AABAAA, VIII=BCAAAA, IX=AAADAA, X=AAAFAA, XI=ACAEAA, XII=ACAABA, XIII=ACABAA, and XIV=ABAAAB. Note: VI, and VII were detected in a separate study and thus are not presented here.

Table 5. Analysis of geographic patterns of heterogeneity in mtDNA haplotypes. A total of 10,000 Monte Carlo simulations were performed; probabilities of exceeding the original χ^2 by chance alone are given.

Comparisons	χ^2	P
Odd-Year Collections		
Among Regions	89.34	0.0000
Within Regions		
Norton Sound	3.75	0.0234
Prince William Sound	9.44	0.1070
Southeast Alaska	6.80	0.0984
Even-Year Collections		
Among Regions	26.18	0.0002
Within Regions		
Norton Sound	3.26	0.4818
Prince William Sound	10.75	0.2649
Duck River Between Years	1.69	0.6653
Even- vs. Odd-Years	72.52	0.0000
TOTAL	255.25	0.0000

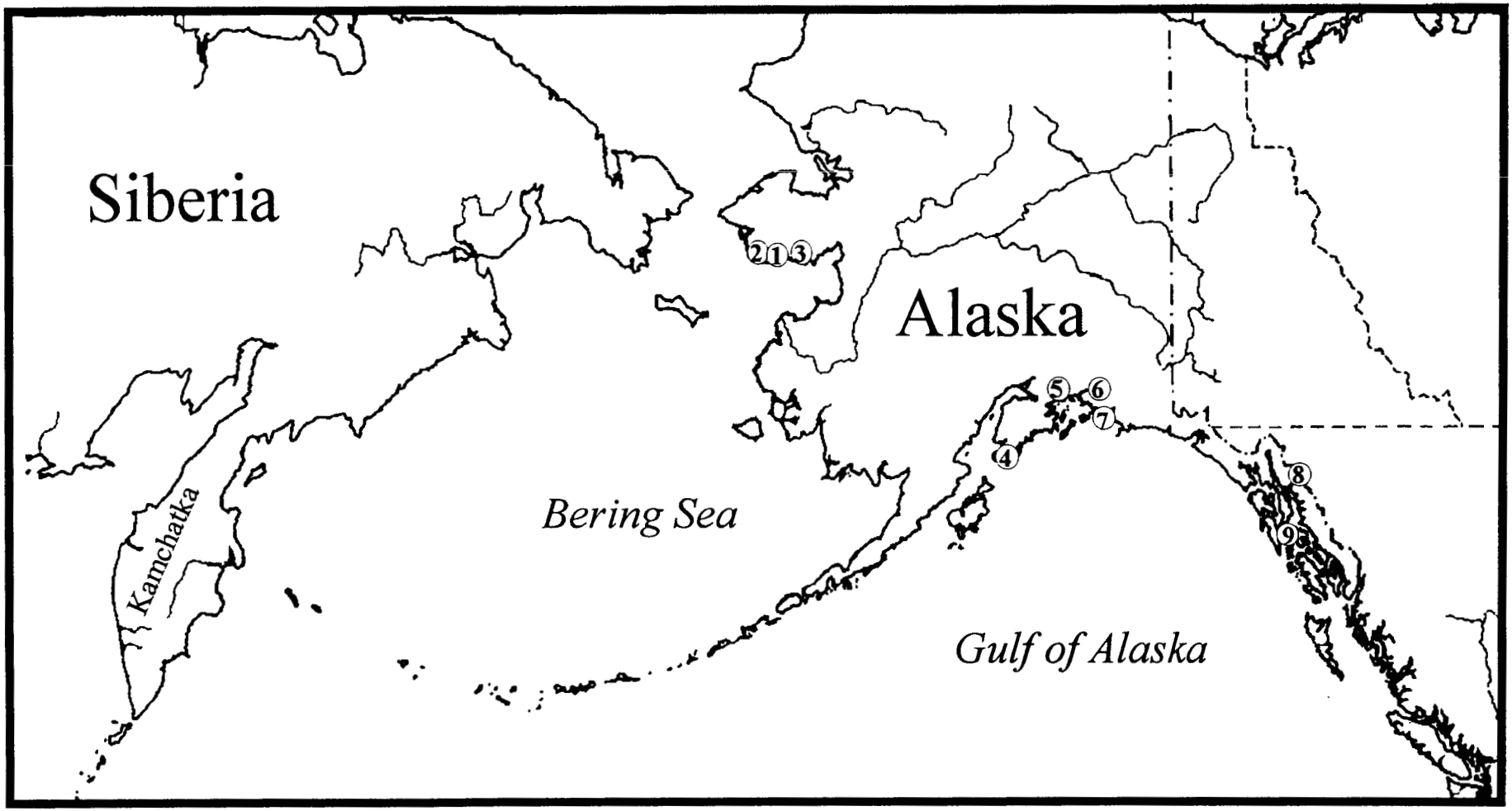
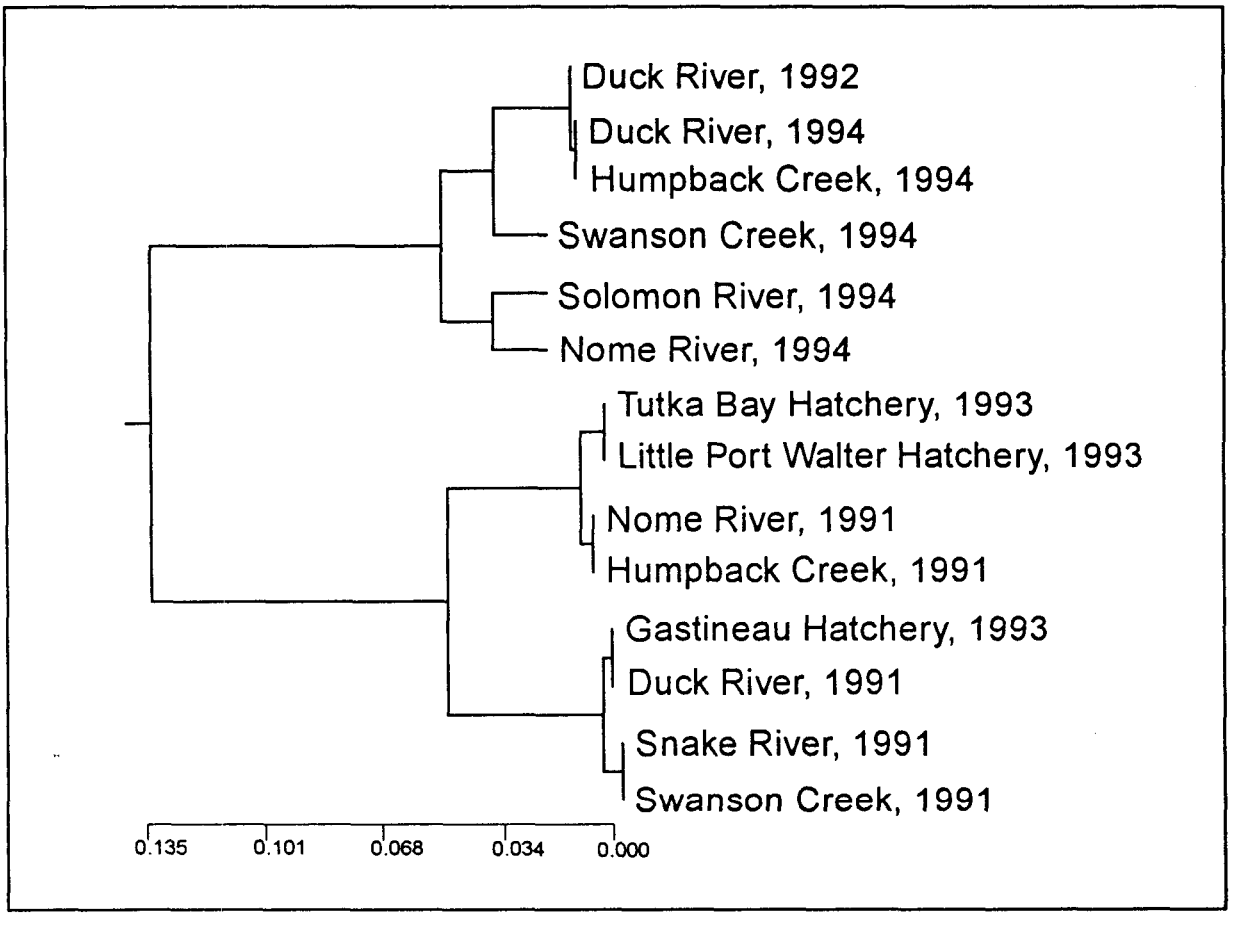


Figure 1. Collection localities of pink salmon populations used in this study. 1) Nome River, 2) Snake River, 3) Solomon River, 4) Tutka Bay Hatchery, 5) Swanson Creek, 6) Duck River, 7) Humpback Creek, 8) Gastineau Hatchery, 9) Little Port Walter Hatchery.

Figure 2. UPGMA phenogram generated from nonlinearly transformed Φ_{ST} distances among populations.



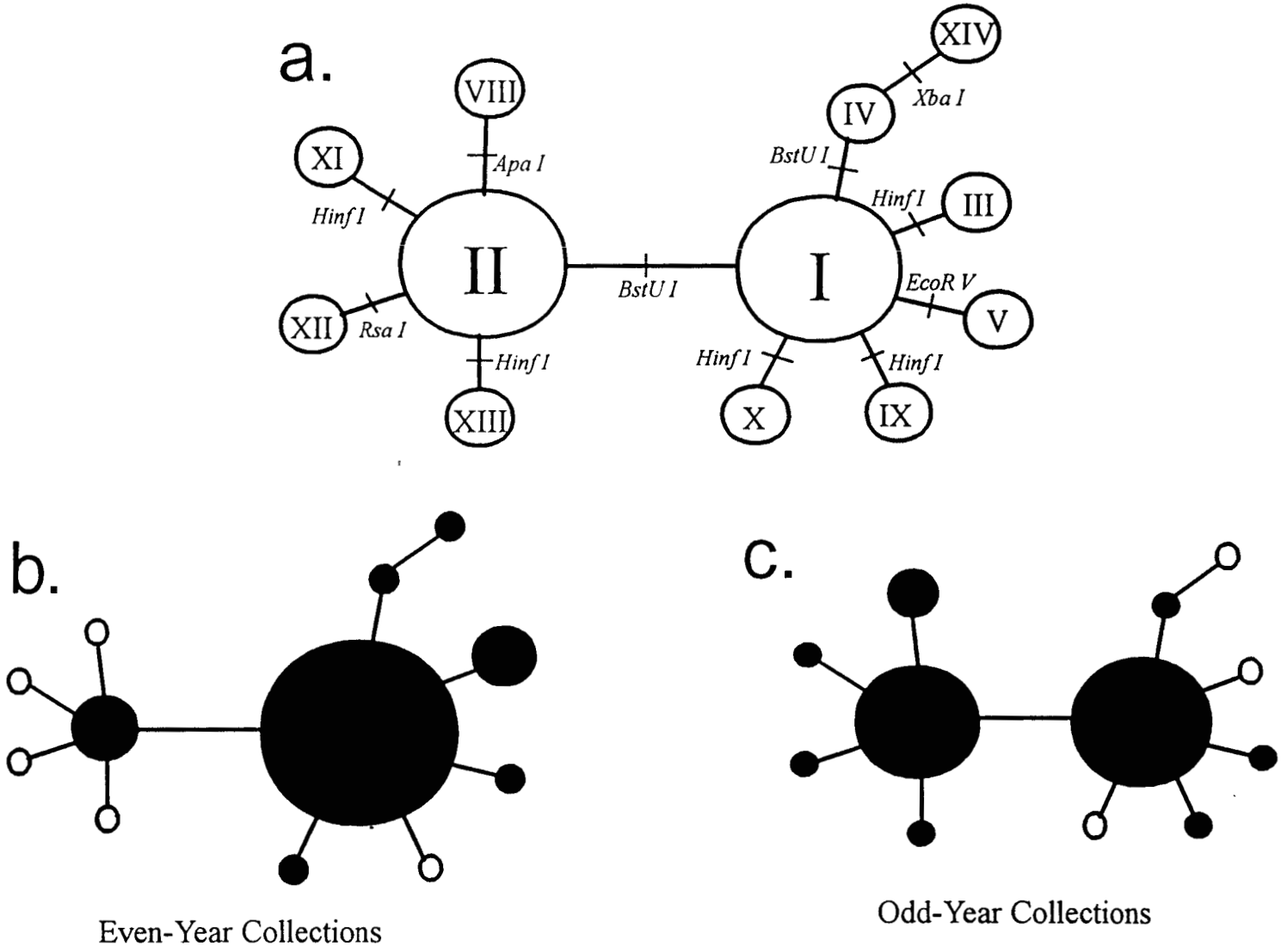


Figure 3. Haplotype network and the distribution of haplotypes within year classes: a. all haplotypes were connected to the network based on single restriction site changes, b. even-year collections, and c. odd-year collections. For the haplotype distributions within the even- and odd-year collections, black circles represent the presence ot that haplotype whereas white circles represent an absence ot the haplotype in that year class. Size of the circles indicates approximate frequencies.

Figure 4. Complete nucleotide sequence of the 2357 base pair (bp) mitochondrial *ND5/ND6* region of pink salmon. The complete *ND5* gene is 1838 bp long (bases 1-1838) and the complete sequence for the *ND6* gene is 522 bp (bases 1834-2357, compliment). There is a four basepair overlap of the two genes at their 3' ends (underlined text). The pink salmon *ND6* sequence contains an 18 bp deletion (starting at position 2105) not found in rainbow trout. This sequence has been submitted to Genbank under accession number U55056.

```
   1 ATGCACCCCA CTACACTCAT CTTAAGCTCA TCCCTTTTAA TAATTTTTAC CCTTCTAATC
  61 TACCCCCTCA TCACCACTCT CACCCCGACC CCTCAACACA AAAACTGATC CCTTACTCAA
 121 GTAAAAACTG CCATCAAAAT GGCCTTCCTC GTAAGCTTAC TCCCCCTTTT TATCTTCCTA
 181 GATCAAGGAA CTGAAACTAT CGTCACTAAC TGGCAATGAA TAAACACCAC AACCTTTGAT
 241 ATTAACCTTA GCTTTAAATT TGACCACTAC TCCATTATTT TTACCCCAAT TGCCCTGTAC
 301 GTAACCTGAT CTATTCTCGA ATTCGCATCA TGGTACATAC ACGCCGATCC AAACATAAAC
 360 CGGTTCTTTA AATATCTCCT CCTCTTCCTG ATTGCCATAA TTATTTTGGT GACCGCCAAC
 421 AATATATTTC AACTATTCAT CGGCTGAGAG GGAGTCGGAA TTATATCGTT CCTCCTCATT
 481 GGGTGATGGC ACGGACGGGC TGATGCTAAC ACAGCTGCCA TACAAGCTGT GATTTATAAC
 541 CGTGTAGGAG ACATTGGACT TATCTTAAGT ATAGCTTGGT TCGCAACAAA CCTTAACTCC
 601 TGAGAAATTC AACAAATATT TGCCTCTTCA AAAGGTCTCG ACCTTACACT CCCTCTTATA
 661 GGCCTCATTC TAGCCGCCAC CGGCAAATCA GCGCAATTTG GACTTCACCC GTGACTTCCC
 721 TCAACGATAG AAGGTCCTAC GCCGGTATCT GCCCTACTAC ACTCCAGCAC CATAATAATC
 781 GCGGGCATCT TCCTGTTAAT TCGACTCCAT CCTCTAATAG AAAACAACCA AACAGCCCTC
 841 ACCACTTGCT TATGCCTAGG AGCCCTAACC ACCCTATTCA CCGCCACCTG TGCCCTAACA
 901 CAAAATGATA TTAAAAAAAT TGTCGCATTC TCTACATCCA GCCAACTAGG ACTTATAATA
 961 GTCACCATCG GACTTAATCA ACCACAGCTA GCCTTTCTCC ACATCTGGCA CTCACGCATT
1021 CTTCAAAGCA ATACTTTTCT TATGTTCCGG TCAATTATTC ACAGTTTAAA CGACGAACAA
1081 GATATTCGAA AAATAGGAGG CATACACAAC CTCACCCCAT TTACTTCCTC CTGCCTTACA
1141 ATCGGGAGTC TTGCACTCAC CGGCACCCCC TTCTTAGCAG GATTTTTCTC CAAAGATGCT
1201 ATTATTGAAG CCTTAAACAC ATCCCACCTC AACGCCTGGG CCCTCACTCT TACCTTACTA
1261 GCCACCTCAT TCACCGCCAT TTATAGCCTC CGAGTTATCT TTTTCGTCTC CATAGGACAC
1321 CCTCGCTTTA CGACAACGGC CCCCATTAAT GAAAATAATC CATCCGTAAT TAACCCTATC
1381 AAACGACTAG CCTGAGGAAG CATCATTGCA GGACTACTAA TTACCTCAAA TTTCCTCCCC
1441 ACCAACACAC CCGTAATAAC TATGCCCACC CACTTGAAAC TGGCCGCTCT CCTAGTTACC
1501 ATCTTAGGCC TTATCATTGC ATTAGAGCTT GCATCACTAA CTAGCAAGCA ATTTAAACTA
1561 CGCCCAACCC TTATACTCTC TAACTTCTCC TACATACTGG GATTCTTCCC CGCTATCATC
1621 CACCGATTAA CCCCCAAACT AAACTTAACT TTAGGACAAG CCATTGCCAG CCAAATGGTT
1681 GATCAAACAT GATTTGAAAA AGTAGGCCCG AAAGGAATTA TTTCAACTCA CCTACCCATA
1741 GTCACAACAA CAAGTAACAT CCAACAAGGC ATAATCAAAA CATACCTCAC TCTATTTTTC
1801 CTTTCGACAA CCCTAGCTGT CCTACTGACA CTAACCTAGA CTGCTCGAAG CGCCCCTCGA
1861 CTCAACCCCC GTGTCAATTC CAGCACCACA AAAAGTGTTA GCAGCAGTAC CCAAGCACAC
1921 GCAATTAACA TTCCCCCTCC ATGAGAGTAC ATCAGCGCCA CCCCGCTCGT ATCCCCACGC
1981 AAGACAGAAA GCTCCTTAAA CTCATCCACC ACTGCTCATG AAGTTTCATA TCATCCACCC
2041 CAAAATAACC CTGCCACTAA TATCACCCCC GCCATGTACA CTACCACATA ACCTAAAACC
2101 GAACGATCCC TTCAAGACTC AGGAAAAGGC TCAGCAGCTA AAGCTGCTGA ATAAGCAAAT
2161 ACCACAAGCA TTCCCCCCAA ATAAATCAAA AATAATACCA AAGATAGAAA AGACCCCCCG
2221 TGACCCACCA AAACACCACA ACCTACACCT GCTGCTACAA CCAATCCCAA AGCAGCAAAG
2281 TAGGGCGCAG GATTGGATGC AACAGTTACA AGCCCTAAAA CCAACCCTAA AAGAAATAAA
2341 GACACAAGAT AAGTCAT
```

Appendix B. Allele frequency estimates of allozymes for pink salmon collected from Prince William Sound, Alaska in 1994. Within the population names, "T" designates collections made in tidal zones and "U" designates collections made in upstream zones.

Population		*sAAT-1,2**			*sAAT-3**	
	N	*100*	*83*	N	*100*	*91*
Rocky Ck T	100	0.9975	0.0025	99	0.7929	0.2071
Armin F. Koernig Hatchery	100	0.9900	0.0100	100	0.8000	0.2000
Cathead Ck T	99	1.0000	0.0000	100	0.7900	0.2100
Herring Ck T	97	1.0000	0.0000	100	0.7200	0.2800
Halverson Ck T	100	0.9950	0.0050	100	0.7850	0.2150
Countess Ck T	100	0.9975	0.0025	100	0.7900	0.2100
Chenega Ck T	100	0.9950	0.0050	100	0.6800	0.3200
Totemoff Ck T	100	0.9975	0.0025	100	0.7400	0.2600
Erb Ck T	100	0.9975	0.0025	100	0.7950	0.2050
Mink Ck T	92	0.9918	0.0082	100	0.8300	0.1700
Mink Ck U	100	0.9975	0.0025	99	0.7626	0.2374
Swanson Ck T	100	0.9950	0.0050	100	0.7450	0.2550
Coghill R. T	100	0.9975	0.0025	100	0.7850	0.2150
Jonah Ck T	96	0.9922	0.0078	96	0.7344	0.2656
Solomon Gulch Hatchery	100	1.0000	0.0000	100	0.8300	0.1700
Duck R. T	99	0.9975	0.0025	99	0.8131	0.1869
Millard Ck T	99	0.9975	0.0025	99	0.7525	0.2475
Lagoon Ck T	96	0.9974	0.0026	100	0.8050	0.1950
Lagoon Ck U	95	1.0000	0.0000	98	0.7296	0.2704
Olsen Ck T	100	0.9975	0.0025	100	0.8350	0.1650
Olsen Ck U	99	0.9975	0.0025	100	0.8000	0.2000
Koppen Ck T	99	0.9949	0.0051	100	0.7750	0.2250
Koppen Ck U	100	0.9975	0.0025	99	0.7525	0.2475
Humpback Ck T	100	1.0000	0.0000	99	0.7576	0.2424
Hartney Ck T	99	1.0000	0.0000	100	0.7550	0.2450
Constantine Ck T	93	0.9919	0.0081	91	0.7857	0.2143
Constantine Ck U	100	1.0000	0.0000	98	0.7704	0.2296

Appendix B. Continued.

Population	N	*sAAT-4** 100	210	290	-10	N	*mAAT-1** -100	-83	-108
Rocky Ck T	100	0.4050	0.5900	0.0050	0.0000	100	0.9900	0.0100	0.0000
Armin F. Koernig Hatchery	100	0.4300	0.5650	0.0000	0.0050	100	0.9900	0.0100	0.0000
Cathead Ck T	100	0.5000	0.4950	0.0000	0.0050	100	0.9900	0.0100	0.0000
Herring Ck T	100	0.5150	0.4650	0.0100	0.0100	100	0.9950	0.0050	0.0000
Halverson Ck T	98	0.4133	0.5663	0.0153	0.0051	100	1.0000	0.0000	0.0000
Countess Ck T	100	0.5100	0.4650	0.0100	0.0150	100	0.9950	0.0050	0.0000
Chenega Ck T	100	0.5500	0.4400	0.0050	0.0050	100	0.9850	0.0150	0.0000
Totemoff Ck T	100	0.4450	0.5350	0.0150	0.0050	100	0.9900	0.0100	0.0000
Erb Ck T	99	0.4091	0.5606	0.0202	0.0101	100	0.9850	0.0150	0.0000
Mink Ck T	99	0.5404	0.4444	0.0101	0.0051	100	0.9950	0.0050	0.0000
Mink Ck U	99	0.4747	0.5101	0.0051	0.0101	100	0.9950	0.0000	0.0050
Swanson Ck T	94	0.4468	0.5266	0.0266	0.0000	100	0.9950	0.0050	0.0000
Coghill R. T	98	0.4439	0.5408	0.0102	0.0051	100	0.9950	0.0050	0.0000
Jonah Ck T	96	0.4479	0.5208	0.0208	0.0104	96	0.9792	0.0208	0.0000
Solomon Gulch Hatchery	100	0.4750	0.5100	0.0100	0.0050	100	0.9800	0.0200	0.0000
Duck R. T	100	0.5250	0.4250	0.0350	0.0150	100	0.9950	0.0000	0.0050
Millard Ck T	97	0.4588	0.5052	0.0206	0.0155	100	0.9850	0.0150	0.0000
Lagoon Ck T	100	0.4700	0.5050	0.0200	0.0050	100	0.9950	0.0050	0.0000
Lagoon Ck U	98	0.5204	0.3827	0.0051	0.0918	97	0.9897	0.0103	0.0000
Olsen Ck T	100	0.4300	0.5350	0.0300	0.0050	100	0.9950	0.0050	0.0000
Olsen Ck U	99	0.5101	0.4798	0.0051	0.0051	100	0.9850	0.0150	0.0000
Koppen Ck T	100	0.5050	0.4900	0.0000	0.0050	100	1.0000	0.0000	0.0000
Koppen Ck U	100	0.4250	0.5550	0.0150	0.0050	100	0.9750	0.0250	0.0000
Humpback Ck T	100	0.4500	0.5350	0.0050	0.0100	100	0.9800	0.0200	0.0000
Hartney Ck T	100	0.4650	0.5000	0.0300	0.0050	100	0.9900	0.0100	0.0000
Constantine Ck T	90	0.5167	0.4667	0.0056	0.0111	93	1.0000	0.0000	0.0000
Constantine Ck U	100	0.4350	0.5500	0.0100	0.0050	100	1.0000	0.0000	0.0000

Appendix B. Continued.

Population	N	ADA-1* 100	86	122	N	ADA-2* 100	110	90
Rocky Ck T	100	1.0000	0.0000	0.0000	100	0.8950	0.0400	0.0650
Armin F. Koernig Hatchery	100	1.0000	0.0000	0.0000	100	0.9450	0.0150	0.0400
Cathead Ck T	99	1.0000	0.0000	0.0000	100	0.9600	0.0150	0.0250
Herring Ck T	100	1.0000	0.0000	0.0000	100	0.9300	0.0400	0.0300
Halverson Ck T	100	1.0000	0.0000	0.0000	100	0.9200	0.0450	0.0350
Countess Ck T	100	1.0000	0.0000	0.0000	100	0.9000	0.0300	0.0700
Chenega Ck T	100	1.0000	0.0000	0.0000	100	0.9350	0.0150	0.0500
Totemoff Ck T	100	0.9950	0.0000	0.0050	100	0.9100	0.0450	0.0450
Erb Ck T	100	1.0000	0.0000	0.0000	97	0.9278	0.0309	0.0412
Mink Ck T	100	1.0000	0.0000	0.0000	100	0.9050	0.0400	0.0550
Mink Ck U	100	0.9950	0.0000	0.0050	100	0.9550	0.0150	0.0300
Swanson Ck T	100	0.9950	0.0050	0.0000	100	0.9650	0.0050	0.0300
Coghill R. T	99	1.0000	0.0000	0.0000	98	0.9388	0.0102	0.0510
Jonah Ck T	96	1.0000	0.0000	0.0000	96	0.9323	0.0365	0.0312
Solomon Gulch Hatchery	100	0.9950	0.0000	0.0050	100	0.9200	0.0550	0.0250
Duck R. T	100	1.0000	0.0000	0.0000	100	0.9250	0.0200	0.0550
Millard Ck T	100	1.0000	0.0000	0.0000	100	0.9150	0.0400	0.0450
Lagoon Ck T	100	1.0000	0.0000	0.0000	100	0.9200	0.0200	0.0600
Lagoon Ck U	98	1.0000	0.0000	0.0000	98	0.9286	0.0561	0.0153
Olsen Ck T	99	1.0000	0.0000	0.0000	99	0.9343	0.0404	0.0253
Olsen Ck U	100	1.0000	0.0000	0.0000	98	0.8929	0.0357	0.0714
Koppen Ck T	99	1.0000	0.0000	0.0000	100	0.8650	0.0600	0.0750
Koppen Ck U	100	0.9950	0.0000	0.0050	100	0.8850	0.0650	0.0500
Humpback Ck T	99	1.0000	0.0000	0.0000	99	0.9343	0.0303	0.0354
Hartney Ck T	100	0.9900	0.0000	0.0100	100	0.9050	0.0450	0.0500
Constantine Ck T	92	1.0000	0.0000	0.0000	92	0.8967	0.0217	0.0815
Constantine Ck U	100	0.9900	0.0000	0.0100	99	0.9141	0.0202	0.0657

Appendix B. Continued.

Population	N	sAH* 100	115	88	N	mAH-3* 100	74	125
Rocky Ck T	100	0.9950	0.0000	0.0050	100	0.9950	0.0050	0.0000
Armin F. Koernig Hatchery	100	1.0000	0.0000	0.0000	100	1.0000	0.0000	0.0000
Cathead Ck T	99	0.9949	0.0000	0.0051	97	1.0000	0.0000	0.0000
Herring Ck T	100	0.9950	0.0000	0.0050	83	1.0000	0.0000	0.0000
Halverson Ck T	98	0.9847	0.0051	0.0102	98	1.0000	0.0000	0.0000
Countess Ck T	100	0.9900	0.0000	0.0100	100	0.9900	0.0050	0.0050
Chenega Ck T	100	1.0000	0.0000	0.0000	100	0.9950	0.0050	0.0000
Totemoff Ck T	100	0.9950	0.0000	0.0050	100	0.9950	0.0000	0.0050
Erb Ck T	100	1.0000	0.0000	0.0000	100	1.0000	0.0000	0.0000
Mink Ck T	98	1.0000	0.0000	0.0000	100	1.0000	0.0000	0.0000
Mink Ck U	100	1.0000	0.0000	0.0000	100	1.0000	0.0000	0.0000
Swanson Ck T	96	1.0000	0.0000	0.0000	100	1.0000	0.0000	0.0000
Coghill R. T	99	1.0000	0.0000	0.0000	100	0.9900	0.0100	0.0000
Jonah Ck T	96	1.0000	0.0000	0.0000	87	1.0000	0.0000	0.0000
Solomon Gulch Hatchery	100	1.0000	0.0000	0.0000	98	0.9898	0.0102	0.0000
Duck R. T	100	1.0000	0.0000	0.0000	100	1.0000	0.0000	0.0000
Millard Ck T	100	1.0000	0.0000	0.0000	100	1.0000	0.0000	0.0000
Lagoon Ck T	100	1.0000	0.0000	0.0000	100	1.0000	0.0000	0.0000
Lagoon Ck U	98	1.0000	0.0000	0.0000	98	0.9949	0.0000	0.0051
Olsen Ck T	100	0.9950	0.0000	0.0050	100	1.0000	0.0000	0.0000
Olsen Ck U	100	1.0000	0.0000	0.0000	100	1.0000	0.0000	0.0000
Koppen Ck T	100	0.9950	0.0000	0.0050	100	1.0000	0.0000	0.0000
Koppen Ck U	100	1.0000	0.0000	0.0000	100	1.0000	0.0000	0.0000
Humpback Ck T	100	0.9850	0.0000	0.0150	100	0.9900	0.0100	0.0000
Hartney Ck T	100	1.0000	0.0000	0.0000	100	0.9850	0.0100	0.0050
Constantine Ck T	91	0.9890	0.0000	0.0110	93	0.9946	0.0054	0.0000
Constantine Ck U	100	0.9950	0.0000	0.0050	100	0.9900	0.0050	0.0050

Appendix B. Continued.

Population	N	*mAH-4** 100	116	81	N	*CK-A2** 100	108	82
Rocky Ck T	100	0.9600	0.0000	0.0400	100	1.0000	0.0000	0.0000
Armin F. Koernig Hatchery	100	0.9700	0.0000	0.0300	100	1.0000	0.0000	0.0000
Cathead Ck T	98	0.9439	0.0000	0.0561	99	1.0000	0.0000	0.0000
Herring Ck T	80	0.9312	0.0000	0.0688	98	0.9949	0.0051	0.0000
Halverson Ck T	95	0.9842	0.0000	0.0158	98	1.0000	0.0000	0.0000
Countess Ck T	99	0.9747	0.0000	0.0253	100	1.0000	0.0000	0.0000
Chenega Ck T	97	0.9588	0.0000	0.0412	100	0.9850	0.0050	0.0100
Totemoff Ck T	100	0.9600	0.0000	0.0400	100	0.9900	0.0000	0.0100
Erb Ck T	100	0.9750	0.0000	0.0250	98	0.9949	0.0051	0.0000
Mink Ck T	100	0.9850	0.0000	0.0150	100	1.0000	0.0000	0.0000
Mink Ck U	100	0.9800	0.0000	0.0200	100	1.0000	0.0000	0.0000
Swanson Ck T	99	0.9596	0.0000	0.0404	99	1.0000	0.0000	0.0000
Coghill R. T	100	0.9550	0.0000	0.0450	100	1.0000	0.0000	0.0000
Jonah Ck T	85	0.9529	0.0000	0.0471	96	1.0000	0.0000	0.0000
Solomon Gulch Hatchery	98	0.9745	0.0000	0.0255	100	0.9800	0.0000	0.0200
Duck R. T	100	0.9400	0.0000	0.0600	96	0.9948	0.0000	0.0052
Millard Ck T	100	0.9850	0.0000	0.0150	100	0.9900	0.0000	0.0100
Lagoon Ck T	100	0.9750	0.0000	0.0250	100	1.0000	0.0000	0.0000
Lagoon Ck U	98	0.9184	0.0000	0.0816	98	1.0000	0.0000	0.0000
Olsen Ck T	100	0.9800	0.0000	0.0200	98	1.0000	0.0000	0.0000
Olsen Ck U	100	0.9550	0.0000	0.0450	100	0.9950	0.0000	0.0050
Koppen Ck T	100	0.9650	0.0000	0.0350	55	1.0000	0.0000	0.0000
Koppen Ck U	100	0.9650	0.0000	0.0350	100	0.9950	0.0000	0.0050
Humpback Ck T	100	0.9750	0.0000	0.0250	100	0.9950	0.0000	0.0050
Hartney Ck T	100	0.9750	0.0000	0.0250	100	0.9950	0.0000	0.0050
Constantine Ck T	93	0.9516	0.0054	0.0430	93	0.9946	0.0000	0.0054
Constantine Ck U	100	0.9350	0.0000	0.0650	100	1.0000	0.0000	0.0000

Appendix B. Continued.

Population	N	*FDHG** 100	132	57	N	*bGALA** 100	111	91	105
Rocky Ck T	99	1.0000	0.0000	0.0000	96	0.9010	0.0625	0.0260	0.0104
Armin F. Koernig Hatchery	100	0.9950	0.0050	0.0000	98	0.8980	0.0816	0.0051	0.0153
Cathead Ck T	96	0.9844	0.0156	0.0000	87	0.9138	0.0517	0.0230	0.0115
Herring Ck T	99	0.9798	0.0152	0.0051	69	0.9348	0.0362	0.0072	0.0217
Halverson Ck T	100	1.0000	0.0000	0.0000	69	0.9275	0.0290	0.0000	0.0435
Countess Ck T	98	1.0000	0.0000	0.0000	94	0.9043	0.0691	0.0053	0.0213
Chenega Ck T	99	0.9949	0.0051	0.0000	78	0.9167	0.0641	0.0000	0.0192
Totemoff Ck T	98	0.9796	0.0153	0.0051	93	0.8763	0.0645	0.0269	0.0323
Erb Ck T	100	0.9900	0.0050	0.0050	97	0.8763	0.0773	0.0103	0.0361
Mink Ck T	100	0.9900	0.0100	0.0000	98	0.8980	0.0612	0.0153	0.0255
Mink Ck U	94	0.9840	0.0053	0.0106	99	0.8889	0.0909	0.0000	0.0202
Swanson Ck T	99	0.9949	0.0051	0.0000	81	0.9074	0.0432	0.0123	0.0370
Coghill R. T	100	1.0000	0.0000	0.0000	96	0.8958	0.0521	0.0104	0.0417
Jonah Ck T	94	0.9894	0.0106	0.0000	94	0.8457	0.0957	0.0160	0.0426
Solomon Gulch Hatchery	95	1.0000	0.0000	0.0000	68	0.9044	0.0662	0.0000	0.0294
Duck R. T	100	0.9900	0.0050	0.0050	99	0.8485	0.0960	0.0101	0.0455
Millard Ck T	95	0.9947	0.0053	0.0000	97	0.8557	0.0928	0.0206	0.0309
Lagoon Ck T	96	0.9896	0.0052	0.0052	89	0.8764	0.0730	0.0337	0.0169
Lagoon Ck U	85	1.0000	0.0000	0.0000	80	0.9125	0.0562	0.0125	0.0188
Olsen Ck T	99	1.0000	0.0000	0.0000	97	0.9278	0.0412	0.0103	0.0206
Olsen Ck U	100	0.9900	0.0100	0.0000	99	0.8889	0.0606	0.0253	0.0253
Koppen Ck T	96	0.9948	0.0052	0.0000	96	0.8802	0.0990	0.0000	0.0208
Koppen Ck U	94	1.0000	0.0000	0.0000	91	0.8407	0.1044	0.0110	0.0440
Humpback Ck T	97	0.9794	0.0155	0.0052	97	0.8402	0.1082	0.0206	0.0309
Hartney Ck T	99	0.9949	0.0051	0.0000	91	0.8516	0.1044	0.0220	0.0220
Constantine Ck T	93	0.9892	0.0054	0.0054	81	0.9136	0.0247	0.0247	0.0370
Constantine Ck U	93	1.0000	0.0000	0.0000	97	0.8608	0.0928	0.0206	0.0258

Appendix B. Continued.

Population	N	G3PDH-1* 100	-151	-52	N	G3PDH-2* 100	120	90
Rocky Ck T	100	0.8100	0.0000	0.1900	100	0.8450	0.0100	0.1450
Armin F. Koernig Hatchery	100	0.8100	0.0050	0.1850	99	0.8586	0.0354	0.1061
Cathead Ck T	100	0.8600	0.0000	0.1400	100	0.8900	0.0400	0.0700
Herring Ck T	100	0.8600	0.0000	0.1400	96	0.8958	0.0365	0.0677
Halverson Ck T	100	0.8100	0.0000	0.1900	96	0.8802	0.0156	0.1042
Countess Ck T	100	0.8450	0.0050	0.1500	100	0.8650	0.0200	0.1150
Chenega Ck T	100	0.8000	0.0000	0.2000	100	0.8800	0.0350	0.0850
Totemoff Ck T	100	0.8300	0.0000	0.1700	99	0.8687	0.0556	0.0758
Erb Ck T	100	0.7800	0.0000	0.2200	100	0.8350	0.1150	0.0500
Mink Ck T	100	0.8600	0.0000	0.1400	99	0.8788	0.0455	0.0758
Mink Ck U	100	0.8300	0.0100	0.1600	100	0.8600	0.0350	0.1050
Swanson Ck T	100	0.8050	0.0000	0.1950	99	0.8636	0.0253	0.1111
Coghill R. T	100	0.8300	0.0000	0.1700	100	0.8300	0.0500	0.1200
Jonah Ck T	96	0.7135	0.0052	0.2812	96	0.8854	0.0260	0.0885
Solomon Gulch Hatchery	100	0.8050	0.0000	0.1950	99	0.8737	0.0354	0.0909
Duck R. T	100	0.7850	0.0000	0.2150	96	0.8750	0.0365	0.0885
Millard Ck T	100	0.8000	0.0000	0.2000	100	0.8400	0.0350	0.1250
Lagoon Ck T	100	0.8250	0.0000	0.1750	98	0.8163	0.0510	0.1327
Lagoon Ck U	98	0.8929	0.0000	0.1071	97	0.8505	0.0052	0.1443
Olsen Ck T	100	0.8150	0.0050	0.1800	96	0.8646	0.0260	0.1094
Olsen Ck U	100	0.7850	0.0000	0.2150	98	0.8724	0.0306	0.0969
Koppen Ck T	100	0.8200	0.0000	0.1800	100	0.8850	0.0150	0.1000
Koppen Ck U	100	0.8600	0.0000	0.1400	100	0.8600	0.0150	0.1250
Humpback Ck T	100	0.7850	0.0050	0.2100	100	0.8350	0.0450	0.1200
Hartney Ck T	100	0.8100	0.0000	0.1900	100	0.8700	0.0300	0.1000
Constantine Ck T	93	0.8172	0.0000	0.1828	92	0.7989	0.0326	0.1685
Constantine Ck U	100	0.7250	0.0000	0.2750	99	0.8333	0.0354	0.1313

Appendix B. Continued.

Population	N	G3PDH-3* 100	90	N	GDA-1* 100	108	113	82	110
Rocky Ck T	100	0.9900	0.0100	100	0.5350	0.4350	0.0300	0.0000	0.0000
Armin F. Koernig Hatchery	99	0.9949	0.0051	98	0.5306	0.4592	0.0102	0.0000	0.0000
Cathead Ck T	100	1.0000	0.0000	100	0.5600	0.4350	0.0050	0.0000	0.0000
Herring Ck T	99	0.9798	0.0202	100	0.5500	0.4200	0.0300	0.0000	0.0000
Halverson Ck T	100	0.9950	0.0050	97	0.4794	0.5052	0.0155	0.0000	0.0000
Countess Ck T	100	0.9900	0.0100	99	0.5000	0.4747	0.0253	0.0000	0.0000
Chenega Ck T	100	1.0000	0.0000	100	0.5800	0.4100	0.0050	0.0050	0.0000
Totemoff Ck T	99	1.0000	0.0000	99	0.4646	0.5101	0.0253	0.0000	0.0000
Erb Ck T	100	0.9950	0.0050	98	0.5000	0.4745	0.0204	0.0051	0.0000
Mink Ck T	100	0.9900	0.0100	93	0.5430	0.4355	0.0215	0.0000	0.0000
Mink Ck U	100	1.0000	0.0000	98	0.5765	0.4082	0.0153	0.0000	0.0000
Swanson Ck T	98	0.9949	0.0051	96	0.4896	0.5000	0.0104	0.0000	0.0000
Coghill R. T	99	0.9798	0.0202	97	0.5258	0.4536	0.0206	0.0000	0.0000
Jonah Ck T	96	0.9792	0.0208	96	0.5833	0.4115	0.0052	0.0000	0.0000
Solomon Gulch Hatchery	100	0.9950	0.0050	100	0.5950	0.3750	0.0250	0.0050	0.0000
Duck R. T	99	0.9899	0.0101	100	0.5050	0.4800	0.0150	0.0000	0.0000
Millard Ck T	100	0.9900	0.0100	99	0.5152	0.4747	0.0101	0.0000	0.0000
Lagoon Ck T	100	0.9850	0.0150	94	0.5053	0.4840	0.0106	0.0000	0.0000
Lagoon Ck U	97	0.9948	0.0052	98	0.6173	0.3316	0.0510	0.0000	0.0000
Olsen Ck T	99	0.9798	0.0202	98	0.5765	0.4082	0.0153	0.0000	0.0000
Olsen Ck U	100	0.9950	0.0050	96	0.5521	0.4375	0.0104	0.0000	0.0000
Koppen Ck T	100	0.9950	0.0050	100	0.5550	0.4150	0.0300	0.0000	0.0000
Koppen Ck U	100	0.9900	0.0100	100	0.5550	0.4250	0.0200	0.0000	0.0000
Humpback Ck T	100	1.0000	0.0000	97	0.5206	0.4485	0.0309	0.0000	0.0000
Hartney Ck T	100	1.0000	0.0000	99	0.5606	0.3939	0.0404	0.0051	0.0000
Constantine Ck T	93	0.9892	0.0108	90	0.5500	0.4389	0.0111	0.0000	0.0000
Constantine Ck U	100	1.0000	0.0000	98	0.5561	0.4031	0.0357	0.0000	0.0051

Appendix B. Continued.

Population	N	GPI-B1,2* 100	200	25	180	N	IDDH-1* 100	134
Rocky Ck T	100	0.9925	0.0025	0.0000	0.0050	90	1.0000	0.0000
Armin F. Koernig Hatchery	100	0.9925	0.0025	0.0000	0.0050	96	0.9948	0.0052
Cathead Ck T	99	0.9924	0.0000	0.0000	0.0076	89	1.0000	0.0000
Herring Ck T	98	0.9949	0.0000	0.0000	0.0051	91	0.9945	0.0055
Halverson Ck T	100	0.9925	0.0000	0.0000	0.0075	83	0.9940	0.0060
Countess Ck T	100	0.9925	0.0050	0.0000	0.0025	72	1.0000	0.0000
Chenega Ck T	100	0.9900	0.0025	0.0025	0.0050	92	1.0000	0.0000
Totemoff Ck T	100	0.9925	0.0000	0.0000	0.0075	93	1.0000	0.0000
Erb Ck T	100	0.9975	0.0000	0.0000	0.0025	92	0.9891	0.0109
Mink Ck T	100	0.9925	0.0000	0.0000	0.0075	90	1.0000	0.0000
Mink Ck U	100	0.9925	0.0000	0.0000	0.0075	92	0.9891	0.0109
Swanson Ck T	99	0.9949	0.0000	0.0000	0.0051	50	0.9900	0.0100
Coghill R. T	100	0.9925	0.0025	0.0000	0.0050	95	0.9947	0.0053
Jonah Ck T	95	0.9921	0.0026	0.0000	0.0053	49	1.0000	0.0000
Solomon Gulch Hatchery	98	0.9898	0.0000	0.0000	0.0102	93	1.0000	0.0000
Duck R. T	96	0.9896	0.0000	0.0000	0.0104	88	1.0000	0.0000
Millard Ck T	98	0.9974	0.0000	0.0000	0.0026	92	0.9946	0.0054
Lagoon Ck T	100	0.9925	0.0025	0.0000	0.0050	94	1.0000	0.0000
Lagoon Ck U	98	0.9949	0.0000	0.0000	0.0051	92	1.0000	0.0000
Olsen Ck T	100	0.9875	0.0000	0.0000	0.0125	90	0.9944	0.0056
Olsen Ck U	100	0.9900	0.0025	0.0000	0.0075	94	1.0000	0.0000
Koppen Ck T	100	0.9975	0.0000	0.0000	0.0025	50	0.9900	0.0100
Koppen Ck U	100	0.9900	0.0000	0.0000	0.0100	94	1.0000	0.0000
Humpback Ck T	100	1.0000	0.0000	0.0000	0.0000	90	1.0000	0.0000
Hartney Ck T	100	0.9975	0.0000	0.0000	0.0025	87	0.9943	0.0057
Constantine Ck T	93	0.9919	0.0000	0.0000	0.0081	79	1.0000	0.0000
Constantine Ck U	99	0.9975	0.0025	0.0000	0.0000	92	1.0000	0.0000

Appendix B. Continued.

Population	N	*mIDHP-1**			N	*sIDHP-2**			
		100	*53*	*69*		*100*	*125*	*134*	*76*
Rocky Ck T	100	1.0000	0.0000	0.0000	100	0.6400	0.3550	0.0050	0.0000
Armin F. Koernig Hatchery	100	1.0000	0.0000	0.0000	100	0.6750	0.3200	0.0050	0.0000
Cathead Ck T	95	1.0000	0.0000	0.0000	100	0.6400	0.3600	0.0000	0.0000
Herring Ck T	100	0.9850	0.0050	0.0100	99	0.6465	0.3535	0.0000	0.0000
Halverson Ck T	100	0.9900	0.0100	0.0000	99	0.6212	0.3788	0.0000	0.0000
Countess Ck T	100	1.0000	0.0000	0.0000	100	0.6550	0.3450	0.0000	0.0000
Chenega Ck T	100	0.9950	0.0000	0.0050	100	0.6850	0.3150	0.0000	0.0000
Totemoff Ck T	100	0.9950	0.0050	0.0000	99	0.5808	0.4192	0.0000	0.0000
Erb Ck T	100	0.9950	0.0000	0.0050	100	0.6000	0.4000	0.0000	0.0000
Mink Ck T	100	0.9950	0.0050	0.0000	100	0.7150	0.2850	0.0000	0.0000
Mink Ck U	100	0.9950	0.0050	0.0000	100	0.6800	0.3200	0.0000	0.0000
Swanson Ck T	100	0.9950	0.0050	0.0000	100	0.6700	0.3300	0.0000	0.0000
Coghill R. T	100	1.0000	0.0000	0.0000	100	0.6750	0.3200	0.0000	0.0050
Jonah Ck T	96	0.9948	0.0052	0.0000	96	0.6250	0.3750	0.0000	0.0000
Solomon Gulch Hatchery	100	0.9950	0.0000	0.0050	100	0.6350	0.3650	0.0000	0.0000
Duck R. T	100	1.0000	0.0000	0.0000	100	0.6650	0.3350	0.0000	0.0000
Millard Ck T	99	0.9899	0.0051	0.0051	100	0.6650	0.3350	0.0000	0.0000
Lagoon Ck T	100	1.0000	0.0000	0.0000	100	0.6650	0.3350	0.0000	0.0000
Lagoon Ck U	98	0.9949	0.0051	0.0000	97	0.7887	0.2113	0.0000	0.0000
Olsen Ck T	100	1.0000	0.0000	0.0000	99	0.7071	0.2929	0.0000	0.0000
Olsen Ck U	100	0.9900	0.0050	0.0050	100	0.6900	0.3100	0.0000	0.0000
Koppen Ck T	100	0.9900	0.0050	0.0050	100	0.6600	0.3400	0.0000	0.0000
Koppen Ck U	100	1.0000	0.0000	0.0000	100	0.6550	0.3450	0.0000	0.0000
Humpback Ck T	100	0.9950	0.0000	0.0050	100	0.5850	0.4150	0.0000	0.0000
Hartney Ck T	100	1.0000	0.0000	0.0000	99	0.5808	0.4141	0.0000	0.0051
Constantine Ck T	93	0.9892	0.0000	0.0108	93	0.6452	0.3548	0.0000	0.0000
Constantine Ck U	100	1.0000	0.0000	0.0000	99	0.5909	0.4091	0.0000	0.0000

Appendix B. Continued.

Population	N	*LDH-A2**		N	*LDH-B2**				
		100	*148*		*100*	*132*	*74*	*151*	*124*
Rocky Ck T	100	1.0000	0.0000	100	0.9950	0.0000	0.0000	0.0000	0.0050
Armin F. Koernig Hatchery	100	1.0000	0.0000	100	1.0000	0.0000	0.0000	0.0000	0.0000
Cathead Ck T	100	0.9800	0.0200	100	0.9950	0.0000	0.0000	0.0000	0.0050
Herring Ck T	100	1.0000	0.0000	100	0.9900	0.0000	0.0000	0.0000	0.0100
Halverson Ck T	98	1.0000	0.0000	100	0.9750	0.0000	0.0000	0.0000	0.0250
Countess Ck T	100	0.9950	0.0050	100	0.9950	0.0000	0.0000	0.0000	0.0050
Chenega Ck T	100	1.0000	0.0000	100	0.9950	0.0000	0.0000	0.0000	0.0050
Totemoff Ck T	100	0.9900	0.0100	100	0.9900	0.0000	0.0000	0.0000	0.0100
Erb Ck T	97	1.0000	0.0000	100	0.9950	0.0000	0.0000	0.0000	0.0050
Mink Ck T	99	1.0000	0.0000	100	1.0000	0.0000	0.0000	0.0000	0.0000
Mink Ck U	100	1.0000	0.0000	100	1.0000	0.0000	0.0000	0.0000	0.0000
Swanson Ck T	97	1.0000	0.0000	100	0.9850	0.0000	0.0050	0.0000	0.0100
Coghill R. T	100	0.9950	0.0050	97	0.9897	0.0052	0.0000	0.0000	0.0052
Jonah Ck T	96	1.0000	0.0000	96	0.9635	0.0000	0.0000	0.0104	0.0260
Solomon Gulch Hatchery	100	1.0000	0.0000	100	0.9950	0.0000	0.0000	0.0000	0.0050
Duck R. T	98	1.0000	0.0000	100	0.9950	0.0000	0.0000	0.0000	0.0050
Millard Ck T	99	1.0000	0.0000	100	0.9800	0.0000	0.0000	0.0000	0.0200
Lagoon Ck T	100	1.0000	0.0000	100	0.9900	0.0000	0.0000	0.0000	0.0100
Lagoon Ck U	97	1.0000	0.0000	98	0.9949	0.0000	0.0000	0.0000	0.0051
Olsen Ck T	100	1.0000	0.0000	100	0.9850	0.0000	0.0000	0.0000	0.0150
Olsen Ck U	100	1.0000	0.0000	100	0.9850	0.0000	0.0000	0.0000	0.0150
Koppen Ck T	100	1.0000	0.0000	100	0.9900	0.0000	0.0000	0.0000	0.0100
Koppen Ck U	100	1.0000	0.0000	100	0.9950	0.0000	0.0000	0.0000	0.0050
Humpback Ck T	100	1.0000	0.0000	100	0.9850	0.0000	0.0050	0.0000	0.0100
Hartney Ck T	100	1.0000	0.0000	100	0.9900	0.0000	0.0000	0.0000	0.0100
Constantine Ck T	93	1.0000	0.0000	93	0.9892	0.0000	0.0000	0.0000	0.0108
Constantine Ck U	100	1.0000	0.0000	100	0.9800	0.0000	0.0000	0.0050	0.0150

Appendix B. Continued.

Population	N	sMDH-A1,2* 100	148	50	126	158	58
Rocky Ck T	100	0.9675	0.0000	0.0300	0.0025	0.0000	0.0000
Armin F. Koernig Hatchery	100	0.9775	0.0000	0.0225	0.0000	0.0000	0.0000
Cathead Ck T	99	0.9798	0.0051	0.0152	0.0000	0.0000	0.0000
Herring Ck T	100	0.9875	0.0000	0.0125	0.0000	0.0000	0.0000
Halverson Ck T	100	0.9900	0.0000	0.0100	0.0000	0.0000	0.0000
Countess Ck T	100	0.9875	0.0000	0.0125	0.0000	0.0000	0.0000
Chenega Ck T	100	0.9700	0.0025	0.0225	0.0025	0.0025	0.0000
Totemoff Ck T	100	0.9875	0.0000	0.0100	0.0025	0.0000	0.0000
Erb Ck T	100	0.9725	0.0000	0.0200	0.0075	0.0000	0.0000
Mink Ck T	100	0.9775	0.0025	0.0125	0.0075	0.0000	0.0000
Mink Ck U	100	0.9825	0.0000	0.0150	0.0025	0.0000	0.0000
Swanson Ck T	100	0.9900	0.0000	0.0100	0.0000	0.0000	0.0000
Coghill R. T	100	0.9650	0.0000	0.0325	0.0025	0.0000	0.0000
Jonah Ck T	96	0.9844	0.0000	0.0130	0.0000	0.0000	0.0026
Solomon Gulch Hatchery	100	0.9875	0.0000	0.0125	0.0000	0.0000	0.0000
Duck R. T	100	0.9875	0.0000	0.0125	0.0000	0.0000	0.0000
Millard Ck T	100	0.9750	0.0000	0.0225	0.0025	0.0000	0.0000
Lagoon Ck T	100	0.9850	0.0000	0.0150	0.0000	0.0000	0.0000
Lagoon Ck U	98	0.9821	0.0000	0.0153	0.0026	0.0000	0.0000
Olsen Ck T	100	0.9825	0.0000	0.0175	0.0000	0.0000	0.0000
Olsen Ck U	100	0.9900	0.0000	0.0100	0.0000	0.0000	0.0000
Koppen Ck T	100	0.9750	0.0000	0.0200	0.0050	0.0000	0.0000
Koppen Ck U	100	0.9875	0.0000	0.0125	0.0000	0.0000	0.0000
Humpback Ck T	100	0.9875	0.0000	0.0125	0.0000	0.0000	0.0000
Hartney Ck T	100	0.9900	0.0000	0.0075	0.0025	0.0000	0.0000
Constantine Ck T	92	0.9755	0.0027	0.0190	0.0027	0.0000	0.0000
Constantine Ck U	100	0.9625	0.0000	0.0350	0.0025	0.0000	0.0000

Appendix B. Continued.

Population	N	*sMDH-B1,2** 100	124	66	N	*mMEP-1** 100	123
Rocky Ck T	98	0.9949	0.0026	0.0026	100	0.6450	0.3550
Armin F. Koernig Hatchery	100	0.9925	0.0075	0.0000	99	0.7424	0.2576
Cathead Ck T	100	1.0000	0.0000	0.0000	99	0.7172	0.2828
Herring Ck T	100	0.9875	0.0125	0.0000	100	0.7100	0.2900
Halverson Ck T	100	0.9975	0.0025	0.0000	100	0.7400	0.2600
Countess Ck T	100	0.9900	0.0075	0.0025	100	0.7150	0.2850
Chenega Ck T	100	0.9925	0.0025	0.0050	100	0.7350	0.2650
Totemoff Ck T	100	0.9875	0.0100	0.0025	100	0.6650	0.3350
Erb Ck T	99	0.9975	0.0000	0.0025	100	0.7200	0.2800
Mink Ck T	100	0.9975	0.0025	0.0000	100	0.7250	0.2750
Mink Ck U	100	0.9975	0.0000	0.0025	100	0.7800	0.2200
Swanson Ck T	100	0.9925	0.0075	0.0000	100	0.7200	0.2800
Coghill R. T	100	0.9975	0.0025	0.0000	100	0.7400	0.2600
Jonah Ck T	96	0.9948	0.0052	0.0000	96	0.7240	0.2760
Solomon Gulch Hatchery	100	0.9925	0.0050	0.0025	100	0.7850	0.2150
Duck R. T	98	0.9872	0.0128	0.0000	100	0.7350	0.2650
Millard Ck T	100	0.9975	0.0025	0.0000	100	0.7600	0.2400
Lagoon Ck T	100	1.0000	0.0000	0.0000	100	0.7650	0.2350
Lagoon Ck U	98	0.9898	0.0102	0.0000	98	0.7908	0.2092
Olsen Ck T	100	0.9925	0.0075	0.0000	100	0.7650	0.2350
Olsen Ck U	100	0.9850	0.0150	0.0000	100	0.7450	0.2550
Koppen Ck T	100	1.0000	0.0000	0.0000	100	0.7450	0.2550
Koppen Ck U	100	0.9950	0.0050	0.0000	100	0.8200	0.1800
Humpback Ck T	100	0.9925	0.0075	0.0000	100	0.7250	0.2750
Hartney Ck T	100	1.0000	0.0000	0.0000	100	0.7500	0.2500
Constantine Ck T	93	0.9919	0.0054	0.0027	93	0.7688	0.2312
Constantine Ck U	100	0.9850	0.0150	0.0000	100	0.7400	0.2600

Appendix B. Continued.

Population	N	*NTP** 100	130	N	*PEP-B1** 100	138	200
Rocky Ck T	100	1.0000	0.0000	100	0.8700	0.1150	0.0150
Armin F. Koernig Hatchery	100	1.0000	0.0000	100	0.8600	0.1200	0.0200
Cathead Ck T	100	1.0000	0.0000	100	0.9200	0.0700	0.0100
Herring Ck T	89	1.0000	0.0000	100	0.8550	0.1200	0.0250
Halverson Ck T	100	1.0000	0.0000	100	0.8450	0.1300	0.0250
Countess Ck T	100	0.9900	0.0100	100	0.8850	0.0950	0.0200
Chenega Ck T	47	1.0000	0.0000	100	0.8750	0.1100	0.0150
Totemoff Ck T	100	1.0000	0.0000	100	0.8250	0.1350	0.0400
Erb Ck T	100	1.0000	0.0000	100	0.8450	0.1150	0.0400
Mink Ck T	100	1.0000	0.0000	100	0.8650	0.0950	0.0400
Mink Ck U	100	1.0000	0.0000	100	0.8950	0.0900	0.0150
Swanson Ck T	100	0.9900	0.0100	99	0.8485	0.1111	0.0404
Coghill R. T	97	1.0000	0.0000	99	0.8737	0.1061	0.0202
Jonah Ck T	95	1.0000	0.0000	96	0.8802	0.0885	0.0312
Solomon Gulch Hatchery	38	1.0000	0.0000	100	0.8750	0.0800	0.0450
Duck R. T	100	0.9950	0.0050	100	0.8400	0.1450	0.0150
Millard Ck T	100	1.0000	0.0000	100	0.8500	0.1050	0.0450
Lagoon Ck T	90	1.0000	0.0000	100	0.8950	0.0850	0.0200
Lagoon Ck U	61	1.0000	0.0000	98	0.9184	0.0612	0.0204
Olsen Ck T	100	1.0000	0.0000	100	0.9150	0.0700	0.0150
Olsen Ck U	91	1.0000	0.0000	100	0.8800	0.0750	0.0450
Koppen Ck T	91	1.0000	0.0000	100	0.8350	0.1350	0.0300
Koppen Ck U	57	1.0000	0.0000	100	0.9050	0.0850	0.0100
Humpback Ck T	100	1.0000	0.0000	100	0.9000	0.0750	0.0250
Hartney Ck T	66	1.0000	0.0000	100	0.8700	0.0850	0.0450
Constantine Ck T	93	1.0000	0.0000	93	0.8925	0.0914	0.0161
Constantine Ck U	99	1.0000	0.0000	100	0.9050	0.0750	0.0200

Appendix B. Continued.

Population	N	PEP-D2* 100	120	80	N	PEPLT* 100	108	90
Rocky Ck T	100	0.5250	0.2350	0.2400	100	0.8800	0.0600	0.0600
Armin F. Koernig Hatchery	100	0.5250	0.2550	0.2200	100	0.8750	0.0850	0.0400
Cathead Ck T	100	0.5500	0.2150	0.2350	99	0.8737	0.0707	0.0556
Herring Ck T	100	0.5600	0.2550	0.1850	100	0.8250	0.1050	0.0700
Halverson Ck T	100	0.5400	0.2250	0.2350	100	0.8100	0.1250	0.0650
Countess Ck T	100	0.5350	0.2400	0.2250	100	0.8650	0.0800	0.0550
Chenega Ck T	100	0.5200	0.2200	0.2600	100	0.8450	0.1100	0.0450
Totemoff Ck T	100	0.5200	0.2200	0.2600	100	0.8150	0.1450	0.0400
Erb Ck T	100	0.5650	0.1850	0.2500	100	0.8600	0.1100	0.0300
Mink Ck T	100	0.5250	0.2250	0.2500	100	0.8300	0.1300	0.0400
Mink Ck U	100	0.6250	0.1900	0.1850	100	0.8300	0.1000	0.0700
Swanson Ck T	100	0.5100	0.2150	0.2750	100	0.8150	0.1000	0.0850
Coghill R. T	100	0.4650	0.2400	0.2950	100	0.8200	0.1250	0.0550
Jonah Ck T	96	0.4740	0.2396	0.2865	96	0.8385	0.0677	0.0938
Solomon Gulch Hatchery	100	0.5000	0.2700	0.2300	100	0.8850	0.0600	0.0550
Duck R. T	100	0.5400	0.2350	0.2250	100	0.8500	0.1100	0.0400
Millard Ck T	100	0.5500	0.2100	0.2400	100	0.8800	0.0500	0.0700
Lagoon Ck T	100	0.5700	0.2550	0.1750	100	0.8600	0.0850	0.0550
Lagoon Ck U	98	0.5102	0.3061	0.1837	98	0.9337	0.0357	0.0306
Olsen Ck T	100	0.5050	0.2100	0.2850	99	0.8788	0.0707	0.0505
Olsen Ck U	100	0.5350	0.2450	0.2200	100	0.8850	0.0600	0.0550
Koppen Ck T	100	0.5600	0.2250	0.2150	100	0.8600	0.0900	0.0500
Koppen Ck U	100	0.5700	0.2200	0.2100	100	0.8750	0.0950	0.0300
Humpback Ck T	100	0.5150	0.2450	0.2400	100	0.8350	0.0950	0.0700
Hartney Ck T	100	0.4700	0.2800	0.2500	100	0.8950	0.0500	0.0550
Constantine Ck T	93	0.5108	0.2258	0.2634	93	0.8495	0.0753	0.0753
Constantine Ck U	100	0.4900	0.2200	0.2900	100	0.8650	0.0650	0.0700

Appendix B. Continued.

Population	N	PGDH* 100	108	96	86	93
Rocky Ck T	100	0.7400	0.0000	0.2200	0.0400	0.0000
Armin F. Koernig Hatchery	100	0.7350	0.0000	0.2300	0.0350	0.0000
Cathead Ck T	96	0.7135	0.0000	0.2604	0.0260	0.0000
Herring Ck T	100	0.7200	0.0000	0.2500	0.0300	0.0000
Halverson Ck T	100	0.6800	0.0000	0.2900	0.0300	0.0000
Countess Ck T	100	0.7700	0.0000	0.2200	0.0100	0.0000
Chenega Ck T	100	0.7300	0.0000	0.2300	0.0350	0.0050
Totemoff Ck T	100	0.7350	0.0000	0.2350	0.0300	0.0000
Erb Ck T	100	0.7050	0.0000	0.2700	0.0250	0.0000
Mink Ck T	100	0.6650	0.0000	0.3000	0.0350	0.0000
Mink Ck U	100	0.6200	0.0000	0.3250	0.0500	0.0050
Swanson Ck T	100	0.7450	0.0000	0.2400	0.0150	0.0000
Coghill R. T	100	0.7100	0.0000	0.2650	0.0250	0.0000
Jonah Ck T	96	0.6823	0.0000	0.2865	0.0312	0.0000
Solomon Gulch Hatchery	100	0.7050	0.0000	0.2600	0.0350	0.0000
Duck R. T	100	0.7200	0.0000	0.2650	0.0100	0.0050
Millard Ck T	100	0.6950	0.0000	0.2800	0.0250	0.0000
Lagoon Ck T	100	0.6850	0.0000	0.2650	0.0500	0.0000
Lagoon Ck U	97	0.5773	0.0000	0.3196	0.1031	0.0000
Olsen Ck T	100	0.7250	0.0000	0.2450	0.0250	0.0050
Olsen Ck U	100	0.6750	0.0050	0.2700	0.0450	0.0050
Koppen Ck T	100	0.6850	0.0000	0.2900	0.0250	0.0000
Koppen Ck U	100	0.7100	0.0000	0.2750	0.0150	0.0000
Humpback Ck T	100	0.6650	0.0000	0.2850	0.0500	0.0000
Hartney Ck T	100	0.6850	0.0050	0.2850	0.0200	0.0050
Constantine Ck T	93	0.6935	0.0000	0.2796	0.0269	0.0000
Constantine Ck U	97	0.7062	0.0000	0.2577	0.0361	0.0000

Appendix B. Continued.

Population	N	PGM-2* 100	155	25	178
Rocky Ck T	100	1.0000	0.0000	0.0000	0.0000
Armin F. Koernig Hatchery	100	0.9950	0.0050	0.0000	0.0000
Cathead Ck T	100	1.0000	0.0000	0.0000	0.0000
Herring Ck T	100	1.0000	0.0000	0.0000	0.0000
Halverson Ck T	100	1.0000	0.0000	0.0000	0.0000
Countess Ck T	100	1.0000	0.0000	0.0000	0.0000
Chenega Ck T	100	1.0000	0.0000	0.0000	0.0000
Totemoff Ck T	100	1.0000	0.0000	0.0000	0.0000
Erb Ck T	100	1.0000	0.0000	0.0000	0.0000
Mink Ck T	100	1.0000	0.0000	0.0000	0.0000
Mink Ck U	100	0.9900	0.0050	0.0000	0.0050
Swanson Ck T	100	0.9950	0.0050	0.0000	0.0000
Coghill R. T	100	1.0000	0.0000	0.0000	0.0000
Jonah Ck T	96	0.9948	0.0000	0.0052	0.0000
Solomon Gulch Hatchery	100	1.0000	0.0000	0.0000	0.0000
Duck R. T	100	0.9950	0.0050	0.0000	0.0000
Millard Ck T	100	1.0000	0.0000	0.0000	0.0000
Lagoon Ck T	100	0.9950	0.0000	0.0050	0.0000
Lagoon Ck U	98	0.9949	0.0051	0.0000	0.0000
Olsen Ck T	100	0.9950	0.0050	0.0000	0.0000
Olsen Ck U	100	1.0000	0.0000	0.0000	0.0000
Koppen Ck T	100	0.9850	0.0100	0.0000	0.0050
Koppen Ck U	100	1.0000	0.0000	0.0000	0.0000
Humpback Ck T	94	1.0000	0.0000	0.0000	0.0000
Hartney Ck T	100	0.9850	0.0100	0.0050	0.0000
Constantine Ck T	93	1.0000	0.0000	0.0000	0.0000
Constantine Ck U	100	0.9950	0.0050	0.0000	0.0000

Appendix B. Continued.

Population	N	*mSOD** *100*	*185*	*118*	*16*	*54*
Rocky Ck T	100	1.0000	0.0000	0.0000	0.0000	0.0000
Armin F. Koernig Hatchery	100	0.9900	0.0100	0.0000	0.0000	0.0000
Cathead Ck T	100	0.9900	0.0000	0.0000	0.0100	0.0000
Herring Ck T	100	0.9950	0.0050	0.0000	0.0000	0.0000
Halverson Ck T	99	0.9949	0.0000	0.0000	0.0051	0.0000
Countess Ck T	100	0.9900	0.0100	0.0000	0.0000	0.0000
Chenega Ck T	100	0.9650	0.0200	0.0000	0.0150	0.0000
Totemoff Ck T	99	1.0000	0.0000	0.0000	0.0000	0.0000
Erb Ck T	100	0.9900	0.0100	0.0000	0.0000	0.0000
Mink Ck T	100	0.9900	0.0100	0.0000	0.0000	0.0000
Mink Ck U	100	0.9950	0.0050	0.0000	0.0000	0.0000
Swanson Ck T	99	1.0000	0.0000	0.0000	0.0000	0.0000
Coghill R. T	100	0.9850	0.0150	0.0000	0.0000	0.0000
Jonah Ck T	96	0.9948	0.0052	0.0000	0.0000	0.0000
Solomon Gulch Hatchery	100	0.9950	0.0000	0.0050	0.0000	0.0000
Duck R. T	100	0.9750	0.0200	0.0000	0.0000	0.0050
Millard Ck T	100	0.9600	0.0300	0.0050	0.0050	0.0000
Lagoon Ck T	100	0.9850	0.0100	0.0000	0.0050	0.0000
Lagoon Ck U	98	0.9949	0.0051	0.0000	0.0000	0.0000
Olsen Ck T	100	0.9950	0.0050	0.0000	0.0000	0.0000
Olsen Ck U	100	0.9900	0.0100	0.0000	0.0000	0.0000
Koppen Ck T	100	0.9850	0.0150	0.0000	0.0000	0.0000
Koppen Ck U	100	0.9900	0.0100	0.0000	0.0000	0.0000
Humpback Ck T	100	1.0000	0.0000	0.0000	0.0000	0.0000
Hartney Ck T	100	0.9900	0.0100	0.0000	0.0000	0.0000
Constantine Ck T	93	0.9839	0.0108	0.0000	0.0054	0.0000
Constantine Ck U	100	0.9950	0.0050	0.0000	0.0000	0.0000

Appendix B. Continued.

Population	N	sSOD-1* 100	176	15	120	140	N	TPI-2* -100	110
Rocky Ck T	100	0.9900	0.0000	0.0000	0.0100	0.0000	100	0.9800	0.0200
Armin F. Koernig Hatchery	100	0.9850	0.0000	0.0050	0.0100	0.0000	100	0.9850	0.0150
Cathead Ck T	100	0.9950	0.0000	0.0000	0.0050	0.0000	100	1.0000	0.0000
Herring Ck T	100	0.9900	0.0000	0.0100	0.0000	0.0000	100	0.9950	0.0050
Halverson Ck T	100	0.9700	0.0000	0.0100	0.0150	0.0050	100	0.9900	0.0100
Countess Ck T	100	0.9850	0.0050	0.0050	0.0050	0.0000	100	0.9900	0.0100
Chenega Ck T	100	0.9850	0.0050	0.0000	0.0100	0.0000	100	0.9800	0.0200
Totemoff Ck T	99	1.0000	0.0000	0.0000	0.0000	0.0000	100	0.9850	0.0150
Erb Ck T	100	0.9850	0.0050	0.0000	0.0100	0.0000	100	0.9850	0.0150
Mink Ck T	100	0.9950	0.0050	0.0000	0.0000	0.0000	100	0.9850	0.0150
Mink Ck U	100	1.0000	0.0000	0.0000	0.0000	0.0000	100	0.9950	0.0050
Swanson Ck T	100	0.9950	0.0000	0.0000	0.0050	0.0000	100	0.9600	0.0400
Coghill R. T	100	0.9850	0.0000	0.0000	0.0050	0.0100	100	0.9850	0.0150
Jonah Ck T	96	0.9896	0.0000	0.0000	0.0104	0.0000	96	1.0000	0.0000
Solomon Gulch Hatchery	100	0.9900	0.0000	0.0100	0.0000	0.0000	100	0.9850	0.0150
Duck R. T	100	0.9850	0.0000	0.0050	0.0100	0.0000	100	0.9900	0.0100
Millard Ck T	100	0.9950	0.0000	0.0000	0.0050	0.0000	100	0.9700	0.0300
Lagoon Ck T	100	1.0000	0.0000	0.0000	0.0000	0.0000	100	0.9950	0.0050
Lagoon Ck U	98	1.0000	0.0000	0.0000	0.0000	0.0000	98	0.9949	0.0051
Olsen Ck T	100	0.9850	0.0000	0.0050	0.0100	0.0000	100	0.9900	0.0100
Olsen Ck U	100	1.0000	0.0000	0.0000	0.0000	0.0000	100	0.9750	0.0250
Koppen Ck T	100	0.9850	0.0000	0.0000	0.0150	0.0000	100	0.9850	0.0150
Koppen Ck U	100	0.9850	0.0000	0.0050	0.0100	0.0000	100	0.9800	0.0200
Humpback Ck T	100	0.9950	0.0050	0.0000	0.0000	0.0000	100	0.9750	0.0250
Hartney Ck T	100	0.9900	0.0000	0.0000	0.0100	0.0000	100	0.9900	0.0100
Constantine Ck T	93	0.9946	0.0000	0.0000	0.0054	0.0000	93	0.9677	0.0323
Constantine Ck U	100	0.9950	0.0000	0.0000	0.0050	0.0000	100	0.9750	0.0250

Appendix B continued

Source of Variation	DF	*GPI-B1,2*	DF	*IDDH-1*	DF	*mIDHP-1*	DF	*sIDHP-2*	DF	*LDH-A2*	DF	*LDH-B2*	DF	*sMDH-A1,2*	DF	*sMDHB-1,2*
Between Sources	2	0.57	1	0.00	2	1.90	1	0.02	1	1.25	2	3.36	3	0.96	2	0.24
Within Sources	50	43.28	25	25.36	50	45.83	25	48.11	25	32.01	50	40.09	75	54.43	50	71.86
Wild	48	41.20	24	24.01	48	44.45	24	47.28	24	32.01	48	38.71	72	53.26	48	70.28
Between elevations	2	0.12	1	0.20	2	0.91	1	3.97	1	3.59	2	0.65	3	2.82	2	5.55
Within elevations	46	41.08	23	23.81	46	43.54	23	43.31	23	28.42	46	38.06	69	50.44	46	64.73
Upstream	8	9.64	4	6.49	8	6.29	4	18.92	4	0.00	8	8.90	12	8.01	8	14.03
Among Regions	4	6.81	2	6.49	4	2.47	2	9.50	2	0.00	4	7.43	6	7.56	4	11.92
Within Regions	4	2.83	2	0.00	4	3.82	2	9.42	2	0.00	4	1.47	6	0.45	4	2.11
East	4	2.83	2	0.00	4	3.82	2	9.42	2	0.00	4	1.47	6	0.45	4	2.11
Tidal	38	31.44	19	17.32	38	37.25	19	24.39	19	28.42	38	29.16	57	42.43	38	50.70
Among Regions	8	3.43	4	2.16	8	10.93	4	3.54	4	11.86	8	7.47	12	12.85	8	12.07
Within Regions	30	28.01	15	15.16	30	26.32	15	20.85	15	16.56	30	21.69	45	29.58	30	38.63
Southwest	12	10.31	6	7.09	12	14.42	6	6.32	6	13.83	12	5.61	18	15.67	12	20.54
North	6	3.10	3	2.90	6	1.75	3	3.64	3	2.73	6	13.74	9	9.60	6	1.55
East	12	14.60	6	5.17	12	10.15	6	10.89	6	0.00	12	2.34	18	4.31	12	16.54
Hatchery	2	2.08	1	1.35	2	1.38	1	0.83	1	0.00	2	1.38	3	1.17	2	1.58

Source of Variation	DF	*mMEP-1*	DF	*NTP*	DF	*PEPB-1*	DF	*PEPD-2*	DF	*PEPLT*	DF	*PGDH*	DF	*PGM-2*	DF	*mSOD*
Between Sources	1	1.33	1	0.59	2	0.63	2	2.02	2	1.99	2	1.10	1	0.04	2	2.02
Within Sources	25	33.22	25	20.70	50	58.38	50	49.02	50	73.40	50	66.84	25	25.20	50	66.38
Wild	24	32.22	24	20.70	48	54.77	48	48.77	48	72.05	48	66.35	24	23.82	48	63.61
Between elevations	1	9.21	1	1.97	2	7.74	2	3.14	2	5.79	2	14.86	1	0.55	2	4.35
Within elevations	23	23.01	23	18.73	46	47.03	46	45.63	46	66.26	46	51.49	23	23.27	46	59.26
Upstream	4	5.11	4	0.00	8	7.34	8	16.79	8	15.99	8	22.59	4	3.08	8	0.82
Among Regions	2	1.74	2	0.00	4	1.31	4	12.48	4	7.99	4	3.67	2	0.86	4	0.41
Within Regions	2	3.37	2	0.00	4	6.03	4	4.31	4	8.00	4	18.92	2	2.22	4	0.41
East	2	3.37	2	0.00	4	6.03	4	4.31	4	8.00	4	18.92	2	2.22	4	0.41
Tidal	19	17.90	19	18.73	38	39.69	38	28.84	38	50.27	38	28.90	19	20.19	38	58.44
Among Regions	4	12.72	4	2.12	8	5.05	8	7.71	8	15.31	8	5.66	4	10.11	8	14.62
Within Regions	15	5.18	15	16.61	30	34.64	30	21.13	30	34.96	30	23.24	15	10.08	30	43.82
Southwest	6	3.45	6	7.41	12	13.64	12	6.89	12	13.03	12	7.63	6	0.00	12	23.42
North	3	0.23	3	5.47	6	2.49	6	2.20	6	10.56	6	4.41	3	2.75	6	4.45
East	6	1.50	6	3.73	12	18.51	12	12.04	12	11.37	12	11.20	6	7.33	12	15.95
Hatchery	1	1.00	1	0.00	2	3.61	2	0.25	2	1.35	2	0.49	1	1.38	2	2.77

Appendix B continued

Source of Variaton	DF	*sSOD-1*	DF	*TPI-2*	DF	Overall	*P*-value
Between Sources	3	4.45	1	0.00	56	55.13	0.50778
Within Sources	75	69.22	25	36.25	1400	1750.02	0.00000
Wild	72	66.11	24	36.25	1344	1691.30	0.00000
Between elevations	3	3.67	1	0.05	56	126.50	0.00000
Within elevations	69	62.44	23	36.20	1288	1564.80	0.00000
Upstream	12	9.05	4	6.05	224	356.90	0.00000
Among Regions	6	2.47	2	3.00	112	184.10	0.00002
Within Regions	6	6.58	2	3.05	112	172.80	0.00020
East	6	6.58	2	3.05	112	172.80	0.00020
Tidal	57	53.39	19	30.15	1064	1207.90	0.00134
Among Regions	12	11.72	4	5.42	224	289.50	0.00207
Within Regions	45	41.67	15	24.73	840	918.40	0.03057
Southwest	18	20.47	6	7.01	336	402.80	0.00717
North	9	8.37	3	11.26	168	175.10	0.33788
East	18	12.83	6	6.46	336	340.50	0.42132
Hatchery	3	3.11	1	0.00	56	58.72	0.37609

Chapter 3

95320E Juvenile Salmon and Herring Integration

Exxon Valdez Oil Spill
Restoration Project Annual Report

Sound Ecosystem Assessment: Juvenile Salmon & Herring Integration

Restoration Project 95320E
Annual Report

This annual report has been prepared for peer review as part of the *Exxon Valdez* Oil spill Trustee Council Restoration program for the purpose of assessing project progress. Peer review comments have not been addressed in this annual report.

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April 5, 1996

Study History: This project was initiated under Restoration project 94320E. An annual report was issued in 1994 by Willette, M., E. Debevec, Jay Johnson under the title Sound Ecosystem Assessment: Salmon Predation. The project effort was continued under Restoration Project 95320E, the subject of this annual report. In 1996, this project will be merged with project 96320A. A final report will be prepared for both projects in FY98.

Abstract: This project is a component of the Sound Ecosystem Assessment program. The project collected data needed to test several hypotheses related to predator-prey interactions affecting the mortality of pink salmon (*Oncorhynchus gorbuscha*) in Prince William Sound. Diel studies were conducted at sixteen study sites during four sampling periods in both May and June, 1995. As in 1994, Age 3+ pollock and herring were found to be important predators on juvenile pink salmon in pelagic habitats. However, sampling with various kinds of fixed gear revealed that age 1-2 pollock, tomcod and benthic fishes (greenlings and sculpins) may be important predators on juvenile salmon in nearshore habitats. It was also apparent that significant interannual variability occurs in seasonal patterns of fish predator abundance and diet composition. We were somewhat surprised to find that the strength of tidal currents in narrow passages may affect the distribution, feeding rate, and diet composition of age 3+ pollock. A period of peak tidal range in mid-May was related to a marked decline in pollock catch per effort in the 0-40m layer, a sharp reduction in stomach fullness, and a shift in diet composition from large copepods to pteropods. Analysis of coded-wire tag data from the 1995 pink salmon return indicated that survival to adult was related to tidal range at the time fry were released from the Wally H. Noerenberg Hatchery in 1994. This result suggests that our observations of pollock distribution and feeding related to tidal range may have important ramifications for pink salmon survival.

Key Words: *Exxon Valdez*, pink salmon, *Oncorhynchus gorbuscha*, Pacific herring, *Clupea pallasi*, walleye pollock, *Theragra chalcogramma*, Pacific tomcod, *Microgadus proximus*, mortality, predation, food habits.

Citation: Willette, M., M. Clapsadl, P. Saddler, M. Powell. 1995. Sound ecosystem assessment: salmon and herring integration. Exxon Valdez oil spill restoration project annual report (Restoration project 95320E), Alaska Department of Fish and Game, Cordova, Alaska.

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Executive Summary

This project is a component of the Sound Ecosystem Assessment (SEA) program. SEA is a multi-disciplinary effort to acquire an ecosystem-level understanding of the marine and freshwater processes that interact to constrain levels of pink salmon and herring production in Prince William Sound (PWS). Pink salmon runs to PWS failed in 1992 and 1993, and herring biomass dropped sharply in 1993. These run failures have drastically affected the economy of the PWS region which is largely based on the salmon and herring resources. This project collected data needed to test several hypotheses related to predator-prey interactions affecting the mortality of pink salmon (*Oncorhynchus gorbuscha*) in PWS. Several other projects within SEA also contribute to this hypothesis testing effort. These hypotheses include the following concepts (1) predation on juvenile salmon and other age 0 fish is inversely related to the abundance of large calanoid copepods, (2) predation risk is related to the daily foraging times of juvenile salmon, and (3) spatial patterns of adult pink salmon production are related to the distribution of large calanoid copepods and walleye pollock during the early marine period. This project was designed to achieve the following objectives: (1) estimate the juvenile salmon consumption rate of fish predators at 16 study sites in northwest PWS, (2) estimate the species/size composition of fish predators at 16 study sites in northwest PWS, (3) conduct preliminary tests of predator/prey hypotheses, (4) examine the relationship between the abundance and diet composition of walleye pollock and tidal range, and (5) estimate the proportion of the diet comprised of juvenile herring as well as total annual consumption of herring for several important fish predators in PWS.

Diel studies were conducted at four study sites during each of four time periods in northwest PWS. Each nearshore study site consisted of an approximately 3000 m long segment of shoreline. Fish sampling was generally conducted at 4 stations located along 3-4 transects perpendicular to the shoreline every 3 hours throughout a 24-hour period. Four vessels were employed to sample fish predators. An approximately 25 m vessel sampled fish in offshore areas using a mid-water wing trawl. Two purse seine vessels sampled fish in the upper 20 m of the water column in nearshore areas with bottom depths greater than 20 m. In nearshore areas shallower than 20 m, variable mesh sinking gill nets, hoop traps, and fyke nets were used to sample fish predators.

As in 1994, age 3+ pollock and herring were found to be important predators on juvenile pink salmon in pelagic habitats. However, sampling with various kinds of fixed gear revealed that age 1-2 pollock, Pacific tomcod and benthic fishes (greenlings and sculpins) may be important predators on juvenile salmon in nearshore habitats. It was also apparent that significant interannual variability occurs in seasonal patterns of fish predator abundance and diet composition. We were somewhat surprised to find that the strength of tidal currents in narrow passages may affect the distribution, feeding rate, and diet composition of age 3+ pollock. A period of peak tidal range in mid-May was related to a marked decline in pollock catch per effort in the 0-40m layer, a sharp reduction in stomach fullness, and a shift in diet

composition from large copepods to pteropods. Analysis of coded-wire tag data from the 1995 pink salmon return indicated that survival to adult was related to tidal range at the time fry were released from the Wally H. Noerenberg Hatchery in 1994. This result suggests that our observations of pollock distribution and feeding related to tidal range may have important ramifications for pink salmon survival.

Introduction:

This project is a component of the Sound Ecosystem Assessment (SEA) program. SEA is a multi-disciplinary effort to acquire an ecosystem-level understanding of the marine and freshwater processes that interact to constrain levels of pink salmon and herring production in Prince William Sound (PWS). Pink salmon runs to PWS failed in 1992 and 1993, and herring biomass dropped sharply in 1993. These run failures have drastically affected the economy of the PWS region which is largely based on the salmon and herring resources. In 1992, pink salmon returns were low in Kodiak, Lower Cook Inlet, and PWS, but pink salmon returns in 1993 were low only in PWS. Low returns of hatchery-produced salmon in both years indicates that the failures were likely caused by processes occurring during the juvenile lifestage. Damage assessment studies on juvenile pink salmon in PWS have demonstrated that growth during the juvenile lifestage is related to survival to adult (Willette et al. 1994). Growth rates of juvenile salmon were estimated in 1991 and 1992 after the fish were released from hatcheries. Juvenile growth and ocean temperatures were low in PWS during the early marine period in 1991. However, in 1992 juvenile growth and ocean temperatures were near average; although, zooplankton abundance was very low. The growth of juvenile fishes is believed to be related to survival, because slow-growing individuals are vulnerable to predators for a longer time (Parker 1971; Healey 1982; West and Larkin 1987). The growth and mortality rates of juvenile salmon released into PWS in 1992 suggests that a change in predation rate may have contributed to the observed run failures.

This is a multi-year project designed to test several hypotheses regarding mechanisms that may regulate predation on juvenile salmon and other age-0 fish in PWS. Regulation of prey population size by a predator requires that prey mortality rate increase with prey population size (i.e density-dependent mortality; Holling 1959). Intense predation immediately after ocean entry may have contributed to poor survival of relatively large release groups of hatchery-reared coho salmon (Bayer 1986, Olla and Davis 1989, Pearcy 1992). Learned behavior or response to environmental cues may cause predators to aggregate in areas where prey are consistently abundant (Ware 1971, Godin 1978). Alternatively, predation on a prey population may increase when the preferred prey of potential predators is not available (Werner and Hall 1974, Ringler 1979, Winfield et al. 1983). In the northern Gulf of Alaska, predators such as juvenile walleye pollock (Armstrong and Winslow 1968) that prefer macrozooplankton (Clausen 1983, Dwyer et al. 1987, Bailey 1989) may switch to age-0 fish when macrozooplankton abundance is low. Macrozooplankton abundance was very low in PWS in 1992 indicating that predators may have switched to juvenile salmon.

The daily foraging time of juvenile salmon and other age-0 fish may be related to predation risk (Walters and Juanes 1993). Juvenile salmon spend much of their time in nearshore nursery habitats (Cooney et al. 1981) that likely provide a refuge from predation. This behavior is common among juvenile fish, and often juveniles must move out of the predation refuge to feed in areas where food abundance is greater (Helfman 1993). The amount of time spent feeding is likely related to food abundance and particularly the abundance of large

calanoid copepods (Parsons and LeBrasseur 1973, Willette et al. 1994). High abundances of juvenile salmon or other age-0 fish may also lead to competition and increased foraging time and predation risk.

This project will provide data needed to test several of the predator\prey hypotheses posed by SEA investigators (See chapter 95320A, Salmon Growth and Mortality).

Objectives:

Original objectives in detailed project description for 95320E:

1. Estimate the juvenile salmon consumption rate of fish predators at 16 study sites in northwest PWS.

2. Estimate the species/size composition of fish predators at 16 study sites in northwest PWS.

3. Conduct preliminary tests of predator/prey hypotheses.

Objectives added as result of new information obtained in FY95:

4. Examine the relationship between the abundance and diet composition of walleye pollock and tidal range.

5. Estimate the proportion of the diet comprised of juvenile herring as well as total annual consumption of herring for several important fish predators in PWS.

Methods:

This study examined the relationship between the daily foraging time of juvenile pink salmon and predation risk modulated by changes in prey abundance and juvenile salmon abundance. This approach required sampling at a number of sites exhibiting a range of both prey abundance and juvenile salmon abundance. Four nearshore study sites were sampled in western PWS during each of four time periods. In FY95, we also sampled at three sites thought to be juvenile herring rearing areas during each of two time periods in southeast PWS (Figure 1). The large releases of juvenile salmon from the Wally H. Noerenberg (WHN) Hatchery provide an opportunity to investigate processes regulating the growth and mortality of juvenile pink salmon. Comparison of survival rates of hatchery-reared salmon and return per spawner of wild salmon suggests that similar mortality processes may be affecting both groups (See chapter 95320A, Salmon Growth and Mortality). Results from the first year of

this project indicate that similar process-oriented studies may not be feasible in areas of much lower juvenile salmon abundance due to difficulties in measuring juvenile salmon consumption rates of fish predators (Willette et al. 1995b).

Objective 1:

Identification of the principal fish predators on juvenile salmon requires estimation of the juvenile salmon consumption rate for each potential predator species. Fish biomass, food consumption rate (daily ration), and diet composition must be estimated for each potential predator species to estimate juvenile salmon consumption rate. Project 95320N estimated fish biomass using hydroacoustic techniques. Project 95320E estimated predator species/size composition, food consumption rate, and diet composition.

Diel studies were conducted at four study sites during each of four time periods in northwest PWS. Each nearshore study site consisted of an approximately 3000 m long segment of shoreline. Fish sampling was generally conducted at 4 stations located along 3-4 transects perpendicular to the shoreline every 3 hours throughout a 24-hour period (Figure 2). Four vessels were employed to sample fish predators. An approximately 25 m trawl vessel sampled fish in offshore areas using a 40 m x 28 m mid-water wing trawl equipped with a net sounder. The cod end of the trawl was lined with 1.5 cm stretch-mesh web to retain small specimens. Each tow was made approximately 1 km offshore parallel to the shore in the upper 40 m of the water column. At the first 4 study sites, a pair trawl (30 m x 30 m; codend 1.5 cm stretch mesh) was also used to sample fish in the upper 40 m of the water column. The pair trawl was fished using two approximately 15 m vessels working in tandem. Data from the pair trawl was used to evaluate the selectivity of the mid-water trawl. Two purse seine vessels sampled fish in the upper 20 m of the water column in nearshore areas with bottom depths greater than 20 m (stations 2-3). Each seiner fished a small-mesh purse seine (250 m x 30 m, 1.5 cm stretch mesh web) holding a hook with the seine open in the direction of the prevailing current for 20 minutes. In nearshore areas shallower than 20 m, variable mesh sinking gill nets (150 m, 1.5 cm to 10 cm stretch mesh), hoop traps (1.5 m diameter, 1.5 cm stretch mesh), and fyke nets (1.5 m diameter, 1.5 cm stretch mesh in codend) were used to sample fish predators. These gear were deployed from an approximately 6 m aluminum skiff at the station nearest to shore (station 1) along each transect. A hotel boat provided room and board for field sampling crews. All sample processing was conducted on board the hotel boat by a single processing crew.

Processing of fish samples from each net set occurred in two stages following procedures outlined by Livingston (1989) and Dwyer et al. (1987). If less than 300 fish were captured, all fish in the catch were enumerated by species. If a large number of fish were caught, species composition was estimated from a random sample of 300 individuals. Fish greater than 150 mm FL were processed differently than those less than 150 mm FL.

Fish less than 150 mm FL were identified to the lowest possible taxonomic level. A sample of 30 individuals from each species was preserved in 10% buffered formaldehyde for later

analysis of stomach contents under project 95163 (Forage Fish Influence on Recovery of Injured Species). The purpose of these studies is to examine diet overlap among forage fish.

For large fish (greater than 150 mm FL), a randomly selected sample (n=60) from each net set and each species was taken. The stomach was excised, placed in a cloth bag, and preserved in 10% buffered formaldehyde for later analysis of stomach contents. Fish showing evidence of regurgitation were not included in the sample. Fork length was measured to the nearest millimeter. Weight was measured to the nearest gram when conditions permitted. Sex and sexual maturity was recorded. Later in the laboratory, total stomach contents wet weight was measured to the nearest .01 gram. Invertebrate prey in the gut were generally identified to the family level. Fish in the gut were identified to the lowest possible taxonomic level, enumerated, and measured to the nearest millimeter. The proportion of total stomach contents in each taxonomic group was visually estimated. Stomach fullness was expressed as a proportion of fish body weight. In cases where distinct size classes occurred within species, stomach contents analysis was conducted for each size class as described above. Size related shifts in diet toward piscivory have been noted in several species of gadoid fishes, including Pacific cod (*Gadus macrocephalus*) (Livingston 1989), walleye pollock (*Theragra chalgogramma*) (Dwyer et al. 1987), Atlantic cod (*Gadus morhua*) (Daan 1973), Pacific whiting (*Merluccius productus*) (Livingston 1983), and silver hake (*Merluccius bilinearis*) (Langton 1982).

Techniques developed by Mehl and Westgard (1983) were used to develop at preliminary order-of-magnitude estimate the juvenile salmon consumption rate of pelagic pollock in northwest PWS in 1994, i.e.

$$C_i = DR \times B_i \times P_i \qquad (1)$$

where C_i is the consumption (grams) of juvenile salmon by pollock during each ten-day sampling period (i), DR is the daily ration (% body weight per day), B_i is the biomass (grams) of pollock during sampling period (i), and P_i is the proportion by weight of juvenile salmon in pollock stomachs during sampling period (i). Acoustic biomass estimates for pelagic pollock were provided by project 95320N. No biomass data was available for the first sampling period in late April, so the biomass estimate from early May was used. Target strength and net catch data indicated that the majority of pelagic pollock were age 3+ fish greater than 40 cm in length. It was assumed that the daily ration of these age 3+ pollock was 0.6 % body weight per day (Lang et al. 1991). Lang et al. (1991) estimated daily ration from annual growth increments of pollock in the eastern Bering Sea and gross growth conversion efficiency. Comparison of size-at-age for pollock in the eastern Bering Sea and PWS indicated that the growth rates of the two stocks were similar.

An analysis of variance was conducted to test for differences in the mean percent of predator diets comprised of juvenile salmon among four time periods in May and June, 1995. Data were arcsin square root transformed prior to conducting the test (Zar 1984). Due to small sample sizes, Pacific cod (*Gadus macrocephalus*) and Pacific tomcod (*Microgadus proximus*)

were pooled in the analysis, as well as, several species of sculpins and greenlings (*Hemilepidotus hemilepidotus, Myxocephalus verrucosus, Hexagrammos decagrammus, Hexagrammos octogrammus, Blepsias bilobus*). All specimens were initially included in the analysis to examine changes in diet for the population within each taxonomic group as a whole. The analysis was also conducted with only specimens found to consume juvenile salmon included. This was done to examine whether individual predators were targeting juvenile salmon. The percent frequency of occurrence of juvenile salmon in predator diets was also calculated to determine the proportion of the population within each taxonomic group that was consuming juvenile salmon. An analysis of variance was conducted to test for differences in mean stomach fullness of juvenile salmon predators among four time periods. Data were arcsin square root transformed prior to conducting the test (Zar 1984). Finally, an analysis of variance was conducted to test for changes in the mean catch per net set of juvenile salmon predators among four time periods. This was done to evaluate differences in the relative abundance of fish predators over time. Data were generally square-root transformed prior to conducting the test. However, trawl data was expressed as natural-logarithm of catch per hour of tow.

Objective 2:

The species/size composition of the fish predators in each study area was estimated from net samples collected as described in objective 1. Species/size composition data from net samples will be used by the Nearshore Fish component of SEA to estimate the species/size composition of hydroacoustic targets. As much as possible, net sampling was paired with hydroacoustic sampling for this purpose. On board each sampling vessel, all fish were identified to the lowest possible taxonomic level. If a large number of fish were caught, species composition was estimated from a random sample of 300 individuals. Length was measured for a randomly selected subsample (n=60) from each species in the catch.

Objective 3:

Sub-hypothesis I-1.

The juvenile salmon consumption rate of age 1+ walleye pollock during the initial 30 days of marine residence was estimated as described in objective 1. An estimate of the juvenile salmon consumption rate of seabirds was obtained from the project 95320Y. The two estimates were summed to evaluate the relative magnitude of juvenile salmon consumption by these two taxonomic groups.

Sub-hypothesis I-2.

Deferred until FY96 when abundance estimates will be available for herring and adult salmon.

Sub-hypothesis II-1.

An analysis of covariance was conducted to test for a relationship between the mean proportion of the diet comprised of large calanoid copepods and the mean proportion of the diet comprised of age-0 fish at each sampling site. Analysis of covariance was also used test for a relationship between the mean proportion of the diet comprised of large calanoid copepods and the mean abundance of large calanoid copepods at each sampling site. Regression analysis was employed to estimate the parameters of the relationships. Diet composition was estimated as described in objective 1. Diet proportions were arcsin square root transformed (Zar 1984). Methods used to collect and analyze zooplankton samples are described in chapter 95320A (Salmon growth and mortality). Only fish species found to consume juvenile salmon in 1995 were used in the analysis.

Sub-hypothesis II-2.

Analysis of variance was employed to test for differences in the mean proportion of juvenile salmon in predator diets in relation to the mean relative abundance (low: <50 m^{-3} , moderate: >50 m^{-3} and <100 m^{-3}, and high: >100 m^{-3}) abundance of large calanoid copepods at each sampling site. Methods used to collect and analyze zooplankton samples are described in chapter 95320A (Salmon growth and mortality). Diet composition was estimated as described in objective 1. Diet proportions were arcsin square root transformed. Only fish species found to consume juvenile salmon in 1995 were used in the analysis.

Sub-hypothesis II-3.

The proportion of juvenile salmon less than 60 mm FL was estimated from measurements of juvenile salmon in predator stomachs. Chi-square analysis was conducted to test for a difference in length frequencies between juvenile salmon found in predator stomachs and those captured alive during each sampling period. The length frequency proportions for the live fry were subtracted from the length frequency proportions for the prey fry in each length interval to evaluate the nature of selectivity.

Sub-hypotheses II-4 and II-5.

See chapter 95320A, Salmon Growth and Mortality.

Sub-hypothesis III-1.

Analysis of variance was employed to test for differences in catch per unit effort of predators in nearshore nursery habitats during four sampling periods in May and June, 1995. Only fish species known to consume juvenile salmon were included in the analysis. Net set was used as the sample unit in the analysis.

Sub-hypothesis III-2.

Deferred until FY96 when ocean temperature, acoustic and net catch data will be integrated.

Sub-hypotheses III-3 through III-5.

See chapter 95320A, Salmon Growth and Mortality.

Sub-hypothesis III-6.

Deferred until FY96 when estimates of daily foraging time of juvenile salmon will be available (See chapter 95320A, Salmon growth and mortality).

Sub-hypothesis IV-1.

See chapter 95320A, Salmon Growth and Mortality.

Sub-hypotheses IV-2 and IV-3.

Deferred until FY96 when otolith thermal marked juvenile pink salmon will be released from all PWS hatcheries.

Hypothesis V.

Deferred until FY96 when the spatial pattern of adult pink salmon production from the 1995 outmigration will be known.

Objective 4:

The relationship between tidal range and seasonal changes in the relative abundance of pollock in nearshore and offshore habitats was examined in a time series plot. Catch per hour of tow in mid-water trawl gear was used to estimate changes in relative abundance of pollock in offshore habitats. The mid-water trawl was generally fished in the upper 40 m of the water column. Catch per net set in purse seines and fixed gear was used to estimate changes in relative abundance of pollock in nearshore habitats. Catch per effort data were natural-logarithm transformed. Tide data was obtained from software provided by NOAA. A regression analysis was conducted to estimate the parameters of the relationship between tidal range and natural logarithm of pollock catch per hour of tow in offshore habitats. Seasonal changes in pollock stomach fullness and diet composition were examined in time series plots. Methods used to estimate stomach fullness and diet composition are described under objective 1. Finally, a regression analysis was conducted to estimate the parameters of the relationship between tidal range and fry-to-adult survival for juvenile pink salmon released from the WHN Hatchery in 1994. The tidal range on the date of fry release was the independent variable in the analysis.

Objective 5:

The percent of the diet comprised of juvenile herring was estimated from analyses of stomach contents of fish predators as described under objective 1. Estimates were derived for northwest and southwest PWS in 1994 and northwest and southeast PWS in 1995. The sites sampled in southeast PWS in 1995 were thought to be rearing areas for herring. The other sites were selected primarily to examine predation processes affecting juvenile salmon. Equation 1 was used to estimate the annual consumption of herring by pollock, arrowtooth flounder (*Atheresthes stomias*), and Pacific cod in PWS. Biomass estimates for these fish predators were obtained from a bottom trawl survey conducted in PWS in 1989. The proportion of the diet comprised of herring for these fish predators was obtained from Yang (1993), and estimates of daily ration were obtained from Lang et al. (1991).

Results:

Objective 1:

Prelimnary estimates of consumption of juvenile salmon by walleye pollock indicated that approximately 5 million salmon may have been consumed prior to the middle of June, 1994 (Table 1). Assuming that all of the juvenile salmon consumed were from the WHN Hatchery, this total consumption accounts for about 3% of the 153 million juveniles released from WHN Hatchery.

The mean percent of the diet comprised of juvenile salmon increased significantly ($P<.001$) over time for age 1-2 pollock and decreased significantly ($P=.041$) over time for age 3+ pollock (Table 2). There was a marginally significant decrease ($P=.063$) in the mean percent of the diet comprised of juvenile salmon for nearshore benthic fish (Table 2). When only specimens consuming juvenile salmon were included in the analysis, there were no significant differences in the mean percent of the diet comprised of juvenile salmon for any of these fish predators (Table 3). The mean percent of the diet comprised of juvenile salmon was greater than 50% for cod, age 1-2 pollock, dolly varden trout, and nearshore benthic fish (Table 3). The percent frequency of occurrence of juvenile salmon in the diet was greatest for age 1-2 pollock, sockeye salmon, and dolly varden trout (Table 4). Significant differences in mean stomach fullness were detected for 5 out of 8 taxonomic groups found to consume juvenile salmon (Table 5). Mean stomach fullness of age 1-2 pollock was greater during early June ($P<.001$) when the percent of juvenile salmon in the diet was high. Stomach fullness of age 3+ pollock and nearshore benthic fish generally increased from early May to mid-June (Table 5). Significant differences in mean catch per net set over time were detected for herring and adult chum salmon (Table 6). Total consumption of juvenile salmon by these fish predators will be estimated in FY96 when acoustic biomass estimates will be available.

Objective 2:

Five of the eight taxonomic groups found to consume juvenile salmon exhibited length modes at about 20 cm (Figure 3). Age 3+ pollock, sockeye salmon, and chum salmon were the only taxonomic groups that generally exceeded 40 cm in length.

Objective 3:

Sub-hypothesis I-1.

Losses of juvenile salmon prior to the middle of June, 1994 may have exceeded 100 million (See chapter 95320A, Salmon growth and mortality). An estimated 2.7-5.9 million juvenile salmon may have been consumed by seabirds near the WHN Hatchery in 1995 (See chapter 95320Y, Variation in local predation rates on hatchery-released fry). Seabirds numbered in the hundreds near the WHN Hatchery in 1995. However, in 1994 we observed perhaps thousands of seabirds (mostly kittiwakes) apparently feeding on fry near the WHN Hatchery. Thus, seabird consumption of juvenile salmon in 1994 may have been as much as ten times greater than in 1995. Pelagic walleye pollock may have consumed approximately 5 million juvenile salmon prior to the middle of June, 1994 (Table 1). However, this is likely a minimum estimate because pollock biomass in the 0-5 m layer and 125-400 m layers was not estimated. In addition, pollock daily ration may be greater if pollock are glut feeding at the surface then descending to depth (Clark and Green 1990). Also, we did not assess pollock biomass or feeding in nearshore areas less than 10 m in deep in 1994. At the present time, data are not sufficient to reject Sub-hypothesis I-1.

Sub-hypothesis II-1.

Results from an analysis of covariance indicated that the intercept and slope of the relationship between the proportion of the diet comprised of age-0 fish and the proportion of the diet comprised of large calanoid copepods was significantly different (P=.001) among the seven species of fish predators included in the analysis. Examination of the parameters of the relationship for each fish species indicated that the proportion of the diet comprised of age-0 fish was significantly related to the proportion of the diet comprised of large calanoid copepods for herring (P<.001) and age 1-2 pollock (P<.001, Table 7). The proportion of the diet comprised of large calanoid copepods was significantly related to the mean abundance of large calanoid copepods at each sampling site for herring, age 1-2 pollock and age 3+ pollock (Table 8). This result supports the hypothesis that herring and age 1-2 pollock switched to feeding on age-0 fish when densities of large calanoid copepods declined. However, for age 3+ pollock other alternative prey were consumed after the seasonal decline in copepod abundance (Figure 9).

Sub-hypothesis II-2.

The mean relative abundance of large copepods at each sampling site was not significantly

related to the proportion of the diet comprised of juvenile salmon for any of the seven species of fish predators included in the analysis (Table 9). This result taken in conjunction with those described under sub-hypothesis II-1 indicates that consumption of juvenile salmon may not be correlated with consumption of age-0 fish in general. This may be because juvenile salmon occupy habitats very near to shore. Predators in these nearshore habitats may experience a different set of alternative prey densities compared to the mean conditions in the area. This is because juvenile salmon occur in dense schools in nearshore habitats, and zooplankton layers in offshore habitats may be disrupted nearshore due to frictional boundary processes (See chapter 95320A: Salmon growth and mortality).

Sub-hypothesis II-3.

The length frequencies of juvenile salmon found in predator stomachs and juvenile salmon captured alive in nearshore nursery habitats were significantly different in the first (P=.001), second (P=.001), third (P=.013), and fourth (P=.005) sampling periods. The length frequency differences indicated that predators generally selected smaller juvenile salmon in all sampling periods (Figure 4). Of the total number of juvenile salmon consumed, 100% were less than 60 mm FL in the first and second time periods (Figure 4). During the third and fourth time periods, 85% were less than 60 mm FL. These results support the hypothesis that predation risk is substantially less for juvenile salmon greater than 60 mm FL. However, we did not sample after the middle of June in 1995 when the mean length of the fry population exceeded 60 mm FL.

Sub-hypothesis III-1.

There were no significant differences in catch per net set for cod, age 1-2 pollock, age 3+ pollock, dolly varden trout, or nearshore benthic predators during four sampling periods in May and June, 1995. Significant differences in mean catch per net set among sampling periods were detected for herring and adult chum salmon (Table 6). No clear seasonal increase in herring catch per net set was detected. This result contrasts with apparent seasonal changes in fish abundance in 1994 (Willette et al. 1995b). These difference in seasonal patterns of abundances may be related to differences in ocean temperature between 1994 and 1995 (Figures 5 & 6). At the present time, we must reject the hypothesis that fish abundances increase substantially from May to June. Rather, it appears that there is significant interannual variability in the timing of these events.

Objective 4:

In 1995, approximately sixty percent of the variance in pollock catch per effort was related to tidal range in offshore habitats of northwest PWS (Figure 7). A peak in tidal range in the middle of May corresponded with a marked decline in pollock catch in offshore habitats. However, pollock catch in nearshore habitats declined only slightly during the period of peak tidal range. Stomach fullness and diet composition of pollock was also apparently related to tidal range. During the period of peak tidal range, stomach fullness declined (Figure 8). Prior

to the period of peak tidal range in mid-May, pollock diets were comprised primarily of large calanoid copepods (Figure 9). During the period of peak tidal range, the proportion of the diet comprised of amphipods and euphausiids increased considerably. After the period of peak tidal range, pollock switched to feeding primarily on pteropods. Consumption of euphausiids peaked again in mid-June coincident with another increase in tidal range and decline in pollock catch. Consumption of age-0 fish was apparently not related to changes in tidal range (Figure 9). However, the proportion of the diet comprised of juvenile salmon declined during the period of peak tidal range. In 1994, survival to adult was significantly related to tidal range on the date each group of fry was released from the WHN Hatchery (Figure 10).

Objective 5:

The percent of the diet comprised of herring was generally very low in both northwest and southwest PWS in 1994 (Table 10). However, 47% of the diet of dolly varden trout was comprised of herring. A similar pattern was observed in 1995 in northwest PWS (Table 11). However, at herring rearing areas in southeast PWS, the percent of the diet comprised of herring was much greater (Table 11). Length frequencies of herring in net catches and those found in predator stomachs indicated that predators likely select juvenile herring less than 170 mm SL (Figure 11). The percent of the diet comprised of herring in southeast PWS was similar to that reported by Yang (1993) for both Pacific cod and pollock (Tables 11 & 12). Estimated annual consumption of herring was greatest for arrowtooth flounder (Table 12).

Discussion:

Sampling with fixed gear in nearshore habitats in 1995 revealed several additional fish species that may be important predators on juvenile salmon. In 1994, fish predators were sampled with a mid-water trawl and purse seines. Age 3+ pollock, herring, adult pink salmon, and dolly varden trout appeared to be important predators on juvenile salmon in 1994 (Willette et al. 1995b). Fixed gear sampling in 1995 consisted of sinking gillnets, hoop traps, and fyke nets deployed on the bottom in nearshore habitats. These gear captured a greater number of age 1-2 pollock, tomcod and a variety of nearshore benthic fishes than was obtained in 1994. The proportion of the diet comprised of juvenile salmon was relatively high for age 1-2 pollock and tomcod (Table 2). Nearshore benthic fish may have consumed a relatively large number of juvenile salmon during early May (Tables 2 & 3). When only individuals found to consume juvenile salmon were included in the analysis, the proportion of the diet comprised of juvenile salmon was relatively high for all three of these taxonomic groups. This result indicates that these fish may at times target juvenile salmon. Bakshtanskiy (1964) concluded that juvenile pollack (*Pollachius virens*) and cod (*Melanogrammus morhua morhua*) were important predators on juvenile pink and chum salmon in the White Sea. He observed that juvenile pink and chum salmon were at times driven from nearshore nursery habitats by large schools of juvenile pollack and cod. We observed a similar event in late May when a large school of juvenile pollock appeared at night and apparently drove juvenile salmon offshore.

Diet data indicated apparent high consumption of juvenile salmon by age 1-2 pollock at that study site (Table 2). The proportion of the diet comprised of juvenile salmon was relatively low for age 3+ pollock and herring (Table 2); however, these species appear to be relatively abundant compared to the other taxonomic groups and their total consumption of juvenile salmon may be high (Tables 4 & 6). Bakshtanskiy (1964, 1965) concluded that predation by herring largely determined survival of juvenile pink and chum salmon in the Barents Sea and White Sea. Dolly varden trout appeared to be an important predator on juvenile salmon in both 1994 and 1995 (Willette et al. 1995b). The juvenile salmon consumption rate of each of these fish predators must be estimated to determine the relative importance of each group. Relative abundance or biomass estimates are needed for each taxonomic group to achieve this objective. It is difficult to accurately assess the relative abundance of the each taxonomic group from net catches due to differences in gear selectivity. Herring were caught primarily in purse seines, age 3+ pollock primarily in a mid-water trawl, and age 1-2 pollock, tomcod and nearshore benthic fish in fixed gear. More detailed analyses of acoustic data and application of video techniques (Collins et al. 1991, Irvine et al. 1991, DeMartini and Ellis 1995) are needed to estimate the relative abundance of each group.

Seasonal changes in fish abundance were very different in 1995 compared to 1994. In 1994, age 3+ pollock were relatively abundant in the 0-40 m layer in offshore habitats in late April and May. In early June, age 3+ pollock abundance in the 0-40 m layer declined, and abundances of herring, salmon, dolly varden trout, etc. increased markedly in nearshore habitats (Willette et al. 1995b). In 1995, catch per effort of age 3+ pollock was not significantly different among four sampling periods (Table 6). Catch per effort of herring was significantly different among sampling periods, but there was not a clear seasonal increase in abundance (Table 6). These differences in seasonal patterns of abundance may be related to differences in ocean temperature between the two years. In 1994, a substantial increase in fish catch in nearshore habitats coincided with a sharp increase in ocean temperature in early June (Figure 5). Ocean temperatures were slightly higher in May and lower in June in 1995 compared with 1994 (Figures 5 & 6). Rogers et al. (1986) noted a substantial seasonal increase in fish species diversity and density in Prince William Sound. In winter, fish distributions shifted further offshore and deeper in the water column (Rogers et al. 1986). Seasonal migrations of fish into deeper water in winter and shallow water in summer are well known (Trout 1957, Alverson 1960, Jean 1964, Heeseen 1983). These seasonal shifts in distribution may be related to temperature, light or food abundance (Laevastu and Hela 1970). Seasonal changes in the vertical distribution and activity patterns of cod have been related to seasonal stratification of the water column (Clark and Green 1990). In the present study, a more detailed analysis of fish distribution and water column structure is needed.

Seasonal changes in the distribution and diet composition of age 3+ pollock appear to be related to food abundance. In May 1994, age 3+ pollock fed primarily on large calanoid copepods in the upper 40m of the water column (Willette et al. 1995b). During June 1994, consumption of large copepods declined, pollock catches in the 0-40m layer declined, and consumption of age-0 fish increased (Willette et al. 1995b). The decline in consumption of large copepods in June coincided with a decline in the biomass of late-stage *Neocalanus spp.*

in the upper 50 m of the water column (Cooney 1995). Increased consumption of age-0 fish coincided with increased net catches of age-0 pollock in all gear types (Willette et al. 1995). In 1995, a different pattern was evident. Age 3+ pollock fed primarily on large copepods in early May, switched to feeding on pteropods in June, and abundances in the 0-40m layer did not decline (Figure 9). It is apparent that choice of pelagic habitats by pollock is strongly affected by the density of zooplankton in the upper layers of the water column. Prey selection is likely a function of the relative profitabilities of alternative prey (Charnov 1976, Mittelbach 1981, Osenberg and Mittelbach 1989). Apparently, large copepods such as *Neocalanus spp.* are not the only zooplankton species that may occur in sufficient densities for profitable exploitation by age 3+ pollock.

Distribution, feeding rate, and diet composition of age 3+ pollock was apparently affected by tidal range in northwest PWS in 1995. During a period of high tidal range in mid-May, pollock catches in the 0-40m layer declined, stomach fullness declined, and diet composition shifted from predominately large copepods to amphipods and euphausiids (Figures 7-9). After the period of high tidal range, pollock catches increased, stomach fullness increased, and diet composition shifted to predominantly pteropods. Cooney (pers. comm.) postulated that these changes may be related to the effect of turbulence caused by strong tidal currents that disrupts zooplankton layers in narrow passages. Such effects may reduce local zooplankton densities to the extent that pollock can no longer profitably feed on zooplankton. Yoshida (1994) found that pelagic pollock feed mainly on macrozooplankton during summer in the central Bering Sea. Measurements of gill rakers indicated that large pollock had the ability to feed on prey greater than 2 mm. However, analysis of stomach contents indicated that pelagic pollock fed mainly on prey greater than 4 mm (Yoshida 1994). These results suggest that pelagic pollock may filter feed, but some prey selection is also evident. The decline in pollock catches in the 0-40m layer during the period of peak tidal range suggests that pollock may (1) migrate out of narrow passages, (2) migrate to depth, or (3) migrate into nearshore habitats during spring tides. Pollock catches in nearshore habitats also declined slightly during the spring tides suggesting that pelagic pollock did not move nearshore. The diets of the few pollock that were sampled during the spring tides indicated increased consumption of amphipods and euphausiids (Figure 9). This result suggests that at least some pollock may have migrated to depth where euphausiids typically occurred in dense layers. Increased survival of juvenile salmon during periods of peak tidal range is consistent with our observations of decreased pollock abundances in the surface layer (Figure 10). This result may support the hypothesis that age 3+ pollock are important predators on juvenile salmon; however, some other processes may also function to increase juvenile salmon survival during periods of high tidal range. Further study is needed to reveal the mechanisms behind these observations as well as any consequences for juvenile salmon survival.

Conclusions:

1. Age 1-2 pollock, tomcod and benthic fishes (greenlings and sculpins) may be important predators on juvenile salmon in nearshore habitats.

2. Seasonal patterns of abundance of fish predators and diet composition exhibited significant interannual variability.

3. Distribution, feeding rate, and diet composition of age 3+ pollock is apparently affected by tidal range in northwest PWS.

4. Survival to adult was related to tidal range at the time fry were released from the WHN Hatchery in 1994.

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Table 1: Preliminary estimates of juvenile salmon consumption by walleye pollock in northwest Prince William Sound, 1994.

Sampling Period	Pollock Biomass (kg)	% Diet Juv. Salmon	Juv. Salmon Consumption
Late April	590,399	4.74	2 ,956,380
Early May	590,399	.96	1,495,247
Late May	802,722	.05	118,452
Early June	460,771	.11	94,229
Total			4,664,308

Table 2: Mean percent of diet comprised of juvenile salmon for several fish taxonomic groups during four time periods in northwest Prince William Sound, 1995. Cod includes both Pacific cod and tomcod. Benthic fishes include various species of sculpin and greenlings. Statistical test for changes in the mean diet percentage comprised of juvenile salmon among time periods. All specimens included in the analysis.

Date	Pacific Herring	Cod	Pollock (age 1-2)	Pollock (age 3+)	Sockeye Salmon	Chum Salmon	Dolly Varden	Benthic Fishes
5/3 - 5/9	.11	2.78	0	.23	33.3	0	0	2.27
5/11-5/17	.01	3.33	0	.01	0	.05	0	.04
5/30 - 6/3	0	.57	14.74	.12	0	.11	12.42	.35
6/8-6/13	.10	4.74	1.38	.01	0	.75	1.45	.72
P-value	.327	.863	<.001	.041	.374	.171	.141	.063

Table 3: Mean percent of diet comprised of juvenile salmon for several fish taxonomic groups during four time periods in northwest Prince William Sound, 1995. Cod includes both Pacific cod and tomcod. Benthic fishes include various species of sculpin and greenlings. Statistical test for changes in the mean diet percentage comprised of juvenile salmon among time periods. Only specimens consuming juvenile salmon included in the analysis.

Date	Pacific Herring	Cod	Pollock (age 1-2)	Pollock (age 3+)	Sockeye Salmon	Chum Salmon	Dolly Varden	Benthic Fishes
5/3 - 5/9	28.7	100.0	-	14.8	100.0	-	-	54.6
5/11-5/17	21.2	100.0	-	1.7	-	2.1	-	4.6
5/30 - 6/3	-	100.0	87.9	26.2	-	6.2	64.8	23.3
6/8-6/13	29.1	77.7	94.0	20.5	-	13.5	63.8	86.8
P-value	.629	.681	.553	.309	-	.470	.776	.154

Table 4: Percent frequency of occurrence of juvenile salmon in the diets of several fish taxonomic groups during four time periods in northwest Prince William Sound, 1995. Cod includes both Pacific cod and tomcod. Benthic fishes include various species of sculpin and greenlings.

Date	Pacific Herring	Cod	Pollock (age 1-2)	Pollock (age 3+)	Sockeye Salmon	Chum Salmon	Dolly Varden	Benthic Fishes
5/3 - 5/9	.39	4.17	0	1.24	33.33	0	0	3.85
5/11-5/17	.04	2.56	0	.57	0	2.56	0	.72
5/30 - 6/3	0	1.41	16.0	.33	0	1.15	16.67	1.75
6/8-6/13	.19	7.58	3.6	.13	0	4.23	4.20	.75
Sample size	5,017	399	872	4,966	17	382	225	638

Table 5: Mean stomach fullness (% body weight) for several fish taxonomic groups found to prey on juvenile salmon during four time periods in northwest Prince William Sound, 1995. Cod includes both Pacific cod and tomcod. Benthic fishes include various species of sculpin and greenlings. Statistical test for changes in the mean stomach fullness among time periods.

Date	Pacific Herring	Cod	Pollock (age 1-2)	Pollock (age 3+)	Sockeye Salmon	Chum Salmon	Dolly Varden	Benthic Fishes
5/3 - 5/9	1.66	1.89	1.81	.91	.21	.35	.90	2.06
5/11-5/17	.42	1.39	.89	.98	.10	.09	.81	2.13
5/30 - 6/3	.44	1.29	1.22	1.13	.52	.72	1.40	2.62
6/8-6/13	.64	.98	.53	1.24	.15	.39	1.82	3.18
P-value	<.001	.106	<.001	.001	.347	<.001	.622	.037

Table 6: Mean catch per net set for several fish taxonomic groups found to consume juvenile salmon during four time periods in northwest Prince William Sound, 1995. Cod includes both Pacific cod and tomcod. Benthic fishes include various species of sculpin and greenlings. Data for age 3+ pollock includes only fish caught in mid-water and pair trawls (catch per hour of tow).

Date	Pacific Herring	Cod	Pollock (age 1-2)	Pollock (age 3+)	Sockeye Salmon	Chum Salmon	Dolly Varden	Benthic Fishes
5/3 - 5/9	25.6	1.4	3.7	36.6	1.0	1.5	1.0	1.4
5/11-5/17	308.7	1.4	3.8	17.1	1.0	3.4	1.0	1.3
5/30 - 6/3	48.4	1.4	18.0	33.1	1.0	11.3	2.5	1.5
6/8-6/13	466.8	2.2	6.0	27.0	1.0	3.3	2.2	1.4
P-value	<.001	.246	.115	.154	-	.002	.533	.157

Table 7: Parameters of the relationship between the mean proportion of the diet comprised of age-0 fish and the mean proportion of the diet comprised of large calanoid copepods at each study site for seven fish species found to consume juvenile salmon in 1995.

Fish Species	Intercept	Slope	P-value
Herring	.85	-.49	<.001
Cod	.82	-.02	.951
Pollock (age 1-2)	1.22	-.87	<.001
Pollock (age 3+)	.31	.02	.856
Chum Salmon	.63	1.94	.409
Dolly Varden	.92	.64	.852
Benthic Fish	.56	-1.18	.241

Table 8: Parameters of the relationship between the mean proportion of the diet comprised of large calanoid copepods and the mean abundance of large calanoid copepods at each study site for seven fish species found to consume juvenile salmon in 1995.

Fish Species	Intercept	Slope	P-value
Herring	.44	.002	.040
Cod	.06	.001	.146
Pollock (age 1-2)	.30	003	.039
Pollock (age 3+)	.27	.002	.056
Chum Salmon	.03	0	.894
Dolly Varden	.02	0	.710
Benthic Fish	.03	0	.747

Table 9: Mean proportion of the diet comprised of juvenile salmon at three levels of relative abundance of large calanoid copepods at each study site for seven fish species found to consume juvenile salmon in 1995.

Fish Species	Rel. Abundance Large Copepods			P-value
	low	moderate	high	
Herring	.02	.02	.07	.835
Cod	2.77	25.00	25.00	.520
Pollock (age 1-2)	13.70	1.61	0	.152
Pollock (age 3+)	.12	.06	.11	.786
Chum Salmon	.56	.10	.04	.485
Dolly Varden	5.53	15.03	.82	.381
Benthic Fish	.72	1.20	.57	.916

Table 10: Mean percent of the diet comprised of juvenile herring for four species of fish predators in northwest and southwest Prince William Sound, 1994.

Fish Species		Number	% Herring
NW PWS	Herring	1,370	.004
	Tomcod	55	.03
	Pollock	2,367	.61
	Chum Salmon	101	.25
	Dolly Varden	57	47.57
SW PWS	Herring	862	.027
	Tomcod	28	0
	Pollock	516	3.04
	Chum Salmon	13	0
	Dolly Varden	45	2.04

Table 11: Mean percent of the diet comprised of juvenile herring for three species of fish predators in northwest and southeast Prince William Sound, 1995.

Fish Species		Number	% Herring
NW PWS	Pacific cod	22	0
	Kelp Greenling	54	0
	Pollock	4,788	.22
SE PWS	Pacific cod	41	54.32
	Kelp Greenling	10	94.68
	Pollock	220	16.05

Table 12: Estimated annual consumption of herring by several important fish predators in Prince William Sound.

Fish Species	Biomass (mt)	Daily Ration (% BW)	% Herring in Diet	Total Consumption (mt)
Pollock	4,788	.01	20	6,426
Arrowtooth flounder	49,842	.01	9	14,899
Pacific cod	1,195	.01	37	1,452

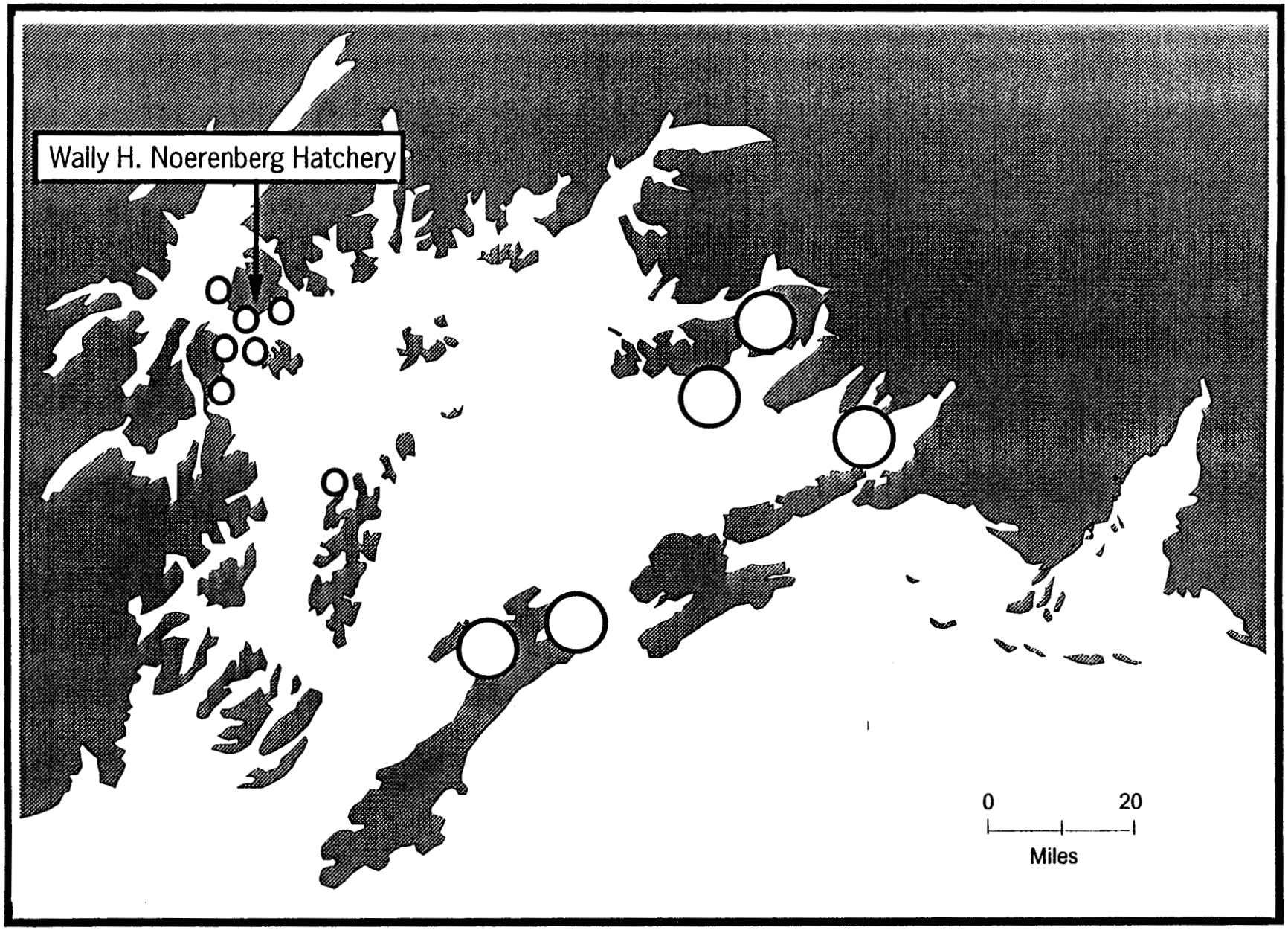


Figure 1: Study sites sampled primarily for juvenile salmon (small circles) and juvenile herring (large circles) in Prince William Sound, 1995.

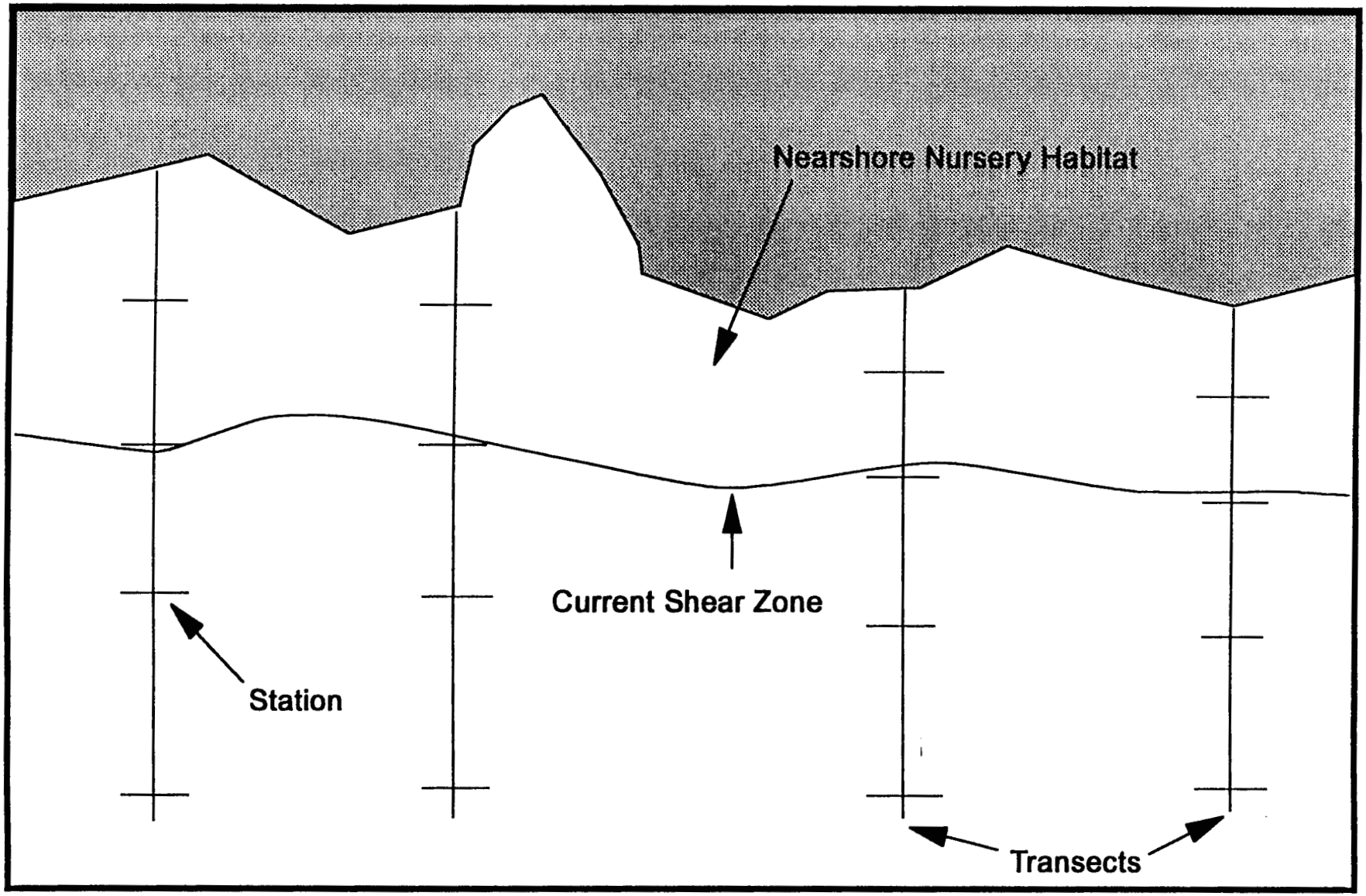


Figure 2: Spatial design for sampling juvenile salmon and their predators and prey at 16 study sites in northwest Prince William Sound in 1995. Each study site was approximately 3000 m in length. Stations were located approximately 100 m apart.

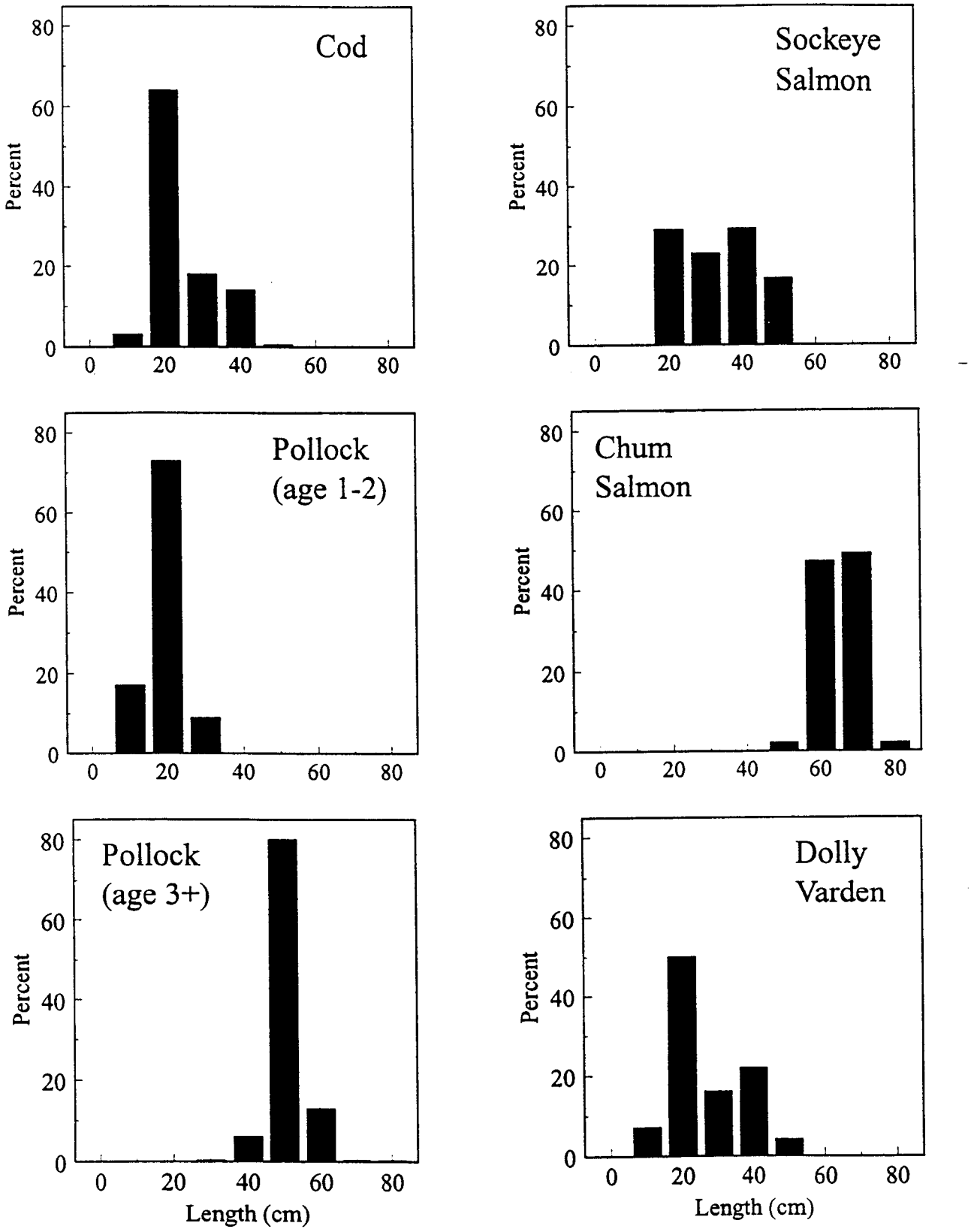
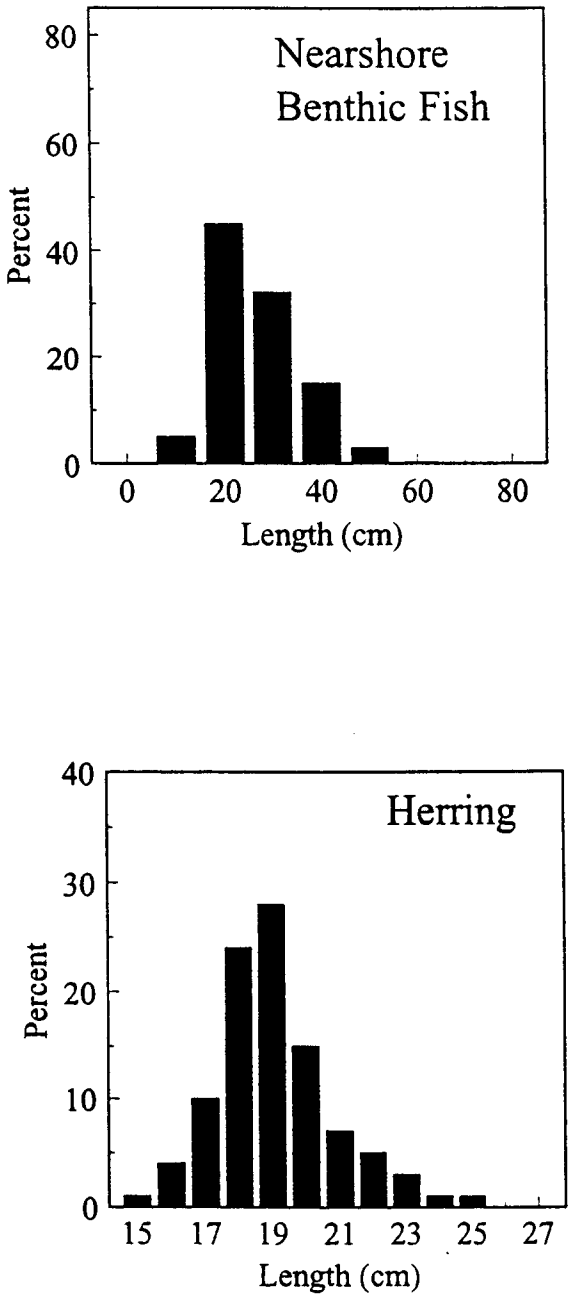


Figure 3: Length frequencies for several fish species found to consume juvenile salmon in northwest Prince William Sound, 1995.



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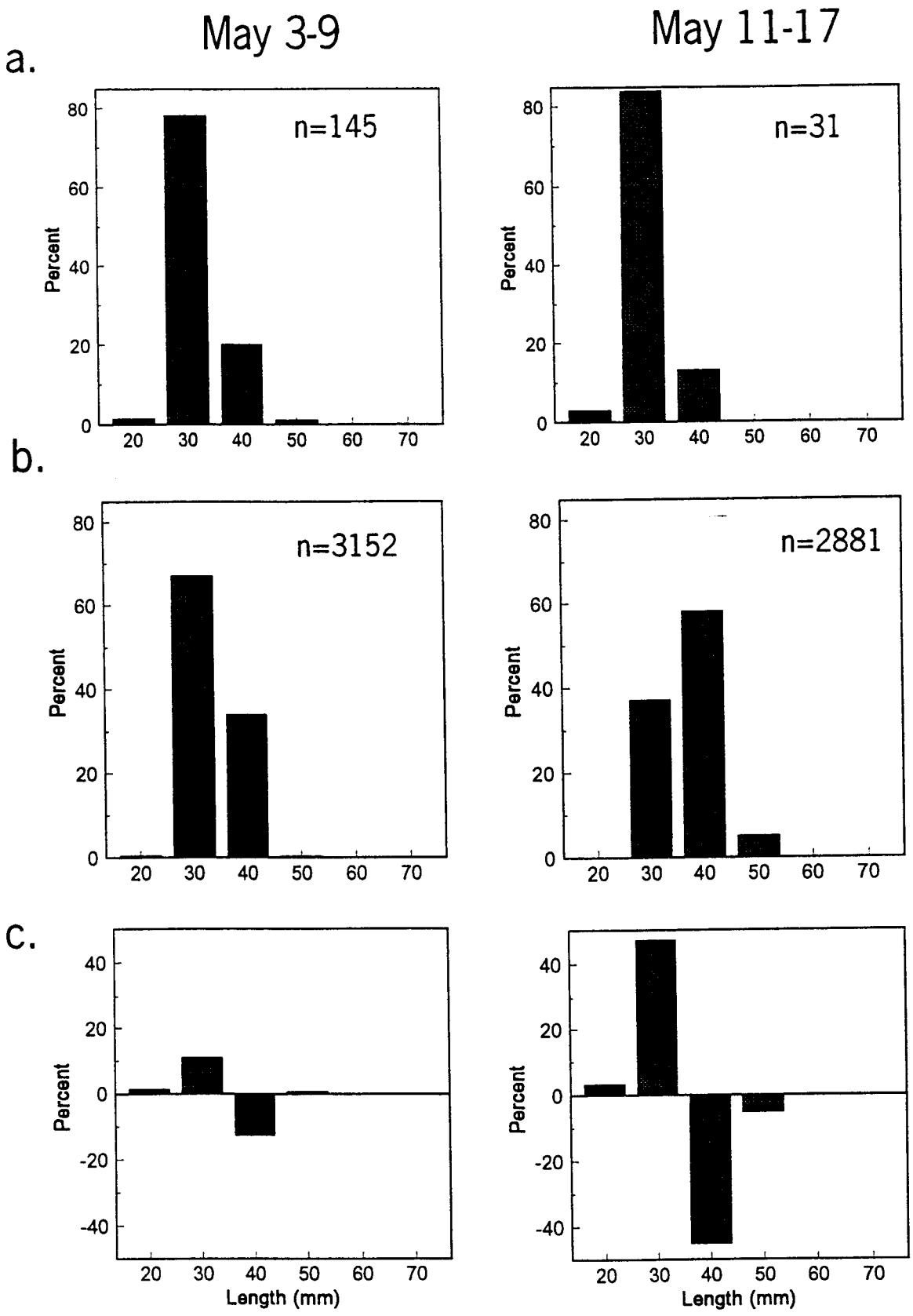
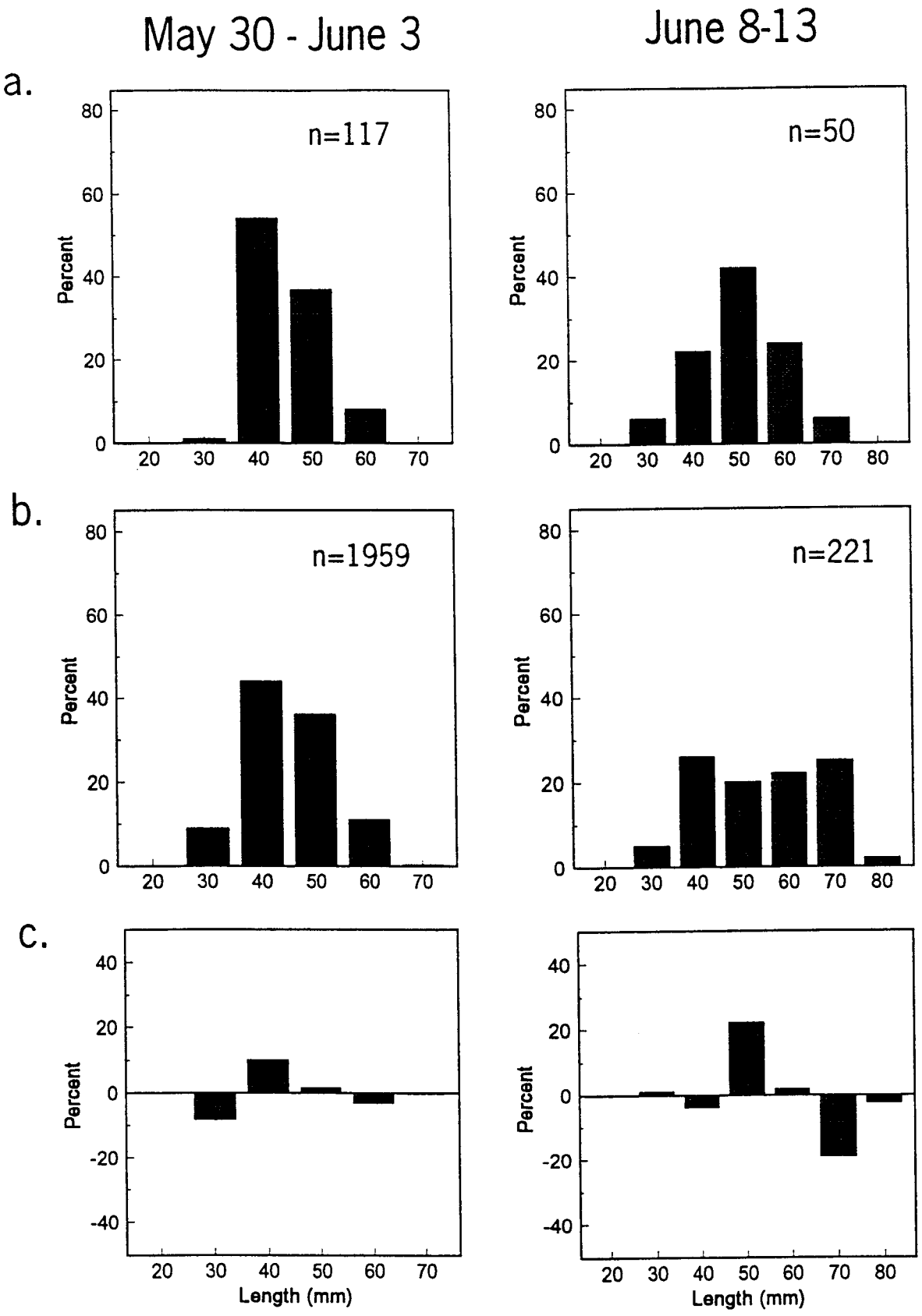


Figure 4: Length frequency of juvenile salmon (a) found in the stomachs of fish predators, (b) captured alive in nearshore habitats, and (c) percent frequency difference.



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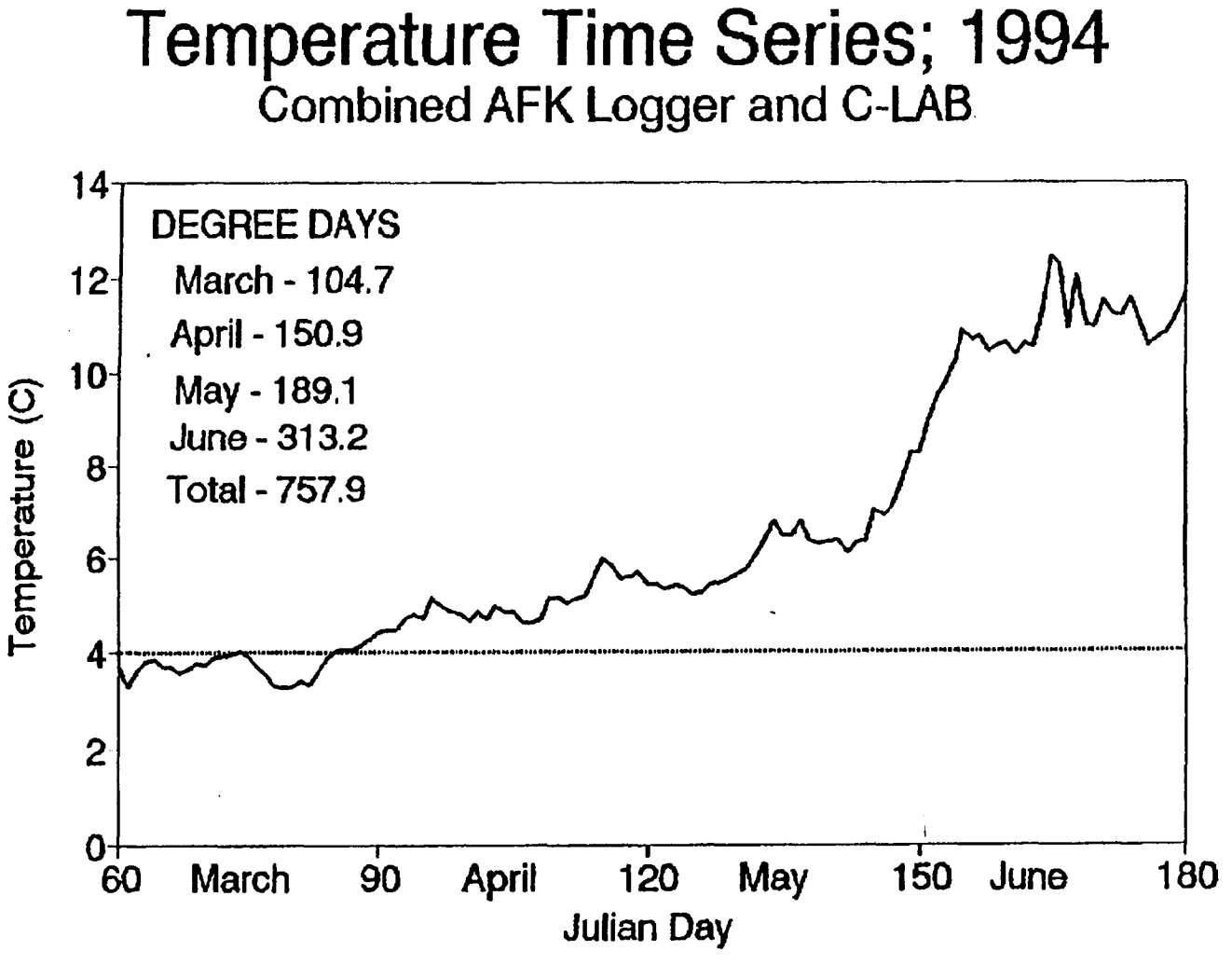
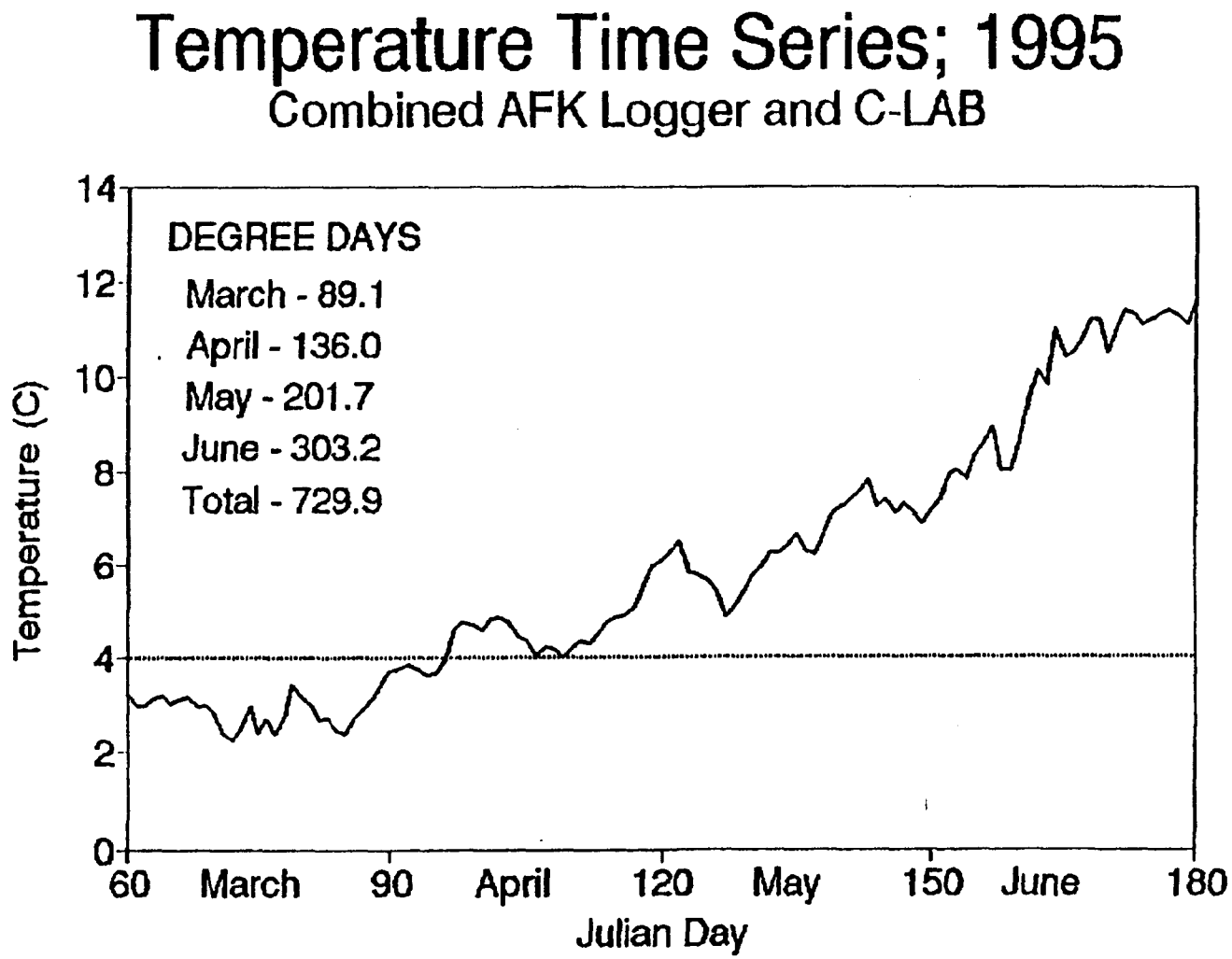


Figure 5: Seasonal changes in ocean temperature at two stations in Prince William Sound, March-June 1994. Data provided by the Prince William Sound Aquaculture Corporation and project 95320-H.



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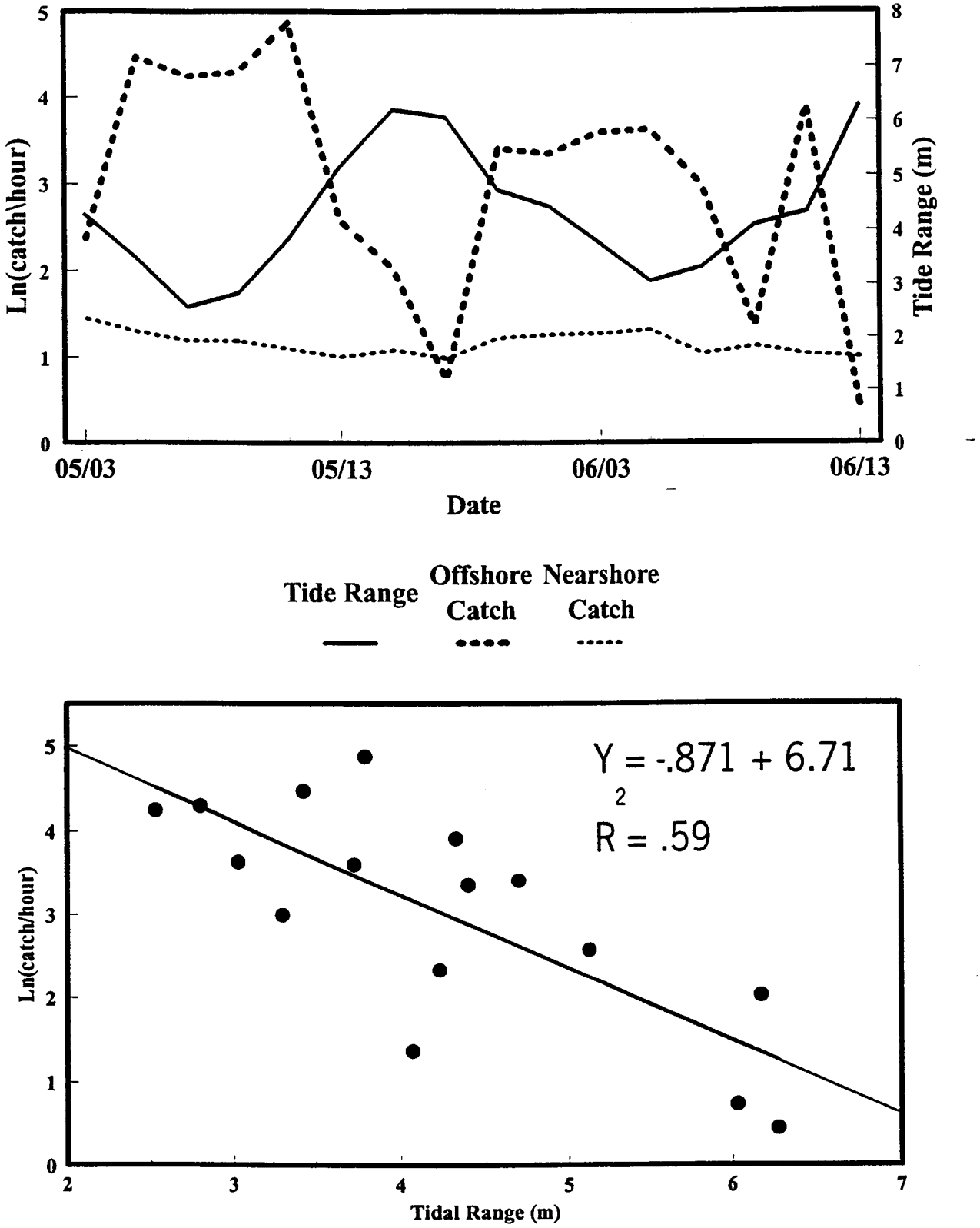


Figure 7: (a) Seasonal changes in tidal range and catch per effort of walleye pollock in nearshore and offshore habitats, and (b) relationship between tidal range and natural logarithm of catch per effort of walleye pollock in northwest Prince William Sound, 1995.

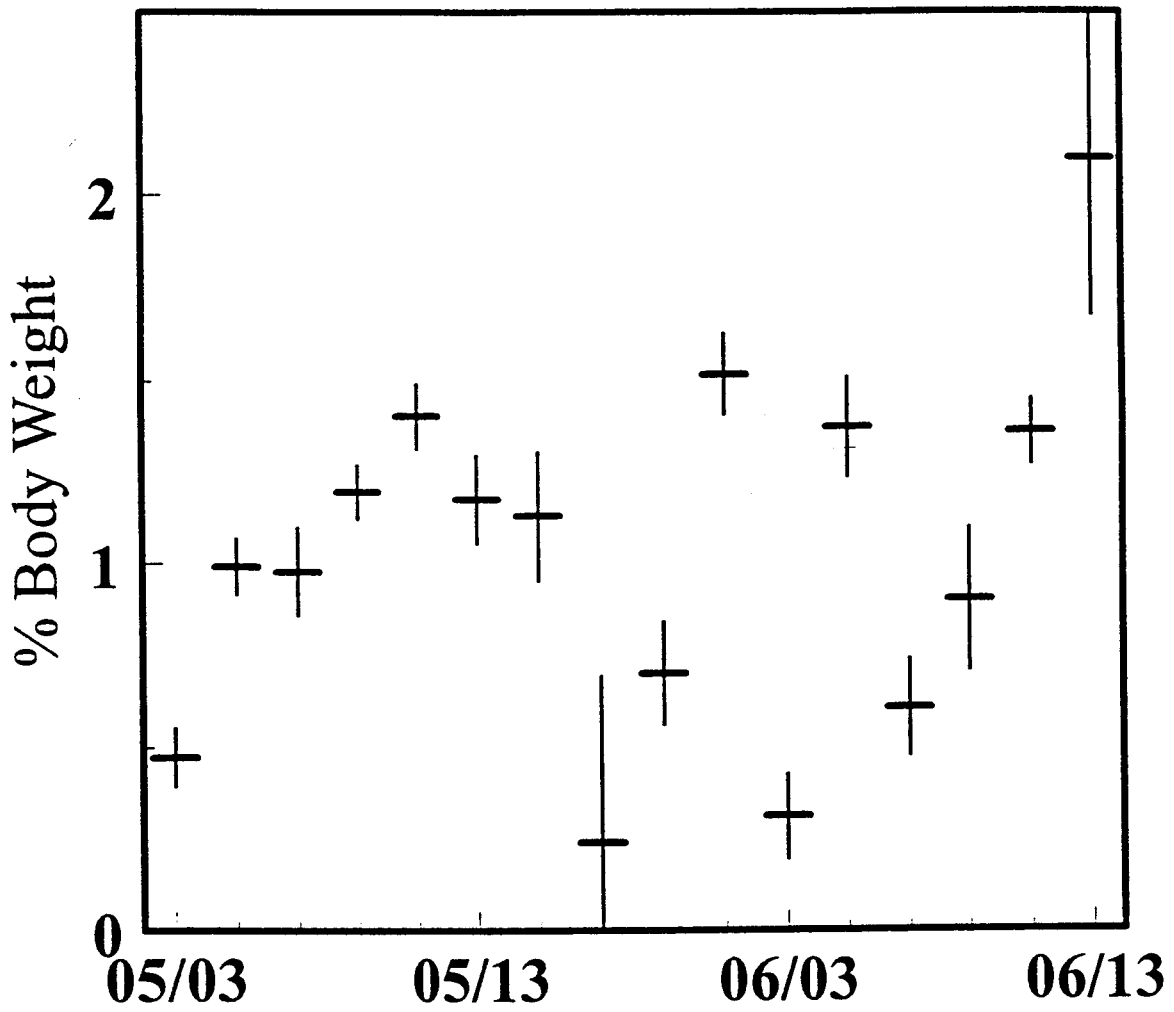


Figure 8: Seasonal changes in stomach fullness (% body weight) of walleye pollock in northwest Prince William Sound, 1995.

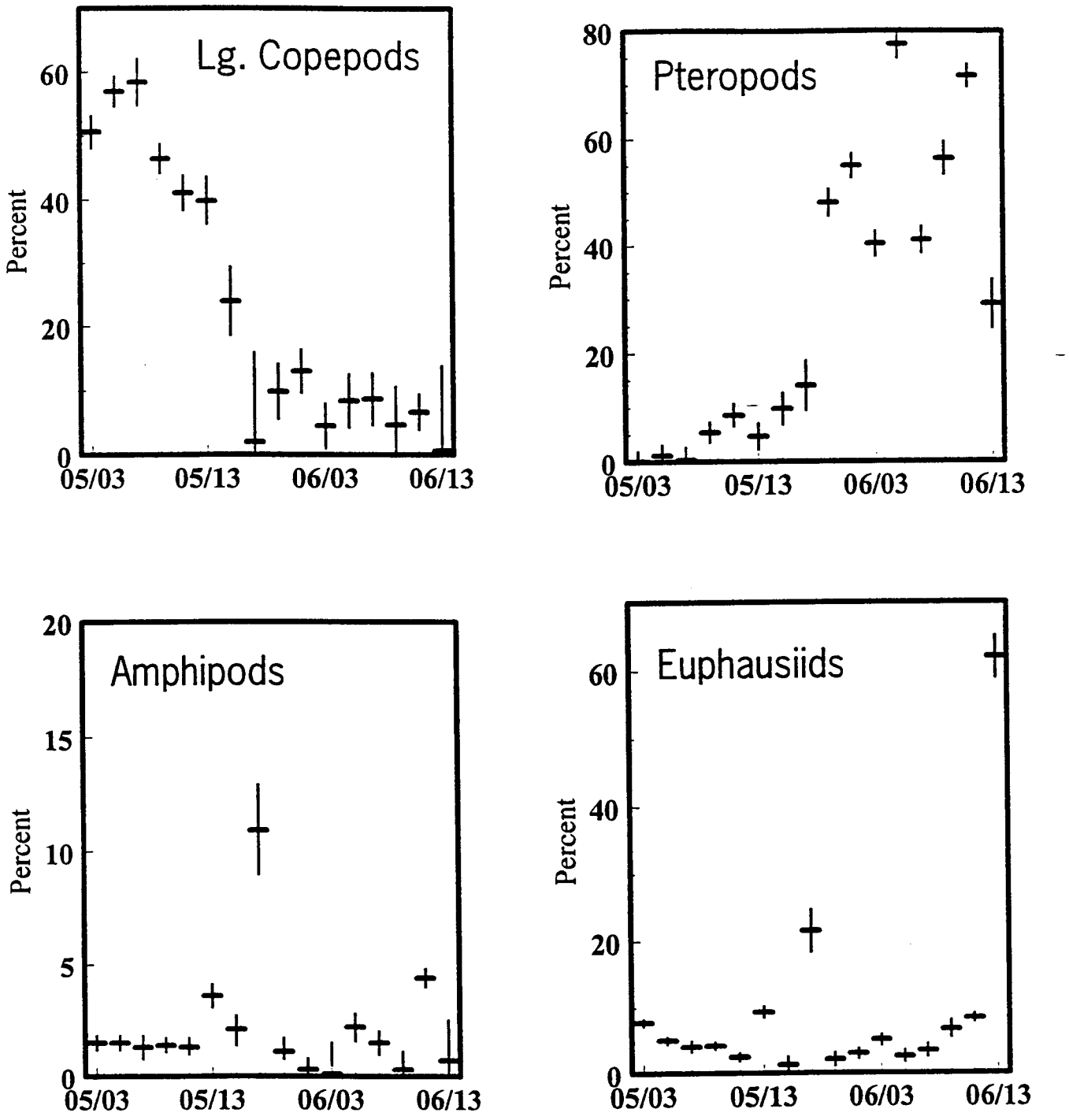
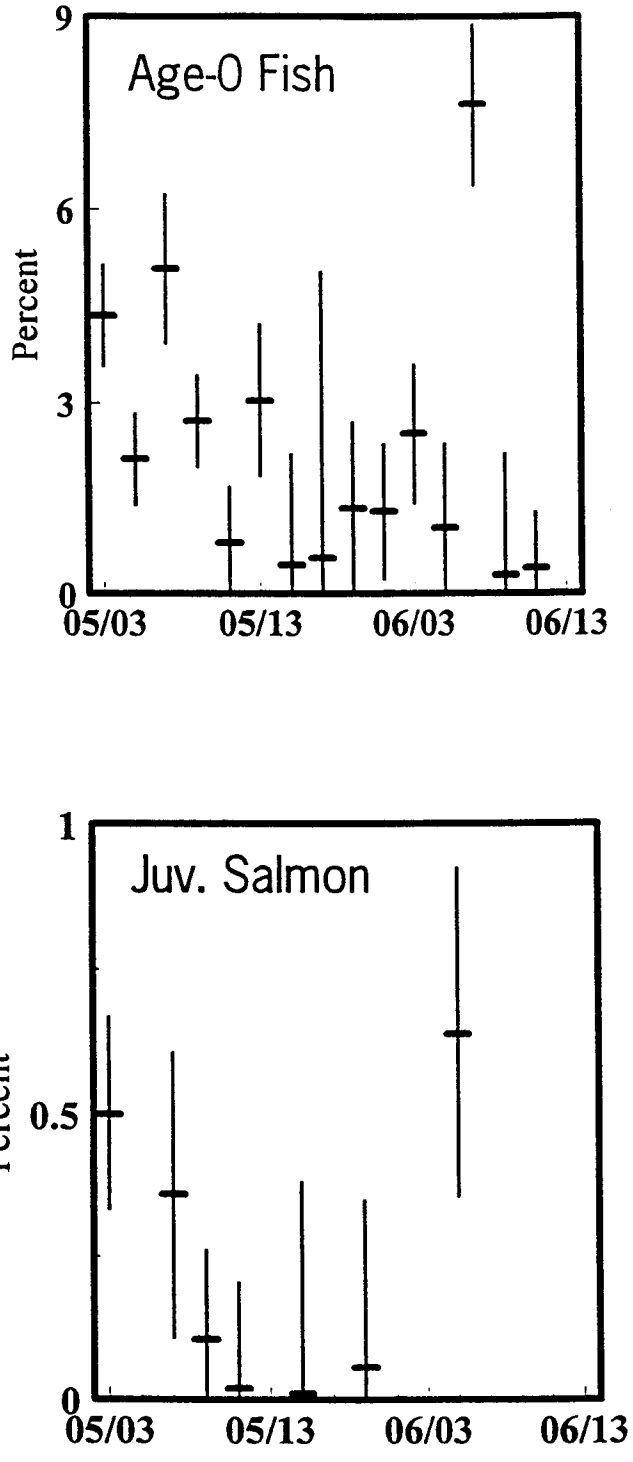


Figure 9: Seasonal changes in diet composition of walleye pollock in northwest Prince William Sound, 1995.



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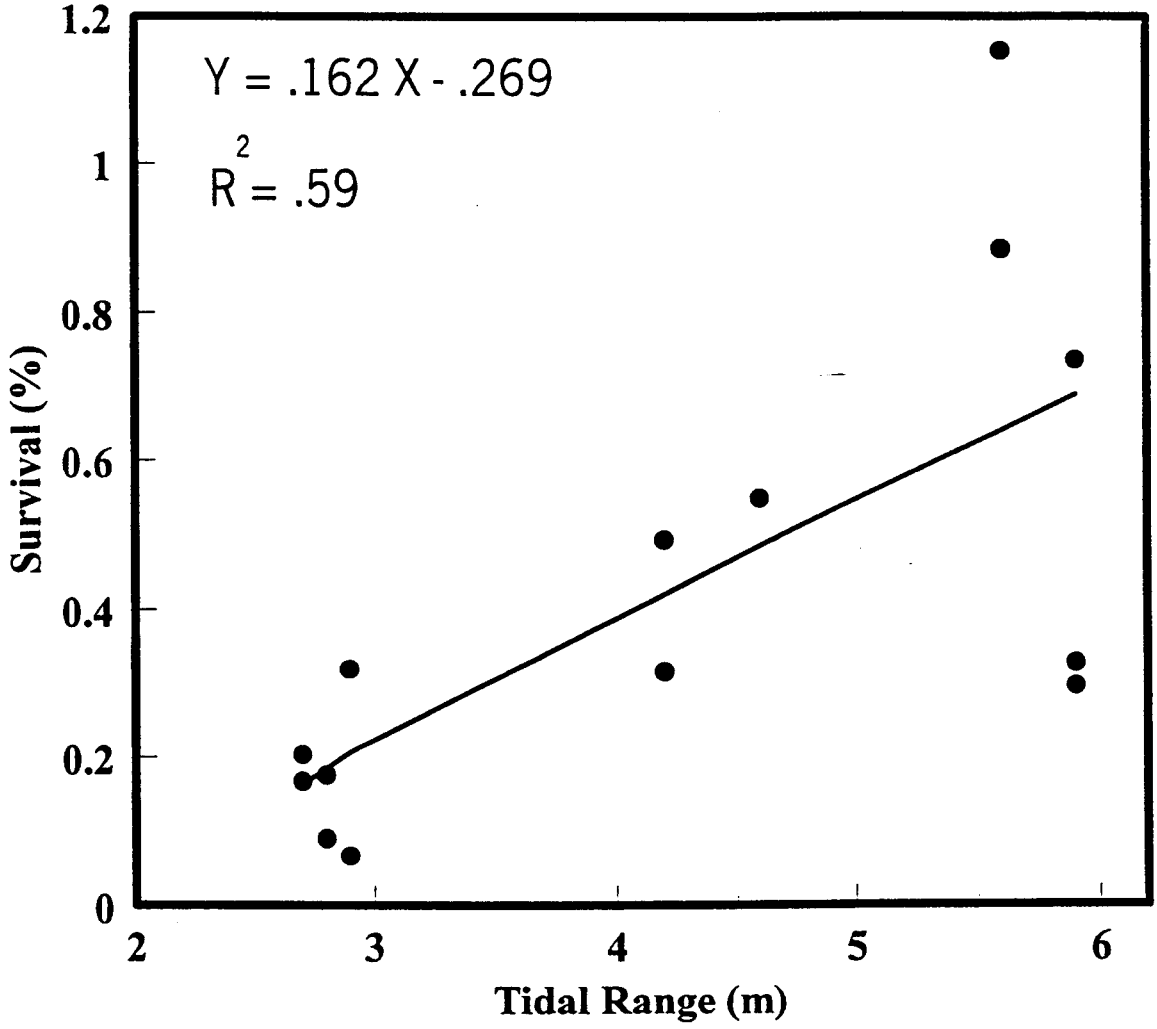

Figure 10: Relationship between tidal range and survival to adult for juvenile salmon released from the Wally H. Noerenberg Hatchery, 1994.

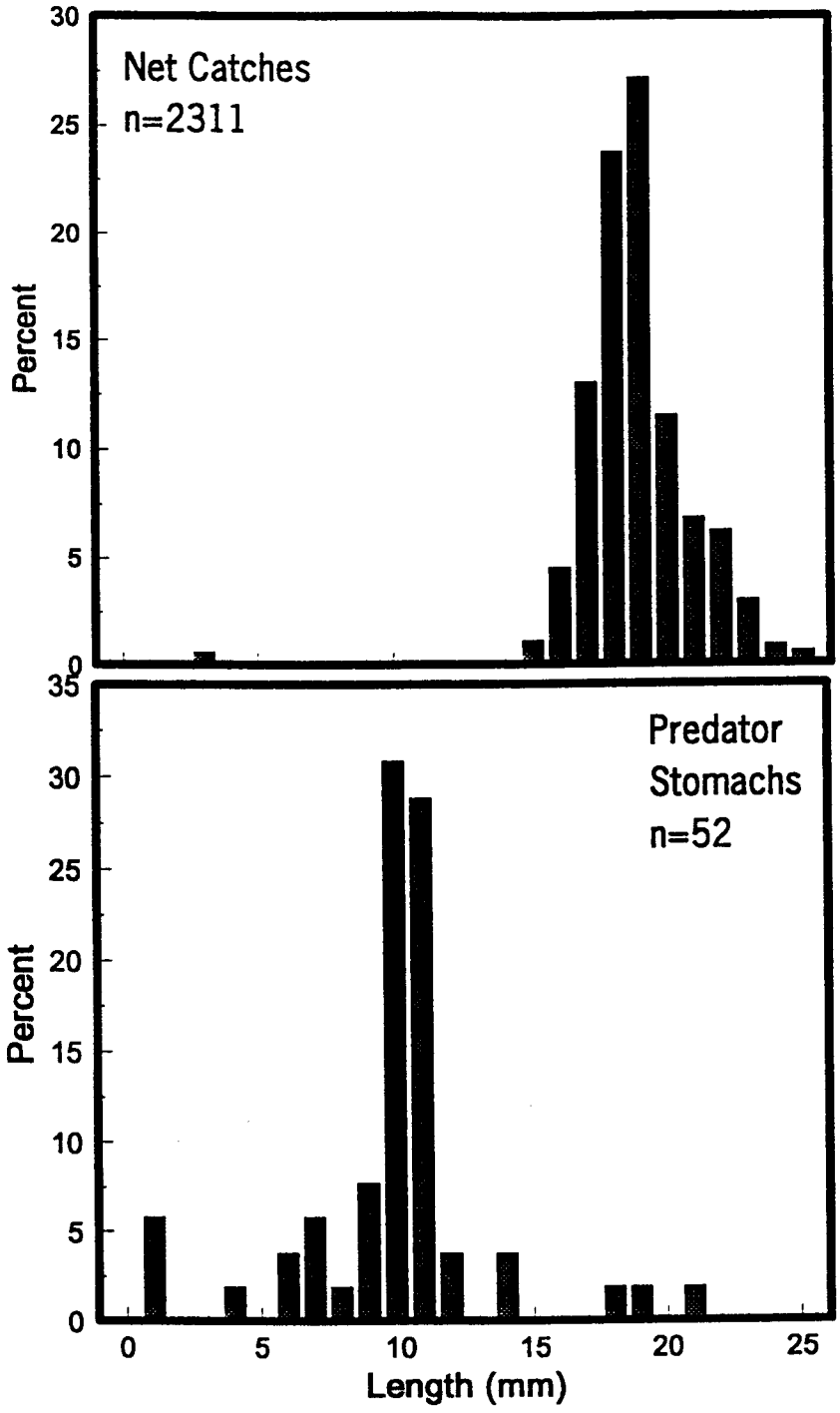

Figure 11: Length frequencies of herring from net catches and herring in stomachs of fish predators in May and June, Prince William Sound, 1995.

Chapter 4

95320G Phytoplankton and Nutrients

Exxon Valdez Oil Spill
Restoration Project Annual Report

Sound Ecosystem Analysis: Phytoplankton and Nutrients
Restoration Project 95320G

This annual report has been prepared for peer review as part of the Exxon Valdez Oil Spill Trustee Council restoration program for the purpose of assessing project progress. Peer review comments have not been addressed in this annual report.

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April 1996

Sound Ecosystem Analysis: Phytoplankton and Nutrients

Restoration Project 95320G
Annual Report

Study History: The project was initiated as Restoration Project 94320G. A "Draft Final Report" was produced as an annual report in 1995 under the title "SOUND ECOSYSTEM ANALYSIS: Plankton Dynamics: Phytoplankton and Nutrients" and continues under the present grant number. Papers were presented at the AAAS Arctic Division Science Conference and the AGU/ASLO Ocean Sciences meeting.

Abstract: In 1995 we collected 1400 samples from several platforms including 5 cruises on chartered vessels and daily sampling at two locations at the AFK Hatchery. The observations (chlorophyll, nutrients, particulate carbon and nitrogen, species composition, CTD, and dissolved oxygen) were supplemented with a moored instrument array (CLAB Buoy) that recorded temperature and chlorophyll (by fluorometry). The geographical coverage of observations was expanded and integrated using satellite images. Field work began in March and was completed in September. This is the first data set for phytoplankton and nutrients that fully includes the spring bloom. The spring phytoplankton increase is strongly influenced by light and mixing. The decline of phytoplankton is a result of nutrient depletion and grazing. In 1995 the limiting nutrient was silicate, in 1994 it was nitrate. In 1995 and 1994 the peak biomass was weeks later than 1993. The timing of the spring bloom is a signal to zooplankton. In all 3 years, the peak of zooplankton biomass occurs 3 weeks after the bloom. Regional coverage confirms the model results showing "river" conditions in the south and "lake" conditions in the north and central sound.

Key Words: Exxon Valdez, phytoplankton, nutrient cycles, primary productivity, algae

Citation: McRoy, C.P., D.L.Eslinger, A. Ward, E.P. Simpson, D. Clayton, B. Bergeron and J. Cameron. 1996. Sound Ecosystem Analysis: Phytoplankton and Nutrients, *Exxon Valdez* Oil Spill Restoration Project Annual Report (Restoration Project 95320G), Institute of Marine Science, University of Alaska, Fairbanks, Alaska

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Introduction

The project seeks to determine the driving force and variability of ecosystem production from a bottom-up point of view. It is our hypothesis in this component that the timing, quantity and species composition of the plant community, that is, the phytoplankton, is the major determinant of annual cycles. Ultimately, physical forces in the ocean play a major role in the dynamics of the phytoplankton community.

The Sound Ecosystem Assessment program (SEA) aims to understand and predict restoration of populations of pink salmon and herring in Prince William Sound. Fundamental to this goal is the understanding of controls of ecosystem processes that nourish the food web at its primary level. This is the goal of this component of SEA. Restoration of marine populations that have been damaged by human activity is usually limited to a few options that focus on controlling loss rate processes, i.e. harvest level, predator control, etc., or minor habitat modification. Pink salmon and herring offer a spectrum of strategies since a large portion of salmon are protected in hatcheries in their early life and herring are completely wild subject to the variance of nature. What then is the role of the annual cycle of primary production in the success of these upper trophic level species? Does the magnitude of the phytoplankton production determine the strength of a year class? Is the phytoplankton species composition an important determinant of the grazing zooplankton community? Does any of this matter or is there always enough food at the right time of the year so that predator populations are determined by the uppermost consumer on the food web? All are questions that are being examined in this study.

One central SEA hypothesis concerns the impact of circulation and physical conditions on the restoration of fish stocks (the Lake-River Hypothesis). This proposes that the circulation of Prince William Sound alternates irregularly between years of strong through-flow, river-like conditions, and relatively stagnant, lake-like conditions. The consequence is a high biomass of large zooplankton (copepods) in 'lake' years that are the major food for target fish (salmon, herring) and their predators (termed 'middle-out' food web control by Cooney and associates). In alternate 'river' years, the large zooplankton are sparse and predation on the target fish species predominates ("top-down" control).

While middle-out or top-down are principal hypotheses being tested by SEA research, the possibility of 'bottom-up' control, where the production of upper trophic level species is modulated by variations in light- and nutrient-driven phytoplankton production. In this hypothesis, the structure and composition of the zooplankton community are determined by variations in phytoplankton primary production and by the species composition of the phytoplankton community. For example, a phytoplankton community dominated by large diatoms can support a high biomass of large oceanic copepods, whereas a phytoplankton population dominated by smaller flagellates results in a reduced number of larger copepods, or in a shift to a zooplankton community dominated by smaller neritic copepod species. Variations in the timing of phytoplankton populations have been previously suggested to be a control of ecosystem events in Prince William Sound (McRoy 1988). A further complication in the interrelationship is that the large zooplankton are one year old when they become major prey for fishes (Cooney, personal communication) so their abundance must be determined by the events of the previous year and their specific biomass by the production cycle of the present year.

In this component, we provide the nutrient and phytoplankton data that are essential to evaluate the influence of phytoplankton dynamics on the food web and to test the bottom-up hypothesis. We will characterize the interannual spatial and temporal variation in nutrient and phytoplankton fields. We will evaluate the role of phytoplankton production in zooplankton recruitment and growth (especially for *Neocalanus* and *Pseudocalanus*). In a general sense we will provide an answer to the question "Is it food?".

A central tenet of the Lake/River Hypothesis is the variable advection of Gulf of Alaska waters into Prince William Sound. This advection affects not only zooplankton populations, but also the Prince William Sound phytoplankton populations and production.

Strong advection may confound the effects of in situ primary production in the Sound. To test the hypotheses further, we use satellite-derived sea-surface temperatures to examine the movement of Gulf of Alaska surface waters into Prince William Sound. In 1995 we assumed the responsibility for maintenance and data collection for the moored instrument array (CLAB) that has been gathering continuous oceanographic data in Prince William Sound since 1992. This platform has provided a valuable data set.

Objectives

This study is designed to investigate the distribution, amount, and type of phytoplankton growth and the major inorganic nutrient fields associated with the growth processes. Our hypothesis is that variations in the phytoplankton production and populations are transferred to the zooplankton and that such variations are a function of oceanographic conditions that control the supply of inorganic nutrients and light. The objectives for 1995 were:

1. Analysis of phytoplankton community ecology in PWS.
2. Determination of basin-wide patterns of temperature, salinity nutrients and chlorophyll from ship-board observations.
3. Determination of temporal patterns of temperature, salinity, nutrients and chlorophyll from AFK Hatchery
4. Provide data for interpretation of CLAB data and integrated modeling.
5. Determination of the linking between phytoplankton and upper trophic levels.

Methods

Phytoplankton Biomass, Spatial and Temporal Patterns:

Phytoplankton biomass is measured using the standard chlorophyll techniques (Parsons et al., 1984) on a Turner Designs Fluorometer. Samples were collected at specific 309 time/space locations on cruises and at a shore-based station. Data allow mapping the areal pattern and description of the water column profile.

Phytoplankton Primary Production

The biomass pattern provides a picture of what is present, but it does not provide information on the phytoplankton dynamics. In 1995 we were unable to make any direct measurements of primary productivity by using isotopes due to the limitations, because of regulatory prohibitions of using radio-isotopes on the available platforms. We can estimate production using dissolved oxygen and nutrient data. Productivity data are also available in our historical database (McRoy, unpublished data). Methods used involved uptake of ^{14}C by phytoplankton in containers under neutral density filters (Strickland and Parsons, 1972; Parsons et al., 1984).

Phytoplankton Community Composition:

The composition of the phytoplankton community can be as important as the total primary production in determining zooplankton species and abundance. We collected 50 ml aliquots from water samples and preserved them in Lugol's solution for species identification. Identifications and cell counts were done using an inverted microscopy method (Sournia 1978). On low (20x) magnification, all visible cells in two transects are counted. On high (40x) magnification, fields are counted until a total of 300 cells is reached. For cell volume calculations and calculation of carbon content, cells identified to genus were grouped according to the maximum cell dimension. At least 20 cells of each species for size class were measured. The procedure is labor intensive and only a portion of the samples collected can be counted.

Nutrient Fields:

Phytoplankton require the major inorganic nutrients (nitrogen, phosphorus and silica) for growth. General oceanographic circulation and land run-off supply nutrients. Since phytoplankton also require light, the problem is understanding how the nutrients are supplied to the illuminated zone of the sea. We routinely collected water samples for quantitative nutrient analysis

In the field, water samples were collected with Niskin Bottles at standard depths over the upper 100 m (deeper if necessary). A small aliquot (250 ml) was filtered and frozen for later chemical analysis. Chemical determination of the quantity of dissolved nitrogen (as nitrate, nitrite and ammonium), phosphate and silicate were measured using prescribed methods with an Alpkem Auto-Analyzer in our laboratory in Fairbanks.

Moored Instrument Array: The CLAB Buoy

We assumed the responsibility for deployment and recovery of the CLAB moored instrument program in 1995 and are working with R.T. Cooney to insure the quality of the data. This mooring consists of a thermistor chain, which measures temperatures at 10 depths from 0 m down to 100 m; an in situ fluorometer at 10 m; and a meteorological package, which measures wind velocity, air temperature, and buoy hull temperature. The buoy continuously acquires wind speed and direction, barometric pressure, air temperature, sea surface temperature, chlorophyll fluorescence, and ocean temperature at 10 depths. Data are relayed via the ARGOS satellite system in near real time. Data from the CLAB buoy are also passed to other groups for use in modeling. The moored instruments provide a mechanism to integrate other discrete observations collected from ships.

Satellite Image Analysis:

Satellite images are a powerful integrative tool. While field samples provide ground truth data, satellite images are valuable sampling mechanisms to examine the pelagic ecosystem on a broad geographic scale and over the entire year. We are currently scanning NOAA Advanced Very High Resolution Radiometer (AVHRR) imagery from the University of Alaska Fairbanks High-Resolution Picture Transmission (HRPT) ground station. The AVHRR data produce sea-surface temperature images of the sound and adjacent regions. We use these images to monitor the inflow of water to Prince William Sound and to determine the spatial extent of water masses identified by the field program.

Personnel

The following people have contributed to sample and data collection and analysis:

B. Bergeron	Technician
D. Clayton	Technician
J. Cameron	Technician
S. Danielson	Graduate Student
L.J. Miller	Graduate Student
N. Pintchouck	Graduate Student
P. Simpson	Graduate Student
S. Speckman	Graduate Student
A. Ward	Graduate Student
C. DeLaca	Student

Results

Samples were collected to document the time series of events in the annual phytoplankton/nutrient cycle as well as to examine spatial variations.

Sample Collection

We collected water samples for analysis from two platforms in Prince William Sound. Short, monthly SEA cruises from March to September (except for August) permitted

regional sampling from the standard SEA ocean stations. This work provides a data set, collected in conjunction with many other SEA components, that is crucial to modeling and synthesis. The second sample site is the AFK Hatchery on Evans Island in the southwestern corner of the sound. We used this shore facility to collect daily samples from mid-April until late June from two nearby locations. These data provide temporal continuity to the ship-board sampling. Additional time series data from the CLAB permanent ocean buoy permit comparison with previous years.

The field season began in March and extended until late September. Platforms for sample collection included ships and shore-based facilities. In 1995 we collected 1400 samples from 5 cruises and 2 shore-based stations from AFK Hatchery, an increase of more than 60% over 1994 (Figure 1). The chartered vessels provided areal coverage of the sound for oceanographic and biological parameters (see Appendices I and II for station locations).

The Phytoplankton-Nutrient Component database includes dissolved nutrients (nitrate+nitrite, ammonia, phosphate, and silicate), dissolved oxygen, CTD (salinity, temperature, depth), chlorophyll a, and particulate carbon (PC) and nitrogen (PN) from all sampling platforms. In addition selected representative samples for phytoplankton enumeration are being processed. We searched daily satellite images showing sea surface temperature from late March to present; of these, 20 are being interpreted for basin-wide patterns and integration with CLAB data. Finally, data from the CLAB buoy (temperature and chlorophyll) are being correlated to the time series data from AFK Hatchery and with satellite temperature images to elucidate basin-wide patterns and processes.

Time Series Measurements: CLAB Buoy

The continuously recorded data from the CLAB mooring presents a detailed time series of phytoplankton biomass (as measured by fluorometer) and associated oceanographic parameters for a central location in the sound. Unfortunately due to necessary maintenance the deployment of the buoy was delayed until May so the data series does not include the spring bloom but only post-bloom summer conditions (Figure 2). A good time series exists for 1994 and 1993. In 1994 the increase was interrupted by storm conditions in April which delayed the spring maximum until the third week of April, two weeks later than in compared to 1993. The fluorometer record is a relative scale so no statement can be made about the absolute level of biomass reached in a year from CLAB data. Such determinations require direct measurement of chlorophyll content in the field.

The phytoplankton biomass at the CLAB buoy (fluorometer) shows a close relationship to the variation in the wind speed as expected. Napp et al. (1996) show that the initiation of the bloom in Shelikov Strait is determined by cloud cover and mixing depth and this probably applies to the start of the bloom in Prince William Sound. The CLAB data missed the early spring increase due to the late date of deployment but the bloom is evident in the AFK data. There is no close correlation between the AFK chlorophyll data and the CLAB fluorometer data for the period they overlap indicating that local conditions dominate.

Time Series Measurements: AFK Hatchery

The best time series data in 1995 were collected from 2 stations in the southwest Sound near the AFK Hatchery (Figure 3).
The data series begins on 18 April 95 and ends on 19 June. In both stations the bloom terminates by the end of April. The pattern is similar in both locations with the differences reflecting the effect of water depth. The deeper station (AFK95.2) more reflects the pattern of the open sound. A high biomass in April is followed in both locations by much lower levels in May and June, with occasional pulses of higher biomass stimulated by mixing events (see the Biological Modeling Component). At AFK95.2 during the bloom an increased biomass occurs down to 100 m, likely a result of the sinking of phytoplankton cells rather than growth in place, but both are possible. The appearance of algal cells at depth is a signal and food source to herbivores in deep water.

Phytoplankton Community

From April 17 - June 20, 1995, phytoplankton samples were collected daily at 0, 5, 10, 25 and 50 meters depths at station SB1 and SB2 at AFK Hatchery. A total of 640 phytoplankton samples were collected for analysis in Fairbanks.

Sixty five samples from the spring bloom (April 17- April 29) were enumerated, identified and measured for cell volume calculations using the Utermohl inverted microscope technique (Sournica 1978). Phytoplankton cells were identified to the lowest possible taxon, genus or species, depending on the condition and orientation of the cells. Small nanoplankton (2-20μm) were classified according to composition and cell size as unidentified flagellates or dinoflagellates. Cell volume calculations will be used to estimate individual diatom and flagellate carbon biomass contributions.

At Station AFK95.2 in Elrington Passage a time-series of physical and biological data were collected from April 18 -June 20. The phytoplankton spring bloom occurred between April 18-28. During this period, the average temperature and salinity were 4.3 °C and 31.45 psu, respectively. The lowest temperature and salinity were 4.1 °C and 30.98 psu recorded on April 19 and April 22. The highest temperature reading of 4.7 °C occurred late in the bloom on April 28. The highest salinity measurement peaked at 31.85 on April 26.

The chlorophyll biomass and phytoplankton cell abundance (cells/ml) at the chlorophyll maximum on the surface were examined between April 18-27. The average biomass was 27.30 (mg/m^3) and the average cell number of flagellates and diatoms was 1287 cells/ml and 1410 cells/ml, respectively. The biomass peak occurred on April 21 followed by a peak in abundance on April 22. The fluctuations in the biomass and abundance observations followed a similar trend especially apparent at the onset of the bloom.

Total cells/ml of phytoplankton was tallied from 18-27 April at all depths to show where the maximum number of cells were found. Totals showed a peak of 25,323 cells/ml at 5 meters depth and a minimum of 15,500 at 50 m. Composition of phytoplankton was dominated by diatoms and flagellates. Phytoplankton counts by taxonomic grouping show a decrease in abundance with depth for all groups (Figure 4). Percentage of diatoms and flagellates didn't vary significantly over time and depth. The average composition from 18/4-27/4 was approximately 55% diatoms and 45% flagellates (standard deviation averaged 8%) for all depths . No significant variations were apparent at deeper depths.

Five genera of phytoplankton composed the majority of the diatom population at all depths (Figure 5). No significant variations in species composition occurred between surface and 50 m throughout the bloom. *Skeletonema costatum* comprised the largest component of the bloom averaging 38%-44% of the diatoms at all depths. *Thalassosira* spp. followed with an average of 30%-34% by depth. *Chaetoceros* spp. was the third largest constituent averaging 10%-12% of the diatom community. *Leptocylindrus* spp. averaged 2%-8% and the small pennate diatom *Nitzchia* spp. averaged 6%-7% of the population. Other diatoms, listed on the species list, averaged 1%-5% of the diatom bloom for all depths.

Nutrient Limitation (Figure 6)

The time series plot of nitrate vs. silicate for AFK 1995 and WNH 1994 indicate a significant shift in the nutrient limitation of phytoplankton growth between 1995 and 1994. In 1994 the system became depleted in nitrogen, but in 1995 silicate is the major limiting nutrient. This difference could result from a shift in circulation, in the herbivore community, in the herbivore predators, or in all of the above.

Phytoplankton-Zooplankton Linking (Figure 7)

A comparison of the seasonal time series of zooplankton and phytoplankton biomass for 1993, 1994, and 1995 is possible using data are from AFK Hatchery (zooplankton data from R.T. Cooney) and from the CLAB buoy (93 & 94 fluorometry) and AFK Hatchery. A key feature of these data is that the peak of the phytoplankton bloom occurs 15 to 20 days earlier in 1993 than in 1994 or 1995. The timing of the bloom in the latter 2 years is nearly

identical. The subsequent peak increase in zooplankton directly reflects the phytoplankton timing. The zooplankton peak is early in 1993 and later in the replicate '94 and '95 seasons. The unavoidable conclusion is that the ecosystem phenology is determined by the timing of the phytoplankton bloom which is itself driven by ocean conditions. While the exact mechanism linking the phytoplankton to zooplankton increase is unknown, we speculate that it is the rain of phytodetritus into the deeper waters that is the signal to the herbivore community to begin grazing and moving up into the surface layers.

Spatial Measurements: Ship-Board Results

Phytoplankton Community Size Fractionation (Figure 8)

For species identification, unfiltered water samples from 5 depths (0, 5, 10, 25, and 50 m) were collected at each phytoplankton station on all 1995 Bering Explorer cruises (see table below).

In addition to phytoplankton standing stock estimates from chlorophyll a fluorescence, additional size fractionation of chlorophyll a was also conducted on all cruises using three filter sizes, 5μm, 20μm and 100μm Nitex netting at the maximum chlorophyll depth. Size fractionation experiments were conducted to roughly determine the composition of the bloom based only on cell size and fluorescence. All fractionation work directly followed the fluorometric studies that determined the depth of the maximum chlorophyll biomass.

Table 1. List of phytoplankton samples collected for size fractionation measurements.

Cruise	Dates	Phytoplankton Samples Collected	Size fractionation
BE503	3/15-3/23	150	50
BE504	4/10-4/16	195	18
BE505	5/4-5/11	150	102
BE506	6/15-6/20	155	93
BE509	9/28-10/3	110	66

Between April 10-16, size fractionation of chlorophyll a was conducted at five stations to determine the dominant phytoplankton biomass based only on cell size. Four stations showed the majority of cell size was between 20 μm and 100 μm with values ranging from 51% to 92% of the chlorophyll biomass. Stations MS6, SEA27 and SEA11 had between 30-33% of the chlorophyll from cells greater than 100μm. Only Station HE12 deviated from the others, showing 53% of the biomass from cells <5 μm and 11% from cells between 20 and 5μm. All the other stations had an average of 7.5% of chlorophyll from cells < 5μm during the month of April.

Phytoplankton Nutrient and Zooplankton Interactions (Figure 9)

In March high nitrate (15-20 μM) concentrations occur throughout the sound along with low biomass of both phytoplankton and zooplankton. In the northern and western sound, in April, the phytoplankton bloom occurs, nitrate declines, and zooplankton begin to increase. In May nitrate is depleted (<2 μM) in the surface waters except the south "river" region, phytoplankton declines, and zooplankton are high. The data show closely coupled spatial and temporal connections for nutrients phytoplankton and zooplankton. The progression is essentially from inorganic nutrients in March to organic biomass (as zooplankton) in May. The spatial pattern is a separation of "lake" type conditions in the northern and western sound and "river" conditions in the south. Nutrient isoclines are

parallel to the flow trajectories described by the SEA physical model (see the modeling component). The high biomass of both plant and animal plankton is confined to 'Cooney's Lake'. These data support the results of the SEA ocean model (Figure 10).

Spatial Measurements: Satellite Images

Eslinger is collecting contemporaneous AVHRR SST data. As part of our 1995 work, we helped in the analysis of remotely sensed sea surface temperature data which was collected prior to the SEA project. We have performed an empirical orthogonal function (EOF) analysis on the SST data covering the spring period for three years. An EOF analysis of satellite images allows one to represent the total spatial and temporal variability in the data set by a temporal mean, a time series of spatial means, and a series of independent (orthogonal) modes or patterns (images), which are sorted from those explaining the most amount of variance down to those explaining the least amount of variance. With each EOF mode, there is an associated time series of unitless eigenvectors, which indicate the relative importance of the associated mode at a particular time. The spatial and temporal variation explained by a particular pattern can be reconstructed by multiplying the spatial pattern by the eigenvector at a particular time; this gives the variation about the mean due to the particular mode at that particular time. The EOF analysis reveals that 40.5% of the variation in the springtime sea surface temperatures, after spatial and temporal means have been removed, is explained by the pattern and time series seen in Figure 11. We maintain that this pattern discriminates the high-flow (river) region from the low-flow (lake) region. The figure shows that temperatures in the early spring are warmer in the Gulf-dominated high flow region, i.e., negative SST variance values (blue) in the southern Sound are multiplied by the negative eigenvectors, which occur prior to day 120, to give a positive overall effect. The SST difference between the two regions decreases through time and, near day 120, changes sign. After day 120, the northern Sound waters are warmer than the southern Sound "river" waters. Interannual variation in the extent and strength of this "lake/river" EOF mode can be seen in by comparing the eigenvectors for the three different years. The largest eigenvectors occurred in 1991, indicating that was the year with the strongest cross-Sound temperature difference and, we maintain, the strongest "river" year. In contrast, 1992 eigenvectors are all very small and the first mode was therefore relatively weak in 1992, i.e., 1992 should have been more of a river year. In 1990, eigenvalues were intermediate, and we maintain it was a mixed year, with more high flow "river" conditions than in 1992, but less than in 1991.

The patterns revealed in this analysis are very similar to the patterns found in our 1995 field data (Fig. 9) and to the dominant circulation patterns observed in the physical modeling work of the SEADATA subprogram. We are greatly encouraged by these findings and since the SEA project as a whole has succeeded in 1) identifying the "river" and the "lake" portions of the sound from physical characteristics, 2) observing the biological effects of the different regions, and 3) implementing a model which contains the necessary physics to reproduce these different regions.

Modeling

As part of our coordination with other SEA groups, we supplied the phytoplankton modeling portion of the SEADATA project with chlorophyll data, for both cruises and hatchery time-series stations, from 1994 and 1995. These data will be important for examining the River/Lake hypothesis and for developing the spatial aspects of the plankton model. In addition, we supplied meteorological and physical oceanographic (water-column temperature) data from the CLAB buoy system to the modeling components. The buoy data is used to force the biological model, and to provide validation data for comparison of the model temperature structure with the actual temperature structure.

Discussion

The general pattern of the time course of phytoplankton biomass is a rapid spring increase followed by an equally sharp decline after about a month. The increase begins in early April unless storm conditions are present, and the decline occurs in May. Summer increases occur if oceanographic mixing events provide new nutrients to the surface euphotic zone. We observed such small scale events both in the buoy data and in the time series from Lake Bay. In 1994 the phytoplankton biomass reached maximum in the last week of April (in 1993 it was early April) and the following minimum occurred in the third week of May (first week in 93). In both years these events in the annual cycle occurred more than a month before those in the phytoplankton cycle reported for Port Valdez in 1987 (Alexander and Chapman, 1980; McRoy, 1988).

The timing of the spring bloom is apparently determined by the interaction of light and mixing in the classic relationship (Sverdrup, 1953). The interruption of the cycle by storms indicates the fragility of the relationship at this time of year and how the ocean conditions can impart an event signal to the food web. The zooplankton data that have been included here show that the delay in the phytoplankton bloom is translated to zooplankton and hence to upper trophic levels.

The pattern of the phytoplankton cycle indicates the classic response of increasing light and stratification in spring followed by nutrient limitation. Such a pattern has been reported for previous studies of Prince William Sound (Goering et al., 1973a, 1973b). The nutrient data we collected generally confirm this as well. It is possible that the end of the bloom period is also influenced by zooplankton grazing since the increase in zooplankton directly follows the decrease in phytoplankton. It is likely that both nutrient limitation and grazing lead to the decrease in phytoplankton biomass. These forces can also have a major impact on the composition of the phytoplankton community.

Alexander and Chapman (1980) report that the phytoplankton community consisted of 97% diatoms in April but by July it was 95 % microflagellates. We found that the diatom fraction in April, 1995 was 55%, with remainder consisting of flagellates. The presence of abundant flagellates is indicative of a mechanism for channeling dissolved organic matter (DOM) that is excreted by phytoplankton through a microbial loop. Such a mechanism retains energy in the food web that might otherwise be lost through excreted DOM. The process is relatively inefficient since at least 3 trophic levels are probably involved (Azam et al., 1983).

Horner et al. (1973) report a detailed list of phytoplankton species for Port Valdez that can also be used for comparison. The shift from nitrate limitation in 1994 to silica limitation in 1995 can have profound impact on the species composition of the phytoplankton community later in the season. Furthermore, such a shift must be the result of changes in ocean conditions that, we hope, can be modeled.

The diatoms present in April and May are expected to be prime food for the large zooplankton, and hence a major energy source for upper trophic level species. On the other hand the picoplankton are a poor food source for these zooplankton but contribute to a microbial food web that can eventually provide energy to the larger consumers.

Particulate nitrogen and carbon are closely correlated with each other and with the chlorophyll values. This is reassuring since it indicates that our chlorophyll techniques are not missing a significant component of the community biomass. Furthermore, nutrient vs. nutrient regressions show a close relationship of nitrogen to silicate, a confirmation of the dominance of diatoms in the system as reported by direct counts.

The close correlation of the phytoplankton and zooplankton increase in biomass in 1993, 1994, and 1995 (Figure 9) indicates more bottom-up forcing than has generally been assumed in this system (refer to the SEA general overview documents.

Do phytoplankton drive the food web? Yes, but. Based on our evidence and that of past studies, the timing of the bloom is a critical event that sends a signal to all trophic levels. Actually, it is an oceanographic event that initiates the signal. The manifestation of such an event in the phytoplankton community could take several forms. It could lead to a different suite of species that may or may not be acceptable zooplankton food. It may simply be a

quantitative event and the early zooplankton could be food limited. The translation of this could then be fewer progeny in the following year.

Conclusions

1. A well-defined spring bloom of phytoplankton occurs In Prince William Sound. The timing of the bloom depends on light and mixing conditions in a given year. Local conditions are important in determining the phytoplankton biomass.

2. Phytoplankton bloom community consists of at least 55% diatoms in the size range of 20 to 100μm, suggesting a direct herbivore link to the food web. An active microbial loop that retains energy in the main food web is proposed for the system.

3. Productivity in 1995 is ultimately silica depleted but in 1994 it was nitrogen limited. This suggests a shift in ocean conditions and ecosystem processes.

4. Spatial patterns indicate that the northern sound has 'lake' conditions and the southern portion is a 'river' of Gulf of Alaska water. The high biomass of phytoplankton and zooplankton occurred only in 'lake' waters in 1995.

5. Phytoplankton and zooplankton are closely coupled in space and time. The timing of the spring phytoplankton bloom sets the timing of the appearance of the zooplankton.

6. The field data support the SEA ocean model, confirming a biological reality of 'lake' and 'river' conditions.

Papers Presented

McRoy, C.P., D.L. Eslinger, B. Bergeron, D. Clayton and A. Ward. 1995. Seasonal patterns of phytoplankton and nutrients in Prince William Sound, Alaska. AAAS, Arctic Division Science Conference, Fairbanks, AK, September 1995.

D.L. Eslinger, R.T. Cooney and C.P. McRoy. Physical forcing of interannual variability in plankton populations in Prince William Sound, Alaska: results of a biophysical model. AGU/ASLO Ocean Sciences meeting, San Diego CA, February 1996.

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Strickland, J.D.H. and T.R. Parsons. 1972. A Practical Handbook of Seawater Analysis. Bulletin 167, Fisheries Research Board of Canada, Ottawa, 310 pp.

Sverdrup, H.U. 1953. On conditions for the vernal blooming of phytoplankton. Cons. perm intl. Expl. Mer 18(3):287-295.

Table 2. Phytoplankton community composition during the bloom (April 18-27, 1995, Station AFK 95.2).

DIATOMS	Size Range (lxw) μm	FLAGELLATES	Size Range (lxw) μm
Asterionella sp.	12x3 -15x4	*Oxytoxum sp.*	25x8 -40x15
Chaetoceros sp.	2.5x2.5 - 40x30	*Peridinium sp.*	20x15 - 65x50
Coscinodiscus sp.	135 -190	Unidentified flagellate	5x2.5 -7.5x5.0
Eucampia sp.	35x20 - 60x15	Unidentified silicoflagellate	
Grammatophora sp.		Unidentified dinoflagellate	10x10 - 80x55
Leptocylindrus danicus	20x7 - 40x10		
Leptocylindrus minimus	35x5 - 40x5		
Leptocylindrus sp.	35x5 - 40x7		
Navicula sp.	40x15 - 60x10		
Nitzschia sp.	35x2 -100x5		
Rhizosolenia sp.	25x14 -500x15		
Skeletonema costatum	5x2.5 -15x2.5		
Stephanopyxis nipponica	40x20 - 60x30		
Thalassiosira sp.	10 - 65		
Thalassionema sp.	30x5 -45x5		
Unidentified centric diatom			
Unidentified diatom	15x10 -130x15		
Unidentified pennate diatom	20x5 - 45x7		

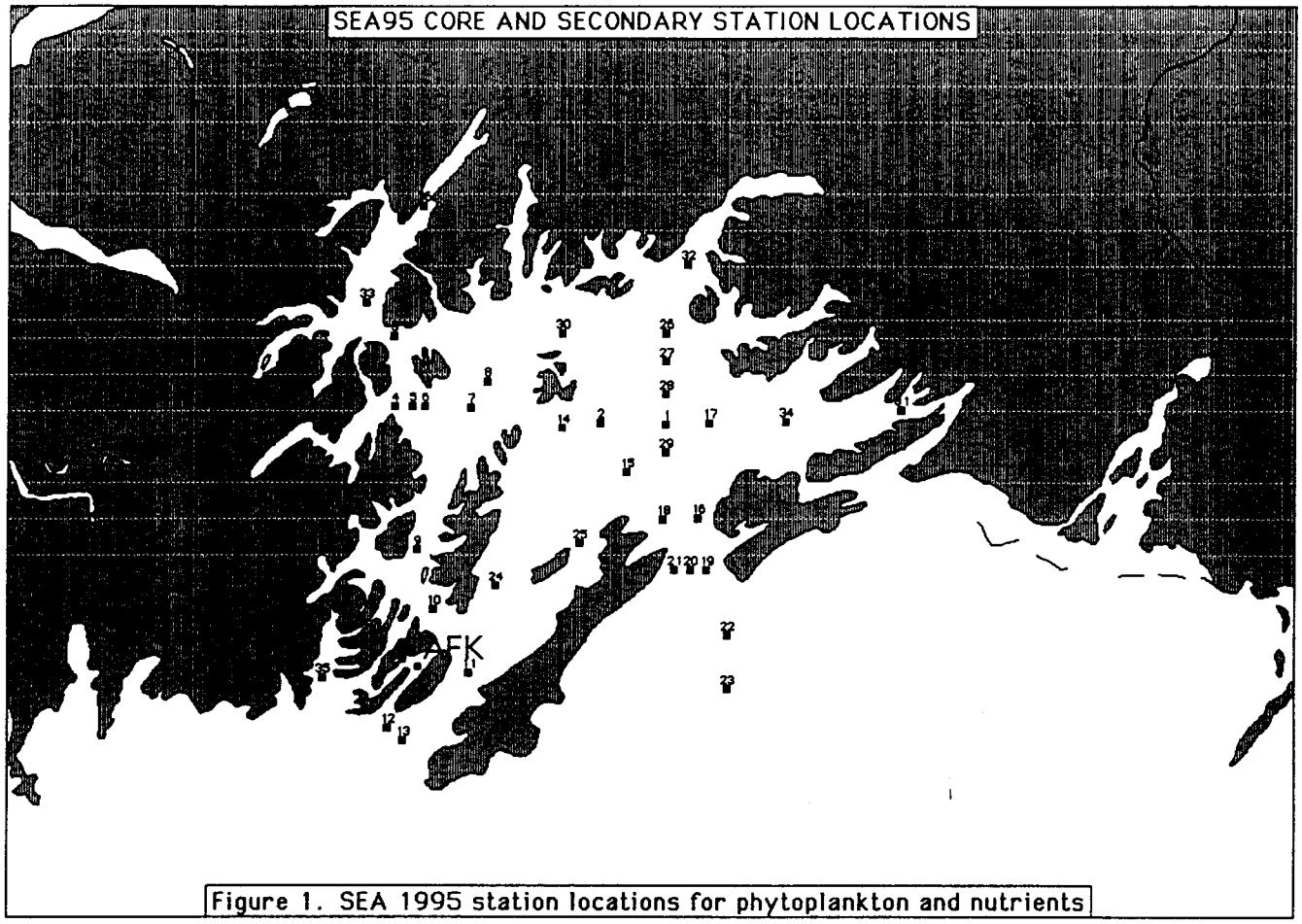


Figure 1. SEA 1995 station locations for phytoplankton and nutrients

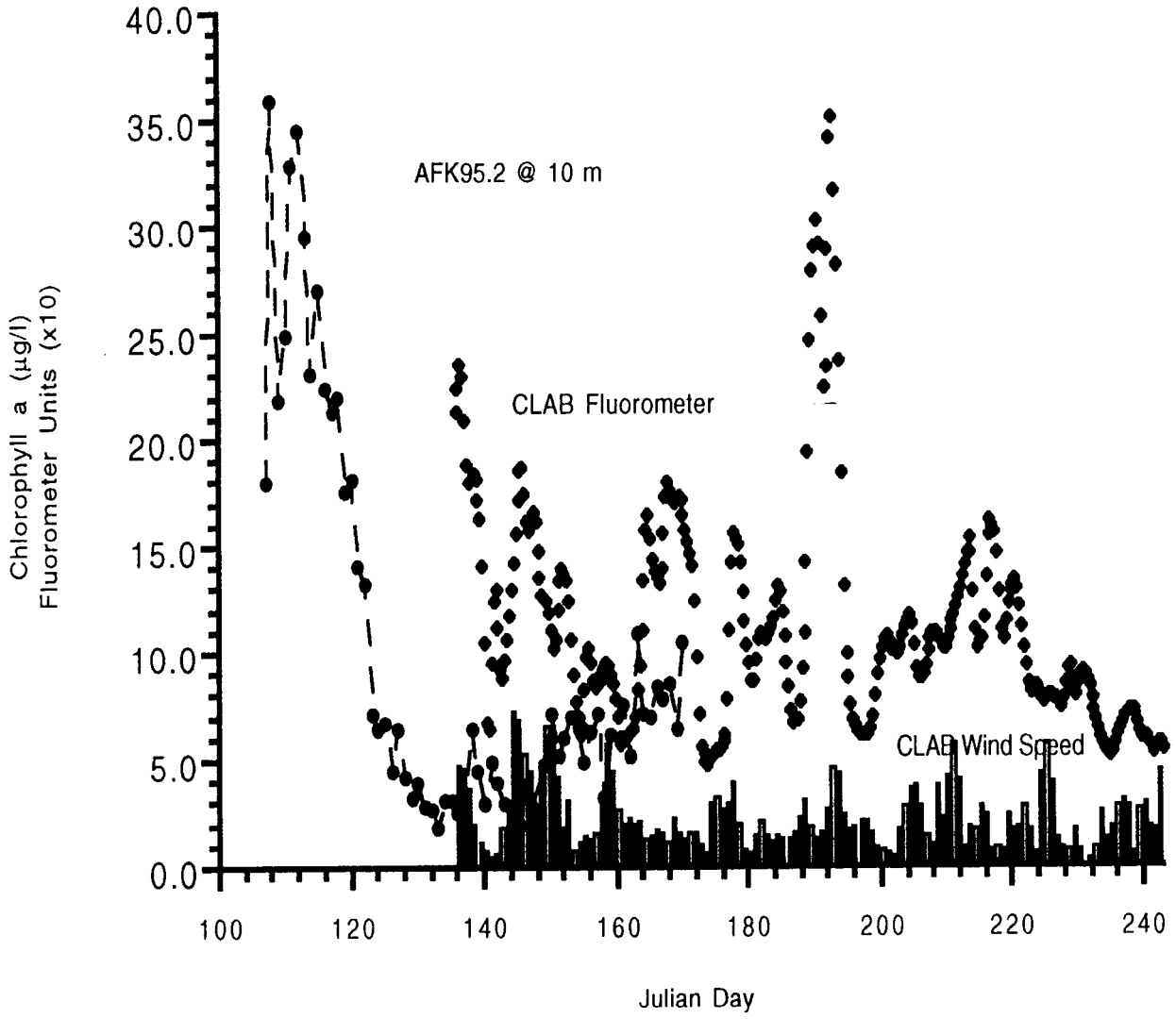
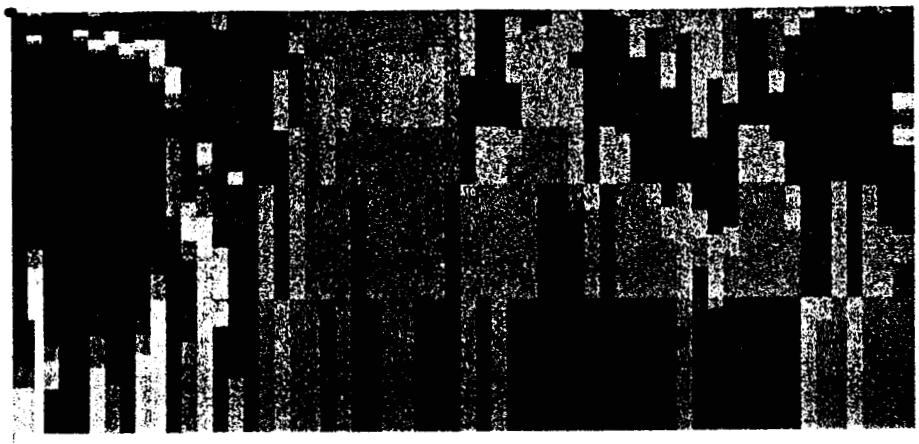
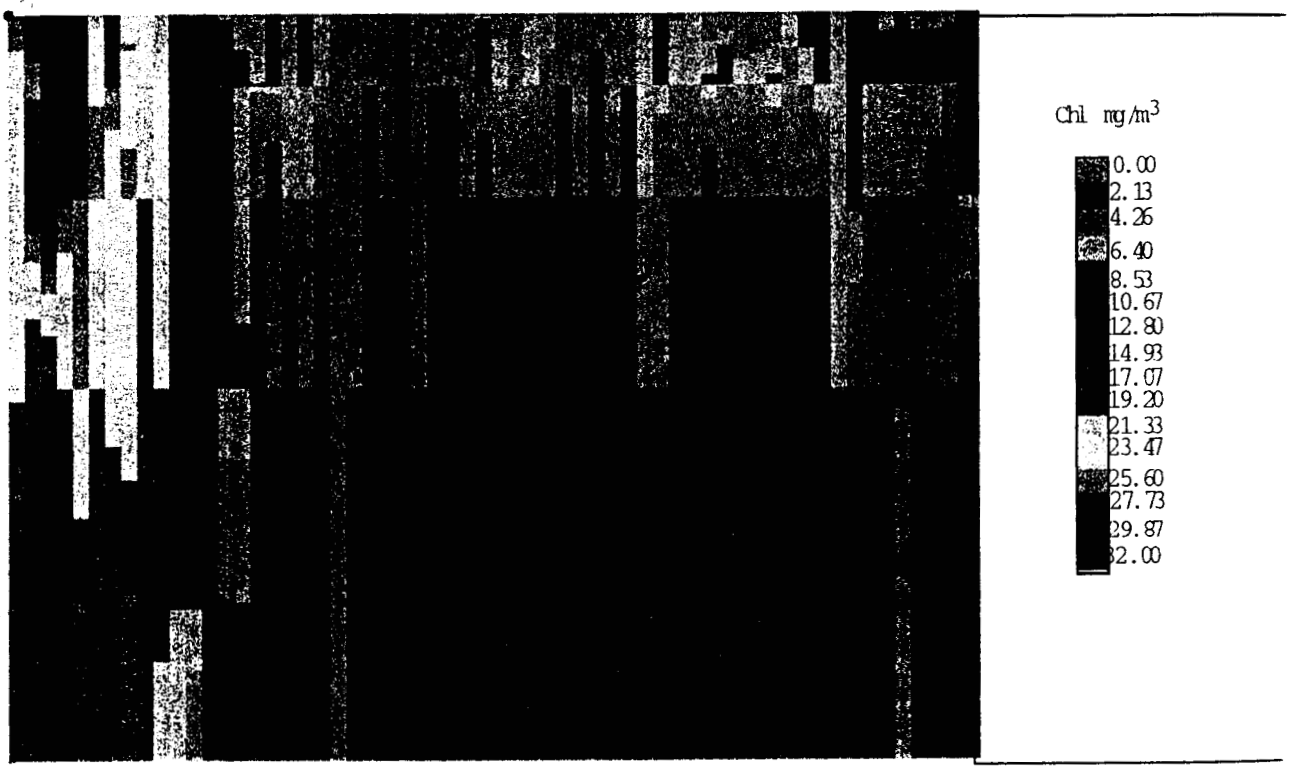


Figure 2. Time series of chlorophyll a at AFK95.2 (10 m) in relation to the 10 m fluorometer signal (x10) and surface wind speed (m/sec) at the CLAB moored instrument buoy.

AFK Station 1 1995



AFK Station 2 1995



18 April — 19 June

Figure 3. Time series of phytoplankton biomass as measured by chlorophyll a for two locations in Prince William Sound in the vicinity of AFK Hatchery.

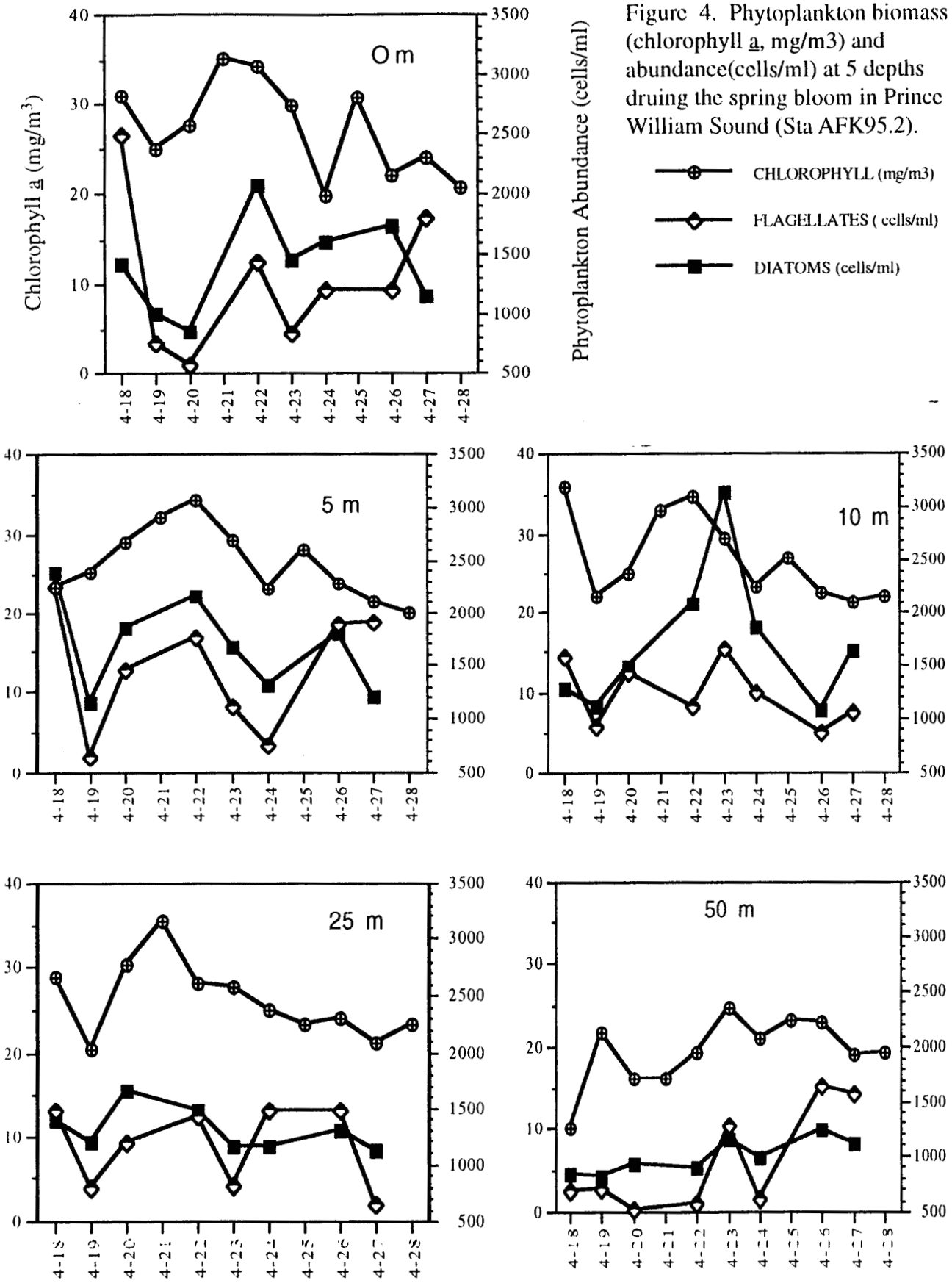


Figure 4. Phytoplankton biomass (chlorophyll a, mg/m3) and abundance(cells/ml) at 5 depths druing the spring bloom in Prince William Sound (Sta AFK95.2).

Figure 5. Major taxa of the diatom community during the spring bloom(18-27 April 1995) in Prince William Sound (Station AFK95.2).

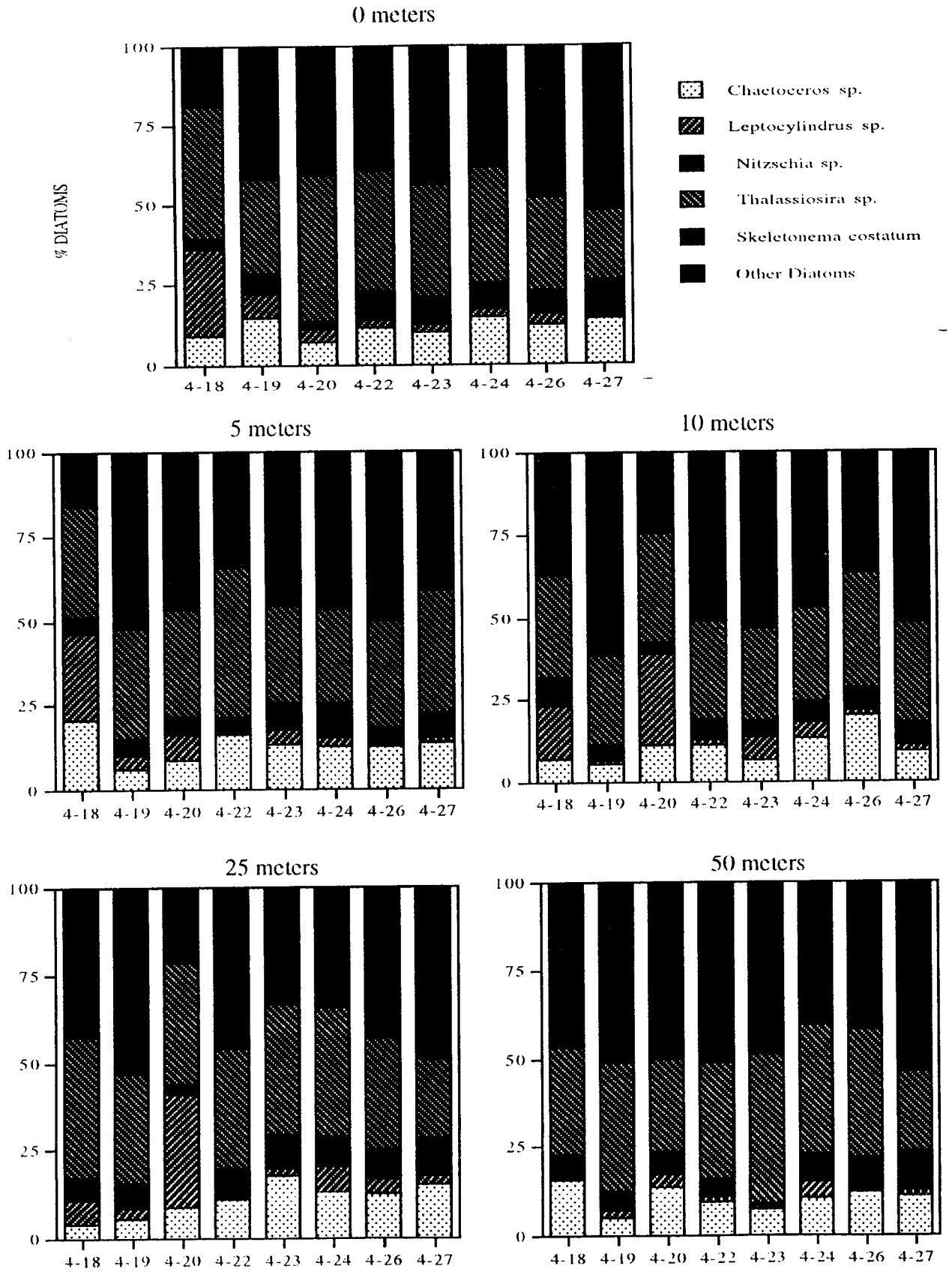
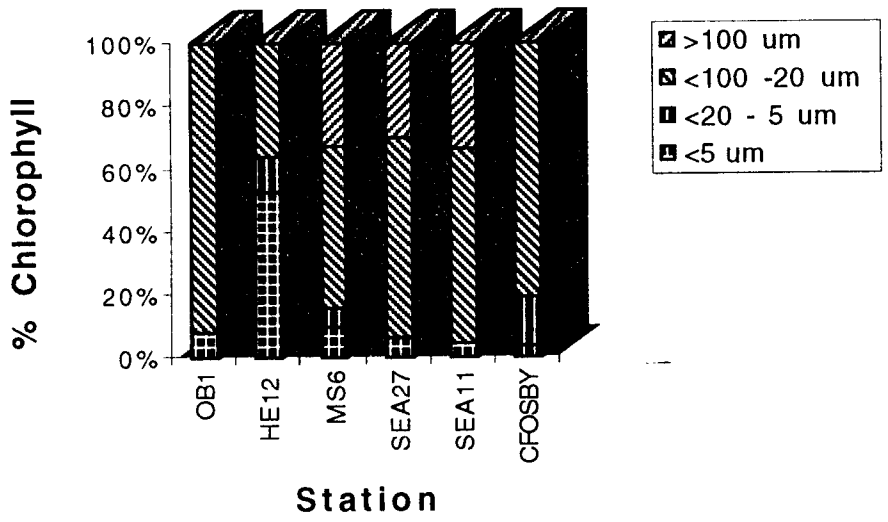


Figure 8. **Size Fractionation of Chlorophyll at Maximum Chlorophyll Depth April 10-16, 1995**



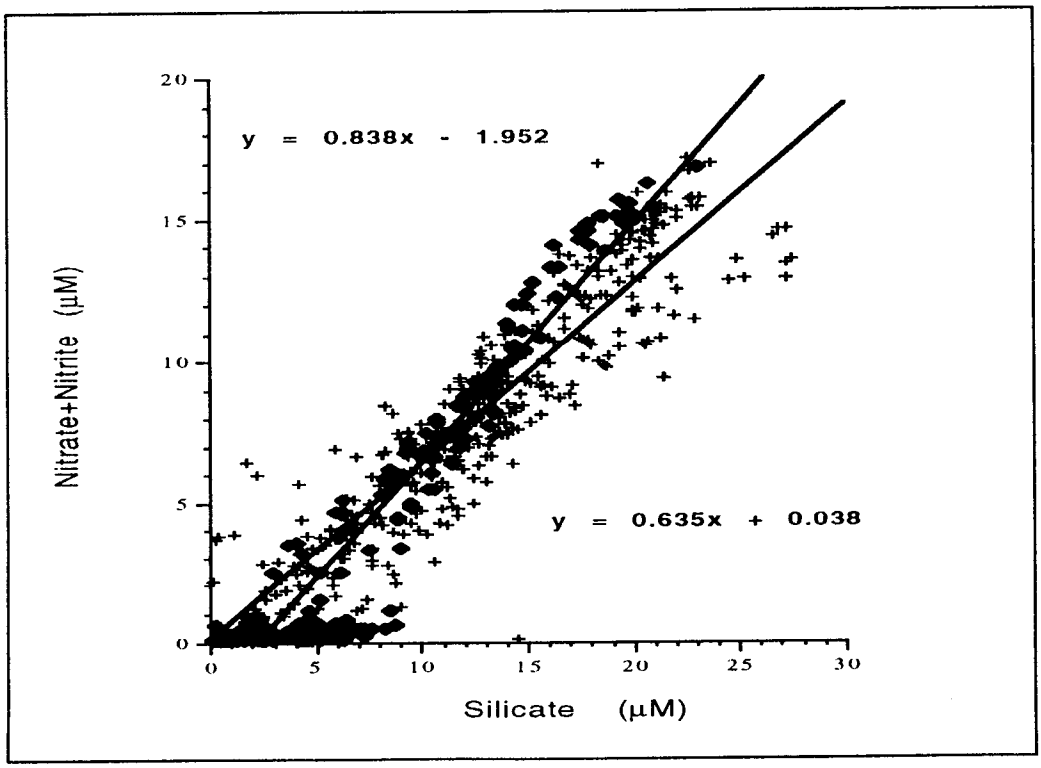


Figure 6. Plot of nitrate vs. silicate for time series data from 1995 (AFK95.2) and 1994 (CWN Hatchery, Ester Island).

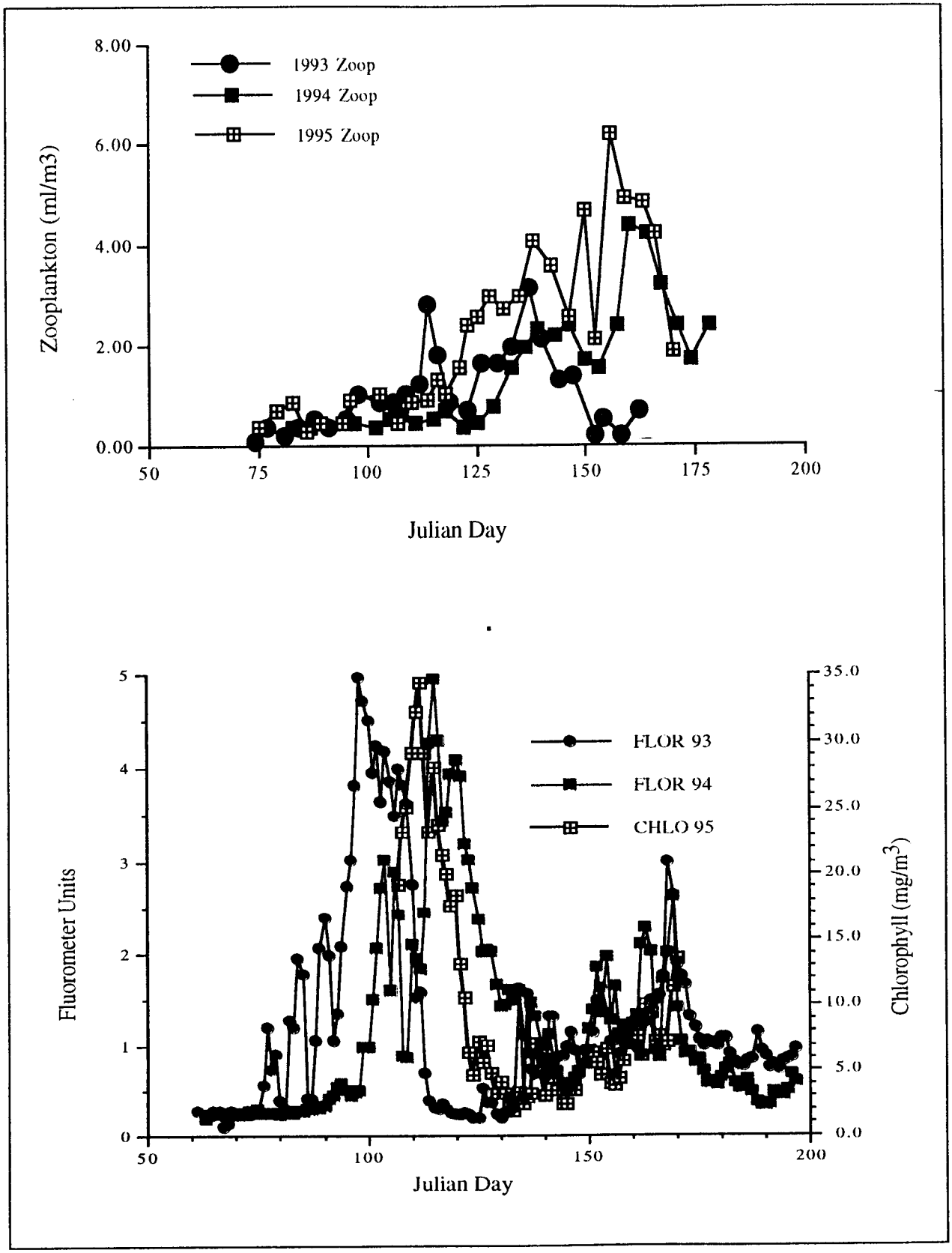
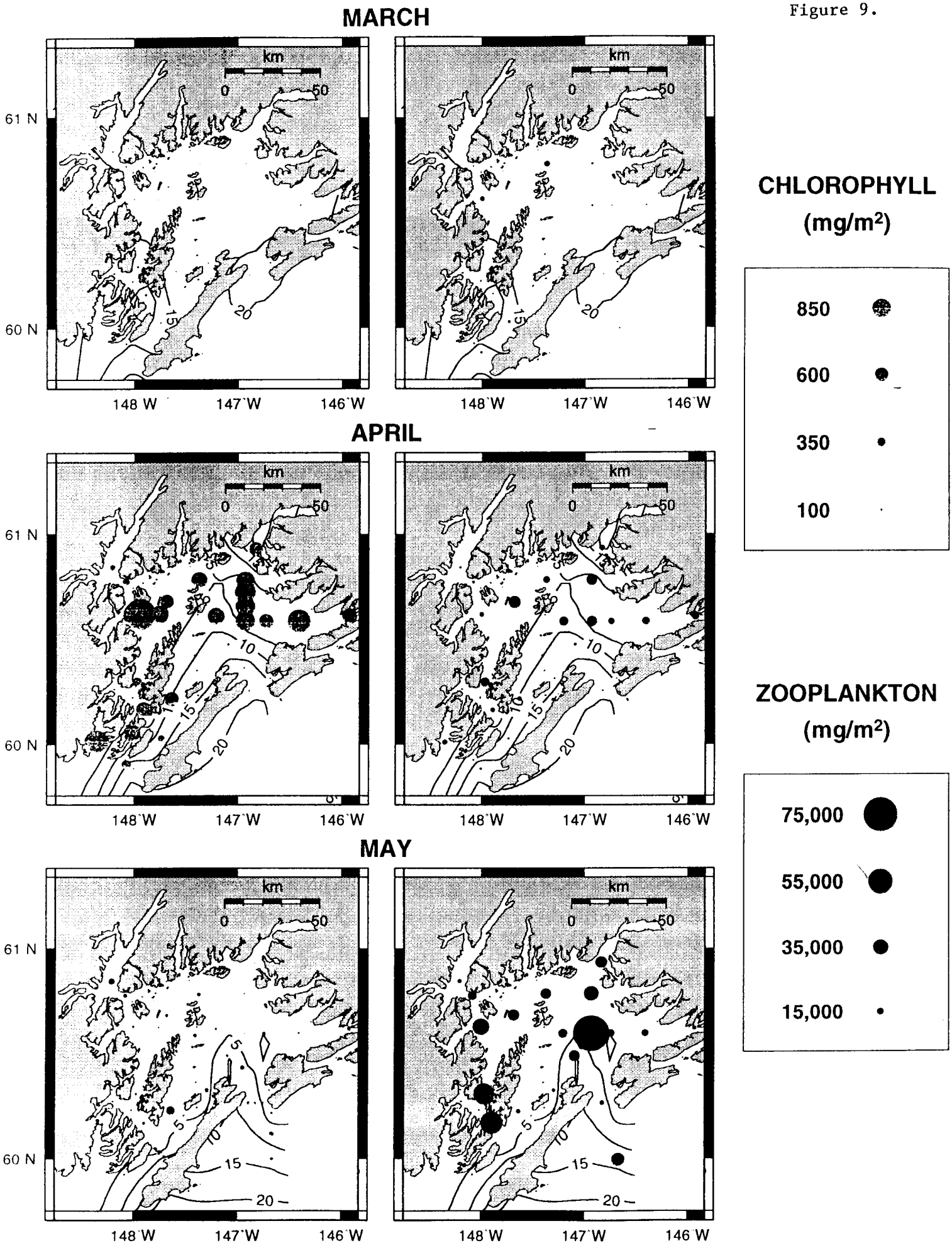


Figure 7. Time series of phytoplankton and zooplankton biomass from AFK95.2 and fluorometer data from CLAB buoy for 1993, 1994, and 1995. In all years the zooplankton peak lags that of phytoplankton by 17 to 21 days, indicating a close food web link.

Figure 9. Spatial fields of nutrients, phytoplankton, and zooplankton for March, April and May 1995 in Prince William Sound.

Figure 9.


MARCH
APRIL
MAY
km
0
50
61 N
60 N
148 W
147 W
146 W
5
10
15
20
CHLOROPHYLL
(mg/m²)
850
600
350
100
ZOOPLANKTON
(mg/m²)
75,000
55,000
35,000
15,000

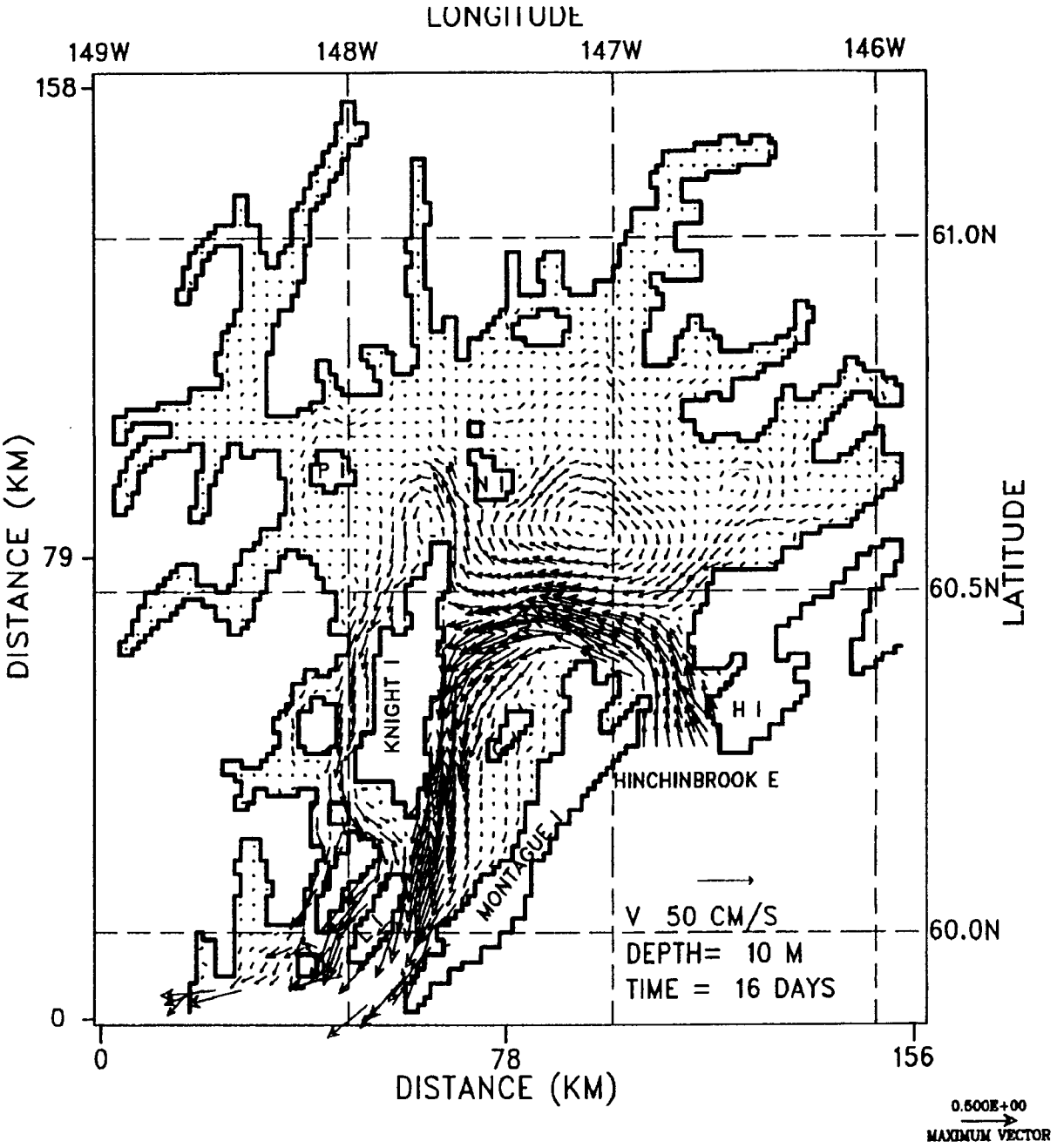
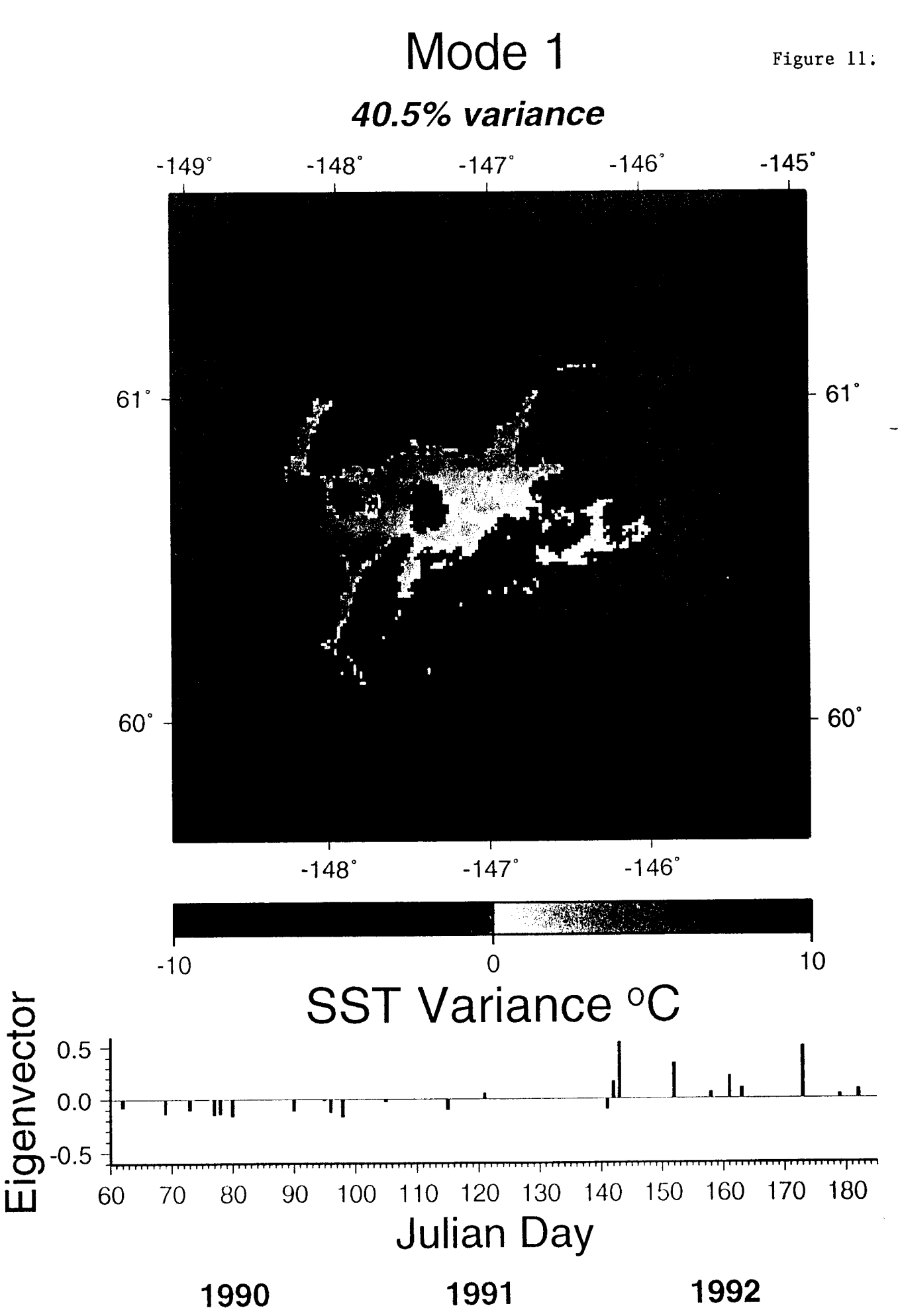


Figure 10. Ocean forcing model showing the "lake" and "river" (strong flow in southern part of Prince William Sound); compare these results to the phytoplankton and nutrient fields in Figure 9. This figure is by V. Patrick and J. Wang, Component 95320J.

Figure 11. First mode of EOF analysis of sea surface temperature patterns. This mode is the empirically derived spatial pattern and its associated time series of eigenvectors, which together explain more of the variance in SST images than any other possible patterns. In this case the first mode explains over 40 % of the variability in SST data from over three years. Eigenvectors are color coded according to year.

Figure 11.


Mode 1
40.5% variance
-149°
-148°
-147°
-146°
-145°
61°
60°
-10
0
10
SST Variance °C
Eigenvector
0.5
0.0
-0.5
60
70
80
90
100
110
120
130
140
150
160
170
180
Julian Day
1990
1991
1992

Chapter 5

95320H The Role of Zooplankton

Exxon Valdez Oil Spill
Restoration Project Annual Report

The Role of Zooplankton in the Prince William Sound Ecosystem

Restoration Project 95320-H
Annual Report

This annual report has been prepared for peer review as part of the Exxon Valdez Oil Spill Trustee Council restoration program for the purpose of assessing project progress. Peer review comments have not been addressed in this annual report.

Robert T. Cooney
Kenneth O. Coyle

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April 1996

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Restoration Project Annual Report

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April 1996

The Role of Zooplankton in the Prince William Sound Ecosystem

Restoration Project 95320-H
Annual Report

Study History: This project was initiated in April, 1994, as part of the multi-disciplinary Sound Ecosystem Assessment (SEA) program studying processes constraining the non-recovery of pink salmon and herring. An annual report was issued in 1995 by Cooney, R. T., under the title The Role of Zooplankton in the Prince William Sound Ecosystem as a chapter contribution to the single compiled report of all SEA94 projects. Two conference presentations were contributed from work sponsored by the EVOS Trustee Council in 1995: 1) Cooney, R. T. and L. Tuttle, Zooplankton in the Prince William Sound Ecosystem: The Role of Size, Species Composition, and Behavior in Mediating Food Web Transfers, Arctic Division, American Association for the Advancement of Science, Annual Meeting, Fairbanks, September, 1995; and 2) Cooney, R. T., Sound Ecosystem Assessment (SEA): Understanding Processes Constraining the Production of Pink Salmon and Herring in Prince William Sound, Alaska, Arctic Division, American Association for the Advancement of Science, Annual Meeting, Fairbanks, September, 1995. 1995 was the second of five anticipated years of funding for zooplankton studies within the SEA program in Prince William Sound. This project is expected to complete its field and modelling work in FY 98, and submit a final report in FY 99 as part of an overall SEA synthesis.

Abstract: Work on zooplankton in 1995 was designed to support studies of juvenile salmon survival in northwestern Prince William Sound, and to examine basin-scale distributions as part of an overall plankton dynamics investigation. Relative to 1994, the early spring zooplankton bloom in 1995 occurred earlier, and stocks at AFK were the highest measured since 1989. For a composite of *Neocalanus plumchrus* and *N. flemingeri*, C1 and C2 copepodite stages were present in March at all locations. By April, all five copepodite stages were present, but stage C3 was numerically dominant. In May, the early stages were diminishing and most of the biomass was centered in the large C5 stage. *Neocalanus* was absent in the surface waters in June except for relatively high numbers at the GOA6 station south of Hinchinbrook Entrance in the open shelf environment. A two-way ANOVA for 6 index stations in the "river region" and 6 in the northern fringe of the Sound demonstrated that *Neocalanus plumchrus/flemingeri* biomass (all stages) was higher in the northern region ($P<0.05$), and that cruises (March, April, May, and June) were also statistically different. In the vertical sense, C4 *Neocalanus* tended to be twice as abundant in the upper 20 m of the water column than between 20 and 50 m. A small sample of full adult pollock stomachs obtained in late April and May were dominated by stage C4 and C5 *Neocalanus,* prompting us to hypothesize that this large fish filter feeds in dense, seasonally occurring layers of large copepods. Pteropod *(Limicina helicina)* biomass in June was much higher than the previous three months ($P<0.05$), but there was no north/south gradient evident. This pteropod was very important in the diets of adult pollock in late May and early June of 1995 (see Herring and Salmon Integration report). Surprisingly, *Limacina* biomass was about the same

in 1994 and 1995 during May, but higher in 1994 during early June.

A biomass ranking of the top 15 taxonomic categories collected during the day and at night in March, April, May, June and September demonstrated a shift from a largely euphausiid-dominated community in late winter to large copepods in April and May, then to pteropods and small copepods in June, and finally to jelly plankton (Ctenophora) and large and small copepods in September. The biomass was usually greater at night for these communities, surface populations being supplemented by diel vertical migrators like euphausiids and the copepod *Metridia.* The March and September biomass was low relative to April, May and June.

Key Words: Zooplankton, *Neocalanus*, oceanography, Prince William Sound, copepod, amphipod, euphausiid, *Calanus*, *Pseudocalanus*

Citation: Cooney, R. T. and K. O. Coyle. 1995 The Role of Zooplankton in the Prince William Sound Ecosystem. Exxon Valdez Oil Spill Restoration Project Annual Report (Restoration Project 95320-H), Institute of Marine Science, University of Alaska Fairbanks, Fairbanks, Alaska 99775-1080.

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Executive Summary

The Sound Ecosystem Assessment (SEA) program was designed to identify and describe the mechanisms influencing survival of juvenile pink salmon and herring in Prince William Sound. This information is presently being used to craft a series of interactive numerical models for management and restoration purposes. Since zooplankton serves as food for young salmon and herring, and for predators (fishes, birds and marine mammals) that also consume juvenile salmon and herring, understanding the interannual, seasonal, and daily dynamics of this important forage element in the pelagic ecosystem is central to the overall SEA synthesis and modelling effort.

A 15-year record of springtime plankton biomass is available from the non-profit salmon hatcheries in the region. For a location in the southern Sound (AFK), annual average settled volumes for April and May are highly correlated (r2=0.70) with the average April/May Bakun Upwelling Index computed for the shelf south of Hinchinbrook Entrance. Zooplankton stocks tend to be greater during years of weak onshore convergence and diminished when the convergence is strongest. This relationship is not apparent for collections at a hatchery in the northwestern part of the Sound (WN). A continuing goal of SEA zooplankton studies is to determine the mechanism(s) establishing this statistical correlation and describe its spatial distribution in the region relative to flow and upper-layer temperature fields.

SEA contends that many of the apex consumers in the Sound, including adult pollock and herring, some sea birds, and marine mammals obtain large portions of their annual rations from zooplankton. This contention is supported by results from diet studies of large fishes captured as part of the SEA salmon and herring predation program. Many zooplankton taxa are important contributors to apex consumer diets including large calanoid copepods, euphausiids, amphipods and pteropods. Some of these taxa occur seasonally as the result of life history patterns keyed to the annual phytoplankton bloom which begins in late March and early April in response to increasing light and shoaling wind-mixed layer (see the SEA modelling and Information Services report). Differences in the timing of the pelagic plant bloom are translated forward to differences in the timing of the spring increase in zooplankton populations. Our examination of a small sample (n=35) of full adult pollock stomachs collected from midwater trawl collections in late April and early May, 1995, demonstrate a remarkable dependence on C4 and C5 *Neocalanus* spp. at this time. This dependence was supplanted in 1995 by Pteropoda (*Limacina helicina*) in late May and early June after the large calanoid populations declined (see Herring and Salmon Integration report).

Our studies of large calanoid copepods in the Sound are aided by the ability to separate and enumerate the different copepodite stages. By applying published (Miller, 1993) molting rates for these stages, it is possible to estimate the time required to develop from the smallest C1 to the largest C5 and adult stages. For a composite of *Neocalanus fleminger/plumchrus*, this period is approximately 50-60 days. This means that the C5 stages comprising the late April and May calanoid bloom in the Sound must be residents for at least this time period, or they arise from adjacent shelf populations with inflow at Hinchinbrook Entrance. Overwintering adult

populations have been documented in the deep water of the region (see Role of Zooplankton 1994 annual report) as have upper-layer populations of the C1 stages in March. On the basis of current patterns, it is probable that south-Sound populations are associated with the Alaska Coastal Current (ACC) intrusion , but that most northern populations are produced by local reproduction from the deeper waters of that region. Direct observations of currents indicate that flow fields in the eastern, northern and western parts of the Sound are less organized, weaker, and more variable than flow in the south (see Physical Oceanography report). One of the first experiments using the 3-dimensional eddy-resolving transport model of the Sound (see Modelling and Information Services report) will be to examine the question of retention times of "local populations" molting from C1-C5 in the northern part of the Sound for evidence that resident reproduction, or seeding, or both establishes population biomass each year.

Studies of the vertical distributions of zooplankton in 1995 centered on the life stages of *Neocalanus* because of its importance in the diet of pollock. C1-C3 stages were generally distributed homogeneously from the surface to 50 m. However, the C4 stage tended to twice as abundant in the upper 20 m than from 20 to 50 m. A stabilizing water column (salinity and temperature) probably focuses the diminishing plant bloom in late April and May at or near the depth of a seasonally developing nutricline. This focus should draw grazers into a relatively narrow zone resulting in "layers and swarms" of zooplankton. An example of this layering was observed for *Neocalanus* C4-C5 size particles in optical plankton counter (OPC) records in late April (see Physical Oceanography report). We surmise this kind of layering may dictate (in part) the distributions of near-surface adult pollock populations during the spring.

A demonstrated influence of tidal range on the survival of hatchery-released pink salmon (see Salmon and Herring Integration report) demonstrates a mechanism that mediates predation on fry. One hypothesis suggests that tidal energy expended in the "edge-zone" of Prince William Sound during spring tides, dilutes layers of zooplankton that feed adult pollock causing a redistribution to deeper-water prey fields and reduced predation on near-surface pink salmon fry. In this regard, we found that *Neocalanus* spp. biomass in the upper 50 m was not correlated with tidal range (May and June), but haste to add our measurements are for integrated densities, not *in situ* concentrations (a consequence of the vertical tow sampling strategy). Layer dilution could have been occurring but not reflected in the integrated vertical tow records. An alternative hypothesis for improved hatchery fry survival during periods of spring tides is that all or most predatory fish cease to feed, or markedly reduce their feeding during periods of strongest monthly tidal currents. This behavior would open "windows" of 3-7 days twice a month during when predation on fry would be minimal.

Differences observed in how alternative prey is utilized each year by adult pollock illustrates the need to monitor zooplankton species composition as one element of future modeling activities supporting nowcasts and forecasts of pink salmon and herring survivals. In 1995, pteropods were more important in diets of adult pollock from mid-May through early June. This switch from large copepods to *Limacina pacifica* may have been initiated by an early decline and low *Neocalanus* biomass in mid-May. May pteropod stocks were about the same in 1994 and 1995

so the switch does not appear to have been initiated by the snails. An examination of the composition of day and night zooplankton communities in the upper 50 from March through September documents seasonal successional patterns. In March, day and night communities were dominated by euphausiids. By April and May, euphausiids were diluted by large calanoid populations, that shifted to small copepods, pteropods, and jelly-plankton in June, and then to jelly plankton (Ctenophora) and large and small copepods in September.

Introduction

The Sound Ecosystem Assessment (SEA) program was designed specifically to determine whether or not the non-recovery of pink salmon and Pacific Herring following the Exxon Valdez oil spill was being constrained partially or wholly by natural mechanisms. Salmon populations in the Gulf of Alaska respond to interannual and decadal-scale variations in environmental conditions influencing their levels of production (Hare and Francis, 1995). The most recent regime shift occurred in the late 1970s and resulted in markedly increased wild production levels in Gulf salmonid populations. This phenomenon is apparent in the production histories of wild pink salmon in the region from ADF&G production records (Figure 1). Wild production peaked in 1984, and with some exceptions, has been declining to pre-regime shift levels each year since. In contrast, the production of hatchery-released pink salmon in Prince William Sound increased steadily after 1976, reaching a peak in 1990. Much of this increase was associated with an increase in the numbers of fry produced each year. A comparison of wild and hatchery marine survivals supports the contention that common factors phased similarly in time effect the production potential for both populations in the region (Figure 2).

The salmon literature proposes that salmon marine survival is established each year during early marine residence, perhaps during the first few weeks in the coastal ocean (Bax, 1983; Hargreaves and LeBrasseur, 1985; Hartt, 1980; Healey, 1982; Parker, 1971). The mechanism for loss is believed to be predation, and the rate of loss is thought to be modified by the growth rate of the fry during this critical period. The slowest growing fish probably experience the highest rates of mortality since they remain at risk longer in the smallest, presumably weakest sizes. Temperature and food have been implicated as the major factors influencing growth rates (Healey, 1991; Mortensen, 1983; Walters et al., 1978) Locally, Willette (1985), and Willette and Cooney, (1991) found that production levels of odd and even year southcentral Alaska pink salmon stocks are sensitive to fry-year spring-time ocean temperatures, and that the odd brood lines are also influenced by ocean temperatures during the late maturing and adult stage.

Wild juvenile pink salmon in the northern Gulf of Alaska begin emerging into the nearshore tidally-mixed zone in late March. The outmigration from natal habitats is usually completed by early June (Taylor 1988). Cooney et al., (1995), demonstrated close correspondence between the timing of wild pink salmon ocean entry and the timing and duration of a coastal springtime zooplankton bloom in Prince William Sound. This correspondence suggested that fry benefit by emerging during this period. In fact, marine survival estimates from the hatchery program

demonstrate that fry released into the bloom perform better than fry released prior to, or after the peak of zooplankton biomass. Until recently, this observation seemed to confirm food-limited growth dependence for fry since juvenile pink salmon are immediate consumers of pelagic food in the deep, nearshore waters of the region (Urquhart, 1979). However, more recent studies (Willette, 1996), demonstrate pink salmon fry growth rates (determined from post-release recaptures of wire tagged fry) are predicted by springtime temperatures, but not by levels of food. This surprising result means that either zooplankton is rarely growth-rate limiting for fry, or that plankton plays some as yet-to-be-determined role in modifying hatchery and (presumably) wild stock production.

The zooplankton component of the SEA program is attempting to understand the coupling between zooplankton as forage for fry and juvenile herring, and zooplankton as alternative prey for predators that also consume juvenile salmon and herring. This work is being undertaken as a SEA collaboration in each of the present field/modelling focus groups; Ocean State and Plankton Dynamics, Pink Salmon Recruitment Dynamics, and Pacific Herring Recruitment Dynamics. In a sense, zooplankton forms one important "bridge" between bottom-up oceanographic forcing and subsequent "effects" at higher trophic levels. Understanding the time/space mechanics of this "bridge" will provide the means to simulate these processes for the kinds of numerical tools SEA is producing for the Trustee Council and its member agencies.

Objectives

Each year, the timing, numbers, biomass and species composition of zooplankton populations are expected to differ in response to interannual and decadal-level shifts in oceanographic and meteorological forcing influencing the northern Gulf of Alaska and Prince William Sound. Understanding how this variability translates to juvenile fish survival requires the careful interpretation of the outcomes of natural experiments staged each year. Accomplishing this requires standardized monitoring techniques and focused process studies. The following represent objectives identified for 1995 zooplankton data bases contributing to collaborative studies of juvenile pink salmon and Pacific herring survival:

1. Use hatchery annual Plankton Watch and SEA shipboard collections to describe the timing, duration, magnitude, and species composition of spring-time upper-layer zooplankton stocks in northern and southern Prince William Sound.

2. Utilize shipboard samples of zooplankton to describe how ontogenetic and diel shifts in distribution and species composition influence trophic coupling between juvenile salmon and herring and their predators.

3. Provide direct measures of community composition, and indices of abundance and size for macrozooplankton occurring in layers and swarms that are also being sampled with optical and acoustic means.

4. Describe how the timing and magnitude of the phytoplankton bloom each year influences the timing and magnitude of subsequent zooplankton populations.

5. Work cooperatively with other components of SEA to test the Lake/River, Prey Switching, and herring Overwintering hypotheses.

6. Provide that means to supply zooplankton for stable isotope analyses.

Methods

Zooplankton stocks in Prince William Sound were sampled in 1995 with simple ring nets, 0.5-m diameter (0.335-mm Nitex) fished vertically in the upper 20 or 50 m of the water column. Hatchery collections follow procedures established for the PWSAC Plankton Watch. This includes twice-weekly sampling at specified locations near each hatchery. Hatchery nets (0.5-m diameter; 0.250-mm) are lowered by hand and retrieved vertically in the upper 20 m. Three or fewer samples are composited in a daily collection at each station to provide sufficient material for settled volume measurements. Following a hatchery determination of settled volume, the samples are stored for processing later at UAF. It is assumed that 3.9 m3 of water are filtered in each vertical tow from 20 m to the surface, although there is no way to determine how much water is actually sampled per tow. Filtering more water (common result with hand-held operations) will result in over estimates of zooplankton stocks.

Zooplankton collections from the R/V *Bering Explorer* and F/V *Alaska Beauty* were obtained in 50-m (occasionally deeper) vertical tows integrating the upper layers of the water column. Samples were collected at SEA core stations and from other locations as needed to support the juvenile salmon work. Aboard ship, the net was fished using a meter-block and light-wire hydrographic winch. The net was weighted with a thirty-lb. canon ball, lowered quickly to depth (20-m, 50-m, deeper sea bed) and immediately retrieved at 1.0 m/sec. A wire angle was estimated during the retrieval to judge error in volumes filtered. Using the winch and heavy terminal weight, most tows from 20 or 50-m to the surface were completed in less than 5 min. However, there were times when excessive wind-drifting introduced large wire angles and increased the volumes being filtered. Samples resulting from extreme wire angles can be excluded from analyses.

Because the schedule of occupying SEA oceanographic stations was not stratified by time of day, zooplankton tows were collected at all hours during the day and after dark. Inspection of data from these different collections indicates a general increase in upper-layer biomass after dark, presumably caused by diel migration of some taxa. This component of variance will reduce the precision of data for certain taxa including euphausiids, amphipods, some copepods and total estimates of biomass, if not taken into account. Most analyses reported here are for daytime collections.

Zooplankton collections are processed in the laboratory using standard oceanographic

procedures. The entire sample is scanned for large or otherwise obvious animals which are counted directly. The remainder of each collection is then subsampled for the numerically dominant species. About 100 to 150 specimens are counted and indentified in subsamples. Subsamples are obtained using a stemple pipet or Folsom plankton splitter or combination of both.

Results

Lake/River Considerations: Macrozooplankton stocks measured in the southern Sound at the AFK Hatchery were the highest since 1989, the last "lake" year (Figure 3). Compared with 1994, the calanoid bloom at AFK in 1995 began and ended earlier than the previous year (Figure 4). The relationship with Hinchinbrook Entrance Bakun wind forcing (upwelling index) remained consistent, Although we found no relationship between the upwelling index and settled volumes measured at the WN Hatchery in the northwest corner of the Sound (Figure 5). A regression of *Neocalanus plumchrus/flemingeri* (all stages combined) on total zooplankton for April and May (slope=0.68) suggested that during the period of wild and hatchery fry entry into the Sound in 1995, about 70% of the measured settled volumes from the hatchery plankton watch was probably *Neocalanus* (Figure 6). With the exception of one measurement, zooplankton stocks at the WN Hatchery at Esther Island were weaker than in 1994, and like AFK, began and ended earlier in the year (Figure 7). These observations have obvious ramifications for juvenile pink salmon survival differences between these two hatcheries in 1995 that may be evident in the 1996 returns.

Results from a two-way ANOVA testing the main effects of month (March-June) and location (north and south) for a subset of six index stations representative of the ACC intrusion and six across the northern deep-water Sound demonstrated seasonality ($P<0.05$) for all categories except euphausiids (composite of all species). North/south gradients emerged for *Neocalanus* spp. (composite of *plumchrus/flemingeri;* all stages) and euphausiids; both taxonomic groups demonstrated higher stocks over the deeper northern region (Table 1). Taxa for this analysis were selected because some are important in pollock diets, while others are abundant but not eaten. The *Neocalanus* pattern is suggestive of consistent north/south differences in the historical plankton records from AFK and WN. While total zooplankton also tended to be higher in the north, observed north/south differences were not statistically significant ($P>0.05$).

Basin-Scale Patterns: A ranking of the top 15 taxa by day and night from all samples collected on Bering Explorer cruises in March, April, May, June and September demonstrates major shifts in near-surface zooplankton community composition from late winter to summer and early fall. In March, the biomass was low and both day and night communities were dominated by euphausiids and the large copepod, *Metridia okhotensis* (Table 2). By April and May, dominance in biomass shifted to *Neocalanus plumchrus/flemingeri* and *Calanus marshallae* (Table 3,4). In early summer, the pteropod *Limacina helicina*, small copepods (*Pseudocalanus* spp. and *Acartia longiremis*), and jelly-plankton were dominating the animal plankton, while by September, jelly-plankton, and large and small copepods were the obvious components (Table 5,6). The

occurrence of CV *Neocalanus flemingri* and *N. plumchrus* in June results mostly from one sample acquired outside the Sound over the adjacent shelf. *Calanus pacificus* was only present in September in the surface waters.

An examination of time-space patterns in the numerical distribution of northern and southern stocks of *Neocalanus plumchrus/flemingeri* by copepodite stage (March-May) illustrates the development from C1 through C5 in approximately 60 days (Figure 8). Seasonal differences for the stages are real ($P<0.05$) but north/south gradients, though suggestive, are not ($P>0.05$). For this analysis, core stations were arbitrarily partitioned north and south of 60.5 degrees north Latitude in the region.

When the inter-stage molt frequency is set at 15 days (a reasonable value), changes in numbers within stages and the overall biomass of the population can simply modeled. Using a daily mortality rate suggested by Miller (1993) and average wet weights for each stage, a hypothetical cohort can be followed in time with a 30-day arrival of CI stages at the surface beginning on March 1(Figure 9). Because the CIV and CV stages are so massive, relative to the younger stages, the bloom in biomass occurs in the older stages despite their much reduced numbers. This crude model accurately predicts the general timing of the macroplankton bloom observed by the hatcheries in the region.

Selected Comparisons Between 1994 and 1995 in the Northwestern Region: Zooplankton studies aboard the F/V Alaska Beauty were conducted to support juvenile salmon survival studies being conducted near the WN Hatchery at Esther Island A late April through mid-June study period was common for both years and was selected for comparative purposes (Figure 10). For Copepoda, the May increase observed in 1994 was not apparent in 1995. Instead, large copepods, including *Neocalanus plumchrus/flemingeri* declined steadily through the period, not peaking in mid-May as generally expected (Figure 11). Larvacean (*Oikopleura* spp.) stocks were higher in 1995, but Gasropoda (Limacina helicina) was not, despite the fact they were apparently more important in the diets of adult pollock in 1995 (Figure 12, 13). The overall relationship between large and small copepods during this period appears to be similar for both years (Figure 14). Amphipods and euphausiids, two taxa found commonly in adult pollock stomachs appear to be more abundant in 1994, but no statistical tests were preformed to evaluate year-to-year differences (Figure 14, 15).

Vertical Distribution Patterns: Although most of the emphasis for zooplankton work has centered on regional-scale patterns, and seasonal and ontogenetic behavior, there was some work undertaken in 1995 to examine vertical patterns in the upper 50 m. Much of this work is being reported as results from Plankton Acoustics and optical plankton counting (OPC) surveys (see Physical Oceanography and Plankton and Nekton Acoustics reports). Plankton samples were collected during OPC and acoustic surveys to describe species composition, numbers and sizes of plankters present in layers and swarms. In addition, 50 and 20-m vertical tows were obtained at all oceanographic core stations in April, May and June. For stages of *Neocalanus* spp. (C1-CV), the abundance of individuals in the upper 20-m was regressed on total numbers in the upper 50 m.

A slope of 0.40 occurs with homogeneous distributions in the upper 50 m. If all the *Neocalanus* are above 20 m, the slope of the regression will be 1.0. For the case of no *Neocalanus* in the upper 20 m, the slope of the regression will be zero. Plots for these regressions suggest that stage CI *Neocalanus* were most abundant below 20 m, that stages CII and CIII were generally homogeneous from 50 m to the surface, but that the CIV stage was about twice as abundant in the upper 20 m (Figure 17, 18, 19). The CV stage demonstrated a tendency to be more abundant above 20 m, but the difference was slight. This approach is crude, but the results hint at movement toward the surface by the younger stages, some compression near the surface in the older stages, and a possible redistribution as older CV animals begin leaving the surface for overwintering depths (reported in FY94). A short OPC record for 28 April along a transect from Hinchinbrook Entrance to Valdez Arm, illustrates a layer of "*Neocalanus* C4 and C5-size" particles in a dense layer centered at about 30 m (see Physical Oceanography report). Net samples taken at this time verified the presence of abundant C4 and C5 *Neocalanus*. The potentially important layers will be the focus of coordinated OPC, acoustic and MOCNESS sampling in May, 1996.

Pollock Stomach Contents: Although it is not the task of this project to undertake stomach analyses of the diets of pelagic fishes in the region, an opportunity was taken in late April and early May to examine a small sub-set (n=70) of full adult pollock stomachs to ascertain which components of the zooplankton community were being utilized at that time. A subsequent laboratory analysis of half of this collection (5 stomachs from 7 different trawl hauls) revealed that large copepods were being taken almost exclusively (biomass), and that the majority of these were C4 and C5 *Neocalanus plumchrus/flemingeri* (Figure 20). An examination of the gill rakers of these fish, suggested they are superbly adapted for filtering large zooplankton (probably in layers).

Tidal Influence on Zooplankton Biomass: A reduction in pollock catch near the middle of May in 1995 near the WN Hatchery appears to be associated with a redistribution of adults during a period of strong spring-tides in 1995. While we do not understand the mechanism for this redistribution, it seems logical that the monthly tidal cycle might influence the amounts and distributions of forage available to the adult pollock. However, we found no relationship between stocks of *Neocalanus* during April and May and tidal range (r2=-0.047). This does not mean that dilution of *in situ* concentrations possibly associated with increased vertical mixing during spring tides was not occurring, only that the vertically integrated biomass (50 m) was not related to the tidal cycle during this time. Since this also seemed to be a period when *Neocalanus* was beginning to leave the upper layers, the ontogenetic migration may have confounded any tidal relationship. The vertical distribution of layered zooplankton relative to tidal range will be examined as part of the FY96 zooplankton field work.

Discussion

After two years of study, our work, coupled with that of others, is demonstrating a complex role for zooplankton in Prince William Sound. Traditionally, zooplankters have been considered a critical link between phytoplankton and higher level consumers, both pelagic and benthic. Our

observations conform to this model, but also provide insight into local mechanisms that create webs rather than simple food chains in the region. The initial carbon budget that SEA prepared as part of its proposal for pink salmon and herring-related studies suggested that zooplankton would serve as food for not only 0-age fish (including pink salmon and herring), but for older juvenile and adult fishes, seabirds and marine mammals as well. These trophic transfers become are because of the life history strategies, behaviors and sizes of the zooplankters in the Sound. Several calanoid copepods, including *Calanus*, *Neocalanus*, *Metridia*, *Eucalanus* and *Euchaeta* are among the largest in the world's ocean. In the Subarctic Pacific, compression of the shelf and coastal production cycles into 2 or 3 month periods each year focuses the reproductive and growth of many species into relatively narrow temporal and spatial (upper ocean) windows. These "peaks" in local population biomass become focal points for all consumers.

We now have evidence that the Prince William Sound pelagic environment is partitioned into two regions; 1) a northern deep-water region of generally weak and variable upper-layer currents, and 2) a southern region associated with inflow of shelf and Alaska Coastal Current water. Since there seems to be no correlation between wind-forced flow over the shelf and zooplankton stocks in the northern area of the Sound, it seems likely the northern Sound is "buffered"from effects associated with the ACC. We now suspect that differences in residence times in these two regions may hold the key for interpreting differences between the generally larger north Sound *Neocalanus* stocks, and those in the south. Understanding these differences, particularly the sources for zooplankton for each region (local reproduction, seeding from outside the Sound) is critical to ideas about interannual differences that were originally described in the context of "lake" or "river". The 3-dimensional circulation model for the Sound is being employed in 1996 to determine relationships between local stock size (*Neocalanus*) and sources and residence times (inside the Sound) of zooplankton in the two main flow regimes.

Mackas et al. (1993) on the large calanoids in the adjacent deep Subarctic ocean finds their distributions are associated primarily with seasonally changing physical strata in the upper-layer of the ocean (0-100 m). Developing *Neocalanus flemingri* and *N. plumchrus* exploit the wind-mixed surface layer above seasonally developing thermoclines. *Neocalanus cristatus* is found below this layer. If these observations are confirmed for Prince William Sound, and we have some evidence to support this now, interannual differences in the timing and degree of upper-layer seasonal stratification should be predictive of the strength of springtime zooplankton layering in the region. We contend that *Calanus* and *Neocalanus* move into the surface layers to feed each spring. Since the photic zone may initially be broad and without nutrient limitation, vertical focusing of the early life stages (C1-C3) may not be as apparent as for the later C4 and C5 stages seeking food during formation of the seasonal nutricline. Most of the annual biomass of these copepods is established in the older, much larger stages, so substantial swarms and layers of "forage" for pelagic consumers will be limited in time and space. Further, since energy for growth comes from each year's phytoplankton bloom, the timing, magnitude and duration of this event is important as well. It is becoming increasingly obvious to us that the alignment in time and space of fry food and fry predators will set the potential for fry losses each year. The evolved strategy of juvenile pink salmon entering the coastal waters in the northern Gulf each year in April

and May is apparently designed to optimize survival relative to fry food, but also to take advantage of predation sheltering by the accompanying zooplankton bloom at this same time.

Identifying the "trigger" mechanisms that couple juvenile pink salmon and their predators has proven to be difficult. If the majority of the large calanoid biomass in April and May is confined to the upper 20-30 m of the water column, stocks in the tidally-mixed nearhsore zone could be diluted by a factor of 2 or more. This gradient may be sufficient to keep adult pollock filter feeding outside fry nursery areas most of the time, but may draw juveniles into deeper water where the risk of predation may be greater. For the case of older juvenile pollock feeding on pink salmon in shallow water nursery areas, the trigger may be associated more with environmental constraints, like water temperature (see Salmon and Herring Integration report). We also wonder whether the practice of feeding fry near hatcheries during the spring may provide cues for pollock and other predators that may follow "scent" trails right to each hatchery.

Since adult pollock can, and do prey on juvenile pollock, the appearance of juveniles in fry nursery zones in mid to late May may also be related to avoiding adult populations near the surface. In years of weak zooplankton, adults may not be able to filter feed broadly in the region and avoid large areas of the Sound. Under these conditions, juvenile pollock could invade the near-shore fry nurseries sooner, and remain longer than years of higher adult biomass.
In a very general sense, the time-series of zooplankton measured at AFK tracks the wild stock success in the region since 1981 suggesting a relationship between fry survival and zooplankton (Figure 21). This new notion transfers the impact of predation on fry mostly away from adults (some impact already demonstrated) to juvenile pollock. It seems reasonable to assume that populations of 1 and 2-year old pollock will generally be more abundant than surviving adults, so that losses of fry to younger pollock could potentially be much greater than losses to adults. If this is true, fry might do better during years of higher-than-average zooplankton biomass because the adult pollock filter-feeding in adjacent waters restrict the timing and duration of juvenile invasions of the nearshore fry nursery areas. These and other complex relationships between juvenile pink salmon and herring, zooplankton, and salmon and herring predator seek collaborative resolution in continuing SEA field and modelling studies.

Conclusions

1. Zooplankton stocks measured at the AFK hatchery in 1995 were the highest since 1989. In this regard, that region was more lake-like than the previous 5 years with obvious implications for salmon returns in the summer of 1996.

2. Regional scale studies of large calanoid distributions demonstrated the presence of all copepodite stages from March through May. The youngest stages were present in both north and south regions. Development from C1 to C5 was completed in about 60 days. For a small set of northern and southern index stations, *Neocalanaus* was found to be higher ($P<0.05$) in the northern region.

3. The biomass structure in the upper 50 m shifted from a euphausiid dominated community in March, to large calanoids in April and May, to pteropods, small copepods and jelly plankton in June. Jelly plankton and large and small copepods (some not observed in other seasons) dominated the September upper-layer zooplankton biomass.

4. Adult pollock feeding at the peak of the *Neocalanus* bloom exhibited a strong selection for C4 and C5 stages in 1995.

Acknowledgements: This work would not have been possible without the able assistance of cooperating SEA personnel in the field and laboratory representing the Prince William Sound Science Center, Alaska Department of Fish and Game, the University of Alaska Fairbanks, and the Prince William Sound Aquaculture Corporation. I am also indebted to the captains and crews of the R/V Bering Explorer and F/V Alaska Beauty. Their inventiveness, professionalism and desire to "get the job done" was crucial to the completion of our work.

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Table 1. Analysis of Variance (ANOVA) for two locations (north and south) and four times (March, April, May, and June) for biomass of seven categories of large zooplankton collected in 1995

	Source of Variability		
Taxonomic Category	Location	Month	Location x Month
Total zooplankton	NS	**	NS
Neocalanus plumchrus/flemingeri	*	**	NS
Pseudocalanus spp.	NS	**	NS
Limicina helicina	NS	**	NS
Euphausiacea	*	NS	NS
Amphipoda	NS	**	NS
Larvacea	NS	**	NS

NS = $P>0.05$
* = $P<0.05$
** = $P<0.01$

Table 2. Ranking the top 15 zooplankton taxa by biomass from samples collected during the daytime and at night in March, 1995; R/V Bering Explorer

Daytime Samples

Taxonomic Category	Rank	Biomass (mg/m3)	Cumulative %
Metridia okhotensis	1	18.15	23.7
Thysanoessa spinifera	2	13.31	41.1
Thysanoessa inermis	3	5.27	47.9
Thysanoessa longipes	4	3.65	52.7
Euphausia pacifica	5	3.51	57.3
Pseudocalanus spp. AF	6	2.50	60.5
Calanus marshallae AF	7	2.37	63.6
Thysanoessa raschii	8	2.16	66.5
Barnacle nauplii	9	1.88	68.9
Metridia pacifica AF	10	1.75	71.2
Sagitta elegans	11	1.72	73.4
Calanidae CII	12	1.32	75.2
Calanidae CI	13	1.19	76.7
Pseudocalanus spp. CV	14	1.06	78.1
Thysanoessa raschii WS	15	0.94	79.3

Nighttime Samples

Metridia okhotensis AF	1	74.40	43.0
Thysanoessa longipes	2	19.15	54.1
Thysanoessa inermis	3	8.10	58.8
Calanus marshallae AF	4	6.25	62.4
Thysanoessa spinifera	5	5.86	65.8
Metridia pacifica AF	6	4.89	68.6
Aglantha digitale	7	4.86	71.4
Euphausia pacifica	8	4.61	74.1
Thysanoessa raschii WS	9	3.63	76.2
Sagitta elegans	10	3.62	78.3
Pseudocalanus spp. AF	11	3.28	80.2
Squid larvae	12	2.29	81.5
Calanus marschallae CV	13	2.21	82.8
Neocalanus cristatus CIII	14	2.20	84.0
Metridia spp. CV	15	1.97	85.2

AF designates adult female; C is copepodite stage; WS is without spermatophore

Table 3. Ranking the top 15 zooplankton taxa by biomass from samples collected during the daytime and at night in April, 1995; R/V Bering Explorer

Daytime Samples

Taxonomic Category	Rank	Biomass (mg/m3)	Cumulative %
Neocalanus spp. CIV	1	55.52	15.7
Neocalanus spp. CIII	2	33.58	25.5
Eirene indicans	3	30.35	33.8
Neocalanus flemingeri CV	4	18.43	39.1
Neocalanus plumchrus CV	5	17.66	44.1
Calanus marshallae AF	6	15.33	48.4
Thysanoessa inermis	7	14.46	52.5
Metridia okhotensis AF	8	12.78	56.2
Clione limicina	9	9.98	59.0
Sagitta elegans	10	7.96	61.2
Thysanoessa longipes	11	7.17	63.3
Calanidae CII	12	6.46	65.1
Octopus larvae	13	5.73	66.7
Barnacle nauplii	14	5.61	68.3
Fish larvae	15	5.29	69.8

Nighttime Samples

Metridia okhotensis AF	1	89.03	19.2
Neocalanus spp. CIV	2	42.01	28.2
Neocalanus spp. CIII	3	31.01	34.8
Calanus marshallae AF	4	21.41	39.5
Metridia okhotensis AM	5	18.35	43.4
Neocalanus plumchrus CV	6	15.36	46.7
Conchoecia spp.	7	14.22	49.8
Sagitta elegans	8	12.54	52.5
Thysanoessa spinifera	9	11.86	55.0
Neocalanus cristatus CV	10	11.57	57.5
Barnacle nauplii	11	9.83	59.6
Euphausia pacifica	12	9.77	61.7
Thysanoessa inermis	13	9.16	63.7
Thysanoessa longipes	14	9.14	65.6
Metridia spp. CV	15	8.42	67.5

AF designates adult female; AM is adult male;

Table 4. Ranking the top 15 zooplankton taxa by biomass from samples collected during the daytime and at night in May, 1995; R/V Bering Explorer

Daytime Samples

Taxonomic Category	Rank	Biomass (mg/m3)	Cumulative %
Neocalanus plumchrus CV	1	250.42	34.9
Neocalanus flemingeri CV	2	73.72	45.2
Pseudocalanus spp. CV	3	39.44	50.7
Neocalanus spp. CIV	4	39.29	56.2
Eirene indicans	5	39.00	61.6
Calanus marshallae CV	6	18.34	64.2
Calanus marshallae AF	7	17.37	66.6
Calanus marshallae CIV	8	14.64	68.7
Coryne princips	9	14.08	70.6
Pseudocalanus spp. CIV	10	14.05	72.6
Calanus marshallae AM	11	10.80	74.1
Pseudocalanus spp. AF	12	10.47	75.6
Clione limicina	13	9.70	76.9
Hippolytidae zoea	14	8.97	78.2
Oikopleura sp.	15	8.12	79.3

Nighttime Samples

Neocalanus plumchrus CV	1	316.01	31.1
Metridia okhotensis AF	2	107.26	41.7
Neocalanus spp. CIV	3	74.59	49.0
Neocalanus flemingeri CV	4	73.72	56.3
Thysanoessa longipes	5	34.76	59.7
Metridia pacifica AF	6	29.99	62.7
Pseudocalanus spp. CV	7	28.51	65.5
Metridia spp. CV	8	23.46	67.8
Neocalanus cristatus CV	9	17.79	69.5
Metridia okhotensis AM	10	17.27	71.2
Pseudocalanus spp. CIV	11	16.64	72.9
Metridia spp. CII	12	15.79	74.4
Metridia spp. CIV	13	15.60	76.0
Calanus marshallae AF	14	15.46	77.5
Neocalanus cristatus CIV	15	14.85	78.9

AF designates adult female; AM is adult male; C is copepodite stage

Table 5. Ranking the top 15 zooplankton taxa by biomass from samples collected during the daytime and at night in June, 1995; R/V Bering Explorer

Daytime Samples

Taxonomic Category	Rank	Biomass (mg/m3)	Cumulative %
Limacina helicina	1	142.32	21.2
Pseudocalanus spp. CV	2	74.46	32.3
Pseudocalanus spp. AF	3	69.95	42.7
Coryne princips	4	53.36	50.6
Sagitta elegans	5	42.45	57.0
Calanus marshallae CV	6	42.09	63.2
Oikopleura sp.	7	41.95	69.5
Limacina helicina SM	8	39.68	75.4
Clione limicina	9	13.88	77.5
Oikopleura sp. SM	10	11.45	79.2
Calanus marshallae AF	11	10.59	80.7
Acartia longiremis AF	12	10.30	82.3
Barnacle cyprid	13	7.82	83.4
Neocalanus flemingeri CV	14	7.37	84.5
Pseudocalanus spp. AM	15	6.62	85.5

Nighttime Samples

Limacina helicina	1	341.44	30.6
Neocalanus flemingeri CV	2	184.30	47.1
Metridia spp. CV	3	117.91	57.8
Metridia pacifica AF	4	64.28	63.5
Sagitta elegans	5	46.16	67.7
Pseudocalanus spp. CV	6	41.67	71.4
Neocalanus plumchrus CV	7	35.32	74.6
Pseudocalanus spp. AF	8	33.89	77.6
Thysanoessa inermis	9	25.05	79.8
Calanus marshallae CV	10	21.73	81.8
Neocalanus cristatus CV	11	15.19	83.2
Calanus marshallae AF	12	13.40	84.4
Eirene indicans	13	11.72	85.4
Thysanoessa longipes	14	11.66	85.5
Metridia spp. CIV	15	10.91	87.4

AF designates adult female; AM is adult male; SM is small

Table 6. Ranking the top 15 zooplankton taxa by biomass for samples collected during the day in September, 1994; R/V Bering Explorer

Daytime Samples

Taxonomic Category	Rank	Biomass (mg/m3)	Cumulative %
Ctenophora	1	16.52	31.6
Pseudocalanus spp. AF	2	13.20	56.9
Euphausiacea JUV	3	2.44	61.6
Limacina helicina SM	4	2.16	65.7
Eirene indicans	5	1.89	69.3
Calanus marshallae CIV	6	1.73	72.6
Neocalanus spp. CIII	7	1.09	74.7
Acartia longiremis AF	8	0.93	76.5
Pseudocalanus spp. CV	9	0.93	78.3
Calanus pacificus CV	10	0.90	80.0
Calanus marshallae CV	11	0.85	81.6
Euphausiid CPS	12	0.81	83.2
Mesocalanus tenuicornis	13	0.80	84.7
Neocalanus spp. CIV	14	0.67	86.0
Calanus pacificus CIV	15	0.65	87.2

Nighttime Samples - no night sampling

AF designates adult female; JUV is juvenile; C is copepodite stage; CPS is calyptopis stage

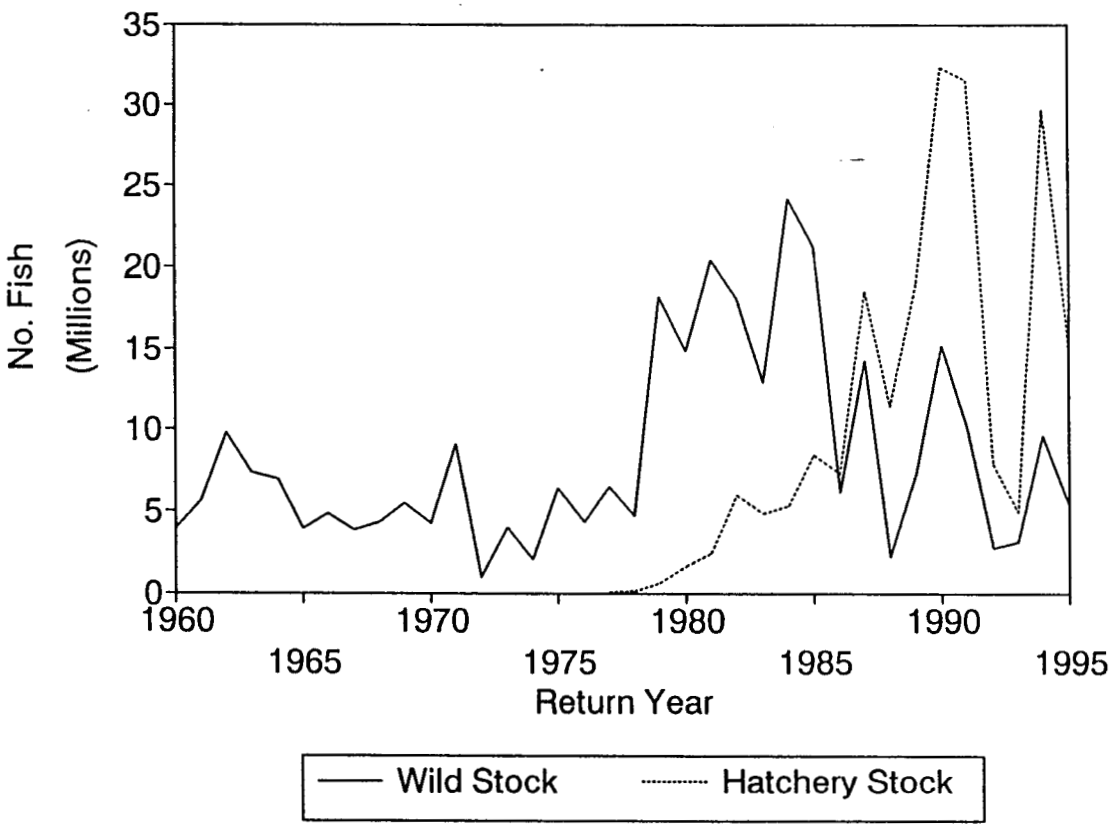


Figure 1. Wild and hatchery historical adult pink salmon returns to Prince William Sound.

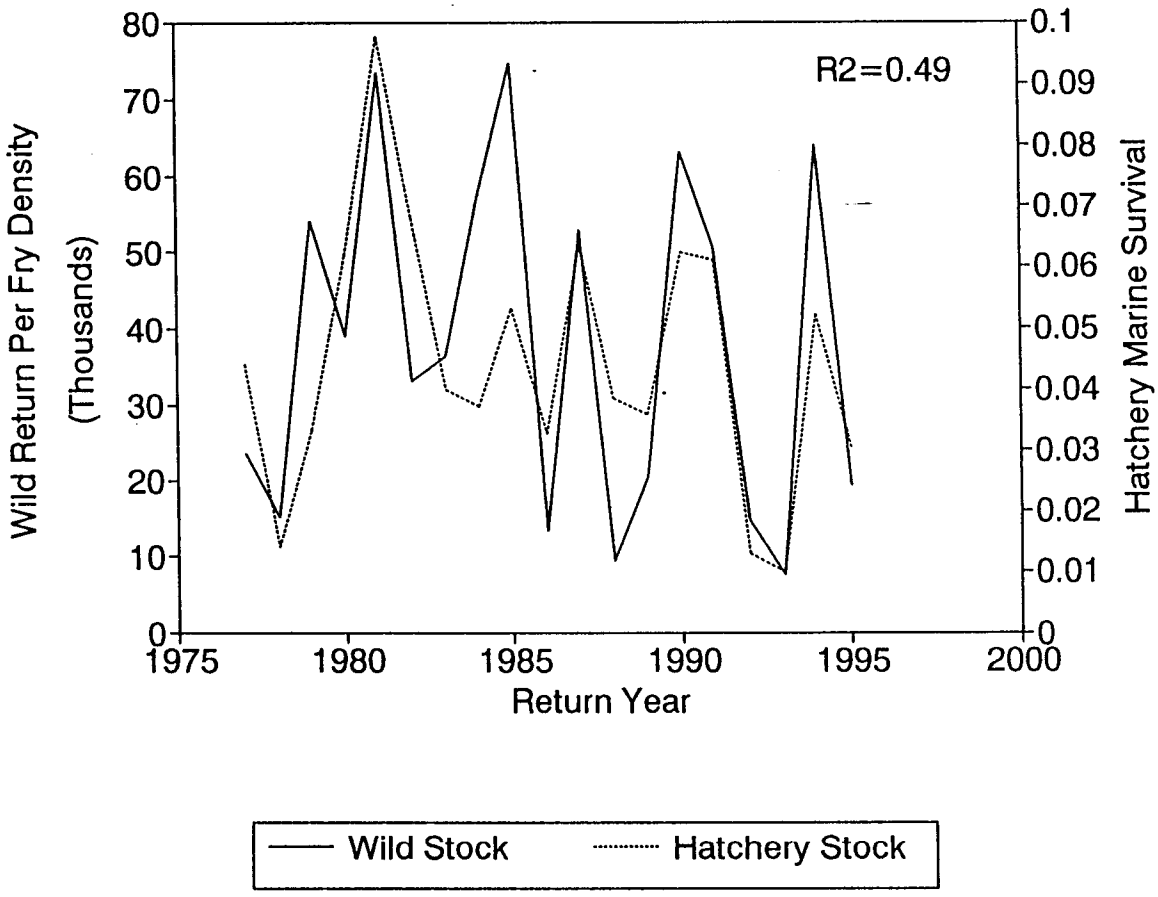


Figure 2. Annual marine survivals for wild and hatchery pink salmon populations in Prince William Sound.

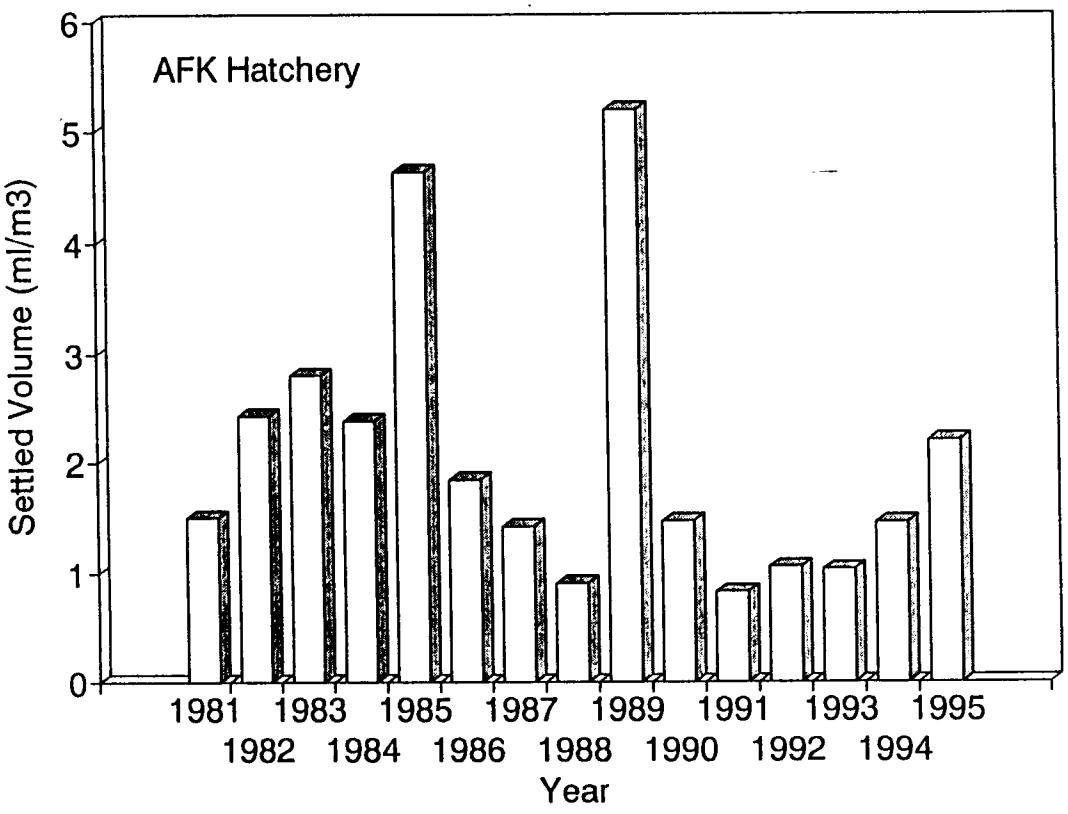


Figure 3. Average annual zooplankton stocks reported for the Elrington Passage station near the AFK Hatchery in Prince William Sound.

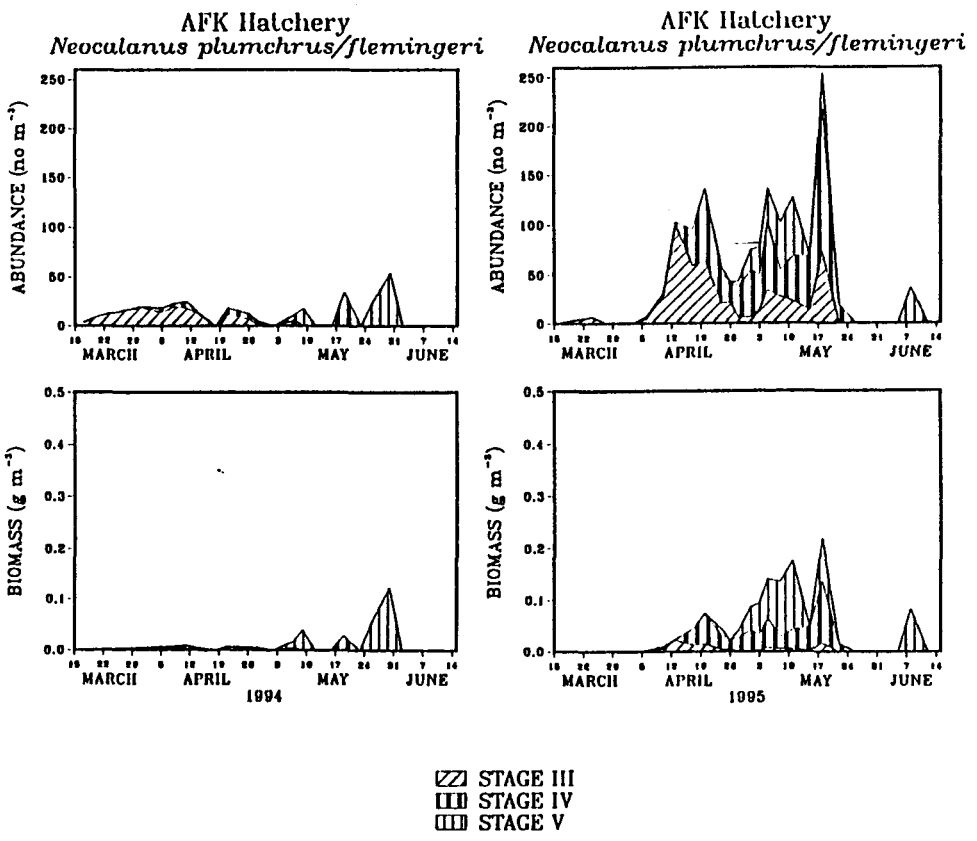
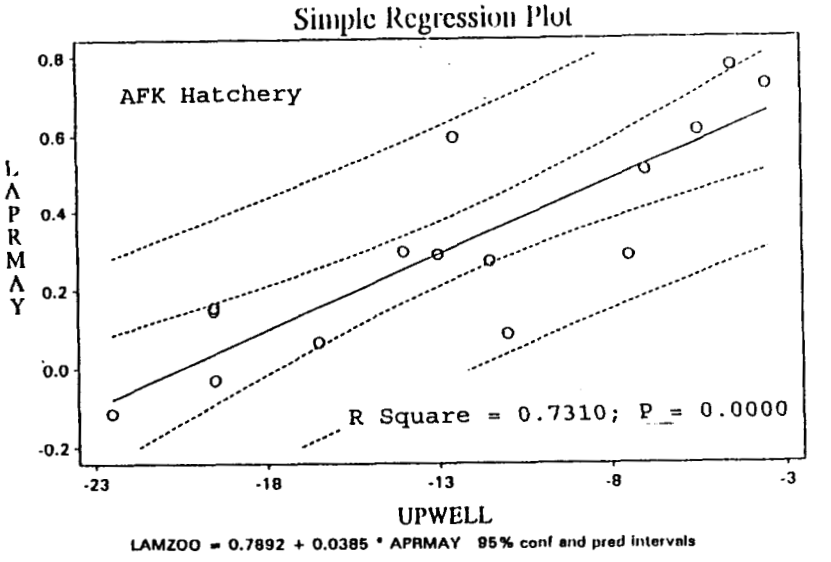


Figure 4. Seasonality in the abundance and biomass of a composite of C3-C4 *Neocalanus plumchrus/flemingeri* from the AFK Hatchery.



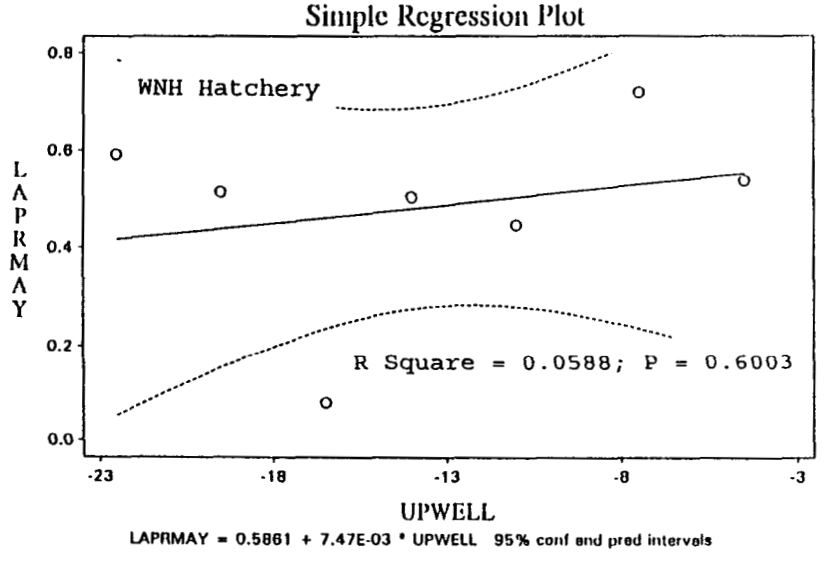


Figure 5. Regressions of hatchery zooplankton settled volumes (April and May) on the average April/May Bakun upwelling index computed for a location near Hinchinbrook Entrance.

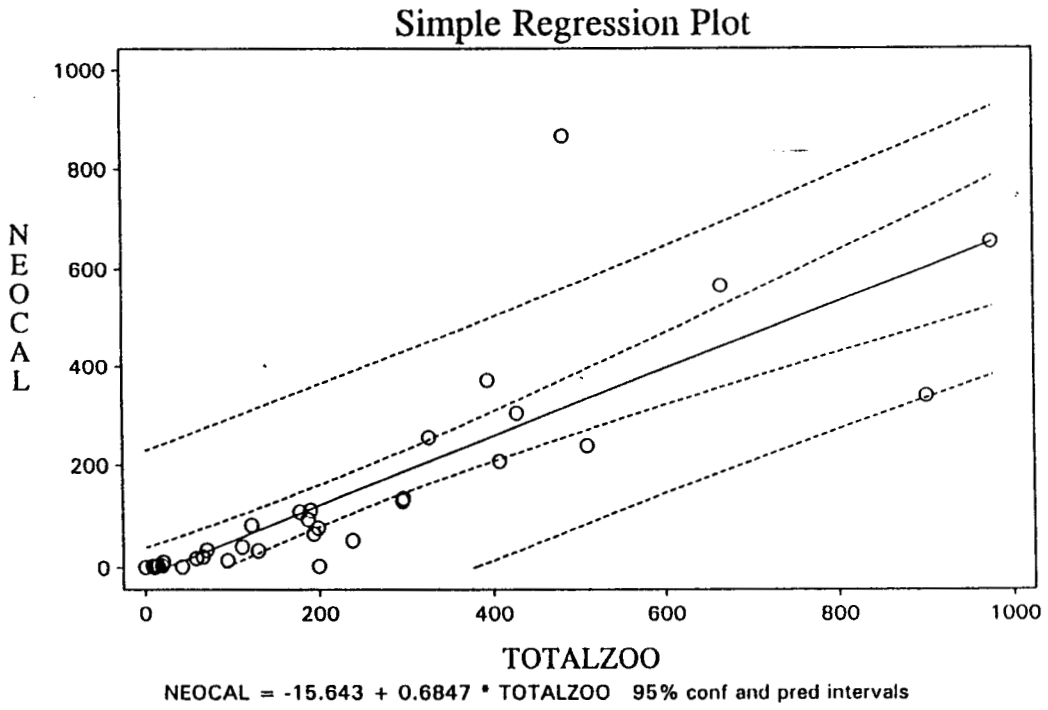


Figure 6. A regression of *Neocalanus plumchrus/flemingeri* biomass (all copepodite stages) on total zooplankton biomass.

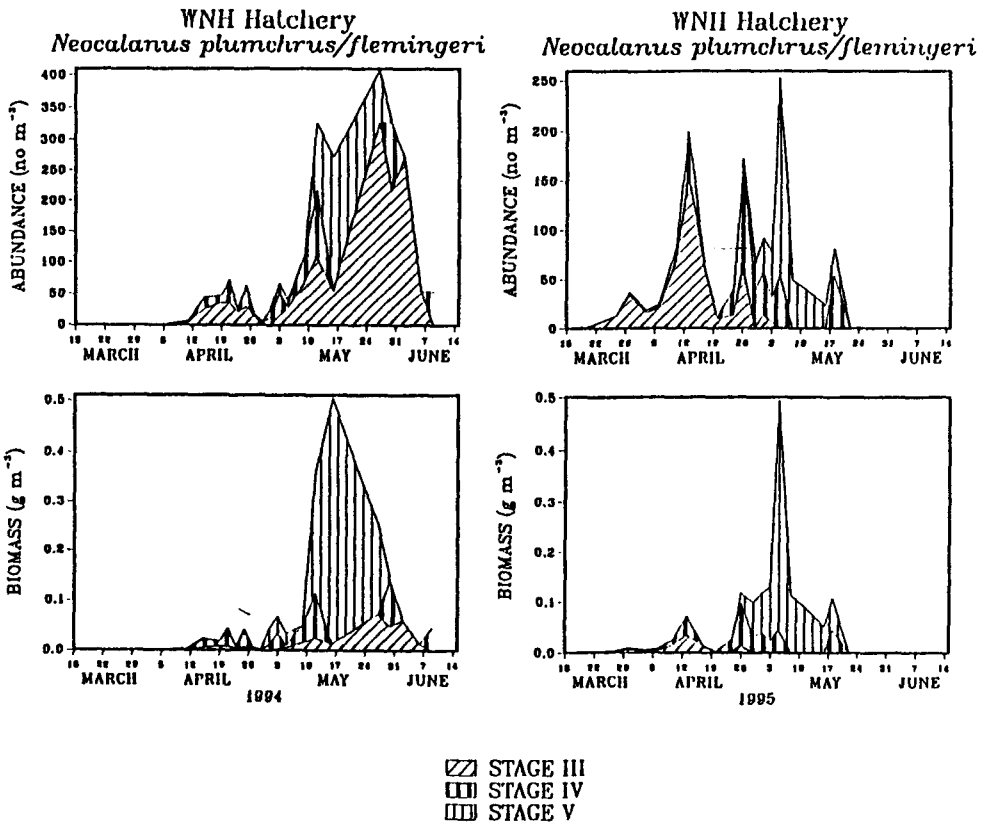


Figure 7. Seasonality in the abundance and biomass of a composite of C3-C5 *Neocalanus plumchrus/flemingeri* from the WN Hatchery.

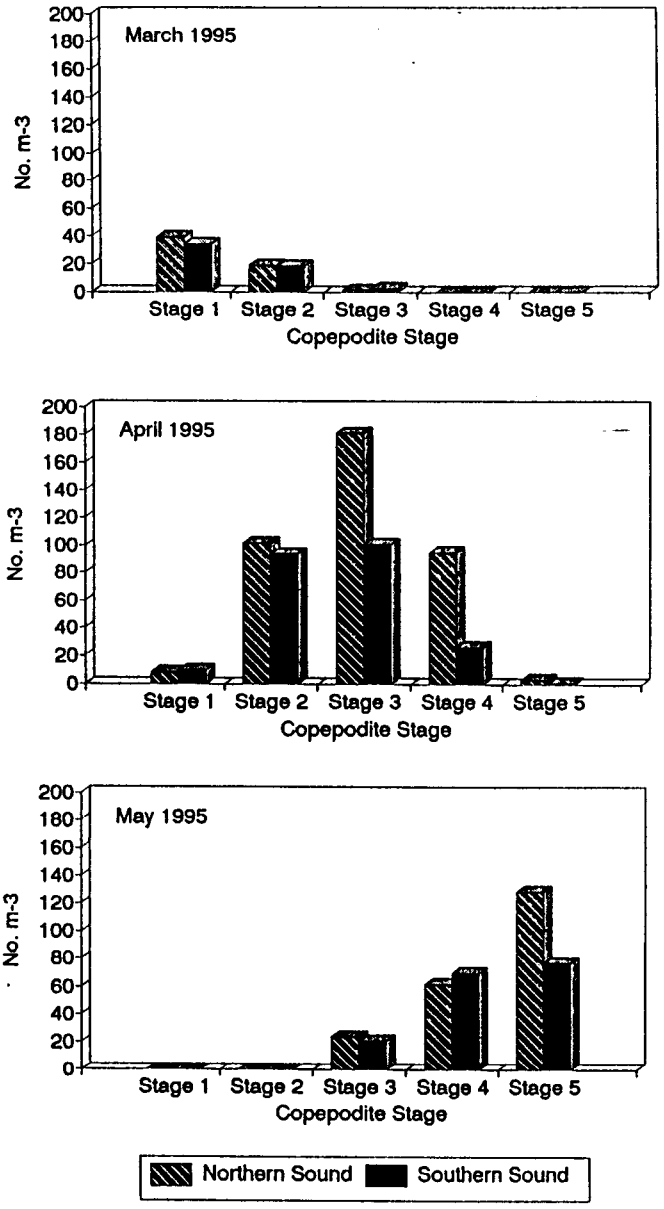


Figure 8. Monthly composition by stage for *Neocalanus plumchrus/flemingeri* partitioned arbitrarily into north and south Sound collections.

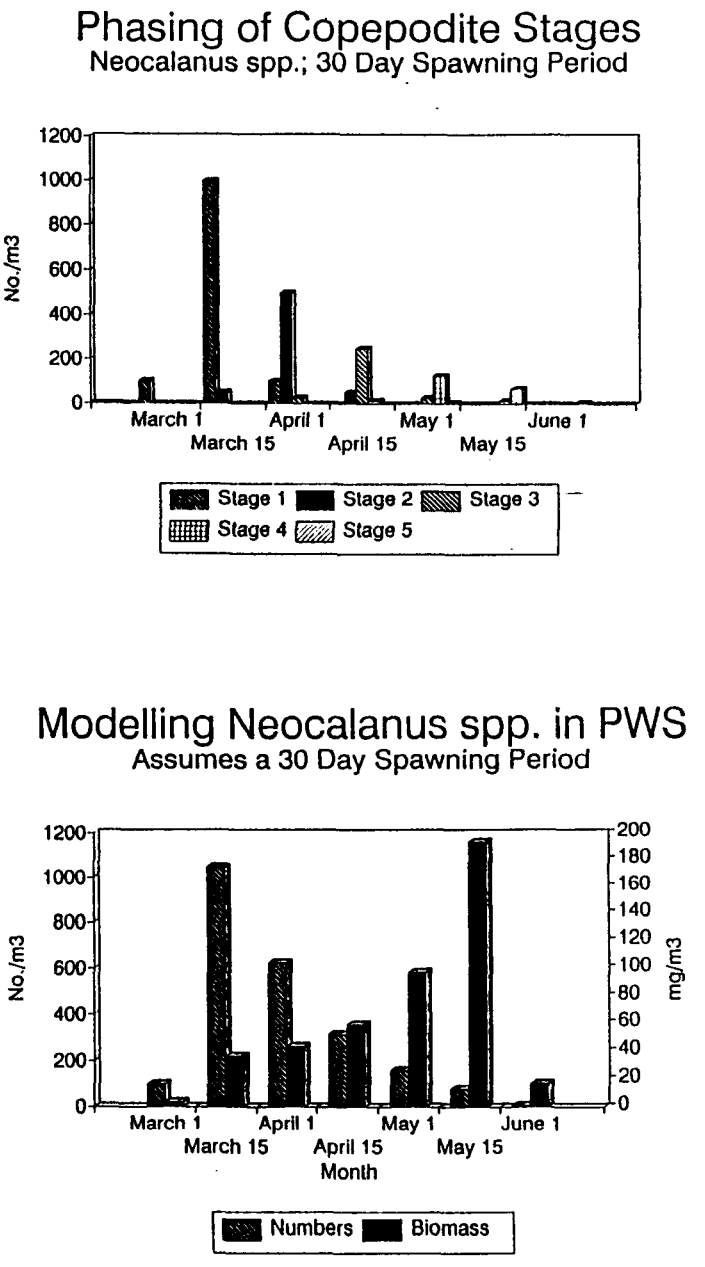


Figure 9. The hypothetical phasing of copepodite stages for *Neocalanus* assuming a 30 day spawning period, C1 stages beginning to arrive at the surface on 1 March, and a 15-day inter-molt period. The bottom panel depicts the composite biomass by month.

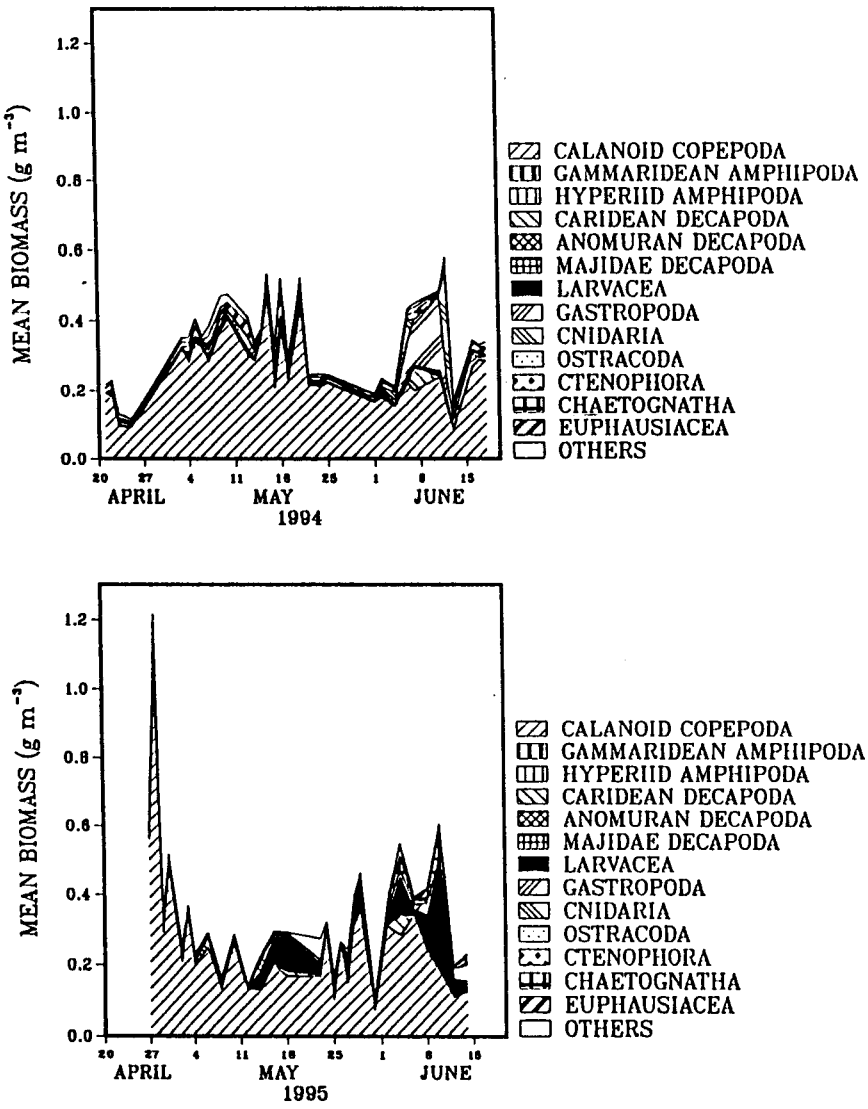


Figure 10. Variability in the community composition of major pelagic taxa during the spring in 1994 and 1995.

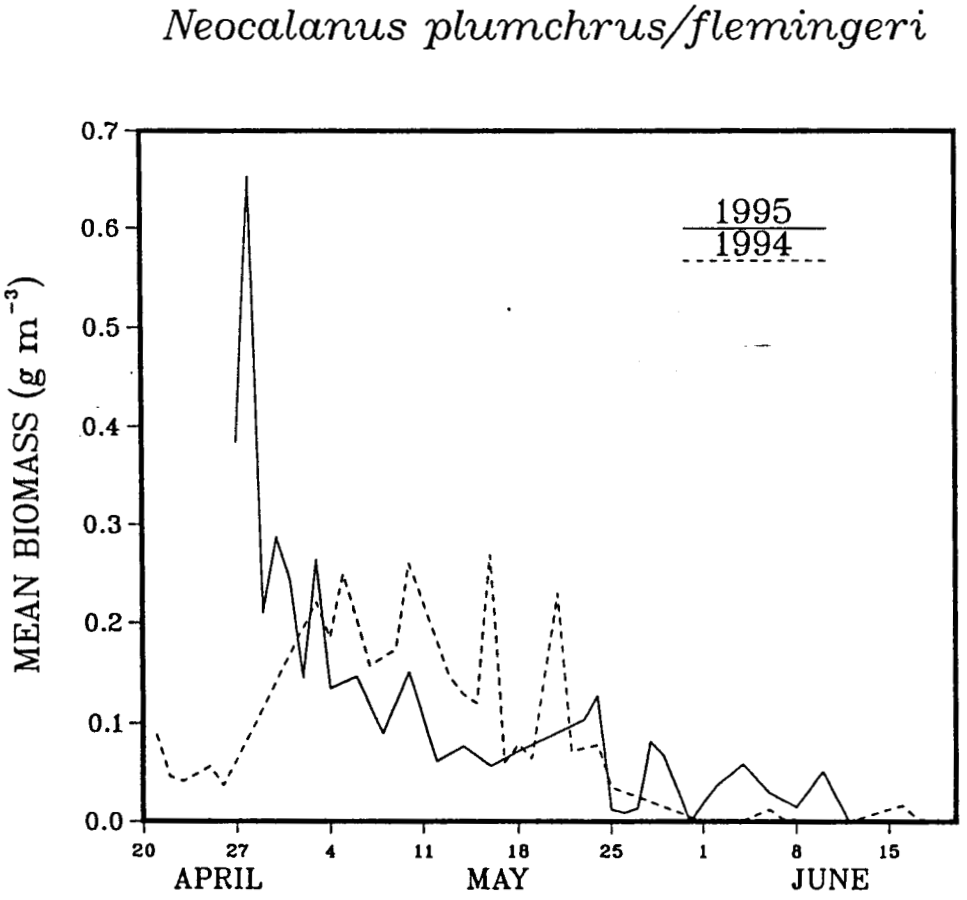


Figure 11. Variability in the *Neocalanus plumchrus/flemingeri* during the spring in 1994 and 1995.

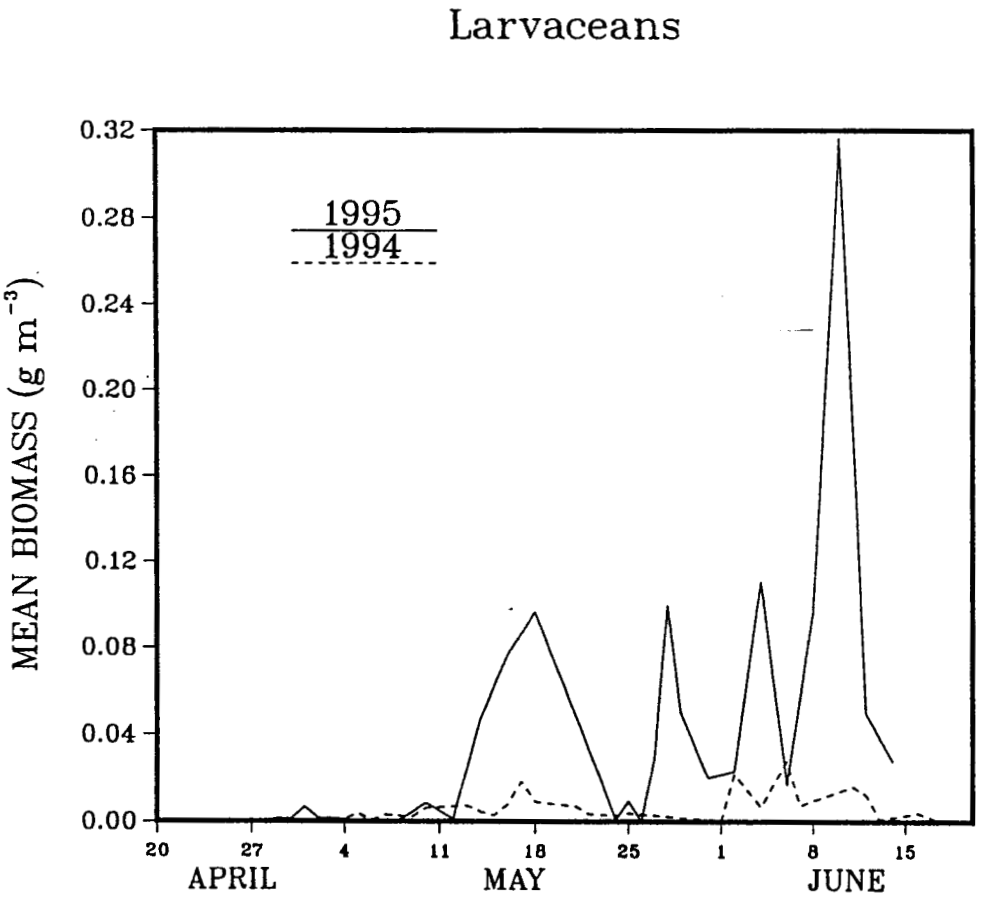


Figure 12. Variability in larvaceans during the spring in 1994 and 1995.

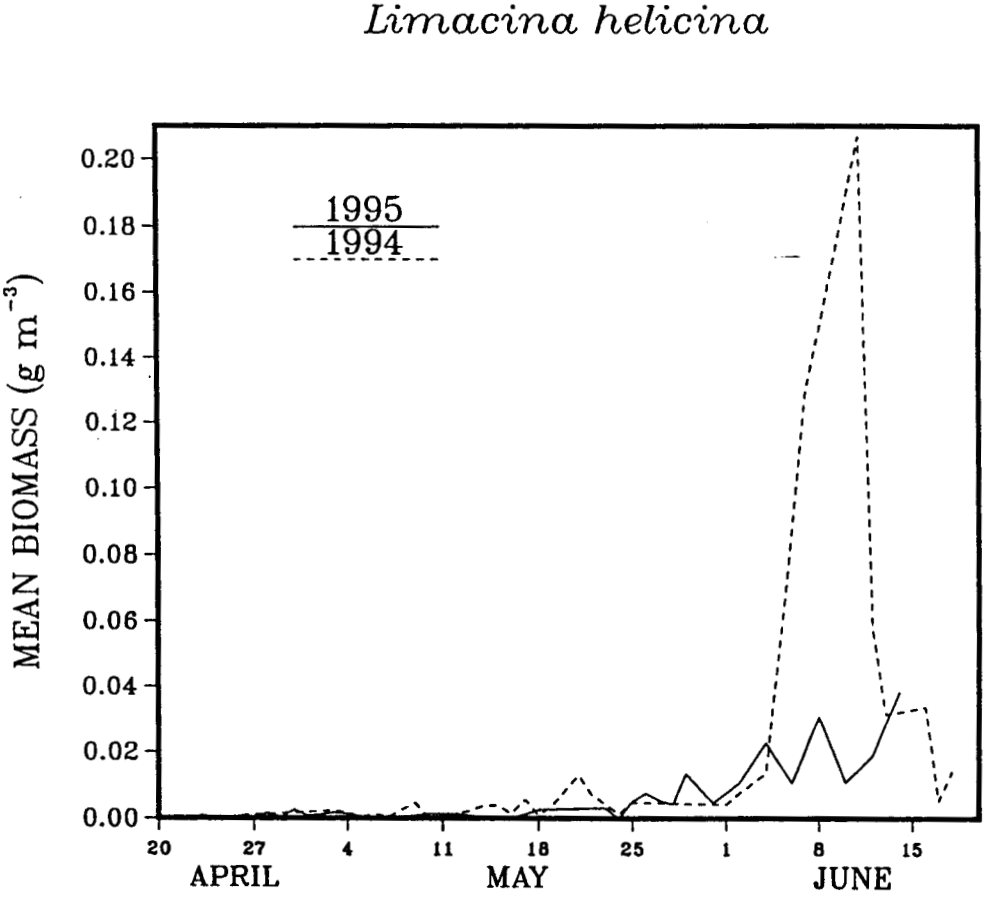


Figure 13. Variability in *Limacina helicina* during the spring in 1994 and 1995.

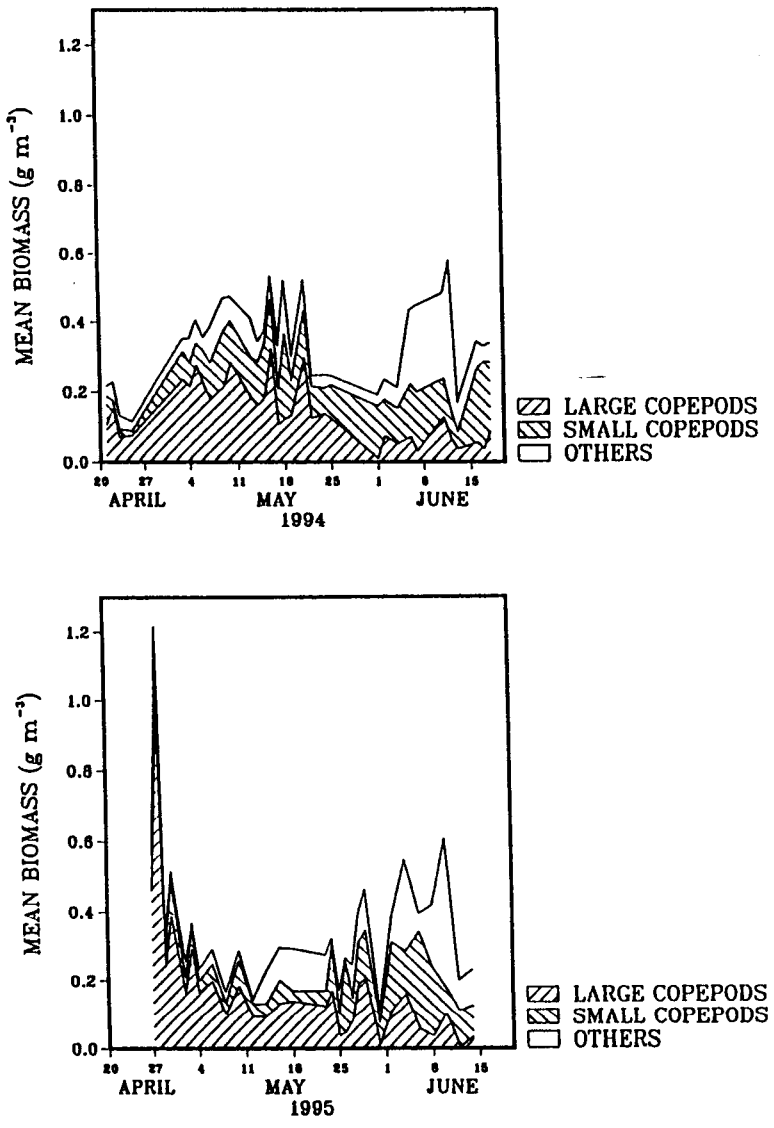


Figure 14. Variability in the large and small copepods and other taxa during the spring in 1994 and 1995.

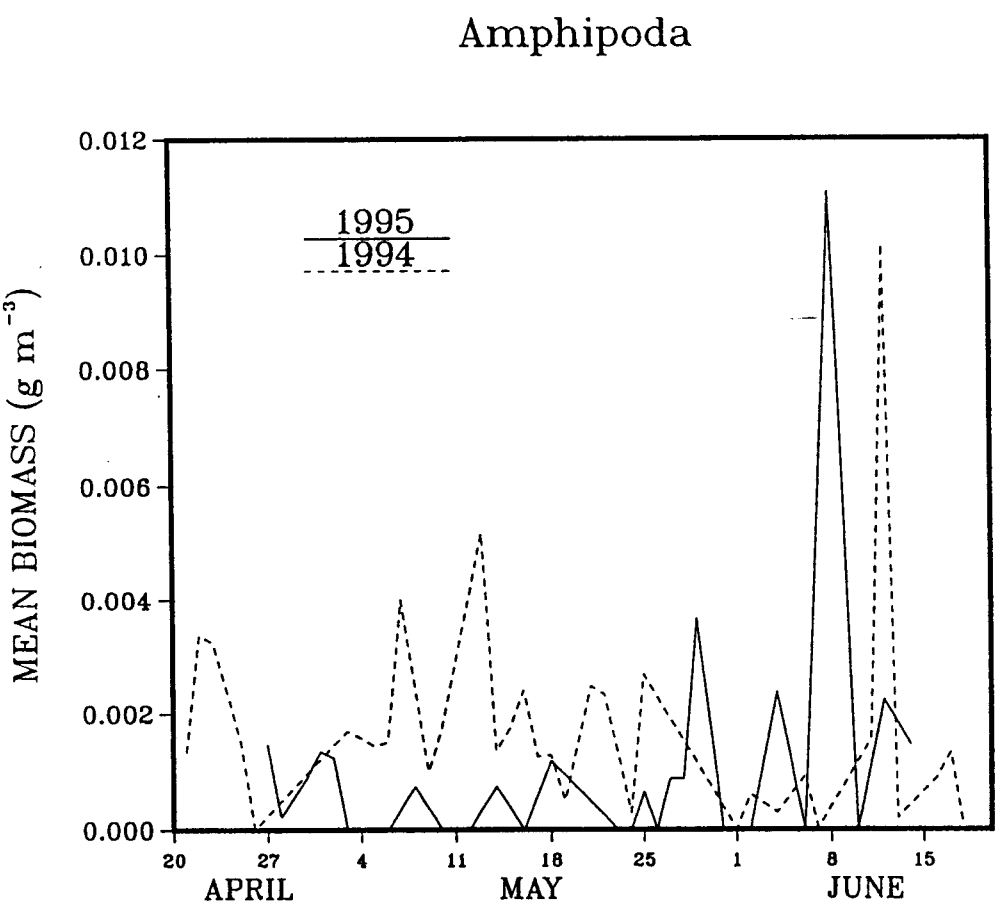


Figure 15. Variability in Amphipoda during spring in 1994 and 1995.

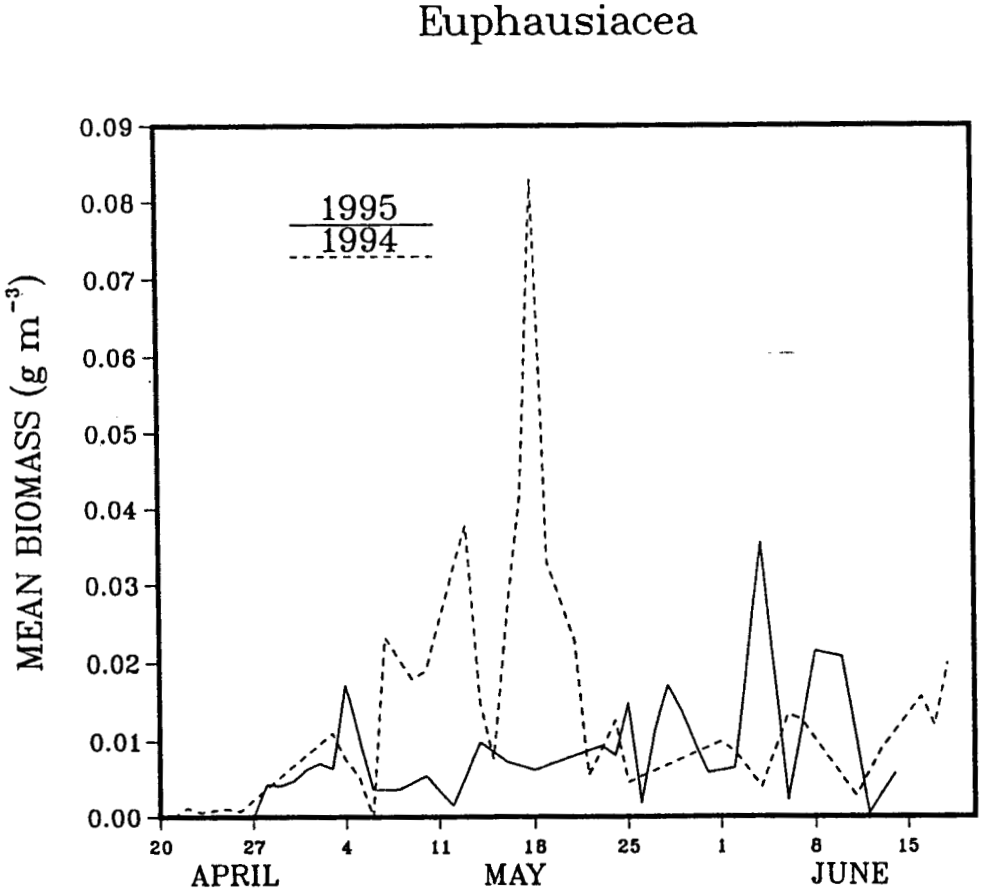


Figure 16. Variability in euphausiids (composite of *Thysanoessa* and *Euphausia*) during the spring in 1994 and 1995.

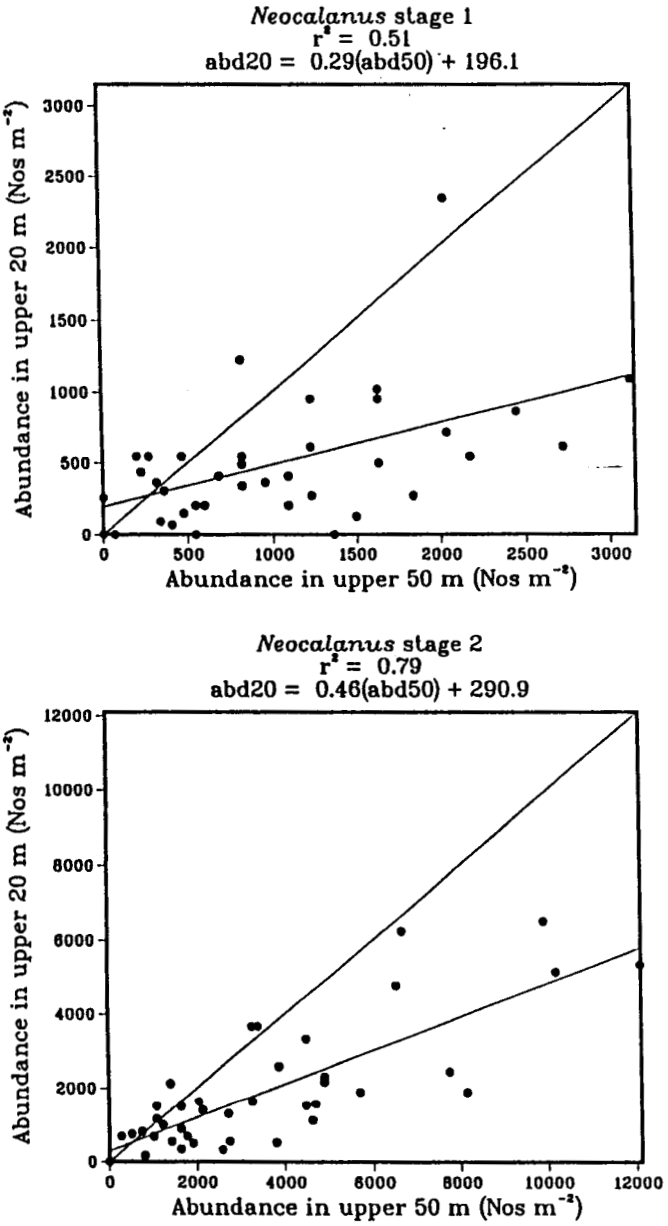


Figure 17. Regressions of 20 m abundance on 50 m abundance for CI (upper) and CII (lower) *Neocalanus* sampled in vertical tows in April and May, 1995.

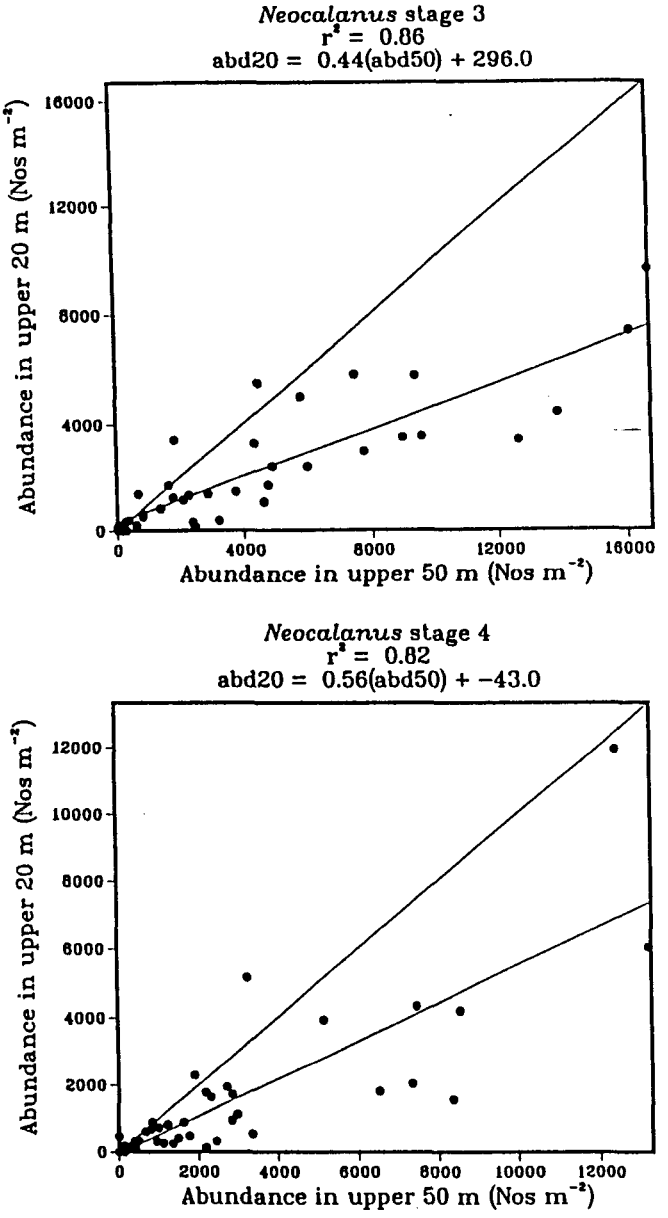


Figure 18. Regressions of 20 m abundance on 50 m abundance for CIII(upper) and CIV (lower) *Neocalanus* sampled in vertical tows in April and May, 1995.

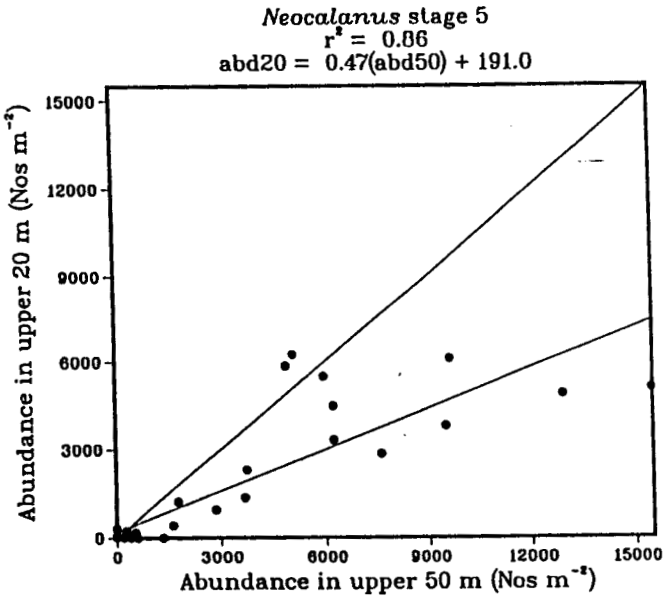


Figure 19. Regression of 20 m abundance on 50 m abundance for CV *Neocalanus* sampled in vertical tows in April and May, 1995.

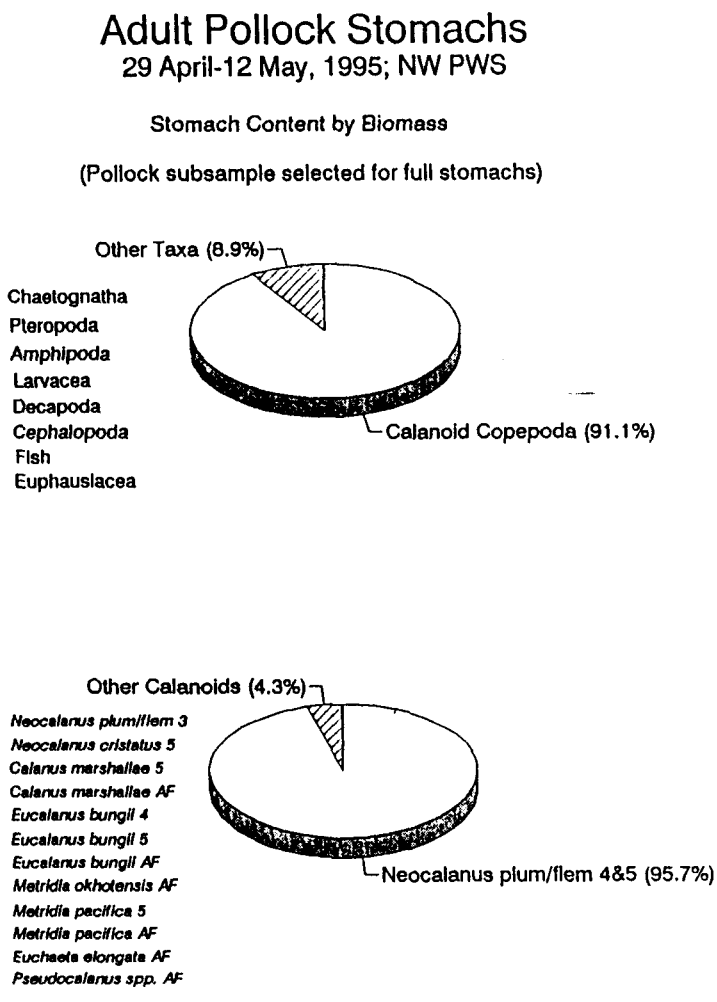


Figure 20. The diet composition by taxa (biomass) in stomachs of adult pollock sampled by midwater trawling in late April and May, 1995; F/V Alaska Beauty

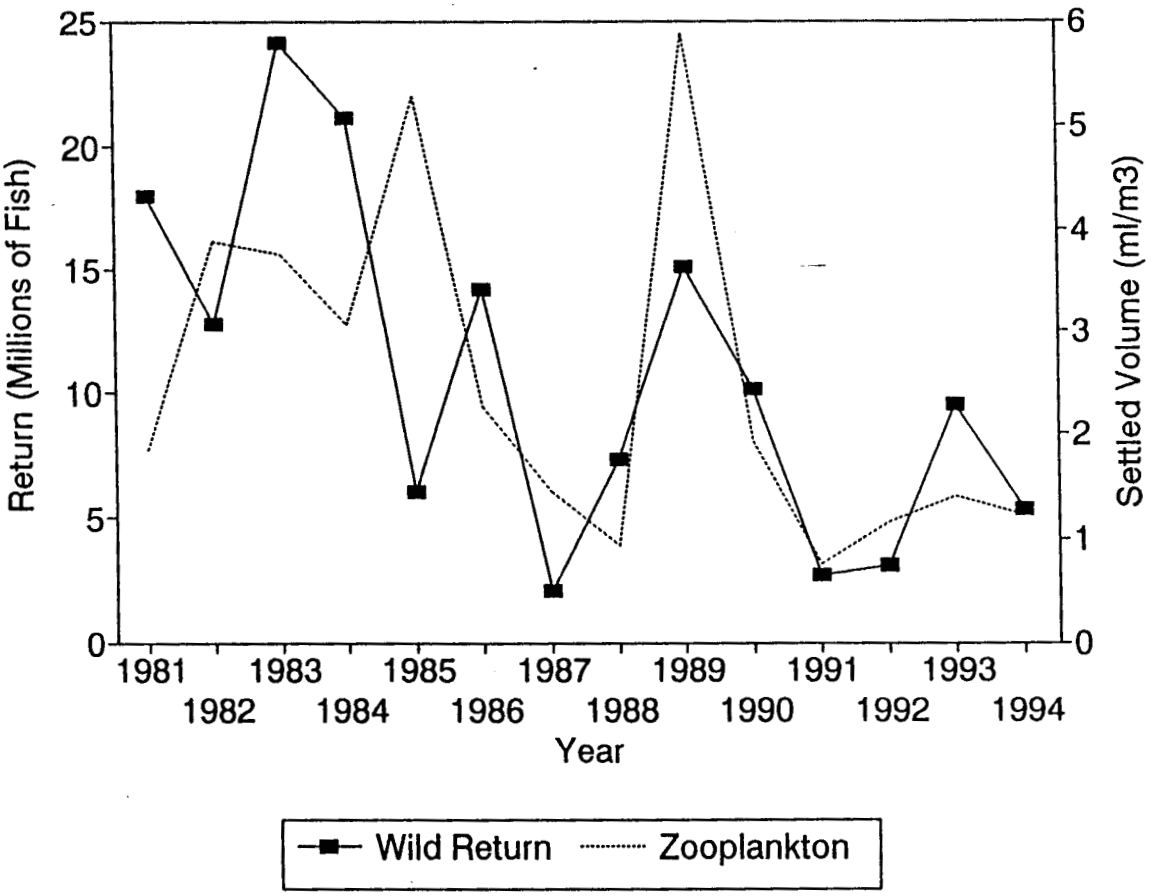


Figure 21. A time-series for zooplankton (AFK) and adult wild pink salmon returns in Prince William Sound.

Exxon Valdez Oil Spill
Restoration Project Annual Report

Isotope Ratio Studies of Marine Mammals in Prince William Sound

Restoration Project 95320I
Annual Report

This annual report has been prepared for peer review as part of the *Exxon Valdez* Oil Spill Trustee Council restoration program for the purpose of assessing project progress. Peer review comments have not been addressed in this report

Donald M. Schell
Amy Hirons

Institute of Marine Science
University of Alaska Fairbanks

for:

Alaska Department of Fish and Game
Habitat and Restoration Division
333 Raspberry Road
Anchorage, Alaska 99518-1599

April 1996

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Isotope Ratio Studies of Marine Mammals in Prince William Sound

Restoration Project 95320I
Annual Report

Study History: This project originated as part of the Sound Ecosystem Assessment program conducted by the University of Alaska and the Prince William Sound Science Center. In cooperation with K. Frost of Alaska Department of Fish & Game, we began a stable isotope study of harbor seals and potential prey species in Prince William Sound. T. Kline, then of University of Alaska Fairbanks, was a co-investigator but upon taking a position with the Prince William Sound Science Center, the project was split into two parts, with Kline collecting data on lower trophic levels and this project focusing on harbor seals and prey species as needed. In FY96, this project was separated completely, although we are still responsible for all of the stable isotope analyses run for the PWSSC and UAF. Other stable isotope ratio users are also accommodated as required.

Abstract: Two components of this project include provision of analytical services for the stable isotope ratio investigations associated with EVOS projects and investigation of food web relationships and trophic interactions of harbor seals and other top consumers of Prince William Sound (PWS). Through the use of harbor seal tissues collected from native harvested animals and tagging programs, seasonal and migrational information was obtained regarding prey utilization and trophic status at differing locations within PWS and adjacent Gulf of Alaska. Preliminary results indicate that within PWS, harbor seals fall at the top of food chains based on in situ primary and secondary productivity. Isotope ratios along whiskers grown over the past year indicate that some individuals migrate into areas (Gulf of Alaska, presumably) wherein the food web structure is very different and seals feed at a full trophic level below that in PWS or that the isotope ratios of prey are considerably lower in offshore pelagic waters than within PWS. Current analyses of potential food items in these locations lead us to believe the latter hypothesis is correct. Experiments with captive seals to determine whisker growth rates and body tissue turnover times are underway to calibrate observed changes in the wild.

Key Words: *Exxon Valdez* oil spill, food webs, harbor seals, $\delta^{13}C$, $\delta^{15}N$, isotope ratios, *Phoca vitulina*, Prince William Sound.

Citation:

Schell, D. M., and A. Hirons. 1996. Isotope ratio studies of marine mammals in Prince William Sound, *Exxon Valdez* Oil Spill Restoration Project Annual Report (Restoration Project 95320I), Alaska Department of Fish and Game, Habitat and Restoration Division, Anchorage, Alaska.

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EXECUTIVE SUMMARY

This report describes the preliminary results of the study on the food webs supporting harbor seals in Prince William Sound. The integrating methodology for this task is the use of natural stable isotope abundances as tracers of carbon and nitrogen transfers through the food webs. During the past three years, vibrissae (whiskers) and other tissues were collected from harbor seals within Prince William Sound and from the surrounding Gulf of Alaska. Samples were obtained from modern animals and from specimens archived at Alaska Department of Fish and Game, the University of Alaska Museum and the National Marine Mammal Laboratory. One to two long vibrissae were cut or pulled from live animals, while harvested or dead animals had all vibrissae removed for analysis. To date, approximately 100 seals have been sampled and most of these have been analyzed. The data from these vibrissae indicate that each has a temporal record of up to several years which may allow comparisons of interannual changes in feeding. When possible, samples from different organ tissues, e.g. muscle and blubber, were also taken. A variety of tissues from a single animal were analyzed to determine isotopic fractionation among the tissues. This has allowed normalization of isotope data to a single tissue type when samples of only a different type were available.

To enable estimation of the time represented by the growth of a whisker, a captive seal was infused with ^{13}C and ^{15}N -labeled glycine in January 1996. A repeat infusion will be made in May 1996 and in fall a whisker will be pulled for analysis. The added label should be visible in the analyzed whisker and will allow estimation of the vibrissae growth rate. In addition, one seal tagged in fall 1994, was recaptured in spring 1996 and whiskers from both time points were analyzed. This revealed that whisker growth is slower than previously thought and is only about 1.5 cm/year. This implies that in the ten cm or so of typical whisker length, several years of feeding are recorded.

Carbon isotope ratios are used as conservative tracers of energy supply between trophic levels (phytoplankton to zooplankton to fishes to top consumers). To establish the required baseline information, we have collected potential prey species of fishes and other organisms from within Prince William Sound and the adjacent Gulf of Alaska. Our findings, although very preliminary at this point include several interesting indications. Stable isotope ratios within harbor seal vibrissae do not appear to fluctuate greatly or with any regular periodicity, although some seals do have large changes between enriched and depleted values. More often there are minor fluctuations in the $\delta^{13}C$ with somewhat larger fluctuations in the $\delta^{15}N$. These shifts in the nitrogen isotope ratios may reflect seasonal changes in prey availability within a small region. Harbor seals tend to have a strong site fidelity and do not migrate extensively, though some have been tracked over many kilometers within a region.

Samples of zooplankton collected by cooperating investigators reveal that primary productivity is much lower in offshore waters as indicated by depletions in both $\delta^{13}C$ and $\delta^{15}N$. These low values provide a distinctive indicator visible in vibrissae of seals that feed in pelagic regions or on prey that have emigrated from offshore areas. Samples of fatty acids from the seals have been analyzed by K. Frost in a collaborating study and have been found to be very different among

regions, supporting the hypothesis that seals in differing parts of the sound have very different food web structures.

Archived samples of harbor seals have been analyzed to determine if the trophic structure of the food webs have changed between the period prior to the decline in population and current years. Our data are show that seals taken in 1995 have a similar range in $\delta^{13}C$ but have split in $\delta^{15}N$ values into two trophic levels. One group of animals remains close to those collected 6 and 20 years previously whereas the other group is almost a full trophic level higher. If food resources are reduced approximately 80-90 percent in going up each trophic level, these seals may be nearing a food-limited base. In contrast, seals from southeastern Alaska showed no apparent change in isotope ratios over the period 1975 - 1995.

A conceptual model of harbor seal feeding has been constructed based on the known isotope ratios in lower trophic levels and fishes, primarily capelin, herring and pollock. Predicted isotope ratios in seals from these food sources match observed $\delta^{15}N$ values closely but the measured $\delta^{13}C$ values are higher than predicted. We hypothesize that benthos, which are usually enriched relative to water column species, are more important than previously believed in the food supply to these seals. Sampling of potential prey species will be a major focus in 1996 and 1997.

INTRODUCTION

This annual report describes the preliminary results of the ongoing study of the food webs supporting harbor seals in Prince William Sound. This project also contributes to the Sound Ecosystem Assessment program being conducted by the Prince William Sound Science Center and the Institute of Marine Science, University of Alaska Fairbanks to describe the food chains supporting important commercial fish species that appear to have been impacted by the *Exxon Valdez* oil spill. In addition, it contributes to the studies by the Alaska Department of Fish and Game personnel to determine the reasons for the decline of harbor seal and Steller sea lion populations in Prince William Sound. The project also seeks to better describe the trophic interactions and trophic status of marine mammals, birds and their prey species. The integrating methodology for this wide range of tasks is the use of stable isotope ratios as natural tracers of carbon and nitrogen transfers through the food webs.

Carbon isotope ratios serve as conservative tracers of energy supply between trophic levels (phytoplankton to zooplankton to fishes to top consumers). Seals, cetaceans, birds, etc., acquire the isotope ratios in proportion to the amount of food derived from each differing source. This, in turn, is reflected in the composition of body tissues and in keratinous tissues (claws, feathers, baleen, whiskers) as a temporal record when multiple sources of food are consumed over time and space. This allows the discerning of important habitats and food resources in animals that seasonally migrate or undergo periods of hyper- and hypotrophy.

Nitrogen isotope ratios reflect both the food sources and the trophic status of that animal. As nitrogen in food is consumed and assimilated by a consumer, the heavy isotope is enriched by

approximately 3 ‰ with accompanying loss of the lighter isotope through excretion. The enrichment occurs with each trophic step and thus allows the construction of conceptual models and food webs and the assignment of trophic status to species for which dietary data are sparse. The data obtained from these measurements are unique in that they trace materials actually assimilated and thus can be used for more accurate ecosystem modeling.

It can be postulated that the natural stable isotope abundances of PWS biota will shift because of changes in trophic level, food web structure, and primary productivity in the context of the SEA hypotheses, thus providing an independent tool to verify, quantify and model ecosystem processes. The tracer nature of the approach will enable the integration of ecosystem components. It will enable us to monitor both "top down" (predation) and "bottom-up" (food supply) controls on herring and salmon production.

The project is composed of three elements:

1. A research component on marine mammals focusing on the trophic energetics and ecosystem dynamics of harbor seals conducted by Dr. Schell, PI, in cooperation with ADF&G personnel working as part of the marine mammal program. A smaller additional effort using captive animals to calibrate the responses to changing isotopic composition in diet and to determine vibrissae growth rates is also currently under way

2. A research effort closely tied to the study focusing on lower trophic levels having direct application to the testing of hypotheses regarding fisheries resources. This work is being conducted by Dr. T. Kline of the Prince William Sound Science Center in cooperation with the marine mammal component and is described in the report accompanying this section.

3. As the major isotope ratio analysis facility, we have provided analytical services for carbon and nitrogen isotope ratios to other PI's involved with EVOS studies and assisted with the interpretation of the acquired data. This task has required approximately 20% of the analytical and research effort and is continuing.

The objectives of our section of the isotope study continue to include:

1. Collect and analyze samples of harbor seal vibrissae through continued cooperative work with the Alaska Department of Fish and Game in Prince William Sound;

2. Collect and analyze samples of harbor seal prey species including forage fishes, salmon and herring in the vicinity of major haul-outs and high population densities. Samples of seal tissues will be collected from native hunters. These samples will be obtained through assistance by ADF&G personnel monitoring harvests and through the efforts of T. Kline.

3. Perform stable isotope ratio analyses on tissues and organisms collected during the sampling program. Through the use of **carbon** isotope data on taxa collected over geographical regions, the presence/absence of **isotopic gradients** useful in sorting out habitat dependencies will be determined.

4. Assist other research programs in the Prince William Sound ecosystem study by conducting stable isotope ratio analyses on samples provided and aid the interpretation of results. This effort will require approximately 20% of the analytical and research effort.

5. Through the use of **nitrogen** isotope ratios in collected taxa, assign **trophic status** to species in each region. Compare trophic status with predictive models based on conceptual food webs.

6. Determine temporal changes in harbor seal trophic status and food dependencies by comparing isotope ratios along the lengths of vibrissae with prey availability and their isotope ratios. Through the use of captive animals being fed known diets, establish the relationships between whisker growth rate and temporal changes and the fractionation factors between the $\delta^{13}C$ and $\delta^{15}N$ values of diet and consumer.

7. Compare the isotope-ratio derived food web models to predictions by the "lake-river" hypothesis and others being tested by the SEA project as an independent means of validation.

METHODS

The analytical methodology for stable isotope analysis are described in detail in the accompanying report by T. Kline. Sampling of tissues for stable isotope analysis has been described for both bulk tissues (muscle, blubber) and temporally variable tissues (whiskers, claws, etc.) (Schell, et al. 1989; Michener and Schell, 1994). This report includes only the pertinent sampling protocols and a synopsis of the analytical methods.

Forage Fishes

Lower trophic level organisms within Prince William Sound were obtained by T. Kline and analyzed within the scope of this project. Stable isotope ratios for these species were used to construct food webs for harbor seal foraging within the Sound. Samples of a few additional forage fishes from areas of harbor seal haul-outs have been provided by ADF&G personnel and combined with other lower trophic level organisms to assist in assigning trophic status. National Marine Fisheries Service personnel provided forage species from inshore and offshore waters in Southeast Alaska. Isotopic values for these species are used to indicate if species originating in food webs in southeast waters are being transported via the Alaska Coastal Current into the Sound and once there, being utilized as food by seals. Pelagic and benthic species were sampled during shellfish surveys conducted by ADF&G personnel in the western Gulf of Alaska. These prey are being used as indicators of regional isotopic differences. These regions are used to help locate areas of foraging for seals traveling outside Prince William Sound. A National Marine Fisheries Service triennial survey of the entire Gulf of Alaska will take place during the summer of 1996 and is expected to provide prey for areas where data are lacking.

A few grams of muscle tissue was extracted from several samples of each species at a sampling site. The tissues were frozen in a standard -10° C freezer and transported to the stable isotope

facility of analysis. Subsamples of the frozen muscle tissues were dried at 60°C, ground for homogeneity and prepared for mass spectroscopy.

Pinnipeds

During the past three years, vibrissae from harbor seals were collected within Prince William Sound and from the surrounding Gulf of Alaska. One to two long vibrissae were cut or pulled from live animals while harvested or dead animals had all their vibrissae removed for analysis. When possible, samples from different organ tissues, e.g. muscle and blubber, were taken for analysis. A variety of tissues from a single animal are analyzed to determine isotopic segregation among the tissues. This will be useful in the future when only one tissue may be available from a seal or sea lion to determine its spatial and trophic distribution.

Vibrissae and tissues from ninety-eight harbor seals have been or continue to be analyzed for stable isotope ratios. Tissues are dried at 60°C, ground for homogeneity and prepared for mass spectroscopy. Vibrissae are scrubbed with steel wool to remove any debris and segmented from base to tip in 2.5 mm segments. Every other segment was analyzed for carbon and nitrogen isotope ratios and the reserved segments were archived for future reference.

Tissues from Prince William Sound, Southeast Alaska and Kodiak harbor seals have been provided by Alaska Department of Fish and Game personnel working as part of the marine mammal monitoring effort. Alaska Department of Fish and Game researchers have provided archived harbor seal tissues, dating from the mid-1970s, for stable isotope comparisons. These comparisons are essential in determining if a dietary shift in harbor seals occurred during the past two decades. The University of Alaska Museum is providing bone tissue from harbor seals from various regions of the Gulf of Alaska from the 1950s to present. The stable isotope ratios of these tissues are being used to compare and contrast to the stable isotope ratios of present samples. By obtaining seal tissues from multiple regions prior to the population decline (pre-1970), any significant changes in these ratios may be an indication of changes in ecosystem productivity over the past several decades.

Analytical Techniques

The samples obtained are dried and powdered for homogeneity and the isotope ratios of carbon and nitrogen determined with a Europa 20/20 mass spectrometer system. The sample is combusted at high temperature and the nitrogen and carbon dioxide gases separated and purified by gas chromatography. These are subsequently led into the mass spectrometer by capillary and the isotope ratios determined. Results are reported in the standard $\delta^{13}C$ and $\delta^{15}N$ notation.

Captive Animal Studies

Vibrissae growth rate studies were initiated with captive harbor seals to determine if growth rates fluctuate with season, age and, ultimately, diet. An adult harbor seal at Mystic Aquarium in

Connecticut was administered 16 ml of doubly-labeled glycine ($\delta^{13}C$ and $\delta^{15}N$) over a two day period. The sudden increase in ^{13}C and ^{15}N, which was expected to be incorporated by the vibrissae, is expected to create a marked peak in these values corresponding to the time of infusion. This will be used as a marker to establish the growth rate after that time. After four months a second dose of doubly-labeled glycine will be administered (May 1996) and after three additional months, one whisker will be removed for stable isotope analysis. The peaks caused by the labeled amino acid additions should be reflected in the stable isotope ratios along the whisker. The positions of these peaks will be measured and a growth rate established. A second whisker will be analyzed after the subsequent six month period to determine if growth rate is constant throughout the year.

A second type of growth rate experiment is being conducted simultaneously at the Vancouver Aquarium on a captive adult harbor seal and subadult Steller sea lions. A short, anterior whisker and a longer posterior whisker from the muzzle have been cut and are being measured periodically. All segments will be analyzed for their stable isotope ratios in the near future. Phocids and otariids are being fed a similar diet. These data are not yet available in sufficiently complete form for inclusion in this report.

PRELIMINARY RESULTS

Isotope Ratio Variations in Harbor Seals

To date, samples from approximately 100 harbor seals have been analyzed and are listed in Table 1 with the average isotope ratio values from vibrissae. In addition, samples of tissue from archived seals have been used to compare isotope ratios in the pre-decline period with those from current samples. The results are described and shown below. The isotopic data from seal vibrissae from collected within Prince William Sound are shown in Appendix A. These illustrate the $\delta^{13}C$ and $\delta^{15}N$ values at 0.25 cm intervals along the lengths of the whiskers. These samples were collected during tagging operations in Prince William Sound and from native harvested seals. At this point only a few generalizations can be made. Male seals show relatively constant values over the time span represented by the length of the whisker whereas several of the female animals showed a marked change in isotope ratios of both carbon and nitrogen during the same length of time. The cause of this shift currently is not known, but data on zooplankton from the Bering Sea indicate that a major geographic gradient exists between on-shelf and deep water regions with samples from deep water having much more depleted values for both $\delta^{13}C$ and $\delta^{15}N$. Whether this is also true of the Gulf of Alaska is not known but the sampling program for summer 1996 is being designed to test if the same is true south of Montague and Hinchinbrook Islands. Data on large calanoid copepods collected by T. Kline in 1995 appear to validate this hypothesis.

Table 1. Harbor seal vibrissae from Prince William Sound and adjacent regions. Age designation refers to adult (A), pup (P) or subadult (SA) seals. Samples for which data are not reported are currently being analyzed.

Harbor Seal	Sample Date	Sex	Age	Range $\delta^{13}C$	avg. $\delta^{13}C$	Range $\delta^{15}N$	avg. $\delta^{15}N$
HSA1PWS7	May	1993	MA	-14.8 to -13.9	-14.5	18.1 to 19.5	18.8
HSA2PWS7	May	1993	F SA	-16.2 to -14.8	-15.4	15.3 to 19.0	17.7
HSA3PWS7	May	1993	MA	-15.8 to -14.8	-15.2	17.3 to 17.9	17.5
HSA4PWS7	May	1993	MA	-16.5 to -15.0	-15.9	15.8 to 17.9	16.7
HSA5PWS7	May	1993	F SA	-16.0 to -15.4	-15.8	15.8 to 18.8	17.1
HSA6PWS8	May	1993	FSA	-16.4 to -15.0	-15.9	15.4 to 16.7	16.1
HSA7PWS8	May	1993	FA	-16.4 to -15.2	-15.7	15.8 to 16.7	16.2
HSA8PWS8	May	1993	MSA	-15.7 to -15.2	-15.4	15.5 to 17.9	16.4
HSA9PWS8	May	1993	MA	-15.3 to -14.7	-15.0	16.9 to 18.7	17.5
HSA10PWS8	May	1993	MSA	-15.6 to -15.1	-15.3	18.1 to 19.2	18.6
HSA11PWS9	May	1993	MA	-15.1 to -14.7	-14.9	16.3 to 17.9	16.8
HSA12PWS9	May	1993	MSA	-15.2 to -14.2	-14.6	16.1 to 19.3	18.5
HSA13PWS9	May	1993	FSA	-16.3 to -16.0	-16.1	15.8 to 17.1	16.4
HSB1PWS26	April	1994	FSA	-17.1 to -15.7	-16.4	14.7 to 16.7	15.8
HSB2PWS27	April	1994	MSA	-16.6 to -15.7	-16.2	15.2 to 17.3	16.1
HSB3PWS27	April	1994	FA	-16.5 to -12.6	-14.6	13.4 to 18.0	16.0
HSB4PWS27	April	1994	MSA	-16.2 to -15.3	-16.1	15.8 to 16.6	16.1
HSB5PWS27	April	1994	MA	-17.9 to -17.0	-17.5	14.0 to 15.9	14.9
HSB6PWS28	April	1994	MA	-17.6 to -15.8	-16.6	13.3 to 16.2	15.0
HSB7PWS28	April	1994	FA	-17.8 to -12.5	-15.2	13.7 to 17.4	15.6
HSB8PWS28	April	1994	MSA	-17.7 to -15.5	-16.3	13.7 to 16.9	15.6
HSB9PWS28	April	1994	MSA	-18.1 to -16.4	-17.1	13.6 to 16.8	15.4
HSB10PWS28	April	1994	MA	-17.7 to -14.5	-15.8	15.2 to 17.8	16.2
HSB11PWS18	Sept.	1994	FA	-17.9 to -16.3	-17.1	14.7 to 17.1	15.4
HSB12PWS18	Sept.	1994	FSA				
HSB13PWS18	Sept.	1994	MSA				
HSB14PWS18	Sept.	1994	MA	-17.2 to -16.1	-16.6	14.8 to 16.1	15.4
HSB15PWS18	Sept.	1994	FSA	-17.0 to -13.2	-15.2	15.8 to 18.9	17.7
HSB16PWS18	Sept.	1994	F SA	no vibrissae			
HSB17PWS18	Sept.	1994	MSA	-16.6 to -15.5	-15.9	15.5 to 16.3	15.9
HSB18PWS18	Sept.	1994	MSA	-16.6 to -16.1	-16.3	15.6 to 17.0	16.2
HSB19PWS18	Sept.	1994	MSA	-16.8 to -16.2	-16.4	15.5 to 16.5	16.0
HSB20PWS18	Sept.	1994	FSA	-16.5 to -15.8	-16.1	16.1 to 17.2	16.7
HSB21PWS18	Sept.	1994	MSA	-17.0 to -14.5	-15.8	15.6 to 17.1	16.4
HSB22PWS18	Sept.	1994	MSA	-18.2 to -13.5	-15.1	15.4 to 19.2	17.5
HSB23PWS18	Sept.	1994	MA	-17.9 to -16.2	-17.6	14.0 to 15.4	14.4
HSB24PWS19	Sept.	1994	FA	-16.1 to -15.6	-15.9	15.6 to 16.8	16.3
HSB25PWS19	Sept.	1994	FP	-17.3 to -14.2	-15.1	16.6 to 17.9	17.1
HSB26PWS19	Sept.	1994	MSA	-16.5 to -16.0	-16.3	15.0 to 16.6	15.5
HSB27PWS22	Sept.	1994	FA	-17.3 to -13.9	-15.4	14.9 to 17.5	16.4
HSB28PWS22	Sept.	1994	MA	-17.5 to -15.6	-16.4	14.6 to 16.4	15.7
HSB29PWS22	Sept.	1994	MP	-16.7 to -15.2	-15.9	17.5 to 19.2	18.4
HSB30PWS22	Sept.	1994	FA	-17.8 to -15.2	-16.9	14.5 to 16.8	15.2
HSB31PWS22	Sept.	1994	FSA	-17.7 to -16.1	-16.8	14.6 to 16.7	15.7
HSB32PWS22	Sept.	1994	FA	-17.8 to -13.8	-16.1	14.3 to 17.1	15.5
HSB33PWS22	Sept.	1994	FSA	-17.8 to -14.3	-16.4	14.7 to 16.6	15.9

Table 1. (Continued)

HSB34PWS22 Sept. 1994MA-17.2 to -14.4-15.314.7 to 17.216.0
HSB35PWS22 Sept. 1994FA-18.1 to -15.6-16.815.0 to 17.415.9
HSB36PWS22 Sept. 1994MA-17.9 to -16.8-17.614.5 to 16.215.1
TATHS1PWS27 Sept. 1994FSA-18.1 t -16.7-17.514.4 to 17.815.8
TATHS2PWS29 Sept. 1994FSAno vibrissae
TATHS3PWS29 Sept. 1994FA-17.5 to -15.5-17.014.3 to 17.114.9
TATHS4PWS30 Sept. 1994MA-16.4 to -16.1-15.616.1 to 18.717.3
TATHS5PWS30 Sept. 1994MA-17.9 to -15.7-16.414.4 to 16.115.6
TATHS6PWS1 Oct. 1994FP-17.8 to -16.1-16.516.0 to 18.316.8
TATHS7PWS1 Oct. 1994MP-17.8 to -14.9-15.714.3 to 19.817.5
HSC1PWS9 May 1995MSA-17.3 to -15.5-16.115.3 to 17.616.3
HSC2PWS9 May 1995MSA-17.5 to -13.4-14.914.6 to 20.017.9
HSC3PWS9 May 1995MSA
HSC4PWS9 May 1995MSA-17.5 to -16.1-16.614.1 to 17.215.8
HSC5PWS9 May 1995MSA-17.5 to -15.6-16.214.6 to 16.915.8
HSC6PWS11 May 1995FSA-17.2 to -15.4-16.215.3 to 16.816.1
HSC7PWS11 May 1995MSA-17.6 to -15.1-16.214.2 to 16.915.7
HSC8PWS11 May 1995FA-17.8 to -14.3-16.414.1 to 16.815.3
HSC9PWS11 May 1995FSA-18.0 to -15.1-15.916.5 to 18.817.8
HSC10PWS11 May 1995MSA-17.2 to -12.8-14.116.0 to 18.917.9
HSC11PWS11 May 1995FSA-15.0 to -13.7-14.316.7 to 17.317.0
HSC12PWS11 May 1995MA-16.5 to -16.0-16.115.6 to 16.916.2
HSC13PWS11 May 1995FSA
HSC14PWS11 May 1995MA
HSC15PWS12 May 1995MA
HSC16PWS12 May 1995MA
HSC17PWS12 May 1995FSA
HSC18PWS12 May 1995MSA
HSC19PWS12 May 1995FSA
HSC20PWS14 May 1995FA
HSC21PWS14 May 1995FSA
HSC22PWS14 May 1995MSA
HSC23PWS25 Sept. 1995FSA
HSC24PWS25 Sept. 1995FP
HSC25PWS26 Sept. 1995FSA
HSC26PWS26 Sept. 1995FA
HSC27PWS26 Sept. 1995FA
HSC28PWS26 Sept. 1995MSA
HSC29PWS26 Sept. 1995MSA
HSC30PWS26 Sept. 1995FA
HSC31PWS26 Sept. 1995MA
HSC32PWS26 Sept. 1995MA
HSC33PWS26 Sept. 1995FSA
HSC34PWS26 Sept. 1995FSA
HSC35PWS26 Sept. 1995FP
HSC36PWS26 Sept. 1995FSA
HSC37PWS27 Sept. 1995MA
HSC38PWS27 Sept. 1995FA
HSC39PWS27 Sept. 1995FSA
HSC40PWS27 Sept. 1995MA
HSC41PWS28 Sept. 1995MSA
HSC42PWS28 Sept. 1995MSA

Benthic and pelagic organisms have been from Southeastern Alaska have been provided by National Marine Fisheries personnel for analysis of isotope ratios. These samples will provide data on the isotope ratios in potential prey from that region which is essentially "upstream" of Prince William Sound in the Alaska Coastal Current. This information may help in the interpretation of the often large excursions in isotope ratios evident in several of the vibrissae collected from female seals. Further samples from offshore waters in the Gulf of Alaska will be available following the NMFS Triennial Groundfish Survey scheduled for summer 1996. Personnel on this cruise have agreed to provide us with subsamples from catches.

Stable isotope ratios within harbor seal vibrissae do not appear to fluctuate greatly or with any regular periodicity, although some seals do have large changes between enriched and depleted values. More often there are minor fluctuations in the $\delta^{13}C$ with somewhat larger fluctuations in the $\delta^{15}N$. These shifts in the nitrogen isotope may be seasonal changes in prey availability within a small region. Harbor seals tend to have a strong site fidelity and do not migrate extensively, though some have been tracked over many kilometers within a region.

Results are still pending in regards to the rate at which vibrissae grow. A harbor seal who had a vibrissae sampled in September 1994 was recaptured and, subsequently, resampled in April 1995 in Southeast Alaska (Figure 3). During that seven month period, the vibrissae on this adult seal grew 1.5 cm. This time period was during the winter months and the seasonal physiology of these animals, excluding parturition, is relatively unknown. Therefore, it is unknown if this measured growth rate is constant throughout the year or if it varies with season. Major inflection points along the length of the two vibrissae match and may be subtle demarcations of season. They may coincide with shifts in the predominate prey species or a change in environmental productivity. Low primary productivity will result in depleted carbon and nitrogen isotope ratios (Laws et al. 1995).

Archived Seal Samples

Archived muscle tissues from Prince William Sound harbor seals were compared with recently sampled vibrissae from animals of the same region (Figure 4). Stable isotope values for muscle tend to most accurately reflect the stable isotope ratios for the whole animal (DeNiro and Epstein 1978). The fractionation of ^{13}C during formation of keratin in the vibrissae was determined by analyzing both muscle and vibrissae from the same animals. Subsistence harvest harbor seals were used from Southeast Alaska. The average $\delta^{13}C$ values for vibrissae were found to be typically enriched by 1.3 ppt relative to muscle from the same seal. The average $\delta^{13}C$ values in seals increased by 0.2 ppt between 1975 and 1989 and by another 0.2 ppt between 1989 and 1995.

Isotope Ratios in Prey Species

The Prince William Sound prey plot (Figure 1) was created using the stable isotope values for phytoplankton, zooplankton, juvenile and adult herring, juvenile and adult pollock and harbor

seals. Hypothetical seals were added to the plot to depict seals feeding primarily on herring and the other primarily on adult pollock. The actual $\delta^{15}N$ values for harbor seals in the Sound represent the anticipated trophic step from herring and juvenile pollock but the $\delta^{13}C$ is greater than expected for either species. Similar to work done in the Bering Sea, areas of the Gulf of Alaska are being refined into smaller, isotopic regions to better define feeding areas for traveling phocids or transport of prey into Prince William Sound (Figure 6).

Harbor seals do not migrate extensively but some have been tracked over many kilometers within a region. Harbor seal isotope ratios are often more enriched in ^{13}C than anticipated from common prey in the regions in which they live. The source(s) of these enriched values may be from offshore prey moving into the environment or from benthic prey which are often enriched relative to pelagic prey. We are currently analyzing potential prey species from these environments.

Figure 6 compares the isotope ratios in the seal whiskers with the values found for potential prey and other species resident in Prince William Sound. The values for most of the samples indicate that the seals fit the expected trophic enrichments in $\delta^{15}N$ and closely match the $\delta^{13}C$ of Prince William Sound prey. However, the most enriched values present in the females do not match prey from Prince William Sound but have depleted $\delta^{15}N$ values that may result from migration into deep water feeding areas outside of the Sound. Determination of the causes for these variations is part of the focus for 1996-7.

Captive Animal Studies

Through cooperation with Keith Hobson of the Canadian Wildlife Service, we were able to acquire whiskers from two harp seals that had been held in captivity and fed known diets of herring. The whiskers from these animals were analyzed along their lengths and are being compared with the isotopic composition of the diets. This work is almost complete. Preliminary results indicate that the seals closely reflect the diet, remaining within 1.5‰ in carbon and within approximately the same range in $\delta^{15}N$ but showing the expected 3‰ trophic enrichment. The data from this experiment has been assembled into a manuscript upon completion of the remaining diet and tissue samples. Other experiments using Steller sea lions are being conducted on a related project and the data from those experiments should prove useful in helping us interpret the seal data as the sea lions continue to grow their whiskers over multiple years. Harbor seals were assumed to shed their whiskers during the annual molt but our data indicates this is not true and that whiskers may represent a multiyear record of feeding.

Isotope Ratios in Potential Prey

The wide selection of potential prey items in Prince William Sound that may be consumed by harbor seals have been collected over the past field seasons or was obtained from archived samples. These data are reported by T. Kline as part of the SEA program conducted by the Prince William Sound Science Center. Samples of harbor seal prey species including forage

fishes, salmon and herring in the vicinity of major haul-outs and high population densities have been collected by us and are currently being analyzed.

Interactions with Other Studies

Our main cooperative work has been with K. Frost of the Alaska Department of Fish and Game in conjunction with their tagging and physiology studies in harbor seal. This work will be reported by that study component and is only briefly described here. Samples of seal blubber have been analyzed for fatty acid composition to estimate the sources of food being passed up the food chain. This work has found sharp changes in fatty acid composition across relatively short geographical distances and between seals captured in Prince William Sound and offshore. As a potentially excellent means of independent validation of the trophic insights gained from stable isotope ratios, we are working closely with this project.

The interaction with the modeling component of the SEA program will intensify during the months ahead. As more data are acquired, we will be able to test model assumptions and predictions by independent comparison using the isotopic model as a validation measure. Although similar carbon isotope labels in different members of the marine community may be indistinguishable, the trophic changes predicted will lead to testable shifts in the isotope ratios of nitrogen.

To date the interaction with other studies on top consumers has been limited to the acquisition of whiskers from archived carcasses of sea otters and sea birds. Following the analysis and interpretation of these samples in 1996, further investigations will be planned linking the top consumers of Prince William Sound into an ecosystem trophic model. Currently available data are being synthesized by the principal investigators and will be reported by them.

CONCLUSIONS

The three aspects addressed by this program are progressing well and there are no perceived reasons for alteration of the scope of work at this time.

Captive seal studies: Data are not yet available to establish absolute whisker growth rates on seals at this time. The experiments are ongoing and will be concluded over the next year. Recapture of a tagged seal has yielded a single animal growth rate of approximately 2 cm/year.

Analytical services for stable isotope ratio determinations: The mass spectrometry service has had full usage by this project, the SEA program and other EVOS projects supporting sea otter and sea bird studies. At six months into the fiscal year, over 4000 samples have been run and a new backlog is building as the spring field season gets underway. No serious machine problems have arisen during the past six months and all data have been made available to the P.I. and collaborators in a timely manner.

Harbor seal trophic energetics: The seal tissues available are now largely analyzed and the data sets are in the process of analysis. The comparisons of archived and modern seal tissues indicate

that modern seals in Prince William Sound have separated trophically into two distinct groups. We are not prepared to state the reasons for this separation until a comprehensive analysis of prey data has been completed. This will require coordination with the stable isotope aspects of the SEA program. From models of trophic transfers we note that there is strong suggestion that the seals are relying on a largely benthic-derived diet, but potential prey data for benthos are still largely unsampled. Summer 1996 sampling will focus on filling this data gap. Fatty acid composition information on the same seals from which our samples were taken is now being compiled by K. Frost of ADF&G. These data will assist in attempting to detail the food web structure in different regions within Prince William Sound.

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Schell, D. M. and S. M. Saupe. (1993). Feeding and growth as indicated by stable isotopes In: (J. J. Burns, J. J. Montague and C. J. Cowles eds.). *The Bowhead Whale* Allen Press, Lawrence, Kansas. 491-506.

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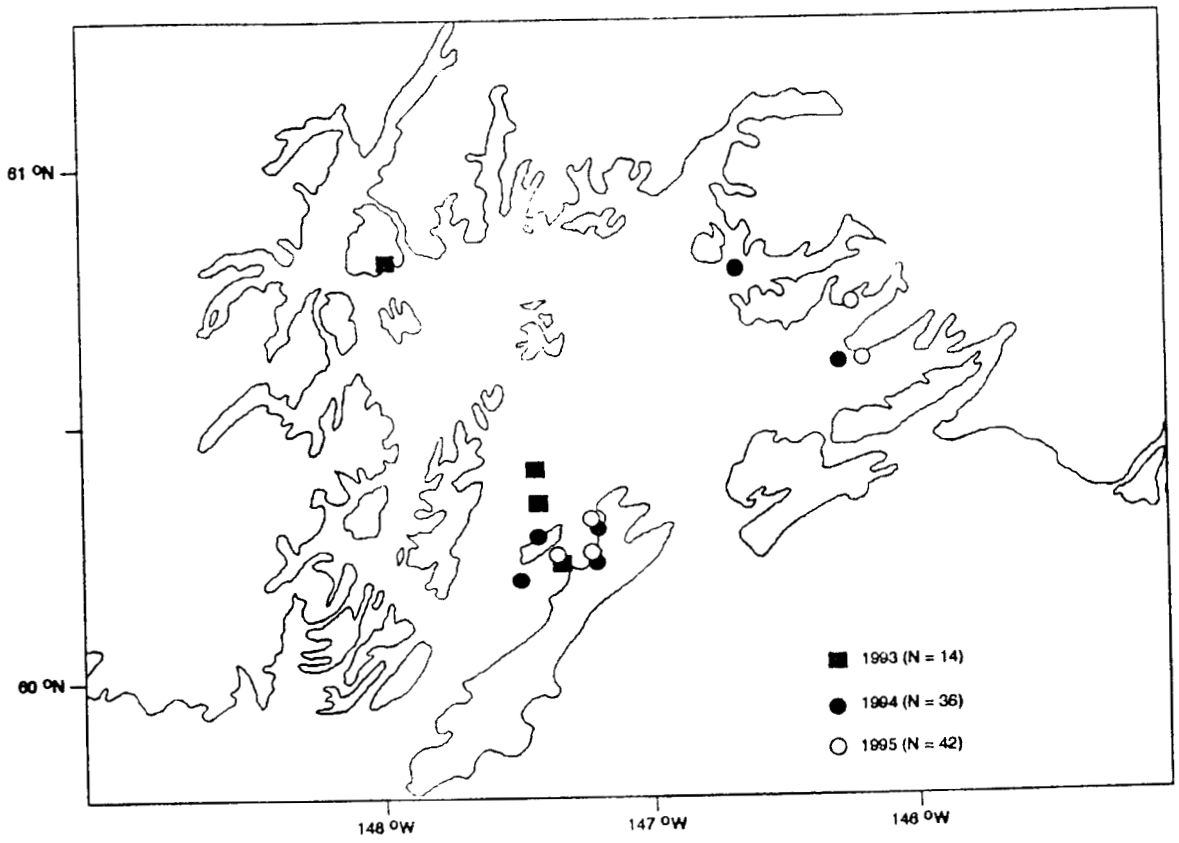


Figure 1. Sample locations for harbor seals in Prince William Sound, 1993-1995.

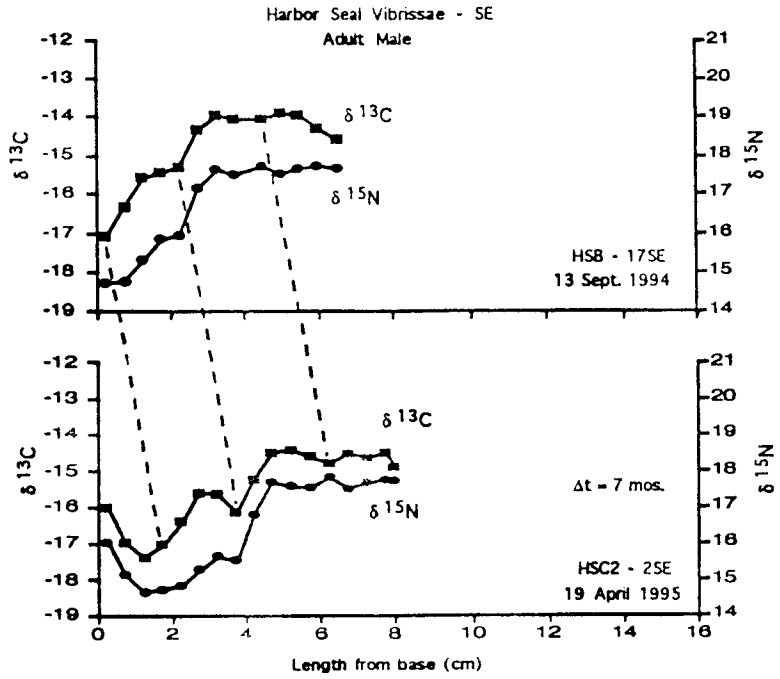


Figure 2. Vibrissae from the same recaptured harbor seal sampled in southeast Alaska. Vibrissae sampled in September 1994 (upper plot) are contrasted with a vibrissae taken from the seal seven months later (lower plot).

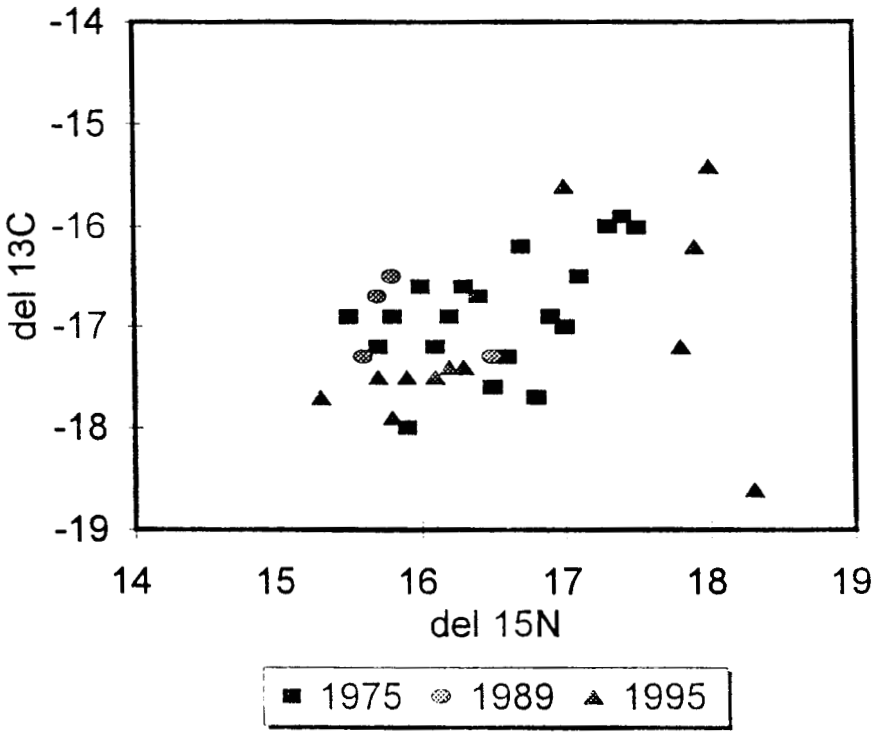


Figure 3. Archived harbor seal muscle tissue (1975, 1989) and vibrissae (1995). Vibrissae have been adjusted to muscle by -1.3°/oo enrichment.

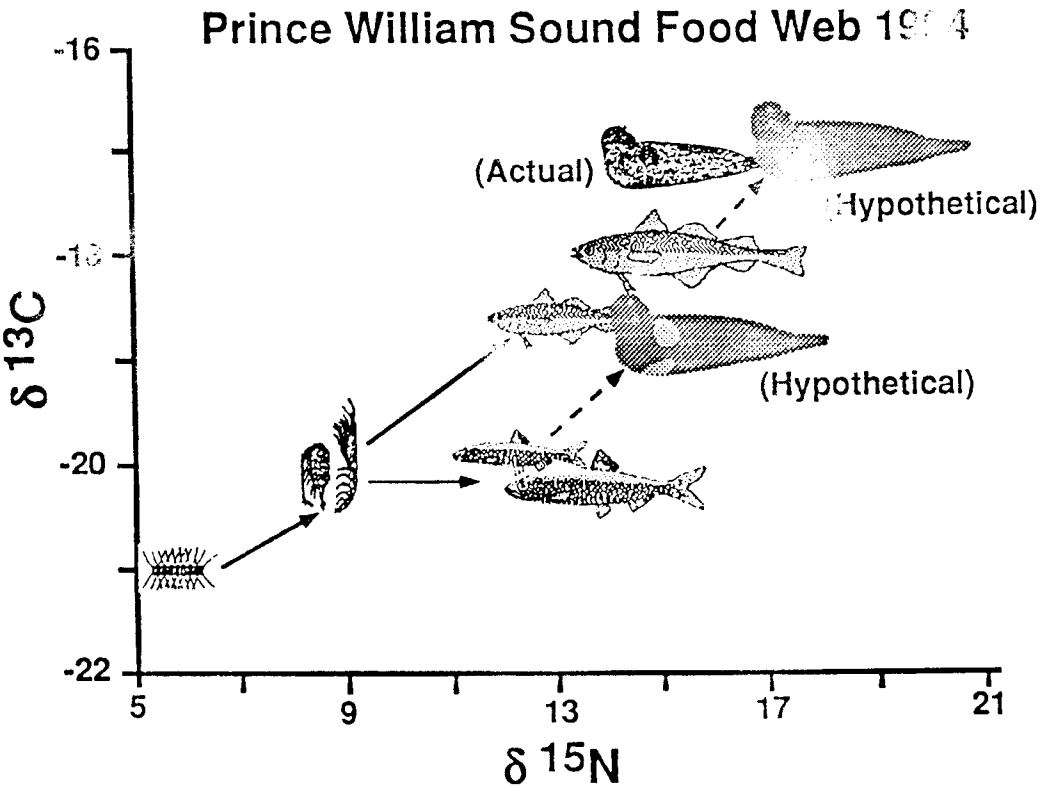


Figure 4. Hypothetical food web for harbor seals in Prince William Sound using carbon and nitrogen isotope ratios for calanoid copepods, juvenile and adult herring and juvenile and adult pollock.

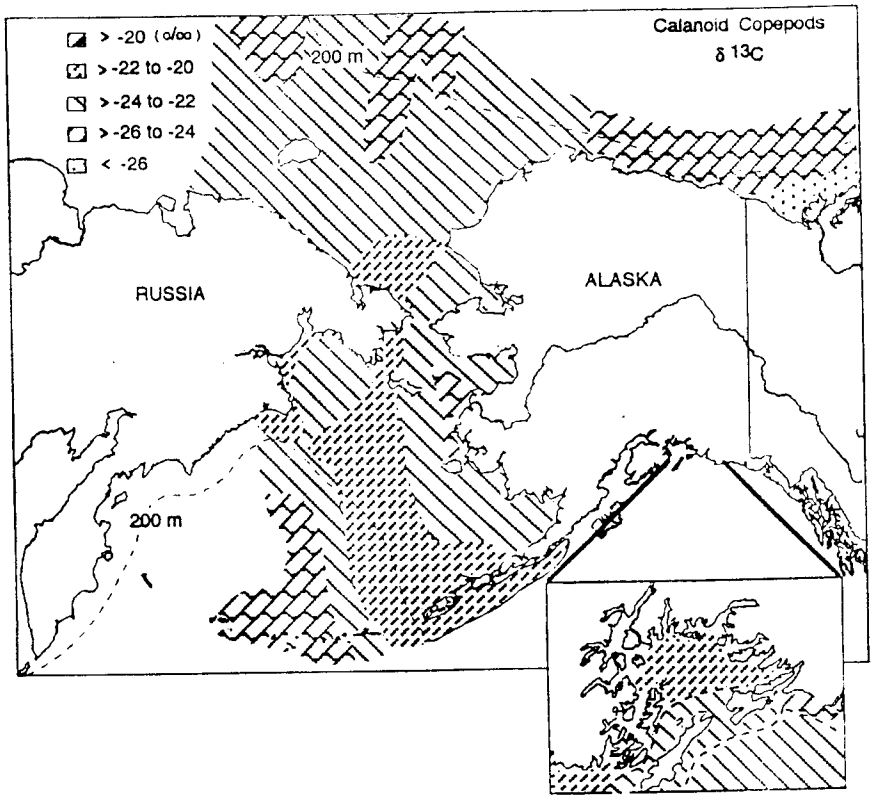


Figure 5. $\delta^{13}C$ isotope contours for calanoid copepods in the Bering and Chukchi seas. The Prince William Sound insert shows estimated contours based on analyzed lower trophic level organisms.

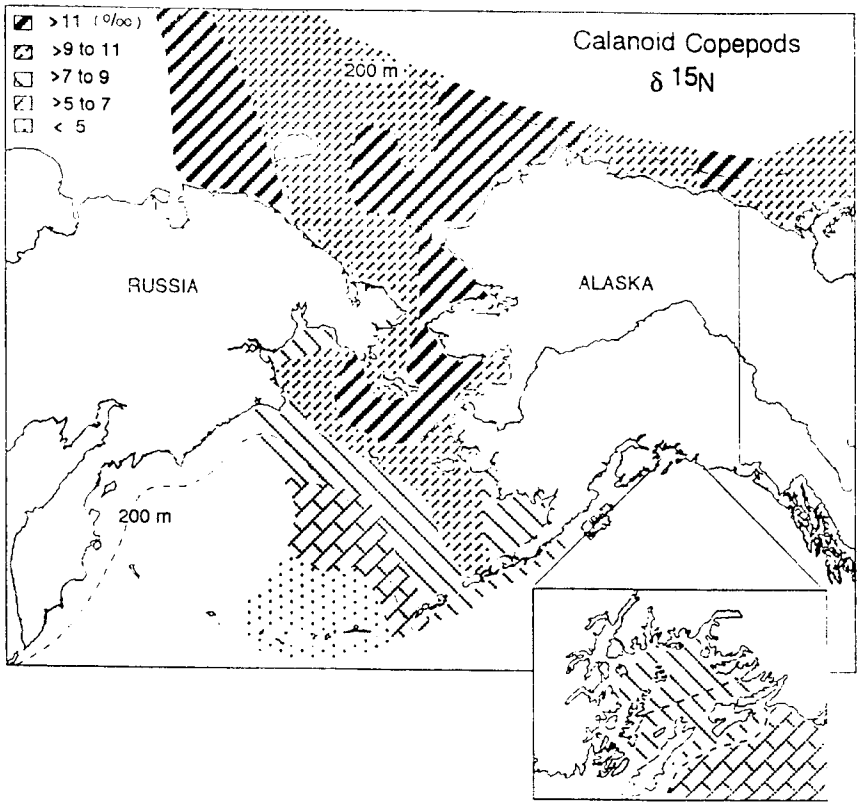
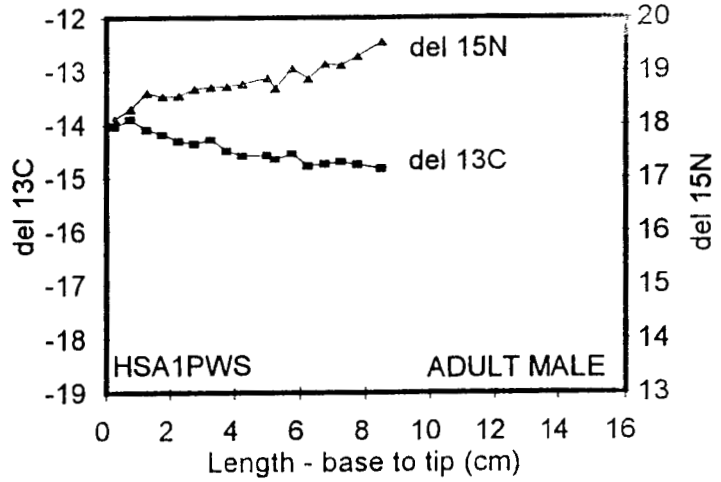
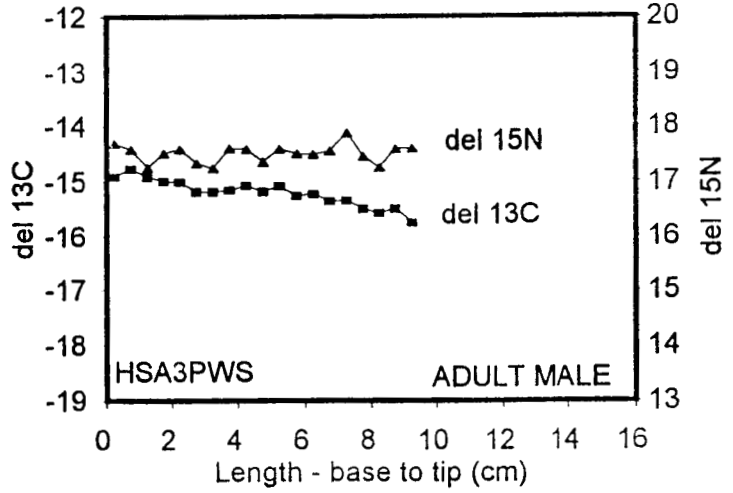


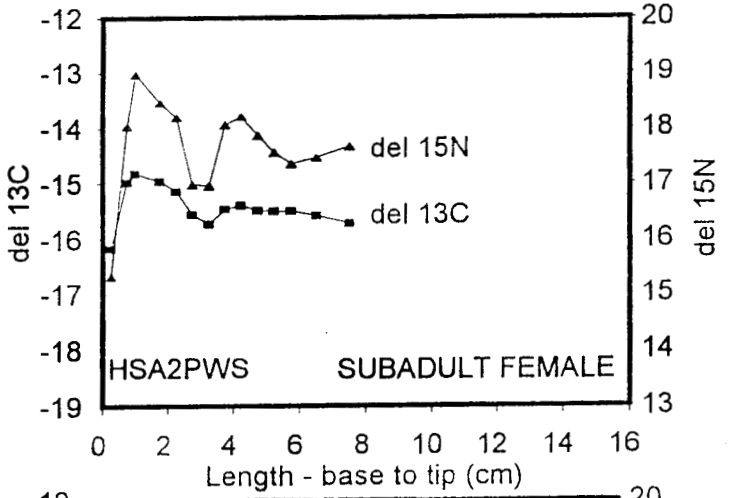
Figure 6. $\delta^{15}N$ isotope contours for calanoid copepods in the Bering and Chukchi seas. The Prince William Sound insert shows estimated contours based on analyzed lower trophic level organisms.

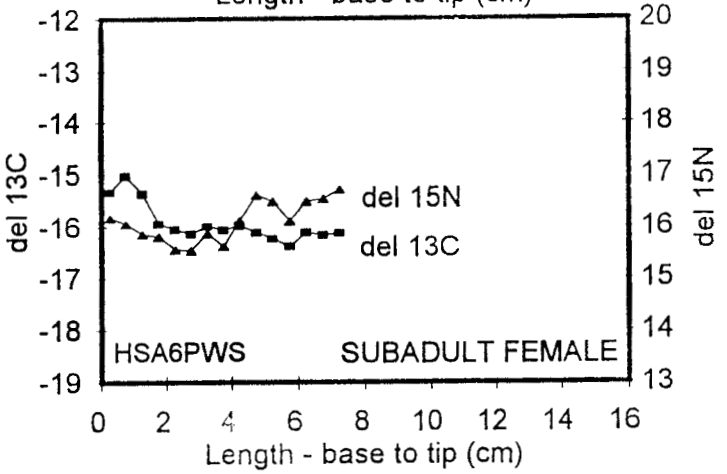
Appendix I.

SEAL ISLAND, PWS MAY 1993



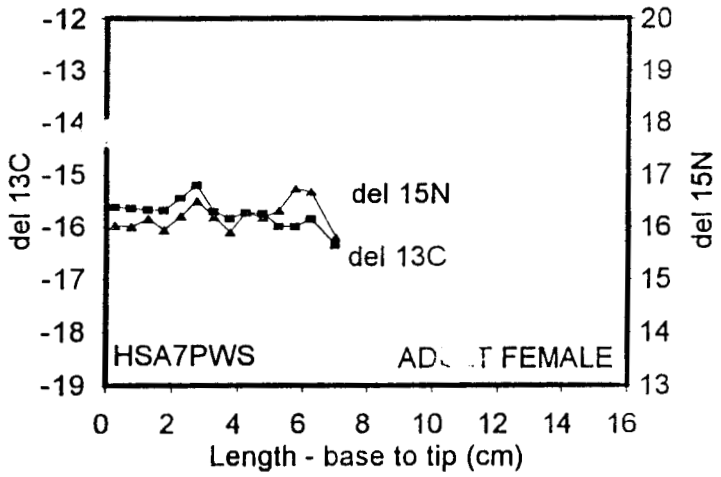


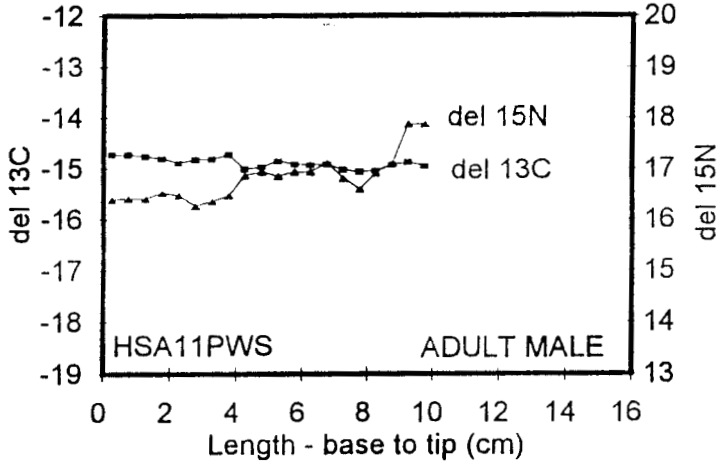


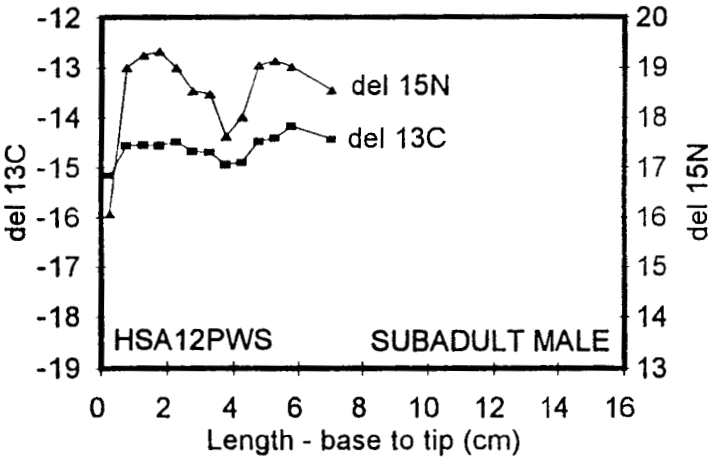


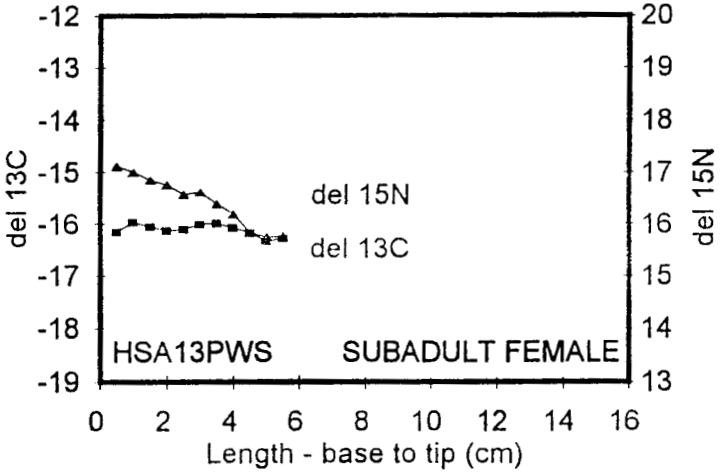
Appendix I.

SEAL ISLAND, PWS MAY 1993



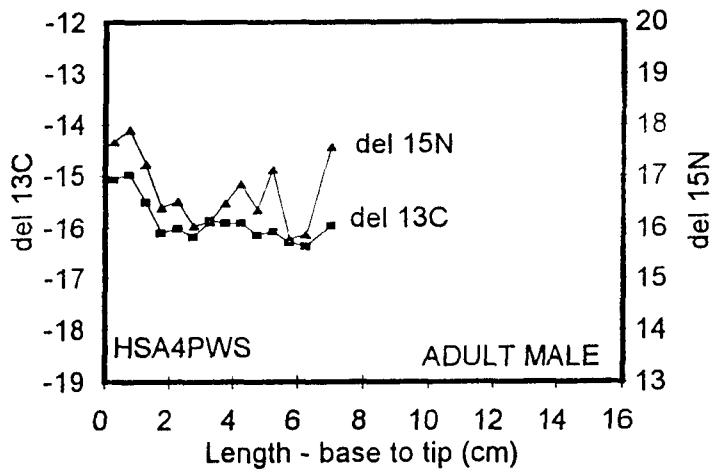


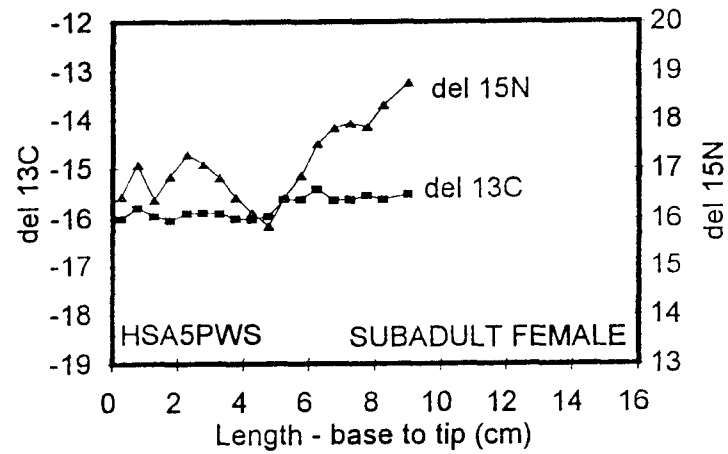


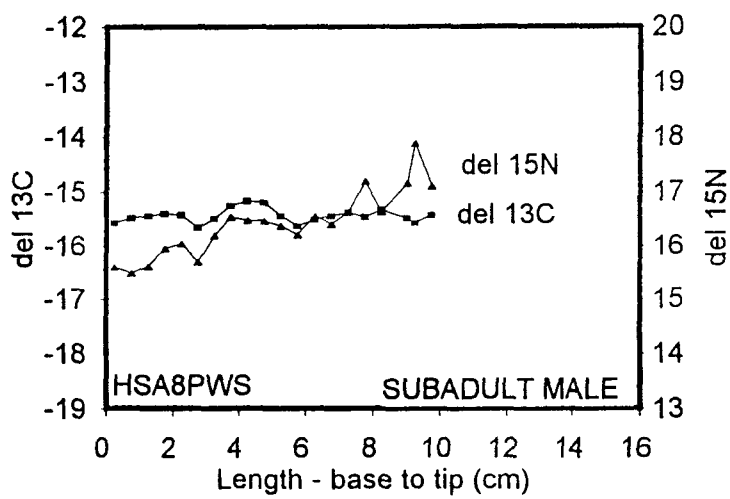


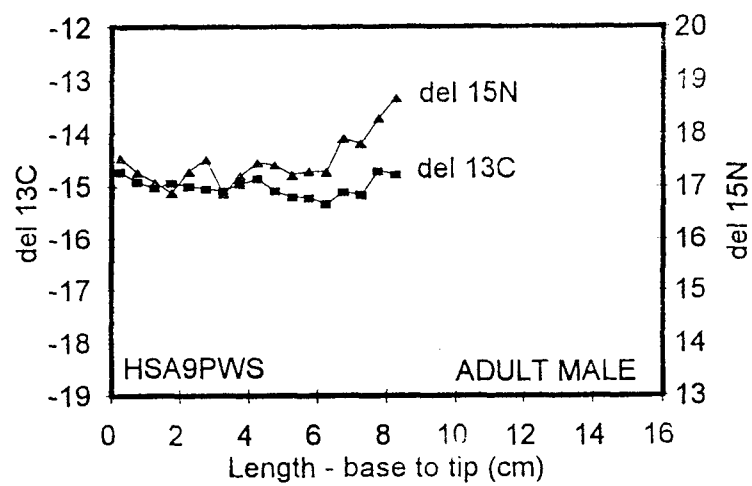
Appendix I.

APPLEGATE ROCKS, PWS MAY 1993



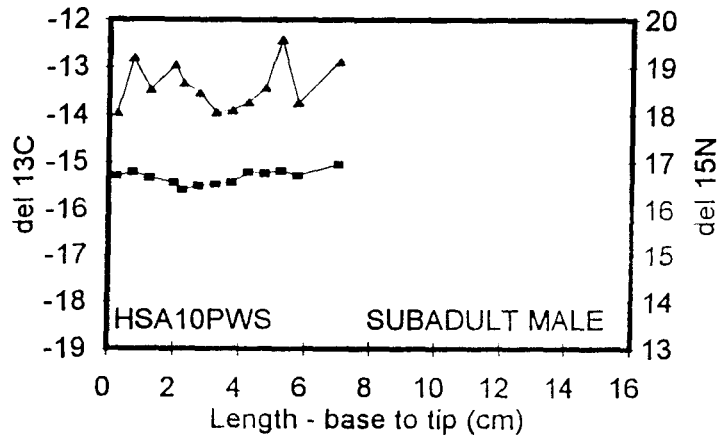






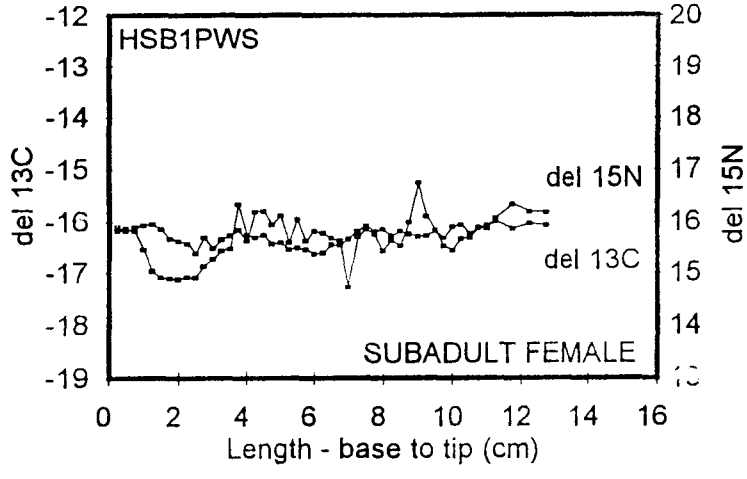
Appendix I.

APPLEGATE ROCKS, PWS MAY 1993



Appendix I.

GREEN ISLAND, PWS APRIL 1994



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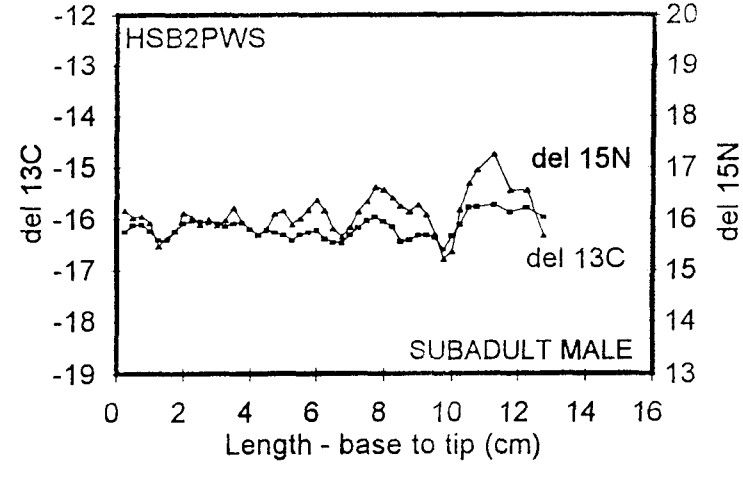

Appendix I.

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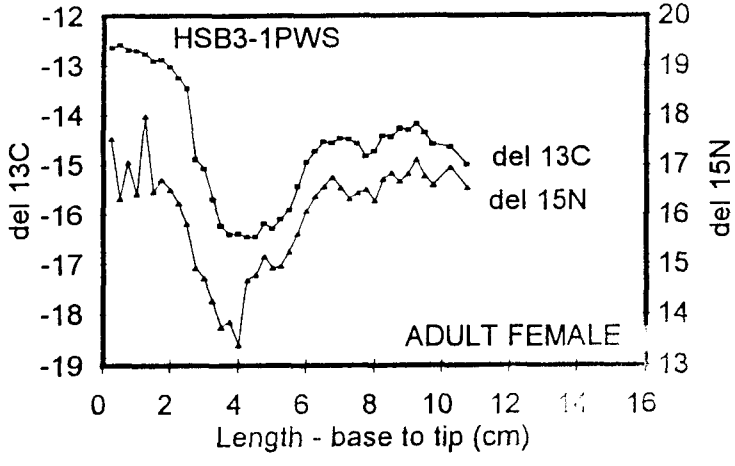
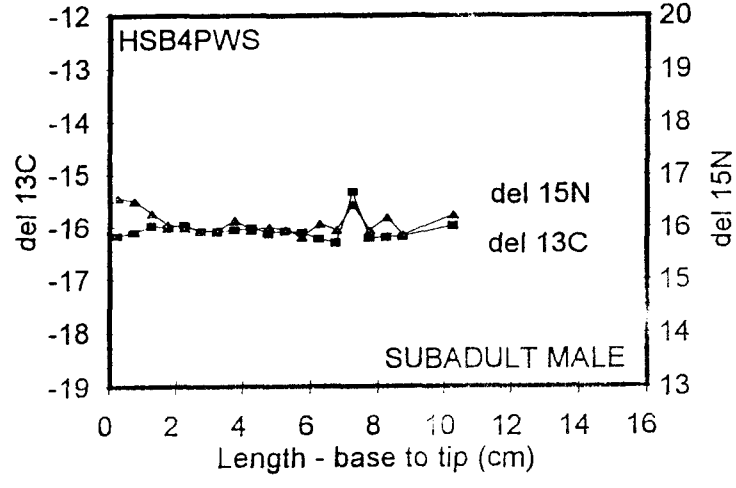
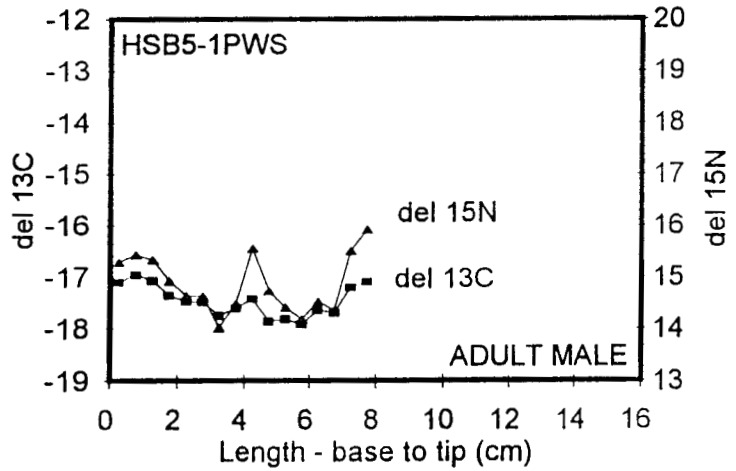
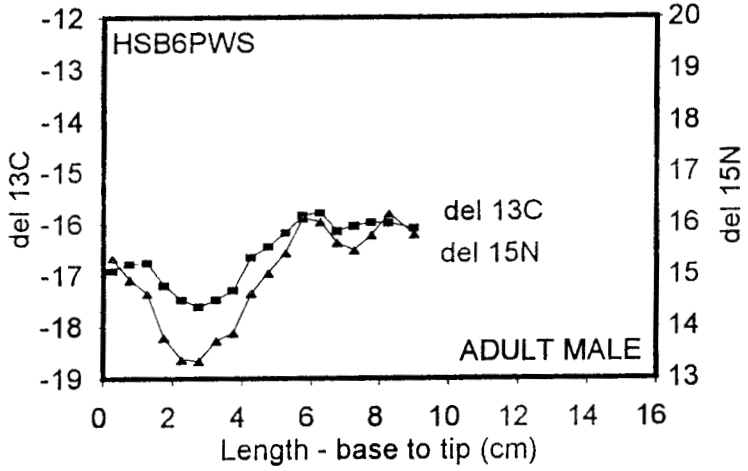


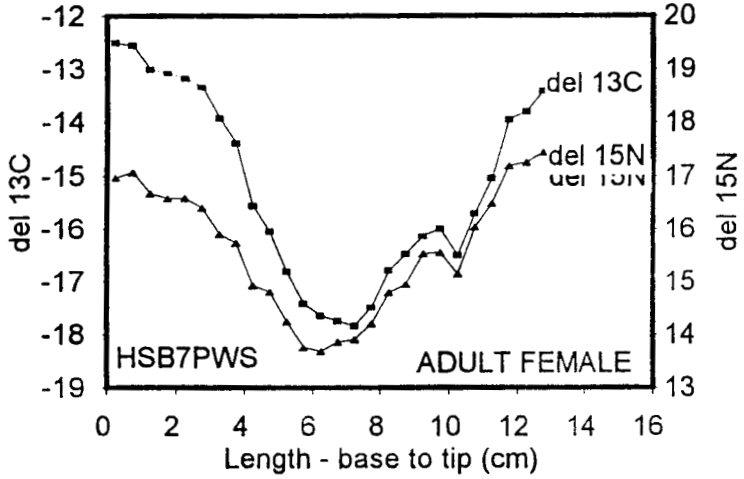


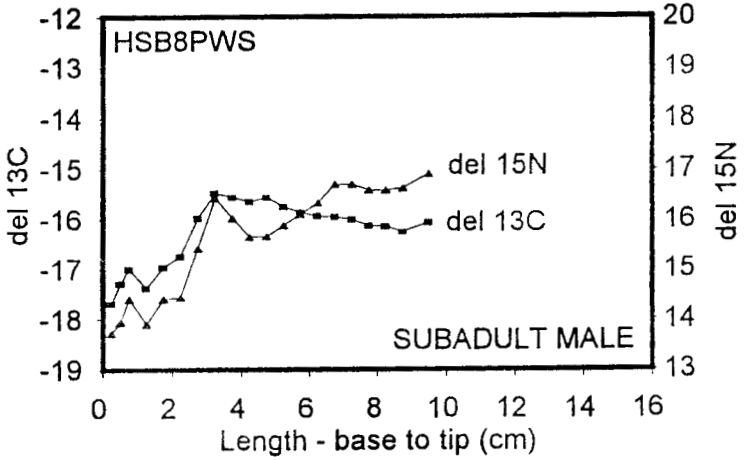
Appendix I.

STOCKDALE HARBOR, PWS APRIL 1994



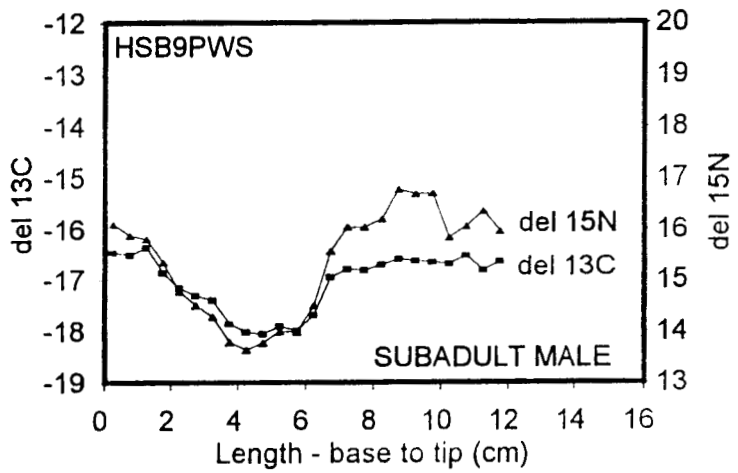


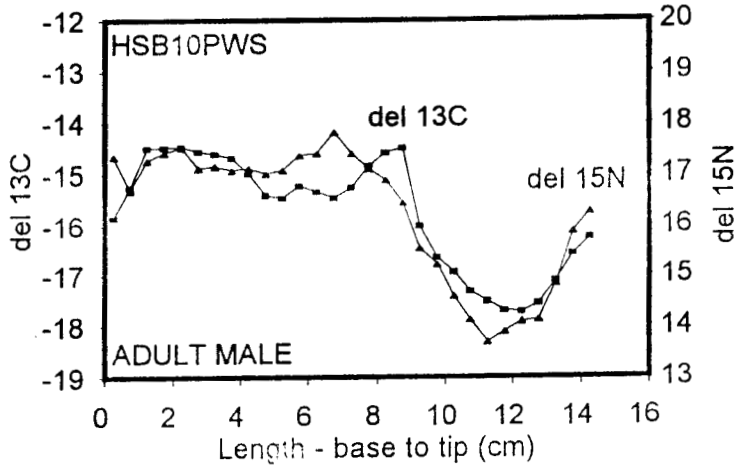




Appendix I.

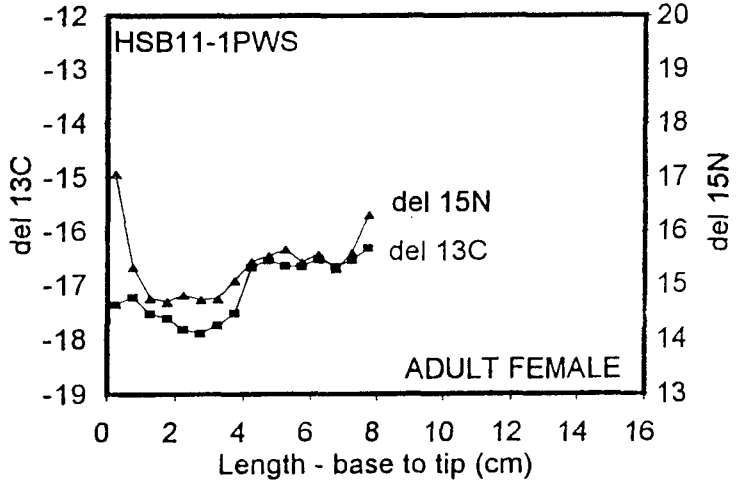
STOCKDALE HARBOR, PWS APRIL 1994

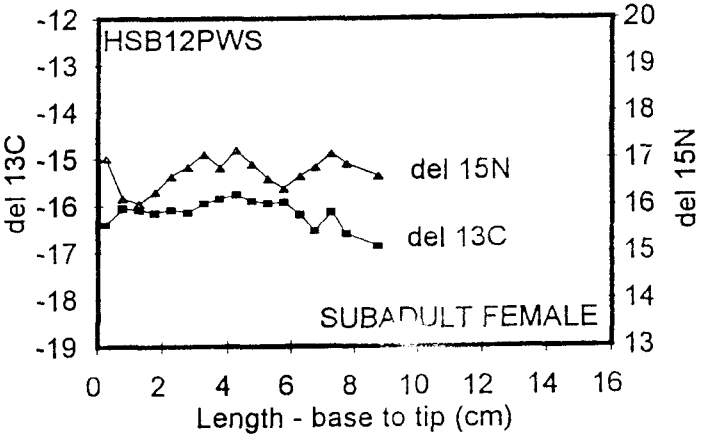


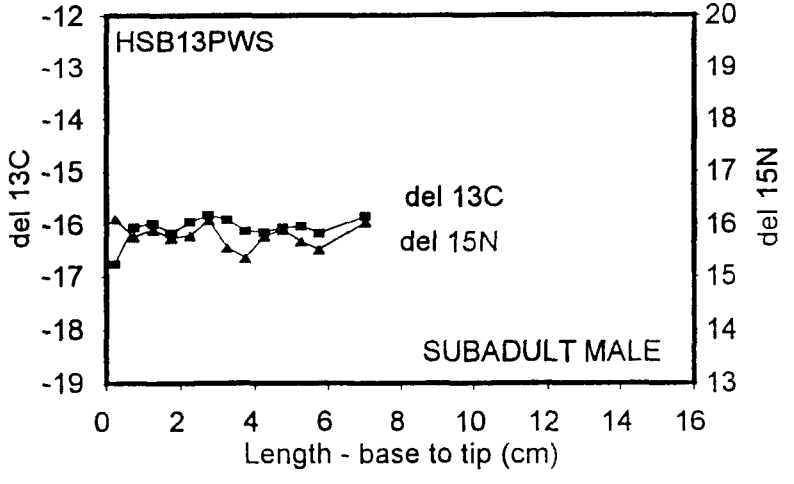


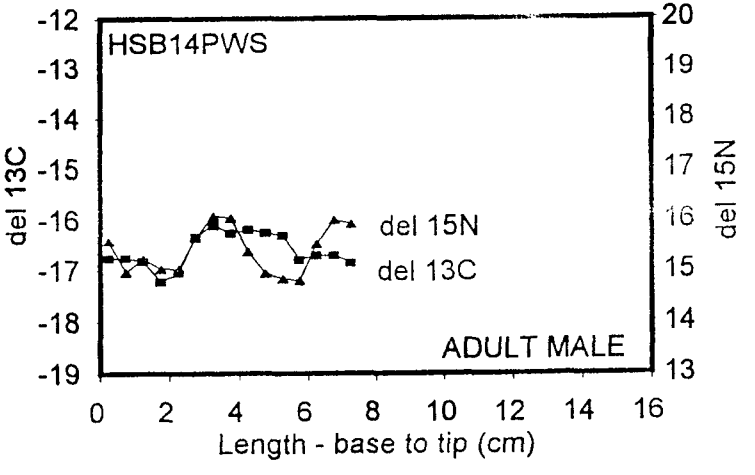
Appendix I.

CHANNEL ISLAND, PWS SEPTEMBER 1994



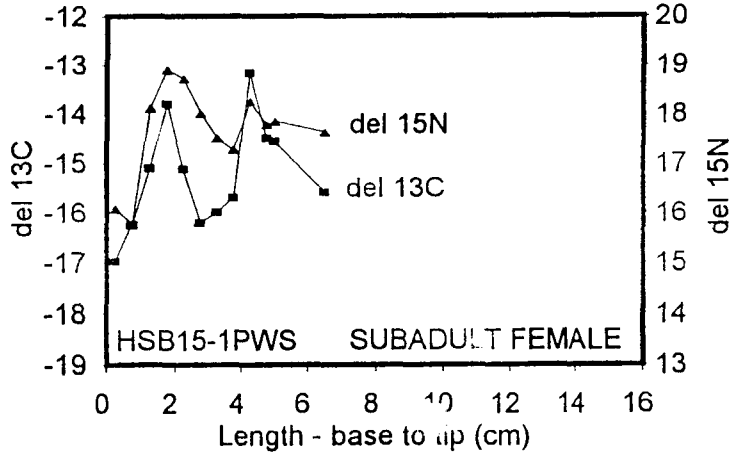


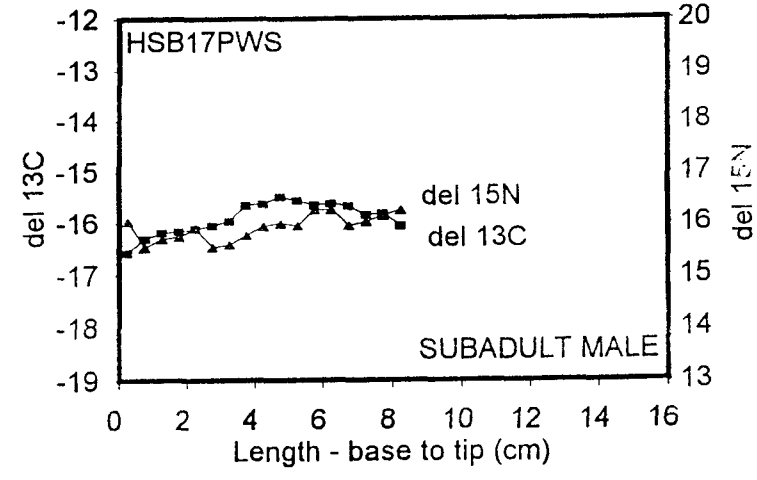


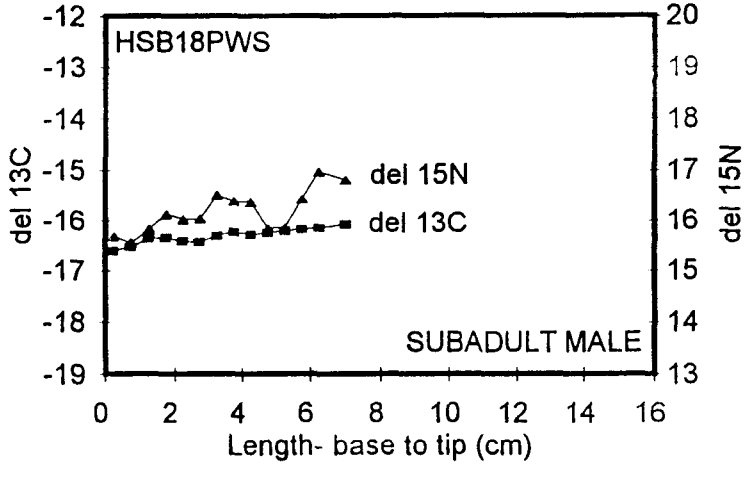


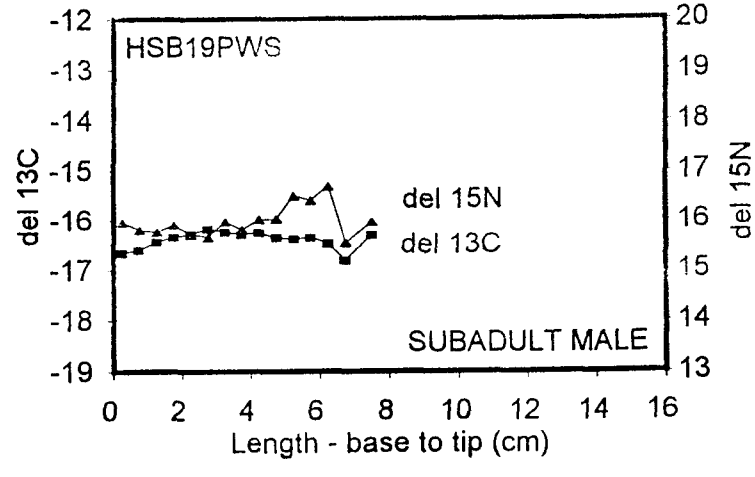
Appendix I.

CHANNEL ISLAND, PWS SEPTEMBER 1994



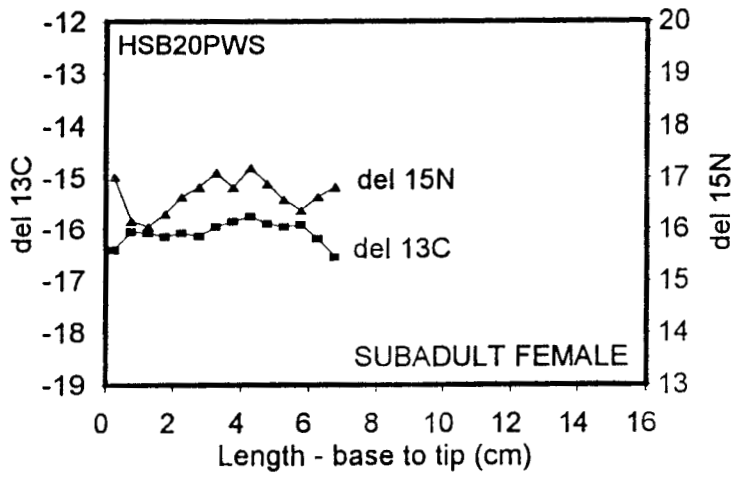


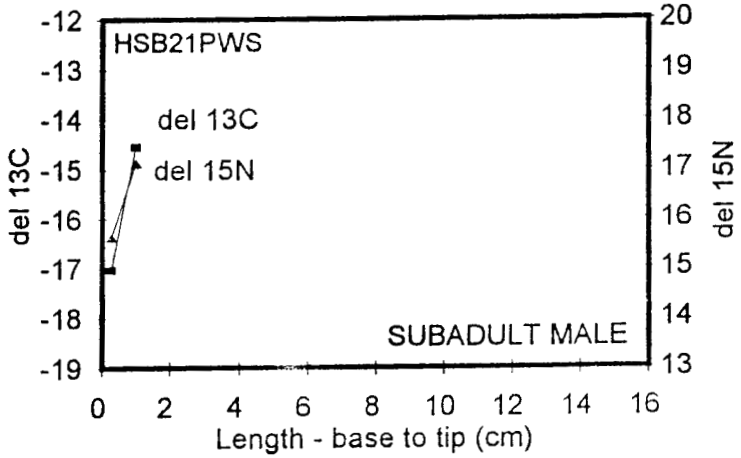


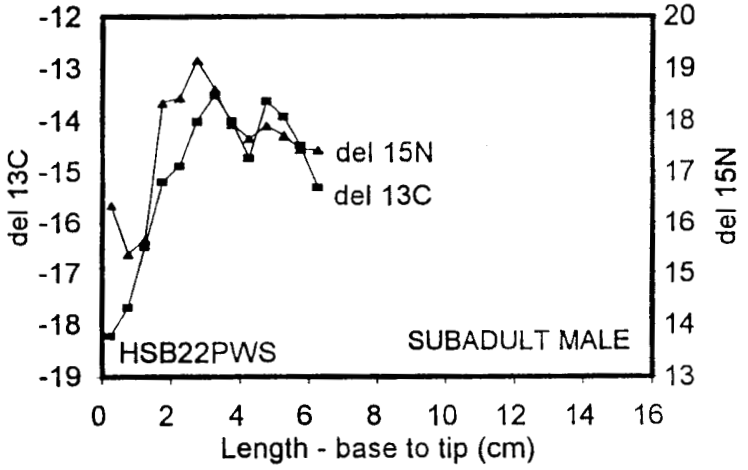


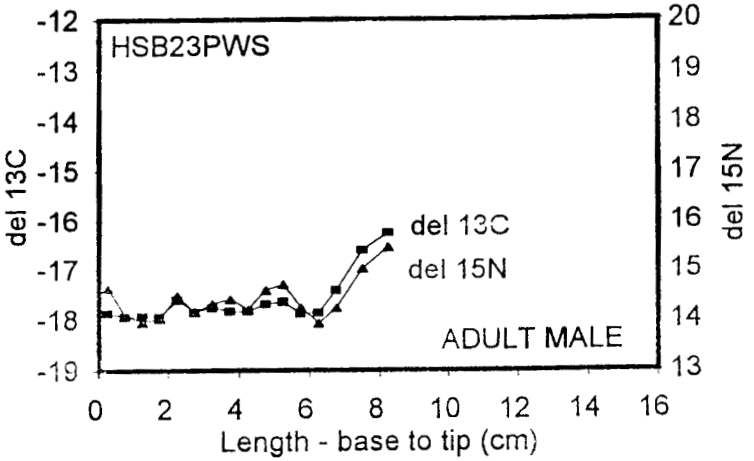
Appendix I.

CHANNEL ISLAND, PWS SEPTEMBER 1994



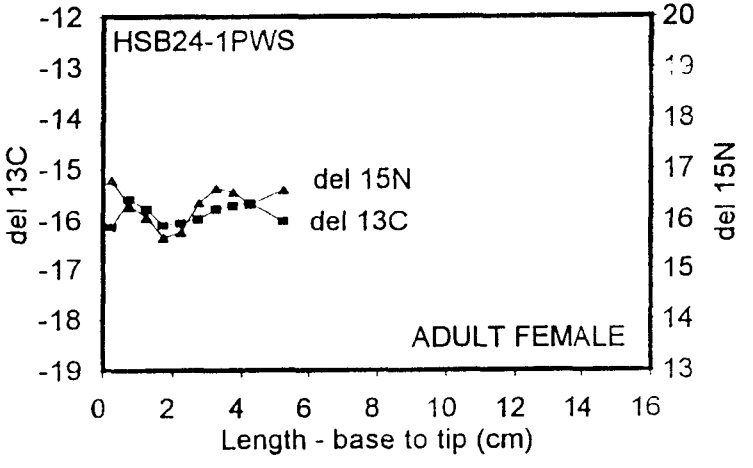


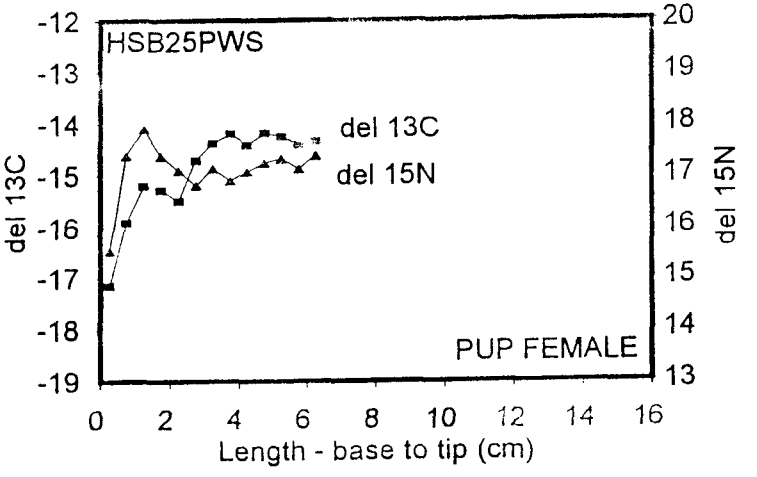


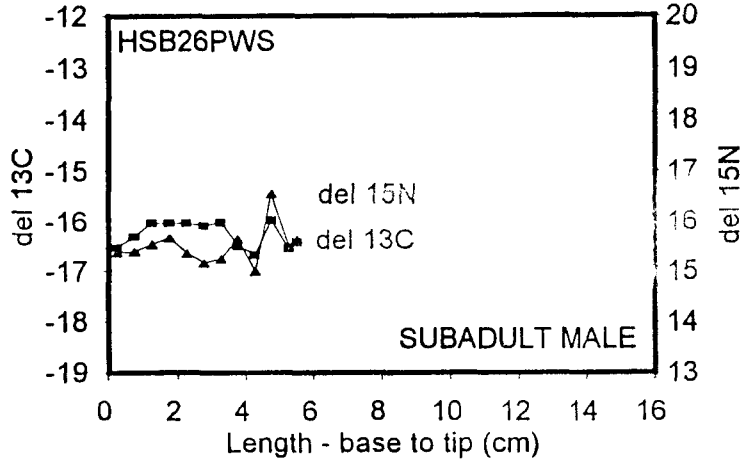


Appendix I.

GRAVINA ISLAND, PWS SEPTEMBER 1994

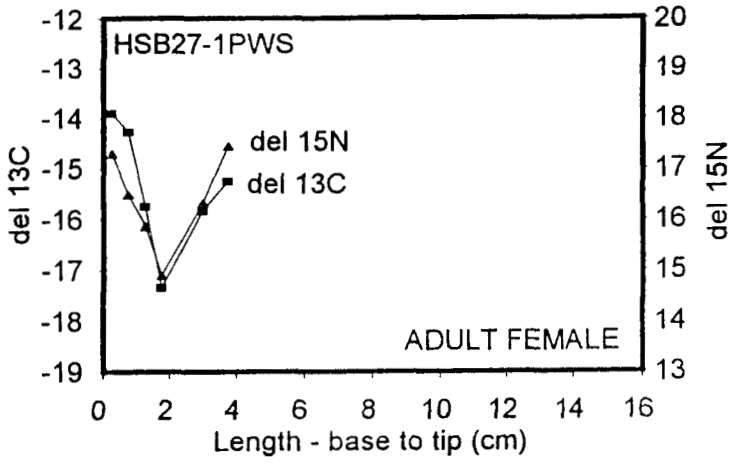


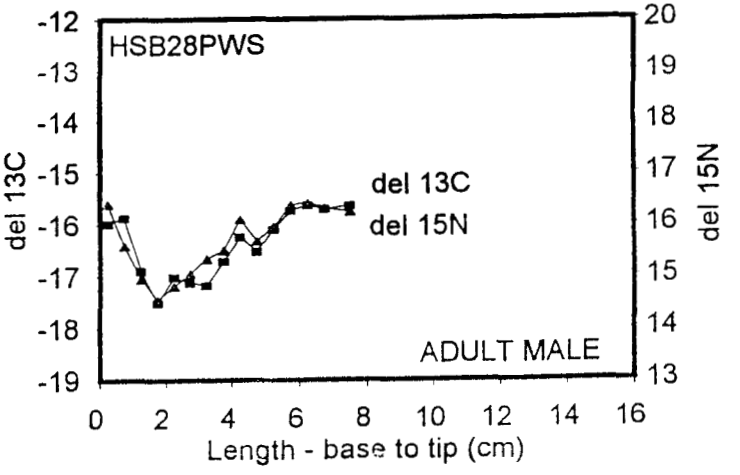


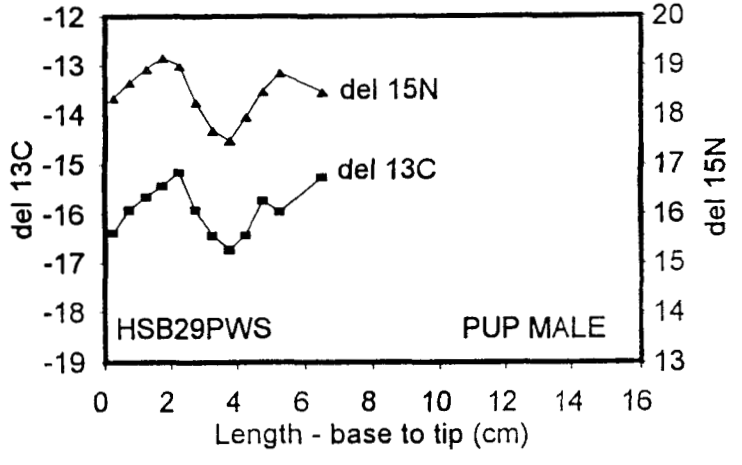


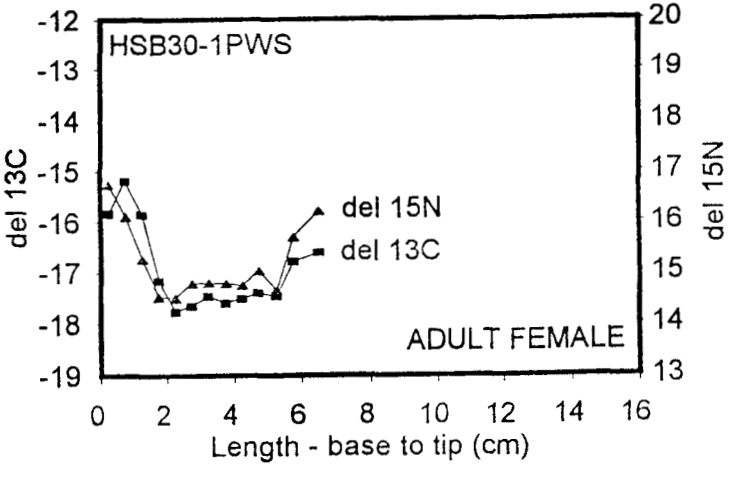
Appendix I.

PORT CHALMERS, PWS SEPTEMBER 1994



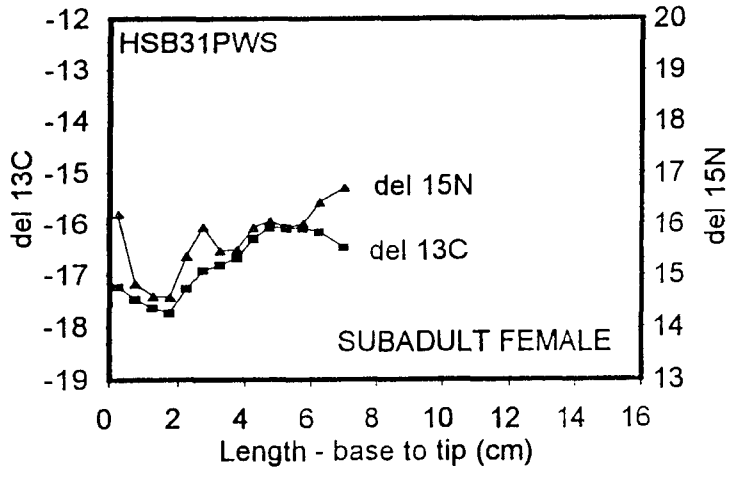


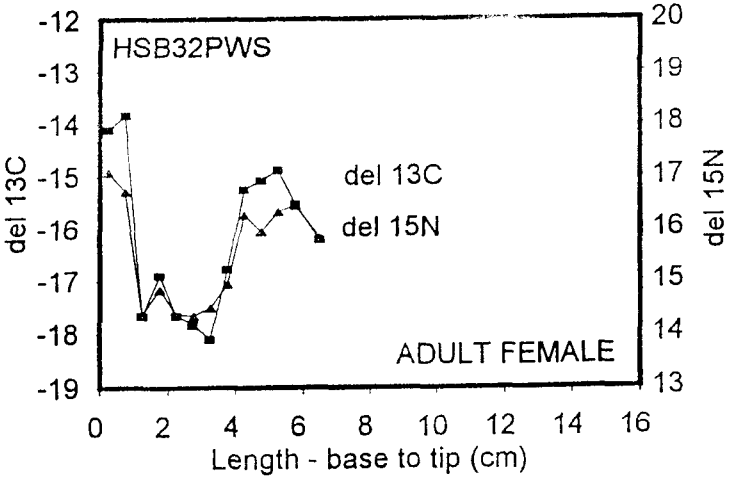


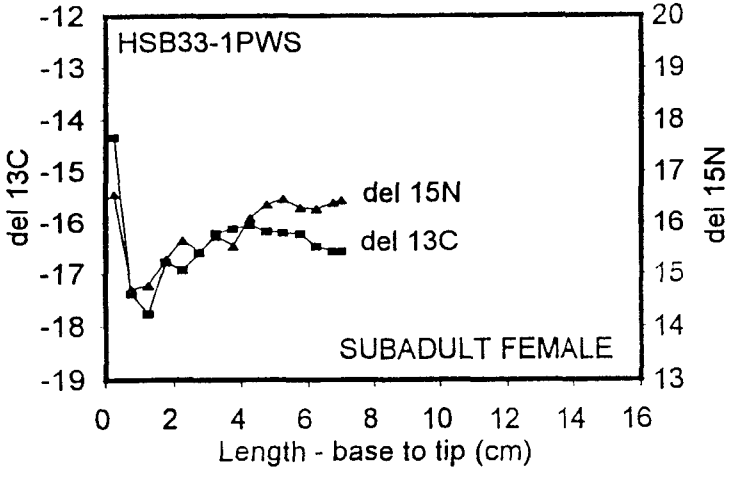


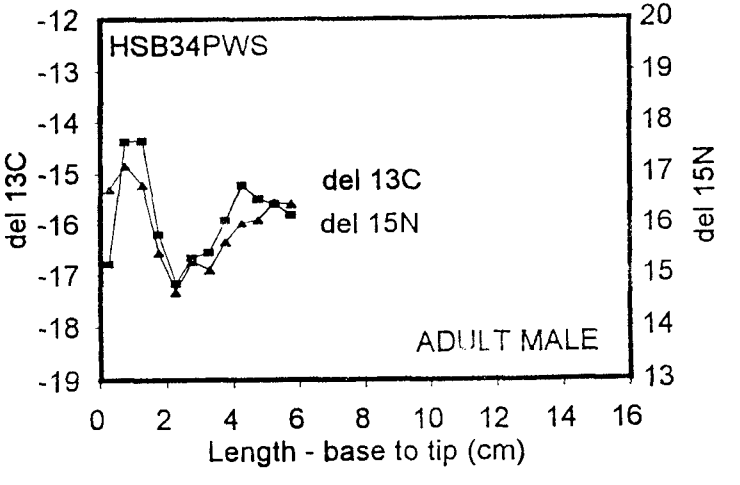
Appendix I.

PORT CHALMERS, PWS SEPTEMBER 1994



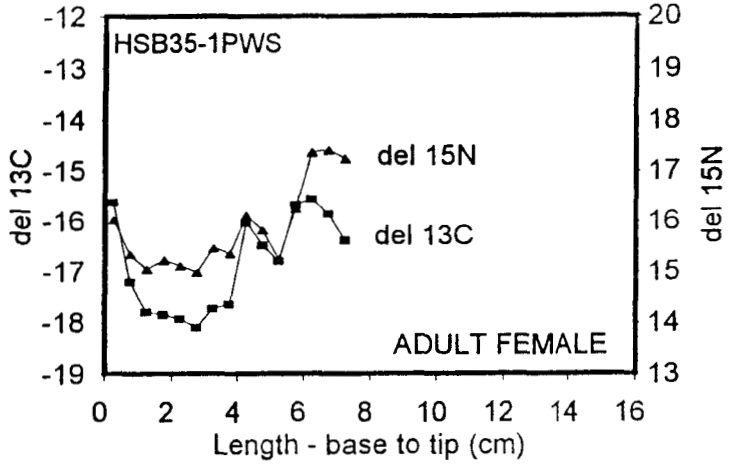


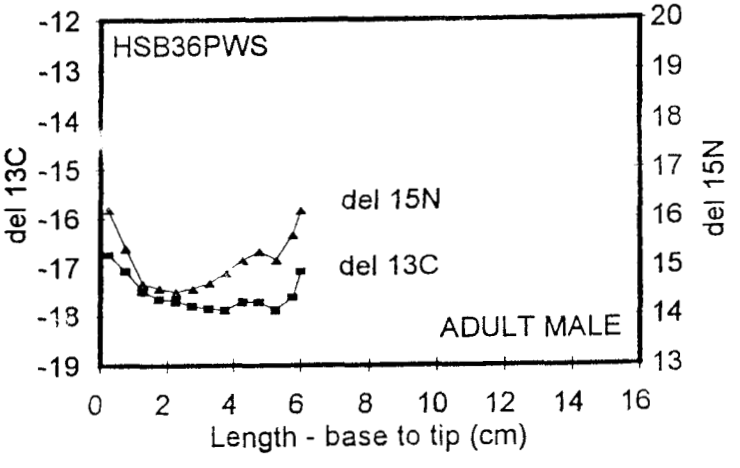




Appendix I.

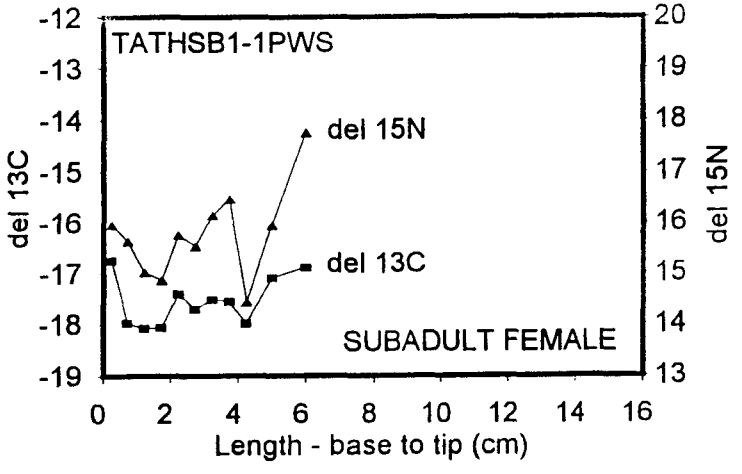
PORT CHALMERS, PWS SEPTEMBER 1994

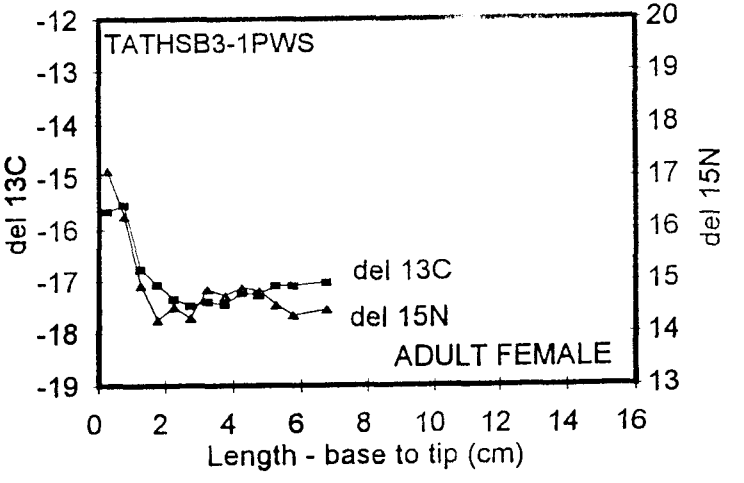


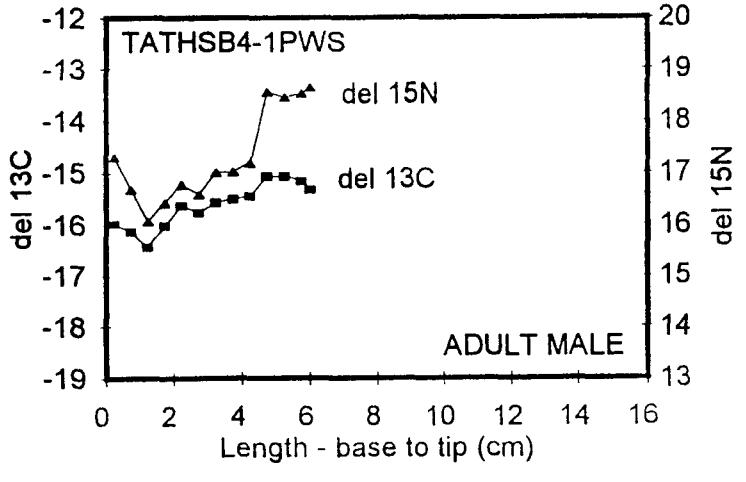


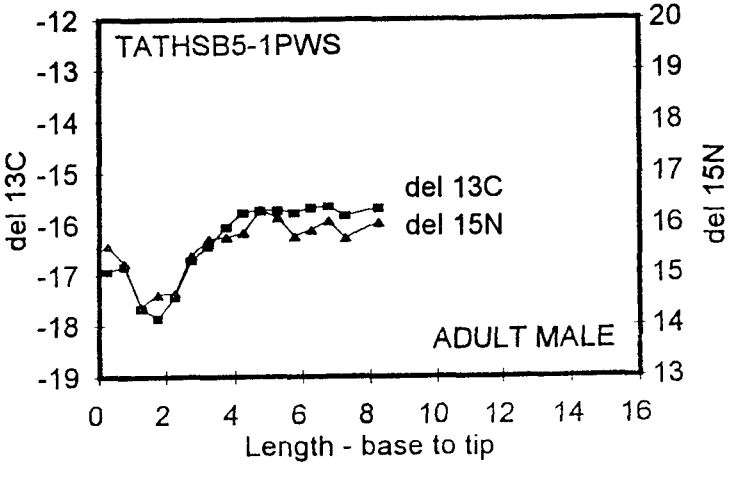
Appendix I.

TATITLIK, PWS SEPTEMBER 1994



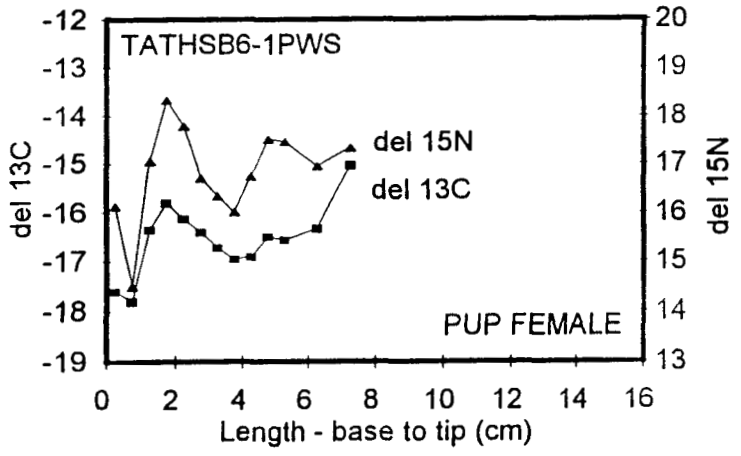


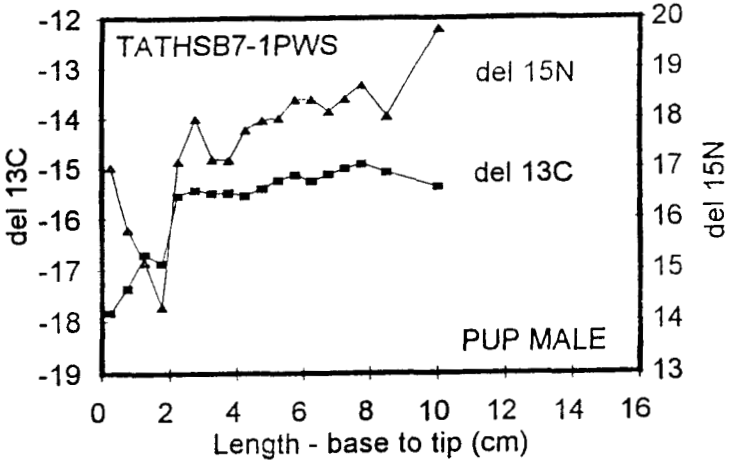




Appendix I.

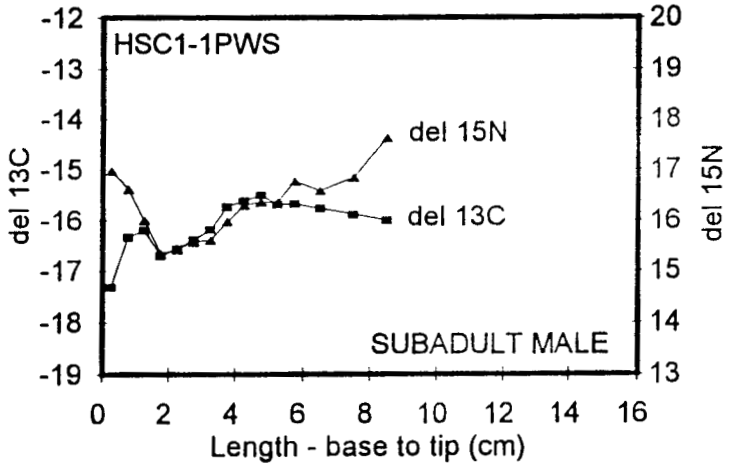
TATITLIK, PWS SEPTEMBER 1994

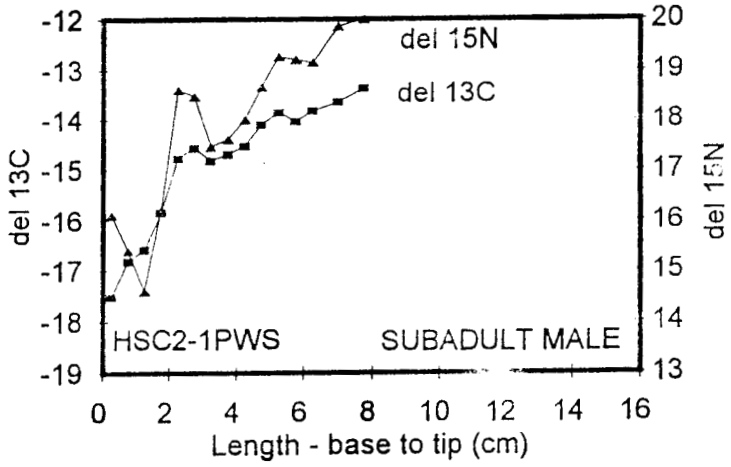


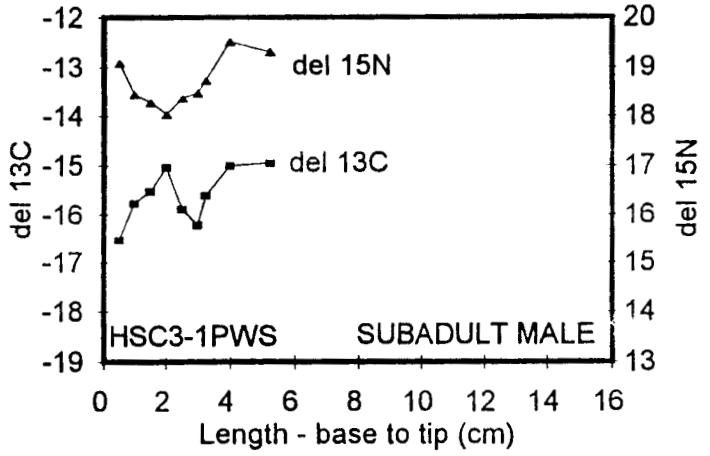


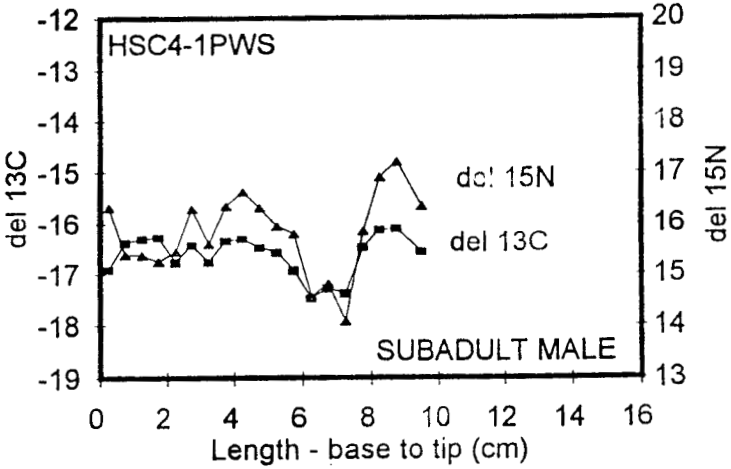
Appendix I.

DUTCH GROUP, PWS MAY 1995



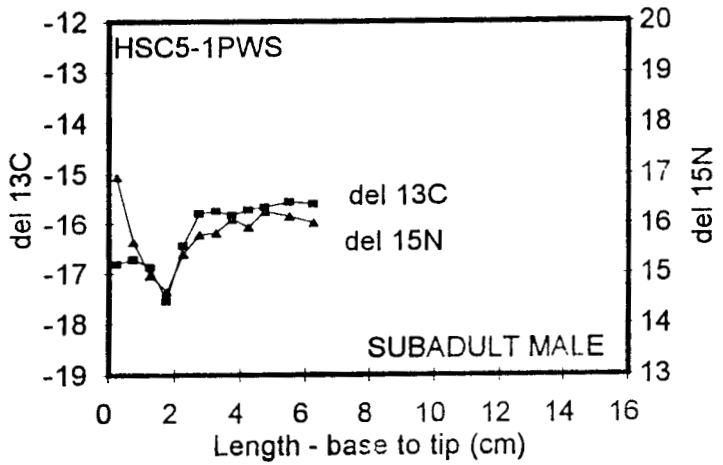






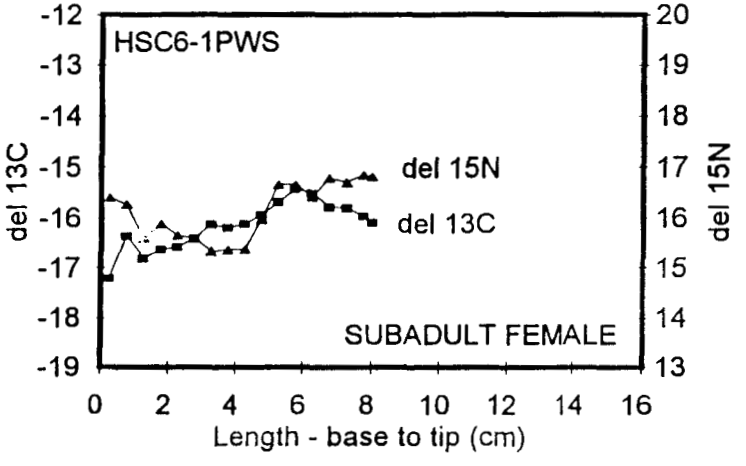
Appendix I.

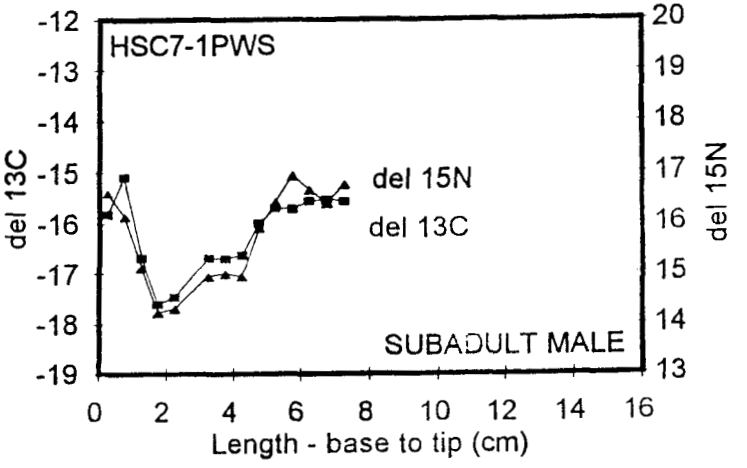
DUTCH GROUP, PWS MAY 1995



Appendix I.

OLSEN BAY, PWS MAY 1995





Exxon Valdez Oil Spill
Restoration Project Annual Report

SEA: Confirming Food Web Dependencies in the Prince William Sound Ecosystem Using Stable Isotope Tracers.

Restoration Project 95320I
Annual Report

This annual report has been prepared for peer review as part of the *Exxon Valdez* Oil Spill Trustee Council restoration program for the purpose of assessing project progress. Peer review comments have not been addressed in this annual report.

Dr. Thomas C. Kline, Jr.

Prince William Sound Science Center
P.O. Box 705
Cordova, AK 99574

April 1996

SEA: Confirming Food Web Dependencies in the Prince William Sound Ecosystem Using Stable Isotope Tracers.

Restoration Project 95320I
Annual Report

Study History: This project was initiated under Restoration Project 94320I(2). An annual report (draft final report) was issued in 1994 by Kline, T. under the title SEA: Confirming Food Web Dependencies in the Prince William Sound Ecosystem Using Stable Isotope Tracers - Food Webs of Fishes. This project effort was continued under Restoration Project 95320I, the subject of this annual report. FY 95 is the second field season for this project that will be closed out with a Final Report prepared in FY 99.

Abstract: It is hypothesized that the availability of macrozooplankton forage for salmon and herring varies in space and time because of changes in physical processes in Prince William Sound (PWS). Under the hypotheses promulgated in the SEA project, it can be postulated that natural stable isotope abundance of PWS biota will shift because of changes in trophic level, food web structure, and primary producer. Thus natural stable isotope abundance can be used to assess these changes.

In project 95320I, biotic samples (including bulk zooplankton, individual macrozooplankters/micronekters and fishes) were analyzed for $\delta^{13}C$ and $\delta^{15}N$, conventional expressions for $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios, respectively. All samples except for smaller macrozooplankters were analyzed in replicate. Data collected during the FY 94 pilot project suggested the existence of carbon sources postulated to correspond to pelagic and neritic organic production sources. Analysis in FY 95 expanded on the FY 94 results with emphasis on verification of the above postulate.

The large herbivorous copepod *Neocalanus cristatus* and net zooplankton samples from the Northern Gulf of Alaska and the area around Montague Island had distinguishable $\delta^{13}C$ values compared to analogous samples collected within Prince William Sound thus confirming the postulated production sources. Isotopic signatures of these sources were conserved in copepods undergoing diapause in PWS and fishes sampled from PWS.

Key Words: carbon, carbon sources, $^{13}C/^{12}C$, copepods, *Clupea pallasi*, $\delta^{13}C$, $\delta^{15}N$, ecosystem process, *Exxon Valdez*, food webs, Gulf of Alaska, Herring Overwintering, juvenile fishes, Lake/River, *Neocalanus cristatus*, $^{15}N/^{14}N$, *Oncorhynchus gorbuscha*, Pacific herring, pink salmon, plankton, predators, Predator/Prey, Prince William Sound, stable isotopes

Citation: Kline, Thomas C., Jr. 1996. SEA: Confirming Food Web Dependencies in the Prince William Sound Ecosystem Using Stable Isotope Tracers, *Exxon Valdez* Restoration Project Final Report (Restoration Project 95320I), Alaska Department of Fish and Game, Anchorage, Alaska.

List of Figures:

Executive Summary The failure of several Prince William Sound (PWS), Alaska vertebrate species to recover from population crashes following the 1989 *T/V Exxon Valdez* oil spill, has raised concerns that shifts in food web structure may have occurred. Of particular concern are post-spill declines in the abundance of *Clupea pallasi* (Pacific herring), and *Oncorhynchus gorbuscha* (pink salmon). It is hypothesized that abundance of large herbivorous copepods of the genus *Neocalanus* and other macrozooplankton, primary sources of food for these fishes and their predators, are controlled by oceanographic processes. When planktonic prey are unavailable, predators switch feeding mode from planktivory to piscivory. Confirmation of these hypotheses are being tested in a large-scale multi-disciplinary project known as Sound Ecosystem Assessment (SEA).

Because of their predictable nature, stable isotope ratios of carbon (^{13}C /^{12}C) and nitrogen ($^{15}N/^{14}N$) are providing an effective method for testing the SEA hypotheses in Restoration Project 320I. Natural stable isotope ratios are useful

for providing empirical evidence of trophic relationships in marine food webs. Stable Isotopes are providing evidence in three SEA hypotheses: (1)"prey switching" through observations of seasonal isotope shifts in predators in combination with a prey database; (2) "river-lake processes" through measurement of related temporal and spatial isotopic effects within the plankton community (with the goal to trace food web carbon sources through to fishes); (3) "herring overwintering" through comparisons of energetic condition with food web carbon source as determined by stable isotope signatures.

In project 320I, biotic samples (including bulk zooplankton, individual macrozooplankters/micronekters and fishes) were analyzed for $\delta^{13}C$ and $\delta^{15}N$, conventional expressions for $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios, respectively. All samples except for smaller macrozooplankters were analyzed in replicate. Data collected during the FY 94 pilot project suggested the existence of carbon sources postulated to correspond to pelagic and neritic organic production sources. Analysis in FY 95 expanded on the FY 94 results with emphasis on verification of the above postulate.

The large herbivorous copepod *Neocalanus cristatus* and net zooplankton samples from the Northern Gulf of Alaska and the area around Montague Island had distinguishable $\delta^{13}C$ values compared to analogous samples collected within Prince William Sound confirming the existence of the isotopic gradient. Isotopic analysis of the copepods undergoing their diapause phase during the winter (1994-95) suggests that half of this population fed exclusively in PWS. 17% fed exclusively in the northern Gulf of Alaska. These proportions are expected to vary between years as a function of river-lake processes. Isotopic analysis of the diapausing copepods can be used to assess river-lake variability and validate model predictions of these processes.

Variability in $\delta^{13}C$ of fishes consistent with shifting from PWS and Gulf of Alaska carbon sources were found to vary spatially and in relation to pre-winter condition of herring. Isotopic shifts in pink salmon fry corresponded to shifts from hatchery diet to carbon from the Gulf of Alaska. $\delta^{15}N$ values were used to assess trophic levels of forage and predator species. These trophic levels are expected to shift in response to ecosystem shifts resulting from river-lake processes that reduce the availability of macrozooplankton forage. Trophic level shifts monitored with $\delta^{15}N$ will enable one to detect these changes.
This study is demonstrating the applicability isotopic chemistry shifts in biota to SEA processes that will be useful in the development of model validation tools obligatory for SEA project ecosystem modeling products.

SEA-FOOD: Confirming Fish Food Web Dependencies in the Prince William Sound Ecosystem Using Natural Stable Isotope Tracers

Stable isotope ratios of carbon serve as effective tracers of energy supply in the study area due to conservative transfer of carbon isotope ratios between the lower tropic levels (phytoplankton to zooplankton to forage fishes, etc.) of Prince William Sound (PWS) and adjacent Gulf of Alaska waters up to the top consumers. The seals, whales, birds, and fishes acquire these isotope ratios in response to the importance of the food sources and record temporal signals in keratinous tissues (claws, hair, feather) and reflect the major sources of their

food in the bulk body tissues (muscle and fat). Isotope ratio analysis of these tissues can provide insight into habitat usage and assist in quantifying amounts derived from various areas. Nitrogen isotope ratios, in turn, provide excellent definition of relative trophic level. The heavy isotope of nitrogen is enriched by about 0.3 % with each trophic level and thus can accurately indicate the relative trophic status of species within an ecosystem.

The availability of macrozooplankton forage for salmon, herring, other forage fish species, and predators vary in space and time because of changes in physical processes in PWS. In the SEA context, the latter is known as the river-lake hypothesis. When macrozooplankton are not available, macrozooplankton consumers are forced to switch prey, thus the predator-prey SEA hypothesis. Mortality of herring during the winters of their early life stages is thought to be the major factor affecting recruitment (overwintering hypothesis). That river-lake processes may affect overwinter survival is being tested by comparison of carbon source (this project) in relation to energetics (95320U). Food web shifts represent fundamental changes in the way the PWS ecosystem produces commercial species injured by the oil spill, i.e., herring and salmon. A better understanding, particularly a quantitative understanding, is a prerequisite to determining protocols for restoration and recovery of these species.

Natural stable isotope abundances reflect (1) trophic level and (2) source of assimilated matter and are thus a proxy for the change in diet specified in the SEA hypotheses. Stable isotope ratios are thus be used as a biomonitor of ocean circulation, salmon and herring production, and shifts in predation as tests of the SEA hypotheses.

OBJECTIVES

Hypotheses

The following hypotheses to be tested were specified in the DPDs:

Hypothesis 1. Carbon and nitrogen stable isotope ratios of biota from Prince William Sound can be used to identify major food sources to top trophic levels and to assign trophic positions to specific consumers of given age classes and habitat.

Hypothesis 2. Isotope ratios in consumers provide a means to validate conceptual food web structures, identify trophic variability by individuals within species, and to validate quantified energy flows in ecosystem models.

Goals

The 95320I study builds upon the existing stable isotope data base and adds new data to construct and test conceptual food webs supporting injured species (and other species for which samples are, or become available) in Prince William Sound and their prey organisms. The goal is to determine the trophic positions and to define the natural history parameters accessible from isotope ratio data in light of the observed declines in their populations. These include changes in trophic level over natural history stages, habitat dependencies, seasonal energetics and trophic dynamics relative to other community organisms. As

part of this goal, this project will integrate analytical results with the field and laboratory studies of other investigators looking at food web structure, productivity of lower trophic levels, and will provide validation data for assessment of conceptual and quantitative models. These models are being developed as restoration tools enabling a predictive capability for resource managers. As these models, are "spun up," *model validation tools* will become necessary in order to test models against field data. The development of natural stable isotope model validation tools will be the principal restoration products of this project.

Specific objectives

Specific objectives of the 95320I project were:

1. To determine the $^{15}N/^{14}N$ and $^{13}C/^{12}C$ of species collected from the Prince William Sound ecosystem with a focus on those components important to man or important in the food webs supporting these species. Herring and salmon collected from PWS are matched with regional isotope abundances in prey species (zooplankton, forage fishes) to allocate food sources and to assess carbon source utilization and trophic transfer efficiencies in specific areas of the sound.

2. Determine isotope ratios on prey species favored by marine mammals in different regions of Prince William Sound. These data will support other Trustee projects (see 95170) concerned with marine mammals and will enable the estimation of seasonal importance of various prey species and the trophic levels of various seal species in the ecosystem. Past data have shown that there are considerable differences between individual animals of a given age and also changes in trophic level over the life span.

3. Synthesize the data obtained in context with conceptual food webs to validate feeding models and expand the natural history information.

4. Contribute stable isotope results to formal tests of the lake-river driven prey switching hypothesis developed by SEA to explain pink salmon and herring production trends.

METHODS

The Stable Isotope Approach

The use of natural abundance ratios of stable isotopes in biological systems has expanded rapidly in recent years and has proved extremely valuable in tracing carbon and nitrogen in both terrestrial and aquatic ecosystems. Most ecosystem studies depend upon two approaches: One is to construct budgets or mass balances of a key element such as carbon and attempt to determine which actions or processes in the natural history of the species of interest dominate these budgets. The second approach is to measure key rates or feeding processes and to relate the findings to the overall goal of assessing energy intake from the habitat. Although the two approaches should ideally coalesce into a coherent and complementary picture, this goal is difficult to attain. There are mismatches between time and space scales of the two approaches. Because stable isotopes can contribute both source (tracer) information and process information, they are ideally suited for identification and

measurement of the movements of carbon and nitrogen in the ecosystem. Since they occur naturally, there are no concerns regarding perturbing the system or the need for experimental manipulations that might alter behavior or ambient conditions.

It is postulated that natural stable isotope abundance of PWS biota will shift because of changes in trophic level, food web structure, and primary producer in the context of the SEA hypotheses, thus providing an independent tool to verify, quantify and model ecosystem processes. The tracer nature of the approach will enable the integration of ecosystem components. It will enable monitoring of "bottom up" shifts (food supply) in consumers such as herring and salmon.

The stable isotope project is an interdisciplinary effort focused on the food web dynamics supporting top trophic levels in Prince William Sound. The study provides an integrating function to projects focusing on several levels in food chains and will employ the stable isotope ratios of carbon and nitrogen to trace trophic transfers of carbon and nitrogen between levels. In cases where regional gradients in isotope ratios exist, it may also be possible to identify critical habitats used by marine biota.

Basis for application of the stable isotope methodology

The natural abundance of stable isotopes, e.g., $^{15}N/^{14}N$ and $^{13}C/^{12}C$, is a very powerful tool for ecological analysis because of the conservative nature of isotopic signatures in food webs (Wada and Hatori 1991). The most extensively measured process that enriches ^{15}N is the trophic level enrichment phenomenon (e.g., the transfer of material and energy from plants to animals or animals to animals). It is now well established that consumers are enriched in ^{15}N by 0.34 $\pm$ 0.10 % compared to their diet irrespective of taxon or ecosystem (Minagawa and Wada, 1984). Although the consistency of the enrichment is not well understood, the virtual universality of its occurrence allows one to determine the number of trophic steps in a food chain from a given producer to consumer (Fry 1988, Wada et al. 1991). Thus change in $^{15}N/^{14}N$ ratio in consumer biota relative to primary food sources over time will reflect change in trophic level (TL). Additionally, shifts in carbon source will be reflected in consumer $^{13}C/^{12}C$ when switching from diets of differing $^{13}C/^{12}C$. For example, shifts in herring and salmon diets that normally consist of macrozooplankton (largely reflecting allochthonous production having been advected into PWS) to autochthonous production (i.e., PWS production) will be evidenced by stable isotope ratios because (1) a greater proportion of PWS production (enriched in ^{13}C) will be needed to make up the deficit and (2) extension of the food web is expected to cause concomitant shifts in $^{15}N/^{14}N$ (reflecting TL shift) with $^{13}C/^{12}C$ (reflecting alternate prey). The shift in $^{15}N/^{14}N$ will be especially notable in predators because of predicted TL shifts. The numerical nature of stable isotope data lend themselves to modeling, e.g., modeling effects of marine-derived nitrogen using ^{15}N (Kline et al. 1993). The data are thus expected to be useful as modeling validation tools.

Sampling design

The sampling effort was broad-scale and consisted of collecting samples at sampling site-times established by the pink salmon and herring projects. These were determined by pink salmon and herring lead investigators and are reported in their respective chapters. Sample sizes consisted of 20 to 50 organisms per taxon (fishes and prey), when available, as these amounts are

required for statistical validation to test for variation with respect to size (Kline et al. 1993), and to determine modalities occurring at a sampling site/time (Kline et al. 1990). Sampled taxa included: (1) pink salmon juveniles (principally from CWT recoveries), (2) herring (principally in conjunction with energetics sampling), (3) macrozooplankton (these include bulk samples and samples of individual zooplankters, principally *Neocalanus cristatus*, and (4) predatory and competitory fish of pink salmon and herring (e.g., pollock, Pacific cod, tom cod, black cod, rockfish, sculpins, sandlance, eulachon, and capelin).

Sample and data integration with other SEA projects

To the extent possible, multiple analyses were made on the same individual organisms. Priority was given to samples for which these other analyses are made. This is accomplished through the integrated field effort as samples must be routed through several procedures such that the all SEA projects needing samples and data from a specific organism can obtain them. In general, tissue collection for stable isotope analysis is done following sampling for length, weight, age (otolith and scale removal), stomach contents, spawning condition, and energetics. This sample protocol can delay the acquisition of some samples until other laboratory processing or analysis is complete. Samples delayed because of post-season laboratory needs and alternate acquisition processes, are presently in process. These samples include herring from which calorimetric analysis was made and coded wire tag (in the future: thermal marked otoliths) recovery pink salmon. Preliminary results from these paired studies are given in this document.

Sampling and Analytical procedures

The methodology used for isotopic sampling, analysis and data interpretation are documented in several publications resulting from prior work (See Kline et al. 1990, 1993). The UAF Stable Isotope Facility, where this project's isotopic analyses were made, has three isotope ratio mass spectrometers including a new automated system which facilitates faster sample processing, allows for more replication and smaller samples, and has greater precision (± 0.1 delta units). This instrument calculates $\delta^{13}C$, $\delta^{15}N$, %C, and %N from each sample per analysis.

Sampling protocols in the field for zooplankton and fishes are well established. Where samples of prey species are missing or few, proxy samples from the same area (zooplankton, benthos), which enable a similar comparison, are taken. After the isotopic values are in hand, synthesis of the data with past unpublished data and with other literature isotope ratio values are used to establish trophic models.

Sample preparation

Removal of non-dietary carbon from bulk plankton samples was effected by removal of shelled pteropods. This approach was used instead of acid treatment in order to avoid potential artifacts (Goering et al. 90, Bunn et al. 95). Shelled pteropods were removed from bulk plankton samples by (1) forcepting, (2) screening with a 2 mm mesh sieve after selective removal of macrozooplanton and (3) decanting after allowing the sample to settle in a beaker.

The gastro-intestinal tract was removed from whole fish samples to remove dietary material from samples.

All samples were stored frozen until freeze dried (Labconco) and ground to a fine powder with a dental amalgamator (Crescent Dental Wig-L-Bug). Replicate (except for individual or composite samples of zooplankters too small for more than one analysis) aliquots of ~1.5 mg were weighed to the nearest µg and placed in combustion boats for loading into the mass spectrometer sample preparation unit.

Isotopic determination

A Europa Scientific model 20/20 stable isotope analyzer equipped with a Europa Scientific Roboprep sample preparation and purification unit was used. Analytical results include $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios in standard delta units, $\delta^{13}C$ and $\delta^{15}N$, respectively, and %C and %N.

Standard delta notation is used to express stable isotope ratios, which are reported relative to international standards (air for N and Vienna Peedee belemnite (VPDB) for C) and defined by the following expression:

$$(1) \quad \delta^{15}N \text{ or } \delta^{13}C = \left(\frac{R_{sample}}{R_{standard}} - 1\right) \times 1000 \text{ per mil}$$

where $R = {}^{15}N/{}^{14}N$ or $^{13}C/^{12}C$ (after Craig 1957). The isotope standards have delta values of 0 by definition, i.e., $\delta^{15}N = 0$ for atmospheric N_2. Naturally occurring $\delta^{15}N$ and $\delta^{13}C$ values observed in biota, range from ~0 to ~ +20 and from ~ 0 to ~ -50, respectively. The negative $\delta^{13}C$ values reflect the relative enrichment of ^{13}C in the limestone standard compared with biota.

Samples were rerun when replication was poor (difference in delta units > 0.6). Typically, replication is < 0.2 delta units. The %C and %N data were used to calculate C/N. Mean of $\delta^{13}C$, $\delta^{15}N$ and C/N replicates were used for data modeling and interpretation.

Lipid normalization

Normalization for lipid composition was by the method of McConnaughey and McRoy(1979) using the C/N ratios derived during mass spectrometry. The C/N atomic ratio is used as a proxy for lipid:

$$(2) \quad L = \left(\frac{93}{1 + \frac{1}{0.246C/N - 0.775}}\right)$$

that is then used to calculate lipid-normalized $\delta^{13}C$ (expressed as $\delta^{13}C'$):

$$(3) \quad \delta^{13}C' = \delta^{13}C + 6\left(\frac{3.9}{1 + \frac{287}{L}} - 1\right)$$

Estimation of trophic level

The enrichment of ^{15}N that results from a feeding process (Minagawa and Wada 1984) enables one to use $\delta^{15}N$ as a good proxy for trophic level (Fry 88, Cabana and Rasmussen 1994). The trophic level reference organism used here is *Neocalanus cristatus*. Trophic level relative to the reference organism based

on the $^{15}N/^{14}N$ trophic enrichment factor, ε_N, was determined using the following expression:

$$(4) \quad \Pi = \frac{\delta_\lambda - \delta_{Neocalanus}}{\varepsilon_N}$$

where δ_λ is the $\delta^{15}N$ of λ, and Π is the number of trophic steps between *Neocalanus cristatus* and λ; $\varepsilon_N = 3.4$ (Minagawa and Wada 1984).

Neocalanus spp. are the dominant herbivores in the plankton community of the north Pacific (Miller et al. 1984). A canonical trophic level for *Neocalanus cristatus* of 2.1 was based on their trophic level being synonymous with the macrozooplankton component in the carbon flux box model of Parsons (1987), i.e., 90% herbivorous and 10% carnivorous (on microzooplankton). *Neocalanus cristatus* are facultatively carnivorous on planktonic Protozoa, but cannot be sustained on such a diet (Gifford 1993). Thus, the *a priori* canonical trophic level (TL) of 2.1 was used as a reference for estimation of TL of other taxa. The *Neocalanus* $\delta^{15}N$ reference value (the mean of the measurements) is applied to (4) which is used in the following expression to calculate absolute TL of λ:

$$(5) \quad TL_\lambda = 2.1 + \Pi$$

Trophic level normalization for carbon source assessment
Each $\delta^{13}C'$ value is trophic level normalized so that the residual value will reflect carbon source. Normalization for trophic enrichment of ^{13}C using the trophic enrichment factor, ε_C, to the reference trophic level (the TL *of Neocalanus cristatus*) was made using the following relationship:

$$(6) \quad \delta^{13}C'_{TL} = \delta^{13}C' - \varepsilon_C \Pi$$

where $\delta^{13}C_{TL}$ is the trophic level normalized $^{13}C/^{12}C$ value of $\delta^{13}C'$ and $\varepsilon = 1$ (DeNiro and Epstein 1978, Fry and Sherr 1984). Trophic level normalized $\delta^{13}C$ without lipid normalization is represented by $\delta^{13}C_{TL}$. $\delta^{13}C_{TL}$ is generally shown as a function of C/N to account for lipid isotope effects (DeNiro and Epstein 1977).

RESULTS

A. Validation of natural stable isotope techniques in the PWS context

1. Net Plankton

The isotopic composition of net plankton from the upper 50m histogrammed in Fig. 1 was extremely variable suggesting the occurrence of isotopic fractionation processes. The lowest 10% of each plot (indicated by differences in shading) tended to occur around Montague Island and in the northern Gulf of Alaska. Low isotopic values suggest minimal fractionation by phytoplankton

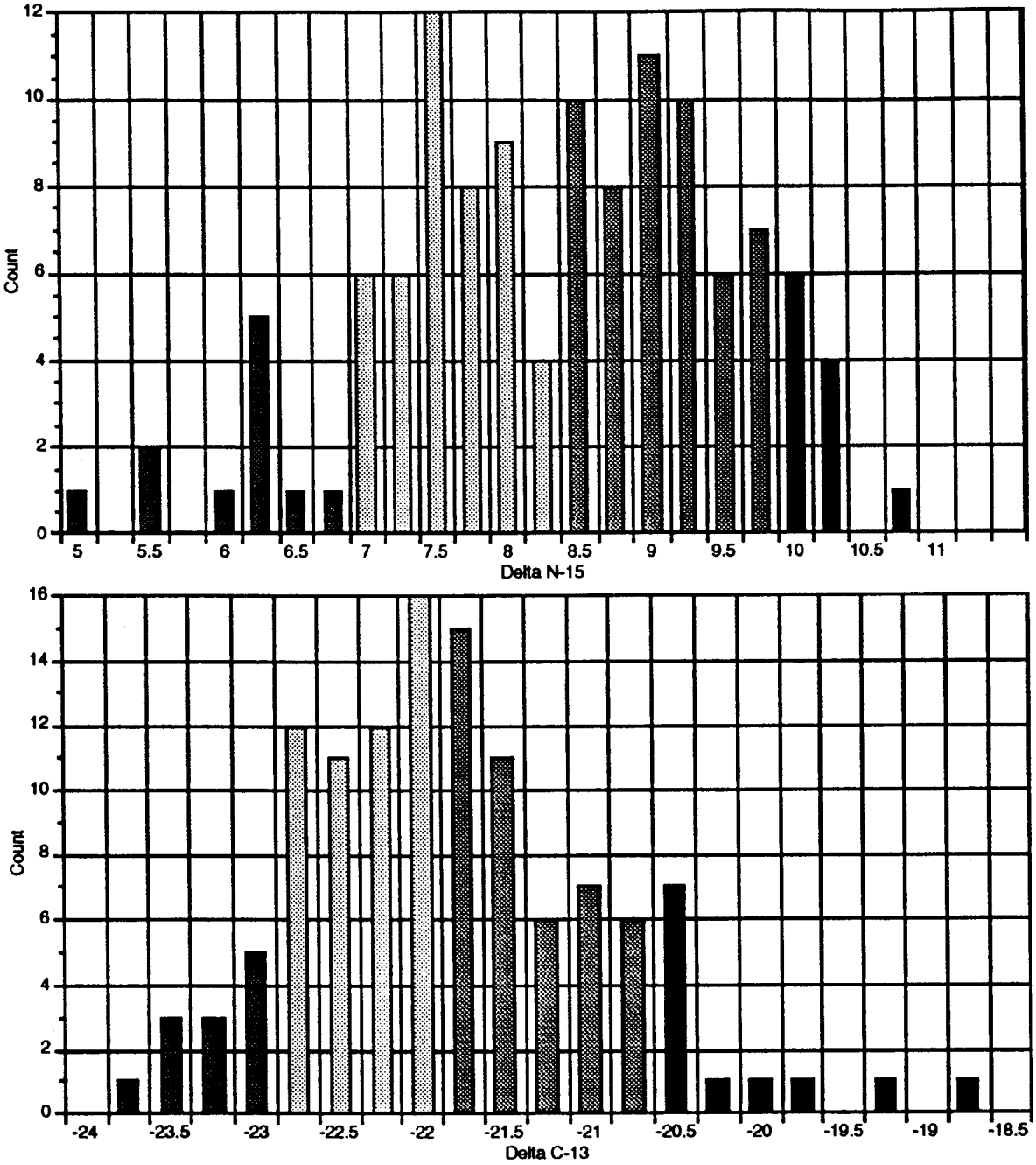


Figure 1. $\delta^{15}N$ (upper panel) and $\delta^{13}C$ (lower panel) of upper 50m net plankton sampled from April 1994 to May 1995 in PWS. Upper and lower 10% indicated by dark shading. Medial value occurred at break in intermediate shading.

because of slow growth, lack of nutrient depletion, or input of isotopically depleted C and N. A portion of the variability might also be explained if differences in zooplankton species composition in the samples reflected differences in trophic level and consumption of different phytoplankton species with differing isotopic discrimination. Differences in species composition of net plankton samples (95320H - this volume - samples taken alongside those for isotopic analysis) cannot account for the isotopic distribution. Differences in isotopic fractionation because of low nutrient

depletion in the northern Gulf (suggesting a high degree of isotopic discrimination by phytoplankton) compared to a high degree of nutrient depletion within PWS (resulting in negligible isotopic discrimination by phytoplankton) was probably a significant factor effecting the isotopic composition of zooplankton (see 95320G - this volume - for nutrient data). However, differential growth rates of phytoplankton or existence of isotopically-depleted nutrients (e.g., upwelled CO_2 consisting of respired carbon is expected to have very low $\delta^{13}C$) are not eliminated as possible factors contributing to the gradient. The significance of the isotopic gradient is that productivity derived from the northern Gulf of Alaska is distinguishable from PWS. The cause of the gradient is beyond the scope of the SEA project. It will be necessary, however to verify the existence of the gradient and to determine $\delta^{13}C$ values associated with GOA and PWS carbon sources. Verification that this source signature is not dependent on zooplankton species was made by examination of a single herbivore of sufficient size allowing for analysis of individual organisms.

2. *Neocalanus cristatus*

Trophic level

Neocalanus cristatus is a common herbivore of the north Pacific (Miller et al. 1984) and stage C5 (see 95320H - this volume - for life history information) is large enough (~2 mg dry weight) to allow isotopic analysis of individuals. *Neocalanus cristatus* stage C5 was found to be an herbivore (TL ~ 2), based on $\delta^{15}N$, when compared with other macrozooplanters large enough for individual isotopic analysis (Fig. 2). $\delta^{15}N$ data collected from 600 C5 and C6 *Neocalanus cristatus* thus far in this project confirms that this organism is virtually a dedicated herbivore (Gifford 1983). Only during March 1995 was there evidence that this species deviates from herbivory (Fig. 3). At this time feeding *Neocalanus cristatus* stage C5 were very rare (n = 7 from 16 stations sampled throughout PWS). The trophic shift in March probably reflects the lack of normal food as this was prior to the phytoplankton bloom (see 95320G - this volume). The value in consistency of TL is (1) that shifts in isotopic composition can be attributed to changes in the isotopic composition of phytoplankton and (2) that the $\delta^{15}N$ value can be used as a reference for TL determination of other taxa using $\delta^{15}N$. TL=2.1 (see methods) was used as the average TL for Neocalanus cristatus C5. This is a good estimation as this species had TL=2.0 during the end of the phytoplankton bloom (May, Fig. 3)

The small inter-site variation in $\delta^{15}N$ (~ 2 delta units) of *Neocalanus cristatus* C5 during May 1995 can be attributed to down-draw of inorganic nitrogen (see 95320G - this volume). The lowest $\delta^{15}N$ values were observed in the northern Gulf of Alaska station (GOA6 - see 95320M, this volume, for station locations) in both *Neocalanus* and net plankton (Fig. 3). Conversely, the highest $\delta^{15}N$ values were observed at station SEA11 in the deep basin located in western PWS. Intermediate values of both *Neocalanus* and net plankton were observed in the central sound (CFOSBY and CS3). Shift in $\delta^{15}N$ resulting from nutrient depletion is relatively small compared to trophic enrichment; note box and whisker plot ranges relative to horizontal trophic level lines (Fig. 3), enabling the use of $\delta^{15}N$ for accurate estimation of trophic level.

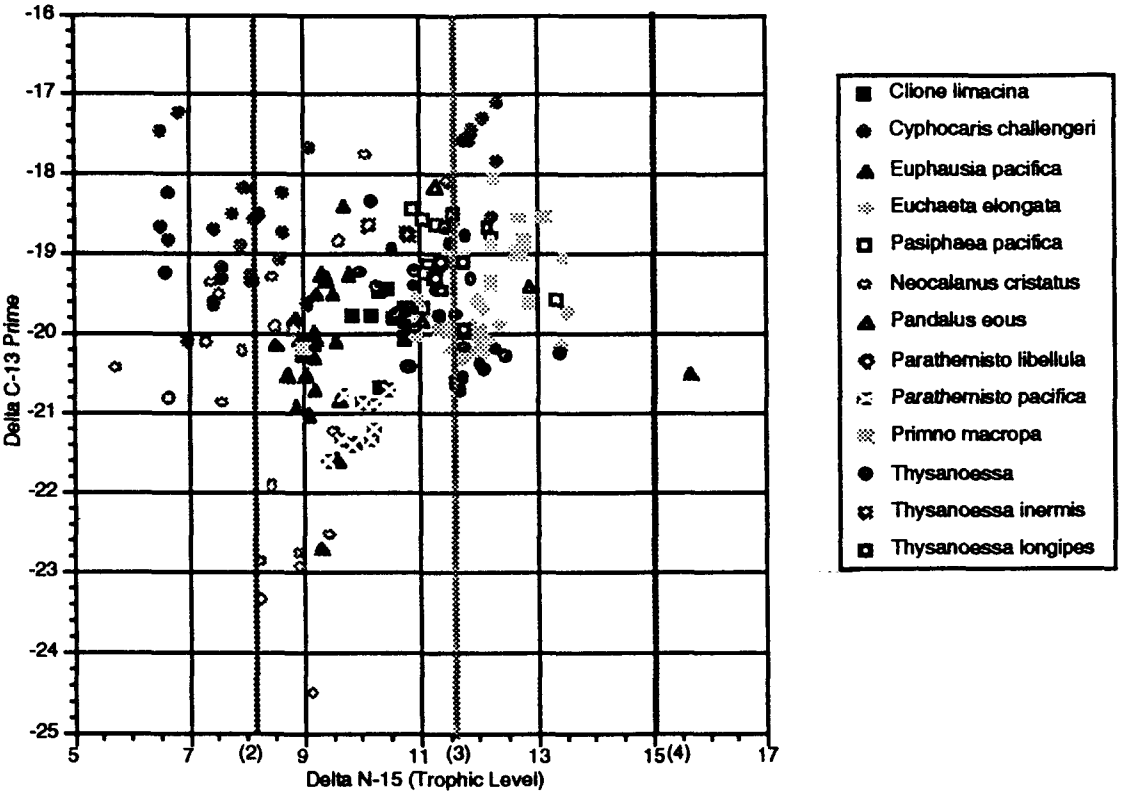


Figure 2. Dual isotope plot ($\delta^{15}N$ and $\delta^{13}C'$) of macrozooplankton collected from September to November 1994 in PWS. Trophic levels are indicated by the thick vertical lines. Many macrozooplankton expected to be herbivorous had shifted to TL~ 3 at this time except *Neocalanus cristatus* that was most consistently an herbivore.

Source effects

The isotopic shifts between northern Gulf of Alaska (GOA) and PWS were greatly magnified in $\delta^{13}C$ compared to $\delta^{15}N$. This probably occurred because the isotopically depleted signatures in GOA plankton are due to factors in addition to inorganic nutrient depletion in PWS (note that isotopic and nutrient depletion are inversely related due to the preference of phytoplankton for the lighter isotopes of N and C during uptake; isotopically depleted = less of the heavy isotope, nutrient depleted = less nutrients), see 95320G - this volume. Determination of the causal factors is beyond the scope of SEA. However, the existence of the isotopic gradient is of supreme importance. Comparison of PWS and GOA $\delta^{13}C$ is shown as a function of C/N in Fig. 4 since C/N correlates to lipid content (McConnaughey and McRoy 1979) and lipids are $\delta^{13}C$ - depleted (DeNiro and Epstein 1977). *Neocalanus cristatus* in diapause show clear isotopic relation to possible feeding in both the GOA and PWS (Fig. 4).This distinction in $\delta^{13}C$ signature allows one to trace carbon originating from these two sources into PWS biota.

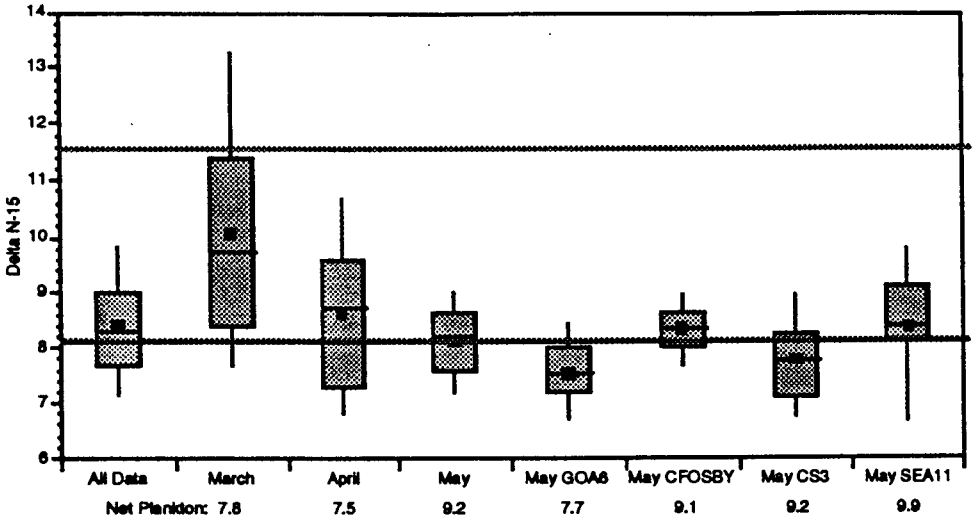


Figure 3. Box and whisker plots *Neocalanus cristatus* $\delta^{15}N$. Plots include: (1) all data from 1994 to 1995, (2) data from upper 50 m collections in March, April, and May 1995, and (3) data from may 1995 stations with $n \geq 10$ in upper 50 m collections. Synoptic net plankton data $\delta^{15}N$ listed below plot. Suggested trophic level 2 and 3 positions are suggested by lower and upper, respectively, thick horizontal lines.

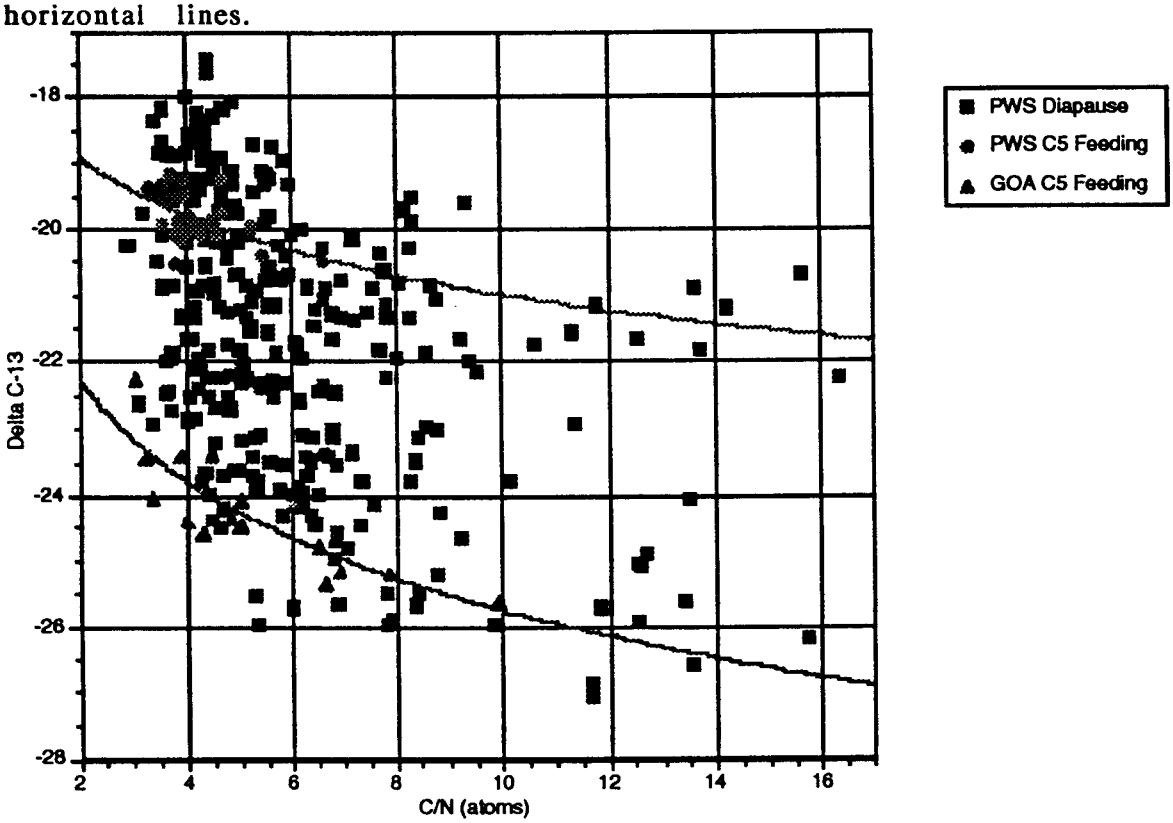


Figure 4. $\delta^{13}C$ of feeding *Neocalanus cristatus* C5 in PWS and GOA (excluding 3 outliers) compared with diapausing *Neocalanus cristatus* C5 collected throughout PWS as a function of C/N. Differences in $\delta^{13}C$ between PWS and GOA conserved in diapause stage, as expected, suggest recruitment of copepods from the GOA as well as PWS into diapausing population.

B. Application of $\delta^{15}N$ and $\delta^{13}C$ in SEA

Stable isotope chemistry was applied to SEA problems by estimation of trophic level and identification of carbon sources.

1. Predation: trophic levels

The high mortality of pink salmon from hatcheries suggested that predation is an important process in PWS (see 95320E - this volume). Pollock and other potential predators were compared to potential forage to determine whether estimated trophic levels (number of feeding steps in a food chain, primary producers are TL=1) using $\delta^{15}N$ is consistent with the predation hypothesis. Piscivorous species are expected to be at TL~4 and those feeding on *Neocalanus* at TL~3. Macrozooplankters can range up from TL~2 (Fig. 2). Thus TL>3 are for mixed TL feeding (including zooplankton and fish, probably more of the later if approaching TL=4). Trophic level estimates are given for several fish taxa and squid comparing Fall 1994 (September to November) with Summer 1995 (late May to July) in Fig. 5.

2. Pink salmon

Shift in pink salmon $\delta^{13}C$ (Fig. 6) concomitant to decrease in C/N (Fig. 7) and increase in weight (Fig. 8) was tracked in CWT (coded wire tag recovery) samples obtained through the pink salmon project (95320A - this volume). These data suggest a rapid turnover from carbon derived from a hatchery diet that was ~-20 (the value of adult salmon (Kline et al. 1993) as hatchery diet consists largely of the carcasses of adult salmon) to a $\delta^{13}C$ diet very similar to the GOA signature. Post-hatchery fish had higher C/N compared to later attesting to the good condition of fry at time of release from the hatchery. Because high C/N is associated with low $\delta^{13}C$, the reverse relationship seen here contraindicates that the $\delta^{13}C$ shift is due to the C/N shift. These chemical composition shifts coincide with the attainment of the critical size that enhances survival from predation (Fig. 8).

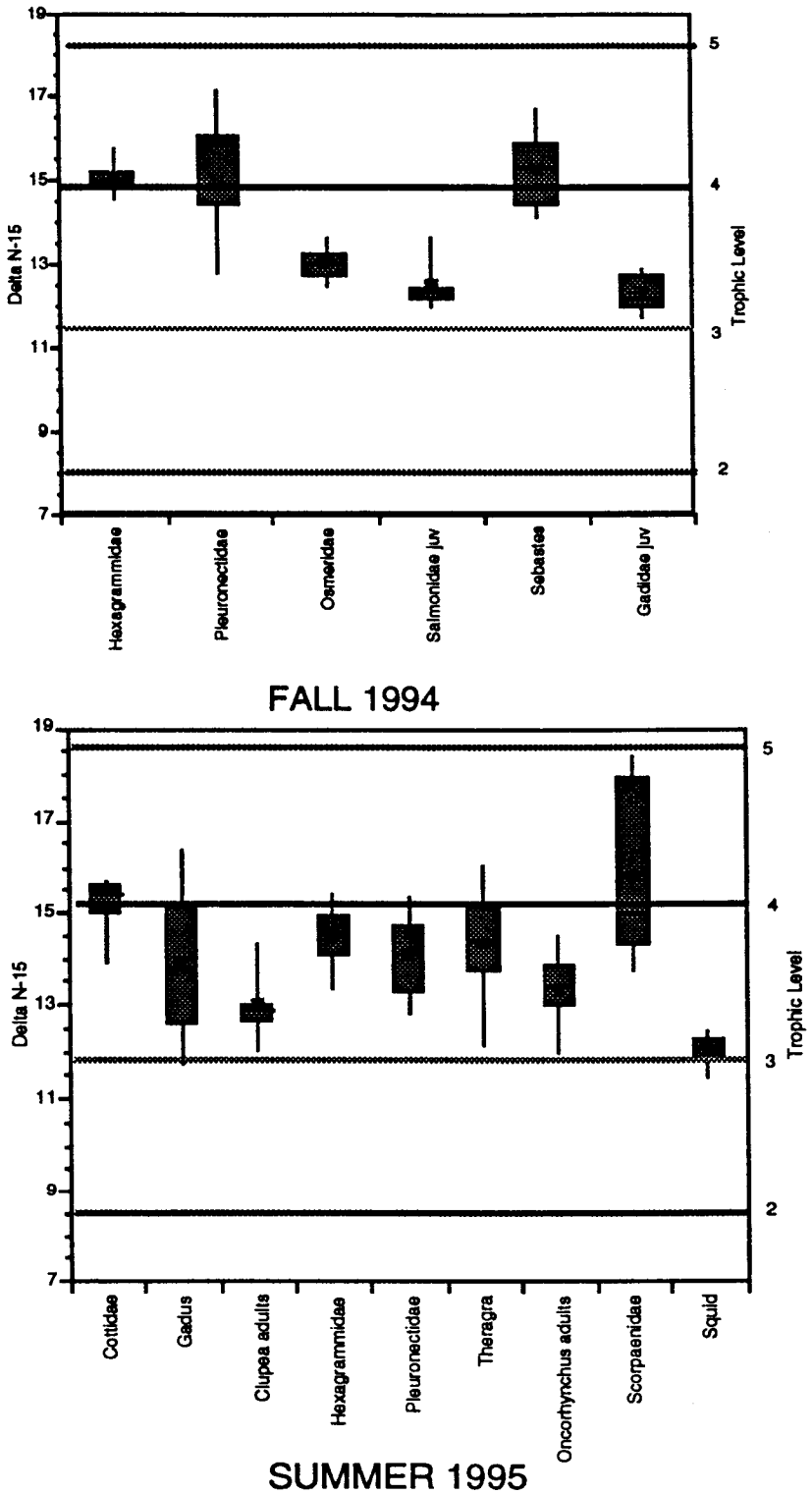


Figure 5. Estimations of trophic level of predator and forage fish species collected in PWS in FY 95 using $\delta^{15}N$. Upper panel shows collections from fall of 1994 while the lower panel shows collections made in late spring to summer of 1995.

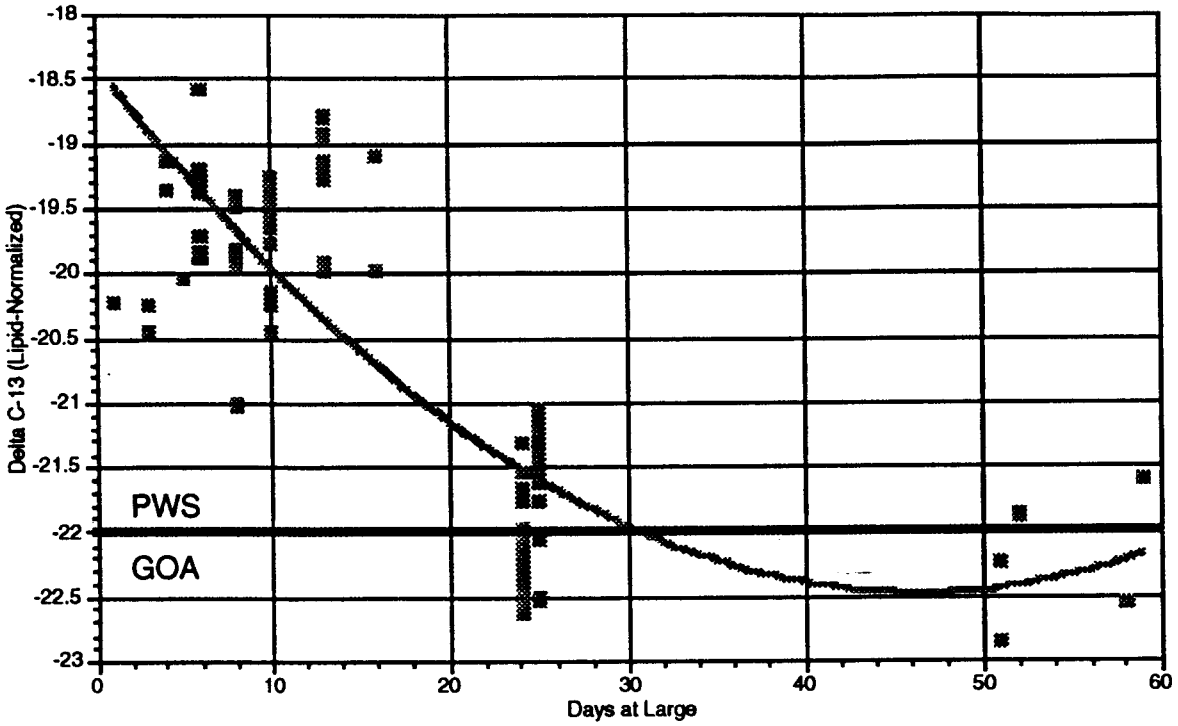


Figure 6. $\delta^{13}C'$ of CWT pink salmon as a function of time since release from the hatchery. The approximate boundary between PWS and GOA carbon signatures indicated.

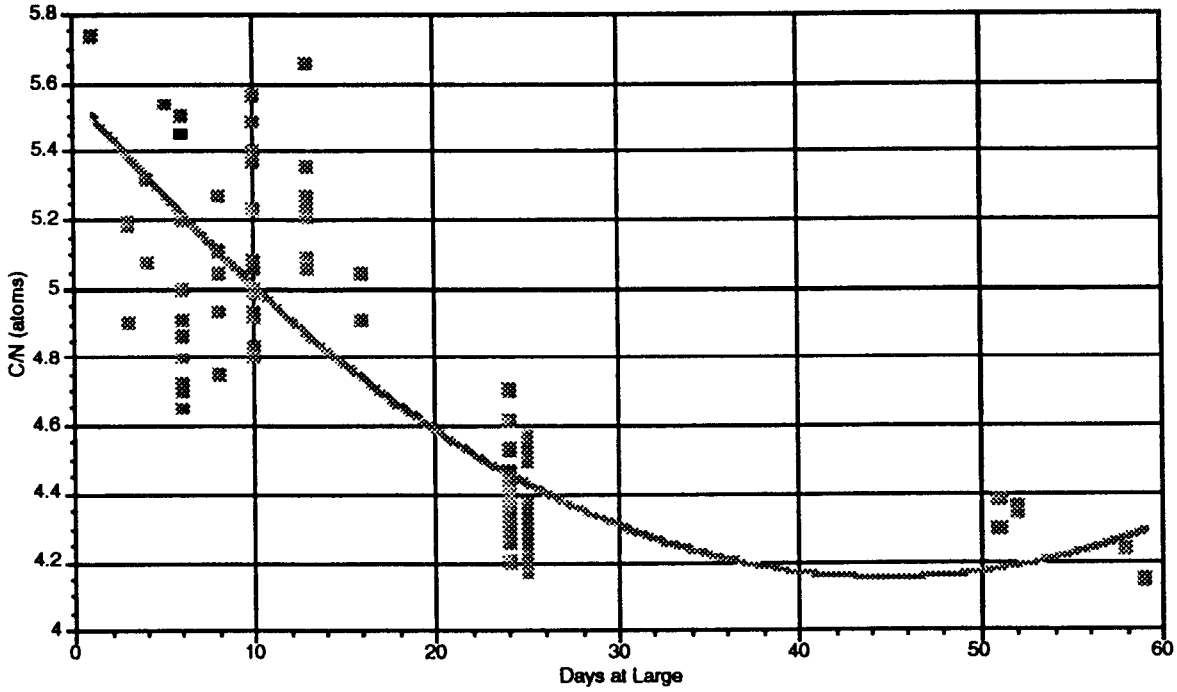


Figure 7. C/N of CWT pink salmon as a function of time since release from the hatchery.

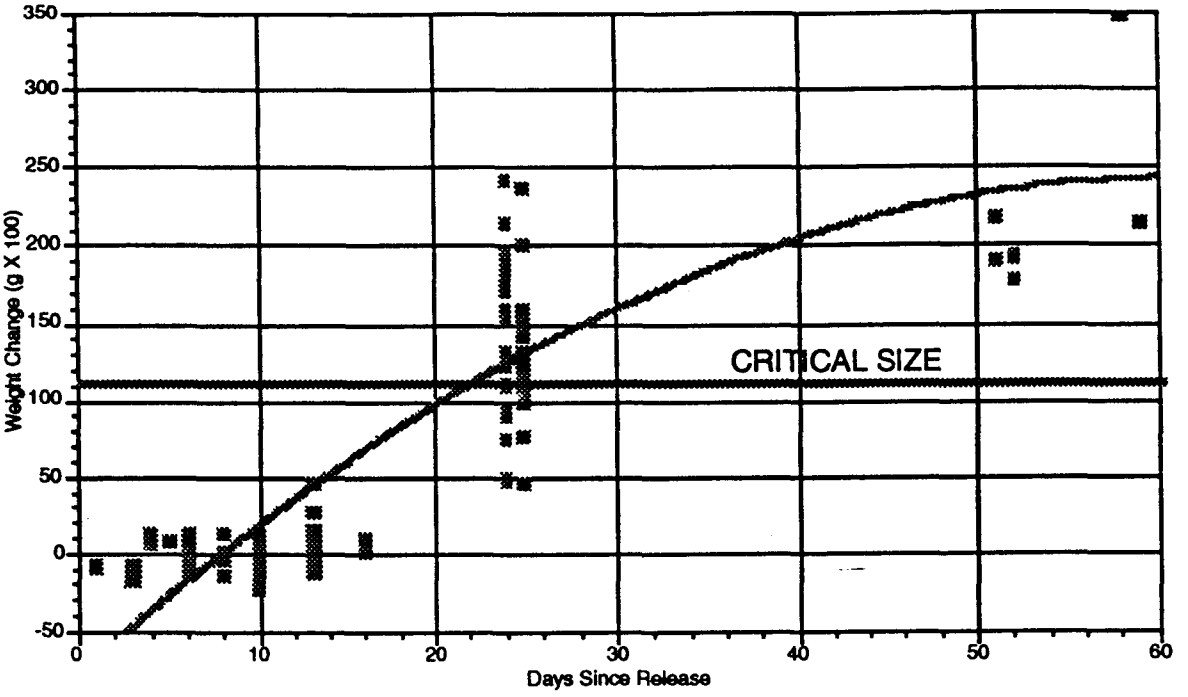


Figure 8. Wet weight of CWT pink salmon (in g x 10) as a function of time since release from the hatchery (data courtesy of 95320A - this volume). The critical size that forms a refugium from predation indicated.

3. Herring

The principal hypothesis vis a vis herring production in PWS is the herring overwintering hypothesis (see 95320T - this volume). Measurement of energetic content addresses this hypothesis (see 95320U - this volume). The isotope technique is being used for parallel analyses. The question being asked is: are there are measurable differences in isotopic signatures of juvenile herring that suggest differences in feeding regime related to winter survival? Maximum benefit of the application of stable isotopes is being attained through paired sampling with the energetics project in order to relate energetic status with past feeding via isotopic signature. This was begun in FY 95 with the analysis of herring juveniles collected in the fall of 1994 (Fig. 9). Two sites in PWS were compared that had different energetic content, Orca Inlet and Port Gravina (see 95320U - this volume). A stratified sample was selected for initial isotope analysis to determine the range in variability. Since Orca Inlet had significantly lower energetic content than Port Gravina, the lower values are more representative of the population whereas the higher values are more representative of Port Gravina (cf. 95320U - this volume). The lower energetic content herring had more positive $\delta^{13}C$ consistent with a decrease in GOA-derived carbon that is corroborated when overlaying the herring data with *Neocalanus* (Fig. 10). The Port Gravina herring appear to consist largely of GOA-derived carbon. The Orca Inlet population was towards the PWS carbon signature although not completely concordant with it (Fig. 10). $\delta^{15}N$ and thus trophic level was very similar in both groups of juvenile herring (Fig. 9) and but slightly lower than adult herring (Fig. 5).

DISCUSSION

Results from FY 95 confirm that stable isotope methods are appropriate to studying processes hypothesized in SEA to be important in affecting fisheries recruitment and thus restoration to those fisheries resources injured by the spill. The emphasis in FY 95 was in validating the relationship between $\delta^{13}C$ and GOA vs. PWS carbon, a requirement prior to exploitation of this natural tracer.

1. Validation of isotopic techniques

Stable isotope applicability hypotheses. The following postulates were confirmed using the data acquired in 1995:

Carbon and nitrogen stable isotope ratios of biota from Prince William Sound can be used to identify major food sources to top trophic levels and to assign trophic positions to specific consumers of given age classes and habitat.

- $\delta^{13}C$ values relate to GOA vs. PWS production sources.
- $\delta^{15}N$ values relate to trophic level, i.e., number of feeding steps from production sources to consumers, in the study area.

Isotope ratios in consumers provide a means to validate conceptual food web structures, identify trophic variability by individuals within species, and to validate quantified energy flows in ecosystem models.

- $\delta^{13}C$ and $\delta^{15}N$ data of representative samples (N=20 to 50 per sampling stratum) of individual biota sampled integrated with results of other SEA projects.

Utilization of an isotopic source effect is contingent on there being a measurable difference in isotopic signature between production sources of concern. This was suggested primarily through comparison of *Neocalanus cristatus* collected during the C5 feeding stage (Fig. 4). It was also determined that this species is principally an herbivore thus eliminating trophic isotope effects (Fig. 3). This species exists at this stage for a very narrow window of time (~2-3 weeks, see 95320H - this volume), thus providing a narrow temporal perspective which is concentrated during the brief phytoplankton bloom (95320G - this volume) thus ideally integrating principal sources of primary production. The signatures appear to be conserved during diapause as expected since energy needs to be conserved for reproduction, which follows the post-diapause molt to the C6 adult stage that, like the diapause period, is non-feeding. The conservativeness of the signature allows one to use stable isotope analysis of diapausing *Neocalanus* to determine where feeding took place. The majority of diapausing *Neocalanus cristatus* appear to have acquired their carbon in PWS (Fig. 4). In a more river-like year (SEA river-lake hypothesis), it is likely that a greater proportion of diapausing *Neocalanus* will have the GOA signature. It will need to verified that the $\delta^{13}C$ signatures of feeding C5 *Neocalanus* for each of the areas are consistent from year to year, particularly during a more river-like year. It is expected that carbon derived from the GOA whether in the Gulf per se or from carbon advected into PWS will have a similar signature.

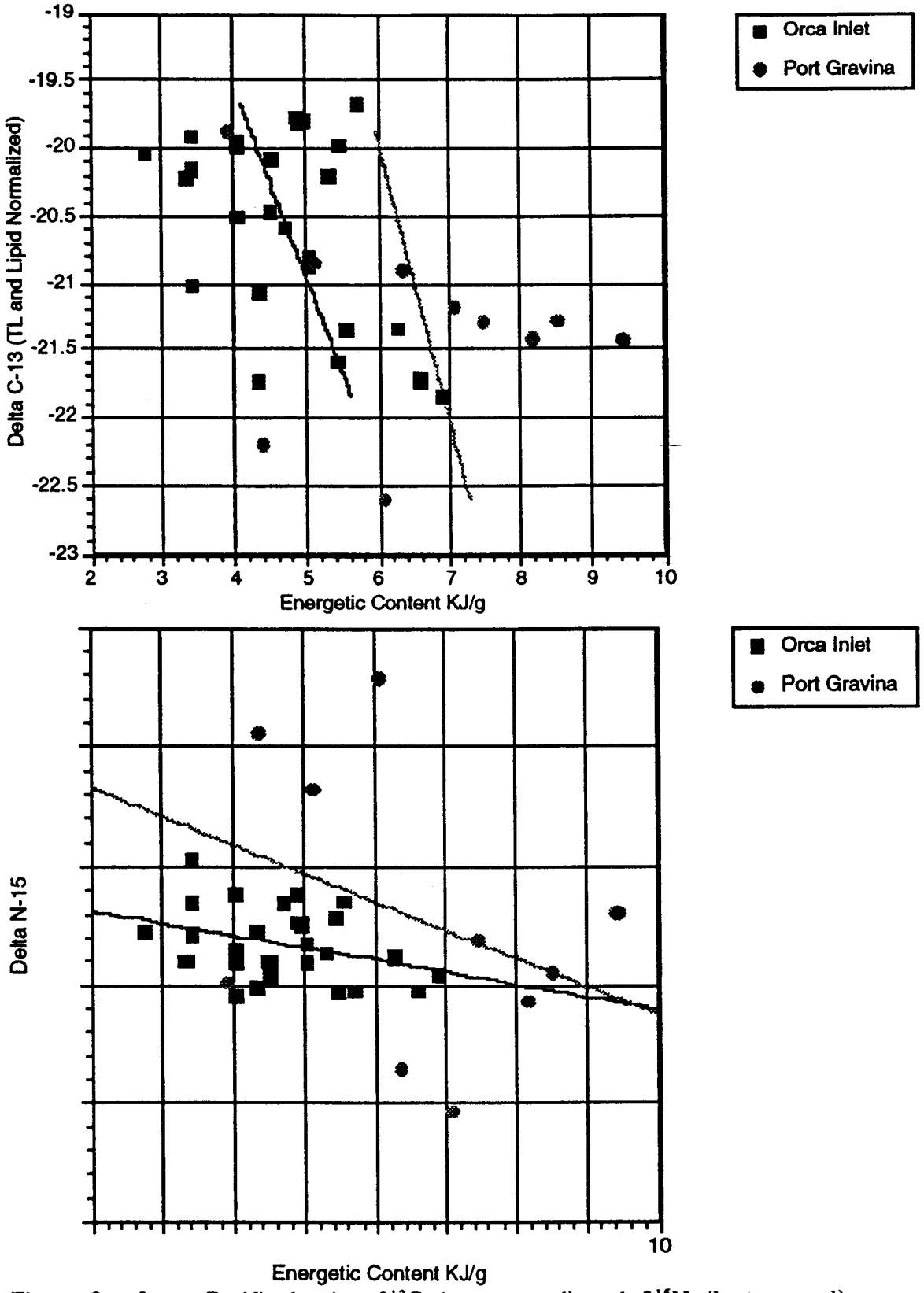


Figure 9. 0-age Pacific herring $\delta^{13}C$ (upper panel) and $\delta^{15}N$ (lower panel) as a function of energetic content (see 95320U - this volume).

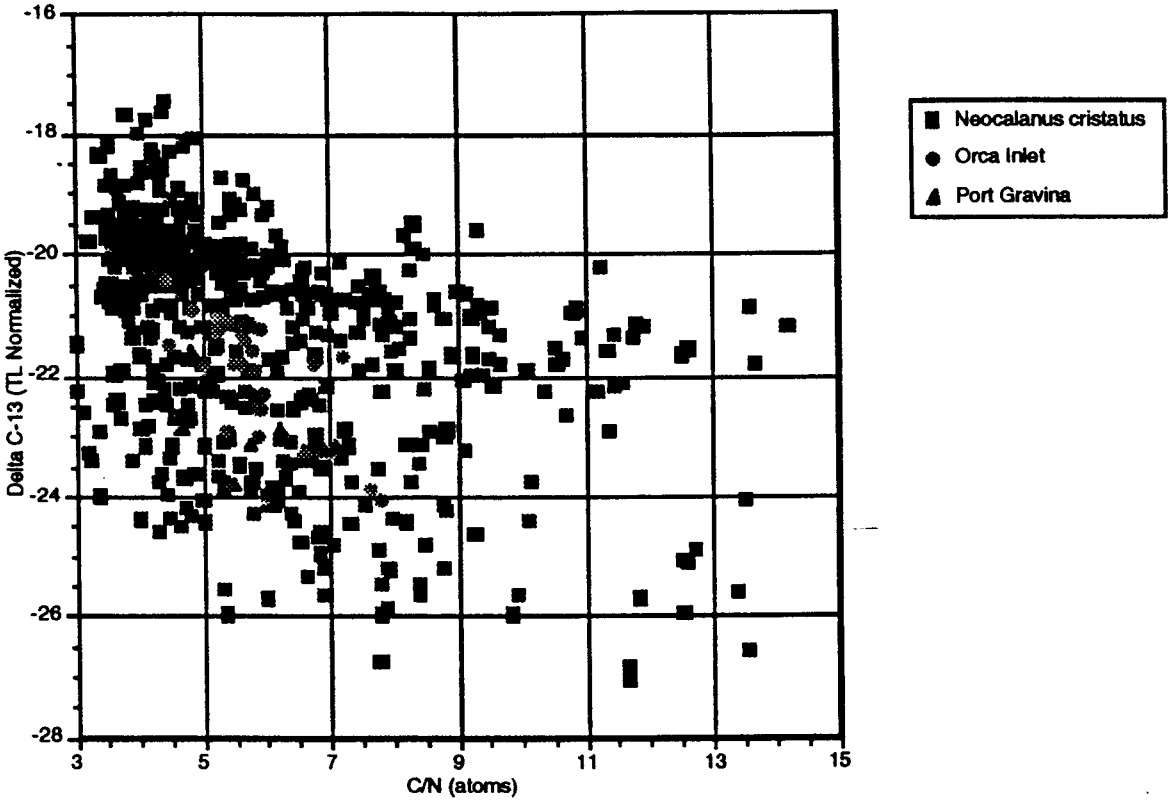


Figure 10. 0-age Pacific herring $\delta^{13}C$ in relation to possible carbon sources.

The boundary where the PWS signature is likely to be found will most likely be within the area subject to nutrient depletion. Oceanographically, The regime between the nutrient deplete and nutrient replete areas (see 95320G - this volume) reflects oceanographic processes thus directly effecting "bottom-up" processes on food webs and indirectly, inducing "top-down" effects through prey-switching on pink salmon and other alternate prey of predators that otherwise consume zooplankton.

2. Isotopic techniques application: SEA hypotheses

The data suggest that stable isotope abundance relate directly to river-lake processes, thus the technique has direct application to SEA hypothesis testing. The evidence from diapausing *Neocalanus cristatus* suggests that PWS has been more lake-like during this investigation. It is predicted that a more river-like year will result in a greater preponderance of diapausing copepods with the GOA signature. If so, the sampling of diapausing copepods would appear to be a useful tool for assessing river vs. lake conditions. SEA also predicts that more lake-like conditions will result in a greater rate of predation on juvenile fishes. In 1992, there were very few *Neocalanus* in PWS (M. Willette. pers. comm.). The reoccurrence of conditions similar to 1992 will be needed to test whether large-scale prey-switching takes place. The predation project (see 95320E - this volume) has determined that prey switching occurs at the seasonal level. A systematic positive shift in predator $\delta^{15}N$ values will suggest that large scale (interannual variability) prey-switching takes place. The

trophic level analysis conducted in this project (Fig. 5) and similar data collected in FY 94 will serve as a baseline for comparison should another low-zooplankton, and presumably lake-like, year such as 1992 occur again. Results of the energetics project (see 95320U - this volume) suggest that there are significant differences in pre-winter energetic condition of herring. These pre-winter conditions appear to be related to feeding on PWS vs. GOA, thus river-lake processes may be influencing pre-winter condition in herring and ultimately their over-winter survival. Differences in the availability of GOA occurring between sites and shifts in the availability of GOA occurring between years may modulate recruitment success through over-wintering mortality differential. Since GOA occurrence in herring can be detected in herring with stable isotope abundance, this tool will enable assessment of this phenomenon (i.e., relation between over-winter survival via pre-winter condition and carbon source) in the development of the herring mortality model.

3. Isotopic techniques application: model validation tools

Model validation tools using natural stable isotope abundance are being developed as the primary restoration tool product of the 320I project. The SEA project is focused on generating models that couple ocean physics to biological productivity, and models that can forecast juvenile pink salmon and herring mortality. A first cut at an ocean physics model dealing with circulation in PWS (see 95320J - this volume) has already been created. This circulation model can be used to predict planktonic trajectories such as macrozooplankton forage. The model will need to be validated using field data. The flux of *Neocalanus cristatus* during diapause recruitment can be model-simulated based on the physical input parameters the model is based on and compared with nature. The recruitment of *Neocalanus cristatus* C5s observed in 1995 resulted from processes taking place in 1994. These processes resulted in ~17% of the population consisting of exclusively GOA carbon compared to ~half consisting exclusively of PWS carbon (Fig. 11). Model simulations need to be able to generate these same results. Interannual variability in circulation is likely to affect diapause recruitment by varying the flux of copepods entering PWS from the GOA. The model should be able to predict such differences while isotopic composition can be used to assess them.

The carbon flow from the GOA into PWS biota other than copepods has also been demonstrated using $\delta^{13}C$ in this project. These biota include net plankton, pink salmon fry, herring, other forage species, and predator species. River-lake processes are hypothesized to modulate the flow of carbon from the GOA into PWS. Differential response in biota to GOA carbon input shifts can be detected by examining the concordance in $\delta^{13}C$ shifts among species between years. Differential response will suggest which species are most sensitive to bottom-up effects of river-lake processes. It is expected that some species will be less sensitive to these shifts with consequence including a diminished degree of predictability based on carbon input. However it is expected that salmon and herring models predicting juvenile mortality will need to predict effects of GOA carbon input shift based on data collected thus far (Fig. 12).

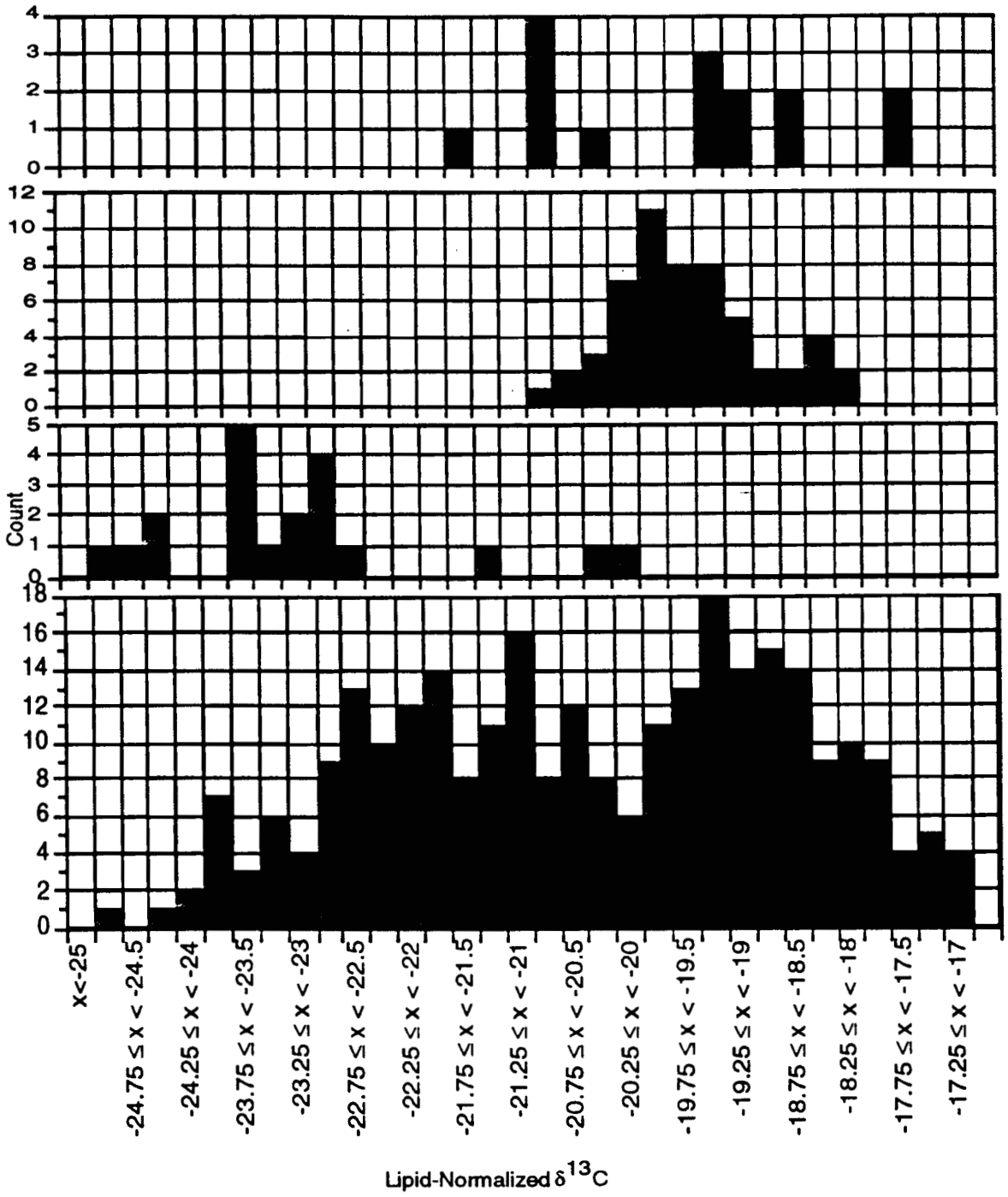


Figure 11. Histograms of $\delta^{13}C'$ of feeding C5 *Neocalanus cristatus* sampled in April and May 1995 in PWS (upper two panels), at station GOA6 in May 1995 (third panel), and diapausing C5 *Neocalanus cristatus* sampled in PWS in March and April. 46 of 277 diapausing C5 *Neocalanus cristatus* had $\delta^{13}C' < -22.5$, i.e., 16.6 % consisting of GOA carbon. 91 of 277 diapausing C5 *Neocalanus cristatus* had $\delta^{13}C' > -22.5$ and < -20.5, i.e., 32.9 % consisting of carbon of mixed origin i.e., these copepods were being advected into PWS during feeding. 140 of 277 diapausing C5 *Neocalanus cristatus* had $\delta^{13}C' > -20.5$, i.e., 50.5 % consisting of PWS-derived carbon.

Positive trophic level shifts are expected in upper trophic levels if low trophic level prey are less available (expected as a consequence of river-lake). Predicted trophic level shifts can be validated using $\delta^{15}N$ since $\delta^{15}N$ relates to trophic level (Fig. 5). Pauly and Christensen (1995) or other Eltonian pyramid models can also be validated by comparing isotopicially-dertermined trophic levels with those predicted by the model (D. Pauly, pers. comm.). Food web reconstruction can include plankton, pink salmon, herring, and other forage species as well as predator species.

Restoration

The goal of the SEA project is to aid in the post-spill restoration effort. Stable isotopes have a major role in understanding the processes, a prerequisite in order to achieve restoration. Isotopically definable carbon sources and trophic levels have been the ways stable isotopes have been used thus far. These relationships can be used as for model validation that will become paramount as models are spun up.

Economical monitoring framework using stable isotopes
Large-scale field efforts are expensive so ways of providing model validation without great costs should be sought out. It is expected that a certain amount of ship time will be needed to collect oceanographic data. However, "samples of opportunity" can allow a low cost sampling approach, for example commercial species arriving at processing plants. Given that catch location can be had, commercial fishery operations could be a source for some data. We are presently seeking out these channels by acquiring predator samples from processors in Cordova. We have been able to obtain intact pollock allowing us to obtain physical dimensions as well as tissue samples for isotopic analysis. We remove otoliths as well for aging.

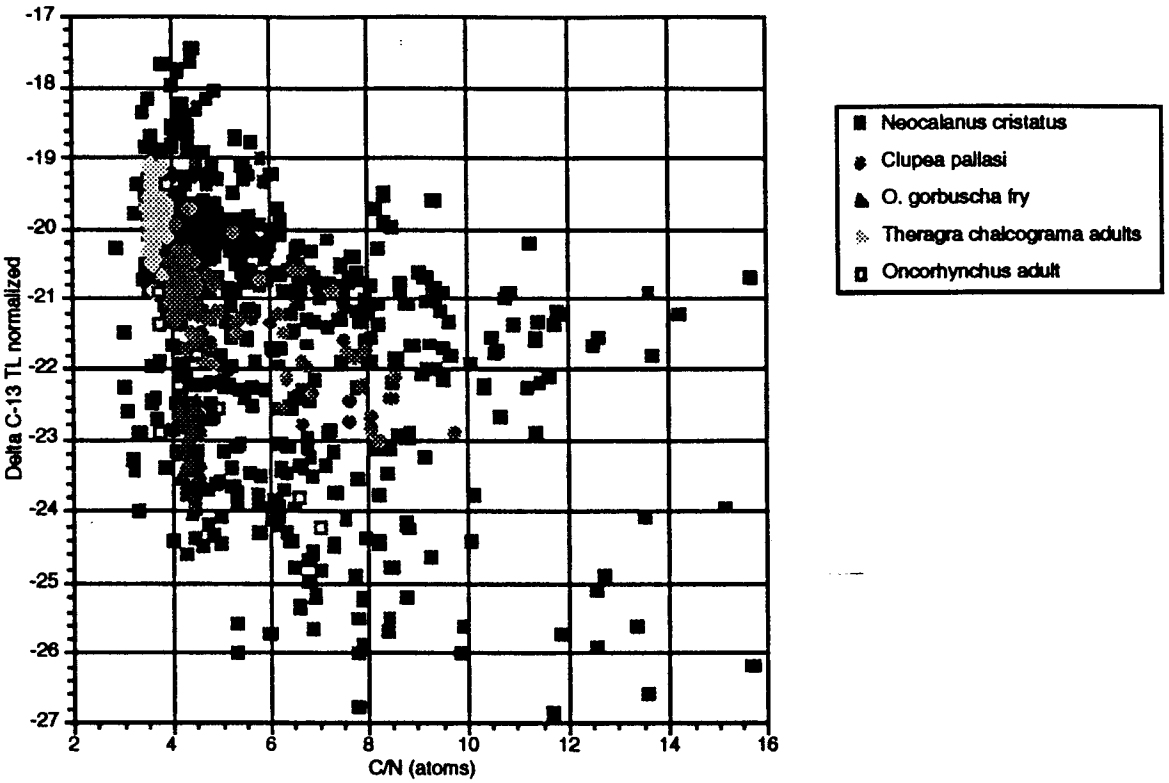


Figure 12. Differential dependence on GOA and PWS carbon sources in principal species suggested by their $\delta^{13}C$. Pink salmon fry are most dependent on GOA carbon (comparable to returning adults), Adult pollock are most dependent on PWS carbon. Herring are intermediate, utilizing carbon derived from both the GOA and PWS. Shifts in the utilization of these carbon sources expected to occur as a result of varying oceanographic conditions can thus be monitored in these and other fishes.

ACKNOWLEDGMENTS

The author is indebted to the cooperation of other SEA P.I.s, other investigators, and project personnel in the present endeavor. Many of these personnel were involved in the collection samples used for the data analysis presented here. Particular acknowledgment must be given to Kim Antonucci, project technician and Bruce Barnette, mass spectrometer technician at UAF for their assistance.

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Exxon Valdez Oil Spill
Restoration Project Annual Report

Information Systems and Model Development

Restoration Project 95320-J (SEADATA)
Annual Report

This annual report has been prepared for peer review as part of the *Exxon Valdez* Oil Spill Trustee Council restoration program for the purpose of assessing project progress. Peer review comments have not been addressed in this annual report.

Principal Investigator:	Vincent Patrick	Prince William Sound Science Center
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	David L. Eslinger	Inst. Marine Sci., U. of Alaska Fairbanks
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April 1995

Information Systems and Model Development

Restoration Project 95320-J
Annual Report

Study History: The Sound Ecosystem Assessment (SEA) Program is based upon the *Sound Ecosystem Assessment Initial Science Plan and Monitoring Program,* Rpt No. 1, Nov. 24, 1993. It began in April 1994 (Restoration Project 94320) and was continued in FY95 (Restoration Project 95320). The Information Systems and Model Development Project (SEADATA) is a Restoration Project (94320-J and 95320-J) within the SEA Program. Progress for FY94 is presented in Ch. 6 of the SEA Draft 1994 Final Report. An paper on the SEA database appears in the *Proceedings of Visualization '95*, IEEE, Atlanta, GA, Nov. 1995 (C. Falkenberg and R. Kulkarni, Using spatial access methods to support the visualization of environmental data). A paper on the ocean circulation model appears in *Conf. on Coastal Oceanic and Atmospheric Prediction*, Amer. Meteorological Soc., Atlanta, GA, Jan. 1996 (J. Wang and C. N. K. Mooers, Modelling Prince William Sound ocean circulation).

Abstract: The four main development areas of SEADATA are reviewed: database, Internet and Web based collaboration tools, circulation model, and the fish model. Each of these has reached important milestones, but each is at midstage and facing ahead tasks that will be equally critical. For the database, the technically demanding tasks of the initial architecture have been resolved. The task ahead is to make everything fit within the available resources. The Web tools that were implemented were a significant accelerator for SEA. But more is required and the technology has now expanded unbelievably. The next steps will be all new terrain. The circulation model has been successfully implemented in a remarkably short time. The task ahead of validation and refinement will become increasingly difficult as the limitations of the available data for Prince William Sound become clearer. The fish model now has an efficient scipting code, a new algorithm, and the major parts of its foraging-bioenergetics model. It now must put these together to get the desired mortality estimates. The one message that came consistently from all quarters in this report is the need for more and better communication and coordination.

Key Words: bioenergetics, circulation model, collaborative software, database, diffusion-taxis, dispersion, *Exxon Valdez,* Mellor-Blumberg, Pacific herring, packet-radio, physical-biological model, pink salmon, Prince William Sound, Princeton Model, SEA, Sound Ecosystem Assessment, visualization, World Wide Web

Citation: Patrick, E. V., J. R. Allen, D. L. Eslinger, C. S. Falkenberg, D. M. Mason, C. N. K. Mooers, R. H. Nochetto, S. P. Rao, and J. Wang. 1995. Information Systems and Model Development Project (SEADATA) of the Sound Ecosystem Assessment Program, *Exxon Valdez* Oil Spill Restoration Project Annual Report (Restoration Project 95320-J), Prince William Sound Science Center, Cordova, Alaska.

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EXECUTIVE SUMMARY

The four main development areas of SEADATA are reviewed: database, Internet and Web based collaboration tools, circulation model, and the fish model. Each of these has reached important milestones, but each is at midstage and facing ahead tasks that will be equally critical. For the database, the technically demanding tasks of the initial architecture have been resolved. The task ahead is to make everything fit within the available resources. The Web tools that were implemented were a significant accelerator for SEA. But more is required and the technology has now expanded unbelievably. The next steps will be all new terrain. The circulation model has been successfully implemented in a remarkably short time. The task ahead of validation and refinement will become increasingly difficult as the limitations of the available data for Prince William Sound become clearer. The fish model now has an efficient scripting code, a new algorithm, and the major parts of its foraging-bioenergetics model. It now must put these together to get the desired mortality estimates. The one message that came consistently from all quarters in this report is the need for more and better communication and coordination.

INTRODUCTION

The Information Systems and Model Development Project (SEADATA) is one of the original projects in SEA. SEADATA was organized to deliver to the Program numerical models, a database, computing resources and networks, data visualization, computer- and network-based resources for remote collaborations and interactions, and selected sensor technologies that were judged to be sufficiently mature to have immediate impact on the cost-effectiveness of the final SEA products for long-term monitoring.

As the SEA Program draws nearer to its climax the success of SEADATA and of the newly formed Trophodynamics project for plankton model development become increasingly important to the goals of SEA. Those goals are a) to quantitatively describe, model, and numerically simulate the time evolution of those parts of the Prince William Sound ecosystem that determine the survival and growth of juvenile pink salmon and Pacific herring; and b) to deliver that knowledge to the Trustee Council in formats suitable for use in the restoration objectives of the Council. SEA has argued that an ecosystem perspective is not only necessary but that it is also sufficient for obtaining at the conclusion of the work a restoration tool that describes the status of the injured resource and how that status is likely to change in response to changes in the ecosystem. In particular, SEA is to deliver a restoration tool that will aid in assessing how recovery may be affected by manipulation of the system through resource management policies. For SEA to reach this goal it is necessary that SEADATA and Trophodynamics deliver the model components, the database, and the connectivity resources whereby the SEA collaboration can produce and validate the SEA coupled ecosystem model.

This report reviews the progress of the collaborators in SEADATA during FY95 toward meeting the goals and objectives of SEADATA and SEA. We present an updated and more complete project plan for the development of the SEA numerical models, and then review each of the parts of SEADATA through manuscripts, reports, and project documentation that have been prepared during FY95. These taken together show that SEADATA is very close to conforming to the schedule originally envisioned in the SEA Plan. At the close of FY95 the numerical models planned in the spring of 1994 were operational realities. The internetworking resources now function such that all of SEA is collectively and continuously collaborating online. And major strides have been made in the development of the SEA database during FY95, with the first key components of the database now operational.

FY95 was a year in which there was substantial cost savings to the project through cost sharing. Approximately twenty and forty percent of the costs for the fish model development and for the database development were provided from sources other than EVOS, respectively. This high degree of leveraging was in part possible because the work addressed fundamental development problems with broad applicability. As these development problems are resolved and the work becomes increasingly focused on issues specific to the restoration objectives, there will not be the same opportunities for cost sharing. Increased effort is now required to identify and secure comparable cost-sharing.

OBJECTIVES

The purpose of SEADATA is to deliver to SEA

1 numerical models;
2 database and network resources;
3 selected *measurement technologies* that were judged to be sufficiently mature to be of probable benefit for realizing low-cost long-term monitoring of recovering resources.

FY95 was the second year for the SEADATA project. For that period the Detailed Project Description identified the following task areas.

1. **Information Systems:** Data management for SEA data; local, wide, and metropolitan area networking; data resources required by SEA (realtime and historical AVHRR, weather, stream gauge); scientific database with ecosystem scale query and visualization tools; and SEA coordination utilities.
2. **Numerical Model Development:** Ocean circulation model for Prince William Sound; physical-biological model (time varying phytoplankton and zooplankton spatial distributions); fish model (time varying distributions relative to space and size of interacting species).
3. **Field Data Communications:** Packet-radio repeater network for near realtime data to and from field surveys.
4. **Sampling Technologies:** Implementation of technologies of critical significance for adequate yet affordable long term monitoring.
5. **Interim Products:** Nowcasts and forecasts from models and model parts that are still in development but that are useful for hypothesis testing, adaptive sampling trials, and model testing and parameterization.

This document reviews four task areas: database development, coordination resources, ocean model development, and fish model development. Areas that are now within the Trophodynamics project will be reviewed by D. L. Eslinger, the principal investigator of the Trophodynamics project, in a separate report. The Trophodynamics task areas are the development of the physical-biological models (i.e., the plankton models) and satellite remote sensing.

Task areas that have required primarily technology implementation will not be reviewed in detail other than to note here their completion. Under Sampling Technologies, during FY95 the Aquashuttle and its onboard CTD and Optical Plankton Counter (OPC) were fully integrated into the SEA field survey design and transitioned out of SEADATA. The physical oceanography project now operates and maintains this equipment, and it processes and delivers to SEA the datasets obtained with these devices. The repeater network objectives of the Field Data Communications area have been completed and the network is operational.

METHODS

Project Plan for the SEA numerical models

The overall plan for the SEA model is shown graphically in Figure 1. The charting method used in the figure is a modification of the standard PERT chart. The first version of this chart was created to document a review of SEADATA conducted by Dr. Andy Gunther in June, 1995, in Cordova. Figure 1 is a second version of Dr. Gunther's chart. It is an attempt to provide a more comprehensive chart that can be used for both planning, reporting, and project tracking. The second version provides in a single graphic the approach, present status, the linkages and dependencies, schedules for development and model output, validation stages, and the major endpoints. In this section the chart is used to review the methods for the model development and to provide a brief status report.

The two major endpoints of the model development effort are shown in Figure 1: (1) the coupled models for juvenile fish and (2) the specifications for a continuously running "facility" whereby data is acquired and the models are used and maintained. The method to realize the coupled model endpoint is to conduct in parallel three separate tracks of model development and then at specified stages of the development to couple the models. The three tracks are the ocean circulation model for PWS, the plankton or physical-biological model, and the nekton or fish model. This method exploits the natural partitioning of the modelling effort that occurs due to different modelling issues, different technical methods, and different human resources for each of the tracks. The final coupling of these tracks is shown in the chart as an endpoint in 1998, but the coupling is in fact carried out in stages that are indicated in the figure.

The endpoint for each of the tracks individually is somewhat different. The ocean circulation model provides information that "forces" the other models but is not dependent upon the other models. Consequently, the endpoint for the ocean model track is in fact a "stand-alone facility" with a wide range of applications. This "stand-alone" aspect of the endpoint is noted in the chart by an entry in the 1998 time period titled *98 online nowcast-forecast system.* The various products that the facility will provide for SEA appear twice in Figure 1: there is an entry at the time it is first implemented and a second entry in the catalog of functions in 1998 titled *ecosystem assessment and tracking tools.*

Both the fish and the plankton models are strongly dependent upon the physical environment. Realistic numerical simulations from the biological models depend upon the availability of realistic simulations of the physical system. The large "X" in 1998 represents how the three parts of the model are "mixed" in the coupled model.

The development sequence shown in the chart reflects a bottom-to-top schedule. This is to accommodate the differing degrees of maturity of the various modelling technologies. Ocean models have been under development for decades, combined physical and plankton models for a decade, and combined fish-plankton-physical models for less than a decade. A second rationale for sequencing the effort from bottom-to-top is to promote securing as soon as possible those milestones most readily achieved. Under this sequencing approach the forcing

effects due to the circulation processes on plankton or larvae are available first, then the impact upon plankton or larvae abundance and distribution, then the coupled response of plankton and fish to the circulation processes. For example, a catalog of candidate river-lake scenarios are available first. This is followed by the consequences to distribution and growth of the macrozooplankton bloom. This is then coupled to fish through growth and predator switching processes. There are similar bottom-to-top schedules for herring overwintering, herring larval drift, and herring summer growth.

Figure 1 is current regarding the status of the models at the end of FY95. The work during FY94 is not shown in detail. Instead for each of the modelling efforts there is a single entry summarizing the general approach and the status at the end of FY94. The progress through FY95 is shown in greater detail. The following is a brief explanation of the status summary shown in Figure 1.

During the summer of 1994 agreement was reached with Dr. Chris Mooers (RSMAS, University of Miami) to collaborate in SEA. Dr. Mooers and Dr. D.-S. Ko were then just completing a numerical simulation facility for the Florida Straits (Mooers and Ko 1994) that was very similar to what was needed in SEA. Work was to begin in October, 1994, to develop a Mellor-Blumberg model for Prince William Sound. The principal features of the approach are shown under *initial selections* for the ocean model in Figure 1.

A funding delay in FY95 moved the start date from October 1, 1994, to late in February, 1995. This delay was then extended to April due to a gap between the departure of Dr. Ko and the arrival of Dr. Jia Wang at RSMAS. Dr. Wang made remarkably rapid strides and the model objectives were all achieved by the end of FY95. The model was setup and configured and running by early summer. By the end of FY95 the ocean model was operating with the features listed in *model config. 95*. Simulations were run, and work then began on the problem of further model realism and model validation.

The principal features of the approach for the fish model are shown under *initial selections* for the fish model in Figure 1. Roughly speaking the fish model extends to the nekton trophic level the model approach used in so-called "physical-biological" ecosystem models. Examples of these latter are McCreary *et al* (1995), Gallacher and Rochford (1995), and Eslinger (1990). Physical-biological models use systems of evolution equations (i.e., partial differential equations in space and time) to describe plankton population densities and growth. This similarity to the structure to the ocean circulation model equations is convenient in combined models. The fish models are evolution equation models suitable for nekton, namely coupled systems of diffusion-taxis equations (Mason and Patrick, 1993).

The project plan for FY94 and the initial brief project plans for FY95 were constructed with more effort allocated to the ocean model. The reviews of the initial SEA Plan in February 1994 had recommended caution regarding the proposed modelling approach. To minimize risk a weighting of 3:1:1 for ocean, plankton, and fish modelling, respectively, was used in the brief project plan and budget for FY95. The October 1994 reviews, however, emphasized

the importance of all of the models. In particular, the reviews emphasized that during FY95 the models, including the fish and plankton models, were expected to provide guidance to the field surveys. Since commitments were in place it was not possible to totally restructure the allocation of effort. However, to the extent possible the work on the fish model was accelerated. On the other hand, because of the allocation of effort it was a near certainty that by mid-year there would be circulation model output that could be used along side the FY95 field sampling. The delay in funding, however, slipped the start time such that the first circulation model results were not available until shortly after the descent of the macrozooplankton.

By the close of FY95 the foregoing development gaps had been greatly reduced. The circulation model was on schedule by late summer. The fish model went through two full implementation revisions for the diffusion-taxis component. Version 4 is an effective and very efficient implementation that enables new scenarios to be set up and analyzed much more quickly. The first version of the bioenergetics component is operational. At the close of FY95 the models have been implemented and the focus will increasingly shift to simulations and validation.

During FY95 no coupling of the three model efforts was scheduled. Model coupling starts in FY96. It is useful to keep in mind that the models are coupled by the SEA hypotheses. The first coupling task is shown in Figure 1: the use of simulations from the ocean model to force the plankton model and herring larval drift. The plankton forcing is for both plankton production (the bottom-up alternative hypothesis) and for macrozooplankton distribution and flushing (river-lake hypothesis). Following this the fish and the plankton models will be coupled in simulations of predator and juvenile fish responses to physically driven changes in the macrozooplankton abundance and distribution. All of the facets of the model coupling will not fit in Figure 1. Some of the ones not shown are juvenile herring overwintering, retention zones, replenishment of zooplankton in embayments, and juvenile herring growth.

RESULTS

The SEA Database

Status

There were four major accomplishments in the development of the SEA database during FY95.

1. The database prototyping phase was completed (at no cost to EVOS); the major questions regarding database architecture and software were resolved; and all of the database software was acquired and installed (with cost sharing across four sites).

2. For the majority of the projects and datasets in SEA the data ingestion steps of *(a)* investigator interview and *(b)* dataset analysis were completed; the subset of the data dictionary consisting of items common to all datasets was completed; and the full specification of the data dictionary for two datasets was completed.
3. For one of the SEA datasets (CTD) a full end-to-end implementation of the database was completed, including a network accessible Web-based (Netscape) query tool.
4. An original software application called the SEA *Cruise Planner* was written and the beta version distributed. The software aids in the design and execution of field surveys that involve multiple vessels, sharing of limited equipment and personnel, and the coordination of dissimilar sampling protocols.

Various aspects of these accomplishments are shown graphically in Figures 2 and 3. Figure 2 shows in a table format a comprehensive list of the SEA datasets (bottom row) and the architecture of the database as reflected in the processing steps (left column) used to ingest a dataset into the database. The table entries are shaded according to the status: blackened indicates completion at the end of FY95, gray indicates completion scheduled for FY96. The complete specification of all processing steps in Figure 2 reflects the accomplishments listed in item 1 above. The accomplishments in item 2 are reflected by the "completed in FY95 status" for interview and analysis processing for most of the datasets. Item 3 is reflected in the completed status for all processing steps for the CTD dataset up through the catalog services query tool. Figure 2 is a working graphic used in project tracking and scheduling and has some imperfections. But it does quickly summarize the scope of both the SEA datasets, the architecture of the database, and the status of the effort to implement the SEA database.

Figure 3 shows the interface to the SEA Netscape query server and reflects the accomplishments of Item 3 regarding network accessible query for CTD data. The Netscape query server is a prototype with respect to ecosystem searches but is fully functional for the CTD dataset. Figure 3 shows a search over a range of positions and time and by station ID. The SEA CTD datasets that are returned by the search are show in Figure 3b, and the position of the CTD cast for each of the returned datasets is displayed on a map (Figure 3c). The query server was implemented using the familiar World Wide Web browser software from Netscape, Inc. This software is available for essentially all computing environments (PCs, macintosh, UNIX, VMS). This means that if a user has the proper login permissions then the query shown can be made from anywhere and from any machine via the Internet. The SEA *Cruise Planner* of item 4 is described in some detail in a later section. As a preview, however, the map used in the Netscape query server is a reuse of the map utility developed for the *Cruise Planner*.

In addition to the four database accomplishments just described, there are two further accomplishments for FY95: *(a)* the substantial cost savings realized by EVOS during FY95, and *(b)* the success of the development group in electing to proceed with Illustra™ as the primary candidate database software for SEA a full four months prior to the announcement that Illustra was selected as the database software for EOSDIS.

EVOS realized substantial cost sharing during FY95 by means of joint efforts and cost sharing. The scope of the joint efforts is shown in Figure 4. During FY95 (as well as during FY94) C. S. Falkenberg, R. Kulkarni, L. Herman, J. R. Allen, and V. Patrick worked on the SEA database. Mark Vallarino of the Herring Project also contributed to the effort. Mr. Kulkarni is a senior developer in the areas of scientific data formats and data models, high performance computer systems, and scientific applications. He participated in the effort throughout FY95 at no cost to EVOS. Mr. Falkenberg is the principle designer of the SEA database. Of his eight months of work on the SEA database half was funded by a research grant from the Advanced Visualization Lab. Throughout all of FY95 we were able to conduct the prototype effort and the initial implementation effort with Illustra under a license grant. The Illustra software was purchased at the end of FY95 with substantial cost savings to EVOS through a multi-user purchase and costs shared by four sites. In total thirty to forty percent of the database development effort was provided by non-EVOS funds.

The SEADATA effort for the development of the SEA database is a collaboration of those with expertise in scientific software and database design with those with expertise in all of the various disciplines of marine biology and oceanography represented in SEA. This collaborative structure provides SEA with a window to the most recent events and trends in the technology as well as to opportunities for substantial cost sharing. In particular, by virtue of this collaboration a quite conservative design approach could be implemented. In particular, it was possible to progress carefully without making premature commitments to an approach that would ultimately prove inadequate and lead to a costly and unsatisfactory conclusion. It was through the prudence of the collaboration that everything was not invested early in the Aurora Dataserver and Xidak, Inc. The design progressed in a manner that maintained the flexibility to easily move to an alternative software. The same approach has been followed with Illustra. Although the database design retains its flexibility and lack of overcommitment to a single solution, it appears increasingly that the selection of Illustra has been prudent. Illustra has been adopted as the database for EOSDIS. In addition, during the spring of 1996 Illustra was purchased by Informix whereby Illustra is now a product of the third largest database vendor.

The remainder of this section provides further details on these accomplishments of FY95, and it references a growing documentation for the database that includes one publication and one non-EVOS funded manuscript that was withdrawn at the eleventh hour.

Xidak and the Aurora Dataserver

Throughout FY94 the primary candidate for the SEA database was an extended relational system from Xidak, Inc. The system consisted of the Orion database with an interface called the Aurora Dataserver. The system was designed specifically to address the problem of managing scientific data. It was at that time unique among commercial-grade databases for the extent of the features it provided for scientific data. Mr. Kulkarni had arranged a co-development agreement with Xidak in which he was to develop a query interface for Xidak using the scientific visualization software AVS™ (from Advanced Visual Systems, Inc.) and

in return Xidak provided no cost software licenses to both AVL and to PWSSC for Orion and Aurora, plus a grant to support the interface development. That grant supported the work of Mr. Falkenberg during FY94. The Prince William Sound bathymetry data was used to demonstrate the interface.

In November 1994 an abstract describing the AVS interface for Aurora was submitted and accepted for presentation at the 1995 AVS Users Conference, April 19-21, in Boston. The proceedings are published in both hardcopy and CDROM, and during the winter the manuscript was prepared for the February submission. However, during the winter Xidak decided based on market considerations not to continue the development of Aurora. Development of Aurora stopped and the paper was withdrawn. The manuscript was revised to accurately reflect the suspended development of Aurora and serves as a technical report documenting the work and the findings of the collaboration of Xidak, Inc., the Advanced Visualization Lab, and SEADATA. The manuscript for the Aurora Dataserver work is attached as Appendix 1.

Illustra and the SEA Prototype Design Study

During that same time period the object-relational database Illustra was announced. Illustra Information Technologies, Inc. was a new startup company formed in 1992 by Michael Stonebreaker. Dr. Stonebreaker had led the Postgres database development project at the University of California, he had founded the company that produced Ingres, and he was currently one of the principle investigators for the Sequoia 2000 project (Stonebreaker 1994), a project to develop a database for the earth sciences. The Illustra software drew upon Postgres with the goal of a relational database that could effectively deal with the rapidly expanding arena of non-traditional data types, which included scientific data as well as the larger market arena of multi-media data. The goal of Illustra had much to recommend it. Moreover, Prof. Michael Franklin of the Computer Science Department at the University of Maryland had been awarded a license grant from Illustra. In January a research study grant was made available to Mr. Falkenberg by the Advanced Visualization Lab, University of Maryland. It was agreed that Mr. Falkenberg, under the supervision of Professor Franklin, would develop a prototype design for a scientific database using Illustra, and he would use the SEA data archive as his test case. Mr. Falkenberg was supported by this grant through June 1995. It was through this collaboration that Illustra was available to SEADATA for prototyping and initial trials.

The report for that study grant is included as Appendix 2. Although the Illustra software had advanced object-oriented features that were tailored to scientific applications, the function of the Aurora Dataserver had to be replaced. The solution chosen in January 1995 is outlined in Figure 5. This solution was described in the Integrated SEA Project Description for FY96. Mr. Falkenberg implemented this solution using Illustra in his prototype design study using the CTD datasets from SEA. It is the result of that study that is shown in Figure 3.

The design study implemented an AVS query interface to the Illustra database and to the SEA datasets. A paper (Falkenberg and Kulkarni 1995) describing the interface was presented at the *Vis95* conference and it appears in the *Vis95 Proceedings.* The manuscript is attached in Appendix 3. (It is included conditional upon the granting of appropriate permissions by IEEE.)

SEA data schemas and data dictionary

From June through September 1995 Mr. Falkenberg was supported by SEADATA. During this period he interviewed each of the SEA principle investigators and began the process of implementing the prototype design for the totality of the SEA datasets. An initial analysis for all of the datasets was completed, and the data dictionaries for CTD and acoustics were completed. Some of the documentation for the SEA database is included. Figure 6 shows the working document describing the collection, routing, processing, and disposition of the biological samples collected by SEA. Appendix 4 contains the documentation for the data dictionaries for the CTD and acoustic datasets.

SEA Cruise Planner

Cruise planning for SEA requires the coordinated efforts of investigators that traditionally use different sampling and measurement methods. The field sampling methods divide largely along the same lines as the models: oceanography, plankton, and fish. SEADATA decided to commit some resources to the development of a software application to more quickly design complicated cruise plans. A first rapid prototype was developed by Sri Rao using the public domain software Tcl. The planner development was then passed to C. Falkenberg and Larry Herman who then completed the development of Version 0 of the SEA *Cruise Planner.*

The user interface of the *Cruise Planner* is shown in Figure 7. The software provides a map in which one can place both fixed stations and transects. There is a data entry and display interface for the two cases. Stations and transects can be entered via the map and the data entry panel, or the *Planner* can read a text file of stations. The file format is shown in Figure 8a, a simple comma delimited list of positions and information about the station or transect. The file is easily maintained or modified with a text editor or word processor. An important application is to use the *Planner* in conjunction with a spreadsheet (Figure 8b) as a means of quickly computing cruise times and costs and comparing alternative plans. The current version of the *Planner* is a beta version and is being evaluated by SEA investigators.

January review

For the January 1996 EVOS Workshop and SEA Review C. Falkenberg and R. Kulkarni prepared a short document reporting on the status of the database development effort at the close of FY95. Their report to SEA and the SEA reviewers is quite useful for it presents the status of the effort from the point of view of the database developer. It is attached as Appendix 5.

Coordination resources

The Internet, Intranets, and the SEA Collaboration

The Internet and the World Wide Web continue to be a major news stories. It is now common knowledge that anyone can retrieve information from resources around the globe by means of the Internet and software utilities called Web servers. However, it is no longer this global aspect of the Internet and the Web that is driving the news. Rather, the real force behind the news stories are the methods of staying in touch with those close by using *Intranets*. The same technology and software that had been developed for information at distance has been recognized to have significant value for coordination and communications within organizations spread over any distance. Because the technology now has applications with significant economic consequences, the rate of development has exploded, especially software development.

These new developments are of particular interest in SEA. The success of SEA requires the closest possible collaboration between all of the SEA projects. New knowledge in one project must become the knowledge of all in SEA as rapidly as possible and with the least overhead. Questions that arise in one project must be posed and discussed across SEA with a minimum time delay. SEA has all of the requirements for coordination and tight collaborations plus the additional burden of significant distances between groups. Awareness of this is not new. What is new is that within as little as the past six months the options for enhancing the collaborative process have increased significantly.

SEA Home Page and the SEA Collaboration

During FY95 SEADATA implemented a first set of network resources intended to enhance and facilitate the collaboration processes in SEA. J. R. Allen is the developer of this new collection of Web-based tools. A Web server (httpd, NCSA) was configured and installed at PWSSC in early September and a SEA Home Page at URL http://www.pwssc.gen.ak.us went on-line.

Some of these new tools are shown in Figure 9 and Figure 10. Figure 9a shows the SEA "home page," the first graphic that appears upon connecting to the SEA URL. The home page has a standard assortment of links to public information about the organization and the

activity responsible for the page as well as links to closely allied sites such as the EVOS OSPIC site and the Web sites for state agencies and universities. At the bottom of the page is the hypertext link to the password-protected section of the Web-site, the "SEA Program Operations" section.

The first page of the Operations section is shown in Figure 9b. The range of the services available are presented as hypertext links. The start-up pages for two of these services are shown in Figure 9c and 9d. The "What's New?" is a listing of those things that have recently changed or newly appeared. As can be seen from the entries in Figure 9c this like most of the items require the regular attention of the site administrator.

Figure 9d is the case of a common and difficult problem in large collaborations quickly resolved. The "Publications in Preparations" area lists the SEA papers that are planned, in progress, or submitted. Postings for the planned papers include a working title, potential contributing authors, and the targeted publication. Postings for papers underway are updated regularly to indicate the current status of the manuscript. Through timely and current postings in each of these categories the problems of unrecognized conflicts and errors of omission and oversight should be nearly eliminated.

The other items in Figure 9b are

Calendar, Deadlines, Announcements
New Proposals Planned
Cruise Plans
Cruise Reports
Data (i.e., access to the online interface to the SEA database (e.g., Netscape server))
Work Groups

Two of these, the Cruise Plans and the Cruise Reports, are to be moved from the SEA operations section to the open access section. The last item of the list is the link to the section supporting the activities of the three work groups (more recently referred to as focus groups). This area contains the majority of the pages supporting the scientific collaborations. The first and second pages are shown in Figures 10a and 10b. Figure 10b shows the four sections used for each of the focus groups. Figure 10c shows how the four sections are used. The "data" area contains links to new results, typically in the form of tables, diagrams or figures with explanatory captions provided by the investigator, plus the cumulative record of the dialogue about that data. This system was used extensively in preparation for the joint presentations made at the EVOSTC Workshop and SEA Review in January 1996. The activities of December and January provided the first test of the potential of these tools for facilitating collaborative work in SEA, and the potential was confirmed and realized.

The Ocean Circulation Model for Prince William Sound

Status

During FY95 Dr. Christopher N. K. Mooers and Dr. Jia Wang, along with Mr. San Jin, have completed the implementation of a first version of a three-dimensional, primitive equation circulation model for Prince William Sound. A manuscript describing this work was submitted by Drs. Wang and Mooers for presentation at the January, 1996, meeting of the American Meteorological Society. The manuscript "Modeling Prince William Sound Ocean Circulation" was accepted and appears in *Conference on Coastal Oceanic and Atmospheric Prediction*, Atlanta, Jan. 28-Feb. 2, 1996, recently published by the American Meteorological Society (AMS). (Wang and Mooers, 1996) That paper is included here as Appendix 6. (Inclusion of the paper is conditional upon receipt of permission from AMS.)

This section is supplementary to the information in Appendix 6. It documents information about the model development effort that is significant to the record of that effort but outside the scope of the conference paper. It also documents some complementary work undertaken to apply early model results and methods developed to assist in model validation.

Initial selections

The general collaborative structure of the ocean modelling effort is quite similar to that of the database development effort. In particular, for the ocean model development there is the following situation:

> The SEADATA effort for the development of the SEA ocean model is a collaboration of those with expertise in numerical simulation modelling of the ocean with those with expertise in all of the various disciplines of marine biology and oceanography represented in SEA. This collaborative structure provides SEA with a window to the most recent events and trends in the science and technology of 4D simulation models as well as to opportunities for substantial cost savings through efficient and effective model development and by building upon recent previous projects that are very similar to the needs of SEA.

There are somewhat limited opportunities for cost sharing for the development of the Prince William Sound circulation model since the set of potential users is very small in comparison with the potential users of a significantly improved scientific or ecosystem database. Instead, there are cost efficiencies due to prior experience and the ability to quickly and efficiently implement the model.

During 1993 and 1994 Dr. Mooers and Dr. D.-S. Ko implemented what they referred to as the "Straits of Florida Nowcast/Forecast System" (SFNFS) (Mooers and Ko 1994). The requirements for that project closely parallel the requirements of SEA. Briefly, SFNFS ingested realtime data and atmospheric model forecasts, produced 0, 1, and 2 day forecasts of the ocean circulation, and delivered these on a continuous basis as part of an online, realtime

resource for use in emergency spill responses and realtime risk management. In addition the system was to provide the capability for "what-if" simulations through the use of hypothetical or historical wind forcing time series. For SEA it suffices that spill response is replaced by plankton and fish models. Indeed, at the American Meteorological Society 1996 Conference on Coastal Oceanic and Atmospheric Prediction, papers on both SFNFS (Mooers and Wang 1996, pp 28-35) and Prince William Sound (Wang and Mooers 1996, pp 36-43) were presented.

Hence, SEA started FY95 with the benefit of Dr. Mooers experience not only with numerical simulation models but also with their application in cross-disciplinary collaborations. Output from SFNFS with the forcing wind fields is still available by anonymous ftp to 129.171.100.26 from the directory /pub/SFNFS. Roughly speaking the SEA circulation model development nearing but not yet half way to the level of refinement represented by SFNFS.

One further note regarding initial selections has to do with the choice of the Princeton Model (alternatively referred to as the Mellor-Blumberg model) for SEA. This model has been used for smaller systems such as Hudson Bay (Wang *et al* 1994) and Tampa Bay (John Wang, RSMAS, personal communication). However, at the end of FY94 some reviewers of SEA expressed varying degrees of concern regarding the suitability and applicability of this selection for a fjord as opposed to the coastal domain in SFNFS. These concerns have apparently been redressed for the FY95 reviews of the ocean model development effort have been uniformly very strong.

Dr. D.-S. Ko left RSMAS in December 1994 prior to the availability of funds in late February 1995. SEA did not have the benefit of his contribution to the Prince William Sound model. In April 1996 Dr. Jia Wang arrived at RSMAS, and he and Dr. Mooers are the developers of the SEA ocean model. Dr. Wang is a valuable addition to SEA. He is highly regarded by all and an effective and congenial collaborator. He brought with him previous experience with the Princeton Model and the modelling of smaller, enclosed systems (Wang *et al* 1994). His curriculum vitae is an accurate indicator of the very substantial contributions that he and Dr. Mooers have made to SEA during FY95 (see the curriculum vitae of Wang and Mooers in the SEADATA Detailed Project Description for NOAA BAA Feb. 1 1996 to Jan. 31 1997).

Setup

The initial "setup" of the model—the selection of the boundaries, grid, depth layers, boundary conditions—was done by Jia Wang during April and May of 1995. A key dataset consists of the exiting bathymetric soundings and the shoreline. The result of this step defines the domain for the circulation model, hence to some extent constrains all of SEA. Figure 12 shows the circulation model domain at zero depth. Figures 16 and 18 show three-dimensional views of the bathymetry data.

The bathymetry data of Figures 16 and 18 are derived from an Alaska Department of Natural Resources (ADNR) GIS data product. The NOAA National Ocean Service (NOS) bathymetry data is too sparse to be used alone. ADNR combined the NOS data with additional depth soundings. From this first generation dataset a second generation dataset was produced consisting of 20m depth contours in the Arc/INFO vector format. This second generation data set is at present the best available bathymetric data for the sound. The bathymetry for the model domain is third generation: the contour data plus shoreline data were interpolated and gridded using the Albers conic equal-area projection (parallels at 55 and 65, origin at -154W, 50) to obtain the needed gridded data product for the model. The first generation data that supplemented the NOS soundings have not been recovered. Only the GIS coverages have been traced.

The limitations of the 20 meter contours can be seen in Figure 18 and in Figures 11 through 15. The 20 m contour is insufficient to retain the shallow water passages into the sound around Hawkins Island. A new first generation bathymetry with coverage of the shallow areas and that extends significantly onto the shelf will be needed for the next version of the model.

Model configuration '95

Three forcing components were implemented during FY95. The dates in parentheses are the approximate time at which the first model output was presented.

inflow at Hinchinbrook Entrance (intrusion of the Alaska Coastal Current) (May, 1995);
the dominant component of the tides, M_2 (June, 1995);
winds (forcing by constant, uniform wind) (August, 1995).
(during the fall 1995 particle advection (tracer) simulations were conducted.)

Additional information on these is contained in Appendix 6.

Simulations using model configuration '95

For all of the following simulations the model output should be understood solely as the product of a simulation exercise; the output should not be expected to closely approximate measured data. Typically here only one or two forcing variables have been applied at one time with only a zeroth order estimate of the proper magnitudes for each.

An example of the type of model simulations obtained first during FY95 is shown in Figure 11. Here a fixed inflow of 0.3 Sv is applied with no other forcing. The model is first run for 17 days to stabilize (spin-up). The days shown in each of the panels of Figure 11 refer to days beyond the first 17 day spin-up. Forcing with inflow of 0.3 Sv is at the upper end of observed the observed range of inflow. It is representative of only the October through December period. Upper layers during the summer can in fact have net outflow at Hinchinbrook Entrance. (Niebauer *et al* 1994). The four panels of Figure 11 are all for

velocities at a depth of 3 meters.

The simulation shows the degree to which the circulation is stable after 17 days. At each time step shown the model has generated some new eddy the near surface flow. In Appendix 6 Jia Wang discusses briefly the fact that the model is eddy resolving.

The model output velocity fields for the combined forcing of 0.3 Sv in-flow and M_2 tide (3 m tide range) is shown in Figure 12 (high and ebb at 3 m depth), Figure 13 (low and flood at 3 m depth), Figure 14 (high and ebb at 100 m depth), and Figure 15 (low and flood at 100 m depth). The addition of the tides has added substantial additional structure to the velocity fields of the central sound. The fields at 3m are different from those at 100m.

In Appendix 6 Jia Wang shows the effects that sustained west and sustained east winds have upon the circulation velocity fields.

Model validation

In the last months of FY95 work was begun to incorporate Prince William Sound data into the model. Work was begun to prepare the hydrographic data and acoustic Doppler current profiler data for use in validating and calibration of the model. The plans for the 1996 oceanography cruises were revisited in an effort to identify optimal allocations of measurement resources. These efforts are continuing they remain the top priority. The validation and calibration of the ocean circulation model must be completed before it can be applied to any of the several SEA projects that are waiting for it.

This first step from concept to initial implementation has been a remarkable one. The achievement of a numerical model where none before existed is a clear and unambiguous major step forward. But it cannot be counted yet as a success; success requires further refinement of the model and the validation of the model. That accomplishment is still ahead.

Preliminary applications

In an effort to quickly utilize during FY95 any new insights that might be obtained from the model, during the winter preparations were made to apply the anticipated model simulations to the problem of the initial conditions of stage one neocalanus arriving at the surface waters in the spring.

Figure 16 graphically summarizes the problem for the initial conditions for neocalanus. In the lower left of the figure a cross-section of a hypothetical region of the sound with depths exceeding 450 m. The notes accompanying the cross section describe the situation and some of the issues. Briefly, the adult neocalanus overwinter at depths below 350 m, and in early March over a period of approximately 15 days they lay eggs. During a second 15 days these eggs rise to the surface and on the way hatch and become stage one copepidites by the time

they reach the surface waters. The notes in the figure identify unknowns regrading the initial distribution of adults at depth.

However, a more substantive question is how the newly hatched early stage neocalanus are distributed when they first reach the surface. If the eggs and nauplii were to rise vertically with no horizontal mixing and if adults at depth were distributed uniformly, then the initial condition would be a population distributed at the surface in proportion to the available volume for overwintering of adults at depth directly below. This is shown in Figure 16 by the surface plot over the sound of the available overwintering volume for adults directly below the position of the plot. Hence, for this hypothetical scenario there would be a very high density of neocalanus directly over the deepest parts of the sound, in particular over the so-called "black hole" in the western sound where bottom depth exceeds 700 m. There would be a very large expanse of midrange density in the east-central sound.

An alternative to the foregoing scenario is substantial advective redistribution of the animals during the 15 day rise to the surface. To illustrate an application of the model, a particle advection simulation prepared by Jia Wang for the velocity fields of Figure 11 is shown in Figure 17. The panels of the two figures correspond. In particular, the velocity fields are due solely to a constant inflow of 0.3 Sv at Hinchinbrook Entrance. The simulation consists of the release of tracer particles throughout the upper 40 m of the model boundary across Hinchinbrook Entrance at a constant rate for the first six days after spin-up. The simultaneous spreading and flushing of the tracer is apparent. It is not shown but the tracer essentially remains within the upper 40m layer.

This simulation scenario, however, does not directly fit the task. According to Niebauer *et al* (1994) the inflow in March approaches zero whereas the simulation assumes 0.3 Sv inflow. However, Jia Wang points out in his paper that the residual tidal flow is approximately that of the assumed in-flow. Hence, it is conceivable that a simulation with tides alone would result in a similar transport but not necessarily along the paths shown. Further model forcing and refinement and model validation is needed before this advective transport feature is meaningful.

A second topic in which there was an effort to pull together field measurements and model results is shown in Figure 18. This shows one of the visualization methods developed to display velocity fields measured by towed ADCP. This picture however is misleading for the time interval over which the measurements were made spans several tide stages. To compare these velocity fields with simulations the time for both the measurement and the model output must be used as well as the spatial position. During FY95 the display method in Figure 18 was further developed so that velocity fields at only a single common tide stages interval are shown. This was then animated so that the display stepped through a tidal cycle.

The Nekton Model for pink salmon and Pacific herring

Status

During FY95 there were many accomplishments, but they all were not achieved at the anticipated time, and for some the need had not been anticipated at all. That is, there were periods in which things were done with temporary solutions until a missing piece was available. There were important pieces that were particularly elusive and the solutions did not all become available until nearly the time of the EVOS Workshop in January 1996. For the fish model development, then, this review of results witll cover the period up through early February 1996. With that warning of some irregularity in the schedule, the key milestones for FY95 are summarized below in approximately chronological order.

During FY95 two versions of the bioenergetic-foraging models were completed. These are referred to as *gut v1* and *gut v2*. The spatial component of the fish model (diffusion-taxis model) has had the name *Alewife* stubbornly remain attached to it (ALaska Experimental Window Interface for Fisheries Ecosystems). This is often shortened to *Aw*. FY95 started with *Aw v1*, a single trophic level, single spatial dimension model. This review will extend to *Aw v4* which allows an unlimited number of interacting trophic levels, one spatial dimension, and implements a new structure that dramatically simplifies all of the coding tasks associated with model development and refinement. The major milestones of the project follow.

1. First foraging-bioenergetics model (*gut v1)* for pink salmon fry. It simulates diel feeding pattern and growth. The model performs well relative to the measurements reported by Godin (1981a, 1981b, 1984) This result was the starting point for *gut v2* below.
   (Feb 95)
2. First results from *Aw v1* configured for three coupled trophic levels and one fixed (forcing) trophic level. This is the code from Tongbio Li from FY94. Although it gave reasonable results the code was too awkward for efficient modelling, it did not scale easily to additional trophic levels, and it did not scale easily to higher spatial dimensions.
   (Apr 95)
3. Limits identified for the taxis to diffusion ratio. These limits are imposed by the numerical implementation of the solver for the partial differential equation; begin process of rewriting the algorithm and the code. Thus begins the need to distinguish the *old* code from the *new* code.
   (Apr 95)
4. Begin work on a graphical user interface intended to facilitate collaborative work with fisheries biologists. This ultimately becomes *Aw v3.*
   (May 95)

The goal here was to have all of the information from Figure 19 readily available. Figure 19 is a list of all possible factors to include in the loss function but typically only a few are used. Even with a few, with Tongbio's code all of the parameters are buried in a large number of small C programs. In addtion, the domain of interest in Figure 20 should be shown. Finally, the interface should facilitate manipulating fish populations with the model as in Figure 21. For example, a linear loss function produces an exponential solution. Hence one can "force" a population to remain at one shore but not another simply by imposing an arbitrary loss function.

5. Arrangements completed with European colleague for collaborative use of recently developed 2-d/3-d mesh generator code.
   (June 95)
6. *Aw v3* with graphical user interface (with *old* code) is ready. See Figures 26 and 27. (Jul 95) Simulation studies performed using fixed *Neocalanus* and coupled pink salmon fry, alternative prey to fry (e.g., juvenile pollock), and adult pollock. See Figures 22 through 25. Present at Fairbanks SEA meeting.
   (Aug, Sep 95)
   One of the September simulations is shown in Figures 22 through 25. This is a four day run starting from atypical distributions in order to see the way the distributions jointly adjust. The loss functions are somewhat forced, and these are described in Appendix 9. In particular, a nearshore preference is applied to fry and a nearshore avoidance to pollock. The feeding rates and rates of encounters with adult pollock are plotted for both fry and age-0 pollock in Figure 23. These are computed during the simulation as part of the calculation of the loss. In Figure 24 the diet of adult pollock on the three prey are shown.
7. Complete second version *gut v2* of bioenergetics-foraging model for both adult pollock and pink salmon fry. Demonstrate the capability to simulate episodic feeding behavior. See Figures 30 and 31. Model performance reviewed within SEA.
   (Dec 95)
8. *Aw v4* (with *new* code) completed. See Appendix 7 and Appendix 8.
   (Dec 95)
9. Prepare updated *Aw* model specification document. See Appendix 9.
10. Conduct simulation studies demonstrating the capability to simulate known or conjectured patterns in fry distribution due solely to current velocity and tides.
    (Jan 95)
11. Conduct first simulations of nearshore interactions of pollock with tidally influenced fry distribution and fixed *Neocalanus* distribution. Present at AGU/ASLO. See Figure 31.
    (Feb 95)

During FY95 the fish model development had the benefit of cost sharing. Dr. Doran Mason and Dr. Ricardo Nochetto, as in FY94, again collaborated with Dr. Vince Patrick on the fish model development. Mr. Sri Rao was added to the group in April 1995. Dr. Mason is

contributing to the biology and structure of both the spatial model (Mason and Patrick 1993) and the foraging and bioenergetics model (Mason and Brandt 1996; Brandt *et al* 1992). He is a Research Associate at the Limnology Lab, University of Wisconsin, as well as as Research Scientist at Prince William Sound Science Center. Because his interest is in the successful development of the model in general as well as for Prince William Sound, his contribution to the model development is significantly greater than would be possible with only SEA support.

Dr. Ricardo Nochetto's contributions address the numerical solutions for the fish model. Dr. Nochetto is an Associate Professor of Mathematics at the University of Maryland and a Research Scientist at Prince William Sound Science Center. He is a numerical analyst working primarily on nonlinear partial differential equations. He is interested in the numerical methods for the fish model in general, and in particular he is interested in the problem of multi-dimensional grids for these classes of problems. These overlapping interests and his use of the findings in other areas are of significant cost benefit to SEA and to EVOS.

Both Dr. Mason and Dr. Nochetto bring to SEA and to EVOS insights, resources, and breadth of participation not available any other way. Dr. Mason is, for examle, an invited collaborator on the Lake Superior Technical Committee, a group just now approaching their first success in their effort to restore a viable population of the once native lake trout. His position at the Limnology Lab places him at one of the crossroads of activity in bioenergetic modelling. Indeed, the Hewett and Johnson bioenergetics software was written at the Limnology Lab and supported and published by Wisconsin Sea Grant.

Dr. Nochetto provided the expertise whereby the modelling effort was never limited by the expertise of any of us who are not numerical analysts. He tended to all of the numeric issues and thereby enabled the rest to focus on the model itself and the results. He is an active researcher (over 40 research articles, an editor of the *SIAM Journal on Numerical Analysis,* and the recipient of the 1993 International Giovanni Sacchi Landriani Prize for outstanding contributions to the numerical analysis of partial differential equations). It is through his involvement in the research community that SEADATA has succeeded with the FY95 objective of finding suitable 3-dimensional mesh generation algorithms for trials with the fish model and the SEA coupled model.

Mr. Rao handled all of the programming. All of the new *Aw* software in the foregoing milestones list is due to him.

Versions completed and simulations of distributions

1. Pollock-Fry interactions in the nearshore regions

The most significant result is Item 11 above, which is shown in Figure 31. The purpose of this simulation was to see how the one-dimensional (spatial) cross-channel model would respond for the following scenario:

1. a nearshore pink salmon fry distribution, as shown in Figure 31b;

2. C5 *Neocalanus* at a relatively high density, as shown in Figure 31a; there is a cross-channel distribution that is approximately uniform but which decreases as the shore is approached; the zooplankton density is assumed to not vary in time;
3. a reduced handling time for adult pollock feeding upon C5 *Neocalanus*, as compared to the handling time for fry, to better represent the apparent switch to filter feeding;
4. the adult pollock following an episodic feeding regime, apporximately four hours feeding and eight hours non-feeding.

All of the possible combinations and ranges have not been run, but from a run such as the one in Figure 31 it does seem that the model is quantitatively discriminating in the ways that previously could only be qualitatively described. Note that the simulation is plotted only for five time steps, each step 0.1 day apart, and that the five time steps shown have been "spun-up" for nearly ten days.

Loose all trace of partitioning during non-feeding. The diffusion and taxis coefficients have been set somewhat high. The assumption is that the predator fish swim sufficiently fast and are sufficiently active that during the non-feeding time that the values are realistic. Their distribution becomes uniform when not feeding. The alternative extreme is to have the diffusivity and the taxis coefficient become small when not feeding. This would "freeze" in place the distribution formed during feeding.

Partitioned between fry and zooplankton during feeding. When the hunger increases for the predator, those that are in "contact" with the fry in the nearshore move strongly further inshore. Those predators that are outside the fry distribution remain with the macrozooplankton. In fact, those that do not come in contact with the fry tend to be held within a "loss function well" in the mid-channel.

The prudence of avoiding gradients. Within the confines of the diffusion-taxis model the pollock arrive at the nearshore either by a random walk (diffusion) or by following a gradient. If the fry distribution tailed off into the channel it would draw in a larger population of predators. In the case at hand there was in fact a shortage of prey and an increase in the loss function which insures no taxis by predators toward the fry from midchannel..

The advantage of a loss function maximum. The only reason the loss function does not monotonically decrease to the nearshore is because the fry are further inshore than a certain macrozooplankton density. If the plankton density were uniform, then the loss would decrease with the additional prey and there would be a continual pressure on the nearshore. This can be avoided by the prey if they can position themselves beyond the "roll-off" of the offshore plankton densities. At some point this is in conflict with their feeding, however. It is an optimization problem, and is dealt with automatically by the model if the fry are free to redistribute in response to predators.

Fry mortality is given at each point by multiplying feeding rate by the adult pollock density.

In this study the pollock loss function was set solely by available feeding rate at a point and the degree of hunger. The fry were responding only to diffusion and tidal current avoidance; they were not responding to predators. The interest here was the relative preference between fry and *Neocalanus*. The answer is that at these densities the fry still are better feeding (in grams per unit time) but all of the pollock cannot fit into the small space occupied by fry.

2. Time varying nearshore densities due to tides

The thinking continues that the nearshore serves as a refuge in some way. Some of the mechanisms were suggested from the simulation above. It is often suggested that the smaller juvenile are "avoiding currents." Hence, simulations were run to determine what the end result looks like from a constant velocity current and then a tidal current. The change was quite surprising.

The redistribution of fry from a point source such as a net pen release was simulated, where it was assumed the fry would attach an increased loss to water with high velocity. The time evolution of the density is shown in Figure 28. When a periodic tide is switched on, the fry distributions tend to "leak" from the shore during slack tides. A new quasi-equilibrium is obtained wherein the fry densities increase during flood and ebb as they move back inshore, then the densities decrease as the population disperses off shore. See Figure 29.

3. Time varying nearshore density due to feeding

In the foraging-bioenergetics component the issue of satiation and its modulation of feeding behavior is of top interest. In *gut v2* better simulations were constructed as alternative mechanisms for modelling satiation and hunger were tried. We can reproduce the intense morning feeding reported in the literature. This is shown in Figure 30. This process is a candidate for modulating the nearshore density of fry.

DISCUSSION

The collaborative process in SEA

Throughout the preparation of this review there was the repeated discovery: there is an immediate and urgent need for a dramatic increase in the communications and collaborations in SEA. Not enough information is changing hands. This is not to say something has not been done, but rather that the tasks have matured in scope and complexity and now require more comprehensive links.

Individual new information must become collective knowledge almost instantly. An unexplained finding must become a global question of which all are aware.

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FIGURES

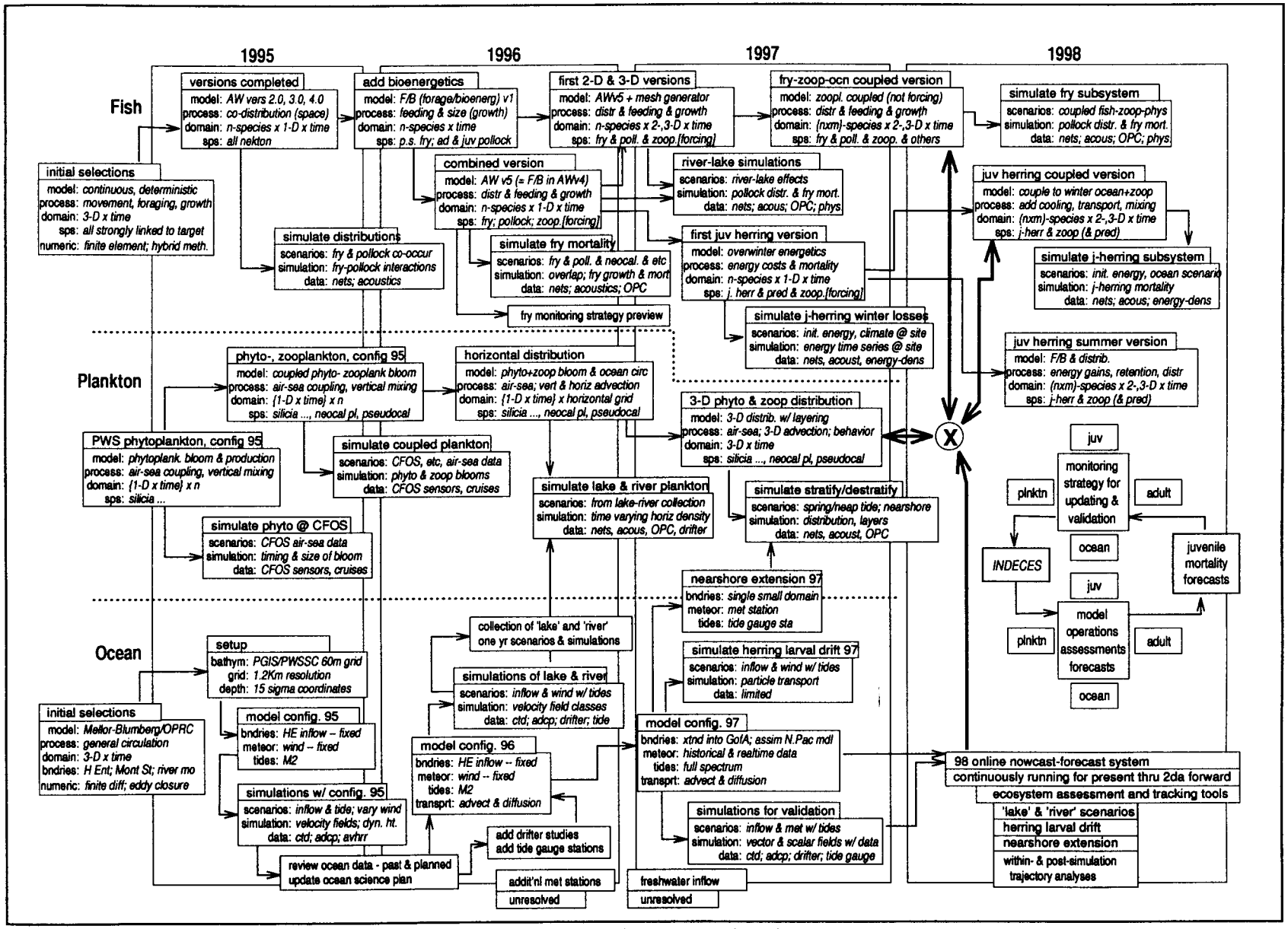


Figure 1. The SEA models: status, development plan, dependencies, and endpoints.

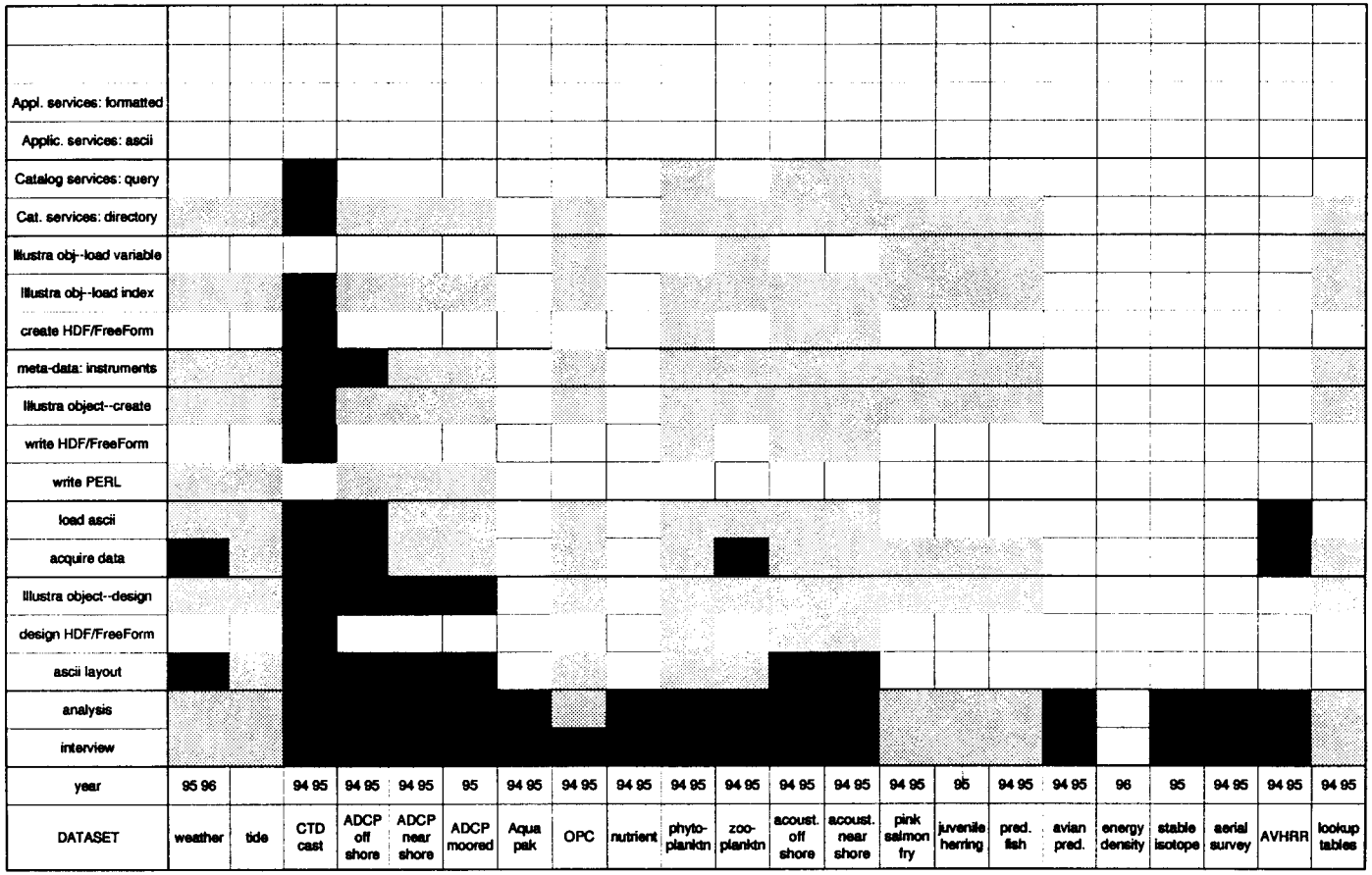


Figure 2. The SEA Datasets and the SEA Database; status for Oct. 95 (black), expected status for Oct. 96 (gray).

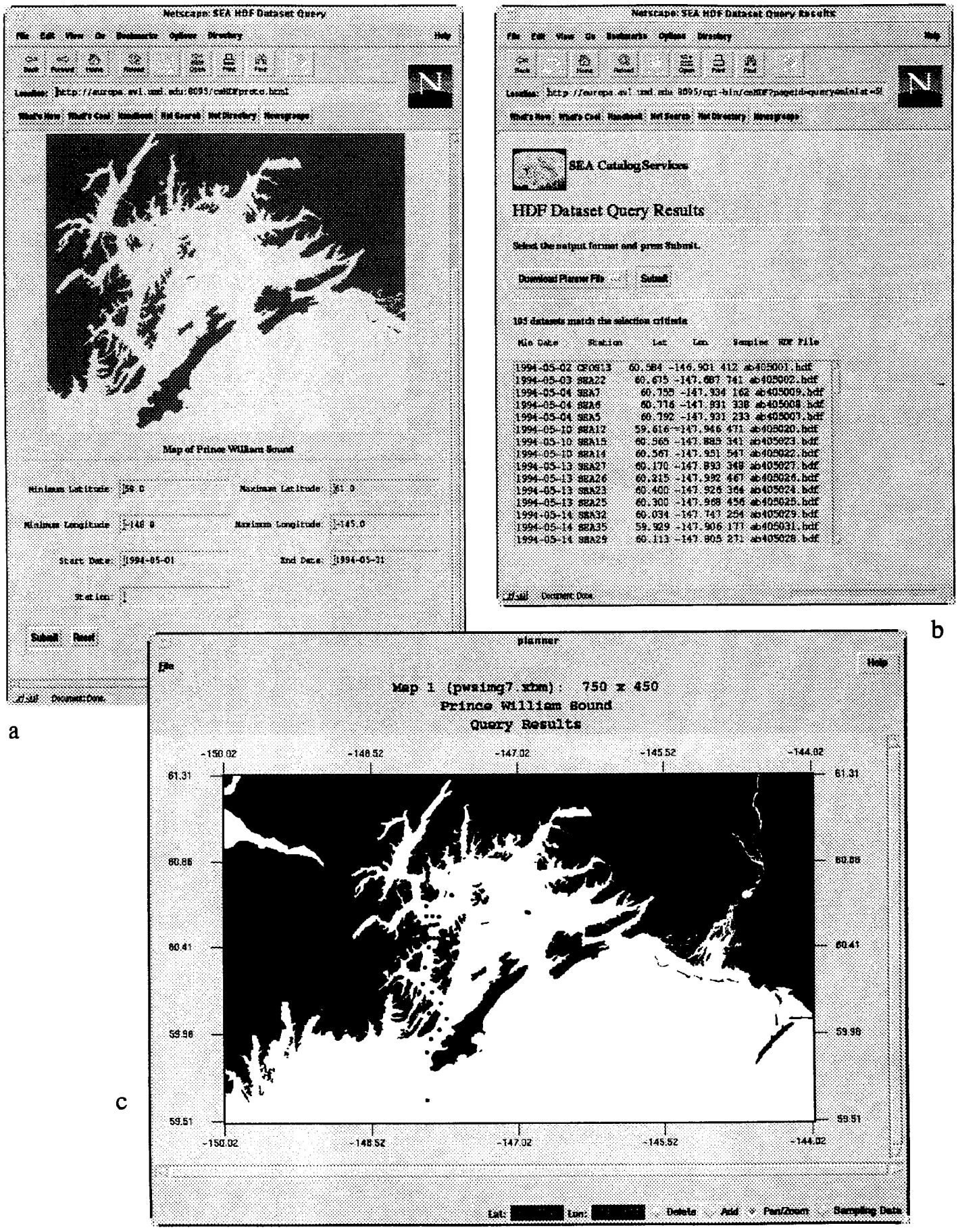


Figure 3: SEA prototype Netscape server: query form (a), query results (b), locations of results (c).

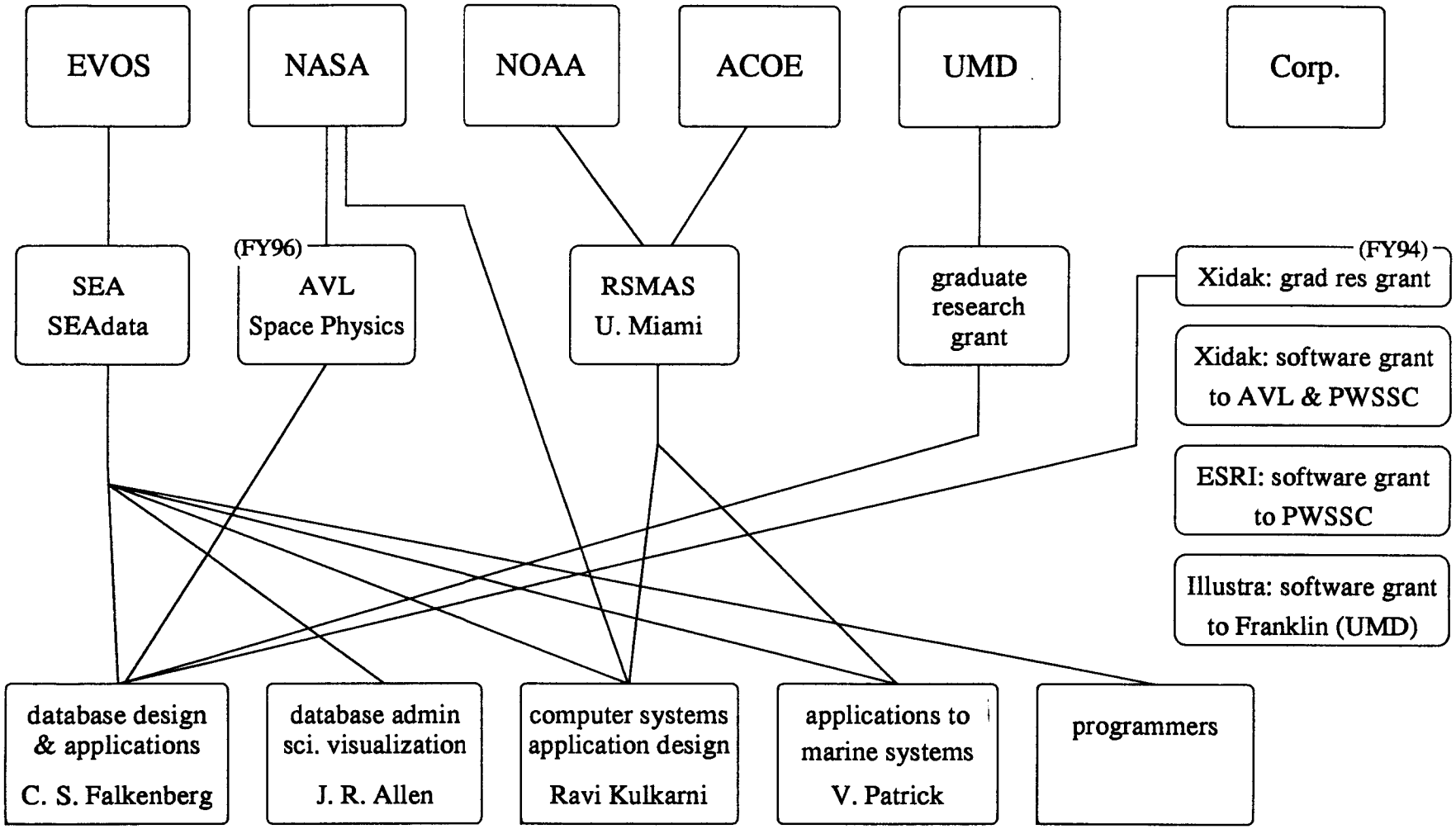


Figure 4. Cost-sharing for the SEA database development during FY95.

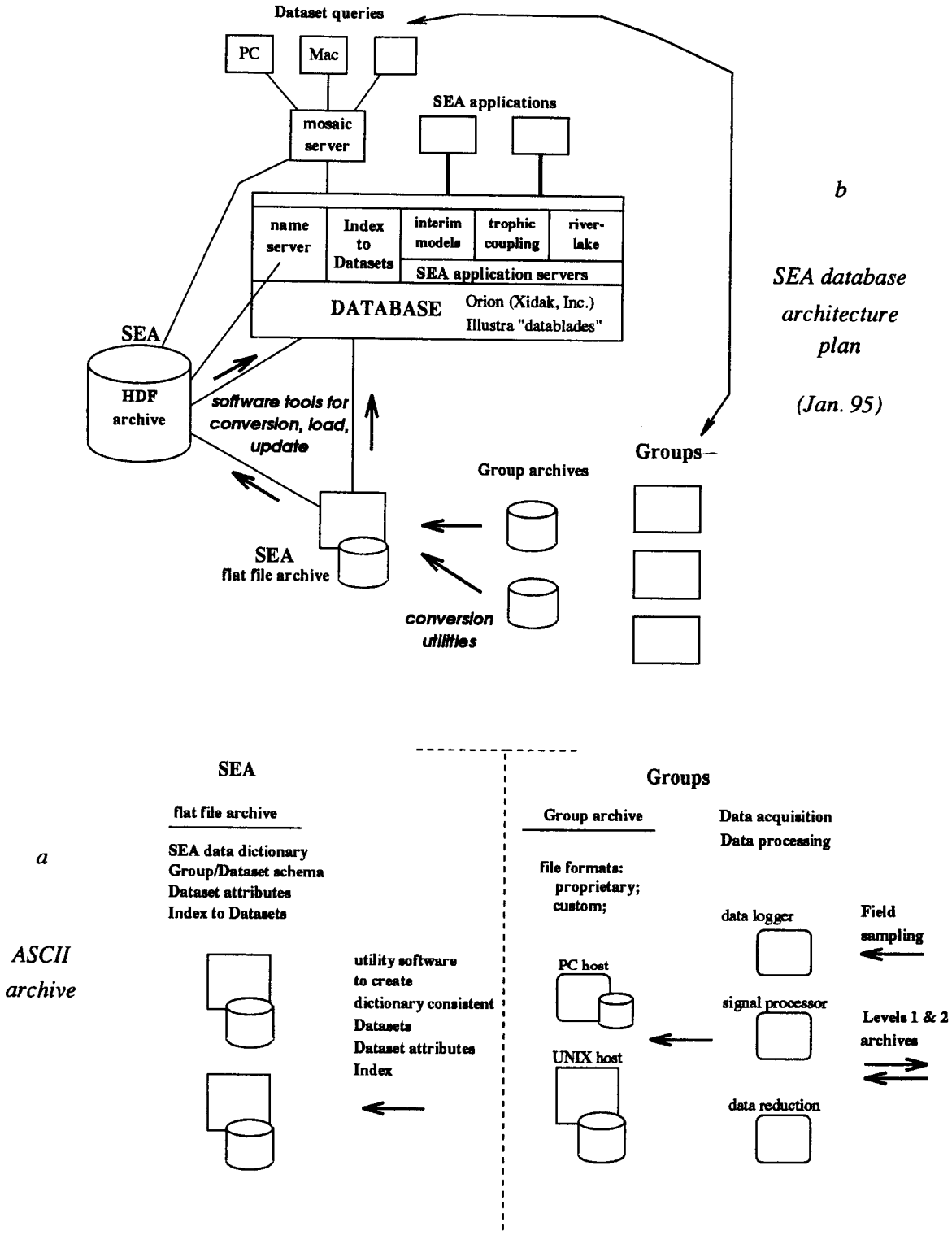


Figure 5. Plan for the SEA database, Jan. 1995.
ASCII archive (a) serves also as first ingestion step.
Database plan (b) show full complement of anticipated services.

Tree Diagram for processing, achiving, and disposition of biological samples

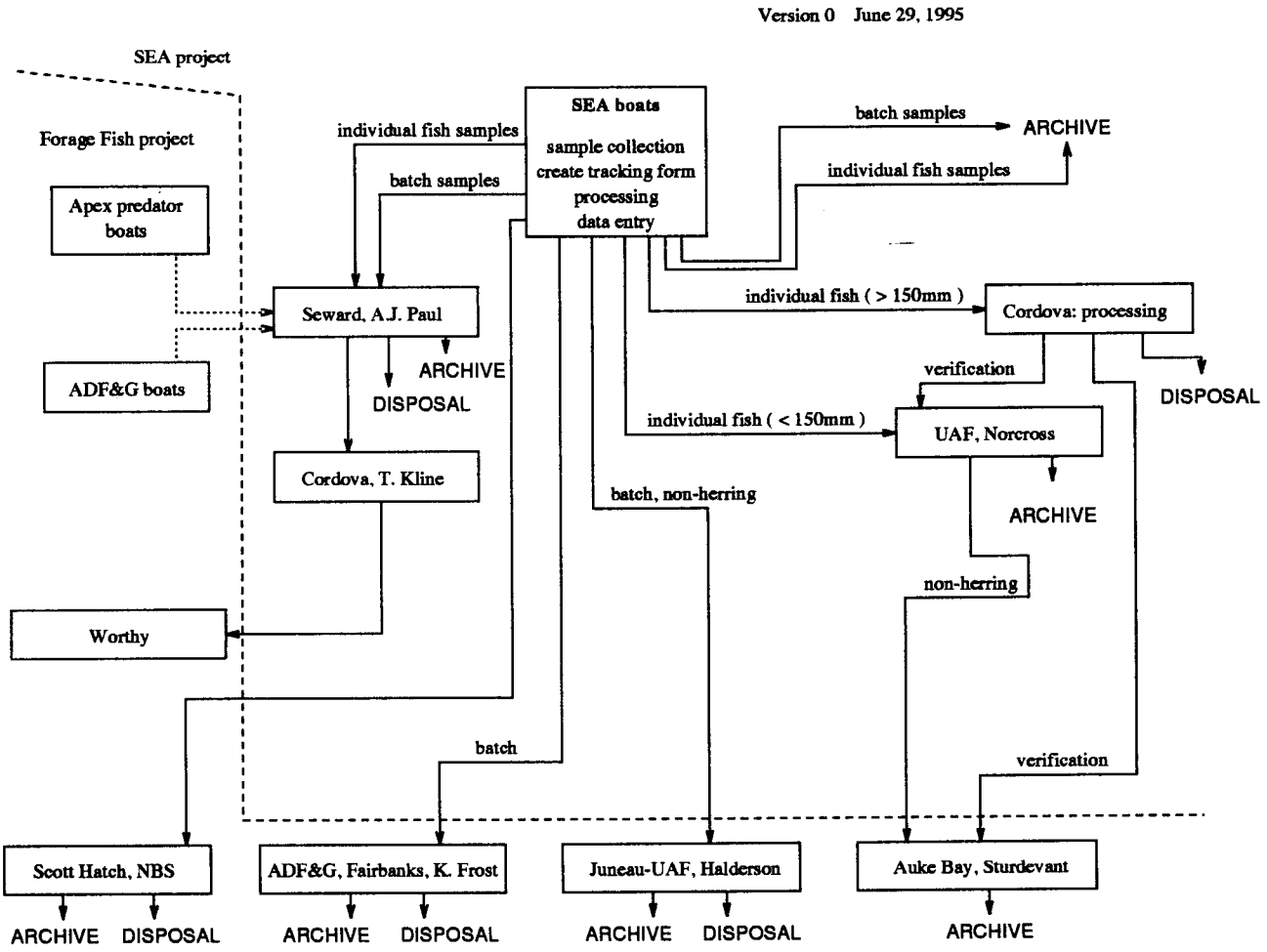
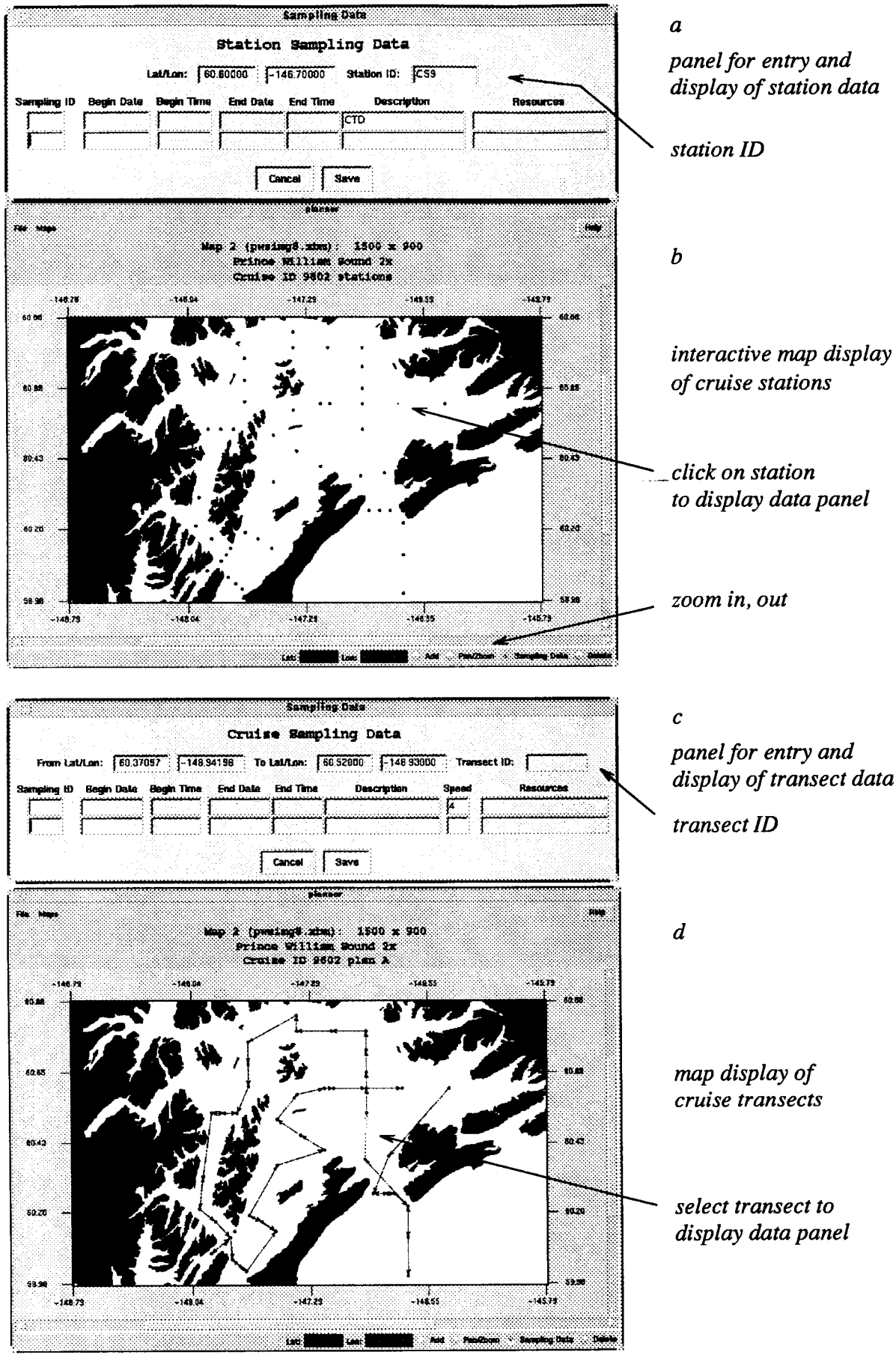


Figure 6. Diagram prepared to guide the development of the data dictionary entries for biological samples.



a

panel for entry and display of station data

station ID

b

interactive map display of cruise stations

click on station to display data panel

zoom in, out

c

panel for entry and display of transect data

transect ID

d

map display of cruise transects

select transect to display data panel

Figure 7. The user interface of the SEA cruise planning software.

```
Cruise ID 9602 plan A
HE13,60.2600000000,-146.8900000000,60.2600000000,-146.8900000000,,,,,CTD,,
HE11,60.2600000000,-146.7550000000,60.2600000000,-146.7550000000,,,,,CTD,,
SEA33,60.0150000000,-147.7000000000,60.0150000000,-147.7000000000,,,,,CTD,,
SEA31,60.0500000000,-147.7850000000,60.0500000000,-147.7850000000,,,,,CTD,,
SEA18B,60.5200000000,-147.7500000000,60.5200000000,-147.7500000000,,,,,CTD,,
SEA16,60.5200000000,-147.9100000000,60.5200000000,-147.9100000000,,,,,CTD,,
SEA26,60.2100000000,-147.9900000000,60.2100000000,-147.9900000000,,,,,CTD,,
SEA25,60.3000000000,-147.9700000000,60.3000000000,-147.9700000000,,,,,CTD,,
   ...

SEA32,60.0300000000,-147.7400000000,60.0300000000,-147.7400000000,,,,,CTD,,
SEA36,59.9100000000,-148.0700000000,59.9100000000,-148.0700000000,,,,,CTD,,
SEA37,59.8800000000,-148.0100000000,59.8800000000,-148.0100000000,,,,,CTD,,
MS8,60.4000000000,-147.2000000000,60.4000000000,-147.2000000000,,,,,CTD,,
MS8,60.4500000000,-147.3500000000,60.4500000000,-147.3500000000,,,,,CTD,,
MS5,60.1400000000,-147.5100000000,60.1400000000,-147.5100000000,,,,,CTD,,
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   ...

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,60.5191323693,-147.9106070710,60.5200000000,-147.8300000000,,,,,,,4,
,60.5191323693,-147.8305537020,60.5200000000,-147.7500000000,,,,,,,4,
,60.5191323693,-147.7505003340,60.5200000000,-147.9100000000,,,,,,,4,
```

a

cruise plan data is stored in a comma delimited test file

planner can read and display cruise lists from text editors and spreadsheets

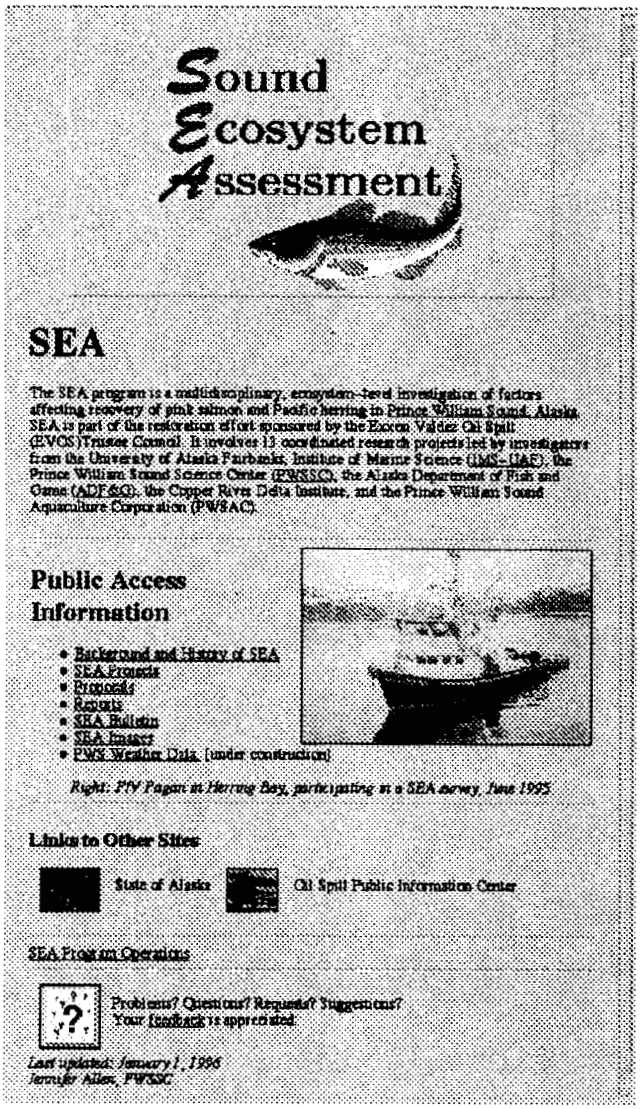
cruise planner file used with spreadsheet to compute transect times and resolve resource conflicts

	A	B	C	D	E	J	K	L	O	P
1								nts/hr	km	hours
2	HE13	60.2600	-146.8900	60.2600	-146.8900		CTD		0.0	
3	HE11	60.2600	-146.7550	60.2600	-146.7550		CTD		0.0	
4	SEA33	60.0150	-147.7000	60.0150	-147.7000		CTD		0.0	
5	SEA31	60.0500	-147.7850	60.0500	-147.7850		CTD		0.0	
6	SEA18B	60.5200	-147.7500	60.5200	-147.7500		CTD		0.0	
7	SEA16	60.5200	-147.9100	60.5200	-147.9100		CTD		0.0	
8	SEA26	60.2100	-147.9900	60.2100	-147.9900		CTD		0.0	
50		60.5992	-146.4016	60.7800	-146.9300		OB2->NS1	4.0	41.3	5.6
51		60.7794	-146.9300	60.7200	-146.9300		NS1->NS22	4.0	6.6	0.9
52		60.7194	-146.9300	60.6500	-146.9300		NS2->NS3	4.0	7.7	1.0
53		60.6493	-146.9300	60.5200	-146.9300		NS3->NS4	4.0	14.4	1.9
54		60.5191	-146.9300	60.3700	-146.9400		NS4->CS12	4.0	16.6	2.2
55		60.3710	-146.9420	60.2600	-146.8900		CS12->HE13	4.0	13.1	1.8

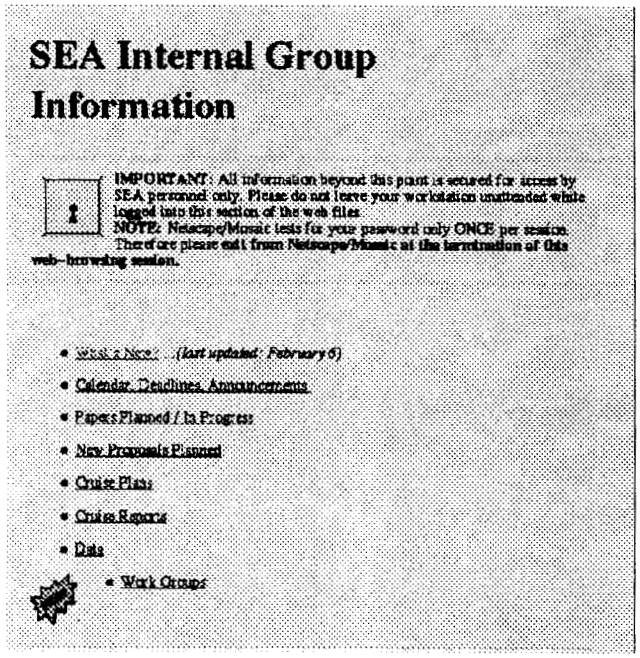
Sheet1

b

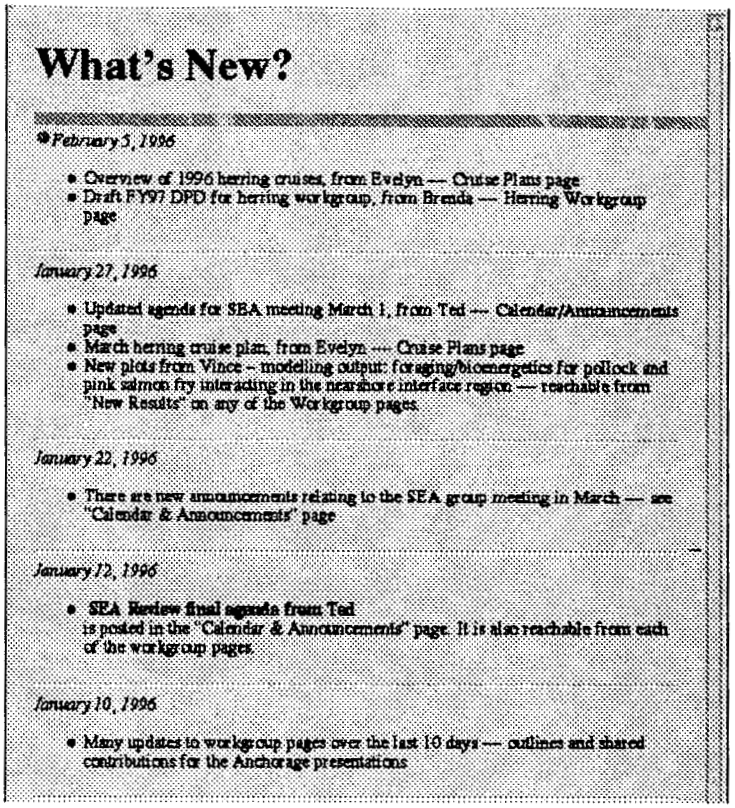
Figure 8. Cruise planner file structure (a).
Planner file used in a spreadsheet for confllict resolution and cruise optimization (b).



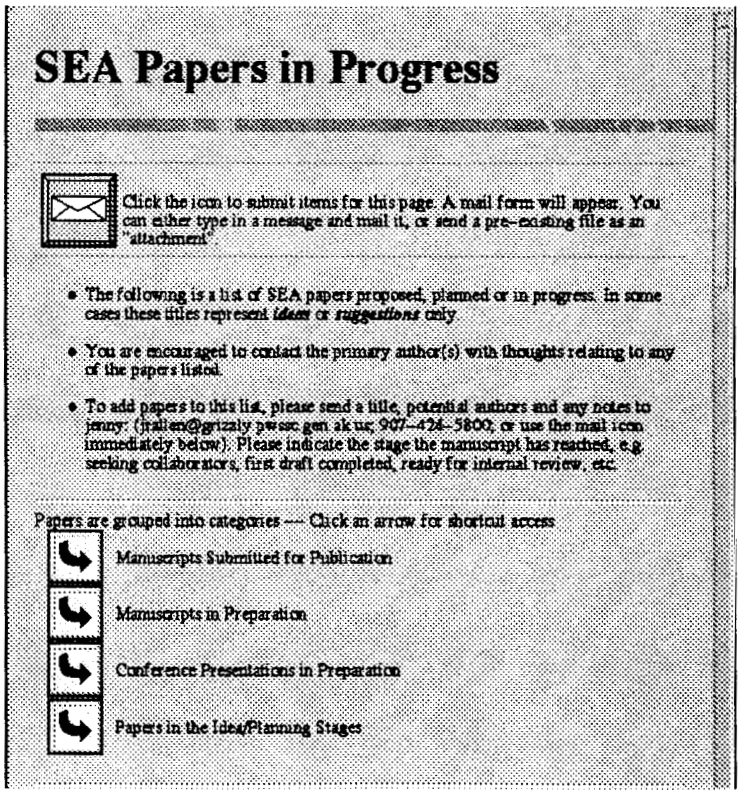
a



b



c



d

Figure 9. The SEA home page (a); samples of the project pages implemented to facilitate the SEA collaboration (b-d).

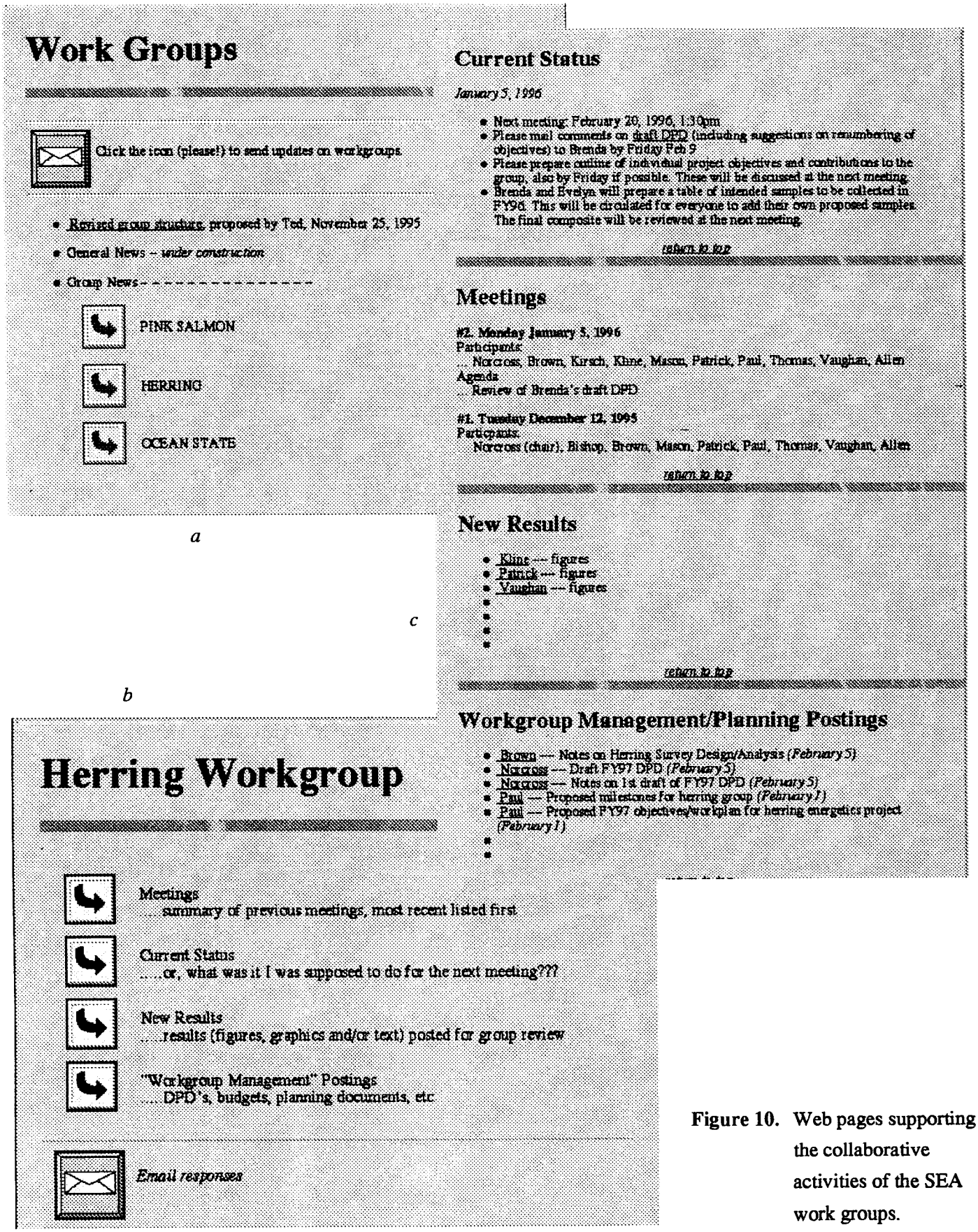


Figure 10. Web pages supporting the collaborative activities of the SEA work groups.

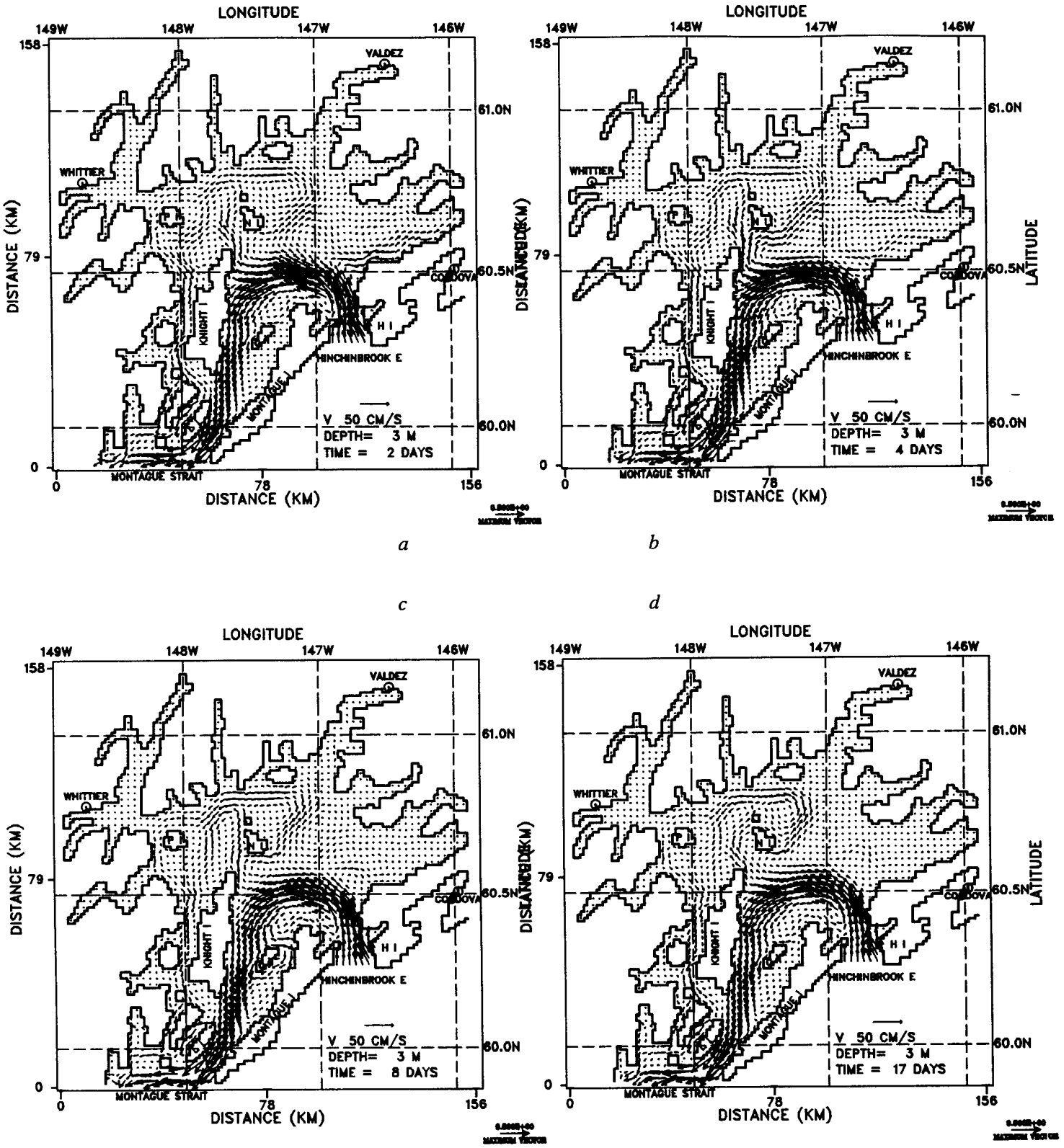
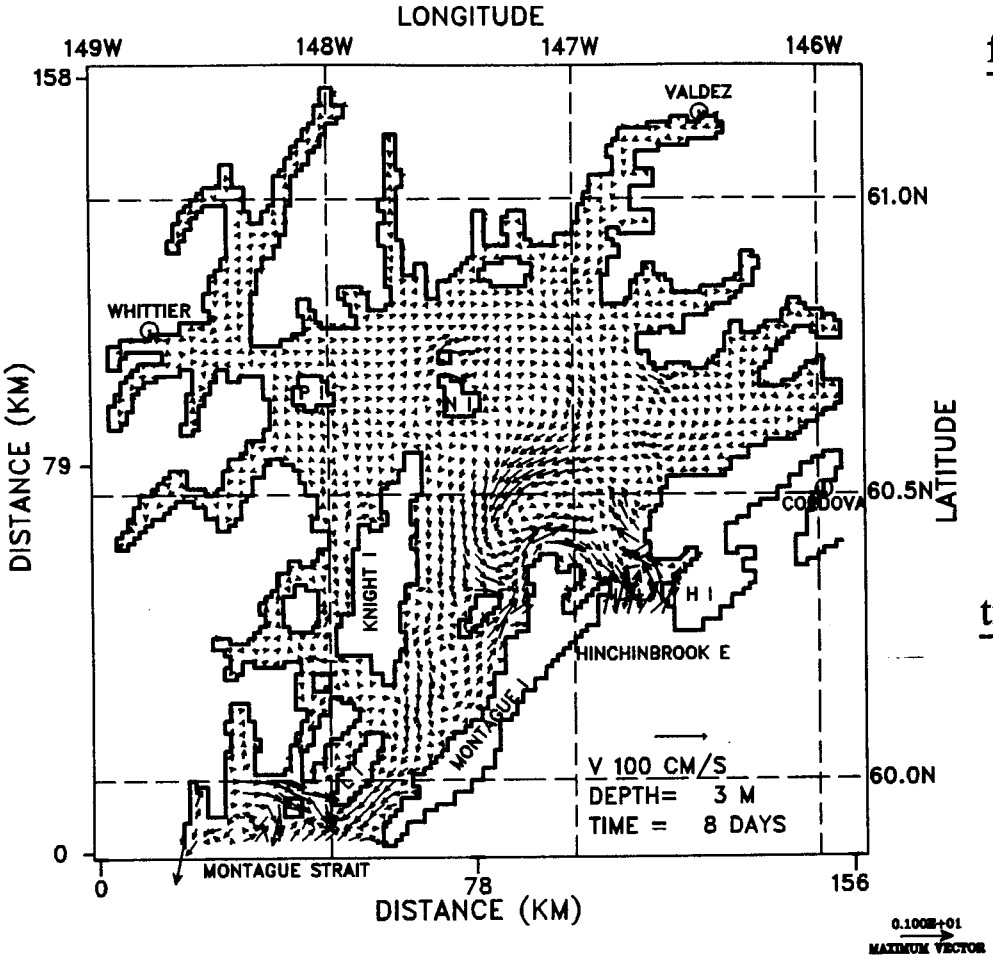


Figure 11. Velocity fields at 3m depth at 2 days (a), 4 days (b), 8 days (c), and 17 days (d) following 17 day spin-up. The sole forcing is in-flow of 0.3 Sv at Hinchinbrook Entrance.



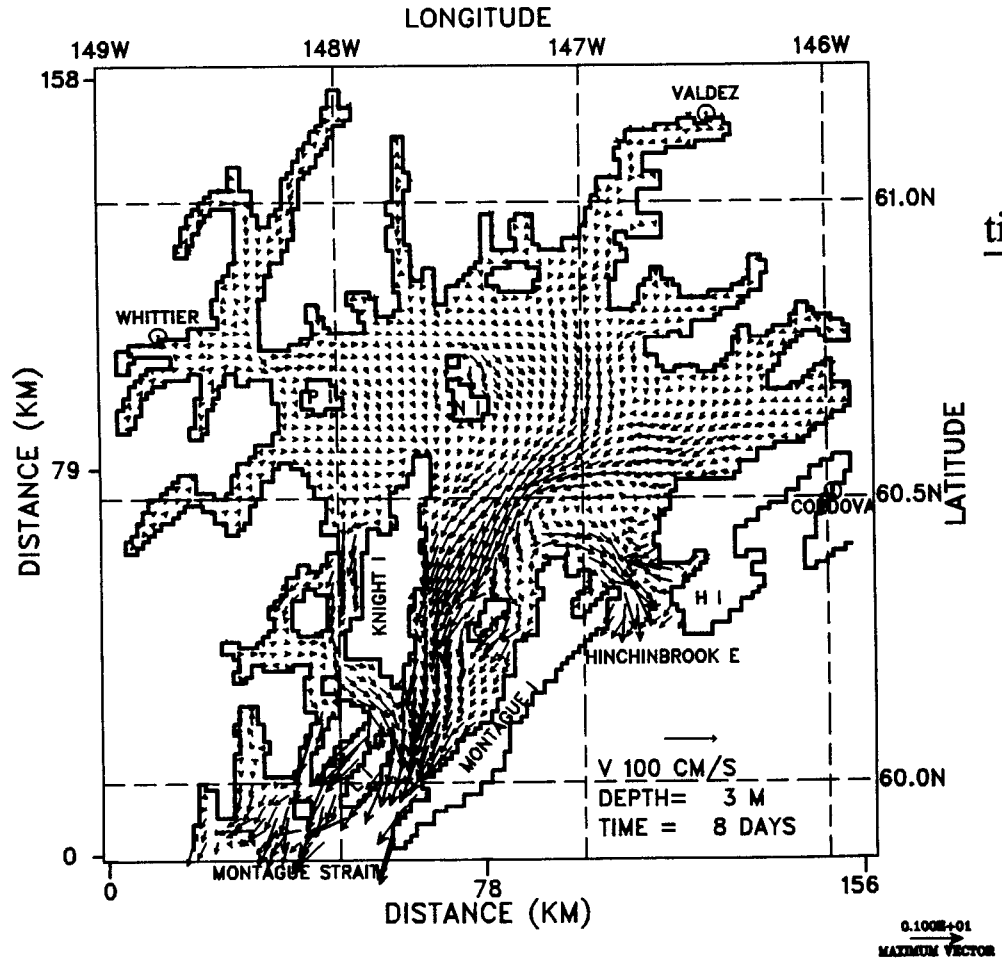
forcing:

in-flow at Hinchinbrook Entrance (HE)

M_2 tide

tide stage:

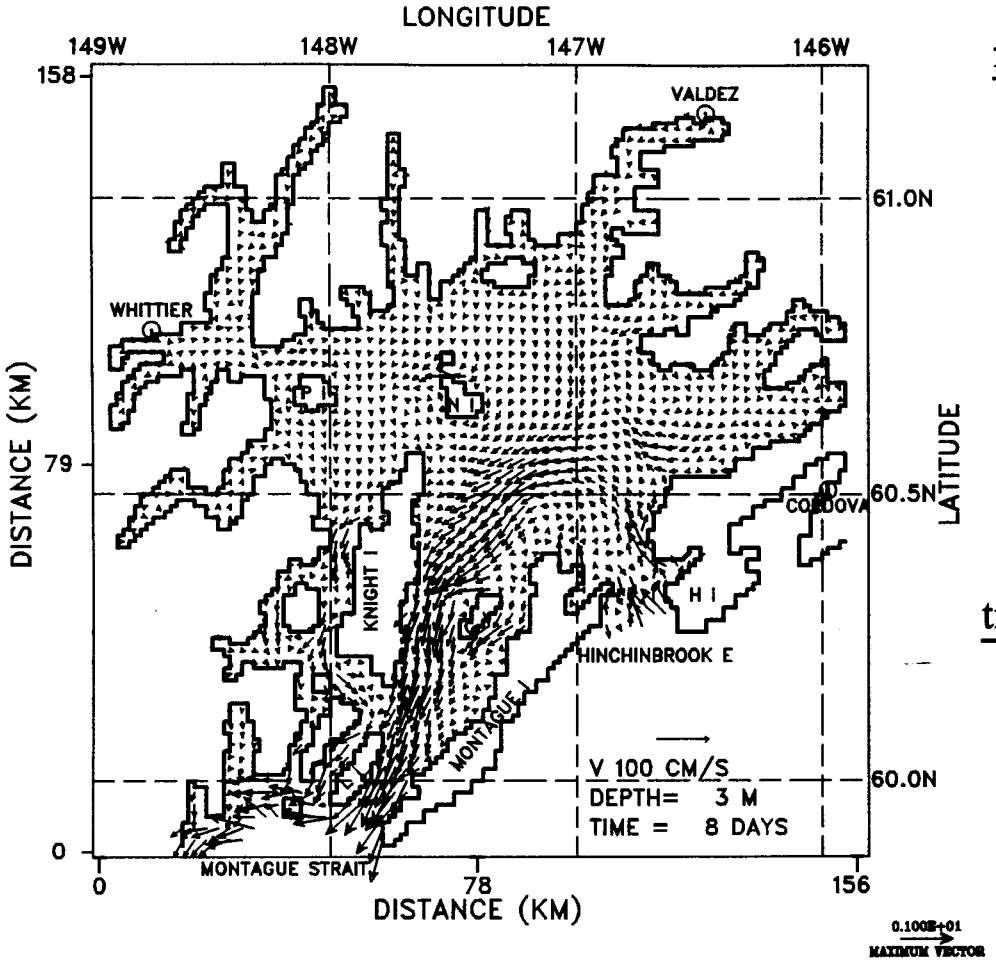
high at HE



tide stage:

ebb at HE

Figure 12. Velocity fields at 3m depth at high tide and at ebb tide.



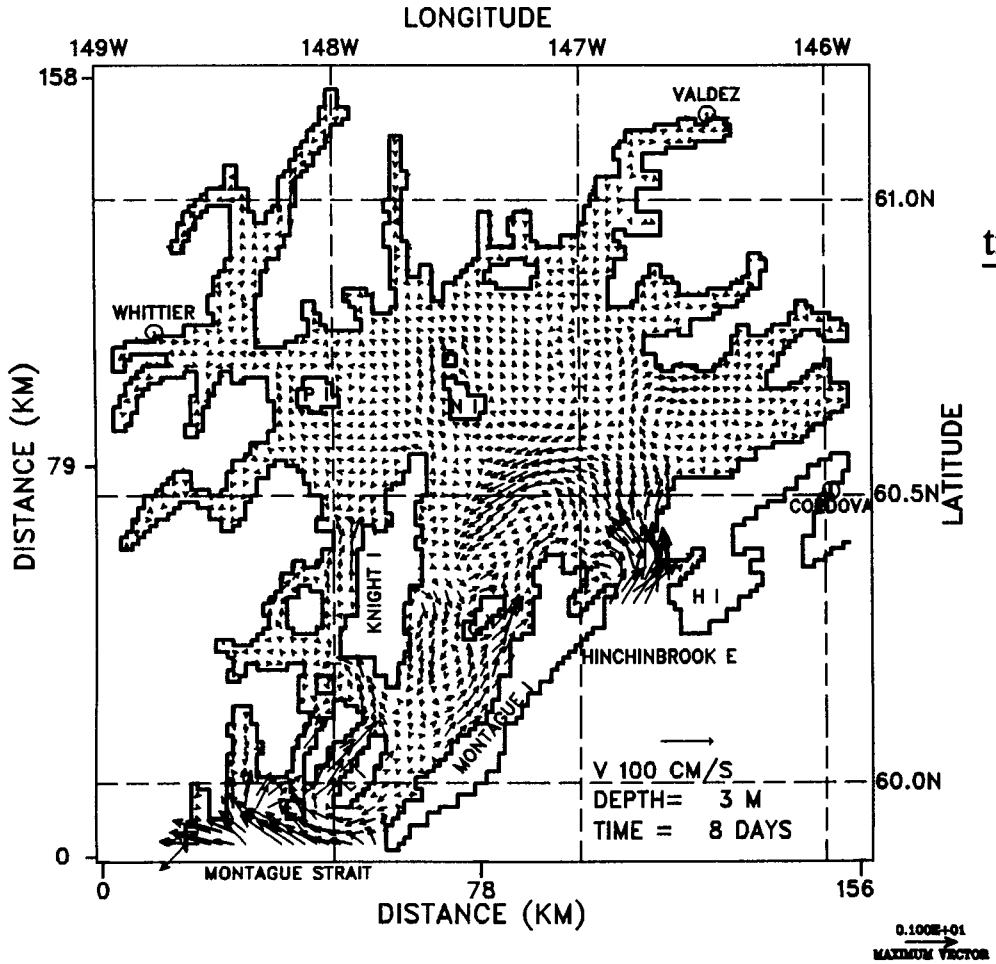
forcing:

in-flow at Hinchinbrook Entrance (HE)

M_2 tide

tide stage:

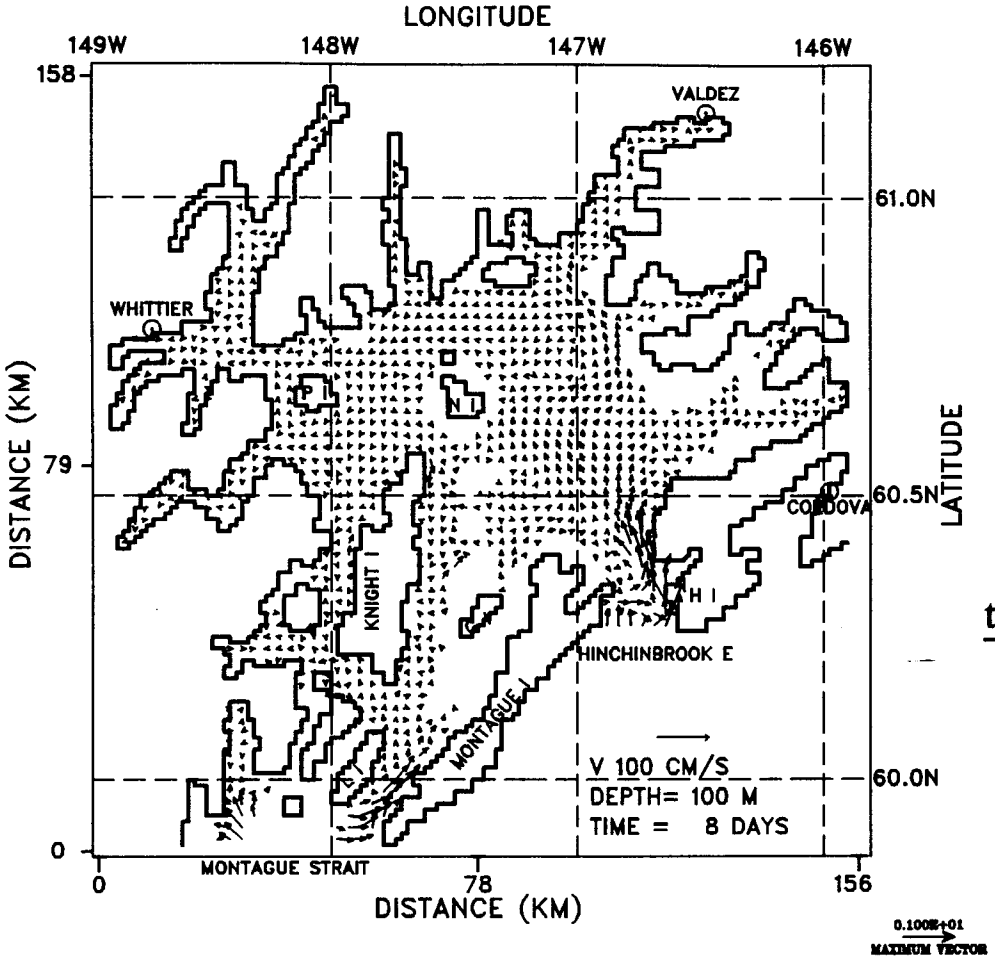
low at HE



tide stage:

flood at HE

Figure 13. Velocity fields at 3m depth at low tide and at flood tide.



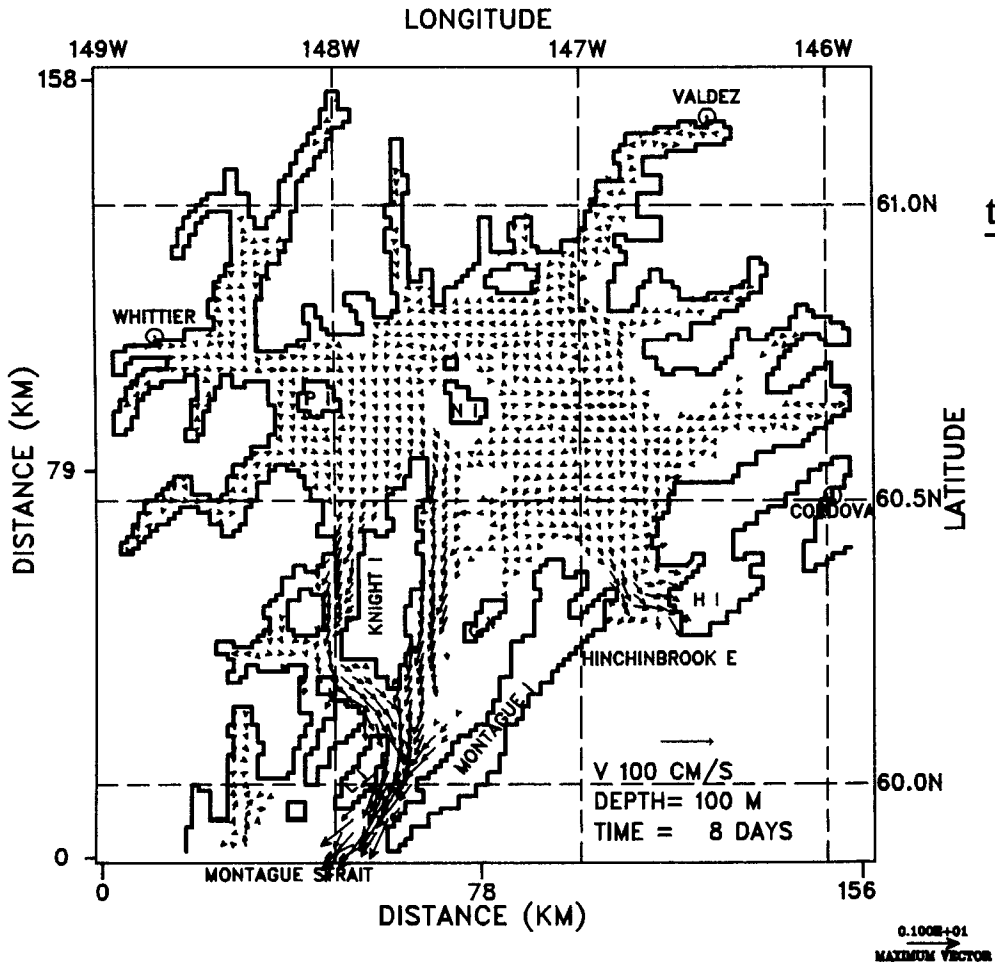
forcing:

in-flow at Hinchinbrook Entrance (HE)

M_2 tide

tide stage:

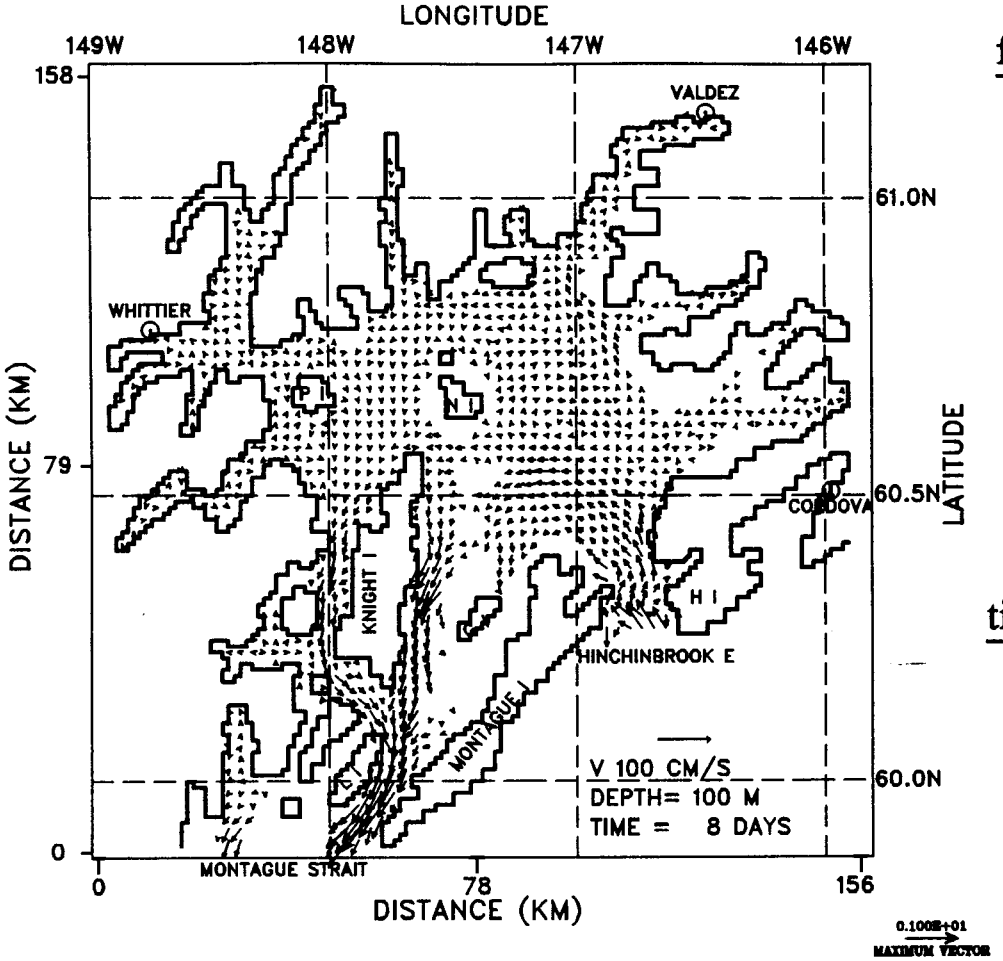
high at HE



tide stage:

ebb at HE

Figure 14. Velocity fields at 100m depth at high tide and at ebb tide.



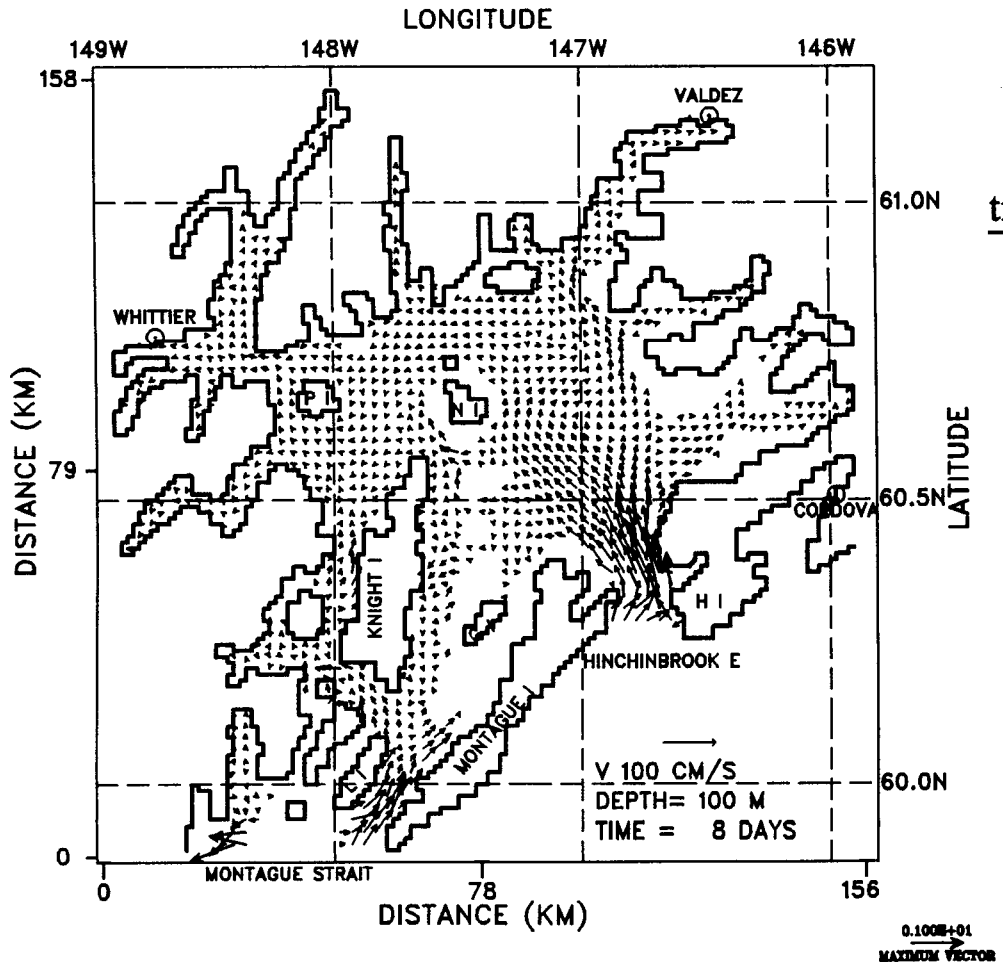
forcing:

in-flow at Hinchinbrook Entrance (HE)

M_2 tide

tide stage:

low at HE



tide stage:

flood at HE

Figure 15. Velocity fields at 100m depth at low tide and at flood tide.

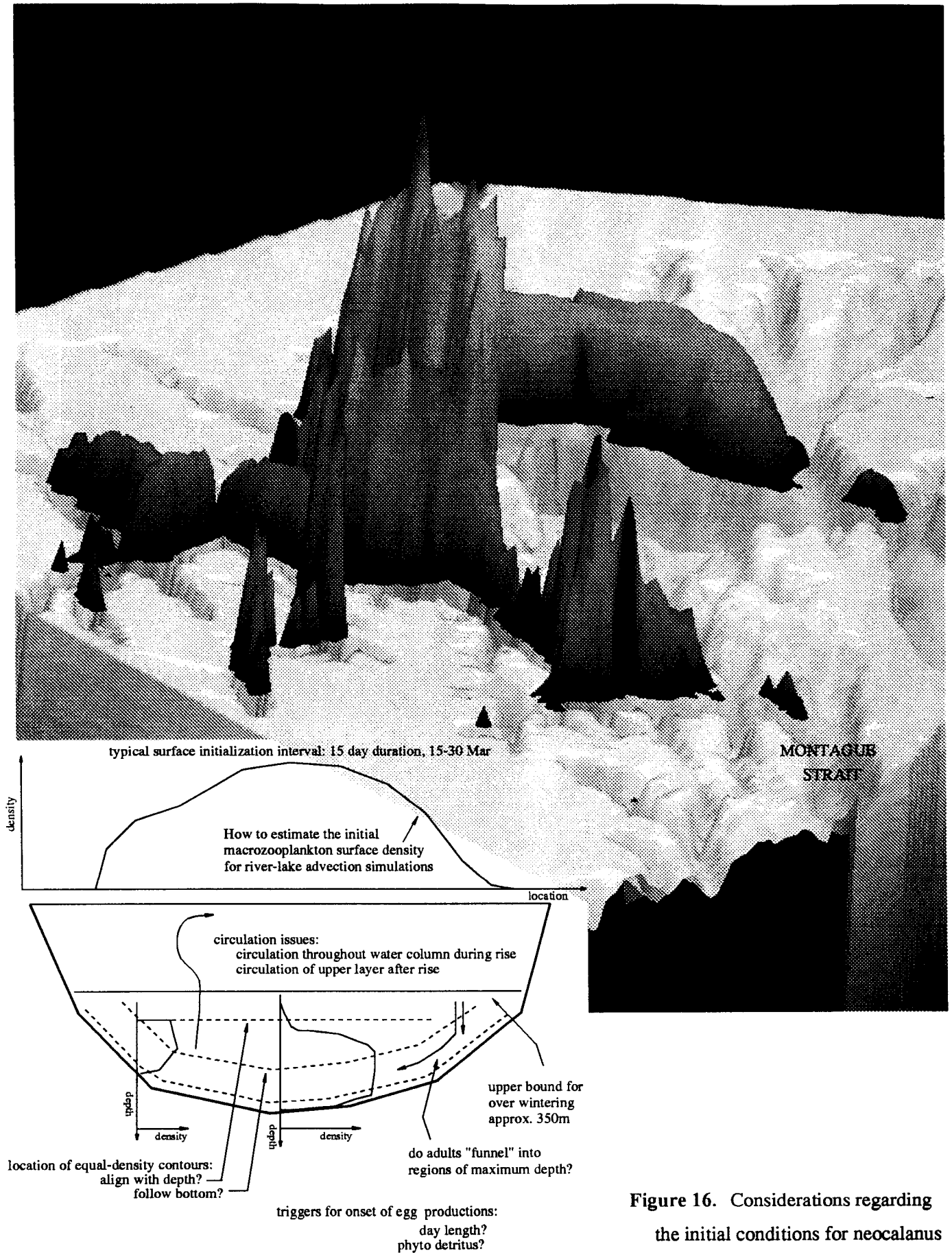


Figure 16. Considerations regarding the initial conditions for neocalanus in river-lake simulations.

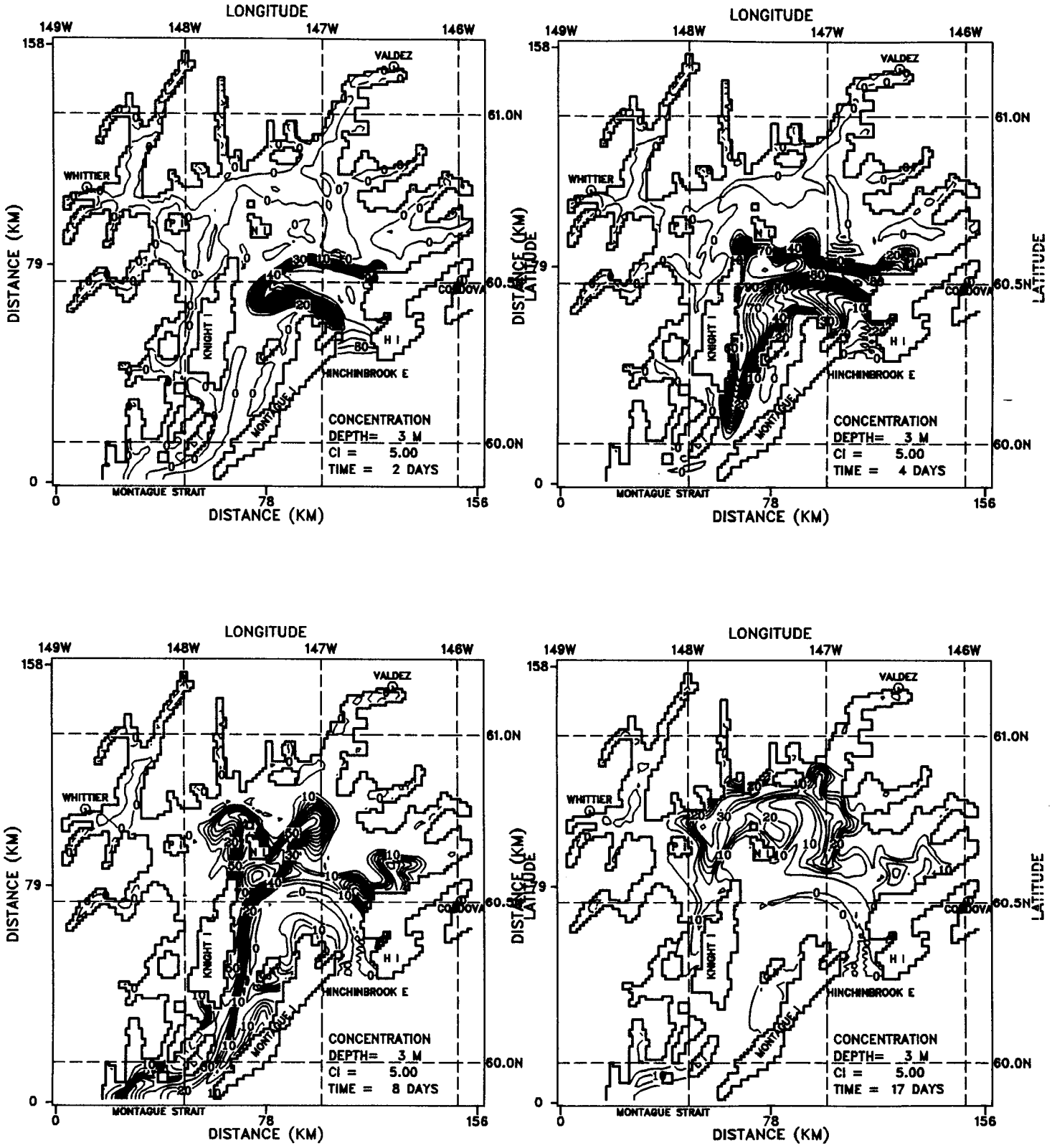


Figure 17. Time varying tracer concentration at 3m depth due to the circulation fields shown in Figure 11. After a 17 day spin-up the tracer is released along the upper 40m of the model boundary at Hinchinbrook Entrance at a uniform rate and for a period of 6 days.

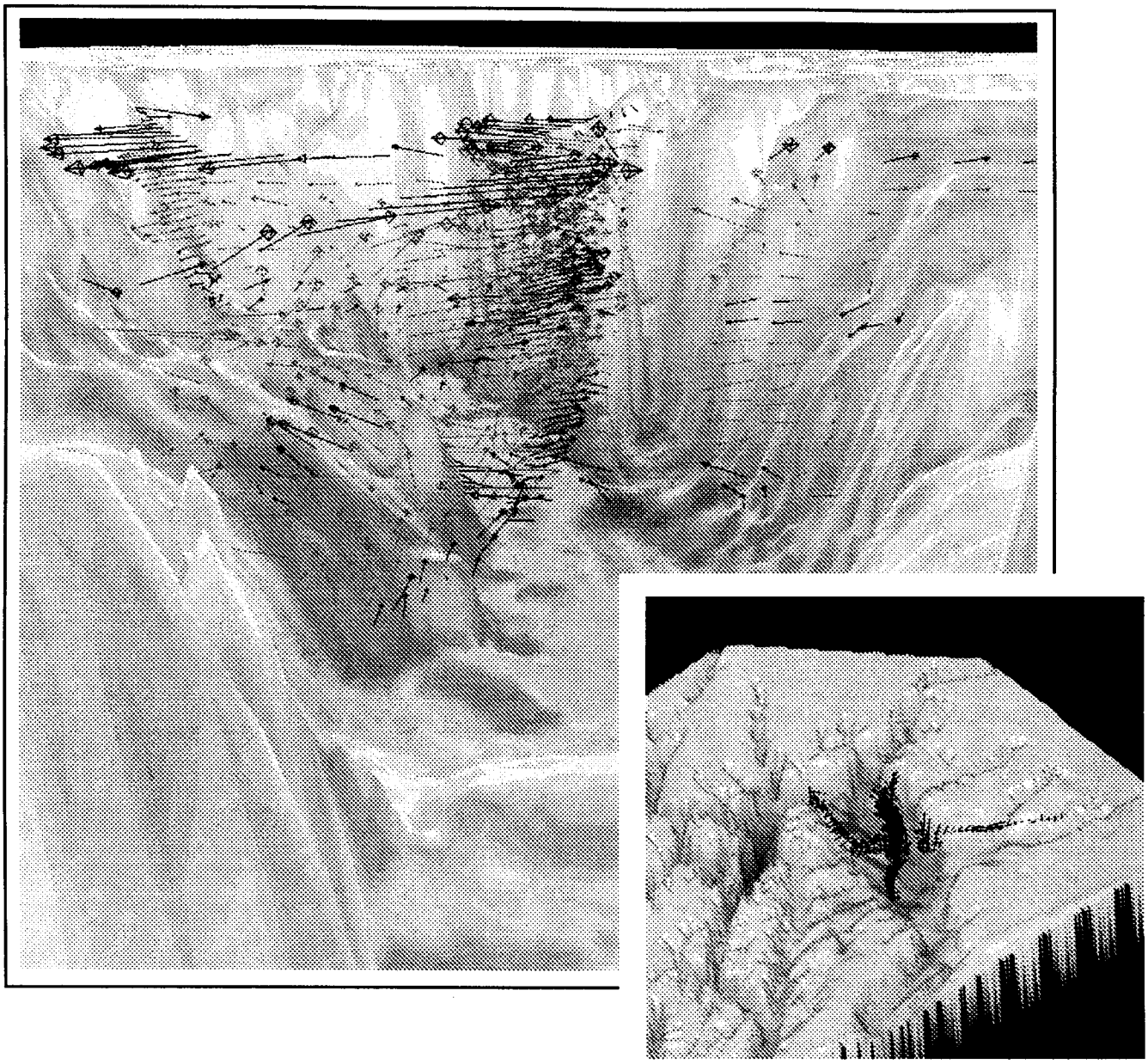


Figure 18. Velocity fields from ADCP transects. The time of the transects extends over multiple tide stages. Both time and position are needed to compare tide model output with towed ADCP data.

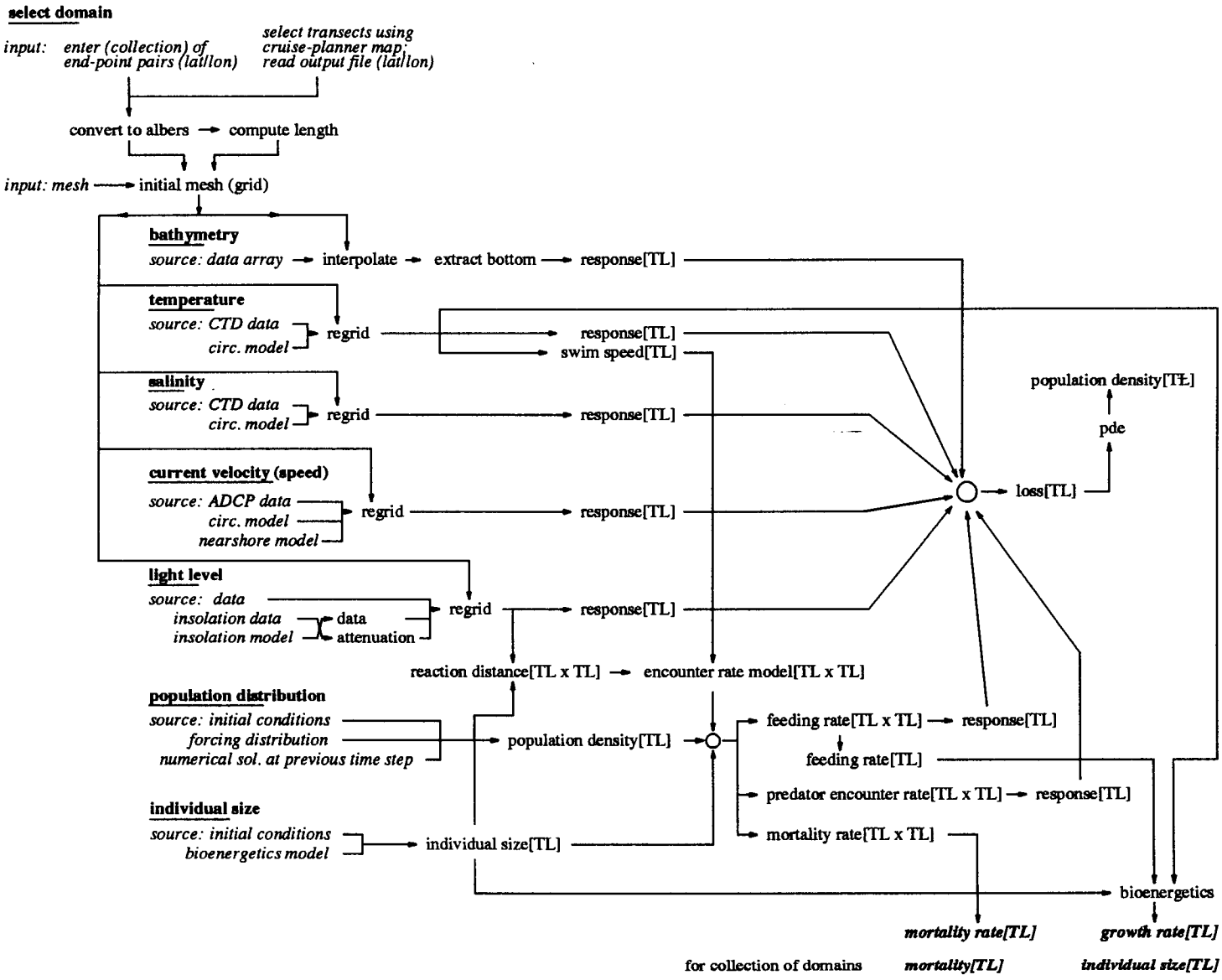


Figure 19. Informal chart representation of the fish model. The arrows indicate the flow of information from "input" to "output."

Figure 20. Set of one-dimensional domains suitable for the one-dimensional fish model.

population density $u(x,t)$

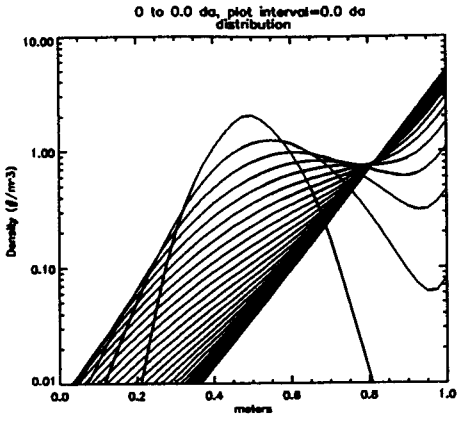
population *flux* (redistribution)

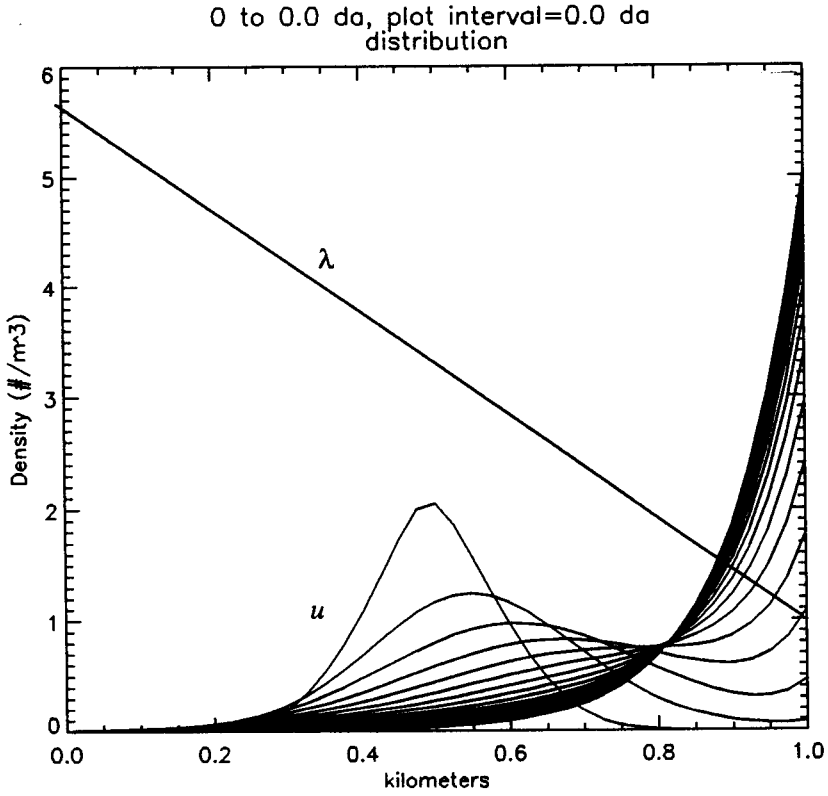
by diffusion $-D\dfrac{\partial u}{\partial x}$ (from high density u to low)

by taxis $-\chi u\dfrac{\partial \lambda}{\partial x}$ (from high loss λ to low)

$$D\frac{\partial u}{\partial x} + \chi u\frac{\partial \lambda}{\partial x} = 0 \quad \text{for } x \text{ on boundary}$$

$$\frac{\partial u}{\partial t} = D\frac{\partial^2 u}{\partial x^2} + \chi\frac{\partial}{\partial x}\left(u\frac{\partial \lambda}{\partial x}\right) \quad \text{for } x \text{ in domain}$$





to increases loss :

decrease feeding rate increase predation pressure

↘ ↙

λ goes up

↗

change environment to increase stress

(e.g., change current velocity, salinity, temperature)

Figure 21. Debugging example: linearly decreasing loss function with distribution initially concentrated near the middle of the domain.

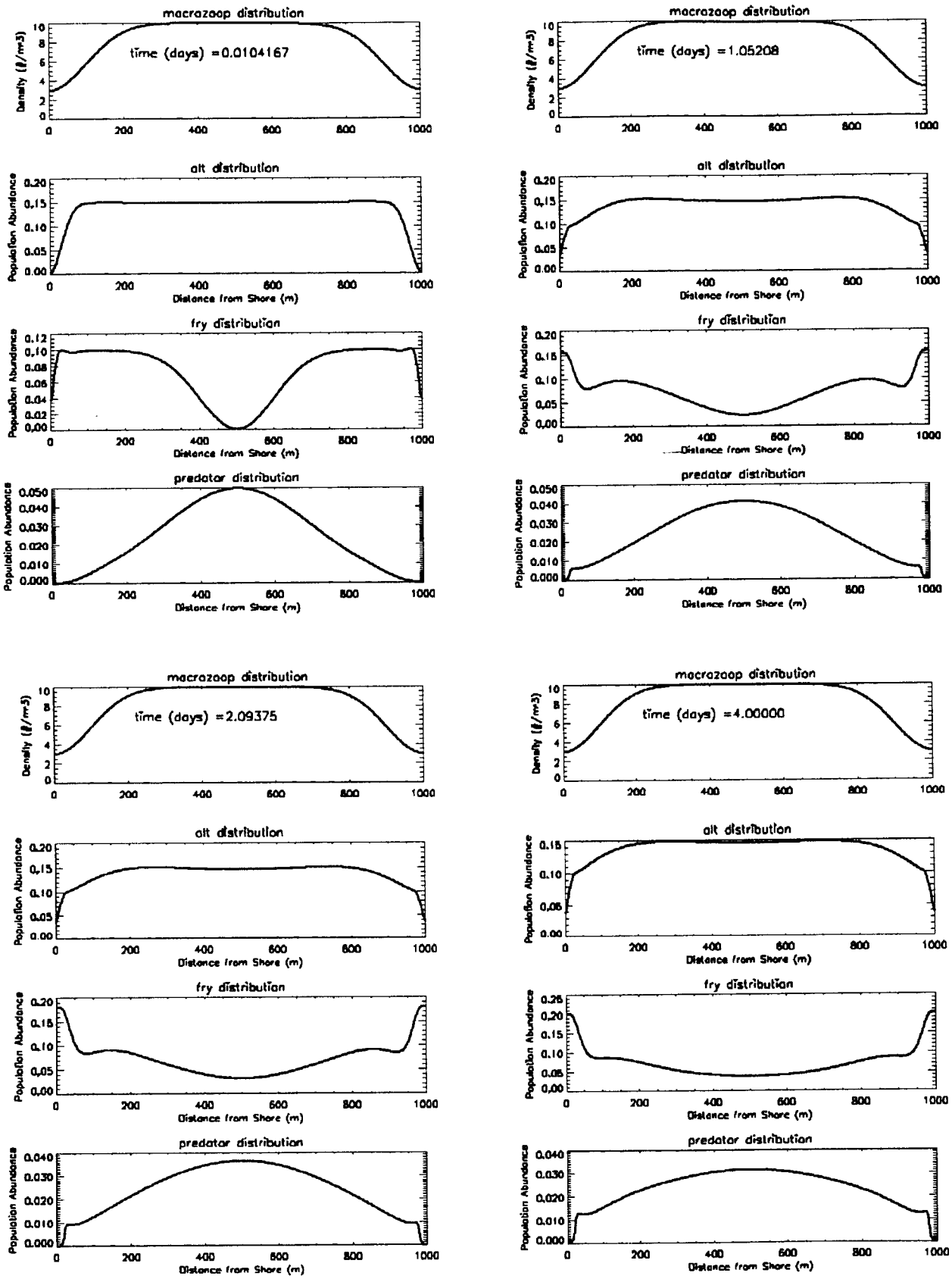
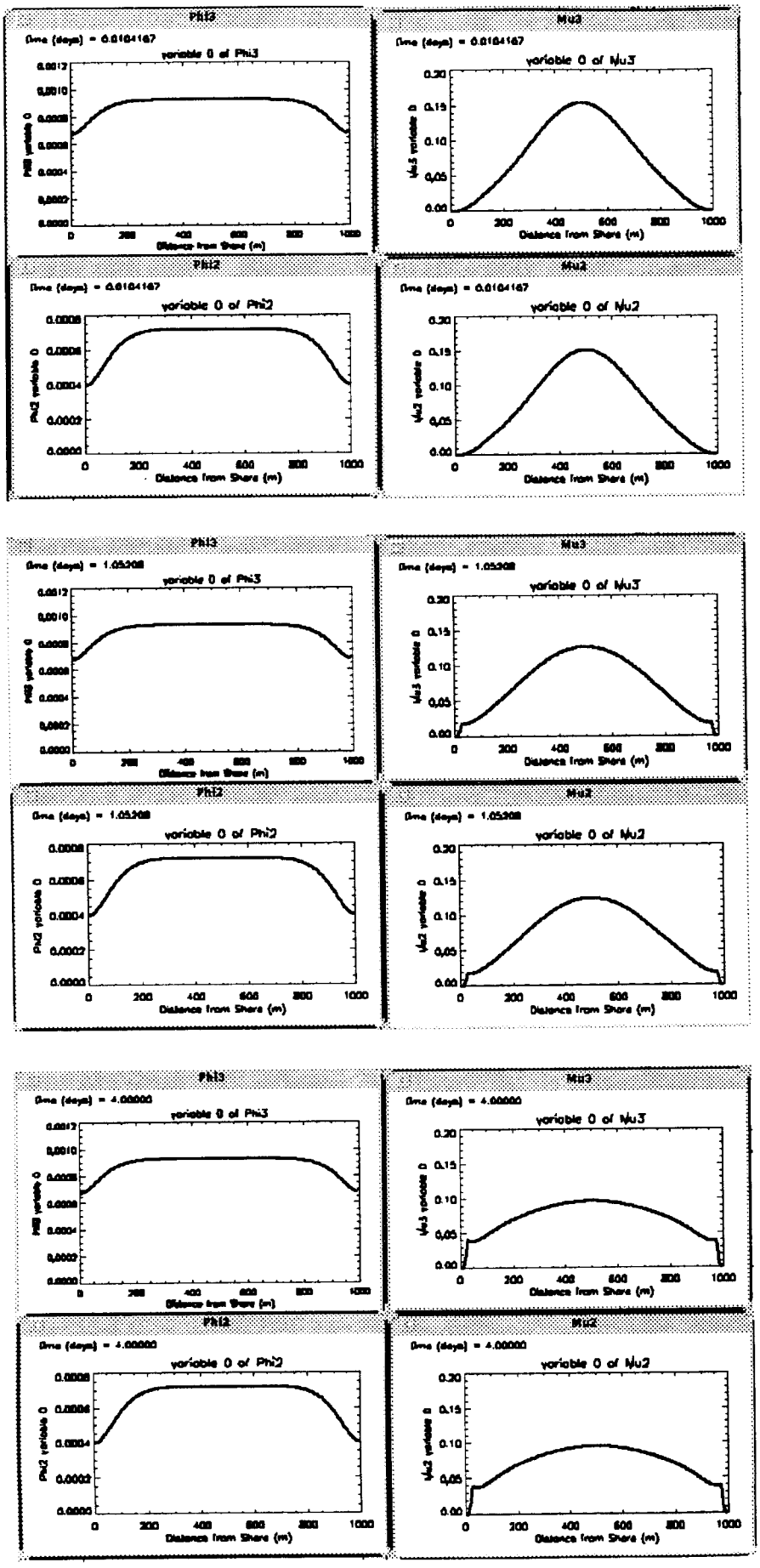


Figure 22. Four day spin-up of coupled system with four trophic levels. The trophic levels are neocalanus, age-0 pollock, pink salmon fry, and adult pollock.



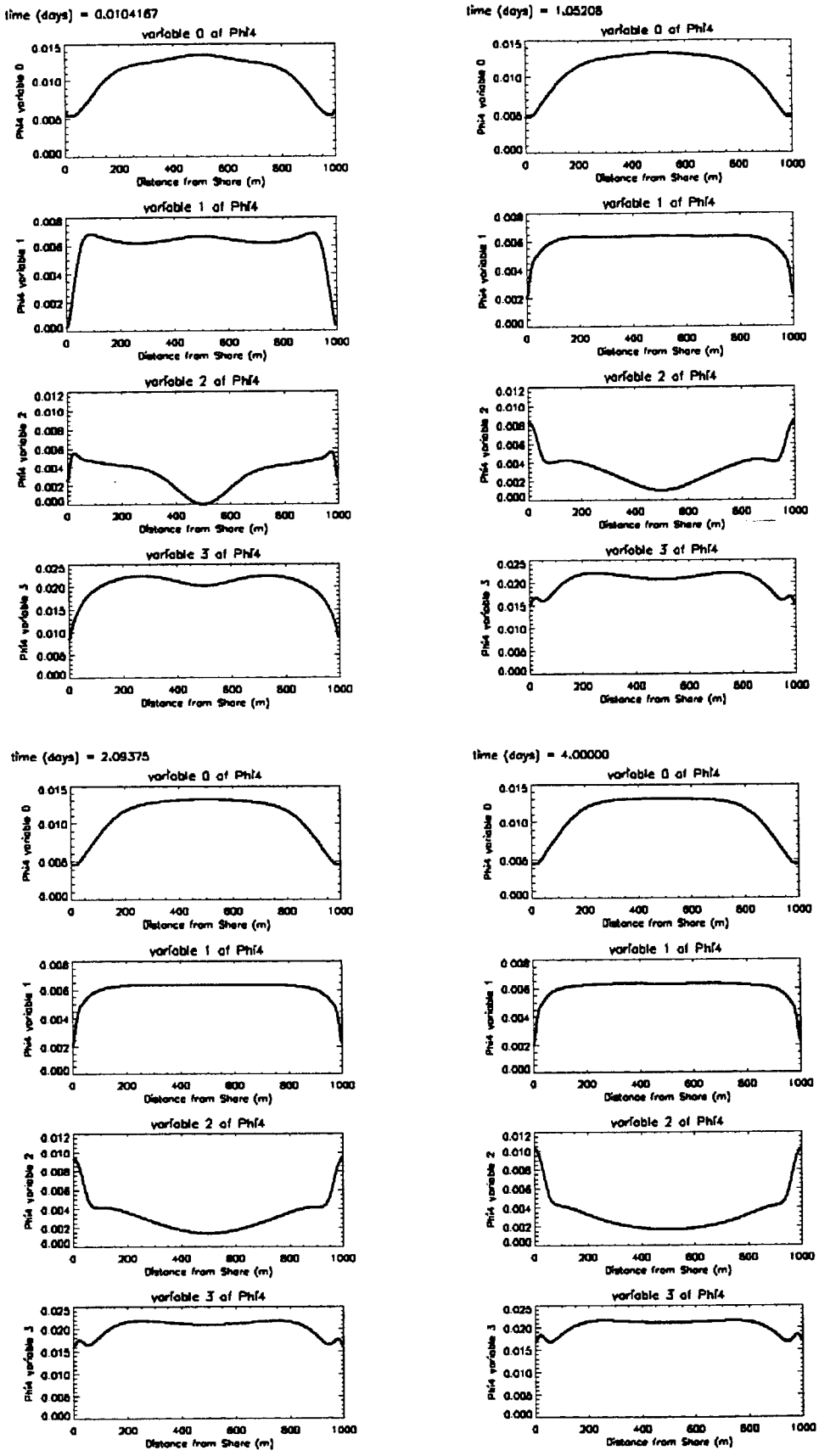
upper panels (xx3): pink salmon fry

left panel: feeding rate

right panel: predator encounter rate

lower panels (xx2): age-0 pollock

Figure 23. The manner in which the rates for feeding and for the encounter with predators change during the four day spin-up



variable 0:
feeding rate of adult pollock on neocalanus.

variable 1:
feeding rate of adult pollock on age-0 pollock.

variable 2:
feeding rate of adult pollock on pink salmon fry

variable 3:
total feeding rate of adult pollock

Figure 24. The change in the species specific and total feeding rates of adult pollock during the four day spin-up.

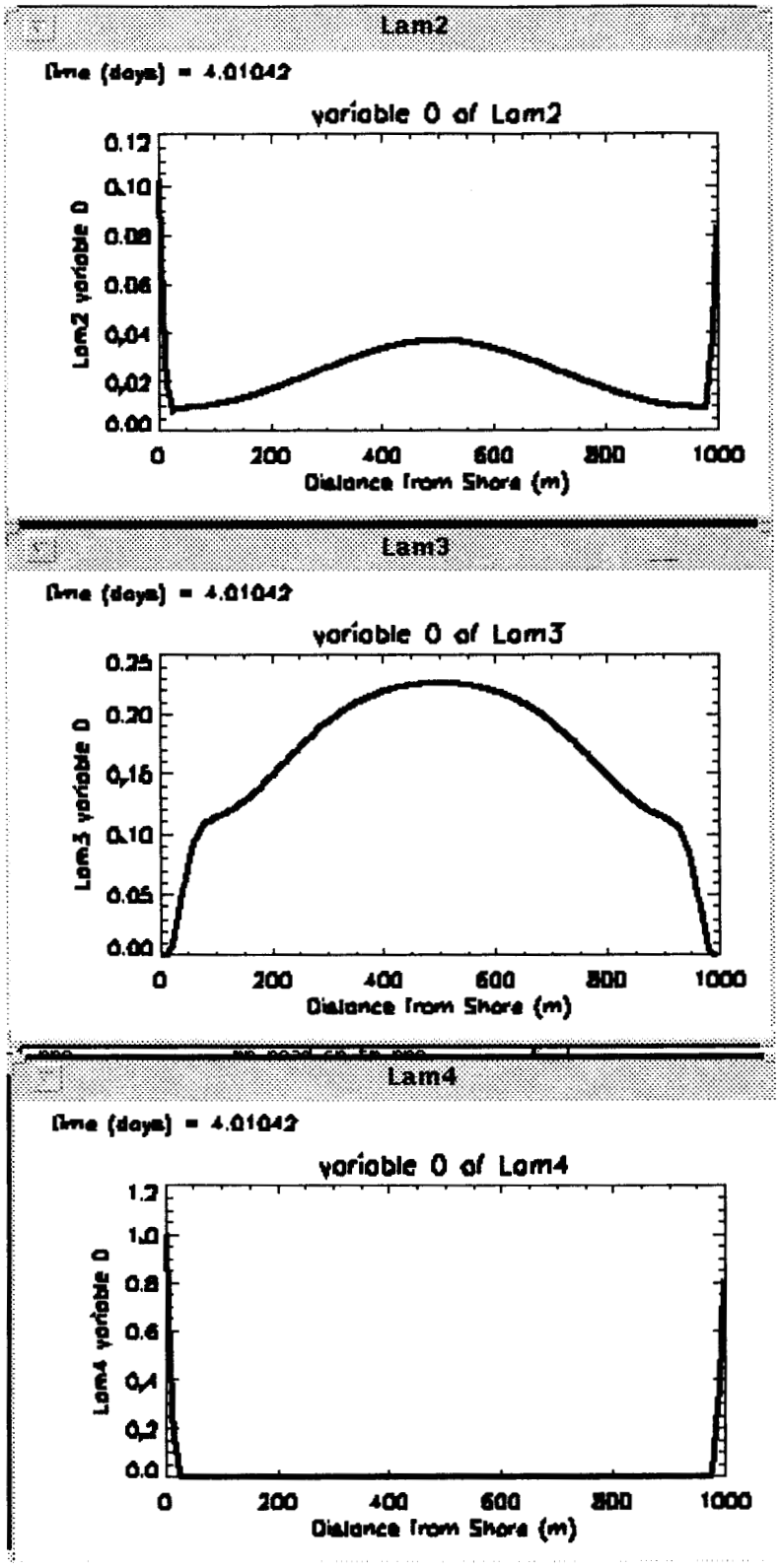


Figure 25. For population densities in a steady state the gradient of the loss function is balanced by the gradient of the density function, up to a constant scaling factor.

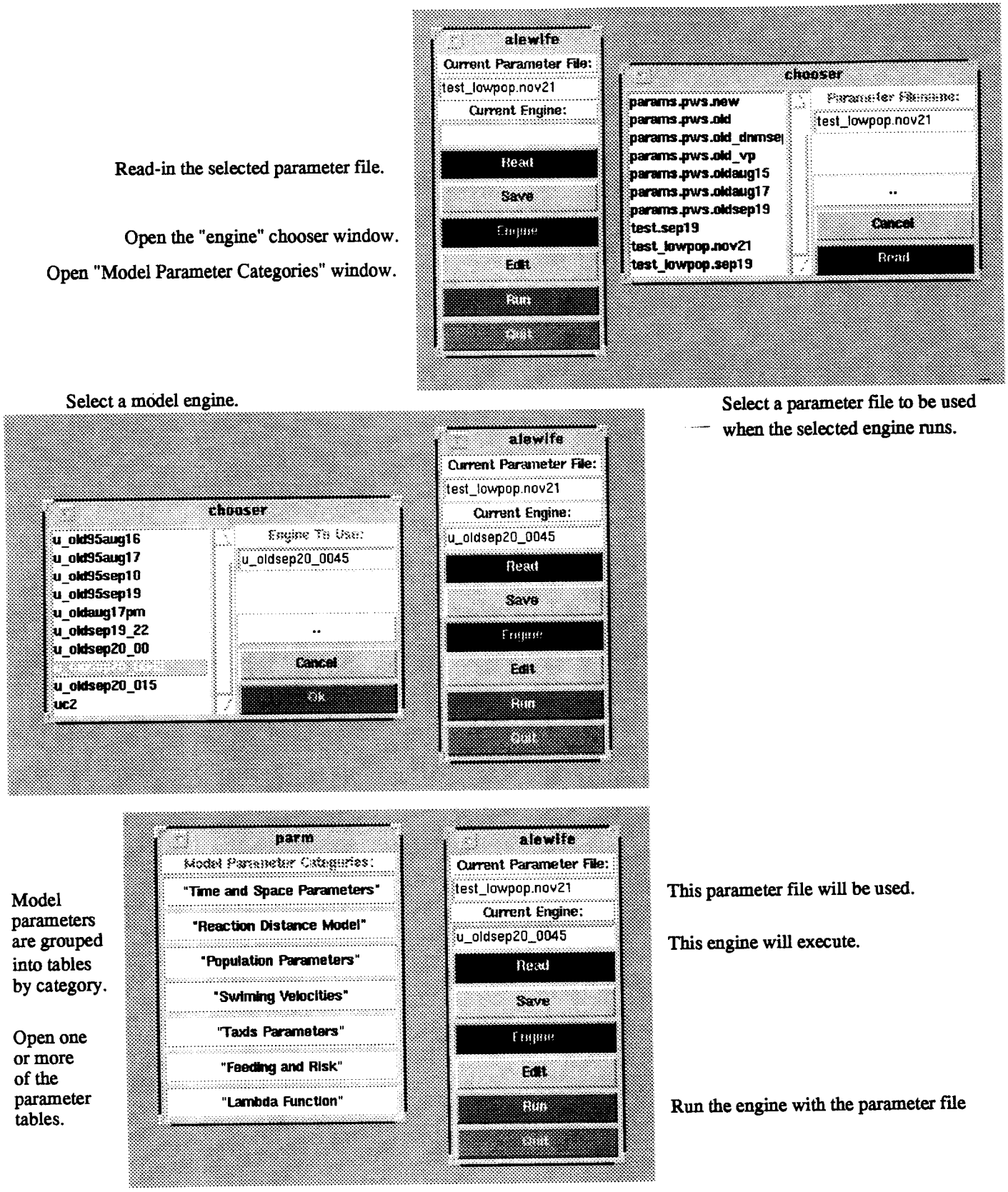


Figure 26. The startup windows of the graphical user interface in *Alewife* Version 3.

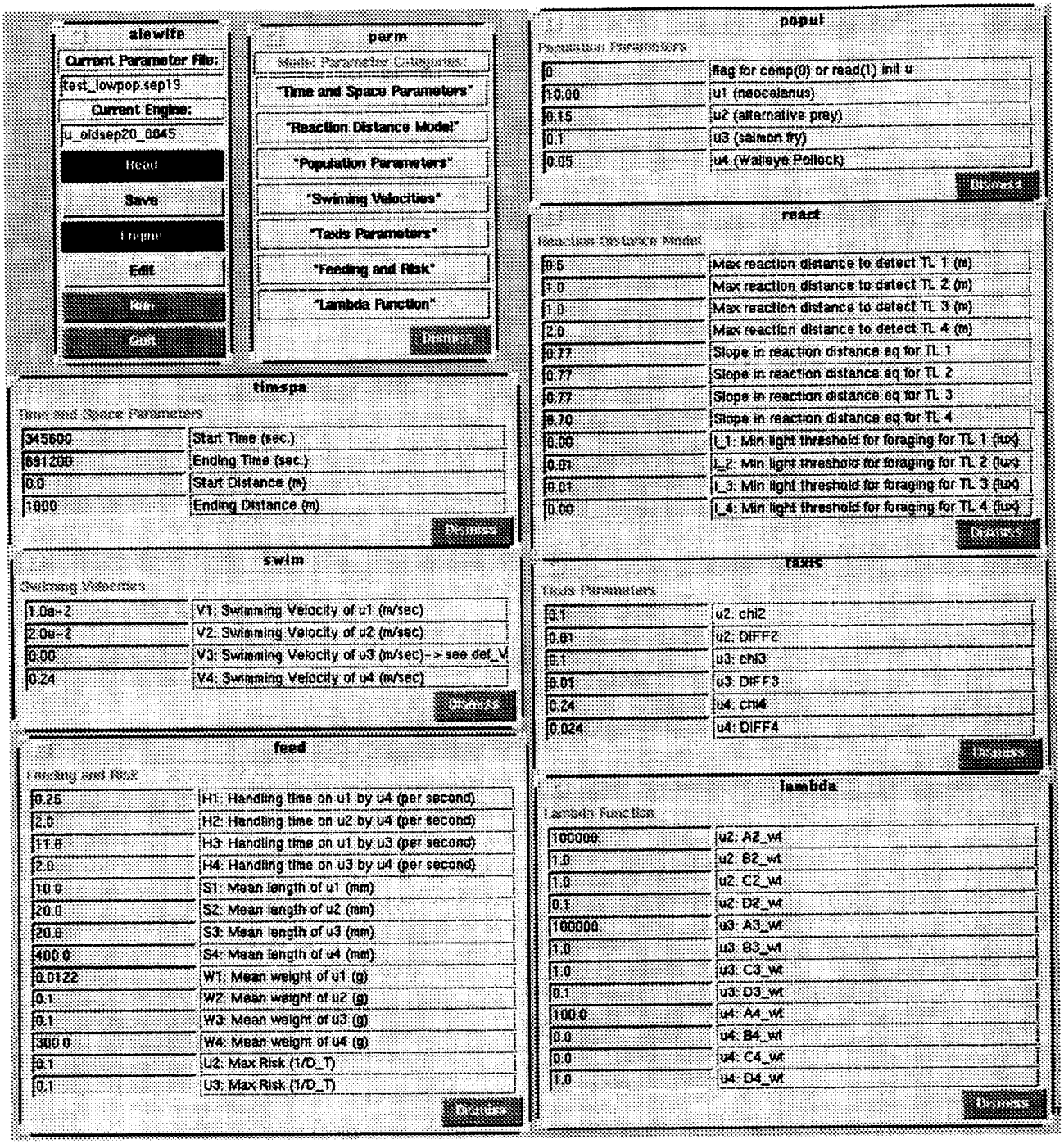


Figure 27. The editable parameter tables in *Alewife* v3.

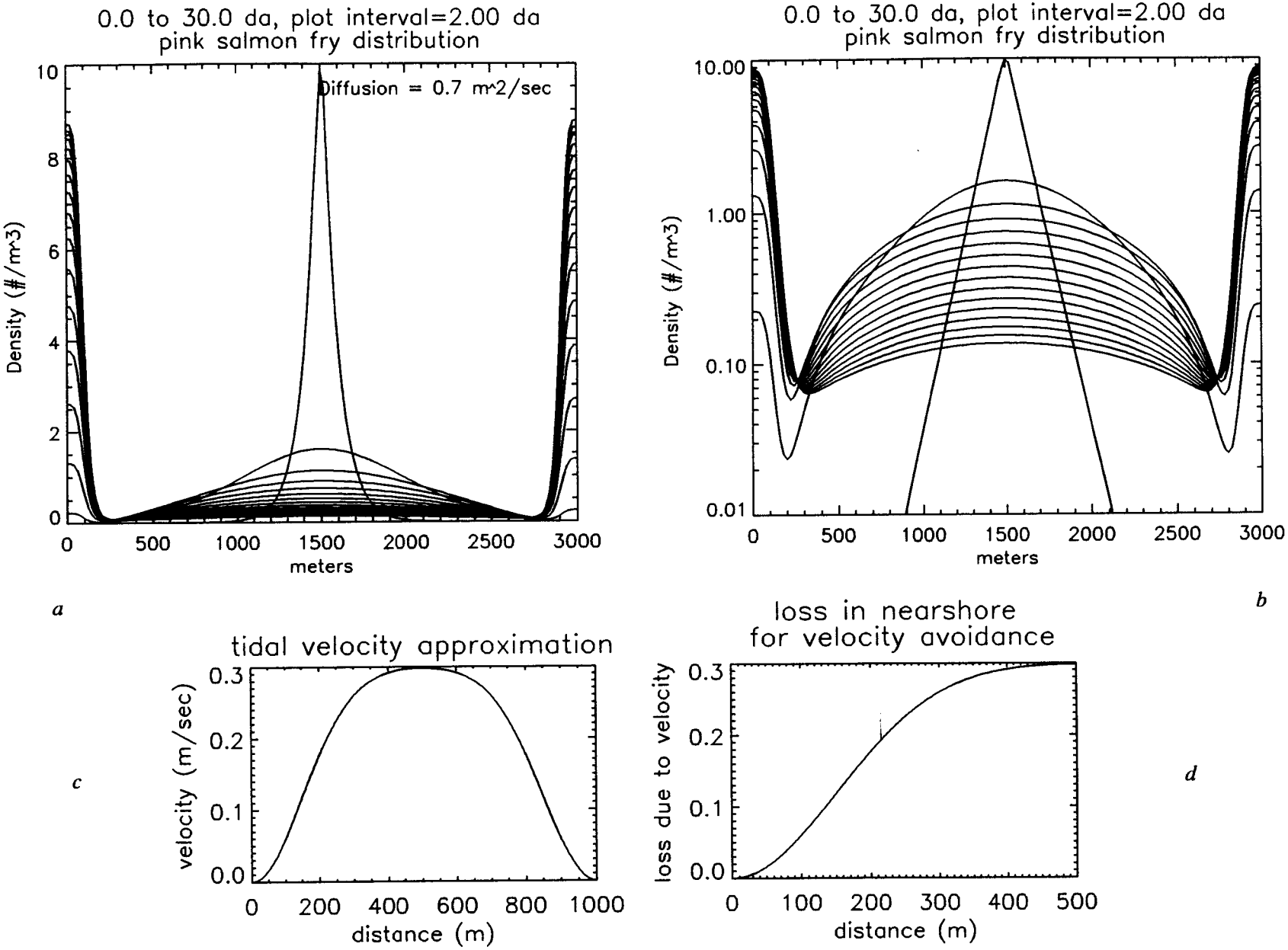


Figure 28. Evaluation of the consequences of avoidance of off-shore currents by pink salmon fry using *Alewife* Version 4. The system consists of a single trophic level (fry) and a loss functiion that depends only on current velocity. A constant (non-tidal) velocity profile as in (c) was used with a loss function as in (d).

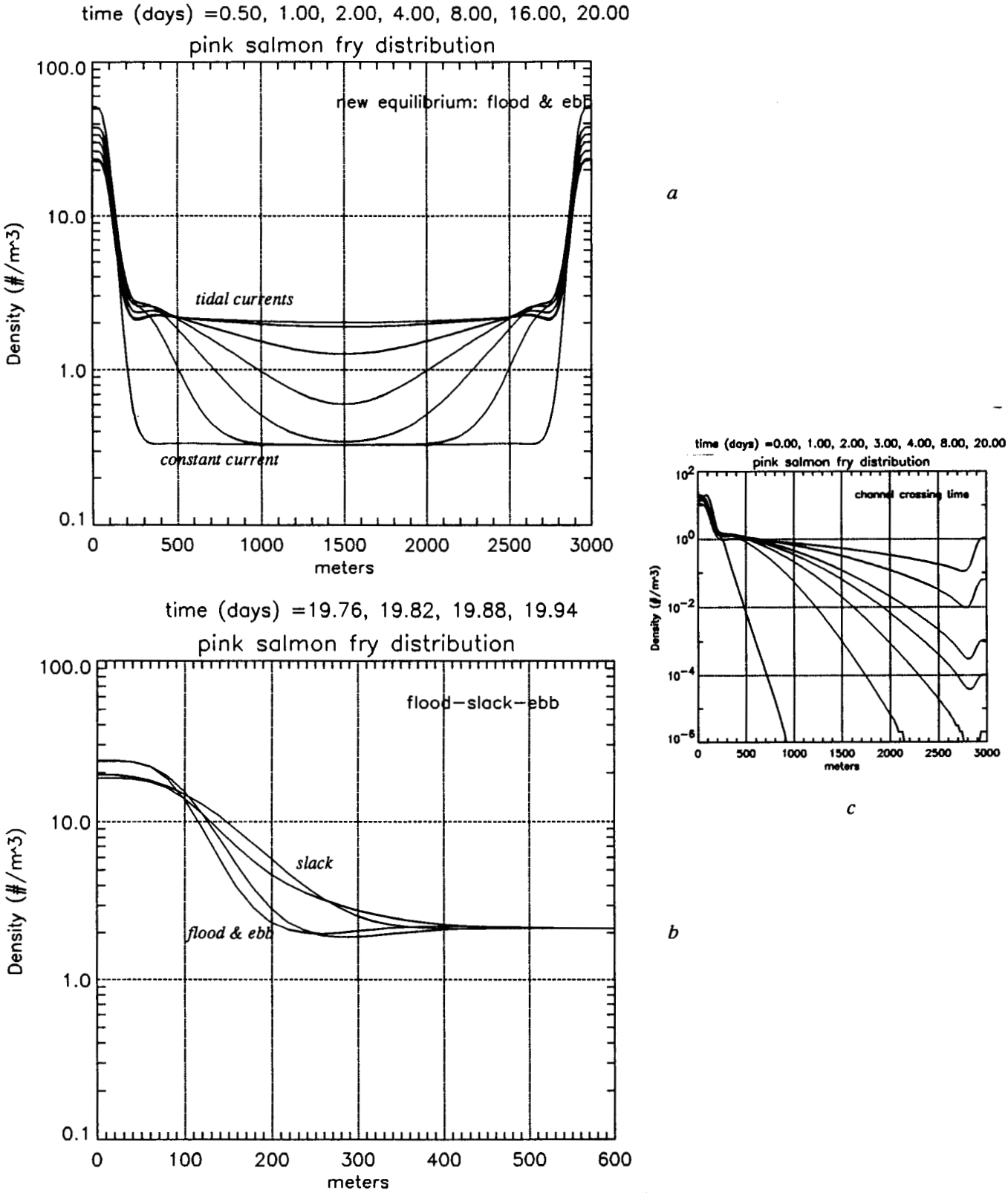
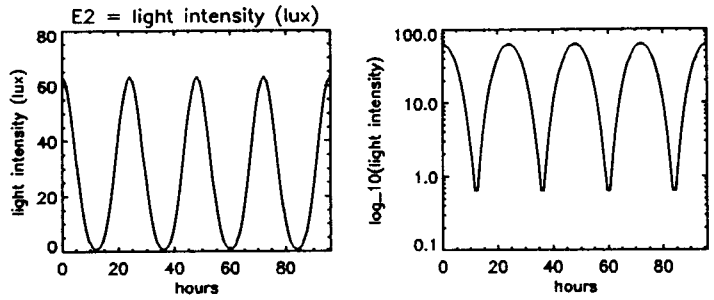
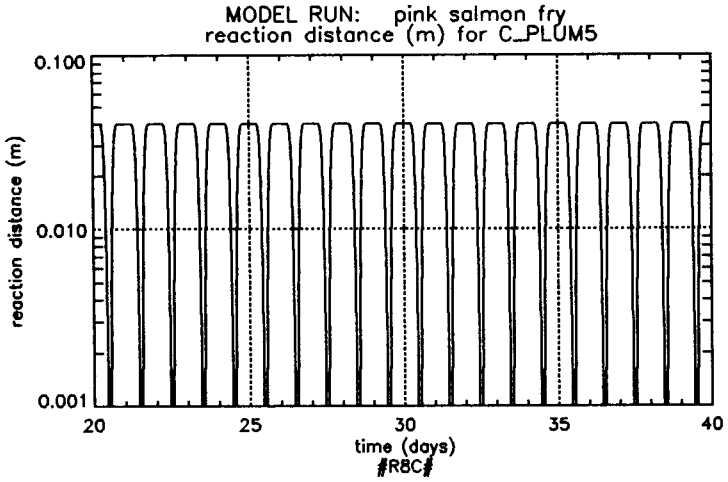


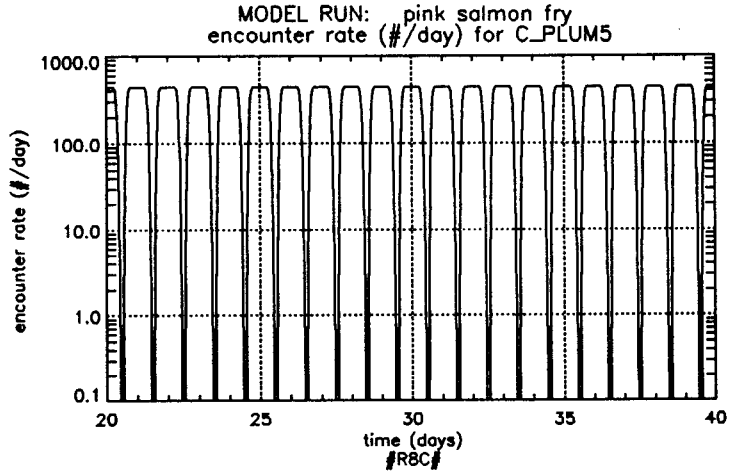
Figure 29. The consequeces of off-shore current velocity avoidance for the case of tidal currents. After spin-up to steady state the constant current of Figure 28 is made tidal resulting in substantial "leakage" from nearshore to off-shore (a) and a tidally synchronized inshore-offshore shuffle (b). Examination of channel crossing time (c) for the same system assumptions.



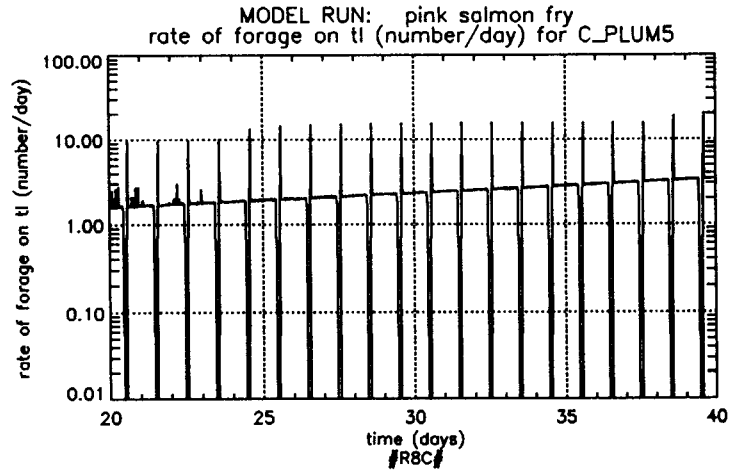
a



b



c



d

Figure 30. Foraging model for pink salmon fry is of sufficient scope to simulate well night fasts, high morning feeding rates, and day feeding that maintains gut fulness.

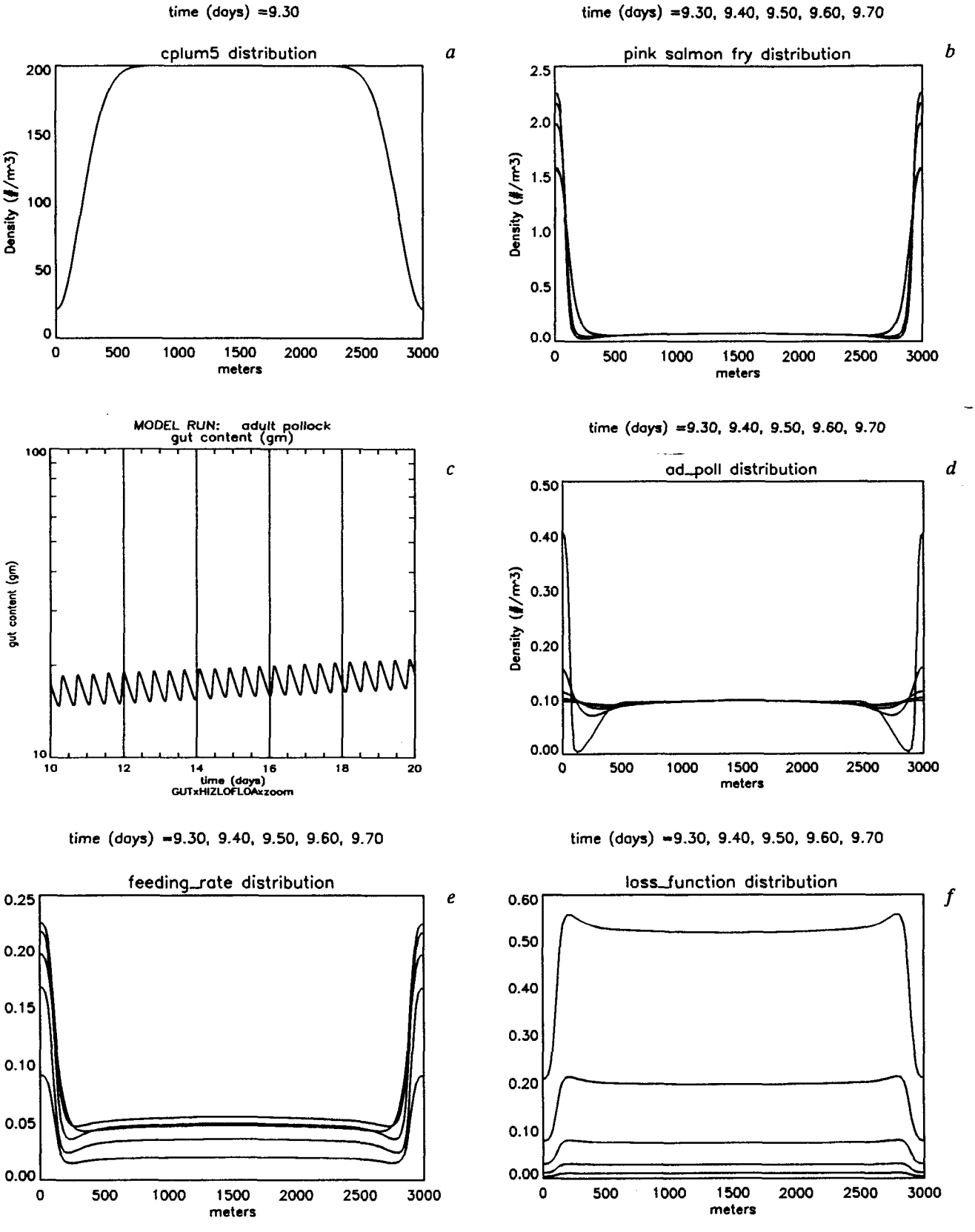


Figure 31. Simulation of pollock - fry interactions using glut-like feeding at 12 hour intervals (c), fixed neocalanus distribution (a), and the tidally shuffling fry distribution (b). Pollock disttribution diffuses to uniform during non-foraging interval. At onset of feeding the distribution quickly becomes disjoint as pollock move to one or the other feeding area.

Appendix 1

An AVS Interface to the Aurora Dataserver

An AVS Interface to the Aurora Dataserver^{TM}

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ABSTRACT: To investigate database support for scientific visualization, we built several AVS modules which provide access to scientific datasets in stored in the *Aurora Dataserver.* Aurora is a scientific database which provides the essential functions to define and access multi-dimensional scientific data. The modules we built allow datasets to be selected from Aurora and converted to the internal representation needed by the AVS scientific visualization system. In addition to providing database support for scientific visualization, the modules were intended to be used by a large research project in Prince William Sound, Alaska. This project, called SEAData, is collecting a wide range of oceanographic and biologic data to assess the long term impact of the Exxon *Valdez* oil spill. In this paper we briefly describe the functionality of the Aurora system, the use of the modules we built, and the tests we ran using scientific datasets from Prince William Sound. Although the Aurora system will not be extended in the future, our work exposes some of the essential database features needed to support visualization.

This research was supported by Xidak Inc. Aurora Dataserver is a registered trademark of Xidak Inc.

Scientific Datasets and the Aurora Dataserver

Most scientific data are characterized by large multidimensional arrays of samples collected during experiments. Modeling these data posses several problems for a database management system. The sample points can fall into a fixed or regular grid, they can be scattered throughout space, or they can represent a mapping from one space to another. For instance, a two dimensional surfaces may be positioned in a three dimensional space. Capturing these geometries is not the only problem associated with scientific data management however. Meta-data which describes the sampling environment, instrumentation and calibration are all critical if the data are to be analyzed properly. Part of this meta-data is the historical record which is needed to trace the evolution of data from a raw state to an interpreted state. These problems all challenge current database management systems.

The *Aurora Dataserver* was designed by Xidak Inc. to provide scientists and engineers with the database functionality needed to deal with scientific data. The Dataserver implements a data model which incorporates many common geometries and it includes the facility for defining meta-data and historical data. The Aurora data model allows scaler sample types, vector and matrix sample types as well as tuple sample types, which are structures of variables for each sample point. The sampling geometry can be a fixed grid, a variable grid, or a warped field which maps points between spaces of different dimension. Warped fields allow lines and surfaces to be positioned in higher dimensional spaces. Together this range of sample types and coordinate geometries describe a large number of scientific datasets.

In addition, the data model allows meta-data (data describing data) and the historical data to be defined. The meta-data pertain to the dataset as a whole, describing all of the samples in the set. These data can include dates, times, and calibration or instrumentation information. The evolution of the data can also be tracked using the history records. Some history records are created automatically by Aurora when a dataset is modified, but history records can also be added by the user.

The functions of the Aurora Dataserver are accessed through an application programming interface (API) which is used with a program to declare and manipulate datasets in Aurora. Each dataset is a member of a 'class' which defines the sample type, sampling geometry and meta-data. The class for a dataset must be created before the dataset is created. Using the API, datasets can be found by navigating through a hierarchical name space or by using an SQL query string. The SQL query also uses the class definition to make a selection based on the values of the meta-data or the dataset history. Whether an SQL query is used or the name space is searched the result is a list of one or more datasets which match the users request.

Once a dataset has been identified, a subset of the samples can be selected. The subset can be based on the value of the samples or the coordinates of the samples. Value based selection can produce a new geometry which includes only those points whose value satisfies the request. Coordinate based selection can specify sub-ranges along each axis or sub-sampling of points at regular intervals. These two types of coordinate based selection can be combined, creating a hyper-slab at a lower resolution. Together these selection operations give users of visualization, or other scientific analysis software, a wide range of functionality.

AVS Access to Aurora Datasets

The AVS scientific visualization system provides visual manipulation and analysis of scientific data. It has its own data model to describe multi-dimensional data which is similar in some respects to the Aurora data model. Our project was to create AVS modules which bring the functionality of Aurora to a user of AVS through a single graphic interface panel. We created two modules, one to select and read an Aurora dataset into an AVS field and another to create an Aurora class and write out an Aurora dataset from an AVS field. This section describes the use of these two modules.

Read Module The read module allows the selection of a dataset and the selection of samples from within a dataset. The samples are converted into and AVS field which is the output of the module. The dataset can be selected by browsing the Aurora name space or by entering an SQL command which queries the meta-data. The name space is an internal hierarchy of Aurora datasets and classes similar to the Unix directory hierarchy. A pattern can be provided by the user and datasets and classes whose names match the pattern are displayed in the AVS user panel. The pattern can contain normal Unix wild card characters. Figure 1 shows the read module as part of a simple AVS network. A space is provided to type in a pattern for a name search or an SQL string for a database query.

The SQL query selects datasets based on the values of meta-data for each dataset. A particular class must be selected first and then the SQL statement can include any of the meta-data defined for that class. The datasets satisfying the query are displayed on the panel for subsequent selection. Whether a name pattern or an SQL statement is given, the user must manually select one of the datasets by clicking on the user interface panel.

Once a dataset is selected several attributes of that dataset are displayed on the panel to allow a user the ability to read all or part of the samples in the dataset. Sliders are provided to select sub-ranges or a sub-sample interval. A mapper string can also by typed in which selects a single variable from a tuple sample type. The dataset can be converted into an AVS field by 'pressing' the button labeled 'load dataset' on the user panel. The Aurora dataset is loaded into memory using the sub-sampling criteria given by the user and converted to the AVS field format. Figure 1 shows the load dataset button and the sliders used to select sub-ranges or the interval for sub-samples.

Write Module The write module allows for the creation of new Aurora classes and datasets. Each new dataset must become a member of an existing class and so the module allows the navigation of the name space to check for existing datasets and classes. A class can be selected from this name space and used to create a new Aurora dataset or a new class can be created from a class specification which is generated from the AVS field. If an existing class is used when a new dataset is created, the existing class and the dataset must be compatible. If a new class is created, the specification is displayed in an editor to allow new meta-data fields to be added to the class.

Aurora Support for SEAData

One of the goals for developing AVS modules which access the Aurora Dataserver was to support a large project analyzing the oil spill in Alaska's Prince William Sound. Over the next 5 years part of this project, called SEAData, will be collecting, analyzing and distributing a large number of scientific datasets. These data include oceanographic and biologic samples from throughout the Sound collected from buoys, ships, and satellites. We implemented some of the Alaska datasets in Aurora and tested remote access to these datasets through the Aurora server. The rest of this section describes how we used our modules with the Alaska datasets.

The earliest dataset collected by the SEAData project was the bathymetry of Prince William Sound. This is a large dataset which was well suited for testing our modules. The dataset could be selected from the read module by browsing the name space or through an SQL command. Once selected, sliders are displayed which allow a sub-ranges and sub-sampling to be specified. The sub-ranges of latitude and longitude can be bounded by the minimum and maximum of the region and the sub-sampling allows up to every 10th point to be selected.

Figure 1 shows the read module after the the dataset has been loaded. The Aurora name space includes the bathymetry dataset and the class associated with that dataset. The dataset has been selected and the class body for the dataset and a set of sliders for sub-setting the samples are displayed. No sub ranges have been specified but an interval of 8 samples has been given to lower the resolution and eliminate the need for a 'down size' module.

The second major test we performed to support the Alaska project was to evaluate the performance of remote access to the Dataserver. We configured a server at the Prince William Sound Science Center in Alaska and a client at the University of Maryland in College Park. Using a query facility which was specially designed by Xidak to minimize network overhead, we read the bathymetry data which was stored in Alaska from an AVS network running in Maryland. One of the significant advantages of sub-setting the data at the server is the reduced amount of network overhead needed to transfer the data. Our performance results were reasonable but more importantly the scaled well as sub-ranges or sub-sampling was specified.

Conclusions

The scientific data model implemented by Aurora greatly simplified the conversion into the AVS field structure. The Aurora data model incorporated most of the AVS field model with the exception of unstructured cell data which is a key feature of AVS. Aurora does, however, provide a much richer set of sample types. Our hope was to build a new set of AVS modules which were tailored the needs of the Alaska project but the development on the Aurora system has been put on hold. It does not appear that Aurora will be expanded to include the additional sampling geometries and class inheritance that we need to support the SEAData project and so we are currently investigating other alternatives. Our current choice is an extensible database management system which provides the necessary class hierarchy and external file references. It will, however, require some additional effort to implement some of the basic functionality provided by Aurora.

Figure 1: AVS network using a read module to select an Aurora dataset containing bathymetry data from Prince William Sound, Alaska

Appendix 2

Prototyping an Architecture for the Management of Scientific Data

Prototyping An Architecture for the Management of Scientific Data

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Introduction

The current state of the art in scientific data management is based on self-describing file formats. Formatting standards like HDF, CDF and FITS, allow scientists a great deal of flexibility in defining and sharing their data. The software needed to read and write data in one of these formats is freely available and the API for defining and accessing data is fairly easy to use. These formats do not, however, provide the functionality available with modern database management systems (DBMS). As the volume of scientific data grows, many scientific applications need the indexing and search features of a DBMS, but modern database management systems do not offer the portability and modularity of data which many researchers have come to depend upon. Since these features are not currently provided with modern DBMS, many sophisticated scientific applications are denied data management functionality which has been available to transaction oriented applications for many years.

To investigate a solution to this problem I built a prototype of a scientific data management architecture which incorporates the advantages of a full function, DBMS, without sacrificing the portability and flexibility of a scientific formatting standard. The prototype uses an extensible database management system to store some of the meta-data associated with an archive of HDF datasets. The DBMS will provide spatial indexing and search facilities for datasets in the archive, and objects which are designed to support specific application. The datasets can also be used independently, without referencing the DBMS.

The prototype is a subset of the data management architecture which is proposed to support a large research project which studying Prince William Sound in Alaska. This project, called SEA-data,is responsible for organizing and disseminating the data collected by 11 groups researching the effects of the *Exxon Valdez* oil spill. In addition, SEAdata is responsible for several scientific visualization applications which will use data from all of the groups to produce visual models of the oceanography and biology of the Sound. The data management architecture proposed for SEAdata includes a large archive of HDF files and a database management system to support the visualization applications. The prototyping activity was tailored to answering the questions

which will reduce the risk of developing this data system by providing information to the design and implementation phases.

The Illustra, extensible, DBMS was used in the prototype to maintain the meta-data for the datasets. Extensions will be created, in Illustra, which support the indexing and access to the HDF datasets. These extensions were designed to support one of the visualization applications which the responsibility of SEAdata with the intent that a generalized model can be drawn from the specifics. Part of the goal of the prototyping exercise is to experiment with the Illustra DBMS and address concerns about its viability for the management of SEAdata's scientific information.

The project lasted throughout the spring of 1995 and meet the requirements of a independent study in scientific data management. This document is intended for the faculty members overseeing this project, the researchers at the Advanced Visualization Laboratory who will be responsible for the SEAdata visualization applications, and the SEAdata information system developers who will be implementing the target data management system. The remainder of this report includes a section describing the HDF standard and the role of scientific data formats and a section describing the SEAdata project in more detail. The proposed SEAdata architecture is presented and the final section includes a description of the prototype and future work.

HDF and Scientific Data Formatting Standards

Like other data formatting standards, the Hierarchical Data Format (HDF) standard is a simple data storage system. It is implemented by a set of accessors which are used to read and write multi-dimensional arrays [RDE93, Nat94]. In addition to multi-dimensional arrays, HDF includes 5 other types of data as well. Multiple datasets of any of the 6 types can be written to a single HDF file in a hierarchical fashion. The possible types are summarized below.

- Two type of raster image data, 8 bit and 24bit
- A text data type for storing documents, annotations and text meta-data.
- Color pallets which are used to assign colors to values when visualizing data.
- Multi-dimensional arrays for scientific data.
- Tables for relational data or complex scientific data.

The software which supports this format is developed and distributed by the National Center for Super Computer Application at the University of Illinois. It includes a library of accessors for each standard data type and a set of low level accessors for extending the system to handle a new type. The library calls are accessible from a C or Fortran program and they are available, along with the documentation, free of charge. Recently, the HDF standard was chosen by NASA as the primary format in which the EOS data will be made available.

The flexibility and portability of a standard format like HDF has a great deal of appeal to scientists collecting or using data. These formats are called *self describing* because they provide accessors which reveal the definition of the sample data or the attributes in any file. Attributes can be defined in a simple way, and given a value, which further describe the data. This internal description, along with a facility to translate data across multiple hardware platforms, make these formats *portable* as well.

The research autonomy enjoyed by scientific community demands this level simplicity and portability of data. A single dataset may be useful to a wide variety of scientists for many different purposes. A research project may demand that data be stored with a minimum of meta-data or, alternatively, with complex catalogs of meta-data. Datasets may also be passed between different types of research projects or revisited years after the initial use. For all of these reasons the scientific community has created these self contained, flexible, formats, which allows the user to define the level of data complexity.

These reasons have also steered the scientific community away from commercial DBMS product. Although there is a great deal of database functionality which would be valuable in managing scientific data, the restrictions imposed by DBMS are to great. The transaction overhead and database administration are unnecessary in most research applications. Primarily, however, committing to a single data and meta-data model which may or may not be shared by another research group is not practical for most scientists. In addition, commercial database systems are seen to need a support staff which will not always be affordable or even available in a research environment. Finally, many of the data used by the scientific community has a more complex structure than can be captured by current relational database systems.

As the volume of scientific data grows, however, the need for database functionality will grow as well. Large numbers of datasets, each of which could be several megabytes in size will require sophisticated indexing and query facilities. In addition, the nature of meta-data is growing more complex as more computer interpolation is applied to raw instrument data. Sophisticated scientific applications are also increasing the demand placed on locating and sub-setting of large arrays. Finally, the performance of these high volume, data intensive applications will suffer without intelligent prefetching and low resolution sub-sampling.

These conflicting demands challenge the designers of database management systems. Our goal, here, is to prototype a system architecture which supports both the functionality of database management systems and the autonomy of a scientific data standard. Although some of this design has been implemented in other research projects we hope to use our prototype as a foundation for further research into scientific database management systems.

SEAdata and Scientific Visualization

The SEAdata project is part of large effort to collect information about the oceanography and biology of Prince William Sound, Alaska. This project is part of the remediation effort following the *Exxon Valdez* oil spill, which, in March of 1989, added 11 million gallons of oil to the sound.

The overall project, called the Sound Ecosystem Assessment (SEA) project is funded by the *Exxon Valdez* trustee council. SEA includes 12 separate research groups, all of which are collecting data on the sound. In addition to the oceanography of the Sound, the groups are researching the phytoplankton, zooplankton, salmon, herring and birds, in and around the Sound.

The goals of the SEAdata portion of the project are to collect a portion of the oceanographic data, produce several scientific visualization applications, and create a data repository for the data collected by all of the SEA groups. The oceanographic data is being collected in a variety of ways. A fleet of ships is collecting a set of measurements across the surface of the Sound and vertically through the water column. Buoys, located around the sound are collecting similar parameters from the water column. SEAdata is also responsible for collecting and analyzing AVHRR data transmitted directly from the AVHRR satellite.

SEAdata is also responsible for creating several scientific visualization applications for analyzing the Sound. One of these will present all of the data collected by SEA, within a given time interval, above a bathymetric surface of the Sound. This interim model will be used to get a picture of the all of the data which has been collected. The final model will allow a time period and sub region to be specified, and the datasets which satisfy the query will be displayed in an image, which can be manipulated by the visualization software. Complex regions, based on shorelines, depths or the spatial extents of existing datasets may also be put into the query.

A second visualization application will focus on the fish populations around an acoustic transect of part of the Sound. By using the transect data, along with any other data collected in the region around the transect, this application will couple all of the trophic levels of the sound in a coherent model of the activity of necton and plankton. The queries needed to support this activity will select several datasets based on specific parameters for this model. The datasets which satisfy the query will be converted into a form which can be manipulated by the visualization software. The modules which prompt for the query, select and subset the data, and convert it into a form which is usable by the visualization software will conform to requirements of that visualization system.

The third goal, of the SEAdata project is to provide a data archive of all of the data collected by the SEA project. As a central repository SEAdata will ingest the data from each of the research groups and providing that data to all research groups in a standard format. This will require a common data dictionary and standards for the HDF files. In addition, SEAdata will maintain a database to support those applications which need mechanisms to locate and process the data in the archive. The architecture of the SEAdata management system as well as a prototype implementation of that system is the focus of the rest of this document.

The Data Management Architecture of SEAdata

An overview of the proposed data management architecture for the SEAdata project is shown in figure 1. The four main components of this architecture are the a DBMS which supports a set of visualization and statistical applications; an archive of HDF files which is indexed by the DBMS;

a mosaic server; and processing required to ingest data into the archive and DBMS.

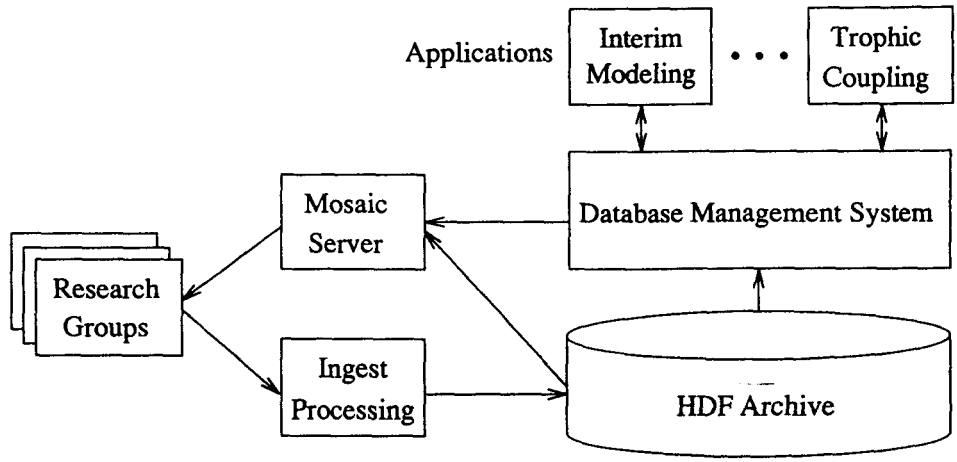

Figure 1: The proposed data management architecture for the SEAdata project

The architecture is designed to maintain an archive of datasets which is essentially independent of the database management system. The DBMS will be used by some of the more sophisticated applications but the HDF archive will be available to any application which does not need the additional functionality provided by the DBMS. The HDF archive could span the disks of several different systems. The DBMS will contain the meta-data associated with each dataset and the pointer needed to locate the dataset in the archive. Some of the meta-data in the DBMS may reference datasets which are not generated by the SEAdata project. The meta-data for NOAA or NASA datasets may be included in the DBMS in order to provide the scientists with a consistent view of relevant data.

HDF Archive The datasets will be stored and retrieved from HDF files stored in the archive. Each dataset will have a unique identifier and a single HDF file may contain multiple datasets. The files can be stored on several different disks, on several different machines, but the DBMS will include a pointer to the disk and directory in which the file is located. If the HDF file is moved, the database can be updated by referencing the unique dataset identifiers in file and in the database. Shadow files might also be used to notify the database that an HDF file has moved.

The system may need to index datasets which are outside of the HDF archive. These dataset could be located at NOAA or NASA or at another research location. The meta-data would be stored in the database and files could be down-loaded when they were requested or copied into the HDF archive and treated like an internally generated file.

Database Management System The database system will provide general functionality, which is available for all applications, as well as functionality which is application specific. Figure 2 shows the two levels of the database management system. The general functionality includes a

name service and a variety of indexes over the meta-data. In addition, some sample data may be replicated inside the DBMS. Specific functionality in the form of query objects will also become part of the high level DBMS interface.

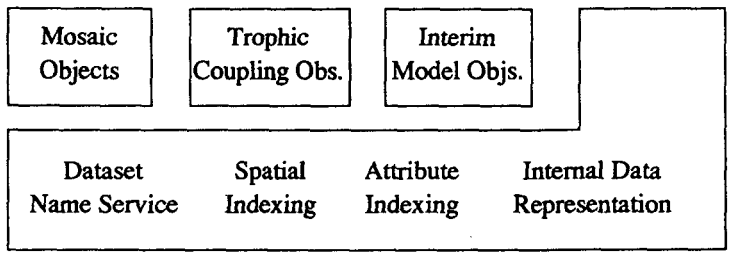

Figure 2: The database components for general and application specific functionality.

The name service will provide applications with simple query facilities to locate files in the HDF archive. This service will also tie a unique file identifier with a disk and directory and file name in the archive. The spatial index will use the meta-data from each dataset to construct a bounding box for that data set. These 2d boxes will be indexed by the database to support spatial queries. Other indexes of the meta-data will be available, also, to speed up queries on non-spatial attributes.

Some applications will need objects which have be created to support that application alone. As an example, the trophic coupling application will tie together several different data sets which are located around the acoustic transect data. The relationship of the data sets to a given transect may be stored in a query object which will accept some parameters and return the list of data sets and the clipping information necessary to execute the application. These query objects would be specific to an application but the would be part of the general interface to the DBMS.

The Illustra database management system is one of the DBMS products proposed for this component of the SEAdata architecture. Illustra is an extensible DBMS which will allow new data types to be defined and optimized by the Illustra kernel [Ill94]. To create a new data type in Illustra, the complete set of functions which are needed to manipulate that type, are created and stored in the database. Illustra can then treat these user defined types as an integral part of the database, and therefore, queries which reference these types can be optimized effectively.

Illustra also provides a several *datablades* which are sets of types for a given application domain. The 2 and 3 dimension datablades provide spatial applications with types for points, lines, polygons, an other shapes in 2 or 3 dimensions. Other datablades provide the functionality for time series analysis or image analysis. These datablades extend the functionality of Illustra, for a given domain of applications, by providing the data types which are standard in that domain.

SEAdata is considering Illustra as the extensible DBMS from which to build the database component. The intent is to create new types in Illustra which represent the datasets in the HDF archive. These would be simple types which are made up of the meta-data of the datasets. The attributes could indexed by standard b-trees and with the 3 dimensional datablade. The query

objects which are needed to support some of the applications also be new types in Illustra.

Data Ingestion The ingestion process in figure 3 will insert a dataset into an HDF file in the archive and update the database with the necessary meta-data. The input to this process will be the ASCII or HDF files which have been generated by the individual research groups. The format of the data from each group will be unique and therefore require processing which is specific to that groups files. If a group chooses to use HDF as the format for the group archive, the ingestion process may need to augmented these HDF files with additional attributes needed for the DBMS.

The update of the database may occur when the datasets are added to the archive or by a separate process, as shown here, which only references the HDF files. In either case, when the process is complete, the database management system will be able to index that dataset and locate it in the HDF archive or across the network.

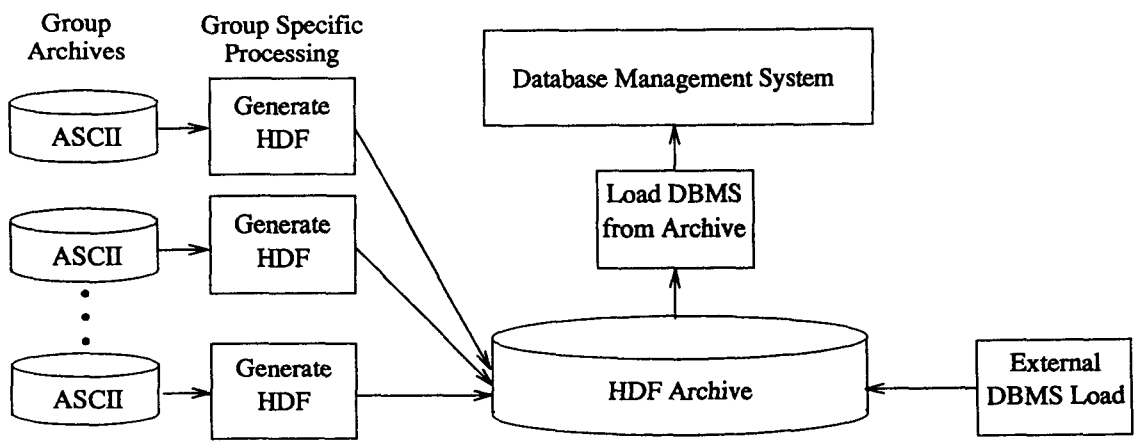

Figure 3: The ingest processing component of the data management architecture

Mosaic Server The last part of this architecture is the mosaic server. This is the interface used by the individual research groups, as well as external researchers, to access the HDF archive. The mosaic interface would display a 3d map of the sound and allow regions and specific datasets to be selected. The database would return a list of HDF files which satisfy the query. The HDF files in which the datasets are located could be down-loaded through the standard mosaic interface and operated on locally.

A group could store its own data at the SEAdata archive and down-load those files which were needed for a given research effort. In addition, groups will have access to any of the datasets which have been generated by another group. The ability to process these standard datasets in HDF format will make a wide variety of data accessible to each group.

This preliminary architecture meets the initial set of requirements outlined by the SEAdata project. Several aspects of this architecture, however, are untried or require new technology and

therefore pose a certain risk to the project as a whole. The goal of prototyping is to identify those risks and build a system which is tailored to reducing risk by providing information to design or implementation phase of the target system.

Prototyping the Data Management Architecture for SEAdata

The goal of prototyping the data management architecture is to provide constructive proof of the design concept, expose the potential problems with the system, and experiment with solutions to those problems. The initial prototype meets the first two of these. It provides a demonstration of the architecture, an experimental interface to that architecture, and it exposes some of the problems which will be encountered with the design of the target system. The prototype can now be used in a second phase, to experiment with potential solutions to problems which have been revealed by phase 1. This section includes a description of the implementation of the prototype, a presentation of the benefits of this prototyping effort, and a summary of open issues and future prototyping goals.

Implementation of the Prototype The prototype is a vertical implementation of the proposed data management architecture for SEAdata. This includes all three phases of data management implemented for a single kind of dataset. The first phase is the ingestion of raw, ASCII data, into HDF format; the second phase is the loading of the database objects from the HDF files; and the final phase is an application which uses the database and the HDF files. The datasets selected contain LTD data which include temperature, salinity and water pressure measurements, sampled at every few meters in a column of water. About 300 such columns were sampled in the 1994 summer season. The prototyping effort included ingesting these 300 datasets and displaying them with a visualization application. The rest of this section describes these phases of the implementation and the data structures and objects which were used.

After a discussion with the PI's of SEAdata, a prototype data structure was designed to hold the meta-data and sample data for all of the SEAdata datasets. This data structure is a hierarchy of inherited attributes which is shown in figure 4. This is the hierarchy of meta-data within the datasets maintained by SEAdata. As an example, datasets collected from ships have particular meta-data attributes but they share geographic attributes with all other SEAdata datasets. A data structure which could describe this hierarchy was designed as used as a model for the meta-data in the HDF files and in the Illustra objects. This data structure and the functions which operate on it make up a *dataset object* within SEAdata. All of the details of this dataset object were not worked out but an initial design was used in the prototype.

The ingestion of the CTD data required the adaptation of a program which read the ASCII files containing header data and sample data. Dataset objects were created using the ASCII input and then passed to a routine which created the HDF files. Some of the HDF functionality is encapsulated in these routines which can be reused when subsequent ingestion programs are written. The ingestion program itself, therefore, contains only the functionality which is specific to the format of the ASCII input files.

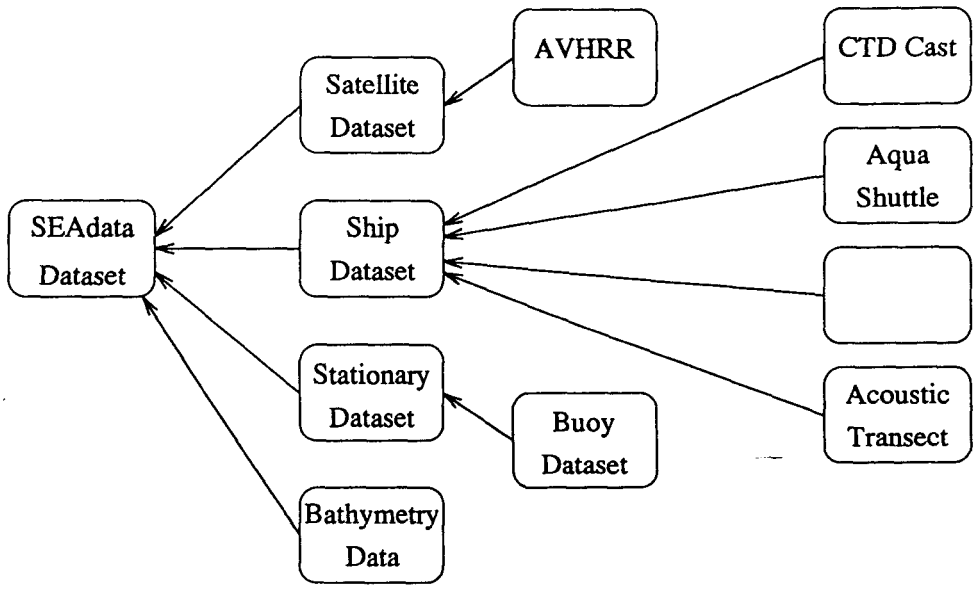

Figure 4: The inheritance hierarchy of the dataset objects in SEAdata

The meta-data for the CTD datasets was read from header files and stored in attributes within the HDF files. These attributed mirror the meta-data members of the SEAdata dataset objects. Four sample arrays were stored in each HDF file. These include the depth, salinity, temperature, and pressure, of each sample. Although depth is a logical coordinate array it would need to be replicated for each of the other datasets and so it was stored separately.

Loading the Illustra database objects was done entirely from the data in the HDF files. This again allows reuse of these routines when other HDF files are used to load the database or when data is loaded from outside of SEAdata. The objects in Illustra mirrored the objects and inheritance of the SEAdata dataset objects. SEAdata objects were created with the meta-data from the HDF files and then passed to a routine which created the Illustra objects. This creation consists of the construction and execution of an Illustra insert statement in SQL. As with the HDF phase, this encapsulates the Illustra functionality within a few routines.

The Illustra objects include a two dimensional bounding box which is constructed from the minimum and maximum of the latitude and longitude of all of the sample points. This additional data member is not in the SEAdata dataset object. The box might be large for some datasets which are collected as a ship moves across the Sound, but for the CTD samples, which all have the same latitude and longitude, it is only a point on the surface. The bounding box data member is a data type from the Illustra 2d datablade. This type is described by the x and y coordinates of two corner points.

An application was created which exercised the access to the Illustra database objects and the HDF files containing the sample points. This application, shown in figure 5, includes a graphic

interface for specifying the a database query and a button which will retrieve and display the selected sample points. This scientific visualization application is written within AVS and provides a working example of how the data management architecture will be used.

The selection of datasets is done with a SQL query of the database in Illustra. The selection criteria include non-spatial attributes like ship name and sample date, as well as the spatial attributes of latitude and longitude. A list of the datasets which meet the selection criteria are shown in a results window which displays some of the meta-data attributes. If the list of dataset meets the users needs a separate 'Load Datasets' button is provided to load the samples into the application and display them.

The application uses some of the previously written routines for loading SEAdata dataset objects from Illustra and HDF. The results of the Illustra query are used to create an internal list of dataset objects which contain no samples. When the button is used, this list of objects is passed to a routine which loads the sample arrays in these objects from the HDF files. The complete list is then passed to a routine which creates AVS fields needed to display the samples. Several other AVS modules manipulate the data including one which projects the sample coordinates from latitude and longitude onto a flat surface.

The utilization of the SEAdata dataset objects is shown in figure 6. Routines were written to convert raw data, HDF data and Illustra objects into a SEAdata dataset object. Routines were also written which create HDF data, Illustra objects and application structures from the SEAdata dataset objects. This provides a high degree of modularity and reuse as well as allowing for the change from HDF or Illustra to another storage manager or DBMS, respectively.

Results of Prototyping The implementation of a prototype provided insight into several aspects of the target system. These include the capabilities the designers can expect from Illustra and HDF, preliminary documentation of the database schema, and a user interface which demonstrates the functionality to potential users. The other major result of the initial prototyping effort is the identification of potential problems with the architecture and the clarification of the experiments needed to reduce the risk which these problems present to the design of the target system.

The capabilities of Illustra which were used in the initial prototype of the SEAdata architecture include type inheritance and spatial access methods. The inheritance hierarchy of the datasets in SEAdata is readily described by the Illustra type system. Although the description of the objects must be replicated in the programming language, the Illustra specification is comprehensive and easier. Several tedious routines had to be written to extract convert the results of the Illustra query into the internal objects but once done these routines can be used any time Illustra is used, within SEAdata or not.

The 2 and 3 dimensional datablades were investigated for use with this prototype. The selection of dataset will nearly always include sample depths and date/time in addition to latitude and longitude. The 2d datablade was used for the prototype, however, because no 4d datablade is

available and the 3d datablade does not include the needed functionality for selecting objects in space. The 2d datablade provides several valuable functions for manipulating and selecting spatial objects. This datablade includes an R-tree index which can be built on boxes and polygons and this feature was used in the prototype. The other parameters can be selected using standard relational selection operations.

The capabilities of HDF are quite limited but the interface is simple and I/O performs well. No inheritance hierarchy can be described in HDF but the meta-data attributes can be represented easily and can mirror the structure of an inherited object. The current HDF API does not provide any type of search facility. Subsections of the arrays can be read but only index values can be used to specify those subsections. No facility is provided to read samples within a range of depth, for instance, or for a range of given values. The API does provide a functions from which a description the attributes and arrays within the file can be displayed. This is vast improvement over the raw, ASCII, representation.

Although the clear understanding of the capabilities of Illustra and HDF is an important result of the prototyping exercise, the refinement of the database schema definition is perhaps the most valuable. The preliminary definition of the meta-data needed for all of the SEAdata datasets includes several different kinds of data. Some of the meta-data summarizes the sample points by giving a minimum and maximum values of the sample arrays. Other meta-data describes the origin of the sample data, providing an audit trail back to the raw data. Finally a third type of meta-data is created to support the administration of the data management system. These data include dataset identification numbers and the bounding box for the R-tree index.

Using meta-data to summarize the sample points will greatly enhance the potential selectivity of the database. As queries get more sophisticated and select dataset based on the values of the sample points, summary data can be used to trim the search space significantly and reduce the number of samples which must be examined. Although summary data is a replication of some of the sample values, these sample data do not change and therefore data consistency is not a concern. One of the benefits of building this prototype was the increased awareness of the value of this technique. Currently we are only using minimum and maximum values for sample points but other methods are being considered too.

Another important benefit of the prototyping activity is the development of a user interface which can demonstrate the functionality of the data management system to the potential users. This demonstration not only provides a framework from which the details of the architecture can be discussed, it allows scientists to consider how they will be utilizing the system for their own applications and challenges them to find ways in which the both the user interface and the basic functionality can be improved. This is a common benefit of prototyping and for good reason.

Finally, the creation of the prototype and a demonstration application exposes several open issues. An initial prototyping step is often followed by several experiments which can address the major risk factors in the target system. This second phase will address the following open issues.

Open Issues and Future Goals Several issues surfaced once the prototype was in place which need to be addressed before the prototyping activity is complete. Perhaps the most important of these is the selection and retrieval of sample points. Other issue include performance evaluation and more sophisticated spatial queries, and the integration of other kinds of datasets.

The basic data management architecture provides extensive selection of dataset based on the meta-data stored in the database. Once the datasets have been identified, however, the samples must be retrieved from the HDF files. This means that the architecture does not support any selection on the values of the sample points. One of the goals was to consider the possibility that sample points could be stored in the database, at least temporarily, giving the user the ability to select on sample values. However, because Illustra does not cache objects at the client, the sample points will need to be down-loaded for each query making solution unworkable as well.

Therefore, additional experiments must be done to see how to provide sample selection on local data. Illustra does provide the ability to run functions at the client, but it is not clear that the basic indexing facility can be provided on local objects. This and other alternative need to be tried and results made available to the designers of the target system.

An evaluation of the performance of Illustra and the 2d datablade is also needed in order to provide confidence. This study needs to quantify the performance of the R-tree index for a large number of datasets as well as quantify the performance of the remote access to the server. The current prototype operates well but some idea of the degradation due to this remote access should be understood before Illustra can be fully recommended.

Part of the performance evaluation should include more sophisticated spatial selection criteria. SEAdata needs to select datasets within an polygon surrounding an irregular region of interest. Additional datasets are being added to the prototype and this evaluation could give insight into the performance of Illustra's spatial access methods.

Finally, as a demonstration tool the prototype does not illustrate the capabilities of the DBMS for integrating different kinds of datasets. The hierarchical structure of the object in Illustra allow the multiplicity of datasets to be searched together and return in a single list of results. This capability is not well demonstrated in the current application which visualizes only one kind of dataset. The addition of another kind of dataset contain biologic parameters will push the application and the interface toward are more realistic view of the final result

Results of the Project As an activity, the constructive prototyping effort replaced an initial set of concerns with a deeper, more informed, set of concerns. No prototype can hope to flush out all potential problems but as a research tool the prototyping activity can provide the design phase with a much clearer idea of the viability of the basic concepts as well as the potential pitfalls.

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Figure 5: The application which allows visual display of the datasets within the SEAdata data management system.

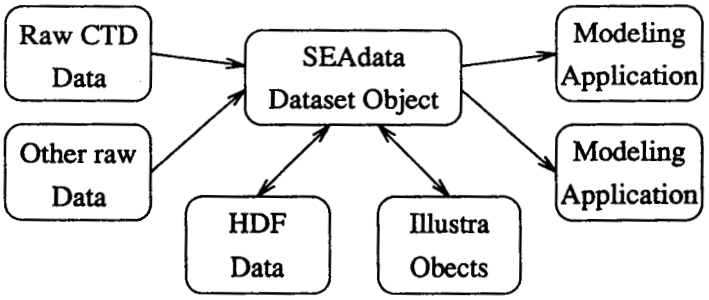

Figure 6: The data flow across the interface to the SEAdata dataset object and the other objects and structures in the system.

Appendix 3

Case Study: Using Spatial Access Methods to Support the Visualization of Environmental Data

Case Study: Using Spatial Access Methods to Support the Visualization of Environmental Data

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Abstract

As part of a large effort evaluating the effect of the Exxon Valdez oil spill, we are using the spatial selection features of an object-relational database management system to support the visualization of the ecological data. The effort, called the Sound Ecosystem Assessment project (SEA), is collecting and analyzing oceanographic and biological data from Prince William Sound in Alaska. To support visualization of the SEA data we are building a data management system which includes a spatial index over a bounding polygon for all of the datasets which are collected. In addition to other selection criteria the prototype provides several methods for selecting data within an arbitrary region. This case study presents the requirements and the implementation for the application prototype which combines visualization and database technology. The spatial indexing features of the Illustra^{tm} object-relational database management system are linked with the visualization capabilities of AVS to create an interactive environment for analysis of SEA data.

1 Introduction

As the quantity and disparity of scientific data increases so do the problems of managing these data. Part of the management goal is to provide consistent methods of access to scientific data which are collected with different techniques for different purposes. Scientific visualization and scientific database technology have both been used to address the problem of managing large numbers of datasets [6, 3]. Meta-databases have been used to index scientific datasets and the problems of combining database technology and visualization techniques are being explored as well [1, 5]. This case study describes the use of the spatial selection capabilities of an object-relational database management system to support queries from a visualization tool. The prototype displays and manipulates scientific data which are being used to analyze biological activity after the Exxon *Valdez* Oil spill.

In 1994, a 5-year effort called the Sound Ecosystem Assessment was begun to analyze the biological impact of the Exxon *Valdez* oil spill in Prince William Sound, Alaska (PWS). As a response to the low production of pink salmon and Pacific herring in the last few years, the SEA project is collecting a wide range of oceanographic and biological data. These data will be used to analyze the ecosystem of the Sound and provide the foundation for simulating sound-wide processes. The project is funded by the Exxon Valdez Oil Spill Trustee Council and includes 13 separate research groups, each of which is specializing in some aspect of the ecosystem [2]. These aspects include the oceanography, plankton, fish, and birds in and around the Sound. Each group is collecting data relevant to its own focus and one group is charged with archiving these data and providing an integrated view of that archive to all groups in SEA. As part of this integration effort we are implementing the data management architecture as well as several visualization tools which take advantage of the spatial capabilities of a database management system.

Our approach to integrating these data includes the use of both visualization and database management techniques. We have built a prototype of our visualization tools which utilizes the Illustra^{tm} [4] database management system. The spatial access methods provided by Illustra become a valuable element in the iterative set of operations for refining the selection criteria within a query and manipulating the results. This case study describes the use of irregular polygons to interactively define regions of interest for selecting datasets collected by SEA. We describe some of the datasets, and then present the motivation for the visualization tools. This is followed by a discussion of how Illustra is being used to implement spatial searches within our visualization tools.

2 The Challenge of SEA Data

SEA is collecting 12 main datasets, each of which has from 3 to 10 measured variables. These datasets are of different sampling geometries and ranks, different resolution in both time and space, and several different levels of interpretation. These data are collected by scientists in different disciplines with occasionally divergent requirements. Each discipline, however, will

need access to most of data, in a format which is suitable to the specific needs of the analysis. Although the total volume of data is not unmanageable, the number and disparity of the datasets presents a data management challenge.

The oceanographic data include remotely-sensed sea surface temperature, Doppler profiles of ocean currents, and vertically and horizontally sampled temperature and salinity. The biological data record phytoplankton and ocean nutrients, zooplankton, herring spawn, salmon fry, adult salmon, herring and walleye pollock, and sea birds. The number, biomass, or feeding behavior are documented for many of the species. These oceanographic and biological data will be augmented with weather data, hydrologic data and tide information which is available from sources outside of SEA. Finally, these data will be analyzed in the context of the bathymetry (water depth) of Prince William Sound.

The level of interpretation of the data will also vary depending on the source instrument or modeling application. Simple values like temperature can be recorded with little or no interpretation. However, acoustic soundings of fish population will require several steps of manual interpretation to produce species counts and biomass. At the highest level of interpretation, simulation models using all relevant data will generate large volumes of 3- and 4-dimensional (space and time) output.

The sampling strategies result in multiple resolutions and geometries. The resolution, or size, of the sample cell varies across each sensing instrument, bottle or net used for collection. The smallest cells are less than a cubic meter and the largest can span hundreds of meters. The sampling geometries may be linear, or planer in 3-dimensional space, or include 3- and 4-dimensional output generated by the models. The geometries may be regular, irregular or scattered.

These data will be used to test several hypotheses about the processes which regulate the population of pink salmon and Pacific herring in PWS. The processes include loss of herring eggs, salmon and herring feeding behavior and predators, the magnitude and direction of the current in the Sound and the overwinter behavior of the herring. Testing these hypothesis requires correlating many of the variables across both time and space. To facilitate this, the collection of the variables is coordinated between all groups to provide simultaneous measurements. Standard locations in the Sound are revisited and the variables are re-measured to allow time series analysis as well.

As an example, understanding the movement and growth of the juvenile fish requires an understanding physical variables such as current, water temperature, the availability of food, and presence of predators. The interaction between these may depend upon the depth, proximity to the shore, and the season. Each scientist will need to correlate a different group of these variables, within given regions, across time.

3 Visualization Tools

In order to provide a visual integration of the disparate datasets within SEA we are creating several tools for selecting and displaying SEA data. The tools use AVS as a visualization engine and allow the selection of data from Illustra and from files which use the HDF standard. Our prototype of these tools includes a set of iterative operations for displaying data in a spatial context as well as in a graph form. The requirements of the interactive visualization tools are driven by the following goals:

- Provide an integrated view of the datasets in SEA in order to support multidisciplinary data analysis and planning for future data collection.
- Allow dataset browsing with various search criteria including space, time, and meta-data attributes.
- Support the selection of individual datasets and alternative displays such as line plots, images, or tables

The data flow architecture of AVS allows iterative cycles of user interaction to be implemented with an upstream flow of data to selected modules. Selecting a geometric object can result in an alternate display of the data or the use of that object to refine the definition of a query. The network can operate as a browse tool or it can assist in more detailed analysis.

For an integrated view we created special subnetworks for each type of dataset with default visuals and a common projection. Alternate visuals were added to allow for detailed views of each dataset. The integrated view provides a perspective on the sampling coverage of each type of dataset in the sound. It also provides the basis for our ability to browse and investigate data in finer detail.

The tools provide several ways to define the regions of interest in space and time by specifying queries visually. A user defined region of interest can be drawn on the geometry viewer. The resulting polygon is used in the selection criteria when the datasets are retrieved. A second method uses a bounding polygon around a selected dataset, or group of datasets, to define a proximity query. These polygons can be entered by the user or generated automatically using the bounds of the selected data. A third approach generates a polygon based on the values of other datasets. Isolines of temperature, salinity, or depth could be used to create a set of polygons used in the selection process. These three types of spatial queries are described more fully later and shown in Figures 2 through 4. Finally, polygons may also be predefined around study areas in which research is being concentrated.

In order to support these types of queries, as well as other types of non-spatial queries, we are implementing a data management architecture which includes an archive of HDF files and a database of meta-data. The database uses Illustra to store meta-data along with other spatial objects including the predefined polygons around study areas. To allow transportability the variables are stored in HDF files. These variables will also be cached in the database in order to answer content based queries.

We chose the Illustra database management system because it includes features of both object-oriented and relational database management systems (DBMSs). Like a relational DBMS the data is organized into tables, views and indexes. Illustra is a commercial database management system which includes a database kernel with a standard set of data types and functions. Illustra is also extensible, allowing the user to create new data types and the functions which operate on those types. Several additional packages, called 'datablades', are available for Illustra, which include a set of types and functions which are tailored to a particular type of application.

Illustra's two dimensional spatial datablade provides a suite of spatial functions and types, including a polygon type. In addition, this datablade provides an rtree index over a subset of the spatial data types. Our meta-database includes a table of datasets with a record for each dataset in the archive. One of the attributes in this table is a 2D polygon which describes the bounds of each dataset in latitude and longitude. An rtree is used to index the set of polygons in the table. A spatial query supplies another polygon which circumscribes the region of interest and the result of the query is a list of datasets from the table which fall within that polygon.

4 Demonstration Prototype

Figures 1 through 4 are images from our prototype application. Figure 1 displays a subset of the SEA datasets over the bathymetry of Prince William Sound. The depth of the ocean floor is exaggerated and all of the topography above sea level has been flattened. The view is looking North East from the gulf of Alaska. The port of Valdez is in the top right hand corner.

Several of the datasets have been lettered in Figure 1. The wavy lines behind the letter A display oceanographic data which is collected with an aquashuttle which moves up and down in the water column as it is towed behind a ship. Acoustic transects of the fish populations appear as blue an green curtain near the letter B. These data represent the echos of objects in the water as the sonar instrument is moved along the surface. The vertical columns display the salinity from the surface to depth. These are called CTD casts and include temperature and oxygen measurements. Finally, the line near the letter D, is the path of an aerial survey (video) of birds and other visible effects like rip tides. The other three figures show different methods of using a polygon to select SEA datasets.

Figure 2 shows a polygon which has been drawn by a user around a local area of interest. This is done to reduce the retrieval time and get a general view of the data collected for a region. Figure 3 displays the selection criteria for a more detailed analysis. Polygons, defined in close proximity to 3 acoustic transects, are used to select oceanographic data which may be correlated with the acoustic data. These polygons could be automatically generated by selecting the transect from the geometry viewer.

Figure 4 shows CTD casts within a polygon which was created from a depth contour. Such an isoline could connect points of equal temperature or salinity rather than points of equal depth. This figure also shows a graph of temperature vs. salinity for a single CTD cast which has been inverted in the display. This type of alternate display provides additional analytic capability from within the visualization application.

5 Conclusion

By combining advanced visualization techniques and database management, our prototype provides a framework for integrating a disparate set of data. The user can visualize the data and specify spatial queries on the display, which then refine the query selection. Our work with the prototype is being followed by a full scale implementation of the data management architecture and several other visualization tools. This will test the scalability of the design with larger volumes of data.

Acknowledgments

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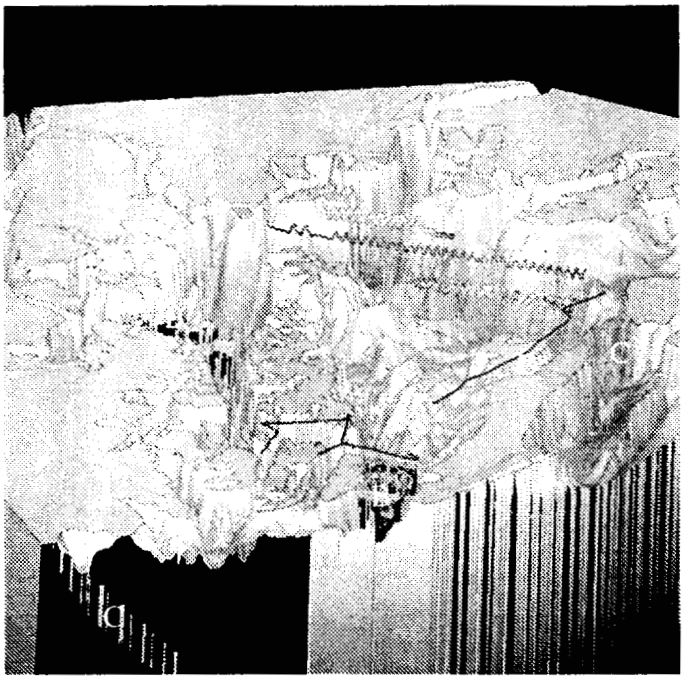

Figure 1: Bathymetry overview of Prince William Sound displaying 4 types of datasets: A) aquashuttle tows; B) acoustic transects; C) CTD casts; D) aerial survey.

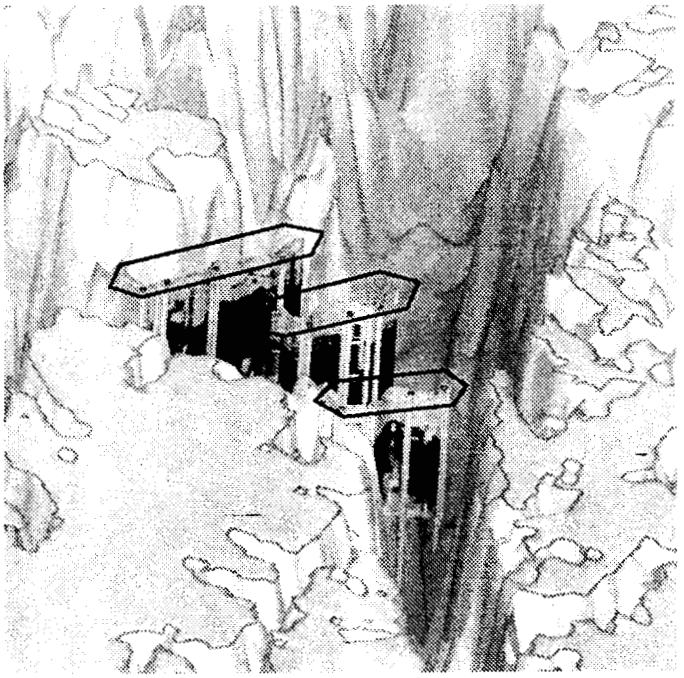

Figure 3: A set of three polygons are used to query within a close proximity of selected transects. The polygons can be drawn by the user or generated automatically from the bounds of the transects.

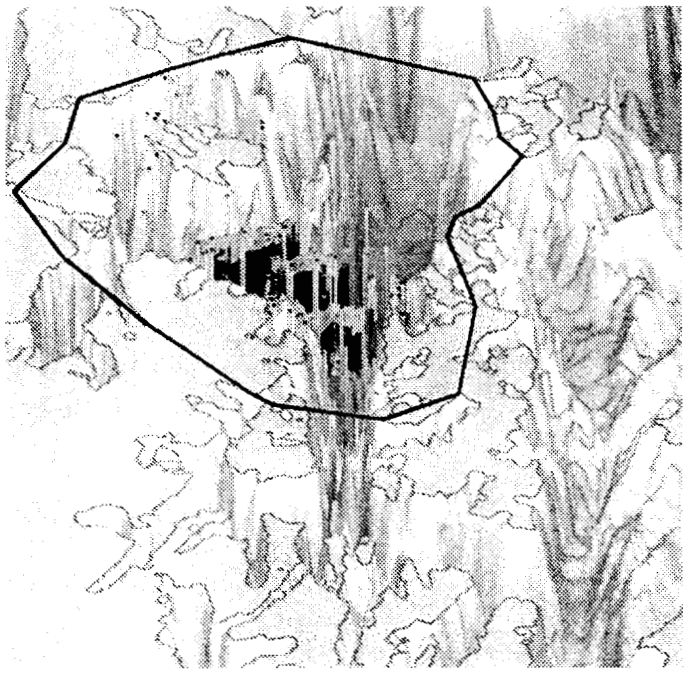

Figure 2: Region of interest specified by the drawing a polygon on the AVS geometry viewer. The polygon is used by Illustra^{TM} to select datasets within the region.

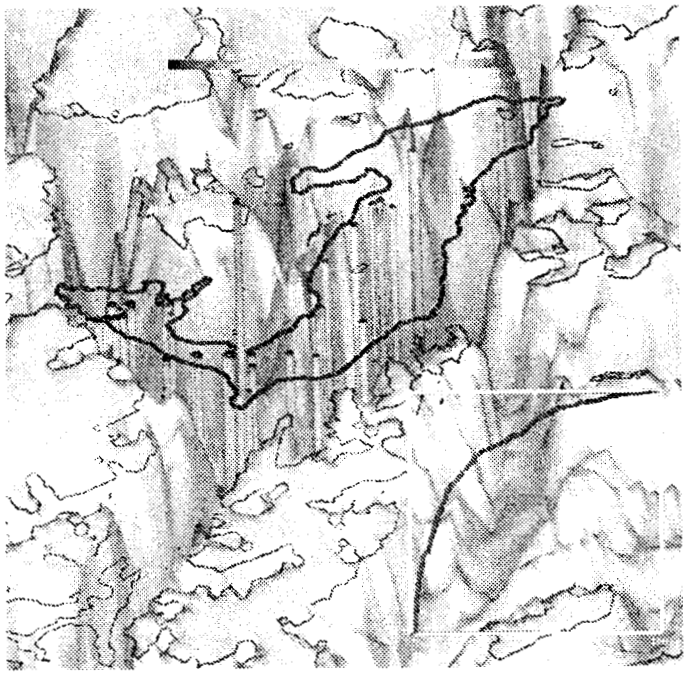

Figure 4: A region is specified using a depth contour of the bathymetry. A single CTD cast has been selected and a graph of the data is displayed.

Appendix 4

SEA Data Dictionaries
CTD casts
Echo Acoustics for Pollock

1.1 CTD casts

General Description

The CTD instruments measure temperature and salinity at a given interval of pressure. Using the salinity value, pressures are converted to depths. CTD cast are made at regular stations during the broad area survey cruise and at index sites. The raw data is averaged and then bined at 1 decibar intervals.

Variables

Temperature	Water temperature in °C.
Pressure	The water pressure in decibars of the measurements.
Oxygen	Some CTD casts include oxygen content of the water in ml/Liter.
Salinity	A unitless salinity derived from conductivity.

Size and Coordinates of Sample Cell

The data are collected for each decibar of pressure from the surface to the bottom. The length of a decibar is very close to a meter for the entire water column The depth array is derived using the pressure a conversion equations. There is only one latitude, longitude and date/time for the entire column of data. A weighted average is applied to the raw data and it is stored for each cell.

The coordinates of the sample include the latitude, longitude, depth and date/time of the cast. In addition, pressure can be used as a coordinate instead of depth. Both pressure and depth are represented as arrays. These coordinates describe a point at the top of the sample cell and the lat/lon is recorded at the beginning of the sampling (some vessel drift may be experienced during the sampling).

Searchable Meta-Data

These data can be used to select CTD casts from the database.

Date/time	Range of dates and times when the data were collected
Location	A bounding polygon in lat/lon
Depth	A range of depth
Station name	The standard station name on which is was collected
Cruise Id	The id given to the cruise on which the data were collected

Ship name	The name of the ship on which the data were collected
Operator	The name of the individual who operated the CTD
Instrument Id	The id of the CTD instrument which collected the data
Temperature	A range of temperature
Pressure	A range of pressure
Salinity	A range of salinity

Estimate of Data Volume

In 1994 there were approximately 1000 ctd cast with an average of about 300 samples each. None of these casts included oxygen. The storage requirement for the HDF archive and the Illustra objects are based on these averages.

In HDF the overhead for the common meta-data (4000) plus the cruise meta-data (4000) plus the ctd meta-data (1000) come to total storage over-head of 9000 bytes. The overhead for 4 arrays (ctd without oxygen) with 300 samples per array is 9600 bytes. Together the average HDF file will occupy 18600 or about 20k per ctd dataset. With a seasonal average of 1000 datasets, 20Mb will be needed to store for the HDF archive of one seasons ctd data.

The storage requirements of Illustra will be much less. Only meta-data will be stored in Illustra and much of the data will not need to be replicated for each dataset. An estimate of 500 bytes per dataset will result in about .5Mb per season.

We also have between 2000 and 4000 historical ctd casts, each with about 300 samples. At 20k per dataset the HDF requirements for these datasets will be about 40Mb to 80Mb of space.

Open questions

- Does the number of data sets look right?
- What is the time zone are the dates and times for?
- Am I right that the coordinates are at the top of the cell?
- What percent of the casts will include oxygen?

1.1.1 ASCII Layout

The ASCII files include a header file, a CTD data file, and a free form text file for instrument calibration or notes. The header file has one line per dataset and the file names of the other two files. All of the dataset meta-data is located in the header file and the text file and the data arrays come from the data file.

ASCII Header data for CTD (id ctd-01) This is a comma delimited header file which contains one line per ctd dataset. Each line includes the dataset type and the format identifier (01). It also includes the information about the location and collection of the dataset. Finally it includes the name of the file which could include a varying amount of text describing the calibration of the CTD or other notes about the dataset.

	Variable	Units/Type	Description
1	Dataset Type	$\leq$ 10 bytes	This dataset type 'ctd'
2	Format version	integer	This file format id: '01'
3	Dataset Id	$\leq$ 16 bytes	A unique dataset reference id set by the submitting group and used to uniquely identify the dataset to the database.
4	Dataset PI	$\leq$ 20 bytes	The name of the PI person who is responsible as the author of the data: 'Dave Salmon'
5	PI Affiliation	$\leq$ 35 bytes	The affiliation of the PI for the data: 'PWSSC'
6	Origin of Dataset	$\leq$ 10 bytes	This is the name of the group which originated the dataset. In SEA this is 'SEA-x' where x is the letter abbrievation for the group: 'SEA-M'
7	Dataset Comment	$\leq$ 25 bytes	A comment used to describe this dataset. (optional)
8	Samples file	$\leq$ 16 bytes	Name of the file containing the sample measurements
9	Samples format id	integer	The file format id number of the samples file

	Variable	Units/Type	Description
10	Cruise id	integer	The id which identifies the cruise on which the samples were collected. The format is xxnn where xx is the year and nn is sequence number within that year.
11	Sampling id	integer	The id which identifies a logical point during the cruise at which several samples were collected.
12	Location name	$\leq$ 8 bytes	The name of the station or transect where the samples were collected.
13	Ship name	$\leq$ 20 bytes	The name of the ship from which the samples where collected.

	Variable	Units/Type	Description
14	Instrument Model	$\leq$ 10 bytes	Instrument model number.
15	Instrument Number	$\leq$ 10 bytes	Instrument serial number.
16	Operator Id	$\leq$ 8 bytes	The id of the person who operated the instrument during the sample collection
17	Log book Id	$\leq$ 10 bytes	The id of the log book used during the sample collection
18	Calibration/Text file name	$\leq$ 16 bytes	Name of the file containing text describing the calibration or other text about the instrument, operation or dataset.
19	Latitude	deg.dec	Latitude of dataset.
20	Longitude	deg.dec	Longitude of dataset.
21	Date	yyyy-mm-dd	Date of dataset collection
22	Time	hh:mm:ss	Time of dataset collection
23	Time zone	4 bytes	Time zone of dataset collection (eg. "YDT")
24	Oxygen Flag	char	Oxygen concentration collected (y/n)
25	Number of samples	integer	The number of samples (lines) in the samples file
26	Salinity Model	$\leq$ 12 bytes	The name of the model used to derive salinity.

ASCII data for CTD casts (id ctd-11) This blank delimited ASCII format includes the variables collected during the CTD drop. The first line of the file will not contain data and is available for column header information.

	Variable	Units/Type	Description
1	Pressure	decibars	
2	Temperature	°C	
3	Salinity	unitless	
4	Flag	none	

1.1.2 HDF and Illustra Schema

A single HDF file will store one CTD dataset. This includes all of the data in the ASCII files along with generic data common to multiple SEA datasets. The data in each HDF file is broken down as follows. The Illustra objects will contain all of the data in the first group, 'dataset attributes'.

- Dataset Attributes
  - Common SEA dataset attributes
  - Names of the HDF file and the ASCII files used for the dataset

  - Cruise level attributes
  - Instrument/Operation attributes
  - CTD specific meta-data
  - Minimum and Maximum values of the CTD arrays
- Dataset Arrays
  - CTD arrays (1-d; size is number of depths)
  - Coordinate array (1-d; depth)

Dataset Attributes

Common meta-data for all SEA datasets

Every dataset will include meta-data which will identify the dataset, describe the data, describe the source of the data, and provide the geographic coordinates of the data. The various meta-data elements shared by all datasets are described below.

Dataset classification and identification

The dataset type, along with the format verion number, can be used to identify the variables and meta-data which are contained in the dataset. Over time, if variables are added or removed from a dataset or the meta-data describing those variables change, a new format version number will be assigned which identifies the new format of the dataset. The type-version is a concatenation of the the dataset type and the version number.

The dataset type and the dataset id unquely identify each dataset in the database. This is independent of the format and can be used to select a single dataset from the database. The dataset id is assigned by the group which submits the dataset in order to prevent duplicate copies of the same data.

Name	Type	Units	Description
dsetSetType	char[11]	none	This is the type of data set. Possible values: {echo, adcpoff, adcpnear, aquapack, opc, avhrr, surbird, surherr, avfry, avspawn, ctd, eggmort, fishcatch, isotope, phynuto, tide, weather, zoops}
dsetSetId	char[17]	none	A unique dataset id established by the submitting group.
dsetFmtVersion	integer	none	This identifies the variables and meta-data in the dataset.
dsetTypeVer	char[15]	none	Dataset type and version combined together to form a unique dataset type id.
dsetGeom	char[13]	none	This is the geometry of the samples within the dataset. Possible values: {relational, uniform, rectilinear, irregular}
dsetLevel	char[4]	none	This is the level of interpretation of this dataset. Possible values: {2, 3, 4, 5}

Dataset geo-location

The following meta-data items describe the space and time in which the data values are located. This includes a bounding polygon projected on the surface of the sound. This polygon is made up of a series of lat/lon points which are stored in the lat/lon arrays. Min and max depth and date/time are also stored for each dataset.

Name	Type	Units	Description
dsetLatArr	real	deg.dec	Array of latitude values for bounding polygon.
dsetLonArr	real	deg.dec	Array of Longitude values for bounding polygon.
dsetMinDepth	real	meters	This is the minimum sample depth of all the points in the Dataset.
dsetMaxDepth	real	meters	This is the maximum sample depth of all the points in the Dataset.
dsetBegDTime	timestamp	yyyy-mm-dd hh:mm:ss tz	Earliest sample time of the variables in the dataset.
dsetEndDTime	timestamp	yyyy-mm-dd hh:mm:ss tz	Latest sample time of the variables in the dataset.

Dataset origin

The following general meta-data items describing the dataset origin of the dataset and include a text comment which can be used for any identifying features. The PI is individual who is responsible for the data in the dataset and the submitting user is the individual who provided the data for the archive.

Name	Type	Units	Description
dsetOrigin	char[11]	none	This is the name of the group which originated the dataset. In SEA this is 'SEA-x' where x is the letter abbrievation for the group. (eg. SEA-M)
dsetPI	char[21]	none	The name of the PI who is the author of the dataset
dsetAffiliation	char[36]	none	The affiliation of the dataset PI.
dsetSubmitId	char[9]	none	The user id of the person who submitted the dataset to the archive.
dsetArrSize	char[18]	none	The size of the data arrays. (eg. 100x230)
dsetComment	char[26]	none	A comment used to describe this dataset.

File names and locations for SEA datasets

The data will be stored in ASCII file archive and in and HDF archive. These attributes provide the pointers to these files. Both types of files could be moved to CD or tape storage. If URL is specified then the directory string contains the URL type (eg. file://) along with the URL directory and the name includes the file name.

Name	Type	Units	Description
dsetASCIIFiles	text	none	A blank delmited list of ASCII files from which the dataset was taken.
dsetASCIIDir	char[129]	none	The name of the directory containing the ASCII files.
dsetASCIILocation	char[13]	none	Storage location of the ASCII files. Possible values: {disk, tape, CD, URL}
dsetHDFName	char[17]	none	The name of the HDF file in which the data is stored.
dsetHDFDir	char[129]	none	The name of the directory containing the HDF files.
dsetHDFLocation	char[13]	none	Storage location of the HDF files. Possible values: {disk, tape, CD, URL}
dsetHDFDTime	timestamp	yyyy-mm-dd hh:mm:ss tz	Date and time the HDF file was created.

Cruise meta-data for SEA datasets

Datasets collected on a survey cruise planned by SEA contain the id of the cruise and the ship name from which the samples were collected. The location is a unique transect or station and the sampling id is used to group together several different datasets which were collected at the same logical point during the cruise

Name	Type	Units	Description
crsCruiseId	long	none	This identifies the specific cruise on which the dataset was collected. The format is xxnn where xx is the year and nn is sequence number within that year.
crsSamplingId	integer	none	This number is attached to all datasets which are collected at the same logical point during a cruise.
crsShipName	char[21]	none	The name of the ship on which the dataset was collected.
crsLocationName	char[11]	none	The name of the station or transect where the dataset was collected.

Instrument/operation meta-data for SEA datasets

Some datasets will include data which describe the operation of the collection instrument. These data document the collection of the data and the calibration of the instrument. The calibration text is a free form description of how the collection instrument was set when the samples were taken.

Name	Type	Units	Description
igoInstrId	char[11]	none	Unique instrument identification.
igoOperatorId	char[9]	none	The id of the person who operated the instrument during the sample collection
igoLogId	char[11]	none	The id of the log book used during the sample collection
igoCalText	char[var]	none	Variable length text for calibration data or notes.

CTD File level meta-data attributes (id ctd-101) These are CTD specific, file level meta-data generated during ingestion of the CTD ASCII files.

Name	Type	Units	Description
ctdSalModel	char[13]	none	The name of the model used to derive salinity.
ctdNbrSamples	integer	none	The number of depths for each array
ctdOxyIncluded	char	none	Oxygen included in dataset (Y/N).
ctdAvgCellHght	integer	meters	Average height of the sample cell over which the data averaged.
ctdCoordLoc	char[8]	none	Location of the coordinate relative to the cell. 'top'

CTD min-max values (id ctd-111) These are minimum and maximum values for the CTD arrays. They are used to answer value based queries.

Name	Type	Units	Description
ctdTempArrMin	real	°C	Minimum value in temprature array.
ctdTempArrMax	real	°C	Maximum value in temprature array.
ctdSalArrMin	real	unitless	Minimum value in salinity array.
ctdSalArrMax	real	unitless	Maximum value in salinity array.
ctdOxyArrMin	real	ml/Liter	Minimum value in Oxygen array.
ctdOxyArrMax	real	ml/Liter	Maximum value in Oxygen array.
ctdPresArrMin	real	decibars	Minimum value in Pressure array.
ctdPresArrMax	real	decibars	Maximum value in Pressure array.

Dataset Arrays

CTD coordinate array (id ctd-111) The only coordinate array for CTD is depth. The depths are derived from pressure and a model of the relationship between depth and pressure.

Name	Type	Units	Description
ctdDepthArr	real	meters	Coordinate array of depths at which the measurements were taken.

CTD data arrays (id ctd-111) These are CTD data arrays. Not all datasets will include Oxygen.

Name	Type	Units	Description
ctdTempArr	real	°C	Array of temprature measurements.
ctdSalArr	real	unitless	Array of salinity measurements.
ctdPresArr	real	decibars	Array of pressure measurements from which depth is calculated.
ctdOxyArr	real	ml/Liter	Array of Oxygen measurements.

1.1 Echo Acoustics for Pollock

General Description

These datasets contain the results of echo soundings collected in offshore or nearshore surveys. The backscatter from echo sounder can be manually interpreted into numeric and biomass density of pollock, and biomass density of herring and zooplankton. The current datasets contain the biomass density and numeric density of pollock. These data were collected from broad area, offshore and nearshore surveys of the sound. Many were collected in the pink salmon migration corridor. The ground truth of some of the acoustic datasets is established from tow net samples.

Variables

Pollock biomass density	The biomass density of pollock in Kg/m^3 for each sample cell.
Pollock numeric density	The numerical density of pollock in $number/m^3$ for each sample cell.

In future, other variables might include the biomass density of zooplankton and herring.

Size of Sample Cell

The variables are retrieved from a given number of pings of the sonar equipment made along a cruise transect. The length of a sample cell depends on the resolution needed but it can range between 4m and 240m, 90m is common. This range is calculated from the ranges shown in the table below.

Pings/cell	8 – 60
Pings/second	1 – 3
Meters/second	1.5 – 4

These data are collected within cells which vary in size with depth. The vertical bin size is standardized during data interpretation to a 1 meter of depth. The horizontal dimension varies from 4m to 240m The average cell size of a dataset could be 90m by 1m.

Coordinates

The coordinates of the sample include the latitude, longitude and depth of each sample. The beginning and ending date and time are available for the the dataset as a whole. The coordinates of the sample values represent the middle of the top of the cell.

Estimate of Data Volume

Each acoustic dataset is approximately 125 by 50, or in the neighborhood of 6250 values. Each value requires 8 bytes or 50k for each array in the dataset.

With one variable in each dataset and an overhead of 8k for the HDF files the average file holding an acoustic dataset would need 58k bytes. In 1995 there were approximately 200 survey datasets and 100 near shore acoustic datasets. If this average holds true for other years, then the HDF files holding the acoustic data will require approximately 17.5Mb of space per season.

The storage requirements of Illustra will be much less. Only meta-data will be stored in Illustra and much of the data will not need to be replicated for each dataset. An estimate of 500 bytes per dataset results in about 150kb for the Illustra objects.

Searchable Meta-data

The following data items can be used to search for acoustic datasets.

Date/time	Range of dates and times when the data were collected
Location	A bounding polygon in lat/lon
Depth	A range of depth
Biomass Density	A range of pollock biomass density
Numerical Density	A range of pollock numerical density
Transect name	The standard transect id used by ADFG
ADFG Set number	The set number assigned by ADFG to net tow.
ADFG Site id	The ADFG numeric site id.
Survey Type	The code used to indicate nearshore, offshore, or broad scale survey.
Cruise Id	The id given to the cruise on which the data were collected
Ship name	The name of the ship on which the data were collected
Operator	The name of the individual who operated the echo sounder
Instrument Id	The id of the echo sounder instrument which collected the data

Tools used for Analysis

These data are acquires and reduced using the BioSonics Echo Signal Processer (ESP); they are processed, regridded and exported with IDL. These data are visualized using AVS.

1.1.1 ASCII Layout

The ascii files include a sampling header file, an instrument operation file, and a variables or samples file. The header file has one line per dataset and there is one samples file per dataset.

The instrument operation file is described below and could pertain to several different datasets. The file names can be 16 characters long and the date/time values are yyyy-mm-dd hh:mm:ss tz in Alaska time.

ASCII Header data for echo acoustic data (id echopol-01) This is a comma delimited header file which contains one line per acoustic dataset. Each line includes the dataset type and the format identifier (01). It also includes the information about the location and collection of the dataset. Finally it includes the name of the file which could include a varying amount of text describing the calibration of the sonar equipment or other notes about the dataset including model parameters used when the data was derived.

The location field is the ADFG transect number which is a number between 1 and 88. The group cruise number is assigned by SEA-N to different cruise legs.

	Variable	Units/Type	Description
1	Dataset Type	$\leq$ 10 bytes	This dataset type 'echopol'
2	Format version	integer	This file format id: '01'
3	Dataset Id	$\leq$ 16 bytes	A unique dataset reference id set by the submitting group and used to uniquely identify the dataset to the database.
4	Dataset PI	$\leq$ 20 bytes	The name of the PI person who is responsible as the author of the data: 'Gary Thomas'
5	PI Affiliation	$\leq$ 35 bytes	The affiliation of the PI for the data: 'PWSSC'
6	Origin of Dataset	$\leq$ 10 bytes	This is the name of the group which originated the dataset. In SEA this is 'SEA-x' where x is the letter abbrievation for the group: 'SEA-N'
7	Dataset Comment	$\leq$ 25 bytes	A comment used to describe this dataset. (optional)
8	Samples file	$\leq$ 16 bytes	Name of the file containing the sample measurements
9	Samples format id	integer	The file format id number of the samples file

	Variable	Units/Type	Description
10	Cruise id	integer	The id which identifies the cruise on which the samples were collected. The format is xxnn where xx is the year and nn is sequence number within that year.
11	Sampling id	integer	The id which identifies a logical point during the cruise at which several samples were collected.
12	Location name	$\leq$ 8 bytes	The name of the station or transect where the samples were collected.
13	Ship name	$\leq$ 20 bytes	The name of the ship from which the samples where collected.

	Variable	Units/Type	Description
14	Instrument Id	$\leq$ 10 bytes	Unique instrument identification.
15	Operator Id	$\leq$ 8 bytes	The id of the person who operated the instrument during the sample collection
16	Log book Id	$\leq$ 10 bytes	The id of the log book used during the sample collection
17	Calibration/Text file name	$\leq$ 16 bytes	Name of the file containing text describing the equipment calibration or other text about the instrument, operation or dataset.
18	Begin Date	yyyy-mm-dd	Begin Date of dataset collection
19	Begin Time	hh:mm:ss	Begin Time of dataset collection
20	End Date	yyyy-mm-dd	End Date of dataset collection
21	End Time	hh:mm:ss	End Time of dataset collection
22	Time zone	4 bytes	Time zone of dataset collection (eg. "AKDT")
23	Group Cruise Id	$\leq$ 8 bytes	The cruise number used by a specific group.
24	ADFG Set Number	$\leq$ 9 bytes	The ADFG set number. This is the set number of the trawl which was underway during echo sounding.
25	ADFG Site Number	integer	The ADFG numeric site id.
26	Survey Type	1 byte	The type of survey (O=Offshore, N=Nearshore, B=Broadscale).
27	Model Name	$\leq$ 12 bytes	A name which describes the models used to derive the data values.

ASCII data for Acoustics datas (id echo-11) This blank delimited ASCII format includes the variables derived from the acoustic soundings. Biomass density of pollock is the variable and the other data items are coordinates.

	Variable	Units/Type	Description
1	Latitude	deg.dec	Latitude of sample
2	Longitude	deg.dec	Longitude of sample
3	Depth	meters	Depth of sample
4	Biomass density	Kg/m^3	Biomass density of pollock
5	Numeric density	$number/m^3$	Numerical density of pollock

1.1.2 HDF and Illustra Schema

A single HDF file will store one Acoustic dataset. This includes all of the data in the ASCII files along with generic data common to multiple SEA datasets. The data in each HDF file is broken down as follows. The Illustra objects will contain all of the data in the first group, 'dataset attributes'.

- Dataset Attributes
  - Common SEA dataset attributes
  - Names of the HDF file and the ASCII files used for the dataset
  - Cruise level attributes
  - Instrument/Operation attributes
  - Acoustic specific meta-data
  - Minimum and Maximum values of the Acoustic arrays
- Dataset Arrays
  - Acoustic arrays (2-d; cols x depth)
  - Coordinate arrays (1-d; lat, lon, & depth)

Dataset Attributes

Common meta-data for all SEA datasets

Every dataset will include meta-data which will identify the dataset, describe the data, describe the source of the data, and provide the geographic coordinates of the data. The various meta-data elements shared by all datasets are described below.

Dataset classification and identification

The dataset type, along with the format verion number, can be used to identify the variables and meta-data which are contained in the dataset. Over time, if variables are added or removed from a dataset or the meta-data describing those variables change, a new format version number will be assigned which identifies the new format of the dataset. The type-version is a concatenation of the the dataset type and the version number.

The dataset type and the dataset id unquely identify each dataset in the database. This is independent of the format and can be used to select a single dataset from the database. The dataset id is assigned by the group which submits the dataset in order to prevent duplicate copies of the same data.

Name	Type	Units	Description
dsetSetType	char[11]	none	This is the type of data set. Possible values: {echopol, adcpoff, adcpnear, aquapack, opc, avhrr, surbird, surherr, avfry, avspawn, ctd, eggmort, fishcatch, isotope, phynuto, tide, weather, zoops}
dsetSetId	char[17]	none	A unique dataset id established by the submitting group.
dsetFmtVersion	integer	none	This identifies the variables and meta-data in the dataset.
dsetTypeVer	char[15]	none	Dataset type and version combined together to form a unique dataset type id.
dsetGeom	char[13]	none	This is the geometry of the samples within the dataset. Possible values: {relational, uniform, rectilinear, irregular}
dsetLevel	char[4]	none	This is the level of interpretation of this dataset. Possible values: {2, 3, 4, 5}

Dataset geo-location

The following meta-data items describe the space and time in which the data values are located. This includes a bounding polygon projected on the surface of the sound. This polygon is made up of a series of lat/lon points which are stored in the lat/lon arrays. Min and max depth and date/time are also stored for each dataset.

Name	Type	Units	Description
dsetLatArr	real	deg.dec	Array of latitude values for bounding polygon.
dsetLonArr	real	deg.dec	Array of Longitude values for bounding polygon.
dsetMinDepth	real	meters	This is the minimum sample depth of all the points in the Dataset.
dsetMaxDepth	real	meters	This is the maximum sample depth of all the points in the Dataset.
dsetBegDTime	timestamp	yyyy-mm-dd hh:mm:ss tz	Earliest sample time of the variables in the dataset.
dsetEndDTime	timestamp	yyyy-mm-dd hh:mm:ss tz	Latest sample time of the variables in the dataset.

Dataset origin

The following general meta-data items describing the dataset origin of the dataset and include a text comment which can be used for any identifying features. The PI is individual who is responsible for the data in the dataset and the submitting user is the individual who provided the data for the archive.

Name	Type	Units	Description
dsetOrigin	char[11]	none	This is the name of the group which originated the dataset. In SEA this is 'SEA-x' where x is the letter abbrievation for the group. (eg. SEA-M)
dsetPI	char[21]	none	The name of the PI who is the author of the dataset
dsetAffiliation	char[36]	none	The affiliation of the dataset PI.
dsetSubmitId	char[9]	none	The user id of the person who submitted the dataset to the archive.
dsetArrSize	char[18]	none	The size of the data arrays. (eg. 100x230)
dsetComment	char[26]	none	A comment used to describe this dataset.

File names and locations for SEA datasets

The data will be stored in ASCII file archive and in and HDF archive. These attributes provide the pointers to these files. Both types of files could be moved to CD or tape storage. If URL is specified then the directory string contains the URL type (eg. file://) along with the URL directory and the name includes the file name.

Name	Type	Units	Description
filSetType	char[11]	none	This is the type of data set. Possible values: {echopol, adcpoff, adcpnear, aquapack, opc, avhrr, surbird, surherr, avfry, avspawn, ctd, eggmort, fishcatch, isotope, phynuto, tide, weather, zoops}
filSetId	char[17]	none	A unique dataset id established by the submitting group.
filFileName	char[17]	none	The file name.
filFileDesc	char[31]	none	The description of the file.
filFileLoc	char[129]	none	The path to the file.
filFileFmt	char[7]	none	The format of the file (ASCII, HDF, DBMS).
filFileCont	char[4]	none	The type of data stored in the file: (hdr, dta, cal, txt, ...).
filFileOnline	boolean	none	(t/f) t: The file is online f: The file is nearline or on tape.
filFileURL	boolean	none	(t/f) t: The file name is a URL f: The file name is not a URL.
filFileDTime	timestamp	none	The date and time the file was last modified.
filFileOnline	integer	bytes	The size of the file in bytes.

Cruise meta-data for SEA datasets

Datasets collected on a survey cruise planned by SEA contain the id of the cruise and the ship name from which the samples were collected. The location is a unique transect or station and the sampling id is used to group together several different datasets which were collected at the same logical point during the cruise

Name	Type	Units	Description
crsCruiseId	long	none	This identifies the specific cruise on which the dataset was collected. The format is xxnn where xx is the year and nn is sequence number within that year.
crsSamplingId	integer	none	This number is attached to all datasets which are collected at the same logical point during a cruise.
crsShipName	char[21]	none	The name of the ship on which the dataset was collected.
crsLocationName	char[11]	none	The name of the station or transect where the dataset was collected.

Instrument/operation meta-data for SEA datasets

Some datasets will include data which describe the operation of the collection instrument. These data document the collection of the data and the calibration of the instrument. The calibration text is a free form description of how the collection instrument was set when the samples were taken.

Name	Type	Units	Description
igoGearName	char[11]	none	Gear/Instrument name.
igoGearNumber	char[11]	none	Gear/Instrument serial number.
igoOperatorId	char[9]	none	The id of the person who operated the instrument during the sample collection
igoLogId	char[11]	none	The id of the log book used during the sample collection
igoCalText	char[var]	none	Variable length text for calibration data or notes.

Echo acoustics file level meta-data attributes (id echo-101) These are Acoustic specific, file level meta-data generated during ingestion of the acoustic ASCII files.

Name	Type	Units	Description
echSurveyType	char	none	The flag indicating the type of survey. (O=Offshore, N=Nearshore, B=Broadscale)
echNetSet	char[10]	none	The ADFG set number.
echSiteNbr	integer	none	The site number used by ADFG.
echGroupCruise	char[9]	none	The cruise number used by a specific group.
echNetSet	char[10]	none	The ADFG set number.
echModel	char[13]	none	The name of the model(s) used to derive data.
echAvgCellLen	integer	meters	Average length of the sample cell over which the data averaged.
echAvgCellHght	integer	meters	Average height of the sample cell over which the data averaged.
echCoordLoc	char[8]	none	Location of the coordinate relative to the cell. vertical:horizontal {{top, mid, bot} : {beg, mid, end}} 'top:mid' for Acoustic.

Echo acoustic min-max values (id aco-111) These are minimum and maximum values for the acoustic data arrays. They are used to answer value based queries.

Name	Type	Units	Description
echPolBioMin	real	Kg/m^3	Minimum value in the Pollock biomass array.
echPolBioMax	real	Kg/m^3	Maximum value in Pollock biomass array.
echPolNbrMin	real	$number/m^3$	Minimum value in the Pollock numeric density array.
echPolNbrMax	real	$number/m^3$	Maximum value in Pollock numeric density array.

Dataset Arrays

Echo acoustic coordinate array (id echo-111) The coordinate arrays for acoustic datasets includes the latitude, longitude, and depth.

Name	Type	Units	Description
echLatArr	real	deg.dec	Array of latitude values of samples points.
echLonArr	real	deg.dec	Array of Lonitude values of samples points.
echDepArr	real	meters	Array of depth values of samples points.

Acoustic data arrays (id aco-111) The acoustic data array maintains the biomass density of pollock for each sample cell.

Name	Type	Units	Description
echPolBioArr	real	Kg/m^3	Array of biomass of Pollock.
echPolNbrArr	real	$number/m^3$	Array of numeric density of Pollock.

Appendix 5

Design and Implementation of the SEA Data Archive

The Design and Implementation of the SEA Data Archive

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Abstract

The data archive for the EVOS Sound Ecosystem Assessment (SEA) project contains a wide range of datasets from several different scientific disciplines. The methodology for developing this archive is broken into three tracks: the documentation of the data, the implementation of the data ingestion system, and the development of the tools used to retrieve the data. This paper outlines the functionality of the data archive and describes the tasks undertaken in each of these three tracks. A brief status as of January 1996 is presented as a conclusion.

Overview

The data archive, or database, for the Sound Ecosystem Assessment project (SEA) is designed to store datasets from the 14 projects which are under the SEA umbrella. In addition, the archive will contain historical datasets and other datasets from outside of SEA. The data include physical variables describing the oceanography, hydrology and weather, as well as biologic variables describing the plankton, salmon, pollock, and herring in the Sound. Currently 18 different types of datasets have been identified and it is estimated that between 4000 and 5000 datasets will be added to the archive each year. The goal is to build a long lasting data archive along with the query tools to retrieve datasets from the archive.

The development of the SEA database is progressing along three tracks:

1. The creation of a dictionary of SEA datasets and data elements
2. The design and implementation of a SEA data management architecture
3. The development of software tools for managing the database and retrieving datasets

Although these tracks are proceeding in parallel the begin dates must be staggered. Some of the data dictionary must be complete before the ingestion portion of the architecture can begin, and some of the datasets must be ingested before the query tools can be fully designed and developed. These three development tracks are described below and together offer an overview of components and functionality of the SEA data archive.

Data Dictionary

The data dictionary for SEA includes the definitions of all of the datasets and data elements in the database. The dictionary entry for each data item includes the item's name, type, unit of measure, and description. The dictionary entry for each dataset includes the following categories:

- A brief description of the dataset
- The list of the variables in the dataset
- The sampling geometry and sample cell size for each variable
- An estimate of the volume of data per year
- A list of the meta-data items which can be used in searches to select the dataset from the DBMS
- The list and layout of the ASCII files which are submitted to the archive as the dataset
- The layout of the HDF files and the DBMS tables which contain the dataset in the archive

One of the prime goals of creating a SEA data dictionary is to identify and standardize shared data elements. These shared data elements will allow query access to multiple types of datasets using a common data definition. Shared elements include the data variables themselves (eg. temperature), geographic coordinates, and dates and times. Shared, collection related, meta-data items such as the survey cruise id, or the name of the PI should also prove to be valuable selection criteria.

The SEA data dictionary will be the comprehensive documentation of the all SEA data. A complete dictionary of the datasets and data elements in the

SEA database will allow effective use of the database by the wide range of research disciplines within SEA. In addition, the long term research potential of these datasets will depend upon comprehensive and consistent documentation.

Data Management Architecture

The structure of the SEA database system is shown in figure 1. It includes an archive of datasets stored in HDF files and an index of those datasets in the Illustra^{tm} DBMS. The ingestion of datasets from ASCII input files includes the creation of the HDF file for each dataset and an entry for the dataset in the DBMS indexes. Queries are submitted to the DBMS through one of several software tools and the result is a list of datasets which meet the search criteria. These components and the relationships between them make up the SEA data management architecture.

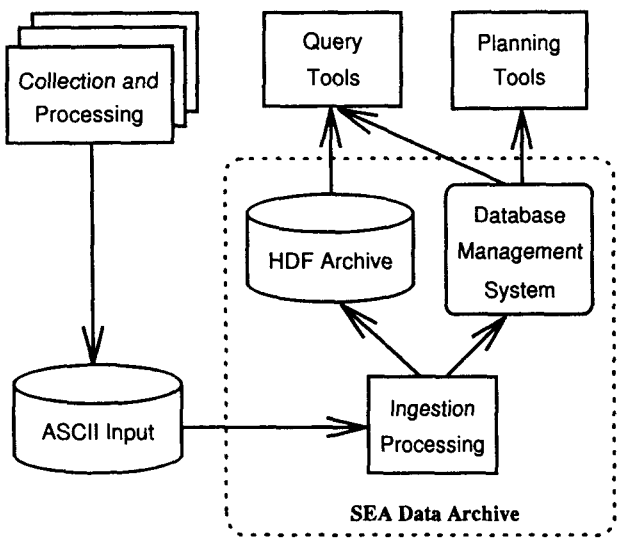

Figure 1: Architecture of the SEA data management system showing the main processing and data components. Arrows indicate the flow of data between components.

The primary storage mechanism in the data archive is the Hierarchical Data Format [4] which was designed and is maintained by the National Center for Supercomputing Applications (NCSA) at the University of Illinois. This scientific data format is called self-describing because each data element is accompanied by a name and definition. This feature, in addition to some other facilities, allow HDF files to be transported to and accessed on different hardware platforms including workstations and PCs.

Encapsulation and portability were part of the HDF specification in order to support long lived data archives. The HDF standard has been chosen by NASA's EOS project as the primary dataset storage technology. This ensures that HDF will continue to receive excellent support and will be available well into next century.

The SEA Software tools

The SEA software tools will utilize the Illustra^{tm} DBMS and will be used to plan the data collection process and query and manage the data archive. The three main tools are:

- Planning tools for organizing the survey cruises.
- Netscape based query tools for selecting and displaying datasets
- Query tools which run at a local workstation or PC

A planning tool is currently running and provides several maps of the Sound on which a survey cruise can be drawn. Once the path of the cruise is laid out, dates and resources can be assigned to each leg or station along the cruise path. The output from the cruise planner can be imported to a spreadsheet program to allow costs to be assigned to each leg of the cruise.

The query tools use the indexing facilities of the Illustra^{tm} DBMS to identify and locate the datasets which meet the given selection criteria. Illustra is an Object-Relational DBMS which allows new data types to be defined and stored using a traditional table structure. A package of spatial data types which includes two dimensional points, lines, and polygons is being used in the SEA database to provide a spatial index of datasets. These spatial queries are the first of the three basic types of queries which the SEA database supports:

- Spatial and temporal queries
- Other meta-data based queries
- Variable value base queries

Spatial and temporal queries result in a list of datasets which are in a given region of space or range of time. Meta-data queries allow selection based on meta-data which describe the collection or interpretation of the data values. An example would be all datasets collected with a given instrument or on a particular survey cruise. The third type of query results in a list of datasets which have variables within a range of values. The result list can be then used to download all or part of the variables in the HDF file or to display the location of the datasets on a map.

The Netscape based query tool allows access to all SEA data from any machine which is able to run Netscape. Once the proper authorization is provided, HDF or ASCII files can be downloaded or displayed using Netscape facilities.

The local query tools have the additional advantage that they can check the local archive of datasets and only download new or changed datasets. In addition, a local tool can be used in conjunction with an application which can accept a list of datasets and process and display the data. This will be an important feature for the large modeling efforts which are part of SEA.

Current Status of Development

Together these three development tracks provide a framework for understanding the design and development of the SEA database. As of January 1996, interviews with all but a few of the groups within SEA have been conducted and 5 of the 18 datasets have been documented. The SEA data dictionary currently includes these 5 datasets and approximately 330 data elements. Most of data elements common to all datasets have been identified and standardized.

A prototype of the data management architecture was implemented last spring and is available for review. The ingestion of the CTD datasets into the production version of the SEA database underway and should be completed in February. A major portion of ingestion processing must be developed as part of the ingestion of this first dataset. A generic data structure is being used which is able to describe most of the datasets within SEA reducing the development effort required for each new dataset.

A planning tool was designed and implemented last summer and fall. It was used to plan parts of the October cruise and is now available for the 96 planning season. This planning tool is also being used as a display tool for the CTD datasets which are in the prototype.

The completion dates for rest of the ingestion process and the query tools will depend upon the resources available. The complexity of the datasets varies widely but a general estimate of bringing a new dataset online is 4 to 8 weeks of individual effort. This includes 2-3 weeks of discussion with each group to define and document each part of the dataset. This time includes the layout of all files and database objects and might include reformatting existing ASCII files to meet the design specifications. Another 2-3 weeks will be needed to create the new programs needed to read in and ingest the dataset. Finally 1-2 weeks are needed to incorporate the new dataset into the query tools. In addition, several weeks will be needed for the completion of the initial ingestion processing and the initial development of the query tools.

The new year begins with the data definition work done for several of the datasets and most of the initial design and development of the ingestion processing. In addition, the planning tool and the database prototype provide a solid set of design criteria for the planned query tools.

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Appendix 6

Modeling Prince William Sound Ocean Circulation

MODELING PRINCE WILLIAM SOUND OCEAN CIRCULATION

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1 . INTRODUCTION

Prince William Sound (PWS) is a combination of multiple fjords, basins, and estuaries along the coast of Alaska. Its spatial scale is approximately 80x80 km with an average depth of about 150m. Its deep basins (with a maximum depth of about 750m) and channels were formed by a combination of preglacial erosion, glacial excavation, and tectonism.

The water exchange between PWS and the coastal Gulf of Alaska strongly influences the circulation pattern and biomass distribution (Schmidt, 1977). The spatial scale of the basin is barely large enough for a recirculation to develop (Niebauer et al. 1994). PWS is very rich in the production of salmon, halibut, herring, and other fish species. Its economic potential strongly depends on how well the fishcatch can be managed and how well the pollution can be minimized in the presence of a major oil tanker route. Therefore, understanding the circulation patterns is basic to understanding PWS ecology and environmental risks. Our goal is to establish a 3-D nowcast/forecast system (and later, possibly a coupled physical-biological (ecosystem) numerical model, including nutrients, phytoplankton, zooplankton, and fish concentrations and their interactions). In this paper, thus, a 3-D model of PWS is presented and the dynamical origin and nature of the circulation are examined.

Tidal currents are very strong in PWS due to the tidal range of 3 to 4 meters. Such a strong tide (with the M_2 constituent being the largest) helps to flush PWS, by mixing coastal waters with those of PWS. The magnitude and pattern of the tidal residual current is another important aspect. These circulation features are not understood. Observations have been conducted since 1973 in PWS (Royer et al. 1979) and along the coast of Alaska (Royer, 1975); it was found that the coastal current usually intrudes into PWS from Hinchinbrook Entrance and drives the basin-scale cyclonic circulation.

A modified Princeton Ocean Model (POM, Blumberg and Mellor 1987), which has been successfully applied to the circulation of Hudson Bay (Wang et al. 1994), is used. It uses the primitive equations (with hydrostatic and Boussinesq approximations) and has the following features: (1) horizontal curvilinear coordinates; (2) an "Arakawa C" scheme; (3) sigma (terrain-following) coordinates in the vertical with realistic bottom topography; (4) a free surface; (5) a level 2.5 turbulence closure model for the vertical viscosity and diffusivity (Mellor and Yamada, 1982); (6) a mean flow shear parameterization for horizontal viscosity and diffusivity (Smagorinsky, 1963); (7) a semi-implicit scheme to solve for surface elevation in the shallow water equations; and (8) a predictor-corrector scheme for the time integration to avoid inertial instability (Wang and Ikeda, 1995).

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The model domain includes the entire PWS with two open boundaries allowing water exchange with the Alaskan coastal water, Hinchinbrook Entrance and Montague Strait (Fig 1). The model grid spacing is 1.2 km, which is eddy-resolving because the internal Rossby radius of deformation is about 5 km (Niebauer et al. 1994). There are 11 vertical sigma levels. The integration time step is 62.1 seconds.

According to the observations in Hinchinbrook Entrance (Niebauer et el, 1994), the coastal inflow varies seasonally: from 0.1 to 0.3 Sverdrup (1 Sv=10^6 m^3s^{-1}). Similarly, the outflow through Montague Strait is of the same order of magnitude according to the conservation of volume transport, though the water volume in PWS may increase or decrease in response to transient forcing. Hence, an inflow of 0.3 Sv was specified through the Hinchinbrook Entrance, while a radiation boundary condition (with self-adjusted outflow of 0.3 Sv) was applied to the Montague Strait. Outflow may also occur through Hinchinbrook Entrance. A coastal M_2 tide with an amplitude of 1.5 m (i.e., the tidal range is 3 m) is specified at both open boundaries.

Section 2 describes the initial simulations. Section 3 presents the future nowcast/forecast system. Section 4 summarizes the present results and the next steps in the future study of the PWS circulation.

2. INITIAL SIMULATION RESULTS

The initial temperature and salinity fields use typical summer profiles with no horizontal variations, because comprehensive spatial data are not yet available. Thus, the results shown below exclude the density-driven component which is smaller than, but perhaps comparable in magnitude to the barotropic current. The model is spun up from these initial conditions for 8 days. At this time a dynamical steady-state is reached, and the restart file is saved for use as the initial condition in the next runs. There are no heat and salt fluxes specified yet at the ocean surface. Vertical viscosity is determined from the Mellor-Yamada 2.5 turbulence closure model with a background viscosity of 10^{-5} m^2s^{-1}, and the horizontal viscosity is determined from the Smagorinsky horizontal mixing closure with C=0.2. The typical computed horizontal viscosity is about 5 to 10 m^2s^{-1}.

2.1 INFLOW-DRIVEN CIRCULATION. The inflow from the Alaskan coastal waters is an important factor in determining the circulation pattern in PWS. The vertical distribution of the inflow decreases linearly from the surface to 150m depth, the sill depth. Tidal forcing is specified at the two open boundaries; there is no wind forcing. The inflow temperature and salinity profiles were taken to be the same as the boundary point values, i.e., the zero horizontal gradient (no heat and salt fluxes) condition was used.

Based on the circulation pattern at depths of 10m and 100m on day 25 (Fig. 2), a coastal inflow of 0.3 Sv enters from Hinchinbrook Entrance and exits through the Montague Strait at 10m, and there is an anticyclonic eddy in the eastern PWS. Inside the cyclonic main stream (inflow), there is a cyclonic eddy (Fig. 2a). At 10m, the outflow is largely channelled through the Knight Inlet (i.e., east of Knight Island).

At 100m, the inflow flows to the north following the deep channel and forms a large cyclonic circulation. The outflow is split between Knight Channel and Montague Strait. Interestingly, the inflow separates into two branches near 60.5N, 147W, perhaps due to two small seamounts (Fig. 1). In the northeastern PWS, there is a cyclonic eddy. These mesoscale eddy features reveal an important fact that PWS, in contrast to typical estuaries, has a large enough horizontal scale to not constrain mesoscale eddy development.

Figure 3 shows an 8-day time series of surface velocity vectors taken from three grid points (see locations in Fig. 1). Over the deepest basin (grid 1), the current is weak, due to the topography and geometry of the interior PWS. In Montague Strait (grid 2), because it is shallow, the tidal current superimposed on the mean southwestward current is very energetic, while the currents near Hinchinbrook Entrance are relatively weak because it is deep (grid 3).

2.2 WIND-DRIVEN CIRCULATION. Wind regimes over PWS vary seasonally with changes in the position and strength of the Aleutian Low. Eastward wind (i.e., Alaskan coastal upwelling favourable wind) tends to occur during summer because of the influence of the North Pacific High, while in winter, there are strong, steady westward winds (i.e., Alaskan coastal downwelling favourable winds) when the Aleutian Low deepens. The surface current fields under eastward and westward wind forcing of 7 ms^{-1} (Fig. 4) (with the same inflow, but no M_2 tide) have the same circulation patterns at 10m, except for small differences. The eastward wind displaces the main stream of inflow to the south through the effects of southward surface Ekman transport, while the westward wind displaces the main stream northward, through the northward surface Ekman transport. There are also numerous, weak mesoscale eddies in the northern PWS. The westward wind produces stronger circulation in the northwestern PWS. Additionally, the eastward (westward) wind piles up the water on the eastern (western) shore by typically 0.3 m (not shown).

2.3 TIDAL-DRIVEN RESIDUAL FLOW. Comparing Figs. 2a and 4, the tide-induced residual current is prominent, modifying the circulation pattern. Because of the presence of many sills, shoals, and seamounts, the interaction of the strong M_2 tidal current and topography leads to a significant tidal-residual mean flow, typically 0.05-0.1 ms^{-1}, i.e., one order of magnitude smaller than the tidal current. This residual flow is comparable to, even though smaller than, the mean current due to the coastal inflow. Therefore, the contribution of the tide to the mean circulation is very important in PWS. It remains to add the other major tidal constituents to the model.

2.4 GENERAL CIRCULATION PATTERN. With tidal, throughflow, and eastward wind (summer case) forcing (Fig. 5), as expected, the main stream is displaced to the south by the surface Ekman transport compared with Fig. 2a. In northwestern PWS, the alongshore current is strengthened.

3. NOWCAST/FORECAST SYSTEM PLAN

To establish the Prince William Sound nowcast/forecast system (PWSNFS), similar to the Straits of Florida Nowcast/Forecast System (SFNFS, Mooers and Ko, 1993; Mooers et al. 1995), the following two initial tasks will be accomplished:

1) Observational System. PWSNFS not only involves the sophisticated 3-D model presented here, but also on a reliable observational network. This network should include the historical temperature and salinity profiles, meteorological stations and buoys, moored current meters, moored and shipboard ADCPs (acoustic doppler current profilers), coastal tide gauges, and surface drifters, as well as other elements. As with atmospheric weather analyses and forecasts, the final stage of PWSNFS will include a robust data assimilation scheme.

The initial observational network (in Fig. 6) commenced from 1994 and will hopefully continue and be extended for several years. These data will be used in PWSNFS as much as possible. For example, the ADCP mooring at the Hinchinbrook Entrance will be analyzed using empirical orthogonal function analysis to determine modal (baroclinic and barotropic) distribution

of variance for the transient flow in order to more accurately specify the inflow profile. The mean and variable inflow transport will be estimated based on the time series. The weather stations and the buoys will provide synoptic winds and other atmospheric variables for the model. The tide gauge data will be harmonically analyzed to determine the tidal amplitudes and phases, and the low frequency variability which is essential to evaluating the model transient response to atmospheric storms, etc. Current meter mooring and surface drifter deployments are anticipated in the future; they will facilitate Eulerian and Lagrangian comparisons between the model and observations.

2) Atmospheric Forcing. A synoptic wind field is essential for PWSNFS, similar to SFNFS (i.e., the atmospheric Eta model winds from the National Meteorological Center will be used). The winds from the new version of the Eta model (which provides a finer horizontal resolution of 28 km) will be evaluated for use in PWSNFS. The six-hourly wind will be used to force the model. Heat and moisture fluxes from the Eta model output will also be evaluated for use as the surface boundary conditions in driving the circulation model, especially the ocean mixed layer and ecosystem dynamics. Due to coastal orographic effects, the Eta model output will probably be combined with local data using an air-sea boundary layer model. The influence of freshwater inflows from PWS coastal orography and the Alaskan Coastal Current, as well as precipitation, are yet to be examined.

4. SUMMARY AND FUTURE WORK

POM has begun to be applied to PWS and some important dynamical factors influencing the circulation pattern have been determined. The initial simulation results indicate that POM has produced basically correct circulation patterns. Inflow/outflow between PWS and the Alaskan coastal waters, wind forcing, and tidal forcing are all essential factors. Vigorous mesoscale eddies are a prominent phenomenon and may be important for biomass distribution, because they influence the biomass abundance, concentration, and residence time. Furthermore, different wind conditions may change the residence time by changing the circulation pattern and stratification.

A seasonal cycle simulation (with and without synoptic forcing and freshwater inflow) will be conducted for both physical and biological interests. The wintertime convection that produces the deep water mass of the major basin will be examined as well. When these steps are accomplished, the physical model will be coupled to the biology, providing as much information for ecological and fishery science as possible in the near future.

ACKNOWLEDGEMENT

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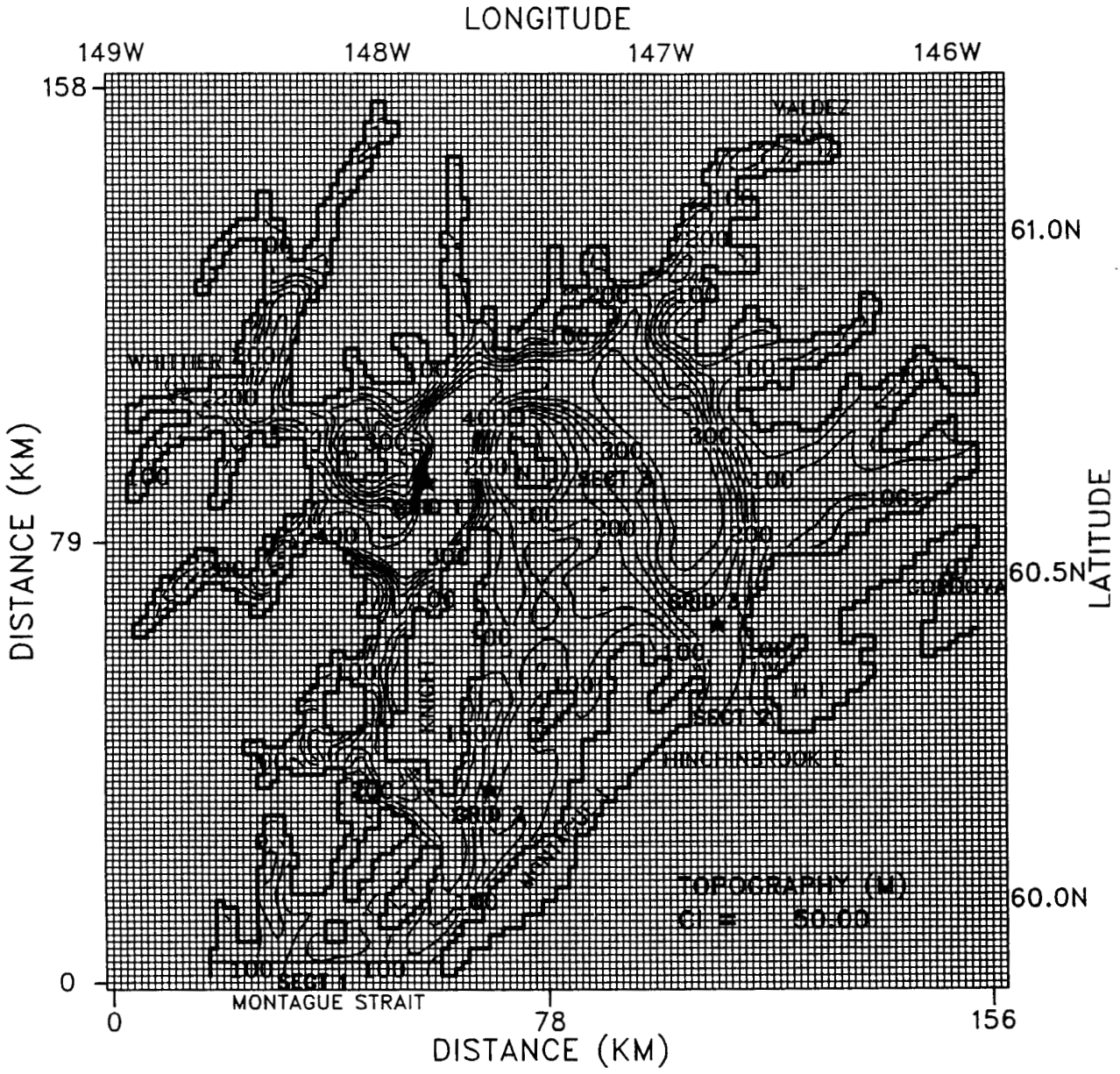
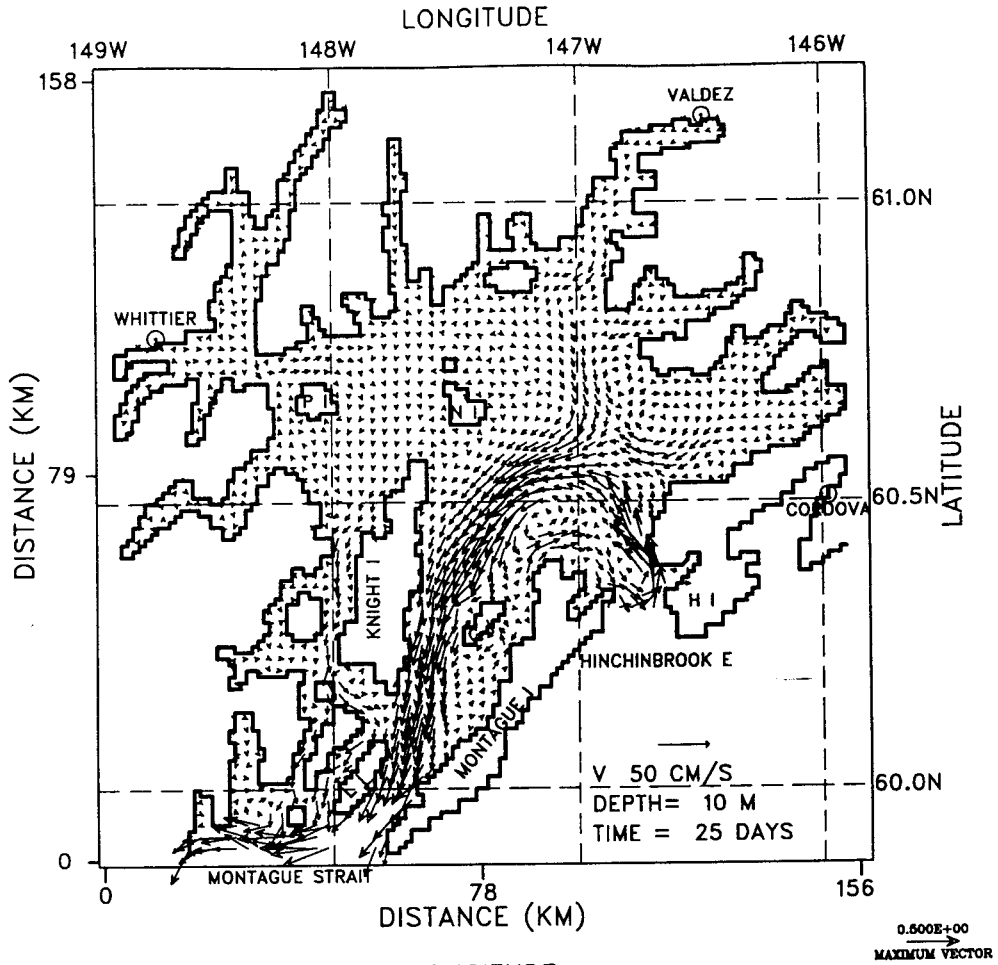
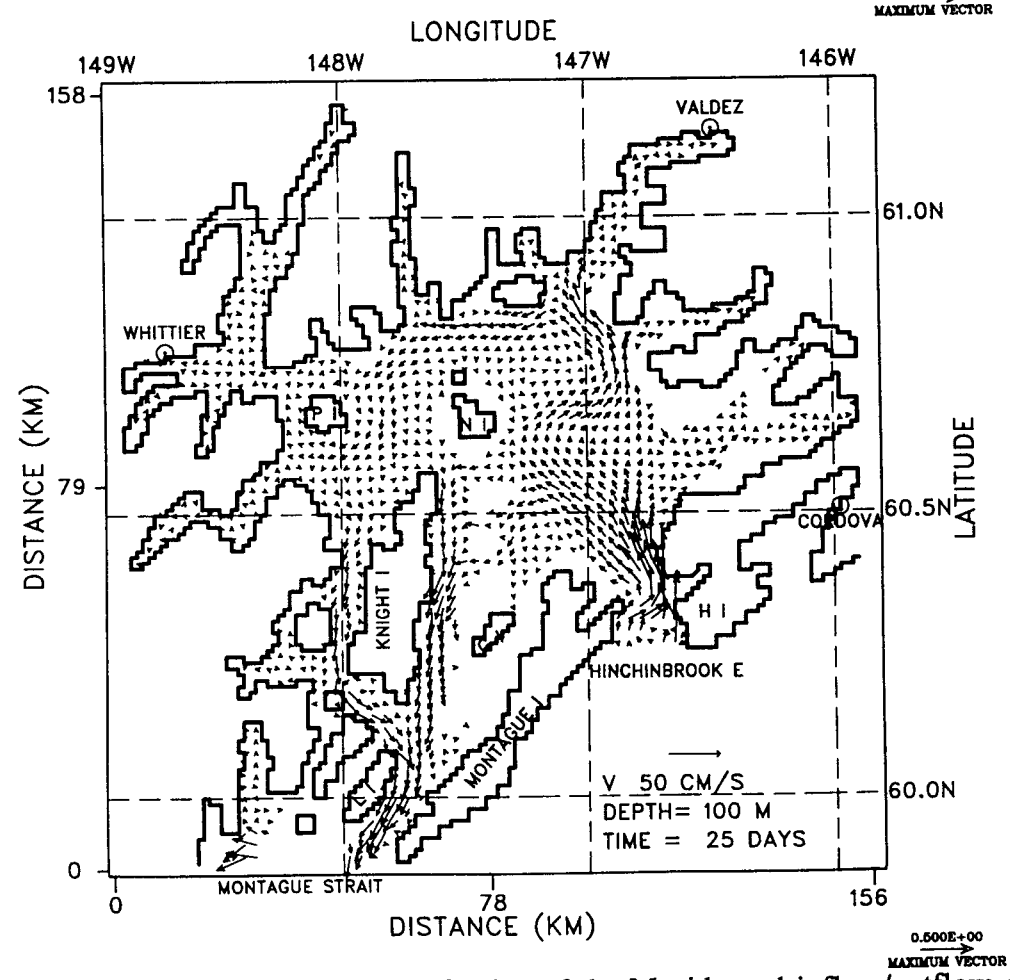


Figure 1 The PWS model domain with topography (depths in meters). The stars denote three grid points from which the time series of current velocity are taken.



a



b

Figure 2 Mean velocity field under forcing of the M_2 tide and inflow/outflow of 0.3 Sv at 10m (a) and 100m (b)

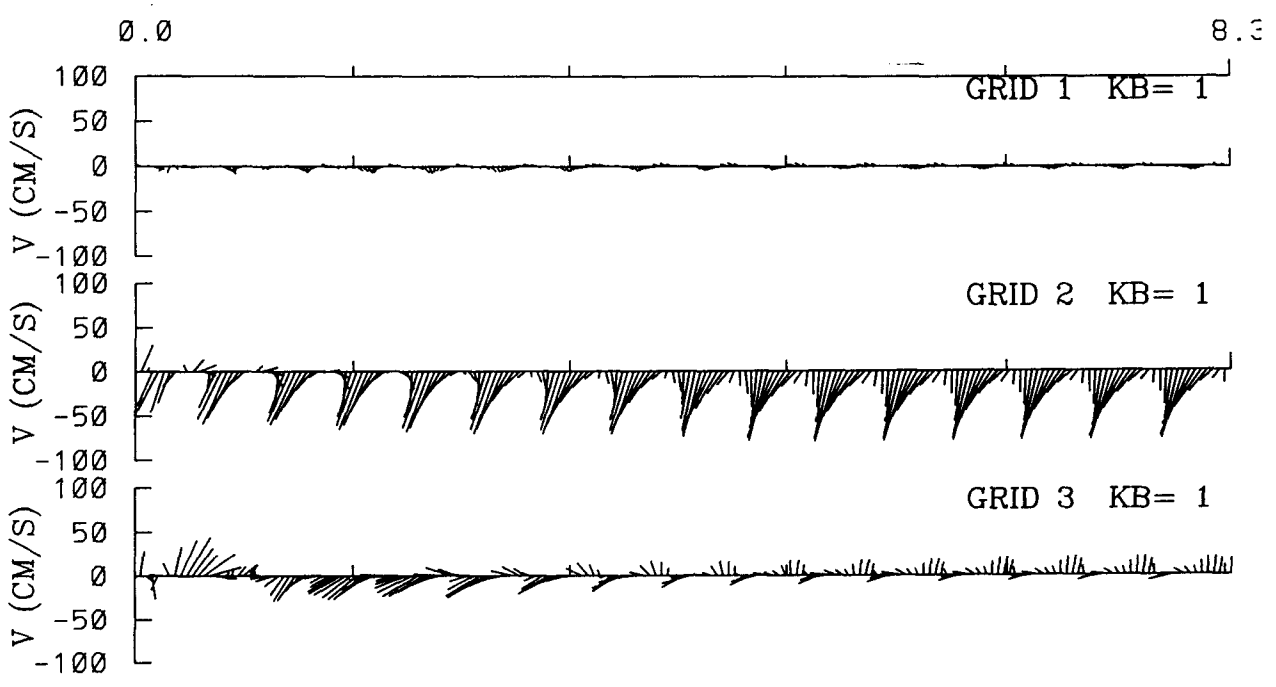


Figure 3 An eight-day time series of surface velocity vectors at grids 1, 2, and 3, as shown in Fig. 1.

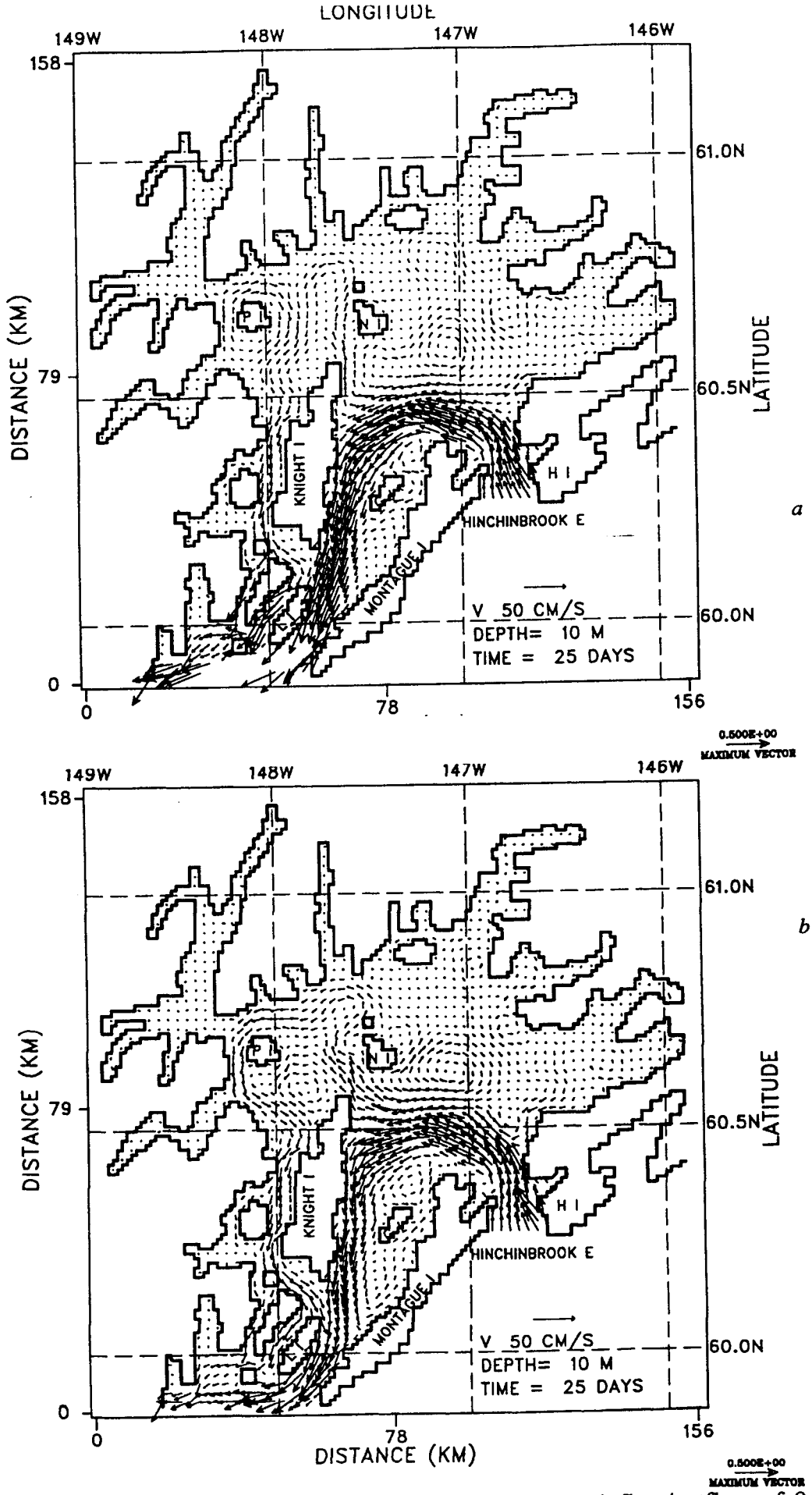


Figure 4 The 10m mean velocity field under forcing of the inflow/outflow of 0.3 Sv and eastward wind (a) and westward (b) wind of 7 ms^{-1}.

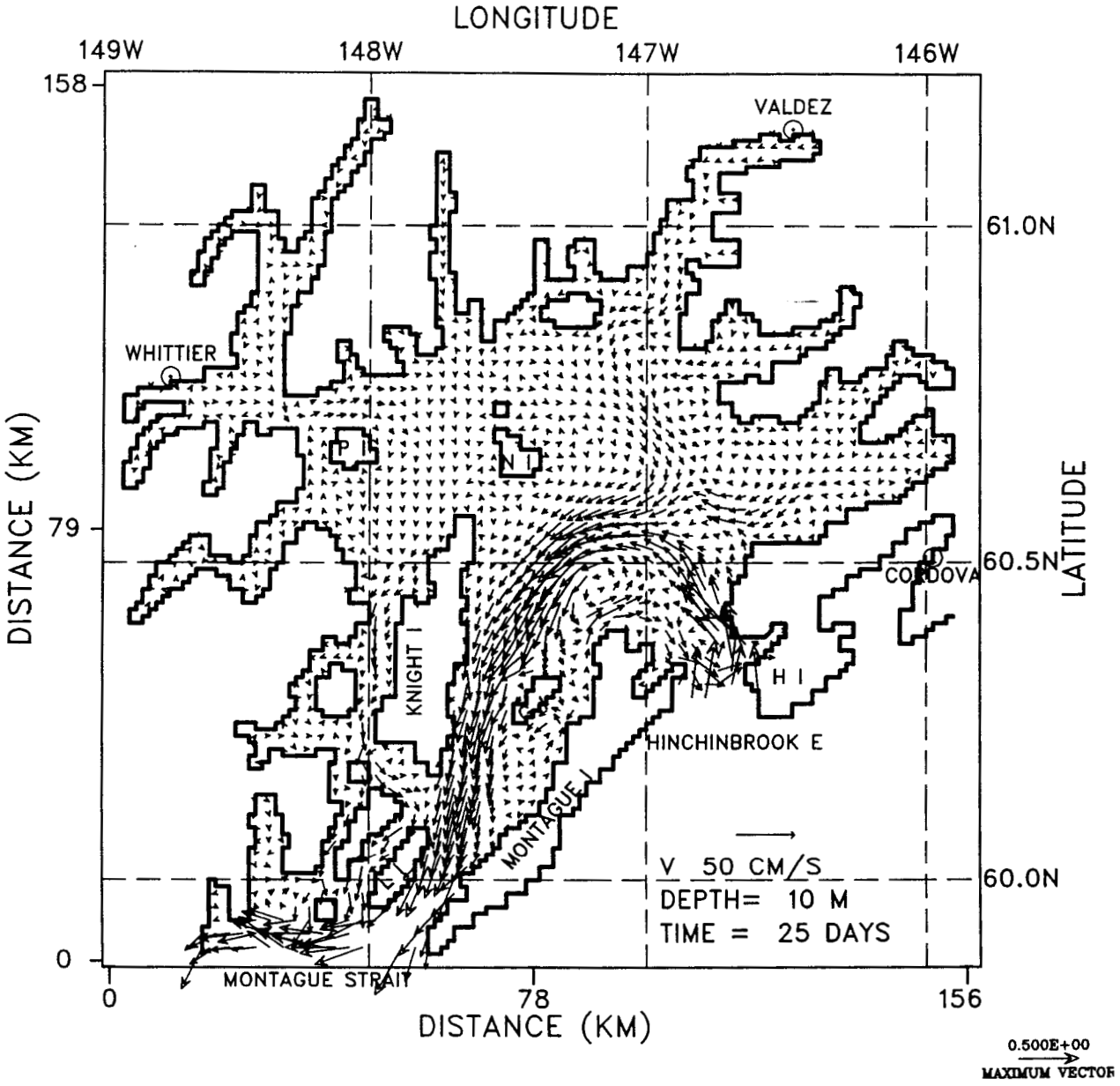
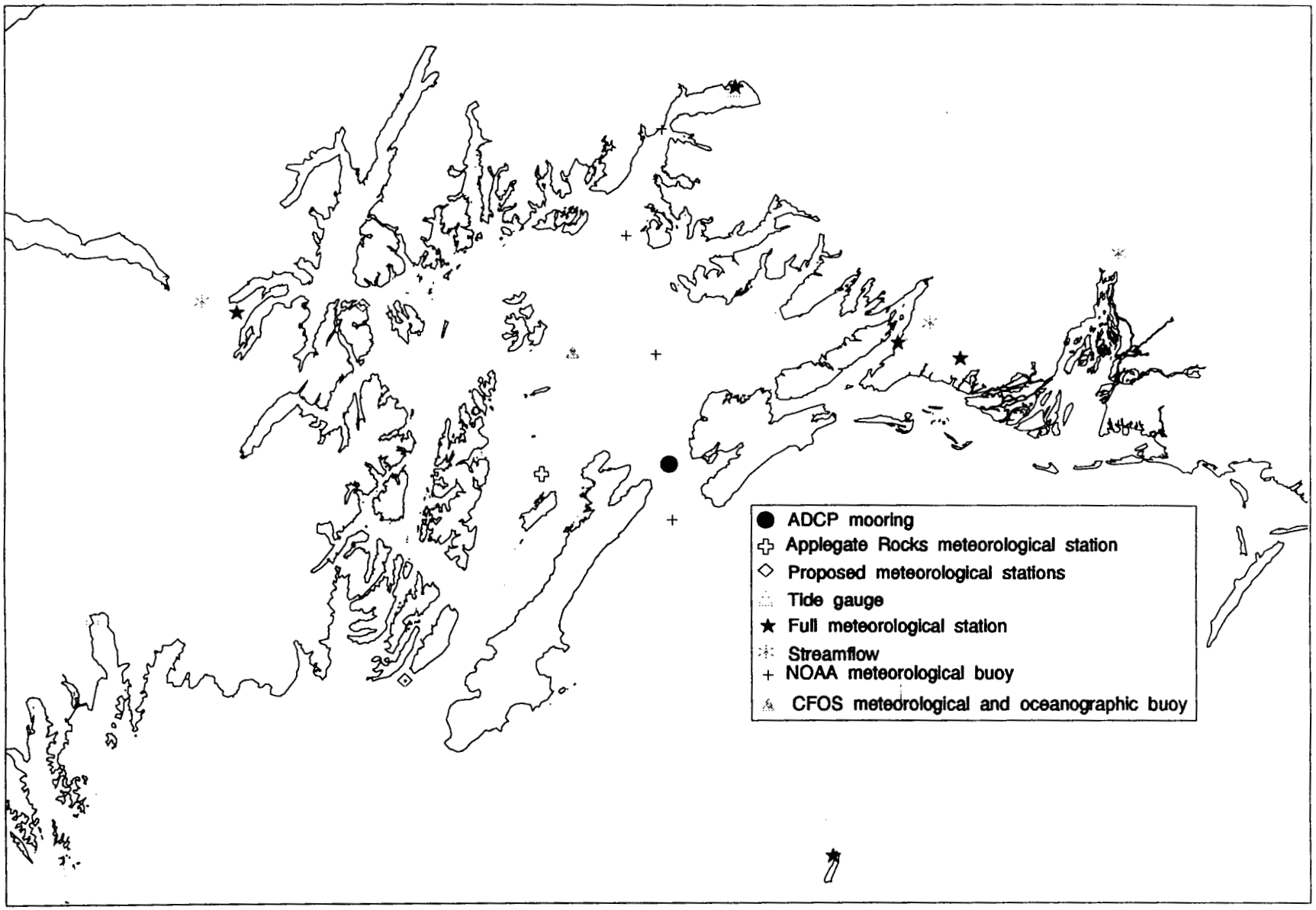


Figure 5 The 10m mean velocity field under forcing of the M_2 tide, inflow/outflow of 0.3 Sv, and eastward wind of 7 ms^{-1}.



Current and Proposed Climatological, Tidal, and Oceanographic Stations, Prince William Sound

Figure 6 The present proposed atmospheric, hydrologic, and oceanic observational stations.

Appendix 7

Finite Element Simulation of a Taxis Model for Population Interactions in 1D

FINITE ELEMENT SIMULATION OF A TAXIS MODEL FOR POPULATION INTERACTIONS IN 1D*

RICARDO H. NOCHETTO† AND SRIDHAR P. RAO‡

ABSTRACT. Predator prey interactions are central in the fields of ecology and biology. A taxis model, which incorporates fundamental processes such as foraging, reducing encounter with predators, and optimizing environmental conditions, is discretized with semi-implicit finite differences in time and mixed finite elements in space. The method exploits the special structure of advection, gradient of a taxis function, and yields a natural upwinding that works equally well in the entire range of advection and diffusion dominated flows. This is essential because either the flow character may vary with the trophic level at a given space-time or the advection/diffusion ratio may be subject to adjustment in the course of this study. The discrete problem is mass preserving, robust to disparate scales of diffusion and advection, and formally second order in space. It consists of a nonsymmetric tridiagonal matrix for each density, assembled from easily computable element contributions, which is solved via LU—decomposition at each time step.

1. Introduction. The taxis model consists of a bioenergetics model for growth coupled with a model for the dispersion of each population and the manner in which they coexist and interact. This yields a system of coupled nonlinear advection/diffusion PDEs

$$\partial_t u_i - \operatorname{div}\left(D_i \nabla u_i + \chi_i\, u_i \nabla \lambda_i(\mathbf{u})\right) = f_i \qquad \text{in } \Omega \tag{1}$$

for density u_i of the ith species and ODEs for size. Here Ω is a bounded domain in $\mathbf{R}^d$ with $d = 1, 2, 3$, constants $D_i, \chi_i > 0$ are to be adjusted so as to match measurements, and $\mathbf{u} = (u_i)_{i=1}^I$; preliminary details are contained in [7]. Function λ_i, so-called *taxis*, is responsible for movement and spatial distribution of organisms. Based on experimental, empirical, and theoretical considerations, taxis incorporates fundamental processes such as foraging (finding food), reducing the encounter with predators, and optimizing physical conditions (water temperature, light, salinity, etc). Prior related, but more restrictive, work includes models for bacteria chemotaxis by Keller and Segal and Kareiva and Odell [4,p.442;5,8].

After discretizing in time with semi-implicit backward differences and (uniform) time-step Δt

$$u_i^n - D_i \Delta t \operatorname{div}\left(\nabla u_i^n + \tfrac{\chi_i}{D_i} u_i^n \nabla \lambda_i(\mathbf{u}^{n-1})\right) = u_i^{n-1} + \Delta t f_i^{n-1} = g_i^{n-1}, \tag{2}$$

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the problem reduces to a space discretization of the system of uncoupled linear advection-diffusion PDE in (2). The Scharfetter-Gummel scheme is very popular to handle that in 1D [9], most notably for the advection dominated regime $D_i/\chi_i \ll 1$. Among the various attempts to generalize this method to 2D and 3D, we are interested in those due to Brezzi et al [2,3,6], because they uncover crucial upwinding and conservation properties by simply interpreting the 1D scheme as a combination of *exponential fitting* and *mixed* finite element methods [1,2,3,6]. Consider a single equation like (2) and set $\rho = u\ \exp(\lambda/\varepsilon)$ for $\varepsilon = D/\chi$. Then each PDE in (2) can be written equivalently as

$$\exp(-\lambda/\varepsilon)\rho - D\Delta t\ \text{div}\ \left(\exp(-\lambda/\varepsilon)\nabla\rho\right) = D\Delta t g. \tag{3}$$

The idea is to discretize (3) with a method that accounts for rapid variations of $\exp(-\lambda/\varepsilon)$ and yield a positive definite M-matrix. For the method to be flux-preserving, that is to enforce continuity of the normal component of flux

$$\sigma = \nabla u + u\nabla(\lambda/\varepsilon) = \exp(-\lambda/\varepsilon)\nabla\rho \tag{4}$$

across interelement boundaries, mixed finite elements are adequate [1]. The method of [2,3,6] uses lowest order Raviart-Thomas mixed finite elements with Lagrange multipliers to relax the continuity constraint of σ. By static condensation, and proper scaling, the problem can be reduced to a *nonsymmetric* positive definite system for the (scaled) multipliers $\mathbf{w}$, which in turn approximate the original density u instead of ρ.

The rest of this report describes the method, the algorithm for local construction of matrices $\mathbf{M}_i$ and right-hand sides $\mathbf{G}_i$, and their assembly to produce $\mathbf{M}$ and $\mathbf{G}$ satisfying $\mathbf{Mw} = \mathbf{G}$.

2. Discretization. Since the discrete formulation is the same for every trophic level, from now on we focus on the *scalar* PDE (2). This second order elliptic self-adjoint PDE can be converted into a first order system by simply regarding both ρ and σ as independent variables as follows:

$$\sigma\ \exp(\psi) - \rho_x = 0 \tag{5}$$

$$\frac{\exp(-\psi)}{D\Delta t}\rho - \sigma_x = g, \tag{6}$$

where we have used the notation $\psi = \lambda/\varepsilon$. Equation (5) is just the constitutive relation between ρ and σ, whereas (6) coincides with (3). The advection dominated regime $\varepsilon \ll 1$ is of special interest.

Let $\mathcal{T}$ denote a partition of the spacial domain $[a,b]$ into intervals (or elements) $[x_i, x_{i+1}]$. Let τ and v be piecewise smooth function whose only discontinuities are located at the nodes x_i, and let τ vanish at a and b. Multiply (5) by τ, and (6) by v, and then integrate (5) by parts to arrive at

$$\sum_i \left(\int_{x_i}^{x_{i+1}} \exp(\psi)\sigma\tau + \int_{x_i}^{x_{i+1}} \rho\tau' + \rho(x_i)[\![\tau(x_i)]\!] \right) = 0 \tag{7}$$

$$\sum_i \int_{x_i}^{x_{i+1}} \left(\sigma_x v - \frac{\exp(-\psi)}{D\Delta t}\rho v \right) = -\sum_i \int_{x_i}^{x_{i+1}} gv, \tag{8}$$

where $[\![\tau(x_i)]\!] = \tau(x_i^+) - \tau(x_i^-)$ stands for the jump of τ across x_i. This is the so-called weak (or variational) formulation of the system (5-6). We stress that the natural boundary condition

$$\sigma = 0 \tag{9}$$

at a and b is implicit in the requirement that τ vanishes at a and b, because τ is an arbitrary member of the space of functions where we seek σ.

2.1 Mixed Finite Element Method. We have to choose appropriate discrete spaces $\mathcal{S}$ and $\mathcal{R}$ to approximate both σ and ρ. However $\mathcal{S}$ and $\mathcal{R}$ must be related to each other for the resulting scheme to be stable [1]. The simplest choice consists of discontinuous piecewise linear functions for $\mathcal{S}$, which vanish at a and b, and piecewise constant functions for $\mathcal{R}$. This choice, however, may not lead to a global M-matrix $\mathbf{M}$. Such a property is intimately related to oscillations, which may occur and should thus be avoided in the advection dominated regime. A simple modification based on [6], which preserves stability and prevents oscillations, introduces suitable piecewise quadratics in $\mathcal{S}$ depending on the flow direction in place of piecewise linears. The resulting scheme reads: seek $\sigma \in \mathcal{S}$, $\rho \in \mathcal{R}$, and $\omega \in \mathbf{R}^N$ (Lagrange multipliers) such that

$$\sum_i \left(\int_{x_i}^{x_{i+1}} \exp(\psi)\sigma\tau + \int_{x_i}^{x_{i+1}} \rho\tau' + \sum_i \omega_i [\![\tau(x_i)]\!] \right) = 0 \qquad \forall\, \tau \in \mathcal{S} \tag{10}$$

$$\sum_i \int_{x_i}^{x_{i+1}} \left(\sigma_x v - \frac{\exp(-\psi)}{D\Delta t}\rho v \right) = -\sum_i \int_{x_i}^{x_{i+1}} gv \qquad \forall\, v \in \mathcal{R} \tag{11}$$

$$\sum_i [\![\sigma(x_i)]\!]\mu_i = 0 \qquad \forall\, \mu = (\mu_i) \in \mathbf{R}^N. \tag{12}$$

Note that (12) enforces continuity of σ across nodes, namely $[\![\sigma(x_i)]\!] = 0$ for all i, as well as the vanishing of the boundary values $\sigma(a) = \sigma(b) = 0$. In matrix form, this system has the following structure

$$\begin{bmatrix} \mathbf{A} & \mathbf{B} & \mathbf{C} \\ \mathbf{B}^T & -\mathbf{D} & \mathbf{O} \\ \mathbf{C}^T & \mathbf{O} & \mathbf{O} \end{bmatrix} \cdot \begin{bmatrix} \sigma \\ \rho \\ \omega \end{bmatrix} = \begin{bmatrix} \mathbf{O} \\ \mathbf{F} \\ \mathbf{O} \end{bmatrix}. \tag{13}$$

This matrix is symmetric but indefinite. However $\mathbf{A}$ is symmetric block diagonal, and $\mathbf{D}$ is diagonal, thereby making elimination of σ and ρ by static condensation feasible at the element level. In fact, elimination of σ results in

$$\begin{bmatrix} \mathbf{B}^T\mathbf{A}^{-1}\mathbf{B} & \mathbf{B}^T\mathbf{A}^{-1}\mathbf{C} \\ \mathbf{C}^T\mathbf{A}^{-1}\mathbf{B} & \mathbf{C}^T\mathbf{A}^{-1}\mathbf{C} \end{bmatrix} \cdot \begin{bmatrix} \rho \\ \omega \end{bmatrix} = \begin{bmatrix} -\mathbf{F} \\ \mathbf{O} \end{bmatrix},$$

with $\mathbf{B}^T\mathbf{A}^{-1}\mathbf{B}$ diagonal, and that of ρ yields the system $\mathbf{H}\omega = \mathbf{G}$ with

$$\mathbf{H} = \mathbf{C}^T\mathbf{A}^{-1}\mathbf{B}(\mathbf{B}^T\mathbf{A}^{-1}\mathbf{B} + \mathbf{D})^{-1}\mathbf{B}^T\mathbf{A}^{-1}\mathbf{C} - \mathbf{C}^T\mathbf{A}^{-1}\mathbf{C} \tag{14}$$

$$\mathbf{G} = -\mathbf{C}^T\mathbf{A}^{-1}\mathbf{B}(\mathbf{B}^T\mathbf{A}^{-1}\mathbf{B} + \mathbf{D})^{-1}\mathbf{F}. \tag{15}$$

Matrix $\mathbf{H}$ is a symmetric, positive definite M-matrix. The multipliers ω approximate the transformed variable ρ at nodes, which is obvious from (7) and (10). We can thus determine a nodal approximation for the density u by simply computing

$$w_i = \omega_i \ \exp(-\psi(x_i)). \tag{16}$$

In terms of the vector of scaled multipliers $\mathbf{w} = (w_i)$ the system reads $\mathbf{Mw} = \mathbf{G}$, with a global tridiagonal M-matrix $\mathbf{M}$ which is no longer symmetric. Matrix $\mathbf{M}$ can be assembled from element contributions and is insensitive to values of ε, the advection/diffusion ratio. This is explained below.

2.2 Element Computations. We define an average exponential $\bar{\psi}_i$ on the interval $[x_i, x_{i+1}]$ as follows ($h_i = x_{i+1} - x_i$):

$$\bar{\psi}_i = \log\left(h_i^{-1} \int_{x_i}^{x_{i+1}} \exp(\psi(x))dx\right).$$

The first equation in (13), namely $\mathbf{A}\sigma + \mathbf{B}\rho + \mathbf{C}\omega = \mathbf{O}$ can be restricted to the interval $[x_i, x_{i+1}]$. If we denote with $\mathbf{A}_i, \mathbf{B}_i$ and $\mathbf{C}_i$ the local matrices, the local system reads

$$\mathbf{A}_i \begin{bmatrix} \sigma_0^i \\ \sigma_1^i \end{bmatrix} + \mathbf{B}_i u_i + \mathbf{C}_i \begin{bmatrix} \omega_i \\ \omega_{i+1} \end{bmatrix} = \mathbf{O},$$

where

$$\mathbf{A}_i = h_i \ \exp(\bar{\psi}_i) \begin{bmatrix} 1 & 0 \\ 0 & \frac{2}{15} \end{bmatrix} \qquad \mathbf{B}_i = \begin{bmatrix} 0 \\ -1 \end{bmatrix}, \tag{17}$$

and

$$\begin{aligned} \psi(x_i) \geq \psi(x_{i+1}) \quad &\Longrightarrow \quad \mathbf{C}_i = \begin{bmatrix} 1 & -1 \\ 1 & 0 \end{bmatrix} \\ \psi(x_i) < \psi(x_{i+1}) \quad &\Longrightarrow \quad \mathbf{C}_i = \begin{bmatrix} 1 & -1 \\ 0 & 1 \end{bmatrix}. \end{aligned} \tag{18}$$

The two different forms of matrix $\mathbf{C}_i$ correspond to the flow from left to right (first case) and the opposite situation. This is a natural built-in *upwinding*.

The second equation in (13), namely $\mathbf{B}^T\sigma - \mathbf{D}\rho = \mathbf{F}$, when restricted to the interval $[x_i, x_{i+1}]$, can be written as

$$\mathbf{B}_i^T \begin{bmatrix} \sigma_0^i \\ \sigma_1^i \end{bmatrix} - \mathbf{D}_i\rho_i = F_i \qquad D_i = \frac{\exp(-\bar{\psi}_i)}{D\Delta t} h_i.$$

2.3 Assembly. The elimination process described above can be performed at the element level in that it is only the third equation in (13) that establishes a link between adjacent elements. We therefore produce an element matrix $\mathbf{H}_i \in \mathbf{R}^2 \times \mathbf{R}^2$ and vector $\mathbf{G}_i \in \mathbf{R}^2$ as follows:

$$\begin{aligned} \mathbf{H}_i &= \mathbf{C}_i^T \mathbf{A}_i^{-1} \mathbf{B}_i (\mathbf{B}_i^T \mathbf{A}_i^{-1} \mathbf{B}_i + \mathbf{D}_i)^{-1} \mathbf{B}_i^T \mathbf{A}_i^{-1} \mathbf{C}_i - \mathbf{C}_i^T \mathbf{A}_i^{-1} \mathbf{C}_i \\ \mathbf{G}_i &= -\mathbf{C}_i^T \mathbf{A}_i^{-1} \mathbf{B}_i (\mathbf{B}_i^T \mathbf{A}_i^{-1} \mathbf{B}_i + \mathbf{D}_i)^{-1} \mathbf{F}_i. \end{aligned}$$

Matrix $\mathbf{H}_i$ is a local stiffness matrix and $\mathbf{G}_i$ represents a local load vector. They have the following expressions depending on the flow direction:

$$\psi(x_i) \geq \psi(x_{i+1}) \Longrightarrow \mathbf{H}_i = \frac{1}{h_i \exp(\bar{\psi}_i)} \begin{bmatrix} \alpha_i & -1 \\ -1 & 1 \end{bmatrix} \quad \mathbf{G}_i = F_i \beta_i \begin{bmatrix} -1 \\ 0 \end{bmatrix}$$

$$\psi(x_i) < \psi(x_{i+1}) \Longrightarrow \mathbf{H}_i = \frac{1}{h_i \exp(\bar{\psi}_i)} \begin{bmatrix} 1 & -1 \\ -1 & \alpha_i \end{bmatrix} \quad \mathbf{G}_i = F_i \beta_i \begin{bmatrix} 0 \\ -1 \end{bmatrix}$$

where

$$\alpha_i = \frac{1 + \frac{17h_i^2}{15D\Delta t}}{1 + \frac{2h_i^2}{15D\Delta t}} \qquad \beta_i = \frac{1}{1 + \frac{2h_i^2}{15D\Delta t}}.$$

An obvious difficulty with these expressions is that they may easily yield over or underflow. If we replace the unknowns ω_i, which approximate $\rho(x_i)$, by the scaled variable w_i given by (16), then we get the following local quantities

$$\psi(x_i) \geq \psi(x_{i+1}) \Longrightarrow \mathbf{M}_i = \frac{1}{h_i} \begin{bmatrix} \exp(\psi_i - \bar{\psi}_i)\alpha_i & -\exp(\psi_{i+1} - \bar{\psi}_i) \\ -\exp(\psi_i - \bar{\psi}_i) & \exp(\psi_{i+1} - \bar{\psi}_i) \end{bmatrix} \tag{19}$$

$$\psi(x_i) < \psi(x_{i+1}) \Longrightarrow \mathbf{M}_i = \frac{1}{h_i} \begin{bmatrix} \exp(\psi_i - \bar{\psi}_i) & -\exp(\psi_{i+1} - \bar{\psi}_i) \\ -\exp(\psi_i - \bar{\psi}_i) & \exp(\psi_{i+1} - \bar{\psi}_i)\alpha_i \end{bmatrix}. \tag{20}$$

The global stiffness matrix $\mathbf{M}$ is obtained by assembling N local stiffness matrices $\mathbf{M}_i$ (N is the number of elements), which combine to form a tridiagonal matrix with negative off diagonals and a positive main diagonal (M-matrix).

2.4 Exponential Scaling. We now show that exponentials occurring in (19) and (20) can be computed safely regardless of the size of $\varepsilon = D/\chi$. We first observe that the taxis function $\lambda = \varepsilon\psi$ depends on previously computed densities, which are known at the nodes x_i. It is thus reasonable to think of ψ as a piecewise linear interpolant of the true scaled taxis function, thereby coinciding with it at x_i.

Computation of the scaled exponentials is dictated by the flow direction. We set

$$\Delta\psi_i = \psi(x_{i+1}) - \psi(x_i).$$

Hence

$$\Delta\psi_i \leq 0 \Longrightarrow \exp(\bar{\psi}_i) = \frac{\varepsilon}{\Delta\psi_i} \exp(\frac{\psi(x_i)}{\varepsilon})\big(\exp(\frac{\Delta\psi_i}{\varepsilon}) - 1\big)$$

$$\Delta\psi^i > 0 \Longrightarrow \exp(\bar{\psi}_i) = \frac{\varepsilon}{\Delta\psi_i} \exp(\frac{\psi(x_i)}{\varepsilon})\big(1 - \exp(\frac{-\Delta\psi_i}{\varepsilon})\big)$$

With these expressions at hand, we can now compute the exponentials in (19) and (20). In fact, if $\Delta\psi_i \leq 0$ we get

$$\exp(\psi_i - \bar{\psi}_i) = \frac{\Delta\psi_i}{\epsilon} \frac{1}{\exp(\frac{\Delta\psi_i}{\epsilon}) - 1}$$

$$\exp(\psi_{i+1} - \bar{\psi}_i) = \frac{\Delta\psi_i}{\epsilon} \exp(\frac{\Delta\psi_i}{\epsilon}) \frac{1}{\exp(\frac{\Delta\psi_i}{\epsilon}) - 1}.$$

Otherwise we obtain

$$\exp(\psi_i - \bar{\psi}_i) = \frac{\Delta\psi_i}{\epsilon}\exp(\frac{-\Delta\psi_i}{\epsilon})\frac{1}{1-\exp(\frac{-\Delta\psi_i}{\epsilon})}$$

$$\exp(\psi_{i+1} - \bar{\psi}_i) = \frac{\Delta\psi_i}{\epsilon}\frac{1}{1-\exp(-\frac{\Delta\psi_i}{\epsilon})}.$$

This method of computing the elements of $\mathbf{M}_i$ eliminates the concern of potential overflow, and shows its robustness with respect to ϵ.

3, Relation to Finite Differences. For uniform meshes $\mathcal{T}$, namely $h = h_i$ constant, the above mixed finite element method can be identified with a suitable upwind finite difference method. This interpretation is revealing in understanding the remarkable approximation and conservation properties of the proposed scheme, which is closely related to the Scharfetter-Gummel scheme for semiconductor device simulation [9].

4. Conclusions. The above approach exploits the special structure of advection due to the taxis, or loss function, via exponential fitting and mixed finite element methods. In doing so, the resulting discrete formulation accounts for both diffusion and advection dominated regimes through the use of inherent upwinding. This is essential in light of the expected high variability of population densities in complementary regions of the space-time domain, which could easily make both extreme regimes coexist for different trophic levels. This extra flexibility suits itself well to the needs of biological and ecological modeling, and is further enhanced by the possibility of using mesh refinement/coarsening strategies, a posteriori error estimation and adaptivity. All these features extend to multidimensional situations.

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Appendix 8

Alewife
A System for Modeling Population Interactions

Alewife

A System for Modeling Population Interactions

Sridhar Rao

Abstract

Alewife is the initial attempt at the design of a language oriented to the analysis of ecosystem behavior - in particular those elements dealing with population interactions.

The initial drive to design such a "language" came from the need to develop an adaptable system of parameter modification for the use in simulations on predator-prey interaction models. This ability to modify parameters quickly became the need to add new parameters as well.

With this adaptability becoming a strong need for the rigourous analysis of models and their relationships, a new approach was devised that was aimed at minimizing the coding and time needed to implement new model characteristics. Further evolution of alewife comes from its continued use and implementation in the real world.

This effort was motivated by the modeling problems being addressed in the Sound Ecosystem Assessment Project for Prince William Sound. The solution presented continues to evolve through this implementation with space and time dependent models for the physical and biological factors affecting the survivorship of juvenile fish. This project is funded by the *Exxon Valdez* Oil Spill Trustee Council Restoration Project 95320.

1.0 Introduction

The modeling process contains many different levels of development. Often the earlier stages are mathematically intensive and also very abstract in relation to the model as a whole. Formulation of the mathematical framework forms the first major step in modeling a system. Once a numerical analysis is complete, the parameters and relationships concerning the system can be applied. The integral formulation, and the use of variational methods to design a solution, is a broader and more general step that contains the actual model relationships and its uniqueness with regards to application.

A process can be developed in which a numerical solution (or formulation) can be designed and used with a set of model relationships. In doing so, different relationships can be added or modified without altering the underlying formulation. This will help to speed up the modeling process by eliminating the need to re-formulate a numerical analysis and help the modeler focus on aspects of the system to be studied.

The process can be expressed as shown in figure 1. The formulation and the model relationship form two parts of the mathematical solution. System

parameters are then fed into this solution which results in data that is to be used for analysis. The formulation is the more abstract of the two and in most cases can be generalized for a certain set of modeling criteria. Model relationships are of primary interest to the scientist dealing directly with the system of study.

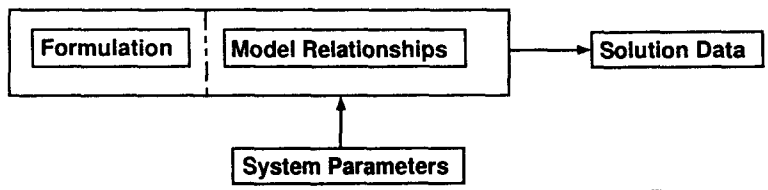

Figure 1: Components of The Modeling Process

Alewife separates these two parts of the solution and creates a standard set of formulations that can be linked with a set of model relationships. This newly linked solution can be used in conjunction with the model parameters to develop data for analysis.

For example, if one wishes to model a system of species using a finite element analysis, it can be separated into two problems: the discretized numerical analysis (formulation), and the population dynamics (model relationships). Using the approach of *alewife* one need only be concerned with the model relationships and how to integrate them into an f.e.m. solution. The numerical formulation will be encapsulated and ready for use from a library of formulations.

This example case formed the groundwork for *alewife* in work done on predator-prey interactions [1]. New attributes were constantly being added to the model relationships causing a large degree of recompile time and modifications to the source code. By separating the formulation and the relationships the addition of new dynamics to the model becomes a speedier process.

2.0 Elements

Alewife consists of various elements that are used to create a working solution called an *engine*. This engine is a compiled executable that takes in parameters and outputs data for the solution. Two main elements form the alewife engine: the *solver* and the *model relationships*.

The solver is a relocatable object file that is stored in a library of solvers and it represents the encapsulated numerical analysis. In the current distribution there is one solver which is a 1 dimensional formulation using a finite element analysis. The second section of the engine consists of the model relationships. These are scripts written by the modeler that are linked with the solver to produce an executable "engine" which is used to generate data.

3.0 Underlying Relationships

For the solver in this distribution the primary relationship that binds all these elements together is a basic advection diffusion form for the space-time behavior of multiple interacting population densities:

$$\frac{du}{dt} = f + D\Delta u + \chi\nabla \cdot u(\nabla\lambda). \tag{1}$$

In the above relationship u, f, and λ are vector valued functions and χ and D are constants. A vector component exists for each of the interacting trophic levels. The primary function of the solver is to solve for the function u which represents densities for populations.

The function λ forms the taxis relationship which is represented by the various model relationships. The forcing term f is also a model relationship and must be coded. Hence, λ and f are the two links provided by the solver to the code written by the modeler. Once these relationships are expressed, they can be joined with the solver to form an engine.

4.0 Modeling Fundamentals

To create a new engine requires a fundamental set of relationships, λ and f, which are to be designed by the modeler as mentioned. These two functions represent the temporal and spatial variations in the interactions between various populations. The function λ expresses the main biological factors and their influence on the densities in the current space-time iteration of the solution.

Due to the solution formulated in the numerical analysis, λ is actually a scaled value when returned. This scaling is related to the constants χ and D. The pre scaled function is the one that is actually used to produce biological results and is called ψ. The function f represents the forcing functions and must also be designated by the modeler.

Alewife "code" is written in a script which is interpreted by the compiler *aw*. *aw* then links the script with the solver and forms the engine which is used in conjunction with a set of model parameters and space time data to produce population dynamics data. ψ and f therefore form the two main modules that must be written in order to link with the solver. This modular approach can be taken to more levels below the top most ψ and f modules to form a series of relationships which allow for a high degree of model complexity.

4.1 Components

Now that we know what functions must be modeled in order to generate an engine, what components are at our disposal to model with? Since *Alewife* is meant primarily for the modeling of biological population interactions, a set of "basic variables" are defined for use:

```
Web
density
psi
tl
rhs
```

Web is the structure that contains variables and data related to the population densities. It contains the variables density and psi. The variable *density* refers to the density of the population that is currently being calculated and psi is the variable that contains the data that is produced from the ψ definitions. Since these are both contained in the structure of Web they are addressed accordingly:

```
Web.density Web.psi
```

The definition of the use of these variables is not yet complete. The values for density and psi are stored in the structure based on time (the current position in time n - and the previous time position n-1, etc.) and space (the solver described here uses a one-dimensional approach). Furthermore the structure Web itself is addressed based on the population or trophic level. Hence, the complete form for using these variables is:

```
Web[tl].density[i][0] Web[tl].density[i][1]

Web[tl].psi[i][0] Web[tl].psi[i][1]
```

The second counter deteremines how far back in time it is being indexed, with lower index values representing previous timesteps. Hence, timestep 0 is prior to 1. The variable tl is also an internal variable that is predifined and managed by *aw*. The variable *i* is also a reserved internal counter for use to represent the indexing through space. Since definitions of Web are only done through space, these definitons will always use i as a counter.

5.0 Coding Method and Example

To describe the method of coding and the syntax, it would be beneficial to begin with a simple example. The following piece of code will intialize all of the spatial elements of the web's density to 0.0 in both time steps (all code written in *alewife* follows a C like syntax except for when a module is declared or ended):

```
!!Module
init
Web[tl].density[i][0] = 0.0; Web[tl].density[i][1] = 0.0;
!!End
```

The first line of this code defines a new module. All *alewife* code consists of modules that are linked together in the processing stage. The next line after a module declare is the module's name, in this case *init*. Following the title is the actual logic for the module. These two lines set the values for the web's density elements for the trophic level tl to zero for all space. This code is automatically handled to iterate over all space and trophic levels. Specifically, there are five required modules for any *alewife* code:

```
init psi rhs post final
```

All of these modules are automatically iterated over space and trophic levels and time (if needed). The trophic levels that are handled can be selected from a data file that is fed to the compiled engine in the run time stage. The module psi is the module that encompasses all the biological relationships that are to be modeled. In essence it is psi that is the most crucial of all the modules. The next piece of code displays how all of the modules are tied together and incorporated into a file:

```
!!Components
lambda
exone
!!End

!!Module
lambda

lambda = (double) i * (1.0) / (N-1);

!!End

!!Module
exone

exone = exp(-1.0 * xx[i]);

!!End

!!Module
rhs

rhs = 0.0;

!!End

!!Module
psi

Web[tl].psi[i][0] = Web[tl].psi[i][1]; Web[tl].psi[i][1] =
Web[tl].psi[i][2];

call lambda print lambda Web[tl].psi[i][2] = lambda;

!!End
```

```
!!Module
init
call exone Web[tl].density[i][0] = exone; Web[tl].density[i][1]
= 0.0;
call lambda Web[tl].psi[i][0] = lambda; Web[tl].psi[i][1]
= lambda; Web[tl].psi[i][2] = lambda;
!!End
!!Module post
!!End
!!Module final
!!End
!!EndEnd
```

The above example shows a host of new terms and functions. Firstly, notice that the five required modules are all defined with the syntax:

```
!!Module
module name

!!End
```

However, prior to the first module declaration is a decalration *!!Components.* This declare states that the following list are the names of new modules that are to be declared and used later in a module declaration. Any new nonstandard module should be decalred here. These components are reserved for use in the final calculation of the value of psi, which is used in the solver to produce a solution when executed.

It is first crucial to recognize the main required modules and their definitions. These are done in the later section of the code. Looking at these definitions we see that they are assigning certain values to variables such as lambda or exone.

The module rhs is used to define the forcing terms previously mentioned as *f*. In this case there is no contribution here. The module *post* is used to do any post spatial calculations needed. This can be useful when certain trophic level relationships are dependent upon the newly attained solution in space. The module *final* is used for any calculations needed after the time iteration is complete (when all the trophic levels have been solved for in time).

When a new module is to be defined by the modeler, it is declared in the first section called the components section. Here, any new modules are named and then their logic will follow somewhere in the code. The fundamental modules, those modules which are required, are *not to be* defined in the components section.

The module lambda is first named in the components section and then the logic is written in the module definition for it. This example also shows lambda using other internally handled variables. When an assignment such as the follwing is made, it calls the module *lambda* and then does the proper assignment thereby returning the value to be assigned:

```
Web[tl].psi[i][i] = lambda;
```

Module definitions can be combined in any way to form a relationship for assignment purposes, hence the following is legal as long as all the modules are defined properly:

```
Web[tl].density[i][0] = foo + bar * (moo / goo);
```

This allows for the rapid modification of model definitons and behavior for the incorporation of new features. All of the above code is entered into the file *defpsi.x* which is one of two files that the *aw* processor takes in. The other is *parameters.x*:

```
!!Module
params

param tau1
param tau2
param tau3
param tau4

set tau4 = webparams->tau1 * (webparams->tau2 + webparams->tau3);
!!End
!!EndEnd
```

Again this file is similar in design in that it follows the standard module definition. The purpose of the parameters file is to define a series of parameters that can be used by any of the modules in defpsi for calculation. In this example four parameters are defined for use: *tau1, tau2, tau3, tau4*. These are all declared by the *params* command as shown. Parameters can *only* be defined within the params module. The last line of this module uses the set command which sets a particular parameter equal to other parameter values. This command is only valid in the params module as well. In this example tau4 is set to the sum of the other tau values times the first tau value. The addressing of parameters that have values is done by the notation:

`webparams->`*parameter name*

The definition of parameters is the most specialized case in the writing of *alewife* code. This definiton merely sets up the proper references to the engine to take in parameters from a data file that contains values for them. Those familiar with C will notice this as the standard pointer usage in structure elements.

This parameter file is fed to the engine upon execution. The data within it must match the assignment order of parameters in the parameters.x file, or else inconsistencies will arise in the calculated results and potential segmentation faults in the executable.

There are two data files, the space time data file and the web parameters data file. The space time data is data pertaining specifically to the engine object used as the solver. This object reads in these space time data and sets certain variables for use as indeces and such. In the examples above, a one dimensional finite element solver was used, and the indicies such as i and N are specific to that solver. When a solver is designed, a set of parameter definitions will be stated so that the proper files can be created. When looking at the engine specification one will notice the order and structure of the predefined parameters neccesary to design a new model.

6.0 Scope and Definition of Fundamental Variables

The variables that are defined (and hence reserved words in *alewife*) internally are called fundamental variables. These must have some definition in order for the model to make sense. Fundamental variables are in two categories: biological, and mathematical.

The mathematical fundamentals are specified by the type of engine one uses. The engine used here implements a finite element method in one dimension (for this solver the variables deal mainly with space time parameters). These fundamental variables are to be specified in the ***engine specification*** (the specification for this engine is located in the source distribution).

These variables are always linked to a solution of the biological side (in the case of this example, it is *psi*). Psi is defined with a given set of relationships (defined by the modeler) and combined into the vector that is fed to the solver for the current time step. The engine does all the handling of storage and recall of previous values and such.

The ultimate goal here is to have the smallest need to adjust and set engine internal variables, and to allow for the maximum effort of defining and coding to go to the biological modeling (although the design of this setup is general enough to potentially have other applications).

In review of the above example the fundamental variables (and their associated modules) are all automatically defined over a given space and time region. Hence when the statement:

```
call lambda
print lambda
Web[tl].psi[i][2] = lambda;
```

Is performed, the value of lambda is distributed over the all i (space) and will automatically be handled through time steps.

7.0 Engine Objects

Engine objects are the heart of the modeling process. These objects are precompiled and ready to link with the biological definitions designed by the modeler. The link between the solver and the defintion files (i.e. defpsi.x) are defined by the solver being used. The one addressed here is a 1d solver that uses finite elements to determine a solution to the advection diffusion relationship from section one.

The engine objects are located in the default library for aw, usually /usr/local/lib/aw. Currently there is only one available solver hence, *aw* does not have a solver choosing option.

The compiled and executable engine will also have a sepcified method of execution dealing with how it ingests data files. In the case of the current one dimensional solver it is done as such:

> `kenobi 21:` **`engine`** *[webparams datafile] [spacetime datafile] [output datafile]*

The first two arguments specify the web and spacetime data respectively, and the final argument is the name of the data file to create for the solutions and any other values that require output. The engine for the 1d case defaults the following data to the *output datafile*:

> `Density` *time step trophic level density*
> .
> .
> .
> `Integral` *integral*

The number of density values printed per trophic levels is specified by the space time parameter pertaining to the number of spatial steps taken (for this solver it is the variable N).

7.1 Compiling an Engine

To actually "compile" an engine the files *defpsi.x parameters.x* and *engine.o* must exist. By typing *aw* the processor looks for these *.x files and builds a

set of *.c files and a makefile that is then passed through gcc to produce the executable engine called *engine*.

The resulting engine can be executed by typing its name and as its arguments: the web parameter data, space time data, and a datafile name for the saving of data to (in that order).

`kenobi 22:` **`engine`** *[web parameter filename] [space time parameter filename] [datafile]*

Figure 2 shows the process of taking multiple files and an engine object, and linking them through *aw* to form an executable engine. The final engine is then fed parameters to produce data that can be used for various applications such as visualization, and comparisons with real world data.

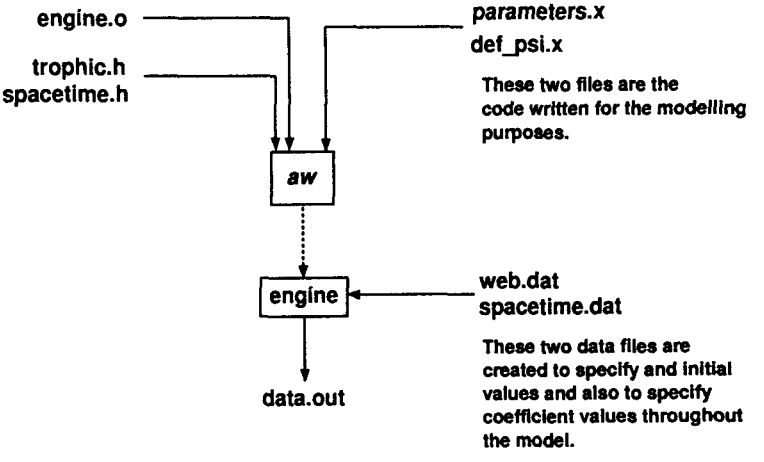

Figure 2: Process for Creating an Engine

7.2 Explanation of Engine Specification

The engine specification consists of four sections, the title, fundamental modules, definitions of structures and variables, and referencing.

The fundamental modules are those modules that *must* be defined when code is written to use this engine. Whatever is done within that module is up to the modeler.

The defined structures and variables are those which are available for the modeler's use in any of the modules. These are listed in their type form (i.e. double precision or integer etc.). The method in which these variables are used is explained in the referencing section.

The engine specification is the key to allowing the modeler knowledge on how the engine can be used in relation to any biological specifications he/she wishss to implement.

8.0 Distribution

The distribution is split into various subdirectories:

```
kenobi 23: ls
Makefile.bsd       docs/          gui/           src/
Makefile.solaris   examples/      interfaces/    testcase/
```

The directory aw contains the source for the compiler *aw*. Parameters is where some template parameter files for the space time and web data are for the 1d solver. These can be used as example building block files to create one's own parameter files. The directory testcase contains various examples for testing out the compiled binaries. Descriptions accompany the various examples. The docs directory is where documentation and help files in relation to this project are (this document should be found there). Interface is the location for the tcl/tk front end interface called the builder. This interface allows for a G.U.I. approach to making and running an engine (help files are located within the interface and in the docs directory). Finally the solver directory contains the source code for the one dimensional finite element solver that is compiled to build the *enigne.o* that is used for the linking process. The makefile should be modified to suit the needs of the installation and the system one is using and a make should be run. To install stuff in system wide accessible locations write privilages to /usr must exist for the user installing the binaries and other files. The examples directory contains expanded examples for use in observing diffusion advection behavior of the engine.

9.0 Conclusion

Alewife is a system designed to help speed up the modeling process for population interactions. By having a set of formulated solvers, one can easily select from this library and link to a modeled scenario. With the process of formulation taken care of, the modeling becomes more concentrated on the aspects of the system to be observed. When focus is shifted to the application, a more thoughtful approach can be given to the design of the underlying model relationships.

References

[1] Mason, D.M., and E.V. Patrick. *A model for the space-time dependence of feeding for pelagic fish population*, Transactions of the American Fisheries Society 122(5):884-901, 1993.

Appendix 9

Diffusion-Taxis Model for Distribution, Mortality and Growth of Multiple Interacting Populations

Diffusion-taxis Model for Distribution, Mortality, and Growth of Multiple Interacting Populations:

The Submodels

Vincent Patrick Doran M. Mason

Abstract

The purpose of this note is to describe the current collection of submodels used in the initial trials of the diffusion-taxis model. A second purpose it to begin to develop a suitable notation.

Construction of the loss function

A loss function is used to model the methods by which physical conditions and biological processes within and between individuals determine the time evolution of the spatial distribution of populations. Specifically, the loss function consists of a choice of how to combine sensory responses of a standardized individual into a single scalar that reflects the net relative dislike of that individual for each point in space and time. Figure 1 is a flow chart for the construction of the loss function currently used to describe juvenile fish in western Prince William Sound along with the other fish populations interacting with those juveniles. It shows each of the physical and biological variables used, with the latter including population density and the size of individuals. The various sources for these space-time dependent variables is indicated. Figure 1 indicates that the loss function is used in a set of partial differential equations. These are described next.

The spatial distribution of freely swimming organisms (nekton) is modelled using equations of the form

$$\begin{aligned} \frac{\partial u}{\partial t} &= \mathrm{div}(D\mathrm{grad}u + \chi u \mathrm{grad}\lambda) + f \quad in\ \Omega \\ u &= g \qquad\qquad on\ \Gamma_0 \subset \partial\Omega \\ D\frac{\partial u}{\partial n} + \chi u \frac{\partial u}{\partial n} &= 0 \qquad on\ \Gamma_1 = \partial\Omega - \Gamma_0 \end{aligned} \tag{1}$$

where f is the reaction term describing mortality and regeneration; the first term in the divergence is the diffusion term describing dispersal by diffusive-like random motions; and the second term in the divergence is an advection term describing directed motions with a drift velocity given by the gradient of a function. The function λ in (1) is the loss function. Note that it is the gradient of the loss function and not the loss function itself that is of consequence. That is, the rate of change and the direction of change of the loss function is the manner by which the loss function influences distributions. In particular, the change of the loss function by the addition of any constant has no effect. However, multiplication by a constant does.

The boundary conditions are typically the zero-flux boundary conditions—the second of the two boundary conditions shown. The first boundary condition is shown for completeness. In exceptional cases there can be the need to specifiy the value of the density on the boundary rather than the flux across the boundary.

The equations are properly interpreted as a set of simultaneous equations. That is, u is a vector valued density function, with each component representing a different tophic level. In this case, there is a different

diffusion constant, taxis constant, reaction term, and loss function for each trophic level. It is this set of equations that is solved in the *Alewife* software.

In (1) the function λ is to be a function of space and time. On the other hand, just prior to (1) the loss was described as a weighting of responses to physical and biological variables. For brevity let the physical and biological variables collectively be referred to as ***habitat variables***. Then the composition of funtions

$$\text{space} \times \text{time} \longrightarrow \{\text{habitat variables}\} \longrightarrow \{\text{responses}\} \longrightarrow \text{loss}$$

specifies how to assign a value $\lambda(x,t) = \text{loss}\,(x,t)$ for each point x in space and time t. To identify explicitly trophic level in this composition a trophic level index TL is added

$$\text{space} \times \text{time} \longrightarrow \{\text{habitat variables[TL]}\} \longrightarrow \{\text{responses[TL]}\} \longrightarrow \text{loss[TL]}\ .$$

The solutions to (1) represent the consequences of each individual in each trophic level being aware at the present time t of the loss[TL](x,t) for the position x that it currently inhabits relative to the loss[TL](x',t) for nearby positions x'. An absolute measure of loss is not needed. Nearby individuals for each trophic level then disperse in the direction that most quickly reduces loss[TL] and at a speed proportional to magnitude of the gradient of loss[TL].

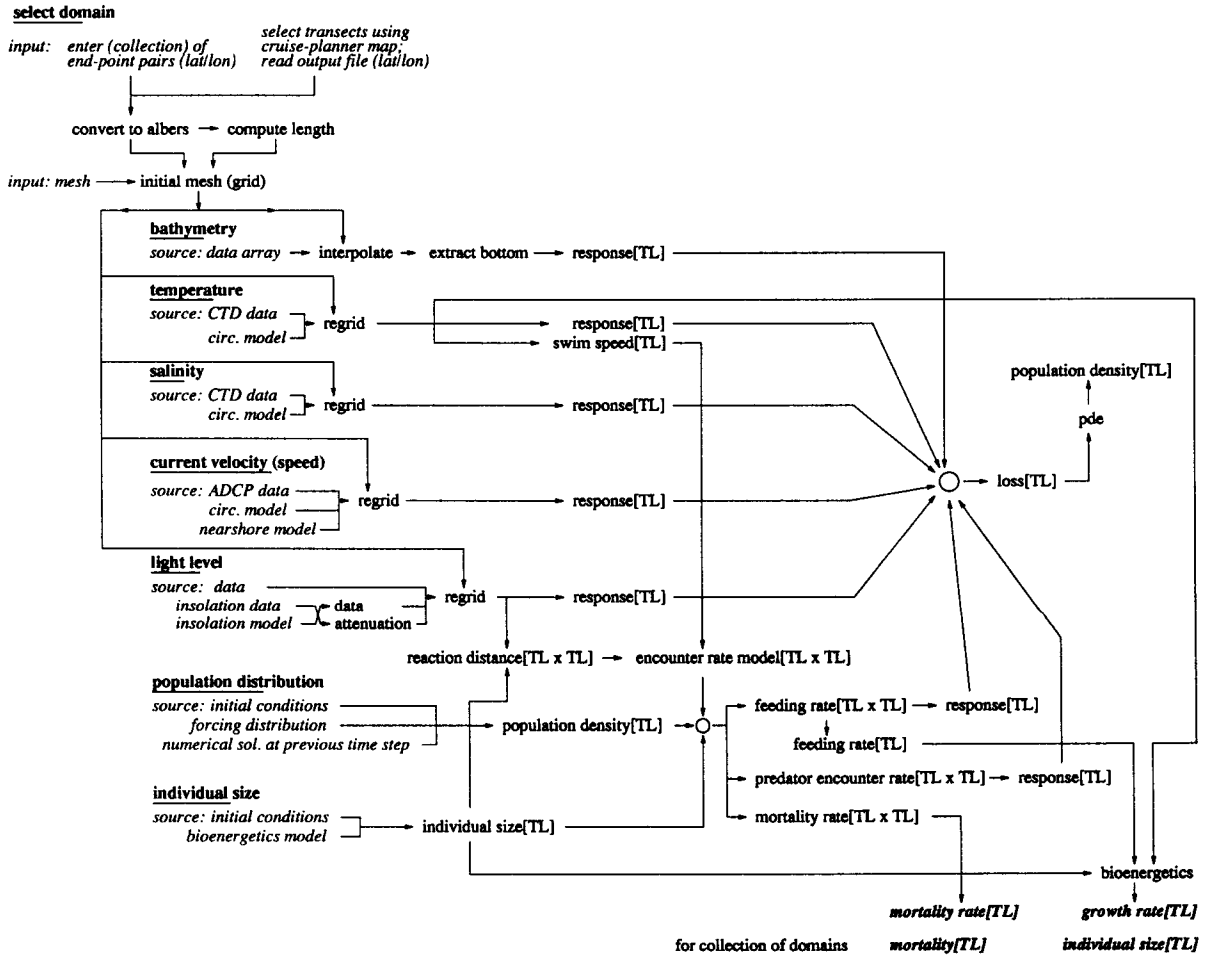

Figure 1 Construction of the loss function for pink salmon fry recruitment model.

In the following sections each of the data sources and sub-models used in the construction of the loss function is described. Each section refers to a node in the flow chart of Figure 1.

Selection of the domains

The first versions of the two- and three-dimension fish models is not ready.

One-dimensional versions of the fish model have been used for the initial developement. The space-time domain for the one-dimensional model is simply a single line and a time interval. The flow-chart in Figure 1 begins with select domain since the use of the one-dimensional model requires a selection of domain location and orientation.

Cruise planner

The cruise planner provides a convenient means to select and display one-dimensional domains for use with the fish model. Pink salmon fry and macrozooplankton occupy the near-surface regions of the water column during the time period of interest. For a zeroth-order approximation these upper regions are assumed to be homogeneous vertically and in the along-shore direction. It is assumed that most of the variation in physical structure and population distribution occurs in the "cross-channel" direction. With these assumptions the one-dimensional model can be used to simulate fry growth and mortality due to a given set of cross channel physical and biological conditions.

An expanded view of the region and of the domains is shown in Figure 2 using the zoom capability and postscript output of the cruise planner.

Length and time

Each of the domains is used separately with the one-dimensional model. For each domain only the length and the time interval is needed. For the alewife interface these data are entered in the Time and Space Parameters table in units of meters and seconds. For initial development a 1 km domain has been used. The tables ask for a beginning and ending position (although unnecessary) and the variables for these are X_A and X_B in meters. The time interval shown corresponds to 4 hr in units of seconds.

Mesh

For alewife the time steps and the spatial mesh are hard coded and a change requires a recompilation. (See CONST.h) The simulations shown here use 250 spatial nodes—a 4 m grid for a 1000 m domain—and 96 time steps—1 hr time steps for a 4 da simulation.

Bathymetry

The variable of bottom depth has two roles in the one-dimensional model. First, a basic issue for pink salmon fry is their reported tendency to prefer nearshore regions. This is in contrast to juvenile gadids that are more likely found in large aggregations off-shore in relatively open water. Although the behavior of salmon fry is often characterized in terms of distance from shore, the variable distance from shore is not likely one for which there is any perception. Consequently the mechanisms for this regulatation of their distribution must be sought amoung other variables. One of these is bottom depth. Once again it would seem that bottom depth itself is not perceived. There are however, two variables that can be perceived and are linked to bottom depth. Thesea are light level and benthic food sources. The bottoms in many nearshore regions are rocky, relatively smooth, and reflective. This bottom source of reflected light is readily detected. Second there are reports of significant benthic feeding for fry, and the illumination level

Figure 2 Cruise Planner map with typical model domains

at the bottom can be a factor for this feeding. For this one-dimensional model study the bottom depth itself is used as a surrogate variable for these potential factors and details for either light or bottom feeding are not made explicit. Conversely, there are populations that tend not to occur nearshore. For these also bottom depth is used as a habitat factor.

A second role for bathymetry is a means for including in the one-dimensional model euphasids. The rise of these animals in the water column during night means that their distribution tends to occur over water of sufficient depth. For these alternative prey the population is treated as forcing, time varying, and with a fixed spatial distribution—that is, their mortality is not included and the model does not attempt to conserve their numbers or account for their dispersal.

Data

The SEA database has a gridded bathymetry dataset for Prince William Sound in Albers 50 154 equal

area projection, with a uniform 60 m grid. The bathymetry along the model domain is to be interpolated from this data set after converting the cruise planner domain data to Albers.

Simulation

For immediate trial the bathymetry is simulated with a functional form. The alewife model has all of these physical simulation functions hard-coded and changes require recompiliing. The bathymetry function is in def_lam.c. For example, $B_{max} = 350\,\text{m}$ and for s the position between X_A and X_B, the bottom depth $B(s)$ is specified very simply by

$$B(s) = B_{max} \sin\left(\frac{\pi(s - X_A)}{X_B - X_A}\right).$$

Responses

The response must be specified for each trophic level. In alewife Version 3 four trophic levels are allowed, with the first (i.e., TL = 1) strickly forcing. For current trials this trophic level is assigned to macrozooplankton, for this population distribution is assumed to be regulated as much by advection as by mortality and responses. Hence, a response specification is needed for TL = 2, 3, and 4.

alternative prey: juvenile gadids For these a response is constructed such that as bottom depth $B(s)$ decreases below $B_{onset}[\text{TL}] = 30\,\text{m}$ then the response Res[TL] gets less favorable (loss increases), with the response varying between 0 and 1.

$$\text{Res}_{\text{bath}}[\text{TL}](s) = \left(\frac{\max\{(B_{onset}[\text{TL}] - B(s)), 0\}}{B_{onset}[\text{TL}]}\right)^2$$

pink salmon fry For fry a candidate response that switches more rapidly is

$$\text{Res}_{\text{bath}}[\text{TL}](s) = \left(1 - \exp\left(\frac{-B(s)^2}{2B_{onset}[\text{TL}]^2}\right)\right)^2.$$

A reasonable value in this case as well is $B_{onset}[\text{TL}] = 30\,\text{m}$.

pollock The same response as for the juvenile gadid alternative prey is used for pollock.

The simulation for the bathymetry and the plots for the two responses are shown in Figure 3.

Temperature

Presently temperature is not used explicity for the one-dimensional model. Swimming speed is specified as an input variable rather than computed using temperature.

Data

The use of measured temperatures requires extensive work to formulate gridded data with sufficiently fine mesh. A better but more difficult solution is to use temperature data that has been assimilated using the ocean circulation model.

Responses

Functional forms have been developed to provide a response that defines a preferred temperature range. This response becomes much more important for spatial domains that extend through the water column.

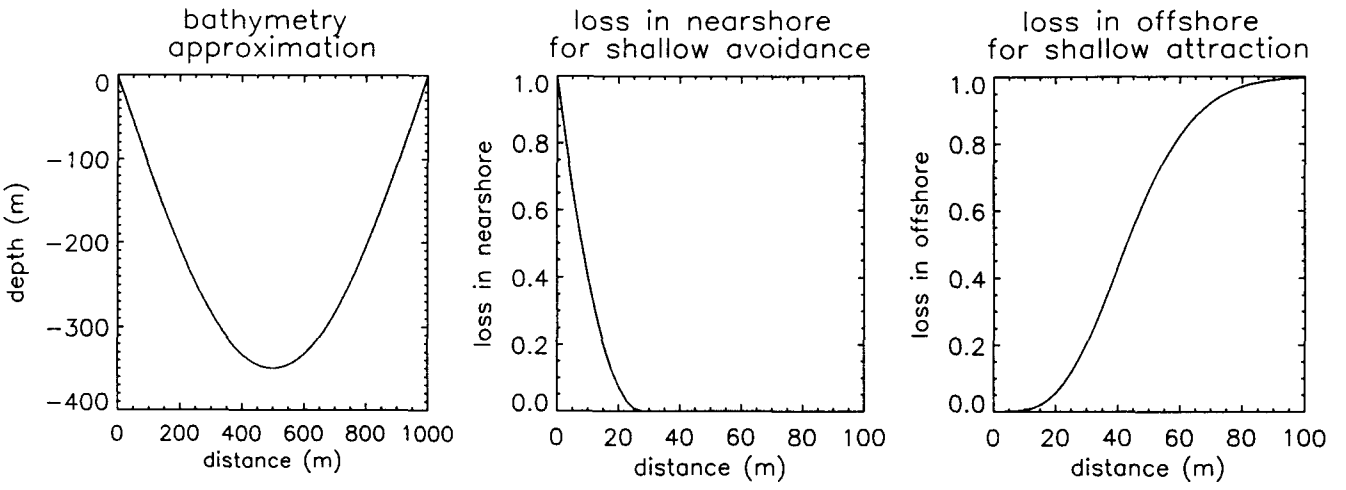

Figure 3 Simulation of bathymetry and plots of loss in the nearshore.

Salinity

Of those species being modelled only the plankton have strong responses to salinity within the range of salinity variation found in the sound. Because the plankton are hard coded and forcing in alewife Version 3, no salinity data, simulation, or response has been implemented.

Current velocity

The magnitudes and the spatial structures of the time varying current velocities, especially the tidal velocities, are hypothesized as significant factors in the spatial distribution of pink salmon fry. To incorporate this variable the time varying magnitude of the currents along the one-dimensional domain are needed.

Data

Soon time varying circulation data on 1.2 km grids will be available from the ocean circulation simulations. At most the data will need regridding for use with the fish model mesh. In addition some alternative approaches will be needed to estimate the current velocities in the near-shore that are not adequately characterized by the circulation model.

Simulations

For the immediate needs the cross-channel structure of the current has been represented by an approximating functional form. One such approximation is the following. Let $V_{max} = .30$ m/sec be the maximum speed. The parameter $\sigma_V = 150$ m is used to set the rate at which the current speed $V(s)$ decreases as s approaches the shore, that is, as s approaches either X_A or X_B.

$$V(s) = V_{max}\left(1 - \exp\left(\frac{-(s - X_A)^2}{2\sigma_V^2}\right)\right)\left(1 - \exp\left(\frac{-(s - X_B)^2}{2\sigma_V^2}\right)\right).$$

Responses

In the current form the responses $\mathrm{Res}_{\mathrm{curr}}[\mathrm{TL}]$ for all except pink salmon fry are neglected.

pink salmon fry For fry the square of the magnitude of the velocity is used for the response,

$$\mathrm{Res}_{\mathrm{curr}}[\mathrm{TL}](s) = V^2(s) .$$

Normalization is not appropriate here for it would eliminate the comparison between different domains (lines). The specification of $V_{max} = .30\,\mathrm{m/sec}$ is for a single domain.

The current speed as a function of distance along the domain is plotted in Figure 4 along with a plot for the loss in the nearshore region.

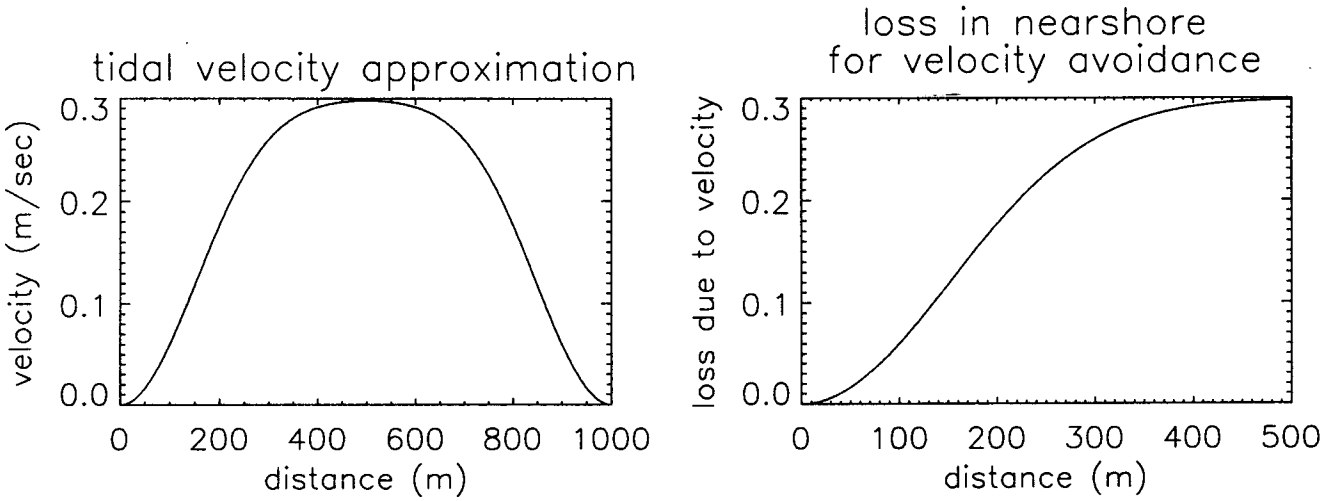

Figure 4

Light level

The light level is driven by the isolation at the surface and the attenuation through the water column. For the one-dimensional study an intermediate value must be used since the depth dimension is not used. However, there is still the diel change in insolation to be accommodated. For the present a functional approximation is used until better time and space data for insolation is available.

Insolation

A time periodic function is used to approximate the diel light cycle. This function is given in the subroutine e2_0.c of the alewife code. The function is somewhat cumbersome for sufficient parameters were included to adjust the insolation range and offset in order to simulate the long days (or nights) in Prince William Sound. A plot of the diel light cycle under this approximation is shown in Figure 5. Both linear and logarithmic plots are shown.

Attenuation

The insolation used here is defined by the fractional power of an affine transformation of a *cosine*. First, let

$$a = 1.03,$$
$$s^* = 4.8,$$
$$s^+ = 3.0.$$

The illumination during a 24 hr (or 86400 sec) May day, with maximum illumination at $t = 0$, is approximated by

$$s^+ + s^* \left[0.25 + a + (0.75 - a)\, cos\left(\frac{2\pi t}{86400}\right)\right]^{0.135}.$$

Responses

For the present study no direct response to the light level is used. All responses are due to the effects of the light level on the reaction distance.

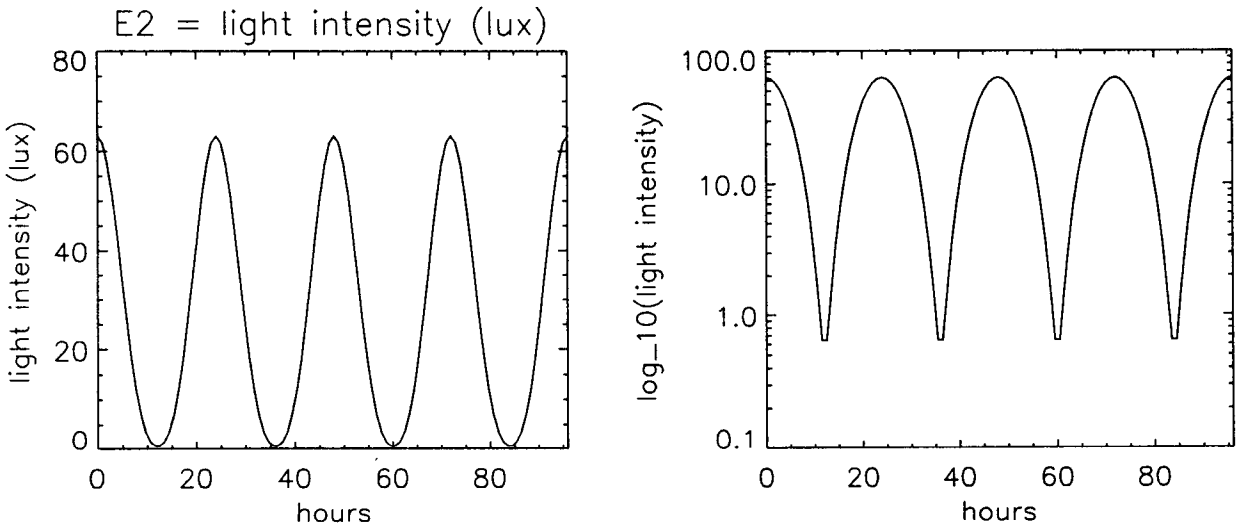

Figure 5

Individual size

This begins the biological components of the model.

Initial conditions

The parameters for individual mean lengths and biomass are shown in the "Feeding and Risk" table.

Mixing assumptions

The values for length and biomass are the initial conditions for the model run. In addition, during the relatively short time intervals of a simulation the individuals are assumed to undergo small changes in size and the effect of this change can be neglected. The net change in size due to feeding or lack thereof during several such intervals is computed at the end of the intervals and the size is changed by use of a bioenergetics model.

Each tropic level is assumed to consist of individuals of common size. If a set of sizes is needed then addtitional tropic levels are used. This is because the use of a size continuum in a single population changes the form of the model to an integral equation. An assumption is needed to have a single population grow at a common rate. It is assumed here that within the distributed population there is sufficient mixing among all individuals such that on average each has the same consumption.

Some work has been done to move to a more complete model that will include size ranges and also time varying satiation within the population.

Reaction distance

The *reaction distance* is the maximum distance at which an individual of the observing trophic level *obs* can detect an individual of the target population *tar*. A functional model [ref] with minimal complexity is used. Specifically, there are four independent variables: *obs*, *tar*, light level, and the size of the detected individual. This dependence is shown in the flow-chart in Figure 1. Reaction distance differs from the foregoing variables in that it does not depend explicitly on space and time.

Model

The function used to model the reaction distance is

$$R[obs, tar](I, s) = R_{max}[obs, tar](s)\left(1 - e^{-\beta[obs]\ \max\{(I - I_0[obs]),0\}}\right),$$

where s is the size of the target individual in *tar*, I is the light level, and . $R_{max}[obs, tar](s)$ is the maximum reaction distance for the detection of an individual of size s regardless of light level. (In Version 3 $R_{max}[obs, tar](s)$ is entered manually in the tables.) It is assumed that nothing is detected by individuals in *obs* for light levels less than $I_0[obs]$, and that the single parameter $\beta[obs]$ suffices to characterize reaction distance for $I > I_0$.

The trophic level names *obs* and *tar* avoid introducing into the relationship those activities associated with the names predator and prey. This reaction distance function applies to the case of an individual prey as observer scanning for predators as targets as well as to the case of a predator as observer and prey as targets.

Swimming speed

For the current development a single average swimming speed is used for each trophic level. For the present trials wherein temperature does not vary within a domain the swimming speed values are manually entered in the tables.

Encounter rate

The *encounter rate* means the rate at which an individual observer of trophic level *obs* has individuals of trophic level *tar* enter the spatial region about the observer defined by the observer's reaction distance $R[obs, tar]$ for targets.

Models

The Gerritsen-Strikler encounter rate model assumes

- all individuals in *tar* are moving at the same "average" swimming speed $\bar{v}_{tar}$, with all directions equally likely
- the reaction distance for the observer does not depend upon viewing angle

Let v_{obs} denote the observer swimming speed and u_{tar} the density of the individuals in *tar*. With the above assumptions and the specification of which of v_{obs} and $\bar{v}_{tar}$ is greater the encounter rate can be computed.

$$\mathcal{E}[obs, tar](v_{obs}, \bar{v}_{tar}, u_{tar}, I, s) = \frac{\pi u_{tar}\, R[obs, tar](I, s)}{3} \frac{\min(\bar{v}_{tar}, v_{obs})^2 + 3\max(\bar{v}_{tar}, v_{obs})^2}{\max(\bar{v}_{tar}, v_{obs})}$$

Feeding rate

The model used for feeding rate is similar in form to a "carrying capacity" model. The feeding rate model uses two parameters to set the feeding rate limits—handling time and "waiting time." Waiting time addresses the feeding rate regulation associated with the hunger. Handling time is used in a slightly extended sense: the time required to complete the physical process of both capture and consumption.

All of the foregoing models applied to all trophic levels. The predator-prey models involve selection among trophic levels. Let $N + 1$ be the total number of trophic levels in the model, and let each trophic level be assigned an integer identifierTL in the sequence $0, 1, \ldots, N$. The index name TL is used whenever there is no need to identify both a trophic level and a process. The index variable $\mathbf{P}$, $\mathbf{P} = 0, 1, \ldots, N$, is used to refer to a trophic level functioning as predator. The index variable $\mathbf{f}$, $\mathbf{f} = 0, 1, \ldots, N$, is used to refer to a trophic level functioning as prey or forage.

Models

Prey For each trophic level $\mathbf{P}$ there is a subset of the $N + 1$ trophic levels that are prey. To provide a notation for this, let K be the function defined by $\mathrm{K}(\mathbf{P}, \mathbf{f}) = 1$ if $\mathbf{f}$ is a prey trophic level for $\mathbf{P}$, and $\mathrm{K}(\mathbf{P}, \mathbf{f}) = 0$ otherwise.

Handling time Let $h(\mathbf{P}, \mathbf{f})$ be the handling time, with $\mathbf{P}$ and $\mathbf{f}$ as above.

Waiting time The feeding rate is known to vary with gut fullness and "hunger." The feeding rate for fry that have been starved and then introduced to prey is initially high then drops to a rate that maintains a constant level of gut fullness [ref]. This study for fry did not address the issue of whether the gut fullness also is initially higher during the first feeding burst. However, feeding in bursts that fill the gut is a known phenomena for other predators.

A separate submodel has been formulated to provide the time varying feeding associated with the above phenomena. The current model does not incorporate this submodel because of the additional complexity. The submodel and the extension to incorporate it are described later and will be implemented in the near future. For now a "mean" waiting time is used.

To accommodate the adaptation of feeding for motivation let $\xi(\mathbf{P}, \mathbf{f})$ be the waiting time that follows consumption of a prey item from $\mathbf{f}$.

Prey preference For the present trials no preferences are assumed. That is, the selection of prey is assumed to be proportional to encounter rates.

Feeding capacity model The model for the feeding rate of a predator in $\mathbf{P}$ is

$$\varphi[\mathbf{P}] = \frac{\sum_{\mathbf{f}=0}^{N} \mathrm{K}(\mathbf{P},\mathbf{f})\,\mathcal{E}[\mathbf{P},\mathbf{f}]\,w(\mathbf{f})}{1+\sum_{\mathbf{f}=0}^{N} \mathrm{K}(\mathbf{P},\mathbf{f})\,\mathcal{E}[\mathbf{P},\mathbf{f}]\,\big(h(\mathbf{P},\mathbf{f})+\xi(\mathbf{P},\mathbf{f})\big)} ,$$

where $w(\mathbf{f})$ is the weight of a prey item in $\mathbf{f}$. Properly $\xi(\mathbf{P},\mathbf{f})$ should depend upon gut fullness and a variable associated with hunger. It is this dependence that is omitted here. However, even in the more complete form it seems adequate to assume that $\xi(\mathbf{P},\mathbf{f})$ and $h(\mathbf{P},\mathbf{f})$ have a similar dependence on the weight of the prey item so that $\xi(\mathbf{P},\mathbf{f})/h(\mathbf{P},\mathbf{f})$ is independent of $\mathbf{f}$. With this assumption define

$$\xi^*(\mathbf{P}) = \frac{\xi(\mathbf{P},\mathbf{f})}{h(\mathbf{P},\mathbf{f})} .$$

It is this variable $\xi^*(\mathbf{P})$ that is needed to account for gut dependent feeding rates. With this the feeding rate is

$$\varphi[\mathbf{P}] = \frac{\sum_{\mathbf{f}=0}^{N} \mathrm{K}(\mathbf{P},\mathbf{f})\,\mathcal{E}[\mathbf{P},\mathbf{f}]\,w(\mathbf{f})}{1+\sum_{\mathbf{f}=0}^{N} \mathrm{K}(\mathbf{P},\mathbf{f})\,\mathcal{E}[\mathbf{P},\mathbf{f}]\,h(\mathbf{P},\mathbf{f})\big(1+\xi^*(\mathbf{P})\big)}$$

Maximum feeding rate If the encounter rate $\mathcal{E}[\mathbf{P},\mathbf{f}_0]$ for a specific prey type $\mathbf{f}_0$ gets large, then the feeding rate approaches

$$\frac{w(\mathbf{f}_0)}{h(\mathbf{P},\mathbf{f}_0)(1+\xi^*(\mathbf{P}))} .$$

An upper bound for feeding rate $\varphi_{max}[\mathbf{P}]$ is then

$$\varphi_{max}[\mathbf{P}] = \frac{1}{(1+\xi^*(\mathbf{P}))} \max_{\mathbf{f}} \frac{w(\mathbf{f})}{h(\mathbf{P},\mathbf{f})} .$$

If gut fullness is included then a further upper bound is the maximum evacuation rate.

Responses

The response is simply that which maximizes feeding. Since response is defined in terms of loss we use

$$\mathrm{Res}_{\mathrm{feed}}[\mathrm{TL}] = \left(\frac{\varphi[\mathrm{TL}]-\varphi_{max}[\mathrm{TL}]}{\varphi_{max}[\mathrm{TL}]}\right)^2$$

Predator encounter rate

The last of the components is simple. It is the previous encounter rate.

Models

For a given prey trophic level $\mathbf{f}$ the total rate of encounters with predators for $\mathbf{f}$ is

$$\mathcal{E}_{\mathrm{Pred}}[\mathbf{f}] = \sum_{\mathbf{P}=0}^{N} \mathrm{K}(\mathbf{P},\mathbf{f})\,\mathcal{E}[\mathbf{f},\mathbf{P}] .$$

Note that the order of the trophic level indeces in K is predator, prey; the order in $\mathcal{E}$ is observer, target. In the summation over predators it is prey $\mathbf{f}$ that is the observer and $\mathbf{P}$ is the target.

Responses

$$\mathrm{Res}_{\mathrm{pred}}[\mathrm{TL}] = \mathcal{E}_{\mathrm{Pred}}[\mathrm{TL}]^2$$

Loss

In the foregoing responses have been specified for bathymetry, current velocity, feeding rate, and encounter rate with predators. The loss for a given TL is the linear combination (i.e., weighted sum) of the responses.

BIOPHYSICAL PLANKTON MODELING
A Component of SEADATA, 95320-J

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Institute of Marine Science
University of Alaska Fairbanks

The SEADATA modeling effort is aimed at achieving three broad goals: 1) creation of a three-dimensional physical current field model; 2) creation of a three-dimensional biophysical model of plankton dynamics, which is coupled to the flow field produced by 1); and 3) creation of nekton models of pink salmon and Pacific Herring. The plankton modeling is being carried out at the University of Alaska Fairbanks. The 3-D plankton model is being built in a structured, step-wise fashion. As a first step, and as a deliverable for FY-95, we stated we would produce an annual one-dimensional coupled biophysical model by the end of FY-95. This model was to include diatoms, flagellates, large *Neocalanus* copepods, and smaller *Pseudocalanus* copepods. We are happy to report this model has been constructed, and is producing very reasonable results. We have presented a portion of these results at two national meetings: the American Association for the Advancement of Science Arctic Science Meeting in Fairbanks, AK, September, 1995; and the American Geophysicists Union/American Society of Limnology and Oceanography Ocean Sciences Meeting in San Diego, CA, February, 1996. These results are presented below.

Description of the model

The coupled biophysical model used in this study is based upon the spring diatom bloom model of Eslinger and Iverson (1996). Their physical model was a 39-layer, one-dimensional mixed-layer model based on the model of Pollard, *et al.* (1973) as modified by Thompson (1976). Meteorological forcing (wind mixing, solar heating, ocean-atmosphere heat fluxes) is applied at the surface and the water column mixes downward until there is a balance between the kinetic energy available for additional mixing and the potential energy cost of overcoming the existing stratification. This balance is determined by examining the Fruode number of the mixed layer. A full description of the 1-D physical model can be found in Eslinger (1990). The biological portion of the Eslinger and Iverson model (the EI model) was designed to simulate spring bloom dynamics over the southeastern Bering Sea shelf. Therefore, many of the biological dynamic processes were simplified. The EI model included nitrogen dynamics, but assumed that all forms of nitrogen are equal. Therefore, nutrient dynamics were modeled using a nitrogen pool containing total nitrate, nitrite and ammonium. Diatoms were the only phytoplankton included in the model, and zooplankton were included only in the sense that there was a grazing loss to the phytoplankton. The grazing loss was a constant fraction of daily production. The EI model was run only for the late winter and early spring period for which it was valid, however, for that period, the highly vertically resolved model reproduced the upper water column phytoplankton, nutrient, and temperature fields with excellent accuracy, Figure BPM-1.

The EI model formed an excellent base for constructing an annual model of upper water column physics, phytoplankton, zooplankton, and nutrient dynamics in Prince William Sound, Alaska. As we modified the EI model to be valid over annual time periods, we increased the

number and complexity of the chemical and biological processes included in the model. We have added ammonium and silicon dynamics; a flagellate component; and three types of zooplankton: large *Neocalanus*-type copepods, smaller *Pseudocalanus*-type copepods, and euphausiids. There is in addition, an unspecified carnivorous nekton component which preys upon the zooplankton. Details of the model are given below.

Model Domain and Forcing Variables:

The physical domain is the upper 100 meters of a significantly deeper water column. Therefore, there are no bottom boundary layer or tidally mixed layer effects. These processes can be simulated by the model, however, they are not included in the present runs. Vertical grid resolution is 2 meters (a 50 layer model), and the time step is two hours. The model was run to examine interannual and spatial variability. When run in an interannual mode, the model was run beginning in late February or early March, depending on the availability of forcing data, and was run through approximately the middle of November for 1993, 1994, and 1995. Although the model was run for the greater part of the year, we will limit the remainder of this report to the spring and summer periods, when the planktonic dynamics are greatest. Meteorological forcing data were obtained from the Cooperative Fisheries and Oceanographic Studies (CFOS) moored buoy system initiated by Dr. Ted Cooney. For 1995, buoy data were unavailable for the early portion of the year, so meteorological data from a National Weather Service (NWS) unmanned station on Middleton Island, Alaska, was used. Wind speeds and air temperatures are shown in Figures BPM-2 and BPM-3, respectively. The spatial variability analysis was performed using 1995 forcing data from two NWS stations: the Middleton Island, AK NWS station, and a NWS station located at Whittier, AK. Wind speeds and air temperatures for the spatial analysis are shown in Figures BPM-4 and BPM-5, respectively. Insolation data required by the model was simulated using the radiation model of Frouin *et al.* (1989).

Phytoplankton Dynamics:

Diatoms and flagellates were included in the model. Maximum possible daily growth rate of both species was determined by temperature (Eppley, 1972), and could be reduced by light or nutrient limitation. Nitrate, ammonium, and silicon were considered as potentially biologically limiting nutrients, and nutrient uptake rate was assumed to follow a Michaelis-Menten relationship (Dugdale, 1967). Ammonium inhibition of nitrate uptake was included (Wroblewski, 1977). Both phytoplankton species competed for the nitrogen nutrients; silicon was utilized only by the diatoms. Photosynthesis was calculated as a function of light intensity, with a possibility of photoinhibition (Platt *et al.,* 1980). See Eslinger and Iverson (1996) for the actual implementation scheme for light or nutrient limitation.

Zooplankton Dynamics:

Three types of zooplankton were included in the model: *Neocalanus*, and *Pseudocalanus* type copepods, and euphausiids. Euphausiids are generally of minor importance in the zooplankton dynamics in PWS, and will not be included in the simulation results presented here. The two types of calanoid copepods differ in two significant ways: the adult *Neocalanus* are much larger than the *Pseudocalanus*, and the two types have significantly different reproductive strategies. The *Neocalanus*-type copepods undergo a dramatic ontogenetic migration, descending in late summer as stage copepidite V (hereafter, CV's) to a depth of 200-400 meters,

where they overwinter. The following spring they mature, reproduce and die. The eggs hatch at depth and the nauplii begin to ascend towards the surface, which they reach at about the time they mature to the CI stage, and generally prior to the spring phytoplankton bloom. They feed and grow in the surface waters for approximately 65-75 days, after which they begin to descend again, as CV's (Fulton, 1973). In contrast, the *Pseudocalanus*-type copepods spend their entire life cycle in the upper water column, and overwinter as adult, fertilized females. They must feed on the spring phytoplankton bloom to begin reproducing, and can reproduce up to 10 times at approximately 5 day intervals (Corkett and McLaren, 1978). Actual embryonic and inter-molt timing was a function of temperature. The above scenarios are representative of the two broad categories of calanoid copepods which dominate the zooplankton biomass of PWS. The life history descriptions given are representative and are a simplification for the purposes of creating this model. For the present set of simulation results, specific life stage and reproductive aspects of *Pseudocalanus* life history were simplified. Total *Pseudocalanus* biomass was modeled, with no attempt made at keeping track of life stage. This introduces two errors into the model: 1) **individual weight-specific** parameters, *e.g.* grazing rate, are constant for all life stages; and 2) egg biomass is included in total *Pseudocalanus* biomass when calculating **population biomass-specific** effects, *e.g.* total phytoplankton biomass consumed by zooplankton grazing. The first assumption is the most critical and is being addressed in current work. The second assumption is less important because, although egg **numbers** may be high at times, total egg **biomass** is never large compared to copepidite biomass.

In the model, *Neocalanus* arrive in the surface (enter the model domain), as three groups of CI's, spaced over a 30 day time period, with the middle group containing one-half of the total biomass the other two groups containing one-quarter total biomass each. *Neocalanus* dynamics include modified Ivlev-type grazing (Ivlev, 1945; Magley, 1990) on both diatoms and flagellates; maturation, fecal pellet production, excretion of ammonium, and natural mortality (6%/day). With the simplification of the *Pseudocalanus* life history simulation, the *Pseudocalanus* dynamics are the same. Actual rates of the various parameters differed between the two zooplankton types.

Results

The model results showed that small differences in the meteorological forcing over a few critical weeks early in the spring phytoplankton bloom could create order of magnitude variations in the standing stock of zooplankton later in the summer. These small changes had similar effects when they occurred at a single location due to interannual variation in meteorological conditions, and when they occurred at different locations in the same year due to small horizontal gradients in meteorological conditions.

Interannual Variability

The model was run using forcing data for 1993, 1994, and 1995. The model simulations began with identical initial temperature and nutrient fields and initial concentrations of phytoplankton and zooplankton. Simulated phytoplankton chlorophyll concentrations are shown in Figure BPM-6a. Also shown is CFOS buoy fluorescence, which is representative of total phytoplankton chlorophyll. In 1993, the model simulated the timing, magnitude, and duration of the spring phytoplankton bloom extremely well. The bloom was fairly short in duration, ~15 days, and maximum chlorophyll concentrations were reached approximately 5 days after the

onset of the bloom. The brief, intense phytoplankton bloom quickly stripped nutrients form the surface layer, and chlorophyll concentrations decreased rapidly. Zooplankton were unable to take full advantage of the brief phytoplankton increase. Biomass of the first *Neocalanus* group increased, while that of the second and third groups, which had less **total** biomass, increased much less. *Pseudocalanus* biomass remained fairly low, even after the phytoplankton bloom (Figure BPM-7a).

In 1994, the model bloom occurred ~5 days earlier than the bloom observed in the fluorescence data. The magnitude of the true bloom was underestimated in the modeled bloom. In both the model results and field data, the initial chlorophyll increase of the 1994 bloom occurred at approximately the same time as it had in 1993. However, in 1994, a strong cooling event occurred in the middle of the bloom, which led to a more protracted phytoplankton bloom (Figure BPM-3b). Zooplankton were able to take full advantage of this longer bloom period, and both *Neocalanus* and *Pseudocalanus* biomass increased substantially, with maximum biomass levels of approximately 1.0 and 0.75 GC wet-weight/m^{-3} respectively occurring in the model Figure BPM-7a.

In the 1995 simulation, the initial chlorophyll increase also began near day 90, but the increase to maximum chlorophyll concentrations took approximately 25 days, due to periodic strong wind mixing events, *c.f.* Figures BPM-2c and 6c. The relatively long, slow phytoplankton bloom allowed the zooplankton to efficiently utilize the primary production. Maximum daily *Neocalanus* biomass was higher in 1995 than in prior years, and *Pseudocalanus* biomass values approached those reached in 1994 (Fig. BPM-7c).

Although CFOS buoy data was not available for comparison with model chlorophyll, we did compare model results with discrete field samples from our 1995 cruises. In Figure BPM-8, phytoplankton and zooplankton biomass simulated in the model (the lines) agree very well with field data.

Spatial Variability

For the analysis of spatial variability, simulations were run using meteorological forcing data from Whittier, AK, located in northwestern Prince William Sound, and from Middleton Island, AK, located at the edge of the continental shelf, south of Prince William Sound (PWS). The Middleton Island data is assumed to be representative of the conditions over the Gulf of Alaska (GOA) shelf, *i.e.*, the "river" which may impact the Sound. The model simulations presented below all began with identical initial concentrations of phytoplankton and zooplankton. Initial temperature and nutrient fields were determined from field data. Figure BPM-8 shows the results of the spatial-variability model runs. CFOS buoy data is not available for this set of simulations.

The phytoplankton bloom began near day, at both locations, however, it was much slower for the GOA location. The maximum spring bloom chlorophyll concentrations were very similar between the two years, reaching peak concentrations of ~20 mg Chl m^{-3}, Figure BPM-9. Zooplankton biomass differed greatly between locations, with maximum values higher by a factor of approximately four for both *Neocalanus* and *Pseudocalanus*.

Discussion

In both interannual and spatial simulations, meteorological factors were responsible for

both the timing of the initiation of the spring phytoplankton bloom, and the **character or nature** of the bloom. By character, we mean whether the bloom was a brief, intense event, or whether it was a more protracted event with a slower increase to, and duration of, maximal chlorophyll values. In cases when the bloom was brief and intense, *i.e.*, the 1993 and the NW PWS simulations, winds calmed, and remained relatively calm, for approximately 10 days (Figs BPM-2-5). This allowed a strong thermocline to develop, and the phytoplankton community responded with a rapid increase in biomass. This increase soon stripped the near surface, stratified layer of nutrients. and the phytoplankton spring bloom ceased (Figs BPM-6,9). Continued production the near-surface layer was driven by recycling through the zooplankton. Zooplankton, whose grazing is a function of biomass, could not take full advantage of these brief intense blooms. Therefore, zooplankton biomass, and *Pseudocalanus* biomass in particular, remained relatively low (Figs BPM 7, 9).

In contrast, when the initial stratification of the water column was periodically interrupted during the bloom period, by either convective cooling, as in 1994 (*c.f.* Fig. BPM-2b, 3b, and 6b), or intermittent strong wind mixing, as in 1995 and the GOA simulations (*c.f.* Fig. BPM-2c, 3c, and 6c; and 4a, 5a, and 9a), the phytoplankton bloom occurred over a longer period of time and was mixed deeper into the upper water column. This deeper mixing caused more nutrients to become available to phytoplankton in the euphotic zone and lead to a longer phytoplankton bloom, with more total primary production. The more gradual bloom gave zooplankton time to increase their biomass at a rate more similar to that of the phytoplankton production. This, in turn, lead to zooplankton biomasses as much as an order of magnitude greater than those found when the phytoplankton bloom was brief and shallow.

This aspect of the system leads to difference in export of fixed organic carbon to the aphotic zone and/or benthos which are summarized in Table 1.

Table 1. Primary production and flux from surface waters

Simulation	Total New Primary Production	% PP Sinking from Surface	Absolute Flux
1993	42.9 GC/m^2	34.8	14.9 GC/m^2
1994	54.9 GC/m^2	26.8	14.7 GC/m^2
1995	51.9 gC/m^2	31.8	16.5 GC/m^2
GOA Shelf	51 GC/m^2	33.6	17.5 GC/m^2
NW PWS	35 GC/m^2	56.6	19.6 GC/m^2

When the phytoplankton spring bloom was short and intense, *i.e.*, in the 1993 and NW PWS simulations, the total primary production during the bloom was minimal. However, because of the poor coupling between the phytoplankton and zooplankton populations, the **percent** of the new production which was exported out of the near-surface waters was maximal. The net effect of these two factors is to increase the absolute amount of primary production exported from the surface layers. This is dramatically illustrated in the spatial simulations, where, although total NW PWS new production was only 69% that of the GOA shelf, the

absolute flux of organic carbon from the NW PWS surface layer was 112% that of the GOA shelf.

Conclusions

Plankton dynamics are a highly non-linear, but deterministic, pathway through which variations in the physical environment can be passed up the food web to both nekton and benthic organisms. Differences in the physical environment, and in meteorological forcing in particular, over a few weeks in early spring, can lead to order of magnitude differences in the upper water column zooplankton biomass throughout the summer. The potential effects of meteorological variations can be simulated, and eventually predicted, using coupled biophysical numerical models. Differences in the zooplankton community throughout the summer can be extremely importance to the planktivorous juvenile pink salmon and Pacific herring populations. We suggest that the models presented here are a critical component in understanding the dynamics of these species. In addition, plankton dynamics determine the partitioning of pelagic primary production between the upper water column and the benthos. Therefore, the type of model presented here will have value to other studies examining trophic structures in Prince William Sound, in particular EVOS groups such as the APEX and NVP programs.

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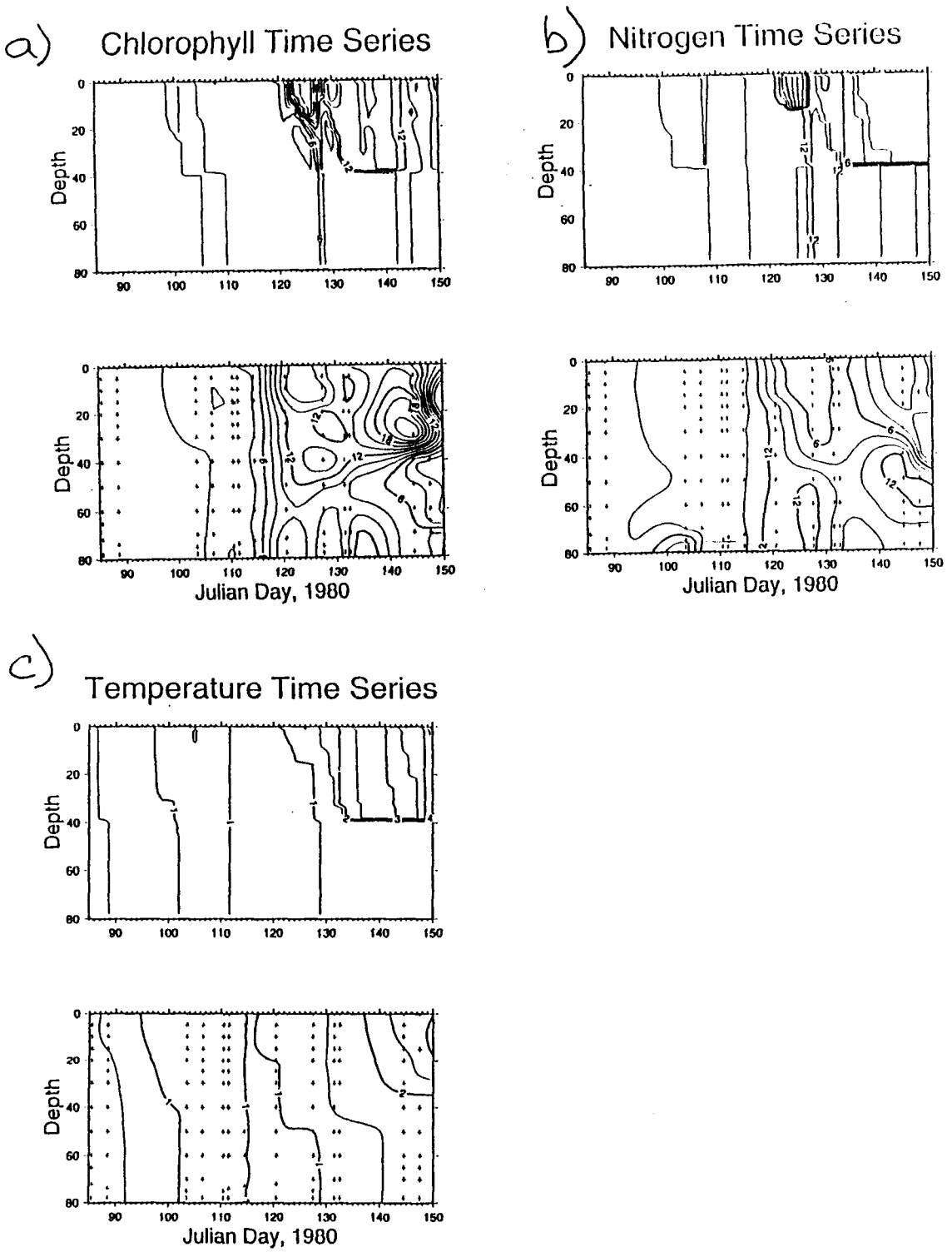

Figure BPM-1. Comparisons of one-dimensional models with field data. a) Depth-time contour of model results (top) and field data (bottom) for chlorophyll concentrations (mg m^{-3}) (Eslinger and Iverson, 1996) in the SEBS; b) as a) but for total nitrogen (μg-at l^{-1}); c) as a) but for temperature (C).

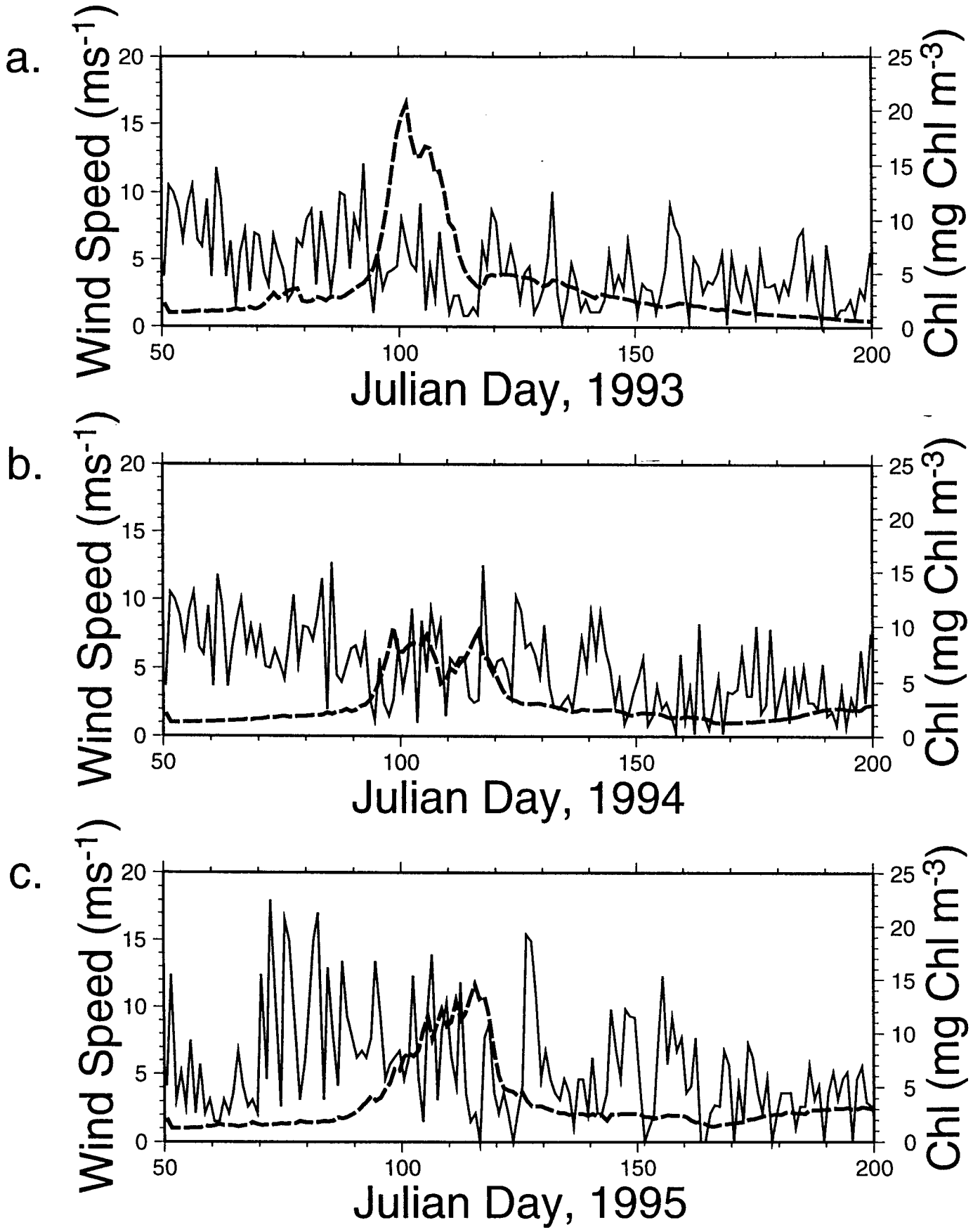

Figure BPM-2. Wind speed and model simulated chlorophyll concentrations for a) 1993, b) 1994, c) 1995.

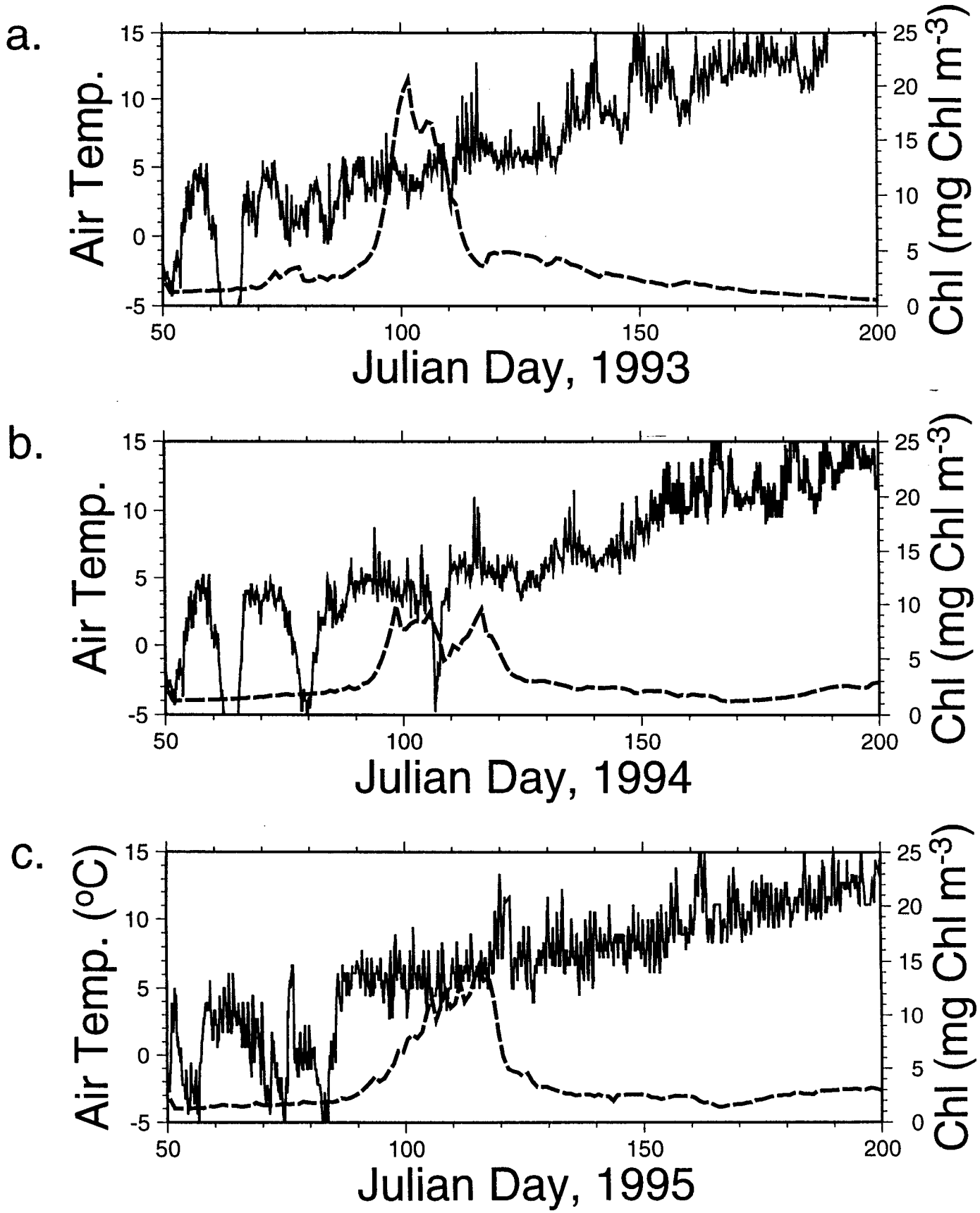

Figure BPM-3. Air temperature and model simulated chlorophyll concentrations for a) 1993, b) 1994, c) 1995.

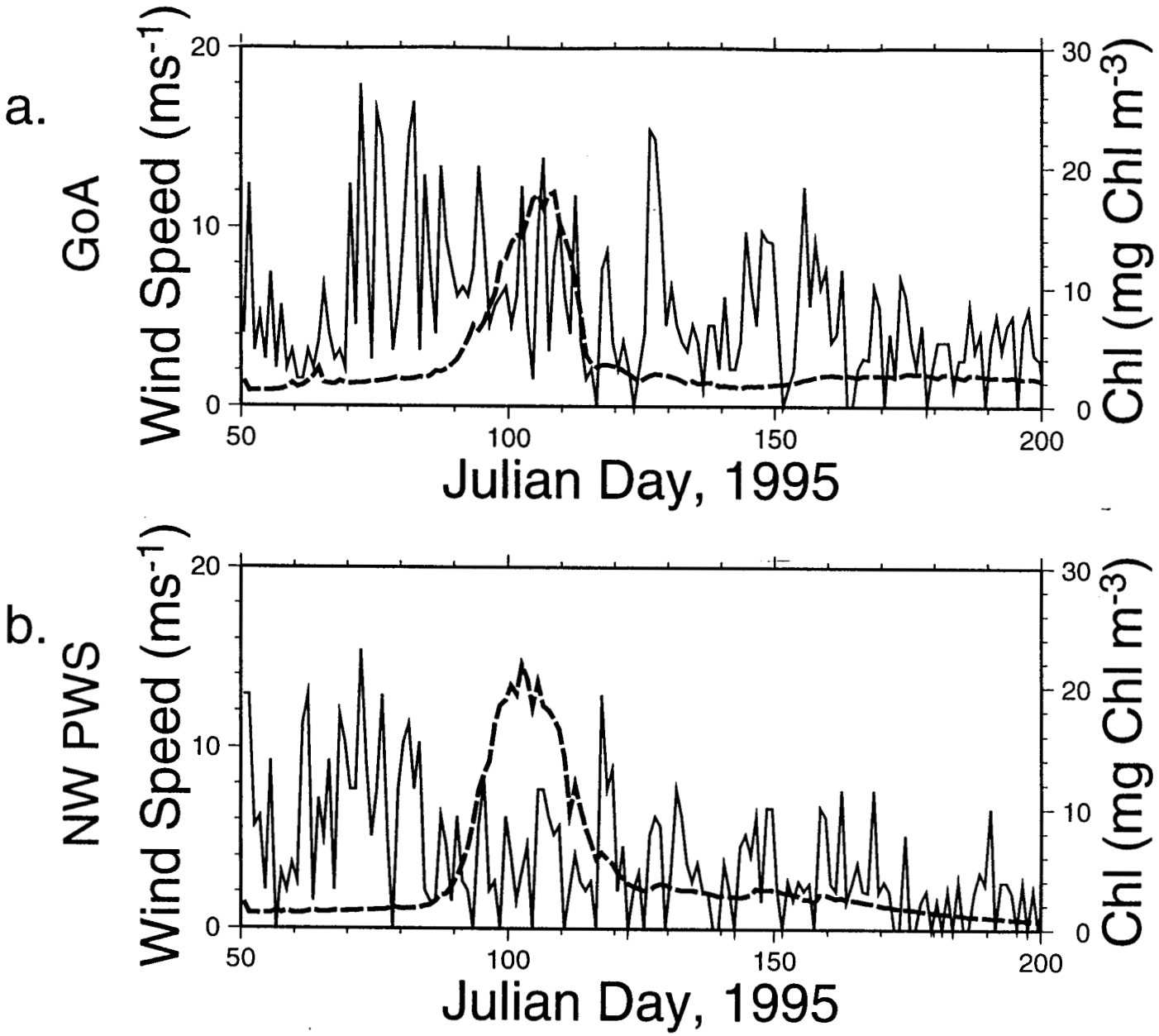

Figure BPM-4. Wind speed and model simulated chlorophyll concentrations for a) Gulf of Alaska shelf, and b) northwestern Prince William Sound, AK.

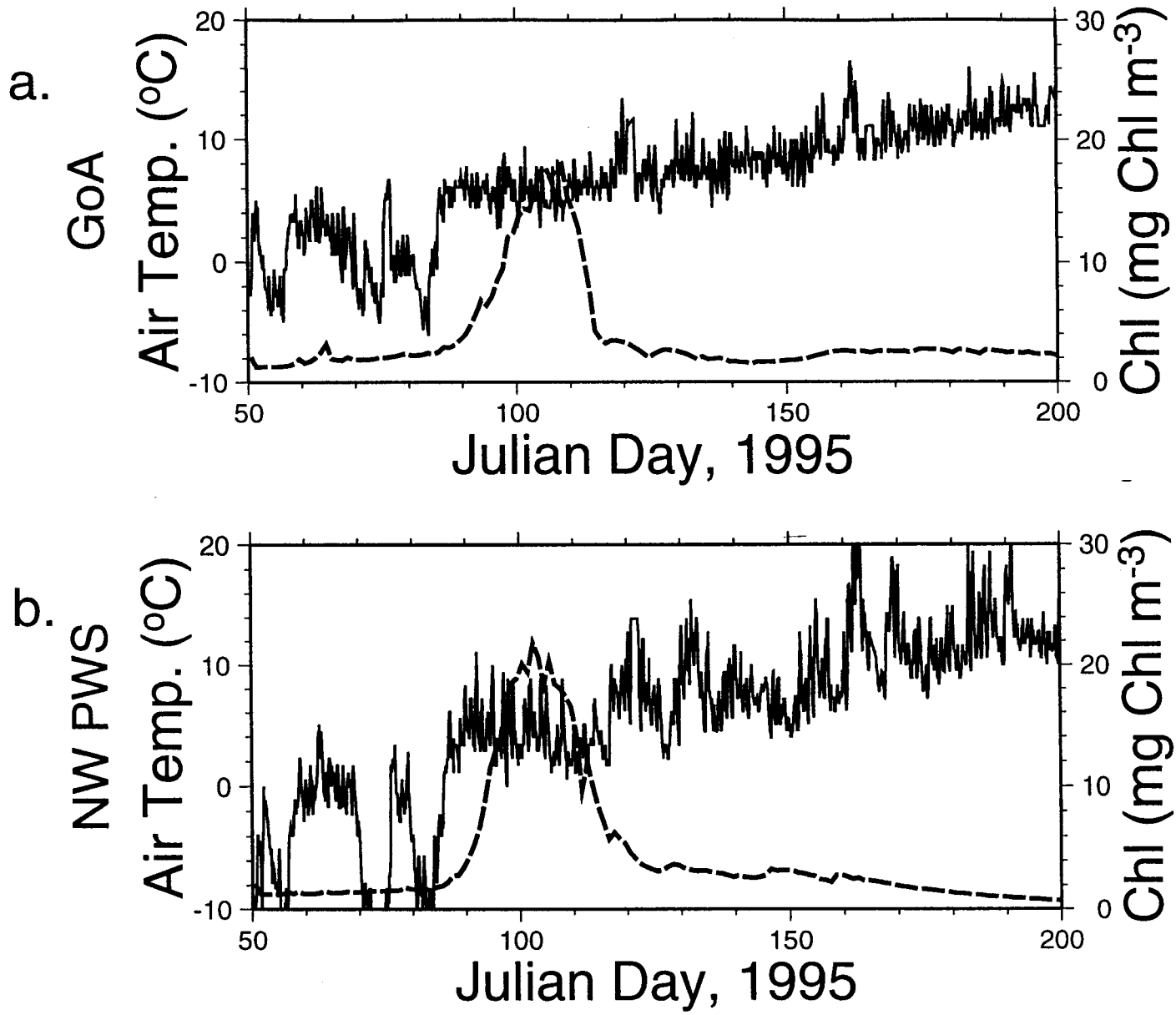

Figure BPM-5. Air temperature and model simulated chlorophyll concentrations for a) Gulf of Alaska shelf, and b) northwestern Prince William Sound, AK.

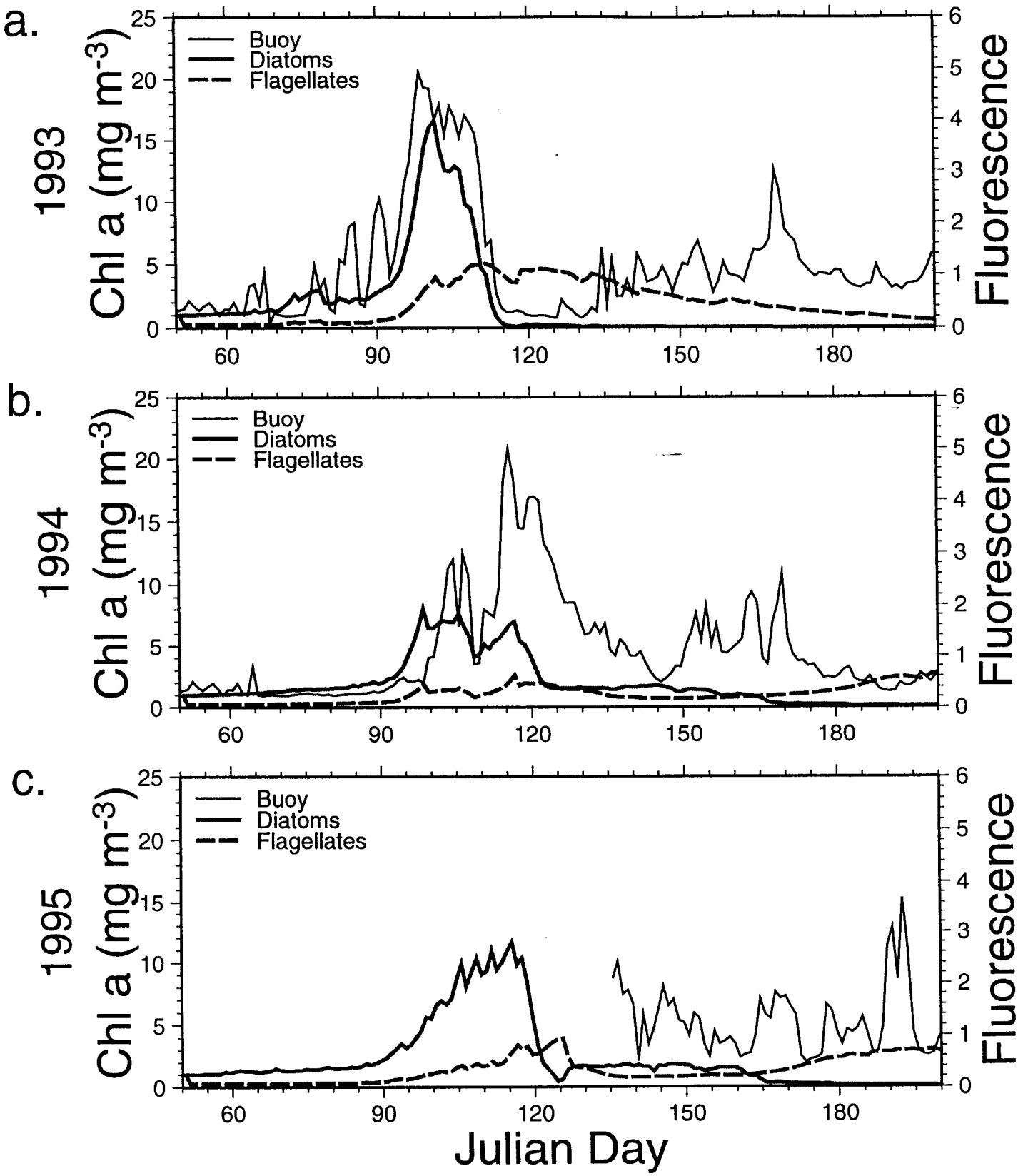

Figure BPM-6. Phytoplankton field data and model simulation results for a) 1993, b) 1994, c) 1995. The thin line is fluorescence measured at the CFOS buoy, the thick line is simulated diatom chlorophyll concentrations and the thick dashed line is simulated flagellate concentrations.

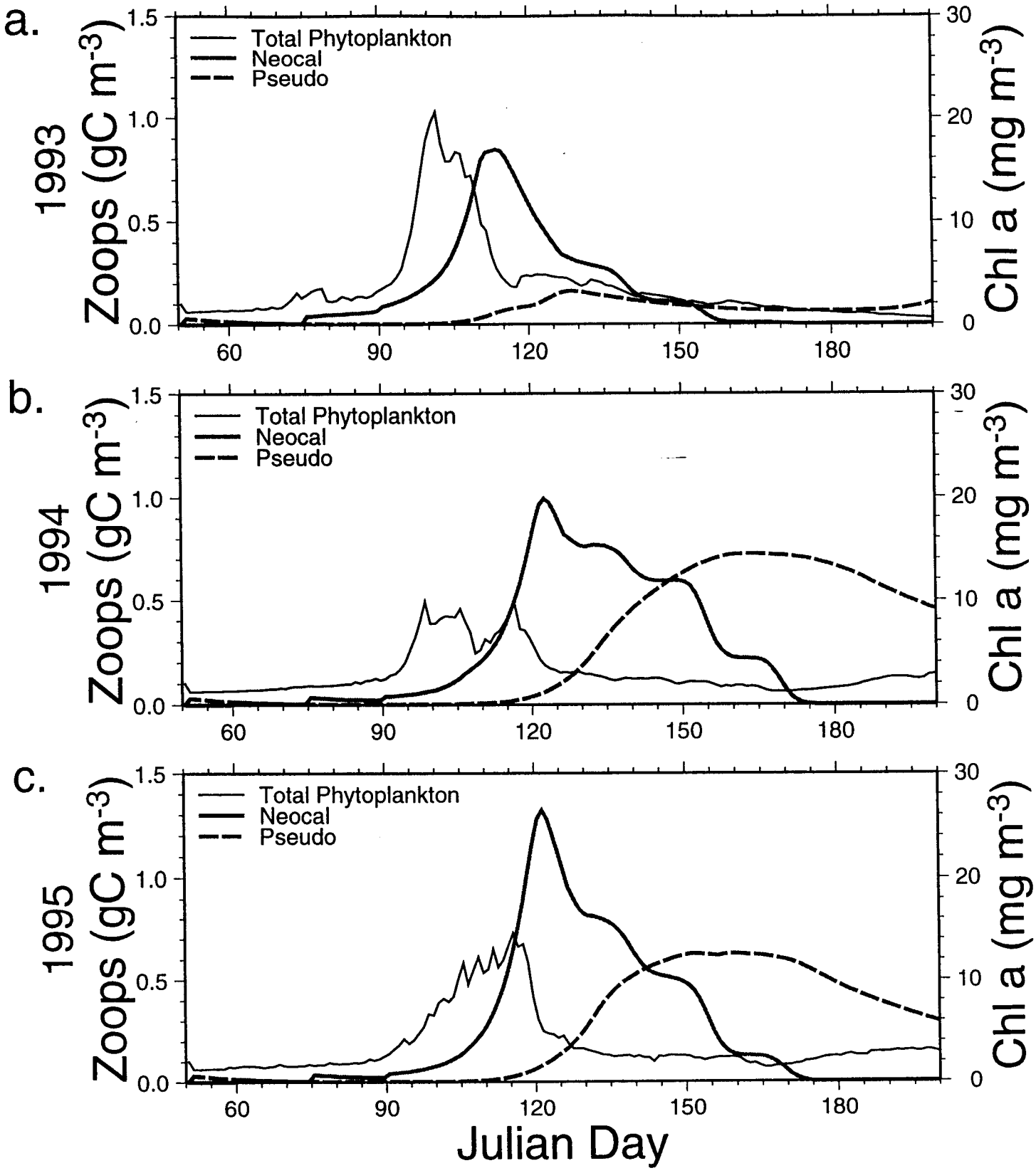

Figure BPM-7. Zooplankton model simulation results for a) 1993, b) 1994, c) 1995. The thin line is total simulated phytoplankton chlorophyll, shown for reference; the thick line is simulated *Neocalanus* wet weight; and the thick dashed line is simulated *Pseudocalanus* wet weight.

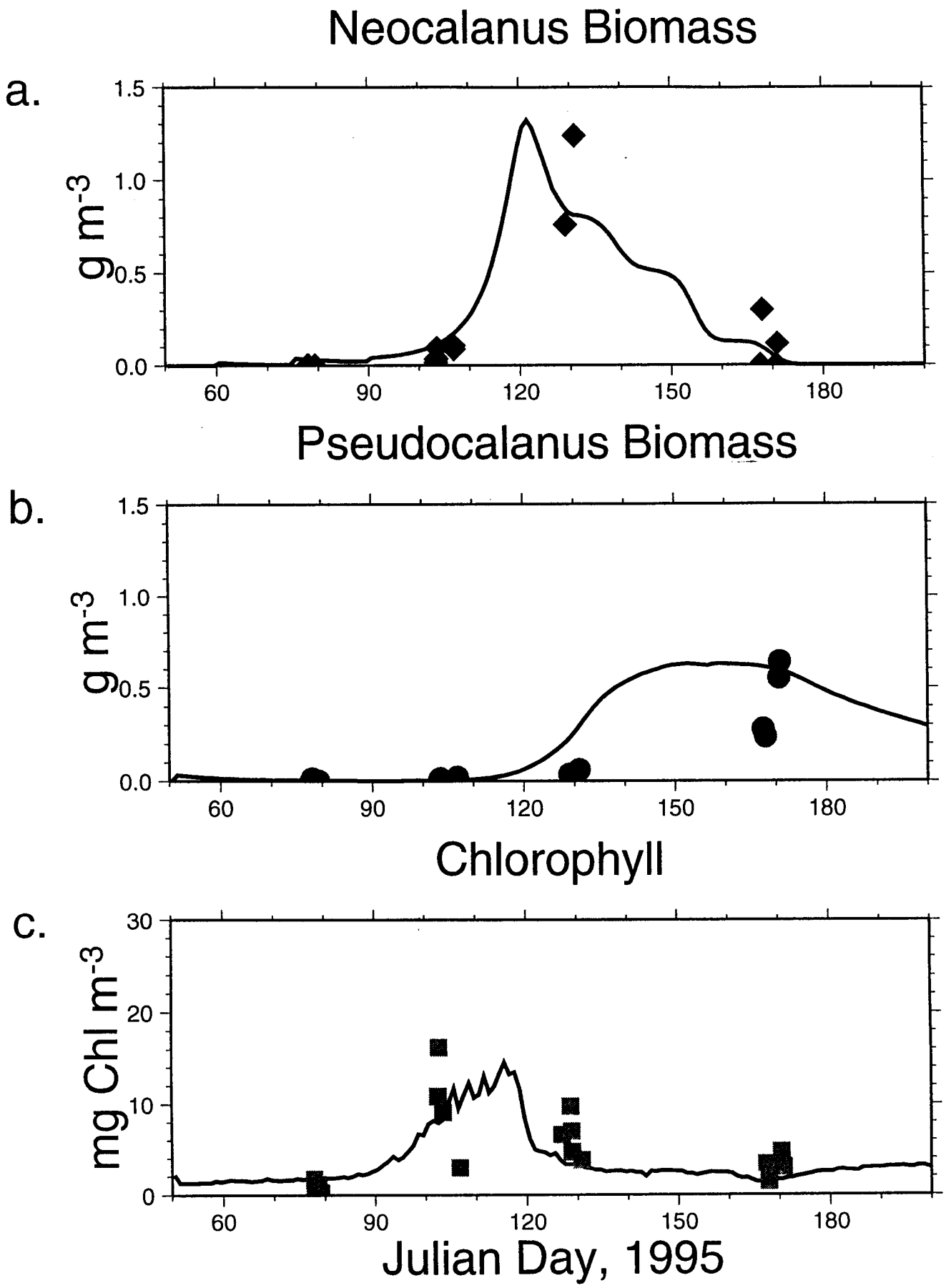

Figure BPM-8. Zooplankton and phytoplankton field data and model simulation results for 1995. The thick lines are model results and the symbols are field data from four cruises made in 1995. a) *Neocalanus* wet weight, b) *Pseudocalanus* wet weight, and c) total phytoplankton chlorophyll.

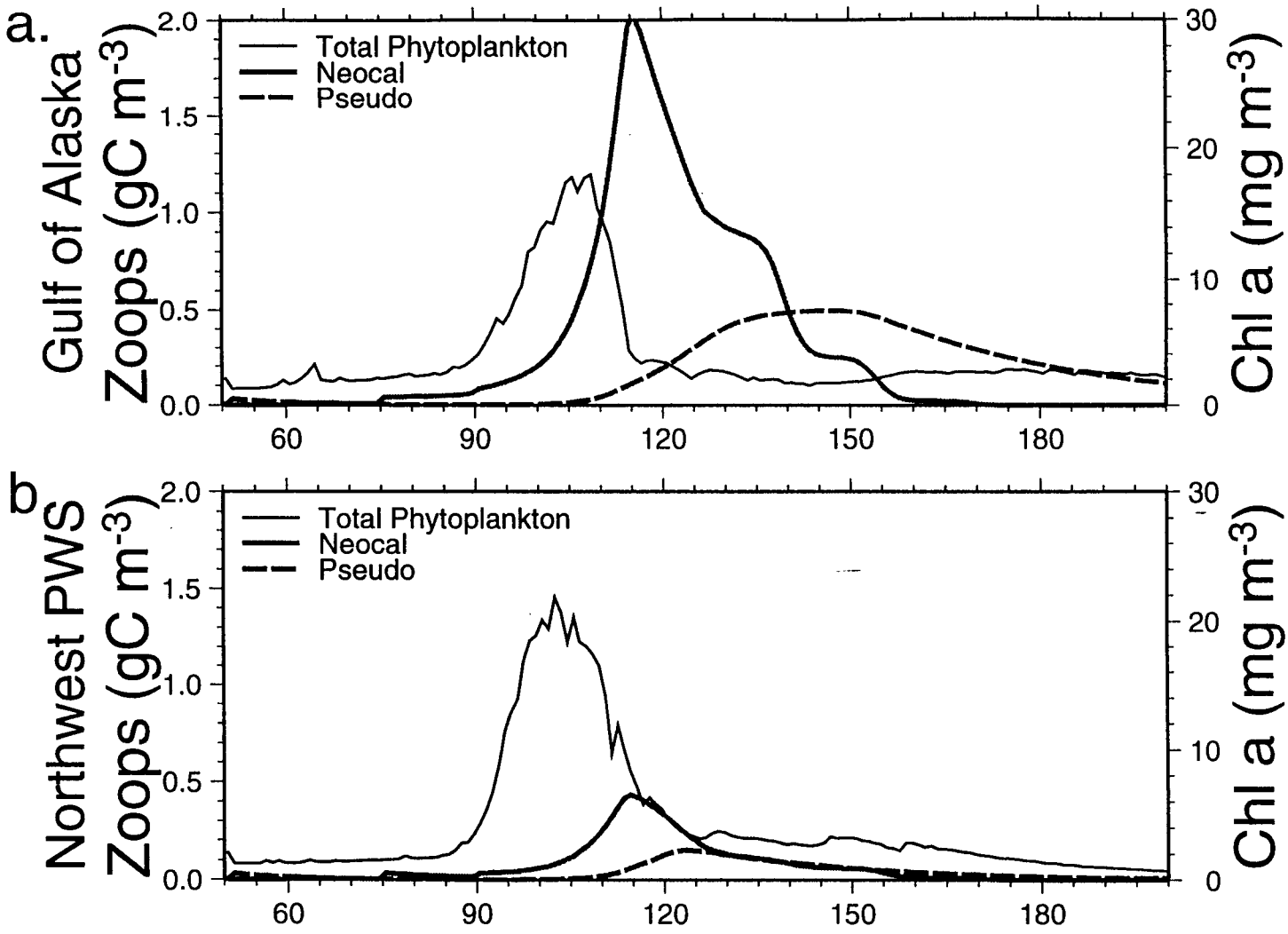

Figure BPM-9. Zooplankton model simulation results for a) Gulf of Alaska shelf, and b) northwestern Prince William Sound, AK. The thin line is total simulated phytoplankton chlorophyll, shown for reference; the thick line is simulated *Neocalanus* wet weight; and the thick dashed line is simulated *Pseudocalanus* wet weight.

Exxon Valdez Oil Spill
Restoration Project Annual Report

PWSAC - PWS System Investigation - Experimental Fry Release

Restoration Project 95320K
Annual Report

This annual report has been prepared for peer review as part of the *Exxon Valdez* Oil Spill Trustee Council restoration program for the purpose of assessing project progress. Peer review comments have not been addressed in this annual report.

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March 1996
PWSAC - PWS System Investigation -
Experimental Fry Release

Restoration Project 95320K
Annual Report

Study History: In support of the needs of the Sound Ecosystem Assessment (SEA) researchers, in 1994 and 1995 combined, Prince William Sound Aquaculture Corporation (PWSAC) released a total of approximately 27.1 million hatchery reared pink salmon fry which were previously reared to approximately 1.2 grams (Restoration projects 94320K and 95320K). Prior to release, the fry were coded wire tagged at known ratios of tagged to untagged fish.

SEA is an ongoing *Exxon Valdez* oil spill (EVOS) Trustee Council program which focuses on the processes and mechanisms that regulate losses of fry and juveniles to predators after emergence from nearshore natal habitats. Hatchery produced pink salmon fry and returning adults may provide a test of the influence of ocean-entry timing and of fry size at ocean entry on losses to predators.

Abstract: In 1995, Approximately 12.4 million pink salmon fry nurtured at PWSAC's Wally Noerenberg (WNH) and Armin F. Koernig (AFK) hatcheries were reared to a size of approximately 1.2 grams each and released on June 15th. Approximately 20,800 of the fry were coded wire tagged prior to release thereby making assessments of early marine growth, life stage mortality and migration patterns possible by other SEA researchers.

Key Words: *Exxon Valdez*, hatchery, marine survival, *Oncorhyncus gorbuscha*, pink salmon, Prince William Sound, Prince William Sound Aquaculture Corporation, Sound Ecosystem Assesment (SEA).

Citation: Ferren, H., J. Milton. 1995. PWSAC - PWS system investigation - experimental fry release, *Exxon Valdez* Oil Spill Restoration Project Annual Report, (Restoration Project 95320K), Prince William Sound Aquaculture Corporation, Cordova, Alaska.

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EXECUTIVE SUMMARY

The Experimental Release Project is an integral component of the Prince William Sound (PWS) Ecosystem Assessment (SEA) studies. Identifiable pink salmon *Oncorhynchus gorbuscha* are an excellent tool to test a central SEA recruitment hypothesis concerning time at ocean entry and fry size at ocean entry. Consequently, SEA researchers requested very large and late fry, relative to typical size and timing upon ocean entry, from the PWS Aquaculture Corporation (PWSAC). Additionally, by utilizing two release sites for the subject salmon fry, an increase in the spatial difference at ocean entry point allows researchers insight into subtle locational differences within the Sound.

Approximately 12.3 million pink salmon fry nurtured at PWSAC hatcheries were released in June 15, 1995 at an average size of approximately 1.2 grams each. About 20,800 large, pink salmon fry were marked and tagged, making assessments by other SEA researchers of early marine growth, life stage mortality and migration patterns possible at a highly reasonable cost.

The justification for the strategy is evidence from Alaska and elsewhere which suggests that fry size is an important determinant of salmon fry survival during early marine residence (Kaeriyama, 1989; Parker, 1971). Faster growing juveniles are thought to enjoy better marine survivals than slower growing fish. As in project 94320K, the fry in this project were targeted for recapture.

INTRODUCTION

The knowledge garnered by SEA researchers, subsequent to *Exxon Valdez* Oil Spill, in evaluating the changes occurring in the PWS ecosystem is vital to evaluating and defining the best approaches to efficiently, and effectively restore the many damaged marine resources and activities. The key to understanding the complex species interactions that occur during critical early marine life stages requires an immense amount of effort and team work by many institutions, agencies, groups, and private individuals. The ecosystem level information that is now being developed will aid the *Exxon Valdez* Oil Spill Trustee Council and others in their restorative tasks assisting injured pink salmon and herring populations in PWS, as well as forming predictive models which will benefit mariners of all stripes on into the future.

Identifiable, as to salt water entry point, time, and size, salmon fry are required by SEA researchers. Consequently, PWSAC has made available its facilities, personnel and expertise. Releases of restoration and enhancement facility-nurtured pink salmon fry is, as indicated by the full SEA proposal, providing "...a powerful test of the influence of ocean-entry timing and fry size at ocean entry on losses to predators".

OBJECTIVES

The goal of this project is, through collaboration with the SEA program, to assist "to develop an ecosystem level understanding of the natural and man-caused factors influencing the production of pink salmon in PWS".

Specific objectives are:

A. Rear 8 million early emerging fry each at the Wally H. Noerenberg (WHN) hatchery on Esther Island and Armin F. Koernig hatchery (AFK) on Evans Island to 1.5 grams live weight for release in mid-June.

B. Determine the marine survivals of fry in experimental releases from coded wire-tagged individuals recovered in corporate escapement and common property fishery the following year.

C. Compare the marine survivals of late-released, larger fry with other releases at these same facilities.

METHODS

Project 95320K took place in PWS at the AFK facility located on Evans Island and the WHN facility sited on Esther Island. Site work commenced in February 1994.

Project pink salmon fry were designated from early outmigrants and weighed on average 0.23 grams, blotted wet weight, each. Volitional outmigration from PWSAC NOPAD incubators insured osmocompetence and optimum developmental fitness. After passing a bank of electronic counters (+/- 1% accuracy), fry were conveyed via flexible hose to 12m x 12m x 3m ($432m^3$) saltwater rearing pens. Approximately three million fry were held in each of a total of four pens, two each at the two facility locations (Table).

Prior to release, 1/2mm Coded Wire Tags (CWT) were be applied to approximately 1 out of every 600 fry. Each pen of fry contained a unique code (Table 1). The CWT fry are integral to identification thus allowing tracking migration patterns of pink salmon fry, and estimation of growth and mortality patterns.

All fry were fed a standard commercial diet of soft, semi-moist fish food for between 75-87 days prior to release. Releases occurred on June 15th at the WHN and AFK facilities. Weights varied (Table) and were the maximum technically feasible given the requested release dates.

Close coordination and communication occurred between SEA researchers and the hatchery personnel during the field season, to assure SEA's sampling efforts were closely timed to releases of facility pink salmon fry. Releases were done in concert with shipboard sampling carried out by SEA research teams. Fry release data from the hatcheries was communicated to biologists stationed on board trawl and purse seine vessels. Thus, nearshore and open water sampling was

targeted on released fry as deemed necessary by collaborating researchers' experimental designs and judgement.

RESULTS

Within the constraints of the state of the science and art of fishcultural technology, PWS pink salmon's genetically determined scope for growth, budgetary reality, and collaborating researchers' experimental designs/timing requirements, the results were as close to planned objectives as are currently feasible. Please see the Table for the exact dates, weights, numbers, number mark/tagged, codes, and untagged: tagged ratios.

DISCUSSION

PWSAC normally releases pink salmon fry in or near the peak of zooplankton biomass abundance after assisting with feeding and predator protection, thus closely emulating what PWS pink salmon fry do when unassisted. Consequently the test releases are not within the normal scope of PWSAC operational strategies. The project delineated herein, however, is intended to provide a tool for SEA researchers assisting increases in understanding of factors affecting survival of juvenile pink salmon fry in PWS.

CONCLUSIONS

Year to year variation in physical and biological oceanographic conditions in PWS are historically evidenced. That the saltwater entry of late, large-sized, marked and tagged pink salmon fry is of value to fellow SEA projects is evidenced by SEA researcher's requests that project 95320K be continued. Given the differences between inter-year PWS ecosystem comparisons, SEA projects require multi-years' data before reliable conclusions can be drawn concerning the many biotic and abiotic factors influencing PWS pink salmon survivals.

Salmon hatchery produced pink salmon fry are a viable tool to test hypotheses regarding the causes of mortality in juvenile pink salmon in PWS. PWSAC has ascertained feasibility of nuturing at least some of its pink salmon to 1.5 grams live weight for release by mid-June.

The feasibility of releasing a 1.0-1.5 gram pink salmon fry using current technology has been ascertained by the 1995 work reported on in this annual report. The objectives appear to be attainable, particularly at the Esther Island facility, allowing that required additional resources are secured and employed.

Project 95320K should be continued as a necessary and important support function to other SEA projects as multiple years of data are needed before reliable conclusions can be drawn concerning factors affecting mortality in PWS pink salmon stocks. With the understanding of the theoretical underpinnings on the dynamics of pink salmon stocks, and their interrelationships with abiotic

and biotic factors, comes the promise of garnering the ability of enduring ecosystem management, thus assuring biodiversity, as well as economic security on into the future.

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TABLE. Project 95320K Results

	FACILITY	
Release Date	**WNH**	**AFK**
1st Pen	June 15	June 15
2nd Pen	June 15	June 15
Weights (g)		
1st Pen	1.06	1.35
2nd Pen	0.95	1.34
# Fry Released		
1st Pen	3,152,969	2,961,191
2nd Pen	3,162,135	3,024,130
# Fry Marked		
1st Pen	5,443	4,949
2nd Pen	5,346	5,056
Tag Code		
1st Pen	13-1-3-5-11	13-1-3-6-11
2nd Pen	13-1-3-5-12	13-1-3-6-12
Untagged/Tagged Ratio		
1st Pen	579:1	598:1
2nd Pen	591:1	598:1

Chapter 9

95320M Observational Physical Oceanography

1995 Annual Report
Observational Physical Oceanography in Prince William Sound
and the Gulf of Alaska
Exxon Valdez Trustee Council Project 95320-M
April 1996

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Prince William Sound Science Center
Cordova, Alaska

ABSTRACT

Hydrographic surveys and current velocity measurements collected during five cruises in 1995 show significant monthly variability in the water mass properties and circulation patterns in Prince William Sound. Temperature, salinity, and potential density in March and April are shown to be fairly homogeneous. The warm, fresh Gulf of Alaska inflow is evident in the upper 20 meter layer in May and June. By September, the water entire column has warmed and become saltier. Baroclinic velocities (20/100 meter) calculated from towed ADCP transects indicate that the northern, western, and eastern portions of the sound are characterized by either weak baroclinic, or barotropic currents. An ADCP mooring at Hinchinbrook Entrance shows weak and variable currents, with mostly inflow above about 150 m and outflow below. Meteorological forcing at one station is shown to mix the water column down to about 50 meters. These measurements, combined with a numerical circulation model, will be used to define 'river' and 'lake' conditions, and to study the effect of these condition on zooplankton abundance and distribution. The goal of this project is to identify physical factors that influence the production of pink salmon and Pacific herring in Prince William Sound.

INTRODUCTION

The Sound Ecosystem Assessment (SEA) is aimed at identifying the primary factors that control the production of pink salmon and Pacific herring in Prince William Sound (PWS). A main hypothesis of SEA is that physical conditions, such as ocean temperature and salinity, flow velocities, and atmospheric forcing, primarily determine the survival of embryos and juvenile fish. Processes that control the physical environment include tidal motions, wind stress, seasonal heating and cooling, precipitation, river/glacial runoff, inflow and outflow of Gulf of Alaska (GOA) water, and longer term events like El Nino and the Southern Oscillation (ENSO). Time scales of these processes range from hours to decades, and space scales range from tens of meters to O(100 km).

The northern Gulf of Alaska (GOA) and the Alaska Coastal Current (ACC) have been studied extensively since the mid-1970's. The seasonal variations of quantities such as baroclinic geostrophic transport, wind forcing, freshwater discharge, and coastal upwelling, have been

described elsewhere (e.g. Royer and Emery, 1987; Johnson et al, 1988; Royer, 1981a,b; Royer, 1979). Several of these papers mention interactions of the ACC with PWS (e.g. Royer et al, 1979; Royer et al, 1990), but attention to the Sound was usually brief.

Niebauer et al (1994) presented the first description of the circulation and water mass properties of PWS, based on data collected between 1974 and 1989. They focused on two periods: 1977-1979, and 1989 (after the Exxon-Valdez oil spill). Hydrographic data were used to created dynamic topographies (0/100 m), and to calculate monthly means of baroclinic geostrophic transport (relative to 100 m) at Hinchenbrook Entrance and Montague Straight. Current meter moorings were deployed over 15 months from 1977 to 1979 in both Hinchenbrook Entrance and Montague Straight. Current velocities (20 m values minus 100 m values) from acoustic Dppler current profiler (ADCP) transects made in 1989 were also presented. Based on estimates of transports in various pressure layers, and estimates of the total volume of the layer, Niebauer et al made estimates of flushing rates of PWS. The concluded that about 40% of PWS was flushed from May through September, and that the Sound was flushed about two to four times from October to April.

Results presented here describe some of the spatial and temporal variability of the large scale circulation and water mass properties of PWS observed in 1995. Basin scale hydrographic surveys, and ADCP transects were conducted on five cruises in 1995. An upward looking ADCP mooring deployed in Hinchenbrook Entrance collected data simultaneously. Two C-MAN stations in PWS collected meteorological data from May to December 1995. The physical measurements presented here may be correlated with the meteorological measurements, and with the biological measurements presented elsewhere in this report. These results demonstrate the monthly variability of the circulation and water mass properties of PWS. Combined results from 1994, 1995, and subsequent field years will be used to create estimates of seasonal mean properties, which will then be used to validate the numerical circulation model described in Chapter 95320-J.

DATA

Large scale oceanographic cruises were conducted in March, April, May, June, and September of 1995. The station locations for the April cruise is shown in Figure 1 as an example. Not all stations were occupied in all cruises, but the spatial coverage was about the same. The cruise dates are listed below.

March	15-23
April	10-17
May	4-11
June	15-20
Sept./Oct.	29- 3

The hydrographic data was collected using a SeaBird 911 CTD. Conductivity, temperature, and pressure (depth) were recorded at 1 dbar intervals. Salinity was calculated from conductivity using

standard SeaBird software. The CTD salinities were not calibrated with bottle samples.

Instantaneous current velocity transects were collected using an RDI 150 kHz broadband ADCP deployed from the stern of the ship in a towed body. Most transects were in water less than 400 m depth so that bottom tracking was available. The bin length was 8 m for most of the data. The ADCP generally measured flows from about 20 m depth to the bottom. Some transects were repeated in more than one cruise, but most were made on an opportunistic basis, generally between hydrographic stations.

A time series of current velocities as a function of depth was collected from an upward looking ADCP mooring (RDI 150 kHz broadband) deployed in Hinchenbrook entrance from mid-June to late September, 1995. Velocities were recorded every 30 minutes using an 8 m bin length. Good velocities were obtained from 43.5 m to about 300 m depth.

Meteorological data from C-MAN stations in PWS are available from the National Data Buoy Center (NDBC). The stations are located at Bligh Reef, Potato Point, Seal Rocks, and Mid-Sound (in the central Sound). Wind speed, wind direction, wave height, barometric pressure, air temperature, water temperature, dew point temperature, and visibility are measured every 30 minutes. The buoys became operational in May 1995, and have collected mostly uninteruped data since then.

Plankton data was obtained from 5 months in 1995 from a towed optical plankton counter (OPC). Some of the OPC cruises were not simultaneous with those listed above. The OPC was mounted together with a Chelsea Instruments CTD (the Aquapak) in an Aquashuttle tow body and deployed from the ship. The combined package cycled vertically from the surface to about 80 m depth.

ANALYSIS

Hydrography

Average values of temperature and salinity were calculated over several depth layers and contoured using GMT version 3 programs (Wessel and Smith, 1995). Examples from May and September 1995 are shown in Figures 2 and 3. Contours of temperature and salinity averaged over the upper 20 meters from May show the intrusion of the warmer, fresher GOA water on the east side of Hinchenbrook Entrance (Figure 2). This intrusion is still present in June (not shown). By September, this water mass contrast is no longer apparent; the layer is cooler and fresher in the north.

Mean temperature and salinity calculated over the 90 to 110 m layer in September 1995 are contoured in Figure 3. Warmer, saltier water outside Hinchinbrook Entrance does not appear to have entered PWS at this depth. A fairly strong temperature gradient exists in this layer between PWS and the GOA.

Potential density was calculated from the temperature and salinity measurements at each station, and averaged over the upper 100 meters. Contours of mean potential density for each cruise are presented in Figures 4(a)-(e). In March and April (Figures 4(a),(b)), this layer is fairly uniform (the contouring interval is .05). By May (Figure 4(c)), the sound has started to stratify zonally (the contouring interval is .1). A mid-Sound (147 W) density gradient between the lighter inflowing GOA water in the east, and a small dense region to the north of Montague Island is present in May. By June (Figure 4(d)), the layer is fairly uniform east of 147 W, but the relatively dense region north of Montague Island persists. By September (Figure 4(d)), the layer density gradients are stronger (the contouring interval is .2) and more symmetric, with the highest densities in the center of the central basin.

To illustrate the monthly variations in vertical stratification, temperature, salinity, and potential density profiles from repeat occupations of the CFOS13 station located in the central sound, are presented in Figures 5(a)-(e). The March potential density (sigma) profiles shows the water column to be completely mixed down to about 200 m (Figure 5(a)). The April salinity profile shows a slight freshening above about 100 m (Figure 5(b)). The April (Figure 5(b)) and May (Figure 5(c)) salinity and sigma profiles are almost identical. Significant warming and freshening has occured by June (Figure 5(d)), but the changes are confined to the upper 100 m. Below 100 m the potential density (sigma) is unaffected. By September (Figure 5(e)), warming has occured at all depths. The September salinities are fresher at the surface (< 15 m) than in June, but at depths greater than 15 m, salinities are greater. Potential density also is less at the surface than in June, and greater below 15 m.

ADCP Transects

Current shear velocities at 20 m relative to 100 m for each cruise are presented in Figure 6(a)-(e). The shear velocities are calculated by subtracting the 100 m velocities from the 20 m velocities. Flow velocities measured by the towed ADCP include a tidal component, and the time for completion of many of the transects covered a large portion of the tidal cycle. Velocities at 20 m relative to 100 m were calculated to eliminate the contribution from the barotropic tide. Neibauer et al (1994) also presented shear velocities over this layer (20/100 m) from data collected in 1989.

Subtracting velocities from two different levels also eliminates any barotropic (vertical mean) component of the flow, along with the tidal component. The vectors in Figure 6 represent the baroclinic (shear) component (the difference in velocities from 20 to 100 m), not necessarily the velocity of the flow. A small shear velocity may result from slowly moving currents at both 20 and 100 m, or from a strong barotropic current (currents of equal magnitude at both 20 and 100 m). If the velocity at 100 m was equal to zero, the vectors in Figure 6 would represent the speed and direction of the flow at 20 m. Vertical sections of ADCP transects show that velocities at 100 m are sometimes not negligable, and are often opposite in sense to those at 20 m. Individual transects must be inspected for the sense and magnitude of the 100 m velocities before conclusions are made about the actual large scale circulation.

In most cruises, the baroclinic velocities are strongest (> 50 cm/sec) at Hinchinbrook Entrance, and and the central sound. Shear velocities north of about 60.7 N (Naked Island), east of about 146.7 W (Knowles Head), and in the western sound (Knight Island Passage and west of Green Island) are generally weaker (although the barotropic component could be strong). This is especially true in May (Figure 6(c)), June (Figure 6(d)), and September (Figure 6(e)), and to a lesser extent in April (Figure 6(b)). The March cruise is an exception. Velocity shears are weakest in the central sound. Strong shears exist outside of Hinchinbrook Entrance in the GOA, in Montague Straight, and in the northwest Sound, south of Ester Island.

ADCP transects were made across Hinchinbrook Entrance on all but the September cruise. Repeat transects were made in May (not shown). The flow was mostly north in March over all depths (barotropic), and mostly southwest in April. Repeat transects in May indicated that the velocities were primarily tidally driven. The velocities were barotropic, and shifted from mostly north to mostly south over approximately a 12 hour period. In June, the velocities across Hinchinbrook Entrance were much less than earlier in the year, and showed considerable vertical structure (baroclinic). The flow was mostly toward the north, with much higher velocities on the eastern side of the Entrance.

ADCP Mooring at Hinchinbrook Entrance

North and east velocity components from an upward looking ADCP mooring in Hinchinbrook Entrance are shown in Figures 7(a) and (b). The velocities represent daily (24 hour) means. Negative velocities (south and west) are shaded. The upper depth bin was centered at 43.5 m. The mooring was deployed from June 22 to September 30, 1995 (julian day 173 to 272).

Above about 150 m, velocities are mostly less than 10 cm/sec toward the southwest. Deeper velocities reach speeds of 15 cm/sec, and alternate between north and south directions. Further analysis is needed to determine if the alternating pattern is an artifact of the averaging (higher frequency components aliased into the daily means), or a real feature of the circulation.

Weekly (7 day) mean velocities were also calculated and contoured (not shown). East velocities were mostly negative (west) and less than 5 cm/sec down to about 200 m. The flow was mostly to the south above 150 m and to the north below. Velocity magnitudes were between 5 and 10 cm/sec.

Meteorological Data

Time series of wind speed and direction were created for the PWS C-MAN stations located at Seal Rocks and in the central sound (Mid-Sound). Mid-Sound winds from September and October are shown in Figures 8(a) and (b). Strong wind events occurred around September 18 (hour 504 in Figure 8(a)), and October 1 (hour 24 in Figure 8(b)). Winds from May to September were mostly less than 10 m/s at both locations.

To examine the effects of wind forcing on vertical stratification, profiles of temperature, salinity, and potential density (sigma) were created for a station in the central sound (NS3) occupied on October 3 (during the September cruise) after the strong October wind event (Figure 9). These profiles may be compared with those of a nearby station (CFOS13) occupied during the same cruise on September 29, before the wind event (Figure 5(e)). After the wind forcing, the temperature at NS3 was uniform down to about 50 m, and is warmer than before the forcing down to about 100 m. Salinities are fresher to about 100 m, and are less than 26 at the surface. Potential densities are decreased as a result of the warming and freshing, down to about 100 m, although differences are small below about 60 m. Below 100 m, all profiles are virtually the same. Between about 10 and 60 meters, the stratification is considerably weaker after the wind forcing.

Plankton Data

An example of OPC data collected in April 1995 from Hinchinbrook Entrance to Valdez Arm is shown in Figure 10. High numbers of neocalanus (or neocalanus sized scatterers) is shown by the light colored region north of Hinchinbrook Entrance from about 5 to 30 meters depth. This high density patch extends from Hinchinbrook Entrance (about 60.25) to about 60.6 N, or roughtly 40 km. A similar plot of total counts (not shown) suggests that the water column down to 80 m was filled with scatterers of various sizes (plankton and others).

ADCP baroclinic velocities (20/100 m) from April 1995 (Figure 6(b)) in the region corresponding to the zooplankton patch are weaker than at Hinchenbrook Entrance, or farther north in the central sound. North of the patch, the velocities at 20 m are greater in the southerly direction than velocities at 100 m. Contours of temperature and salinity averaged over the upper 20 meters from April (Figure 11) show a relatively warm, salty region at the patch location. A zonal density front centered at about 60.5 N separates the warm, salty region from the rest of the Sound. Whether the location and extent of the neocalanus patch is related to the current velocities at 20 m, or to a particular water mass is uncertain.

DISCUSSION

The potential density contours in Figures 4(a)-(e) may be used to make some inferences about the sense of the baroclinic (shear) velocities in this layer. The thermal wind relationship (based on geostrophic and hydrostatic balance) states that horizontal gradients in density are balanced by the vertical gradient of velocity (vertical velocity shear). The horizontal gradients of mean density of the 0 to 100 m layer (Figure 4) are proportional to the difference between the velocities at 0 and 100 m (shear). If the horizontal density gradient is zero, then the baroclinic component of the flow is zero, but the barotropic component may be non-zero. A barotropic current (constant with depth) could exist in the absence of a horizontal density gradient. In some cases, ADCP transect current data indicate that the velocity at 100 m is much smaller that the velocities above, so that the horizontal density gradients may be interpreted as proportional to the speed and direction of the

actual flow (not just the baroclinic component). In other cases, the 100 m velocities are not negligable, and the relationship is more complex.

The density contours in May (Figure 4(c)) and June (Figure 4(d)) show a region of increased density north of Montague Island. Assuming thermal wind balance, this pattern is consistent with a cyclonic (counterclockwise) baroclinic flow. The velocities would be increasing from 100 m to the surface in the cyclonic direction. The weak horizontal density gradients in the eastern sound (east of 147 W) especially in June, indicate the absense of a baroclinic flow. Currents from Hinchinbrook Entrance north could be barotropic.

The density contours in September (Figure 4(e)) show a maximum density in the central sound. This pattern is consistent with a cyclonic baroclinic circulation in the central basin. Without knowing the flow velocities at 100 m, it is impossible to determine the magnitude and sense of the actual circulation. The density contours in September indicate only that from 100 m to the surface the current velocity is increasing cyclonically.

The results presented here may be compared with those of Niebauer et al (1994). Maps of 0/100 dbar dynamic topography from June 1976 and September 1978 (their Figure 7) are used to infer the large scale circulation pattern in the Sound. They state that the June 1976 dynamic heights 'hints' at the inflow through Hinchinbrook Entrance and cyclonic interior circulation. The closed contours in the central sound in September 1978 also suggest a cyclonic circulation similar to that indicated in September 1995.

Light and variable current velocities from the ADCP mooring at Hinchinbrook Entrance are consistent with the results of Niebauer et al (1994). A current meter mooring with instruments at 50, 100, 200, and 320 m recorded velocities in Hinchinbrook Entrance from June through September 1978. From 50 to 200 m, flows were generally around 10 cm/sec in no preferred direction, with occasional bursts to 25 cm/sec or greater. Only at the 320 m level were strong (> 25 cm/sec) northward velocities observed during this period. Strong (> 50 cm/sec) northward velocities were observed in the upper levels (30 and 50 m) starting in October (Niebauer et al, 1994).

CONCLUSION

Characteristics of the large scale water mass properties and circulation of PWS in March, April, May, June, and September, 1995 have been briefly described. Considerable variability in the spatial temperature and salinity structure exists between months. The sound is nearly homogeneous in March and April. Intrusions of GOA water is first evident in the upper 20 m layer in May, and remains present in June. By June, warming and freshening has occurred in the upper 100 m. Below 100 m, the water column is still weakly stratified. By September, the entire water column has been modified. Temperature and salinity were greater at all depths, except for a fresh layer at the surface. In all profiles, the density seems to be governed primary by salinity.

Current velocities also vary throughout the sound between March and September. Baroclinic current velocities were presented to illustrate the seasonal changes in circulation without the effects of tides. The flow in the northern, western, and eastern Sound is either weak or mostly barotropic. Current velocities at Hinchinbrook Entrance were shown to be weak and variable over the June to September period. The upper 150 m at Hinchinbrook Entrance was dominated by outflow. Below 150 m, alternating inflow and outflow was present.

Wind forcing from one brief event in October was shown to affect the vertical stratification down to about 60 m. The mixing was sufficient to remove the thermocline in the upper 50 m, but not the halocline. Some freshening was present down to about 100 m. With the NDBC C-MAN meteorological buoys in place, further investigation into the relationship between longer wind events, vertical stratification, and current velocities will be possible.

The results presented here represent work in progress. Future efforts are aimed at using the hydrographic and current velocity data to define 'river' and 'lake' conditions, and to study the effect of these condition on zooplankton abundance and distribution. The 1995 data will be combined with SEA data from 1994 and 1996, and with historical data, to make estimates of seasonal means and seasonal variability. The seasonal mean properties, along with the flow velocities at Hinchinbrook Entrance, will be used to verify and refine the numerical cirulation model of PWS.

ACKNOWLEDGEMENTS:

James Murphy (formerly at PWSSC) prepared the ADCP velocity transect figures, and post-processed the ADCP mooring data. Steve Bodnar (PWSSC) located and downloaded the meteorological data from NDBC. The software used to generate the OPC figure was developed by Eddy Jin (UAF).

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LIST OF FIGURES

Figure 9: Vertical profiles of temperature, salinity, and potential density (sigma) at station NS3 located in central PWS occupied on October 3, 1995, just after a period of increased winds.

Figure 10: OPC transect from Hinchinbrook Entrance to Valdez Arm in April 1995 showing counts/sec of Neocalanus as a function of depth.

Figure 11: Temperature and salinity averaged over the upper 20 m for April 1995.

Figure: 1

CTD Stations - be504

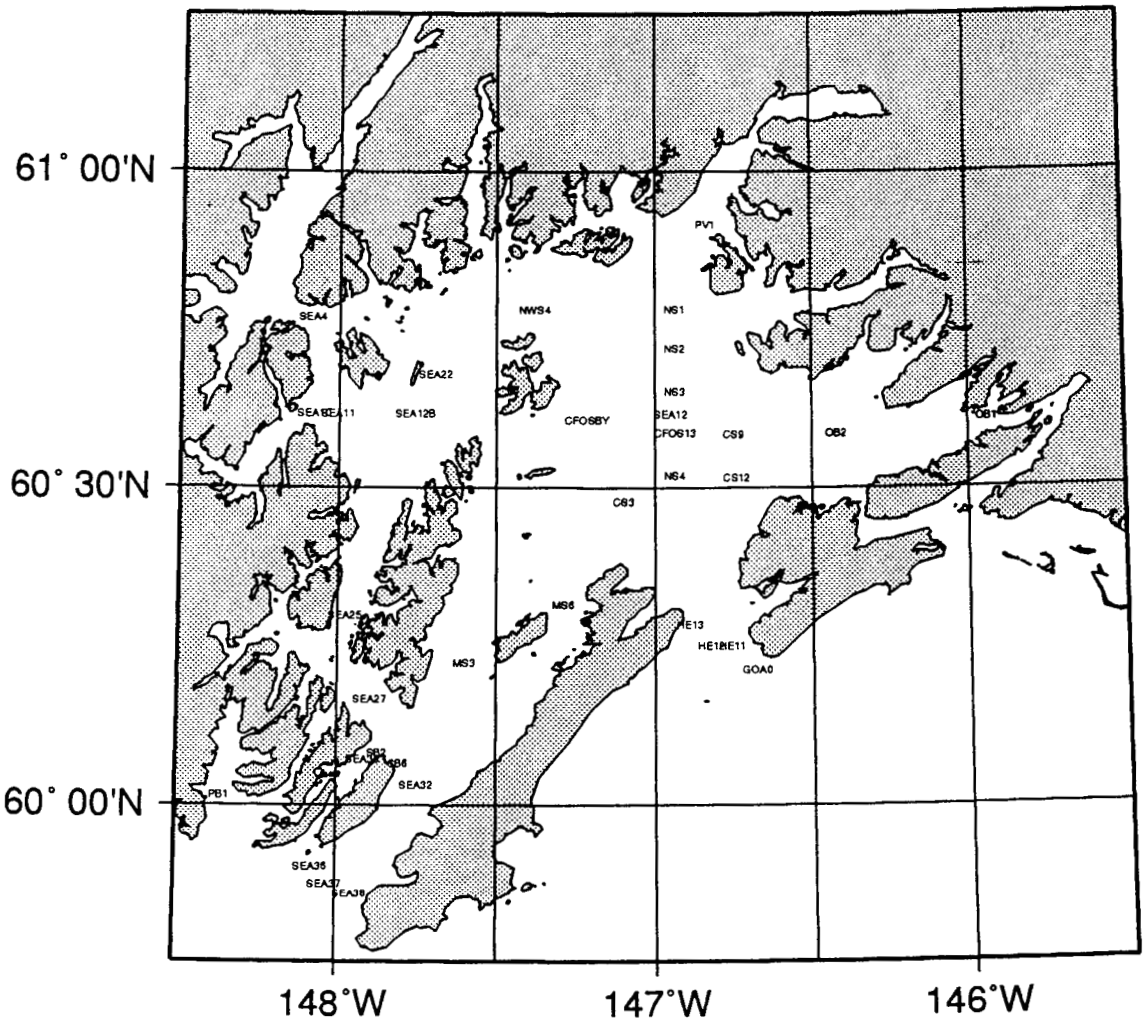

Figure: 2

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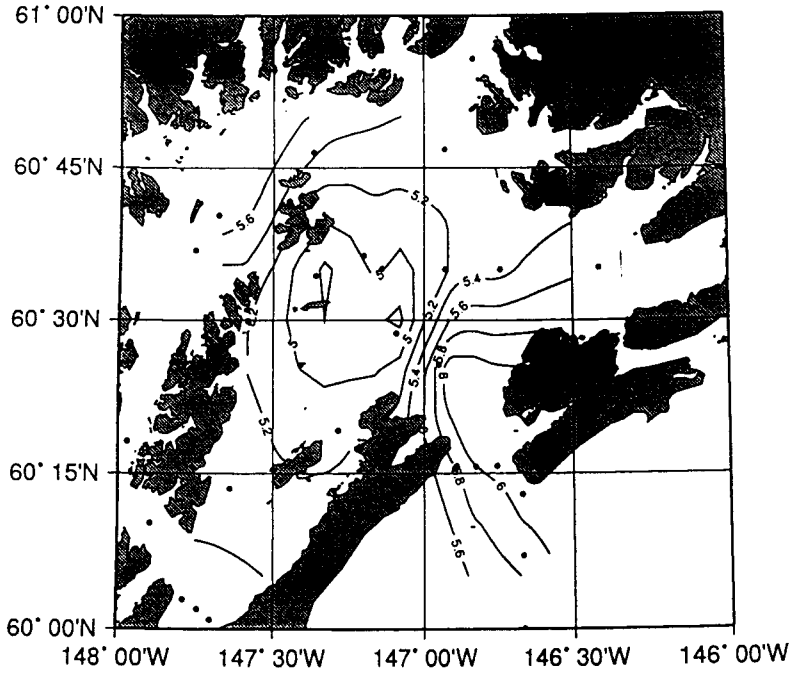

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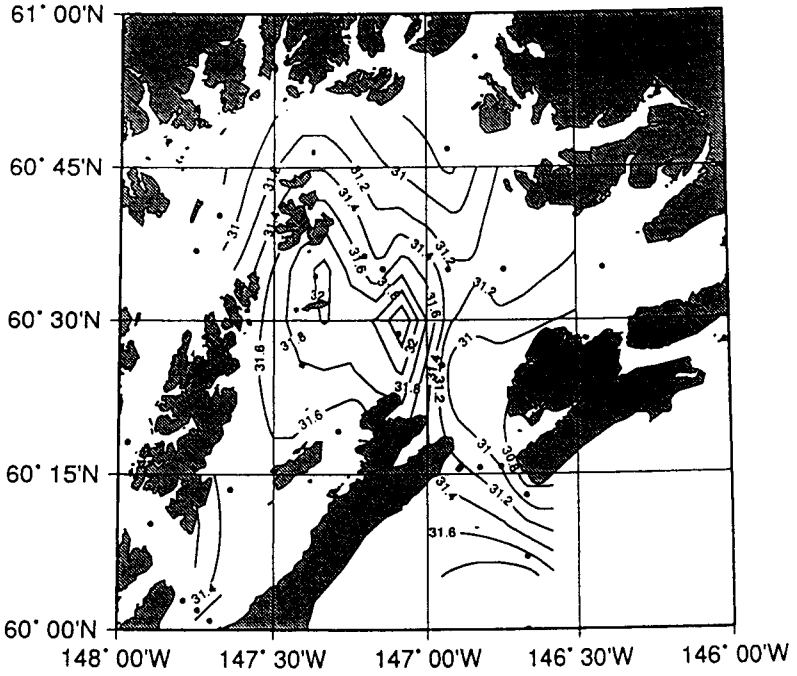

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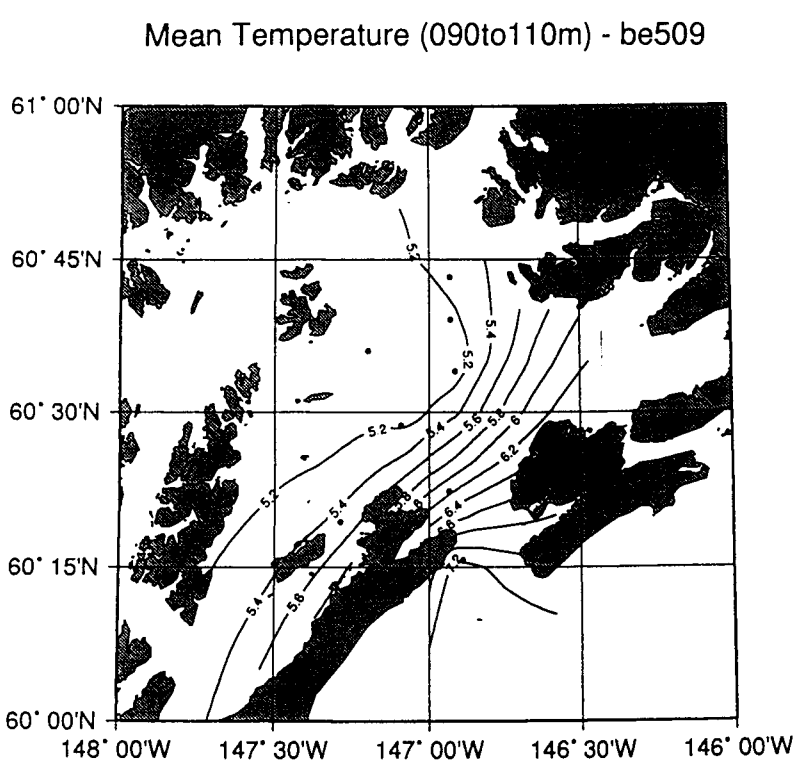
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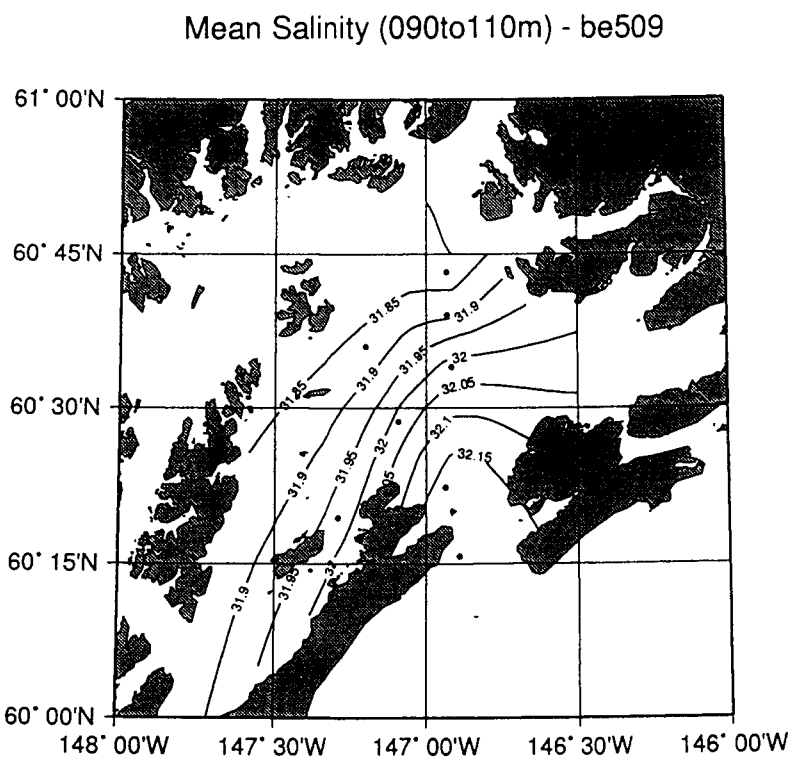
147° 00'W
146° 30'W
146° 00'W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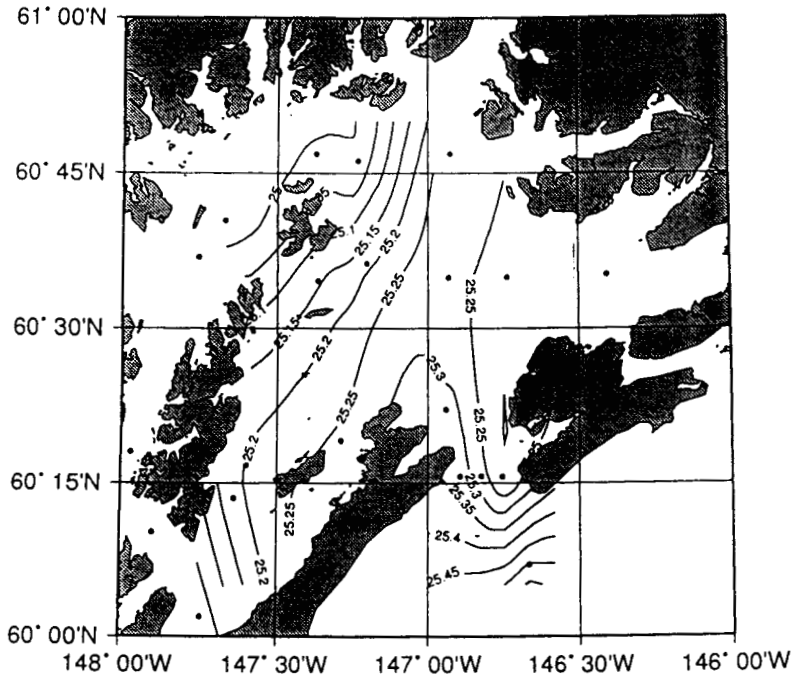
60° 30'N
60° 15'N
60° 00'N
148° 00'W
147° 30'W
147° 00'W
146° 30'W
146° 00'W

Figure: 4

(a)

Mean Pot. Density (000to100m) - be503



(b)

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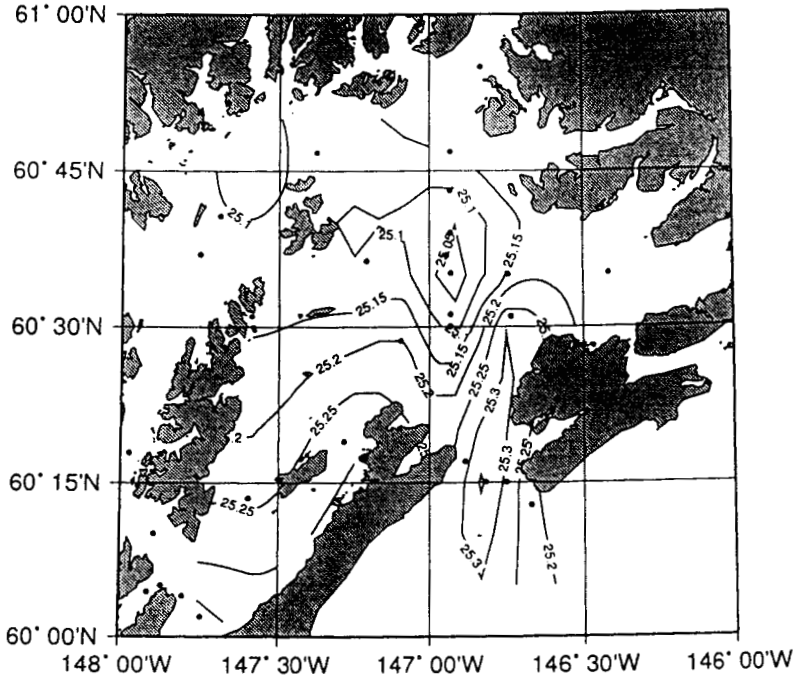
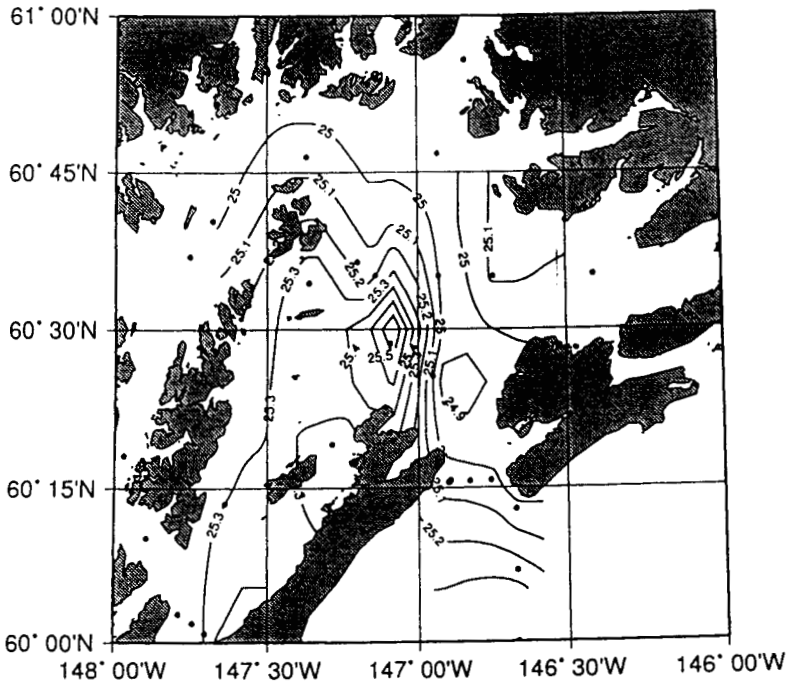


(c)

Mean Pot. Density (000to100m) - be505



(d)

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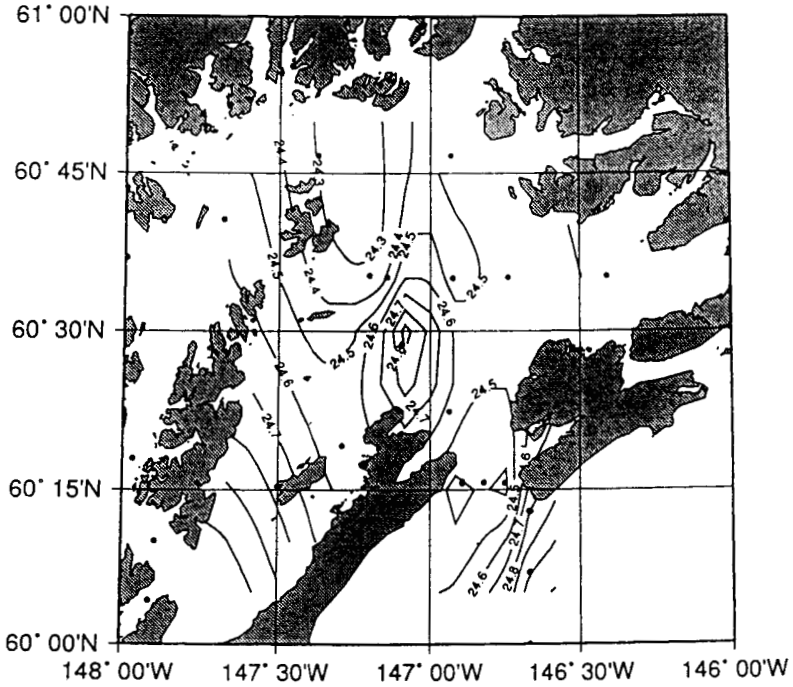

(e)

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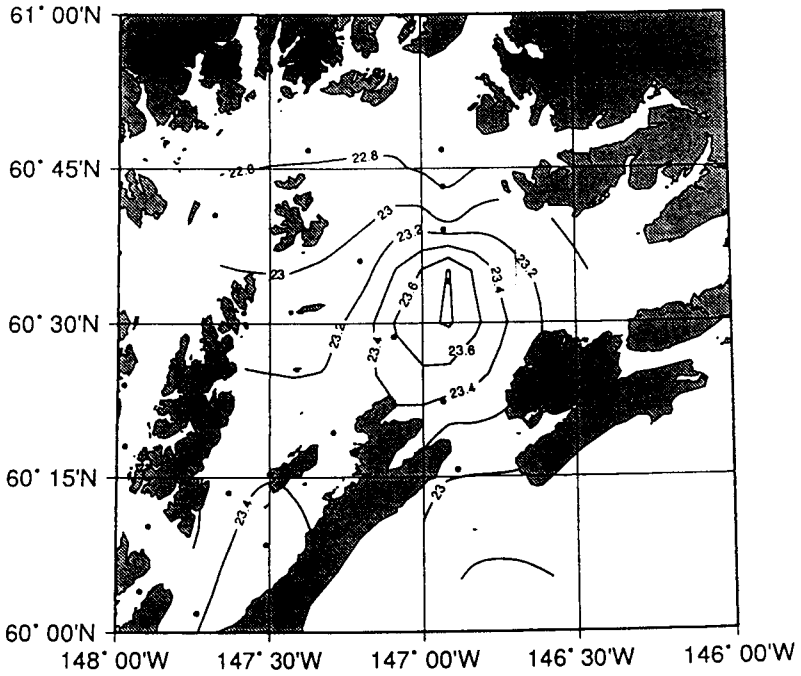

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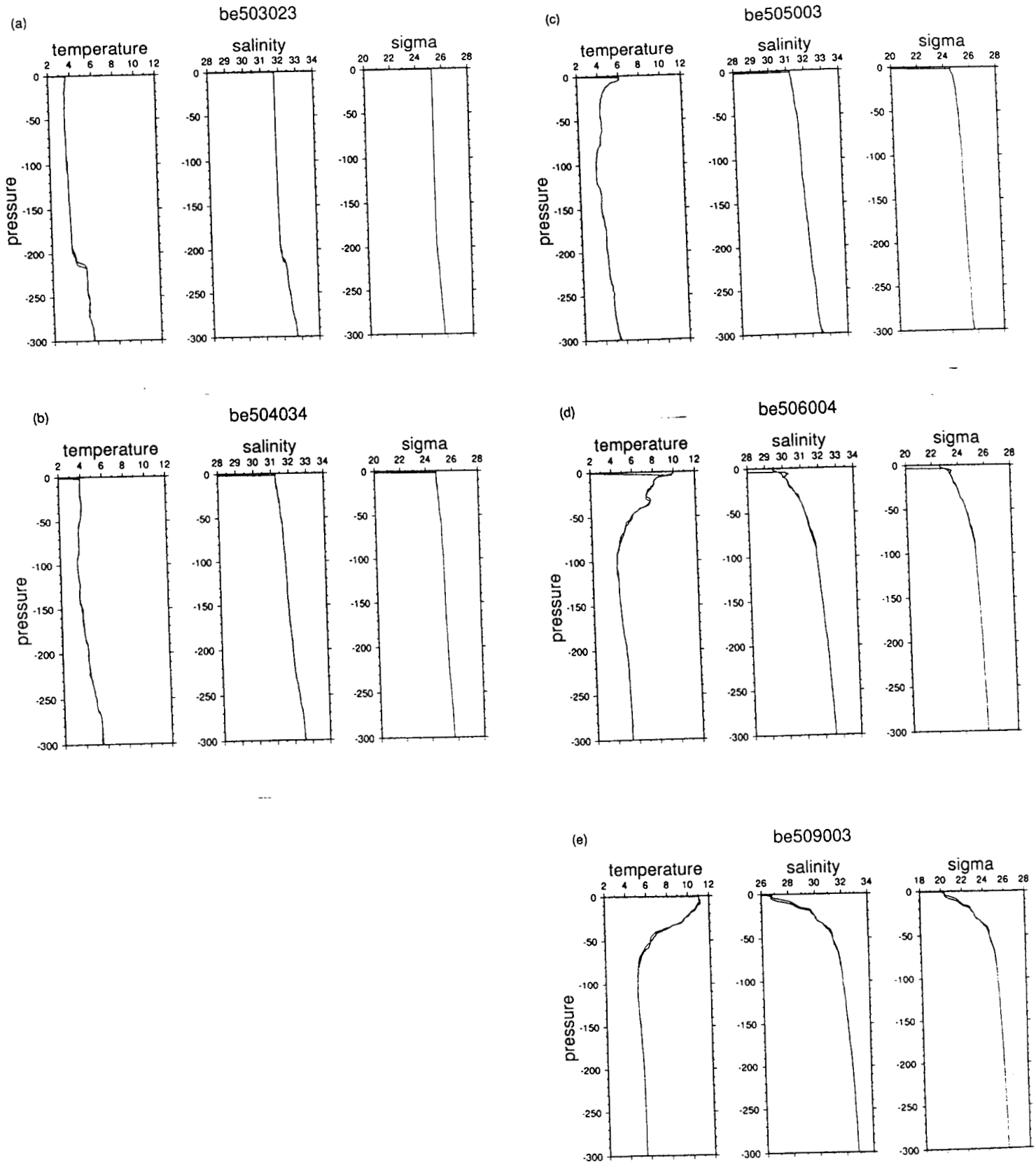
(a)
be503023
temperature
salinity
sigma
pressure
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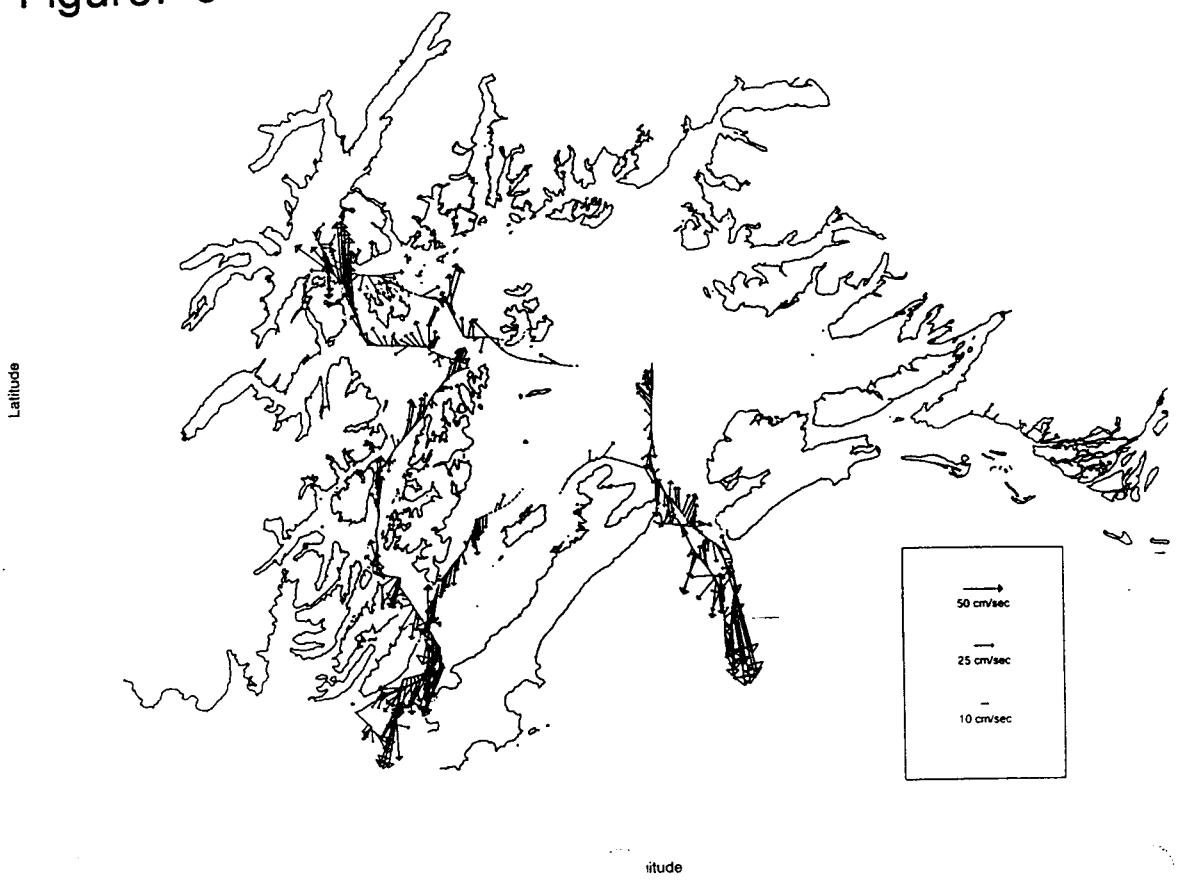
temperature
salinity
sigma
pressure
(c)
be505003
temperature
salinity
sigma
pressure
(d)
be506004
temperature
salinity
sigma
pressure
(e)
be509003
temperature
salinity
sigma
pressure

Figure: 6

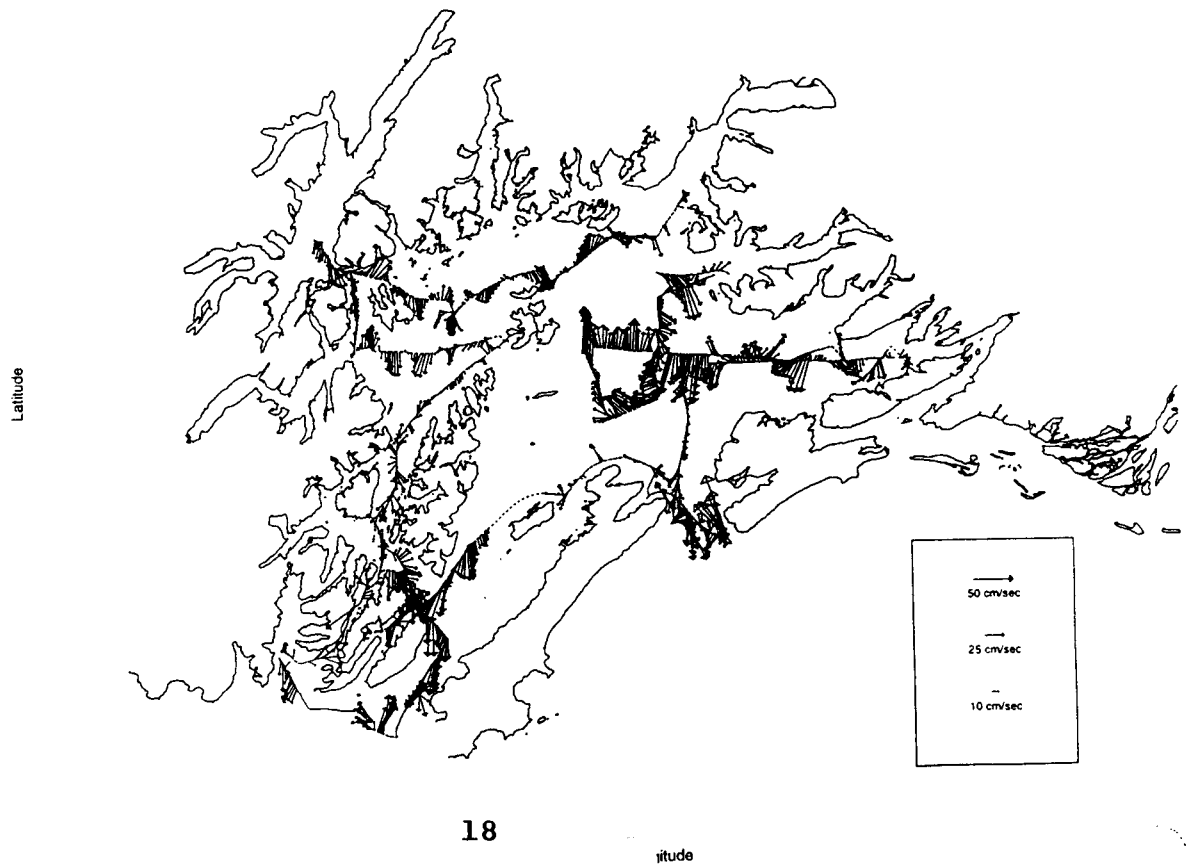
March 1995 ADCP Data
20/100 meters

(a)



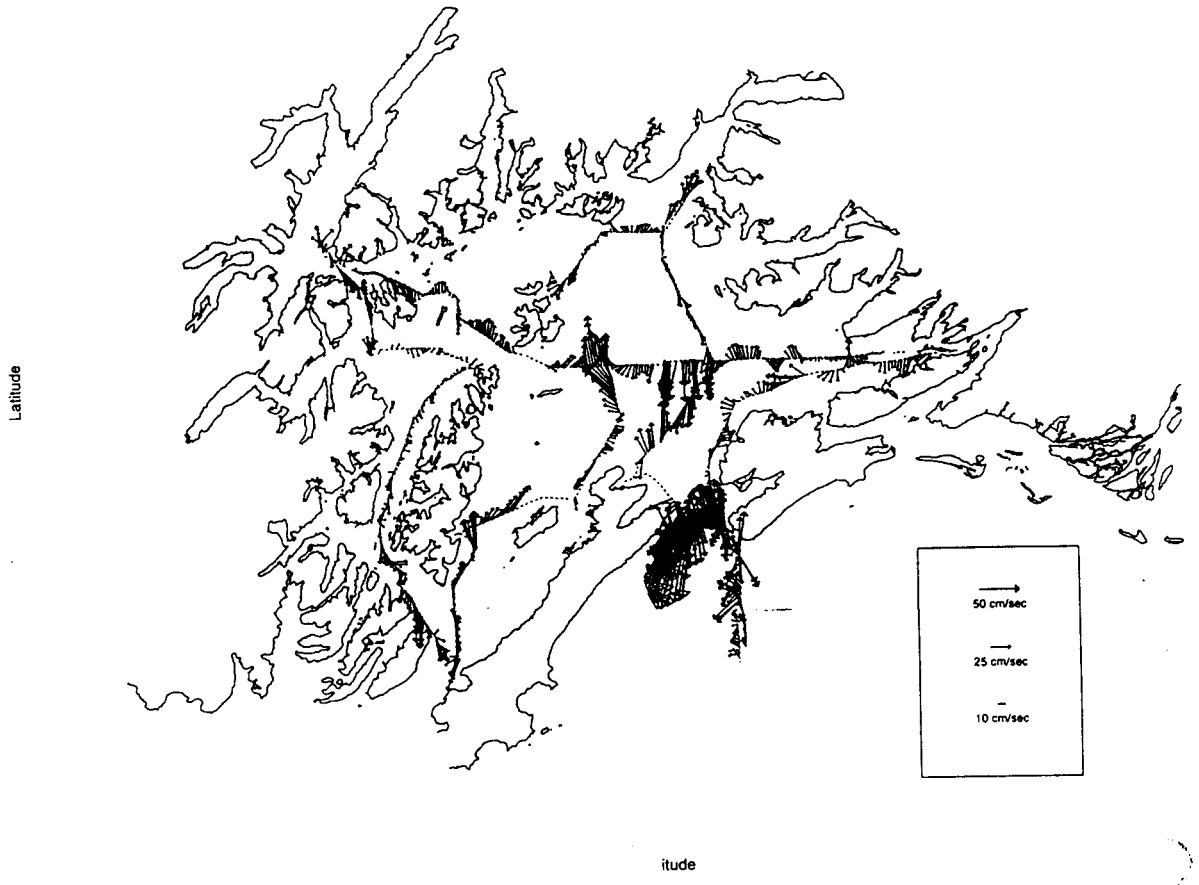
April 1995 ADCP Data
20/100 meters

(b)



May 1995 ADCP Data
20/100 meters

(c)



June 1995 ADCP Data
20/100 meters

(d)

September 1995 ADCP Data
20/100 meters

(e)

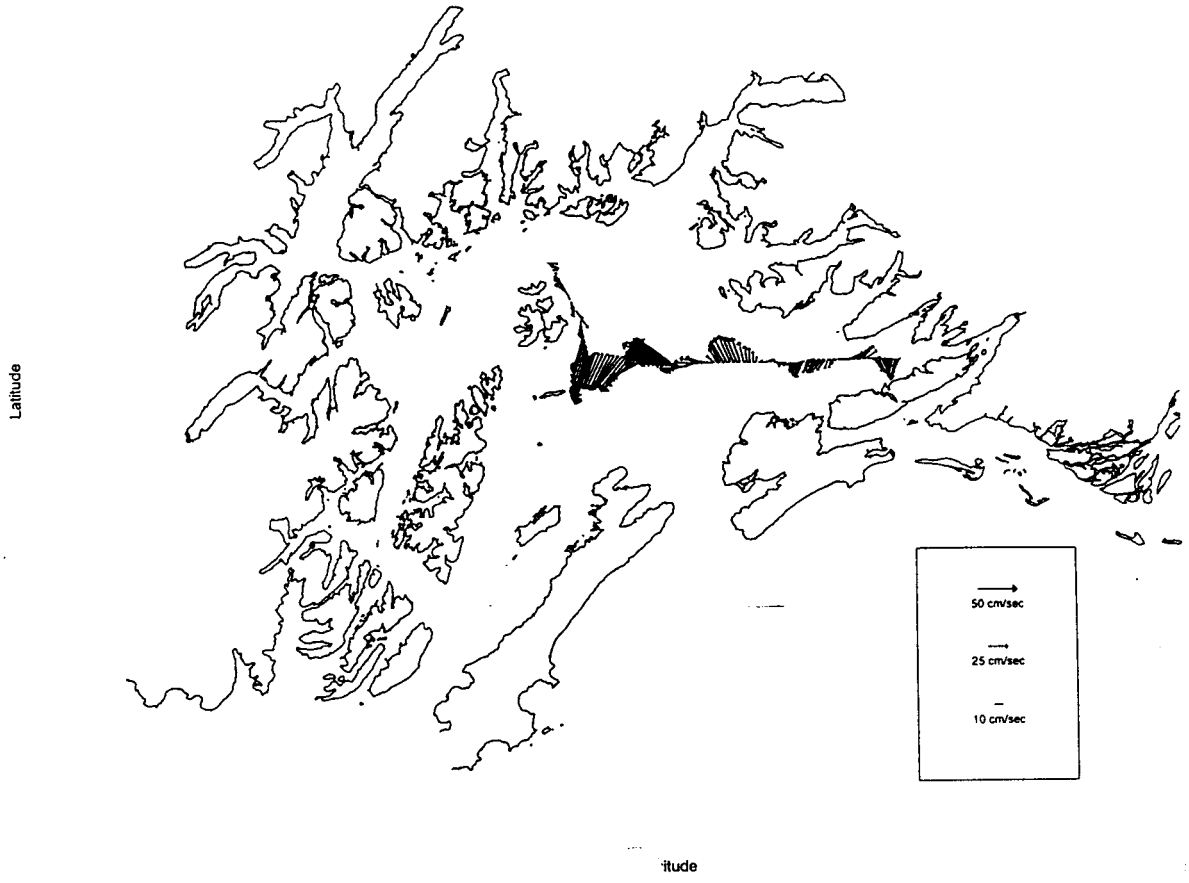
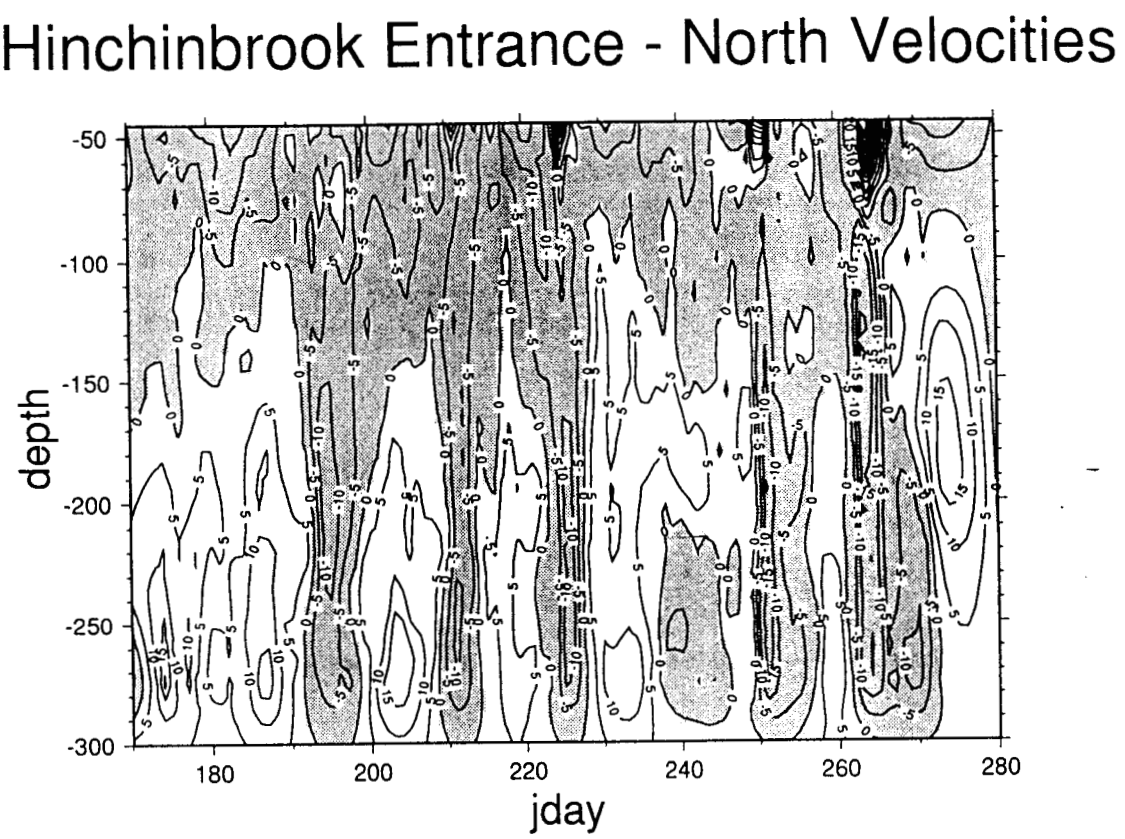


Figure: 7

(a)


Hinchinbrook Entrance - North Velocities
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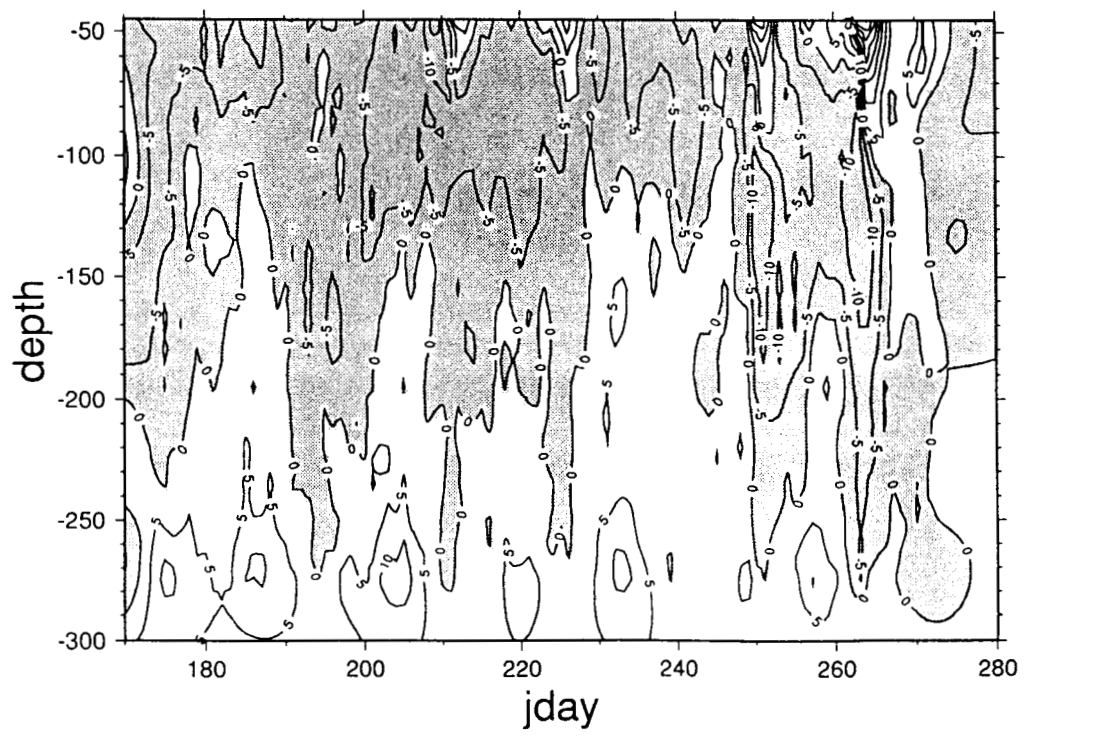
-50
-100
-150
-200
-250
-300
180
200
220
240
260
280
jday

(b)


Hinchinbrook Entrance - East Velocities
depth
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-100
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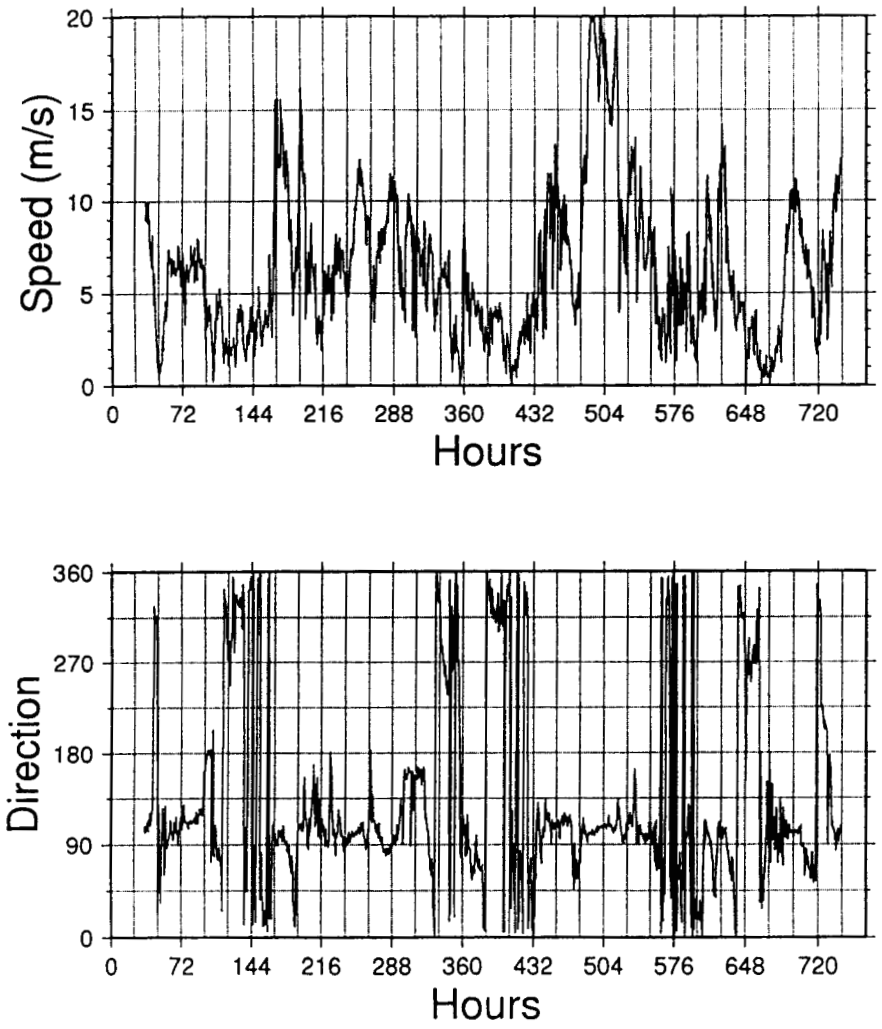
-300
180
200
220
240
260
280
jday

Figure: 8

(a)


Mid-Sound Wind - September
Speed (m/s)
0
5
10
15
20
0 72 144 216 288 360 432 504 576 648 720
Hours
Direction
0
90
180
270
360
0 72 144 216 288 360 432 504 576 648 720
Hours

(b)

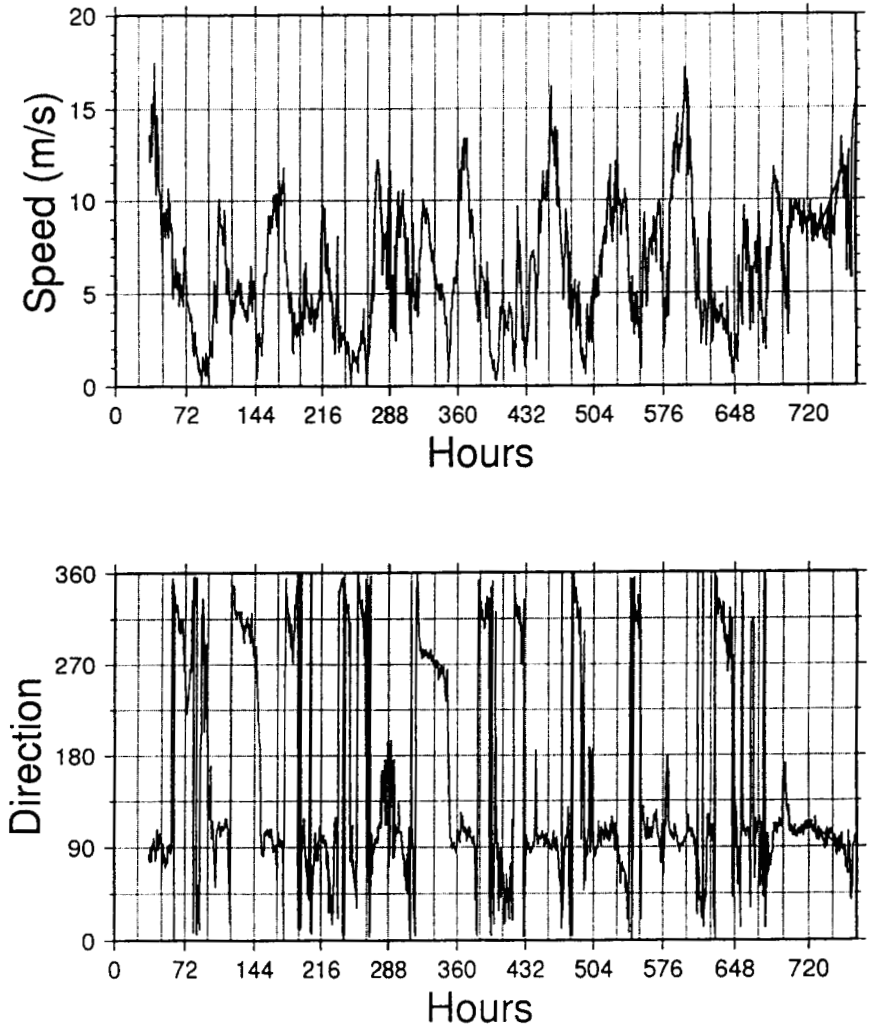
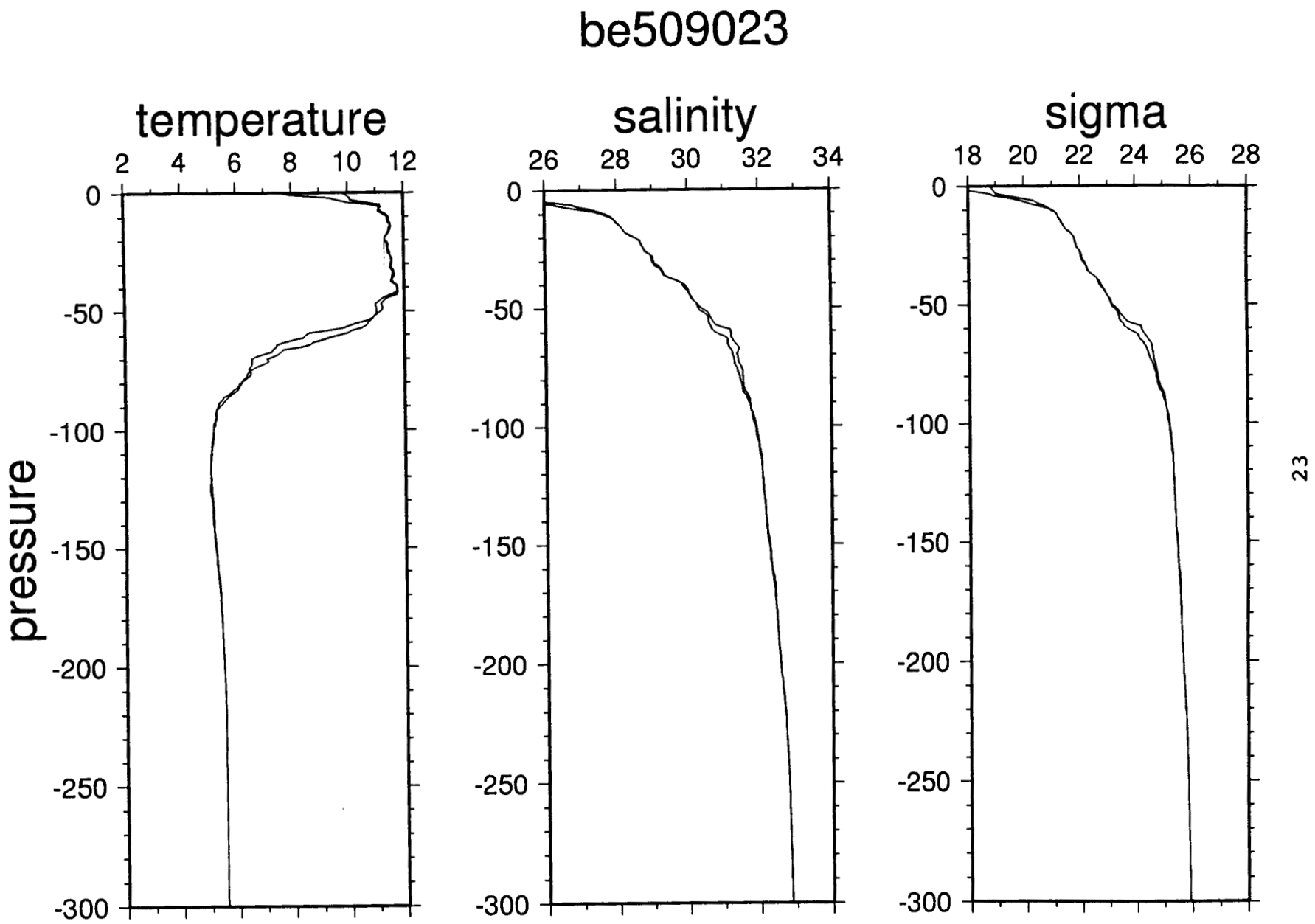

Mid-Sound Wind - October
Speed (m/s)
0
5
10
15
20
0 72 144 216 288 360 432 504 576 648 720
Hours
Direction
0
90
180
270
360
0 72 144 216 288 360 432 504 576 648 720
Hours

Figure: 9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salinity
sigma
pressure
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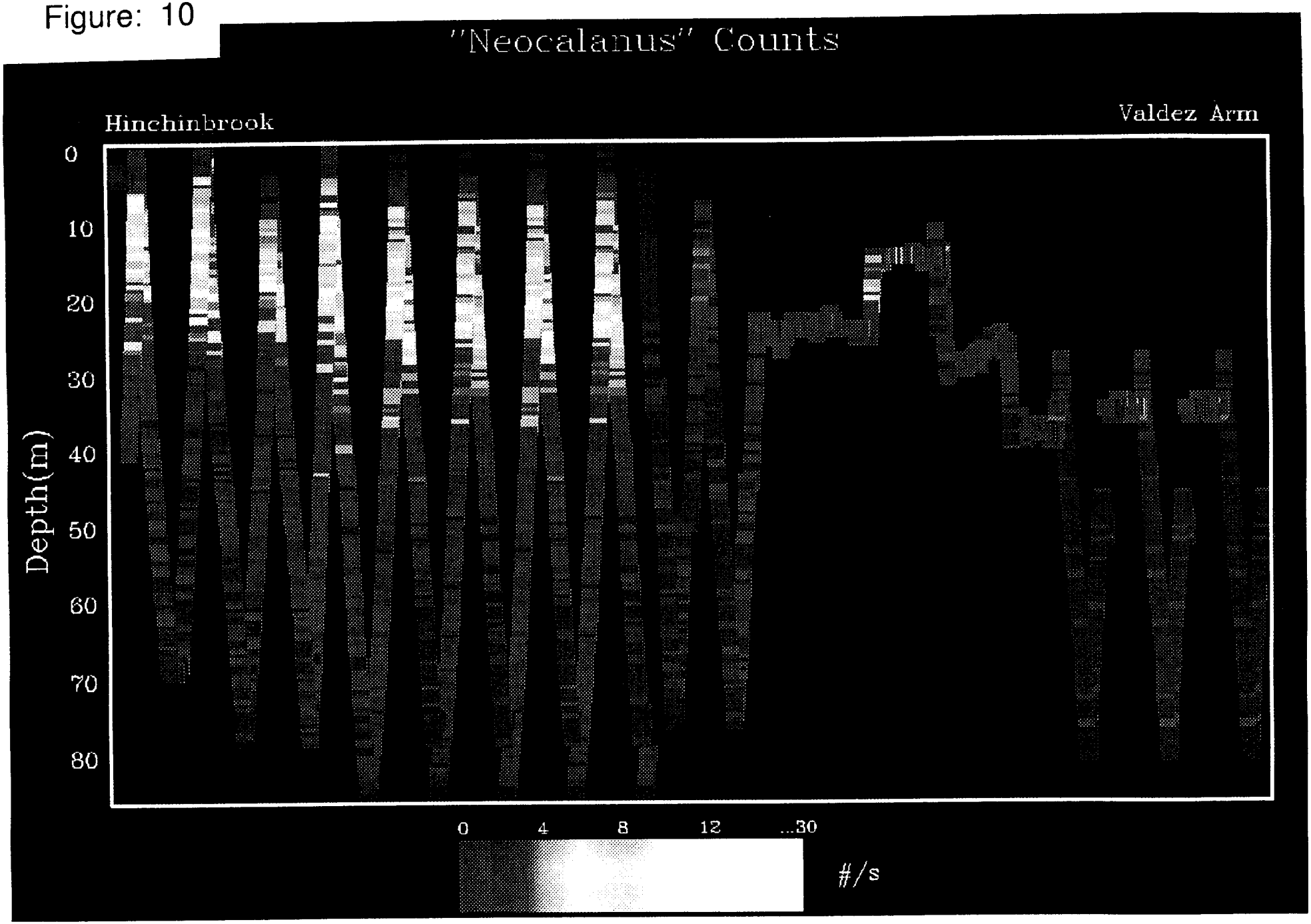
-250
-300

Figure: 10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Valdez Arm
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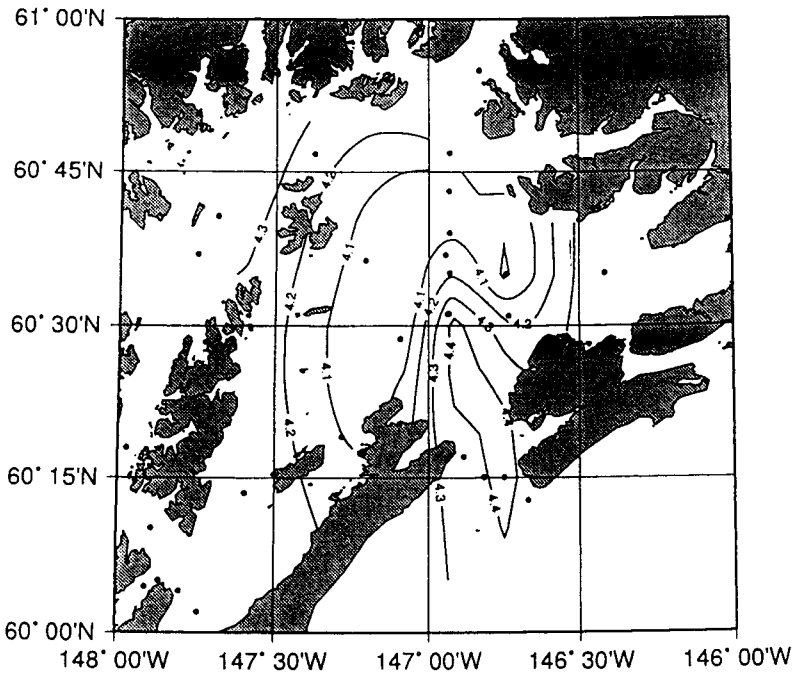
10
20
30
40
50
60
70
80
0
4
8
12
...30
#/s

Figure: 11

Mean Temperature (000to020m) - be504



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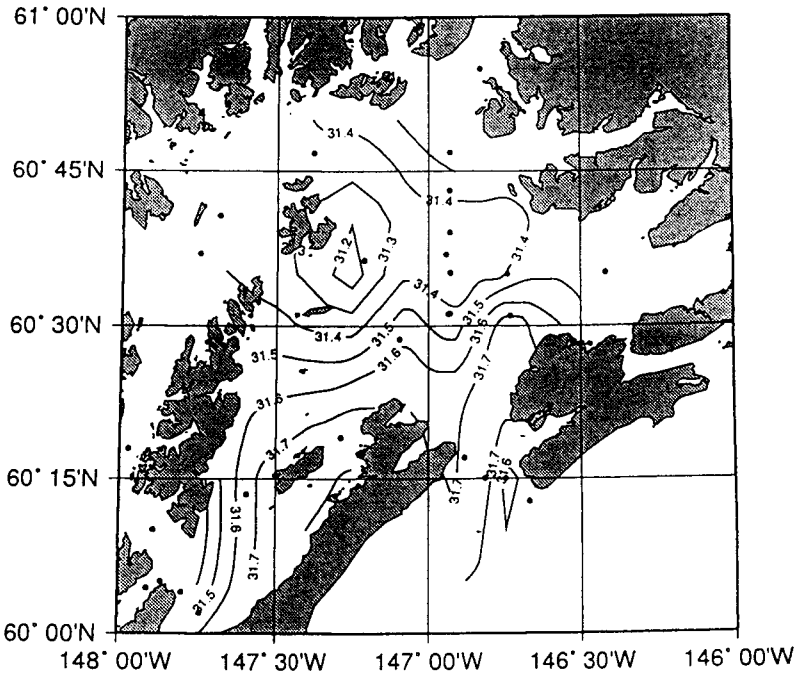

Exxon Valdez Oil Spill
Restoration Project Annual Report
Nekton-Plankton Acoustics Project 95320N

Nekton-Plankton Acoustics

Restoration Project 95320N
Annual Report

This annual report has been prepared for peer review as part of the *Exxon Valdez* Oil Spill Trustee Council restoration program for the purpose of assessing project progress. Peer review comments have not been addressed in this annual report.

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April 28, 1996

Nekton-Plankton Acoustics (SEAFISH)

Restoration Project 95320N
Annual Report

Study History: The small runs of Prince William Sound pink salmon in 1992 and 1993, and the collapse of the herring population in 1993, prompted the EVOS Trustee Council to initiate the ecosystem-level studies to improve existing predictive tools. In 1993, the Sound Ecosystem Assessment science plan was developed using the GLOBEC program as a guide. Funding of research began in the spring of 1994. The Nekton-Plankton Acoustics project (SEAFISH) is evaluating and applying acoustic measurement technology to collect information on fish and macrozooplankton distribution and abundance.

This is the second annual report for the Nekton-Plankton Acoustic project. Three technical reports and four abstracts have been published to date, and the chapters in this report are being prepared for submission to journals this year. The Sound Ecosystem Assessment program was recommended to be a 8-10 program. Funding for the third year is in place and preliminary budgets have been projected through FY98 (five years).

Abstract: The primary contribution of the Nekton-Plankton Acoustics project is to estimate animal abundance and distribution information for testing of the river-lake and prey-switching hypotheses and the development of predictive numerical models. The results are spilt between preliminary and completed products. The preliminary products are the estimates of nekton predators and macrozooplankton prey along the outmigration corridor for the pink salmon and the fall and winter density and distribution of the juvenile herring population. The completed products are the stock assessments of adult pollock biomass in Feb-Mar 1995 (37 thousand mt), and adult herring biomass in Oct-Nov 1993-94, April 1995, Oct-Nov 1995, Mar and April 1996 (20, 13, 13, 24, 23 thousand mt).

Key Words: *Clupea harengus*, *EXXON VALDEZ*, hydroacoustics, macrozooplankton assessment, salmon fry predators, *Oncorhynchus gorbuscha*, Pacific herring, pink salmon, population trends, stock assessment, *Theraga chalcogramma*, walleye pollock.

Citation: Thomas, G. L., Jay Kirsch and T. McLain. 1996. SEA: Nekton-plankton acoustics second annual report, 1996. Restoration Project 95320N. *EXXON VALDEZ* Trustee Council. Anchorage, Alaska. 120 pp.

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EXECUTIVE SUMMARY

The Nekton-Plankton Acoustics Project (96320-N) is evaluating and applying acoustic measurement technology to collect accurate information on the distribution, density and size of specific animal populations. These data are essential for the development and operation of numerical models to improve the prediction of animal population change and the testing of the river-lake and prey switching hypotheses under the Sound Ecosystem Assessment Program (SEA). Improving the prediction of animal population change is a prerequisite for accurate assessment of anthropogenic influences and restoration from damage.

This is a multi-tasked project that relies on: (1) cooperative model development to assist in sampling design, data analysis, and interpretation, (2) shared vessel and facilities for data collection and logistical support, (3) data sharing with the agency, university, public and commercial interests, and (4) remote sensing with acoustical and optical technologies. We use the existing knowledge and skills of commercial fishers in the design and implementation of surveys. Salmon hatcheries in the region provide support for field crews and the hatchery releases of pink salmon are treated as an experimental manipulation of the marine ecosystem. Because of the multi-tasking nature of this project, we have relied on partnerships with other funding sources to accomplish tasks to fill in some of the gaps between SEA projects.

This annual report includes: (1) near-field predation studies in Sawmill Bay, (2) far-field predator-prey studies with an emphasis on northwestern Prince William Sound, (3) winter assessments of walleye pollock biomass, and (4) fall, winter and spring assessments of Pacific herring biomass. Predator and prey acoustic assessments are major components of the pink salmon investigations and incremental stock assessments are a primary part of the herring research.

Pink Salmon: We have partitioned the predation of pink salmon fry into near- and far-field and near- and offshore events. The primary focus of this report is on far-field and offshore predation.

Nearfield: In 1992, we measured a gradient of fish density that increased towards the AFK hatchery net pens in Sawmill Bay, southwest Prince William Sound (PWS). These fish were aggregated in large schools at the bottom of the water column during the day and in a diffuse surface (0-20 m) layer at night. They were identified as juvenile gadids and herring. In spring 1993, we made repeated measurements of juvenile pink salmon schools after their release into Sawmill Bay. The pink salmon fry target strength mode was at -55dB and near-surface schools of fry were easily detected using a 200 kHz side-scan sonar. Also in 1993, larger fish targets considered potential fry predators were observed at higher densities in the vicinity of the pink salmon releases.

These observations in the southwest Sound have not been observed in our northwest Sound study area (Lake Bay). The northwest Sound was selected as the primary study area for the pink salmon predation because of the annual release of over 500 million salmon fry from the Arim Koernig hatchery in Lake Bay. Also, the predation events in Sawmill Bay appear to be a late-season event by juvenile and subadult pollock which are more abundant in the southwest Sound.

Far-field, offshore: In 1994 -95, our acoustic-midwater trawl surveys described adult walleye pollock as the dominant offshore predator and competitor with salmon fry along the outmigration route in western PWS. Adult salmon and pollock were the dominant nearshore fry predators. Plankton densities were highest in the offshore sampling areas. The high concentrations of plankton found offshore overlapped with the vertical distribution of pollock at the surface. This made the use of echointegration to estimate pollock density inaccurate. In 1995, we used a counting technique which provides accurate relative densities of walleye pollock numbers along acoustic transects run in 1994 and 1995. We assumed pollock target strength is length dependent: TS = 20 log L -66 dB.

Our preliminary estimates of walleye pollock biomass in the Wells-Perry Island passages in May-June to be 160,722+/- 29,798 kg in 1994 and 85,484 +/- 5,619 kg in 1995. In 1994, the Wells-Perry Island estimates were 173,327+/-32,422 in May and 148,117 +/- 27,146 in June. This represented around 15% of the offshore pollock in the western Sound study area. In 1995, the Wells-Perry Island estimates were 26,437 +/- 3556 kg in May and 144,117 kg in June. This represented approximately 20% of the offshore pollock in the western Sound study area. There appeared to be a northward movement of pollock in the Sound between May and June of 1995. We have evidence to suggest that the counting procedure underestimates of pollock numbers because of the presence of multiple targets, the small sample unit volume near the surface and boat avoidance. Currently investigations are being conducted to evaluate and correct for bias.

The offshore population of pollock in the northwest Sound is primarily comprised of adult fish averaging 480 mm in length in the spring of 1994 and 515 mm in 1995. These adult pollock are found primarily in the top 40 meters of the water column where they co-occur with macrozooplankton prey. In general, smaller pollock ranging from 150 to 400 mm were observed in the southwest Sound. In 1995, we found the adult pollock distribution around the Sound to be highly patchy and mobile. In winter 1995, we measured 37+/- 7 thousand metric tons of

prespawning pollock in the Knight Island and Port Bainbridge areas of the southwest Sound. It is likely that the prespawning biomass measured on the winter survey had migrated from both the Gulf of Alaska and Prince William Sound.

The winter stock assessment of prespawning pollock in the lower Knight Island passage area in 1995 was 10,587+/- 2,193 metric tons. Springtime acoustic surveys in 1995 suggested that the biomass in the western outmigration corridor was about 1,000 metric tons. It is likely that the 10,000 metric tons of pollock seen at Knight Island in the winter was migrating to Port Bainbridge and had originated from throughout Prince William Sound. By collecting this information we have independent support that the spring pollock numbers may be underestimated. This is important because the initial estimates of predation using the springtime pollock numbers suggest that less than ten percent of the fry are eaten by pollock.

Macrozooplankton: We have made preliminary estimates of the relative abundance and distribution of macrozooplankton layers in the top 50 meters of the water column in 1994 and 1995. Initial examination of the acoustic measurements show 0.1-10.0 kilometer horizontal patchiness in the surface plankton layers and 3-4 orders of magnitude variability in density. Net catches suggest the species composition of the layers to be over 90-99% calanoid copepods early in the season. Pteropods, which have a significantly higher TS than copepods, were not found at the surface in high numbers until later in the season. The data also suggested that high densities of pollock often co-occurred with high density patches of macrozooplankton prey.

In 1994-95, we discovered that the adult walleye pollock population in the Sound was targeting the spring macrozooplankton bloom. Measurement of the density, size, and distribution of macrozooplankton patches may be a key aspect of the Sound's capacity for fish production since both juveniles and adults are dependent upon it. We suspect that extreme tidal conditions can disrupt the plankton patchiness and as a consequence predators such as the pollock move down in the water column and feed on other "more" available organisms. This mechanism would support both the river-lake and the prey-switching mechanisms since the physics affects the macrozooplankton prey availability (a patch response hypothesis), which then affects the feeding of predators (a patch dependence hypothesis). Preliminary analysis suggests the role of turbulence may be more important as a mechanism than flushing rate for testing the river-lake and prey-switching hypotheses.

Pacific herring: The adult herring schools observed in the fall-spring surveys between 1993-96 maintain a unique high density and a distinct vertical distribution at night. Juvenile herring mix with juvenile pollock and form a relatively predictable surface layer at night. Echointegration-seine estimates of adult herring biomass in fall 1993, fall 1994, spring 1995, fall 1995 and spring 1996 were 20, 13, 13, 24 and 23 thousand metric tons. The distribution of adults in the spring and fall have been consistent with the primary concentration of fish found around Green Island in the fall and around Zaikof Bay in the spring. When the fish population was sedentary, repeated surveys indicate a precision level of +/- 20%. The fall and winter-spring estimates of herring biomass (13/13 in 1994/5 and 24/23 in 1995/6) indicated low over-winter mortality for adult

herring and was excellent repeatability of the stock assessment. We assume that target strength is length dependent: TS = 20 Log L - 71.9.

Seasonal changes in the abundance and distribution of adult herring were observed from 1993 to 1996. Adult herring have been seen to migrate from the Gulf into the Green Island region of the Sound by mid- October. By late winter they move into the Zaikof and Rocky Bays at the east end of Montague Island where they remain until mid-April. In mid-April they appear to spread out along the beaches to spawn. Juvenile herring have been seen in the same areas as adults in the fall, but perhaps not in the same proportion. Juvenile herring appear to be more inshore and nearer to the surface than the adults.

Multi-species management and restoration: SEA has shown that the pink salmon, herring and walleye pollock populations are dominant competitors and/or predators in the Sound. Since the EVOS Trustee Council is a unique entity in the fact that it represents the agencies that are responsible for establishing harvest strategies for pink salmon, Pacific herring and walleye pollock management, the continued investment in monitoring these populations creates an opportunity to evaluate the use of multi-species harvest strategies to assist the restoration of damaged species. The key to making multi-species management decisions is having reliable estimates of the abundance of each species and knowledge of how they interact. The opportunity to evaluate a multiple-species approach to fisheries management in the Prince William Sound is unique and could be a major contribution to fisheries science by the EVOS Trustee Council.

CHAPTER 1

Near-field pink salmon predation: Predator co-occurrences with pink salmon fry in Sawmill Bay, Prince William Sound, Alaska, 1992-93. G.L. Thomas and J. Kirsch. Note: This paper is not to be cited without permission from the first author

ABSTRACT

We made acoustic observations of pink salmon fry predators near a salmon hatchery in Sawmill Bay, Prince William Sound. Shortly after the fry-release season in 1992, we observed an increasing gradient in fish density in Sawmill Bay as we approached the hatchery. Early in the fry-release season of 1993, we made acoustic measurements of larger fish in the water column after fry were released in the central Bay but large fish densities were relatively low and no gradient was apparent. Sample fishing showed the assemblage of fish in the Bay was dominated by adult Pacific herring *Clupea pallasi*, juvenile walleye pollock *Theragra chalcogramma*, and tomcod *Microgaduus proximus*. The average length of pacific herring was 232 mm (n=36), walleye pollock 270 mm (n=57) and tomcod 241 mm (n=17). No pink salmon fry were found in the herring stomachs, but the juvenile gadids had an average of 6.2 fry per stomach (n=64). One tomcod had 82 pink salmon fry in its stomach. Future sampling to document the magnitude of pink salmon fry predation by the juvenile gadids needs to be either continuous or late in the fry release season.

INTRODUCTION

From 1989-93, the Prince William Sound Aquaculture Corporation (PWSAC) released an average of 431 million pink salmon, *Oncorhynchus gorbuscha*, smolts annually into the nearshore marine waters of Prince William Sound (Thomas and Mathisen 1993). From 1990-1994, these releases produced runs of 44, 23, 14, 11, and 27 million adults (ADF&G recorders). This variability in marine survival of pink salmon is of great concern to hatchery and fisheries management in the Sound.

The major source of mortality for larval and juvenile fish in the marine environment is assumed to be predation (GLOBEC 1991; SEA 1993). It is generally accepted that decreased growth of pink salmon in the nearshore marine environment results in higher mortality because it prolongs the period that the fish are highly vulnerable to predation (Heard 1991; Parker 1962, 1964, 1965, 1968, 1971; Mortensen et al. 1991). Several researchers have shown the number of adult pink salmon that return to be affected by mortality during the early marine period (Parker 1968; Ricker 1976; Bax 1983).

In Prince William Sound, the processes of migration, growth and survival begin anew each spring for hatchery pink salmon when the fry are released from hatchery net pens. Although we understand some of the processes that determine the ultimate strength of the pink salmon return, our predictions of returning adults are poor because of the absence of adequate predictive tools

(GLOBEC 1988) and a general weakness in monitoring processes in the marine environment. Despite such weakness, researchers have shown predators are attracted to hatchery release sites to feed (Bayer 1986; Collis et al. 1995). Thus, PWSAC hatcheries have manipulated the time and location of the release of fry in an attempt to minimize the near-field mortality of fry from predation.

Past PWSAC release strategies primarily involved manipulating time of fry release to promote growth as a method to reduce predation: (1) with high zooplankton densities to promote fast growth, (2) when fry are large (the late spring releases) and (3) during times of the day and at places where near-field fry predators are fewest (such as at night to avoid bird predation). Since we assume that predation is the principal source of fry mortality and that survival is size dependent, fast growth or the release of large fry should minimize predation, and therefore maximize survival.

Historical observations suggest that fry releases at night, during high spring zooplankton densities had high survival. Recently, such releases have been met with mixed results. Although these past release strategies are believed to affect processes throughout the life cycle of pink salmon, there has recently been an increased emphasis to reduce predation near the hatcheries shortly after fry release. Observations of high fish predator abundance in the vicinity of the release site (net pens) and plankton/predator densities along the outmigration route are causal mechanisms that have been advanced to explain the recent observations of lower or inconsistent survival of fry.

Objectives

In spring of 1992 and 1993, we conducted surveys to evaluate near-field predation on pink salmon fry from Armin F. Koering (AFK) hatchery in Sawmill Bay. Specifically, the present study was conducted to document: (1) the presence of salmon fry predators near the Arim F. Koernig hatchery in Sawmill Bill and (2) to monitor predator response to fry releases.

METHODS

Data acquisition

Hydroacoustic surveys were conducted in 1992 and 1993 in Sawmill Bay, Prince William Sound to evaluate fish predation on salmon fry. In 1992, a reconnaissance survey was conducted after the fry release season in Sawmill Bay to determine the relative fish density and distribution. In 1993, a hydroacoustic survey was conducted in Sawmill Bay (AFK hatchery) during fry releases to collect quantitative measurements of fish density and target strength for fish sizing.

Six sonar surveys were conducted between May 22 and 25, 1992 in Sawmill Bay after the fry release season. These surveys were designed to describe the distribution of fish relative to the hatchery net pens. The surveys were conducted to cover morning, midday, evening, night, low tide and high tide conditions. In 1992, a 70 kHz Simrad EY-M scientific echosounder was used

to measure fish density.

Twelve acoustic surveys were conducted between April 24 and May 6, 1993 in Sawmill Bay early in the fry release season. These surveys were conducted before and after fry released to measure predator response. In 1993, BioSonics 120 and 200 kHz, models 101 and 102, dual-beam echo sounders, BioSonics Echo Signal Processor (ESP), Sony DAT and DVT recorders, BioSonics model 171 tape interface, BioSonics model 111 thermal chart recorder and a portable oscilloscope were used to estimate fish density and target strength. Transducers were mounted in a V-fin and towed at a rate of approximately 7 knots and at a depth of approximately 1 meter. Data collection, real time processing and georeferencing was conducted and recorded with a 486-66, Compac portable computer, a Magellan GPS and BIOMAP/ESP software. Equipment performance was monitored in the field with oscilloscope and chart recorder.

In 1992, predator identification was limited to hook and line and visual observations. In 1993, fish were captured with a variety of gear types that included horizontal variable mesh gill nets, a commercial herring purse seine, long line and by angling. Information on fish length, weight, and stomach contents was derived from captured fish from all gear types. All fish were identified, weighed, measured and examined for stomach contents. For the purposes of this study only pink salmon fry diet items are reported.

Data analysis

In 1992, fish density and distribution was recorded on echograms. In 1993, densities, distribution and target strength of pink salmon fry and potential predators were determined from voltages obtained from the BioSonics ESP software. Acoustic data were post processed using programs coded in IDL (Interactive Data Language) on a Unix workstation. Sample fishing catch data, target classification data on the paper echograms and target strength data on the target echograms were used to identify fish targets to species.

RESULTS

1992 Sawmill Bay Survey

A series of 38 acoustic transects were run perpendicular to the shoreline encircling Sawmill Bay. Day and night surveys were conducted. Refer to Figure 1 for subsample of transects (13, 15, 17, 22, 24, 26, 28, 31, 33) and design.

There was an increase in fish density along the north shoreline as the survey approached the hatchery net pens, and a corresponding decrease in fish density along the south shoreline as the survey left the hatchery net pen site. Figure 2 illustrates the echograms from a subsample of the night-time transects (13, 15, 17, 22, 24, 26, 28, 31, 33). This gradient in density was also present during the daylight transect, however fish were concentrated in schools (Figure 3, transects 24 and 26, day versus night).

Juvenile walleye pollock (about 230 mm) were captured by hook and line around the net pens. Juvenile walleye pollock and large Pacific herring (>220 mm) were observed in the area at the surface during the night survey.

1993 Sawmill Bay Survey

Twelve acoustic surveys were conducted between April 24 and May 6, 1993 in Sawmill Bay, early in the fry release season. Table 1 demonstrates the density of large fish (>-50 dB) in Sawmill Bay for each survey. Figure 4 depicts the target strength histograms for the twelve surveys. Note that on surveys 4271, 4292 and 5032 there is a mode at -55 dB which corresponds to the presence of pink salmon fry released in the center of Sawmill Bay on three occasions, hence the selection criteria of -50 dB for larger fish (potential predators).

Table 1 and Figure 4 correspond in terms of the presence and absence of these large targets. First, note the absence of a mode above -40 dB in surveys 4292, 427h1, 4291, 4293, 5011, 5031, 5032 and 5061 in Figure 4. Large fish were at very low abundance or absent on these surveys (Table 1). Second, note the large modes above -40 dB on surveys 4261, 4271, 4301 and 5021. Large fish were present at moderate to high densities on these surveys. On survey 4271, the mode for the larger target strength is -32 dB, survey 4301 reflects a mode at -40 dB and possibly another at -32 dB, and survey 5021 shows a mode at -40 dB.

Sample fishing with a purse seine, gillnets, long line and hook and line showed that the schooling fish were adult Pacific herring *Clupea pallasi*, juvenile walleye pollock *Theragra chalcogramma*, and tomcod *Microgaduus proximus*. The average length of pacific herring was 232 mm (n=36), walleye pollock 270 mm (n=57) and tomcod 241 mm (n=17).

No pink salmon fry were found in the herring stomachs. In addition, a number of large (600 mm) Pacific cod, *Gadus macrocephalus*, were captured which did not have pink salmon fry in their

stomachs. In contrast, the juvenile gadids had an average of 6.2 fry per stomach (n=64). One tomcod had 82 pink salmon fry in its stomach. The most abundant juvenile gadid, walleye pollock, had an average of 4.9 pink salmon fry per stomach. Other less abundant small fish caught by hook-and-line, such as starry flounder, *Platichthys stellatus*, and a black rockfish, *Sebastes melanops*, also had pink salmon fry in their stomachs. Besides pink fry, the fish stomachs contained a variety of marine invertebrates (Euphasiids, oligocheates, copepods).

DISCUSSION

In 1992, after the pink salmon were released from the Sawmill Bay hatchery, we saw large aggregations of schooling fish (potential predators) that increased in density towards the hatchery. The target strength modes of -40 dB and -25 dB support the fish catch data that there were schools of Pacific herring, juvenile and subadult gadids (pollock and tomcod) and large gadids (Pacific cod) present in the area. Early in the 1993 fry release season, we did not see this aggregation of fish. However, near the end of the fry-release season, hatchery personnel observed predators in high concentrations feeding on fry at the surface immediately after releases (R. Korker and others, AFK Hatchery, personal communication). Similar observations were reported in 1991, 1992 and 1994. It appears that major predation events occur late in the fry release season or are episodic. A continuous monitoring program at the hatchery is needed to document the magnitude of the near-field predation.

In contrast, these major predation events by juvenile gadids are not reported for the Lake Bay hatchery where we have concentrated our research efforts. This could be due to the fact that Lake Bay does not have the amount of rearing habitat preferred by the juvenile gadids at Sawmill Bay. The shoreline slope drops off faster and the average depth is greater for Lake Bay than Sawmill Bay. There are also differences in climate with the northwest Sound having a cooler and later springs (Thomas et al. 1991). Spring, summer and fall surveys have shown that the subadult year classes of pollock are more abundant in the southwest passages than in the northwest study areas (Chapter 2). Thus, the characteristics and magnitude of near-field predation may be significantly different between northwest and southwest areas of the Sound. Scheel and Hough (1996) reported high bird predation events on fry released at the Lake Bay hatchery.

Juvenile gadids were observed and caught within Sawmill Bay on all sampling occasions. The greatest proportion of fish captured with conventional gear (89 %) were 175-350 mm. This size of fish was not seen in abundance in the northwest Sound. However, feeding rates for these smaller fish were a lot higher than for the large (400+mm) pollock seen in the offshore sampling areas in the north. Although the higher numbers of fry eaten by the smaller gadids were probably due to the higher nearfield fry concentrations, it is also likely that the smaller gadids may target salmon fry to a greater extent, especially in the shallower nearshore areas. Because of the sharp dropoff in the Lake Bay area, future nearshore sampling will focus within a 50 m distance from the shoreline to determine a possible concentration of juvenile predators missed by the past nearshore surveys that covered 300 m from the shoreline.

In 1996, the number and size of pink salmon fry schools along the shoreline near Boca de Quadra, southeast Alaska, was estimated using side scan sonar (Marino and Stables 1996). In 1992, we used side-scanning sonar to observe the pink salmon fry at the surface and along the shorelines of the Bay and independently used echosounders to estimate the number of predators under the fry schools. In 1996, we will deploy both side scan sonar and down-looking echosounders to collect distribution of pink fry and predators within 50 m of the shoreline.

ACKNOWLEDGEMENTS

This research was funded by a grant from the Alaska Science and Technology foundation and by the Prince William Sound Aquaculture Association. We thank the PWSAC hatchery personnel for logistic support during this study. We also thank BioSonics Inc. for the generous grant towards the purchase of the 101 echosounder system.

We also acknowledge the EVOS Trustee Council and all who have supported our efforts to improve conservation of fish populations by monitoring with new technologies.

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Table 1. Parameters of the acoustic equipment used during the fall 1994 herring survey in Prince William Sound.

SYSTEM	FREQUENCY	SOURCE LEVEL	SYSTEM GAIN	TRANSDUCER DIRECTIVITY	PULSE DURATION
BioS. 101	120 kHz	-225.075 dB	-165.264 dB	.0010718	0.4 ms
BioS. 102	200 kHz	-221.655 dB	-155.756 dB	.0006515	0.4 ms

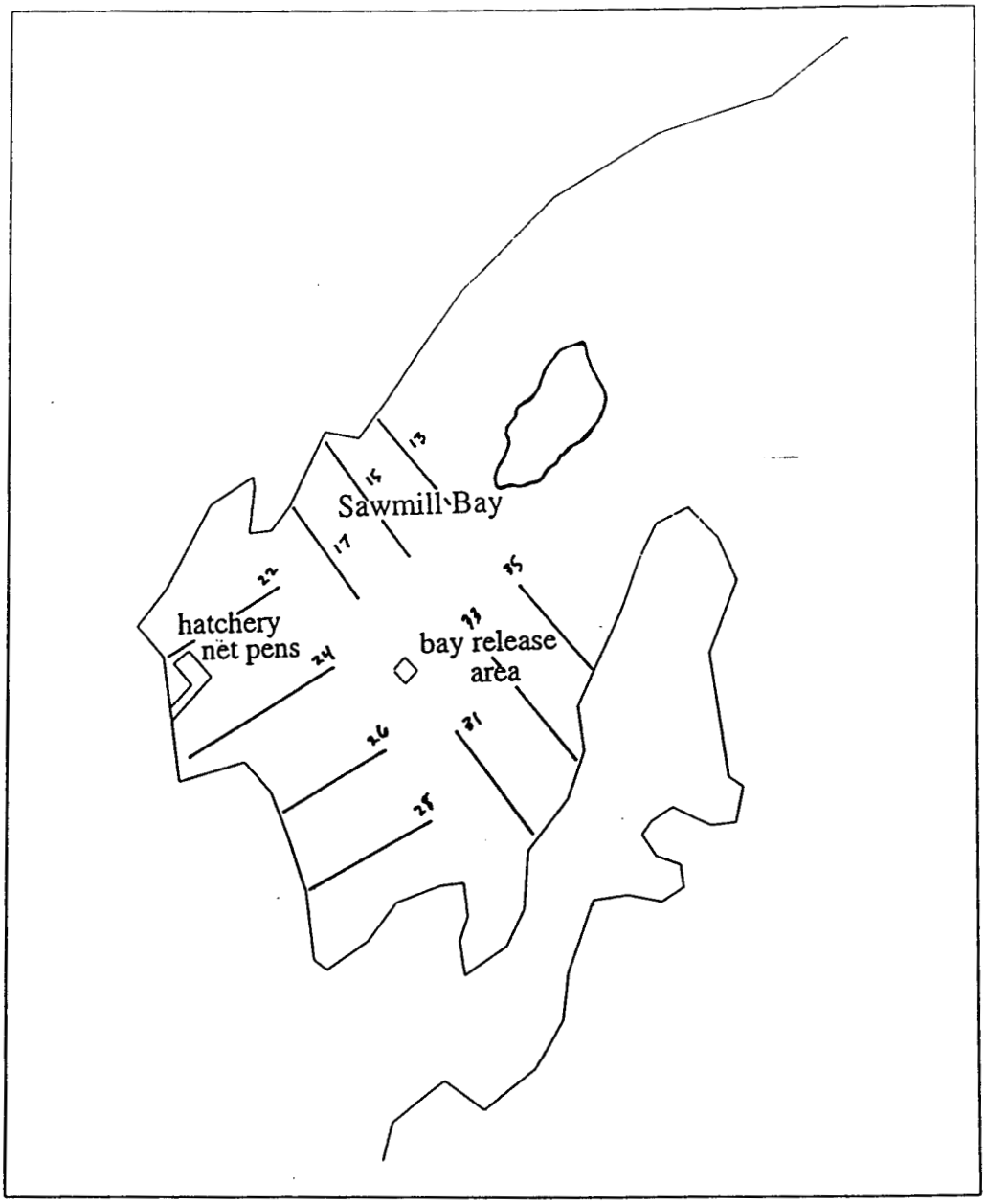

Figure 1. Map of Sawmill Bay showing a subsample of the transects that were run to acoustically measure fish density around the hatchery net pens, May 1992.

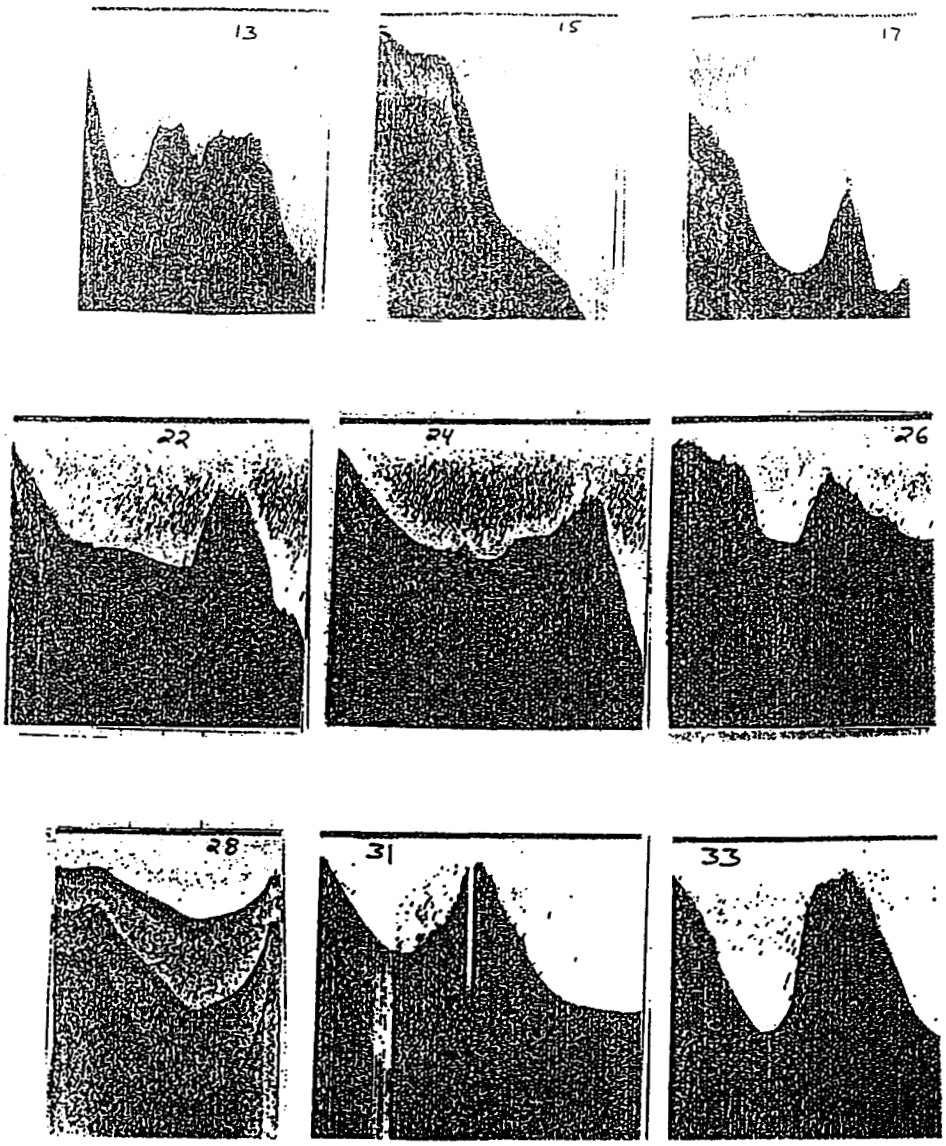

Figure 2. Nine echograms showing the acoustic measure of fish density by depth along a subsample of transects in Sawmill Bay, May 1992. Top line is surface, second line is bottom contour (ranges from 5 to 50 meters). The relative length of transect is shown by width of echogram. Fish targets in the water column are represented as single or clouds of black speckles.

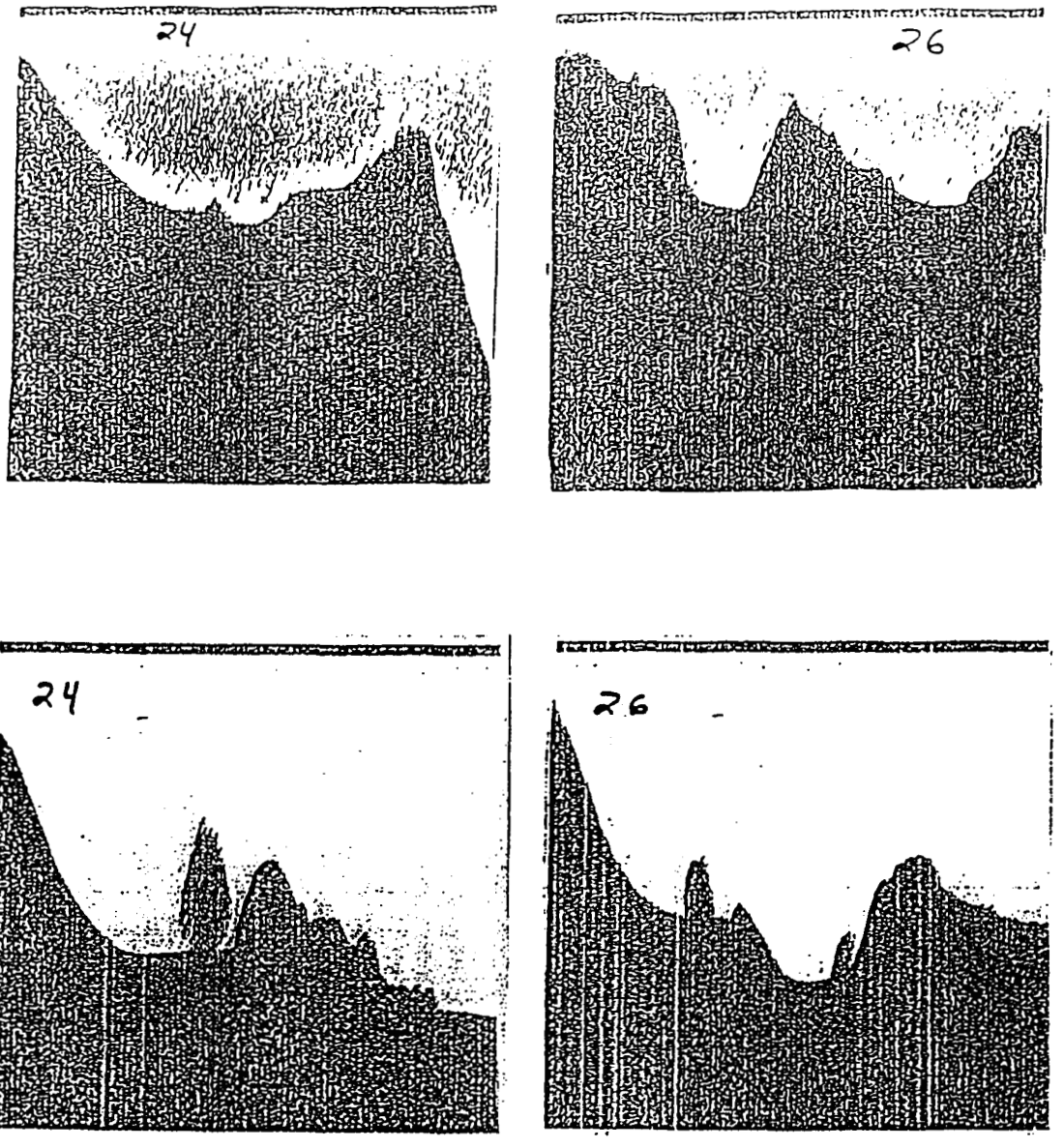

Figure 3. Four echograms showing the acoustic measure of fish density by depth along two transects (24 and 26) in the night (top) and day (bottom). Top line is surface, second line is bottom contour (ranges from 5 to 50 meters). Relative length of transect is shown by width of echogram. Fish targets in the water column at night are represented by single or aggregations of small black speckles, whereas fish targets in the day are large blotches (schools) near the bottom.

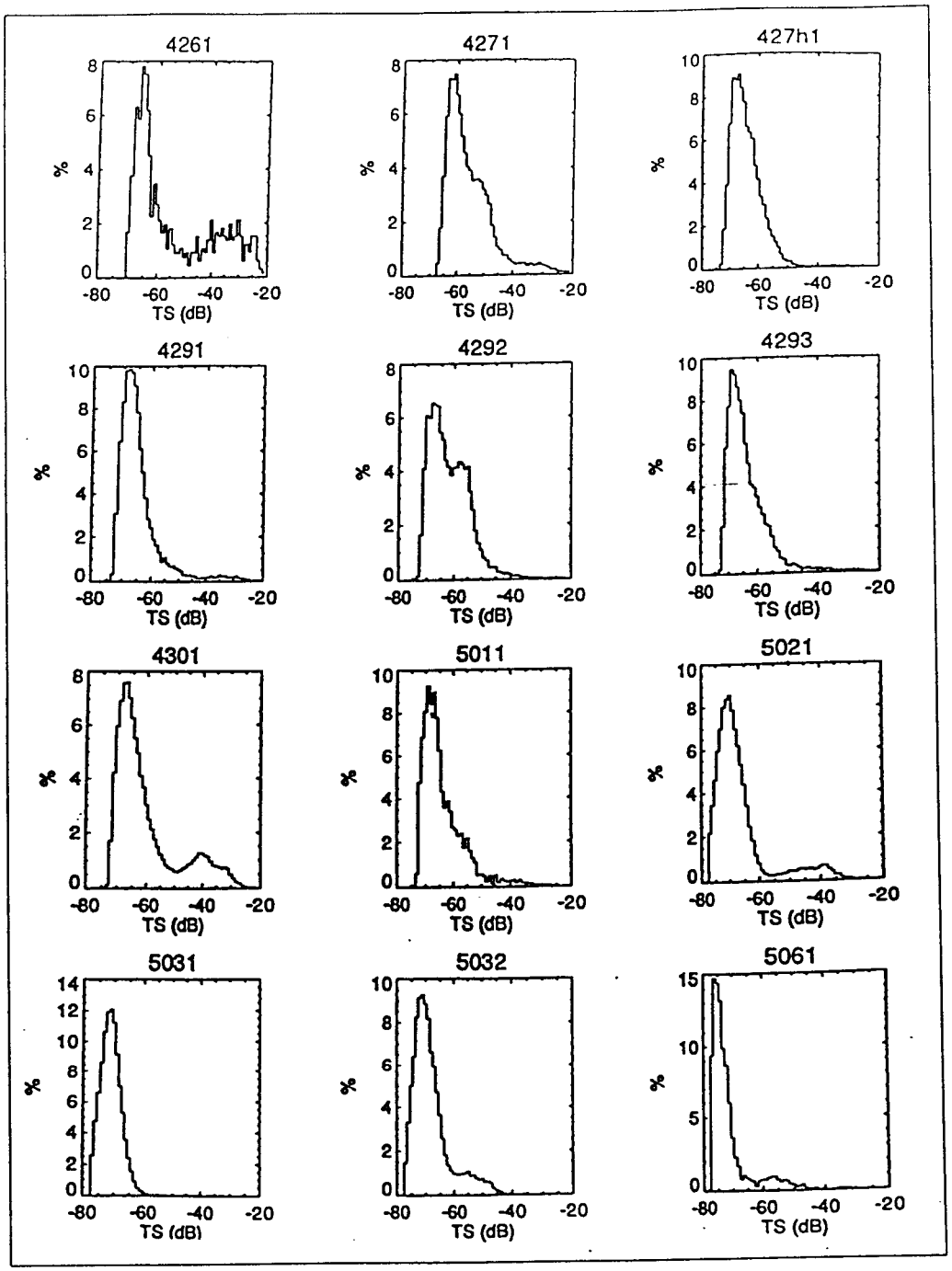

Figure 4. Histograms of fish target strengths by survey in Sawmill Bay, April 26 to May 6, 1993.

CHAPTER 2

Far-field predation: Predator and prey densities along the outmigration path of the juvenile pink salmon in Prince William Sound. G.L. Thomas, Jay Kirsch, Tom McLain, Mark Willette and Ted Cooney. Note: This paper is not to be cited without permission from the first author.

ABSTRACT

We made acoustic observations of nekton and plankton along the outmigration corridor of pink salmon fry in western Prince William Sound in the springs of 1994 and 1995. The greatest emphasis on observations was made in the Wells-Perry Island passages to observe conditions present during and after the release of 500 million pink salmon fry from the Ester Island hatchery in Lake Bay. The nearshore nekton was sparse in contrast to the offshore pelagic assemblage. Adult walleye pollock dominated the offshore pelagic nekton assemblage. Preliminary estimates of walleye pollock in the Wells-Perry Island passages were 160,722 +/-29,798 kg in 1994 and 85,484 +/- 5619 kg in 1995. Due to the fact that over 90% of the walleye pollock were found in the top 40 meters of the water column we are currently investigating the possibility of underestimating abundance as a result of near-surface errors (sample unit volume and boat avoidance).

Larger scale acoustic surveys of the Sound were conducted in 1994 and 1995. In 1994, surveys of the western Sound showed gradients in pollock density between the north- and south-western regions of the Sound. Smaller walleye pollock were concentrated in the southwestern region where several passages lead to the Gulf of Alaska. This is also the location of the Sawmill Bay salmon hatchery. In 1995, acoustic surveys over the entire Sound showed that the density of pollock were notably patchy.

Meso-scale patches of plankton were measured in the top 40 meters of the water column with acoustics in the spring of 1994 and 1995. Vertical net tows at this time of year showed that the species composition of the macrozooplankton was over 90% calanoid copepods. The patchiness of pollock appeared to be correlated with high density patches of plankton. The primary diet of the walleye pollock at this time was calanoid copepods. We are currently investigating the sources of error (species composition, target strength, etc.) in estimating the abundance and distribution of plankton using acoustics.

INTRODUCTION

The stocks of pink salmon in Prince William Sound experienced large fluctuations in survival to adulthood after the *Exxon-Valdez* oil spill. At the present time, it is not clear to what extent the oil spill or natural environmental conditions have affected run strength. Efforts to determine EVOS damages were confounded by the poorly understood effects of climate, food and predators which typically account for 76.9-99.8% of marine mortality (Ellis 1969; Taylor 1983). Recruitment to adult salmon populations is strongly affected by high mortality during the early

marine period (Parker 1968; Ricker 1976; Hartt 1980; Bax 1983, Willette 1993). During this period, slow growing individuals sustain a higher mortality because they are vulnerable to predators for a longer time (Parker 1971, Healey 1982; West and Larkin 1987).

This research is a component of the Sound Ecosystem Assessment (SEA) program, a multi-disciplinary effort to acquire an ecosystem-level understanding of the marine and freshwater processes that interact to constrain levels of plankton, fish, mammals and birds. Primary SEA hypotheses are that: (1) climate forcing indirectly influences survival of juvenile salmon through control of the available macrozooplankton prey (Cooney 1993), and (2) during periods of low macrozooplankton prey abundance predators shift to juvenile fish. The SEA program adopted the GLOBEC assumption (GLOBEC 1991), that all mortality of juvenile fishes results in a predation event, either by active or passive predation. Passive predation exists when fry no longer have the food reserves (energy) to escape a predator. Considering survival of the juvenile salmon is partially a function of predation and growth rate dependent upon prey availability, knowledge of predator and prey densities along the migratory outpath are essential.

The assessment of predators and prey along the outmigration path is complicated not only by the lack of complete information concerning how the juvenile salmon outmigrate, but also measurement difficulties. In general, areas frequented by salmon fry during the season were known and sampling effort was therefore stratified to appropriate nearshore and offshore areas. However, the salmon fry use of shoreline and offshore areas in response to tidal and diel changes, and behavior around high prey and predator concentrations, is largely unknown.

Accurate measurement of animal abundance and distribution is crucial to SEA. Measurements of animal abundance are needed to initialize and verify the models being developed. Also, simultaneous measurement of ocean physics and animal distributions is one of the keys to advancing our understanding of marine production processes. Underwater acoustics and optics appear to be the appropriate tools for quantitative assessment of fish and zooplankton (GLOBEC 1991). It is evident that development and implementation of these technologies are of critical importance to the success of SEA.

The objectives of this ongoing study are to: (1) acoustically measure nearshore and offshore nekton and plankton densities, their target strengths and characteristics along the outmigration route of the pink salmon, (2) identify acoustic targets using signal processing, net catches and life history information, (3) evaluate techniques to estimate predator and prey population abundance and distribution along the outmigration path, and to use this information in the testing of SEA hypotheses and model development.

In this paper, we present some of the results of these investigations, develop specific criteria for discriminating pollock which appeared as large, single targets within the water column, and were the most abundant offshore predator along the salmon fry migratory path. In addition, the initial assessment of offshore prey density and distribution relative to the walleye pollock population is presented.

METHODS

Prince William Sound (PWS) is a complex fjord/estuary (Schmidt 1977) located at the northern margin of the Gulf of Alaska (Figure 1). Prince William Sound covers an area of about 8800 square km with approximately 3200 km of shoreline (Grant and Higgins 1910). High mountain peaks in excess of 4000 m border the Sound and receive the brunt of the seasonally intense cyclonic storms from the Gulf of Alaska. Much of the shoreline is bordered by coastal rainforest which receives in excess of 7 m of rain annually (Thomas, et al. 1991). Freshwater input to the Sound occurs as runoff from glaciers, icefields and streams. Large scale surface currents are driven by the wind and buoyancy forcing. Depths exceeding 400 m occur in the western and central portions of the Sound which support overwintering populations of oceanic copepods.

Survey design

Acoustic-trawl/seine surveys were designed for nearshore (within about 300 meters of the shoreline) and offshore (>300 m) areas, with emphasis placed upon the Wells-Perry Island passages in the northwestern corner of the Sound near a large source of outmigrating fry (Ester hatchery). Nearshore and offshore areas were stratified because even though schools of outmigrating juvenile salmon have been sampled in shoreline rearing areas since 1989 (Willette 1993), there are several locations that require juvenile salmon to cross large expanses of open water. Since the portion of salmon fry using the offshore versus the shoreline for migration purposes was unknown, a nearshore and offshore strata were established to determine predator fields along the migratory path.

The western corridor of PWS was stratified in north-south and nearshore-offshore directions (Figures 2 and 3). A time series of surveys were conducted over the season to determine the density and distribution of potential predators and prey along the outmigration route. In 1994, the primary effort was to describe conditions in Wells-Perry Island and upper Knight Island passages but some effort was made to describe the nearshore and offshore conditions along the western corridor from Wells Passage to Montague Straits. In 1995, the primary effort was made to describe conditions in the Wells-Perry Island passages (Figure 4), but some effort was also made to describe the predator-prey densities in offshore areas throughout the Sound (Figure 5).

Acoustic transects

Due to the large offshore area, sparse sampling, and unknown predator population, a systematic transect design was chosen over a random design to provide better representation of the north-south gradient in densities. As a result, some precision was sacrificed for accuracy (Cochran 1977). Precision was estimated by assuming the transects to be independent samples and computing the weighted mean densities and biomass (Seber 1973). The offshore sampling was conducted on parallel transects that ran orthogonal to the passage being sampled.

In 1994, a zig-zag transect design was adopted to sample nearshore fish and collect predator

density information along extensive shorelines by bottom depth. Post-stratification of the shoreline by bottom depth was conducted to define near from offshore habitat and zigs were treated separately from the zags to establish independent units for computation of precision. In 1995, we used a series of systematic transects perpendicular to the shoreline because the horizontal extent of the nearshore area sampled was reduced to about 4 by 0.3 km.

Transecting was conducted between 2 and 3 meters per second using transducers mounted on a fin which was towed off the side of the vessel at a depth of approximately 2 meters. Boat speed was estimated at 2.5 m/sec. Night-time navigation in shallow littoral areas was hazardous due to the presence of rocky pinnacles and large tidal fluctuations. Consequently some transects were modified for safety purposes.

Survey timing

Both trawling and purse seining are most efficient at night when lower visibility reduces fish avoidance. In general, acoustic surveys are also best conducted at night because the fish are more evenly distributed in the water column (Houser and Dunn 1967; Burczynski and Johnson 1986) which improves the precision of the estimates. However, because latitude creates less than optimal night lengths and light levels, several compromises were necessary. In 1994, acoustic surveying was conducted during the day and midwater trawling was conducted at night. Acoustic sampling was also conducted during each trawl for signal classification purposes. In the nearshore strata, acoustic surveying and purse seining was conducted during both day and night. In 1995, a 24 hour sampling schedule included day and night efforts with acoustics and nets. All large scale surveys of the Sound were conducted in daylight.

In 1994, a time series of six surveys were conducted: late-April, early-May, late-May, mid-June and mid-July (Legs 1-6). Four surveys were conducted in northwest PWS nearshore and offshore strata to assess early season predation at the beginning of the outmigration on Legs 1-6. Two surveys were conducted in the southwest PWS nearshore and offshore strata to assess late season predation at the end of the migratory corridor on Legs 5 and 6. Two offshore surveys to assess predator distributions along the length of the outmigration corridor were conducted with the acoustics-midwater trawler on Legs 2 and 6. Two 24-hour diel surveys were conducted to assess temporal trends in fish behavior.

In 1995, two surveys (Legs 7 and 8) consisting of a series of 24 hour nearshore and offshore transects and one broad-Sound survey with the acoustic-midwater trawler were conducted to describe the predators and plankton during the fry outmigration season.

Acoustic equipment and processing

Geo-time coded acoustic data was collected using BioSonics 101-120 kHz and 102-200/420 kHz dual beam echosounders, processed in real time with ESP and BIOMAP software on a 486 laptop computer. Each sonar system is equipped with a Magellan DX5000 GPS receiver and external

antenna to measure geographic position. Data were collected in 1 meter depth strata from 0-20 meters, 2 meter strata from 20-50 meters and 5 meter strata below 50 meters. Sample process distances were established as every 45 pings in offshore strata and every 45 seconds on the inshore strata. Echo integration, dual-beam target strength and GPS data were stored on hard drives and backed up on optical or magnetic disks/tapes. Unprocessed data were stored on DAT recorders. A block diagram of the data acquisition system is shown in Figure 6.

The entire system was calibrated before, during and after the field season using standard calibration techniques. Standard technique included pre- and post- field season tank calibration by the manufacturer, dockside calibrations with standard targets (tungsten-carbide spheres and ping pong balls) and field tests during the survey with ping pong balls. During the cruise the system receiving characteristics and transmit power was routinely monitored using the calibration oscillator.

Noise peaks at 100 meters were approximately 10 mr on the narrow and wide beams, respectively, with 40 log R amplification. A 5.08 mm tungsten carbine ball was used as a standard target for dockside calibrations which produced a constant mean target strength of -41 dB with a SE of 0.3. Standard tungsten-carbide targets which are accurate within 1 dB (Foote and MacLennan 1982) were used for dockside calibrations. Dockside calibrations were made by collecting a large sample of positions in the beam by allowing the target to swing freely within the acoustic beam. Using the known TS of the sphere, the peak in the target strength distributions was used to calculate the combined source level and receiver gains (SL+RGn andSL+RGw). Subsequently, TS distributions are generated for possible values for the wide beam dropoff (w), and w is chosen from the distribution with the minimum variance. The determined calibration parameters are then set so that the expected TS value of the sphere can be obtained regardless of position in the beam. Ping-pong balls that were calibrated against the standard targets at the dock were used in the field on extended cruises to monitor for through-system changes in sensitivity (Foote and MacLennan 1982). Important system parameters and calibration data are presented in Table 1.

Reference voltages and TVG curves are systematically recorded at the beginning of every 2-hour DAT tape, measured on a digital voltmeter, and written in the field log book. This allows us to calibrate the tape playback output in the laboratory to match the echosounder's output from the field. This routine also allows for detecting changes in the receiver gain while in the field (amplifier drift and TVG curve).

Considerable post processing was necessary because of equipment malfunctions in the field which interrupted real time processing. Post processing to collect missing target strength, echo integration and echogram data, and to correct for system parameter changes was conducted by playback of DAT tapes on personal computers and BioSonics processing equipment. All processing of echo-counting, echointegration, target strength determination, and biomass estimation was done in accordance to standard techniques (Traynor and Ehrenberg 1979, Thorne 1983, etc.). Acoustic data for Echointegration was received on the narrow beam element only,

and amplified by a 20 Log R time varied gain (TVG). Dual beam processing data were received on both wide and narrow elements of the transducer and amplified by a 40 log R TVG.

After transferring the data to a UNIX workstation, batch processing of data was conducted to correct acoustic data for temperature and salinity, bottom integration, classify and transform acoustic targets from dB to kg and numbers,and estimate and visualize biomass were conducted. All data are stored in the appropriate format for post processing using ARCINFO, interactive data language (IDL), and automated visual systems (AVS) software. ARCINFO is the geographic information system software being used to store and process electronic map information. The Interactive Data Language (IDL) is an array-oriented programming environment which has been chosen for visualization, signal processing, and statistics. Advanced Visualization System software allows for 3D visualization, data I/O, and statistical functions.

In the spring, we expected a multi-species environment, where schools and single targets are interspersed and are difficult to catch, so a combination of dual beam (Traynor and Ehrenberg 1979; Dickie et al 1983; Burcynski and Johnson 1986; and Foote et al 1986), and multifrequency (Holliday 1972; Saetersdal et al. 1984; Zakharia 1990; Simmonds and Armstrong 1990) measurements represented technological considerations in the survey design. Collaboration with other SEA projects was necessary to conduct the net sampling needed to collect the biological information for sea-truthing the acoustics.

Measured target strengths of individual fish were compared with length data of fish captured by the nets. A power function (Traynor and Ehrenberg, 1979) is currently used to simulate a transducer's beam pattern (ideally a Bessel function) so as to estimate TS. The target strengths are therefore compensated for off-axis location, and targets with angle greater than the mode in the angle distribution (usually about 3 degrees) are excluded so as to remove size bias (since off-axis targets require higher noise thresholds). The empirical formula derived by Love (1977) relating target strength to length was used to convert lengths of captured fish into predicted values of target strength. To establish a fish size-target strength relationship, we used the relationships advanced by Thorne (1983) for target strength per kg versus length, and Traynor and Ehrenberg (1979) for target strength versus length of individual fish.

Identification of targets is a problem in Prince William Sound because of the diversity of marine life. While pollock, salmon, and herring are the dominant fish species, other organisms including zooplankton, squid, and jelly plankton are plentiful and capable of reflecting sound. A first step in the identification of targets was to classify target types on the echogram: schools, layers, aggregations of large targets, large single targets (nekton<-60dB) and small single targets (plankton >-60dB). The second step was to code the appropriate echo integration cells and outline the targets in the processed electronic files with the type of classification or mix of classification that represent each. Third, the species composition for target classes was determined and applied to each of the coded integration cells. At this step the lengths of the fish in the net catch was also compared to the target strength data from the dual beam analysis. Those cells which had mixed classifications received prorated estimates of composition. The integration

cells were then assigned a species and size, and a meta file for level of reliability was developed to reflect the consistency of the decision from the various data sets. The target strength for the acoustic to biology transformation (dB to weight) was then chosen from the literature values, in-situ target strength measurements and length of fish in the net catch. Weight of cells then was expanded to density per unit volume or surface area for visualization and estimation of biomass. The area estimates of the strata used to expand density to biomass were derived from maps generated in ARCINFO. Weighted mean densities and their variances were computed and extrapolated to biomass and 95% confidence limits via the delta method (Seber 1973). Biomass estimates of predators were then combined with estimates of salmon fry predation by the same technique to estimate total fry consumption. A block diagram of data analysis is shown on Figure 7.

Sampling equipment and data analysis

Purse seining, midwater trawling and 0.5 m vertical ring nets were used by other SEA projects to collect biological information on nekton and plankton. Purse seining was used along the shorelines and conducted in conjunction with the nearshore acoustic transects. Commercial seiners were chartered to deploy 250 by 30 m purse seines with 1.5 cm stretch mesh and a sink depth of 20 meters. Both round hauling and 20 minute hook-set procedures were used. A midwater trawler of 25 m was chartered to fish the 40 by 28 m wing trawl with 1.5 cm mesh in the bunt. The trawl was equipped with a net sounder to determine depth to head rope and measure fish entering the mouth. Time of trawl ranged from 20 minutes to two hours dependent upon catch rates. In 1995, a pair trawl was used for a brief period for nearshore sampling. Vertical ring nets were used to sample surface plankton from 50 m to the surface. All nekton catch information from the trawls and seines was provided by the Alaska Department of Fish and Game (Mark Willette). All plankton catch information from the vertical ring nets was provided by the University of Alaska at Fairbanks (Ted Cooney).

RESULTS AND DISCUSSION

In 1994, three acoustic-midwater trawl surveys, Legs 2-4, showed relatively high densities of nekton and plankton in the offshore sampling areas of Wells-Perry-Upper Knight Island passages. Nearshore surveys in these areas showed low densities of nekton and plankton. In 1995, similar results were observed on Legs 7 and 8. The following results concentrate on these findings.

Species and size composition

In 1994, the offshore sampling with a midwater trawl at night on Legs 2-4 indicated that northern smoothtongue, walleye pollock and squid dominated the nekton assemblage (46%, 36% and 17% of the catch, respectively, Figure 8). In 1995, the offshore sampling with a midwater trawl at night on Legs 7-8 indicated that walleye pollock and squid dominated the nekton assemblage (87% and 12% of the catch, respectively, Figure 9). However, most trawling in 1995 was conducted during daylight hours and walleye pollock dominated the catch (91% of the catch,

Figure 10). Upon closer examination, over 80% of the northern smoothtongue in the 1994 catch came from two back-to-back trawls.

In contrast, the nearshore sampling with purse seines indicated that Pacific herring dominated the nekton assemblage (56% of the 1994 catch, Figure 11, 56% of the 1995 catch, Figure 12). Sampling the nearshore area in 1995 with a pair trawl on Leg 7 supported that the nearshore assemblage was dominated by Pacific herring (91% of the catch, Figure 13).

The dominant mode of the length frequency of the offshore pollock was 480 mm in May 1994 and 515 mm in 1995 (Figures 14 and 15). There was no significant trend in the size of pollock caught in the midwater trawl by depth (Figure 16). The mean size of pollock in the Montague-Passages areas in southwestern Prince William Sound was considerably lower than the Wells-Knight Island pass areas (Figures 17).

The length frequency of non-pollock targets and juvenile pollock show that the midwater trawl retained large numbers of smaller nekton (northern smoothtongue and squid, Figures 18 and 19) with modes at 100 and 220 mm (Figures 20 and 21). Some adult salmon between 400-700 mm were captured each year by the trawl but their numbers were less than 1% of the midwater trawl catch.

The highest catch rates of adult pollock by the midwater trawl were made between 50 and 70 m but there were a limited number of samples at these depths (Figure 22). In contrast,the highest catch rate for squid was at 10 m (Figure 23) which was the shallowest depth that the midwater trawl could fish (squid were only caught at night). In 1994, the catch rate for northern smoothtongue was highest at 40-60 m (Figure 24). Figure 25 shows a biomodal vertical distribution for juvenile pollock.

The midwater trawl was fished offshore and captured primarily pollock, although it was designed as a herring trawl and readily captured smaller fishes. The purse seines were fished nearshore and captured primarily herring, even though they are effective at capturing larger fish such as adult salmon and pollock. We observed few herring schools offshore and believe that the assemblage is dominated by pollock. We observed fewer pollock inshore and believe that the inshore assemblage is dominated by herring. The small midwater trawl sample effort to the below 40 m layer could have introduced some bias to the average catch by depth, therefore care should be taken when interpreting these results.

Target classification

The echograms revealed three major classifications of acoustic targets: loose aggregations of single and multiple nekton targets, dense layers of plankton targets, and an occasional dense school target. The loose aggregations of single nekton targets had a target strength mode of about -32 dB (Figures 26 and 27), consistent with the observed mean polluck length of 480 mm, assuming a target strength of 20 log L -66 (Traynor and Ehrenberg 1979; McLennan and

Simmonds 1992, Figure 28). The depth distribution of these large targets indicated the highest density of pollock targets were at 20 meters, which is higher in the water column than peak trawl catch rate of 60-80 m.

There are several explanations for the discrepancy between the pollock target and the trawl catch rate depth distributions. First is the small sample size of the trawl effort below 40 meters. These trawl hauls were made at these depths to deliberately target a concentration of fish. Normally, the fish observed in real time were targeted at depths of 20-40 meters, hence the trawl effort distribution by depth. Also, the frequency of midwater trawl malfunction increased closer to the surface, which decreased the actual fishing time and underestimated the catch rate. It is also common for catch efficiency of the trawl to decline towards the surface because of the increase in available light (Barranclough and Robinson 1976). Finally, we recognize that there is some contamination by adult salmon, which are large, near-surface, single and multiple targets like the pollock. However, no changes in the vertical distribution of pollock were discerned at times when large concentrations of salmon were known to be present.

The layers of plankton targets had target strength modes of about -60 to -54 dB which may be high relative to the backscatter from the dominant macrozooplankton at this time of year, calanoid copepods (Figures 26 and 27). This is presumed to be due to failure of the target strength discriminator, i.e. the targets are pooled. This is supported by the fact that when the density of the plankton layer was highest the mode of the target strength distribution shifted to about -55 dB, and when it was lowest the shift was towards -65dB. The smallest targets measured at 40 m (the deepest plankton layer) was -67 dB in 1994 and -71 dB in 1995 (Figures 29 and 30).

The occasional dense school target was limited in numbers and primarily observed in the nearshore areas during the day. This behavior is typical for herring which dominated the nearshore catches. The depth distribution of school targets and highest midwater catch rates coincided between 20- 40 m (Figure 31).

Although their target strengths are relatively low the squid and jelly plankton present problems to acoustic assessment via echointegration. First, the midwater and pair trawl catches of squid suggest it is low in abundance relative to the pollock and only at the surface at night (Figures 8-13, and 23). Since squid have a low target strength (-58.6 dB, Jefferts et al. 1987) and the pollock numbers actually declined slightly at the surface at night (Figure 32), we assumed that the effect of squid was negligible (Figure 32). However, the density of the jellyfish in the pair trawl show that despite their low target strength (which is largely unknown) their abundance may confound echointegration. Target strengths of jellyfish and quantitative information on their vertical and horizontal distributions are needed.

Echointegration and counting of targets

Acoustic samples were collected simultaneously with the midwater trawls in 1994. Trawl catches that were dominated by pollock were converted to c/f (kg/min) and compared to the

echointegration of acoustic backscatter (Figure 33). Because of the uncertainties of estimating the pollock abundance by echointegration in a surface layer where they mixed with sometimes heavy layers of plankton of unknown backscatter, we investigated the feasibility of thresholding out the plankton and counting the pollock. We established the threshold by choosing the antimode between pollock targets and plankton targets (Figures 26 and 27). Applying this threshold, we made manual counts of pollock (from tape playback to a storage oscilloscope) and auto-counts of pollock using the BioSonics ESP-DB program for the same transects. We compared manual and auto-counts from 0-50 m which included the plankton layer (Figure 34) and we separated the counts into a plankton layer and sub-plankton layers for comparison (Figure 35 and 36). The auto counts underestimated the manual counts by 13-28% and the manual procedure underestimated pollock densities approximately 5% of the time when multiple targets were encountered. Comparisons between the predicted echointegration from autocounts and observed echointegration values in transects containing low plankton densities suggest the counts may be underestimated (Figure 37).

Another possible source of underestimation is the sample unit size relative to the size of the fish. Given the small sample volume of the narrow beam transducers we use to estimate density and target strength, a problem can occur if the sample unit size is not sufficient to adequately ensonify the fish. This relationship can be viewed as a fish volume to cone frustrum volume or a fish length to cone frustum diameter function (Figure 38). The Central Limit theorem suggests that when a sample unit with sufficient size to reduce the possibility of incomplete ensonification to 5% or lower is absent, the estimate may be biased. This should apply to both the estimation of density and target strength. Observations of slight declines in target strength and densities have been made which could be biological or artificial. Hence, additional research is required.

Several sources of error contribute to this relationship. First, the trawl catch per unit effort is far from perfect. The trawl is open as it is deployed and retrieved which changes the effort. It was not uncommon for the trawls to become imbalanced and alter the fishing effort, especially when fished near the surface. The water flow through the net was unknown. Second, the echointegration measures all the backscatter from nekton and plankton. This includes the plankton, jellyfish, squid and other non-pollock targets. Given the accumulation of these errors and more, the relationship observed suggests that our sampling was robust, yet, not adequte to estimate biomass with certainty. Thus, we developed and implemented an echo counting procedure which could be automatically applied to digital files for numerical assessment. The fit between manual and auto-counts is poorer in the plankton layer, but preferable to echointegration, so it was utilized to estimate pollock abundance. Our analysis of echocounting suggests that the pollock numbers are underestimated by approximately 18-33%. Routines for correcting the underestimation of pollock from auto-counting and multiple targets need to be developed.

Preliminary estimates of pollock abundance

We estimated the average biomass of offshore adult pollock in the Wells-Perry Island passages at 160,722+/- 29,798 kg in May-June 1994 and 85,484 +/- 5,619 kg from three acoustic-midwater

trawl surveys of the northwest Sound in May-June 1995. Also in May 1994, we conducted an acoustic-midwater trawl survey of the western Sound to determine how the Wells-Perry Island area compared to the rest of the outmigration corridor of the pink salmon fry. We estimated that 15% of the offshore pollock in the western Sound were in the Wells-Perry Island Passages. During the large-scale survey of the western Sound, we estimated the offshore pollock biomass in the Wells-Perry Island Passages to be 138,641+/-40,585 kg and the entire pass to be at least 1,886,147 +/-1,295,713 kg. The biomass estimate for the Wells-Perry Island portion of the large-scale survey agreed with the estimate from the three northwest Sound surveys.

In 1995, we conducted two acoustic-midwater trawl surveys of the Wells-Perry Island Passages in May and June. The Wells-Perry Island estimates were 26,437 +/- 3556 kg in May and 144,117 kg in June. We also conducted a large-scale survey of the Sound after each of the above surveys. We estimated the offshore pollock biomass in the Wells-Perry Island Passages to be 30,786 kg in May and 251,237 kg in June. We estimated that 8% of the offshore pollock in the western Sound were in the Wells-Perry Island Passages in May and 31% in June. The biomass estimate for the western Sound was about 400,000 kg in May and 800,000 in June. There appeared to be a northward shift in the pollock population from May to June on both the east and west sides of the Sound suggesting a possible post-spawning feeding migration in the spring.

The numbers of pollock are important because they allow evaluation of population-level predation on juvenile salmon in the marine environment. We feel that the present counts of adult pollock are underestimates of the fish present for a number of reasons. First, we feel that the auto-counting technique is about 80% as effective as manual counting. This is probably due to the dual beam target discriminator being more rigorous and excluding valid targets. Second, manual counting is an underestimate of the pollock because of the multiple targets, which are also excluded in the auto-count. Estimates of the number of multiple targets that are excluded from the echo-counting are needed to control quality of the procedure. Third, the large (0.5 m) size of the pollock reduces the probability that they will be completely ensonified in the beam at short ranges. In accord with the Central Limit theorem, the diameter of the beam should be 20 times the length of the fish (10 m) to insure that 95% of the fish are completely ensonified. Since the beam is not 10 m in diameter until a depth of 100 m, the target strengths and densities of the fish may be underestimated. This bias needs to be investigated since the depth distribution of the pollock is 0-40 m. Finally with the near-surface depth distribution of the pollock, boat avoidance may be another source of underestimation. The last two problems will be investigated in 1995 with side-looking sonar measurements of pollock depth and distance distributions.

Plankton and nekton patchiness

Initial estimates of plankton density were made by scaling the echocounts into three density bins (high, med and low). Net samplin during the May-June cruises revealed that calanoid copepods represented over 95% of the plankton catches in vertical ring net catches. The vertical distribution of plankton and pollock were both nearsurface, displayed significant overlap and showed the pollock to be slightly deeper (Figures 39). Initial 3D visualizations of plankton and

pollock patchiness have demonstrated some co-occurance of patches in horizontal space, but this is difficult to see without color viewgraphs (Figures 40, 41, 42 and 43).

The quasi-continuous nature of acoustic measurement data allows for quantitative descriptions of patchiness. Hjort (1914) advanced the patchiness hypothesis by concluding that if all the food in the ocean was evenly distributed all the fish would starve to death. Lasker (1988) has proposed a climate-driven, food-patch model to explain anchovy larvae survival in the Southern California Blight. We have proposed a similar hypothesis under the guise of the River-lake hypothesis. Presently, the plankton acoustics project is examining the nature of plankton patches relative to physical forcing events such as tides and storms, and predator abundance. The examination of the predator and plankton patches should reveal insights to the predator behavior in response to prey availability which is congruent to the prey switching hypothesis.

ACKNOWLEDGEMENTS

This project was funded by the EVOS Trustee Council. Special thanks go to the commercial fish experts for assisting in developing and implementing the survey design. The Alaska Department of Fish and Game and the University of Alaska Fairbanks were responsible for subsampling and analyzing the purse seine catches for biological information on the fish targets (Evelyn Brown, Mark Clapsaddle, Brenda Norcross and many more). Finally, the EVOS Trustee Council and staff (Molly McCammon and others) deserves significant thanks because of their long-term commitment to research and development of better methods. We thank all who have supported our efforts to improve conservation of fish populations by monitoring with new technologies.

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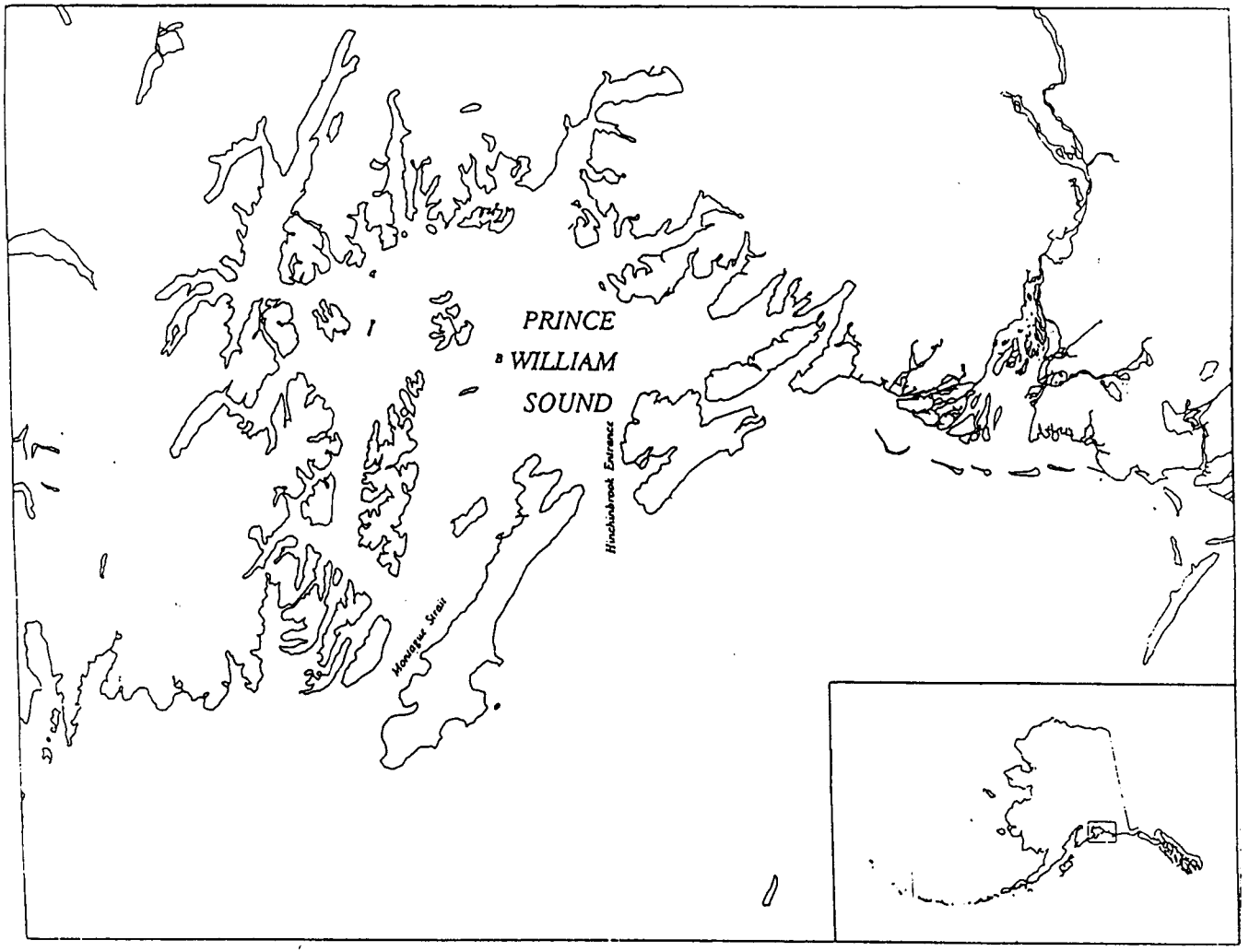

Figure 1. Map of Prince William Sound with a State of Alaska locator.

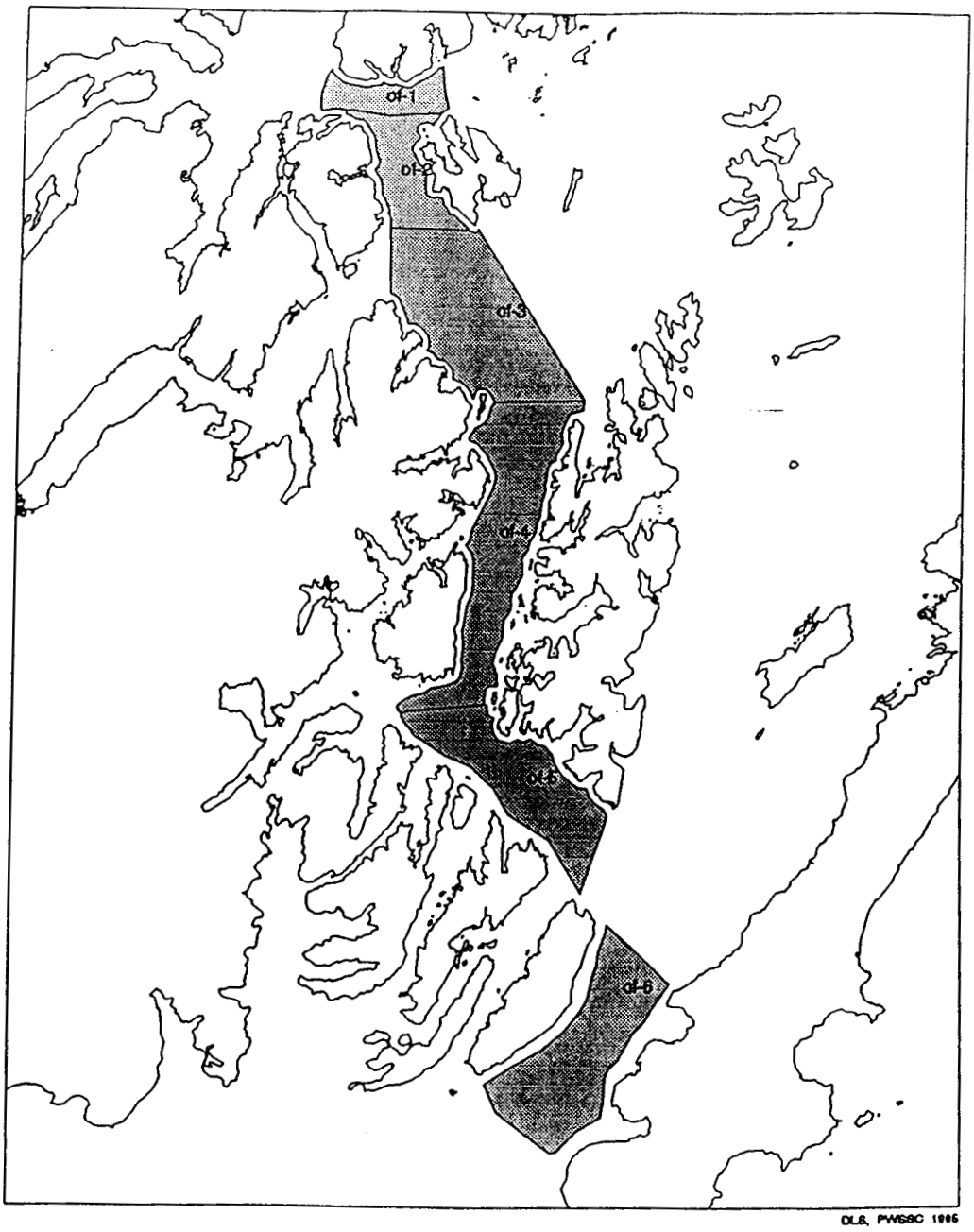

Figure 2. Map of Western Prince William Sound showing offshore areas surveyed with acoustic and midwater trawls. (Codes are: of-1 = Wells, of-2 = Perry Is, of-3 = North Knight Is, of-4 = Chenega Is, of-5 = South Knight Is, of-6 = Montague Is).

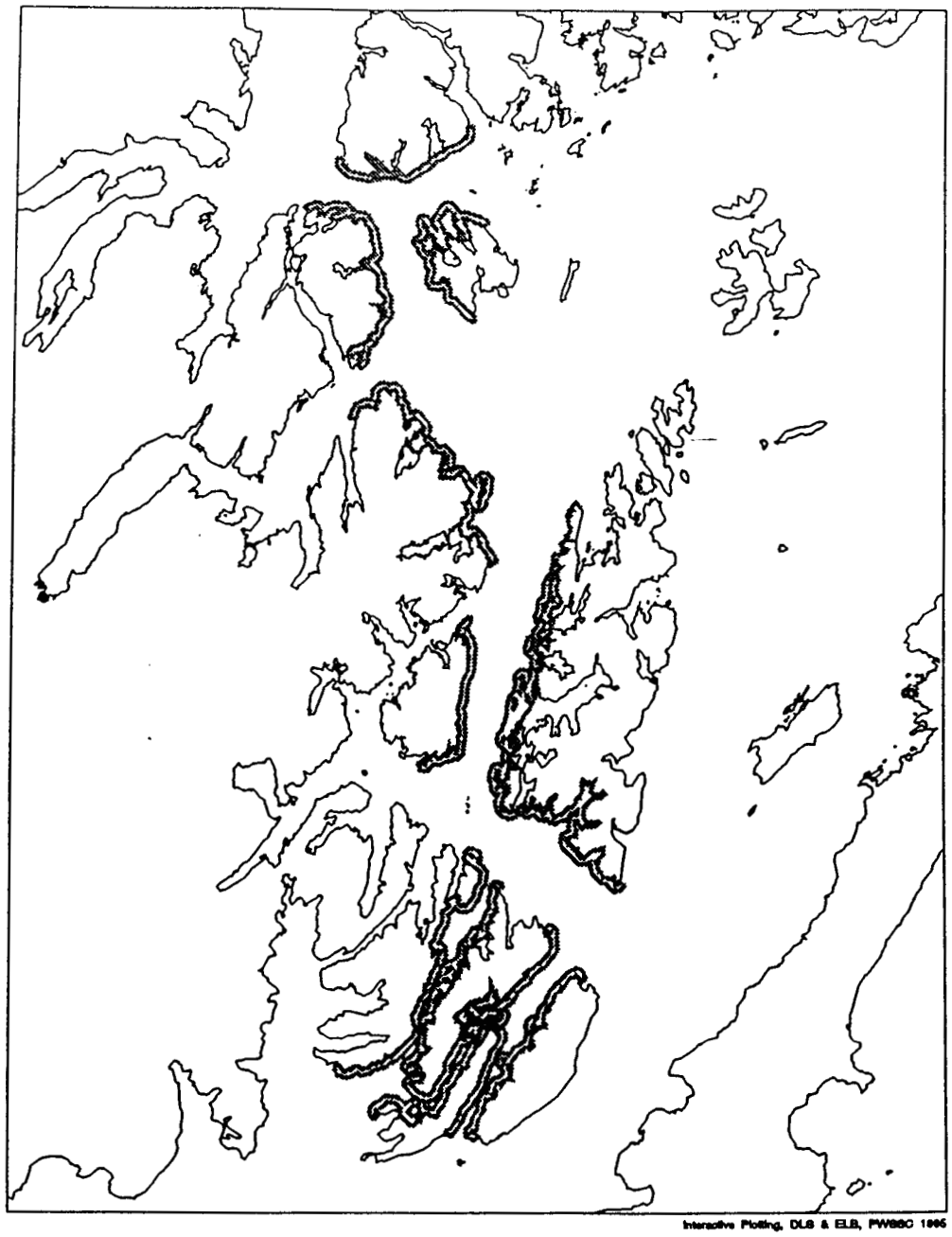

Figure 3. Map of Western Prince William Sound showing nearshore areas surveyed with acoustics and purse seines in 1994.

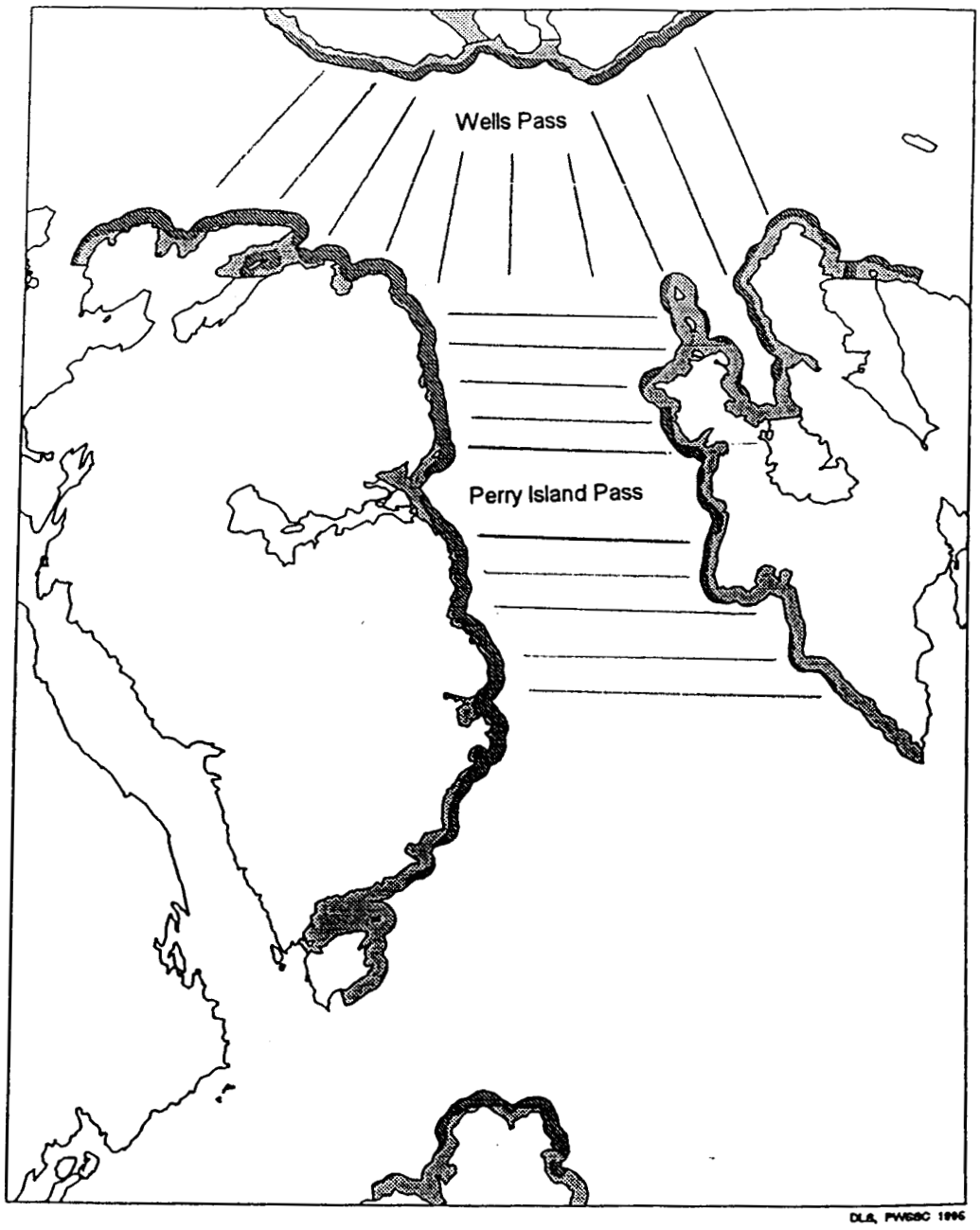

Figure 4. Map of the Wells-Perry Island passages which were surveyed intensively with acoustics, trawls and purse seines in 1994 and 1995.

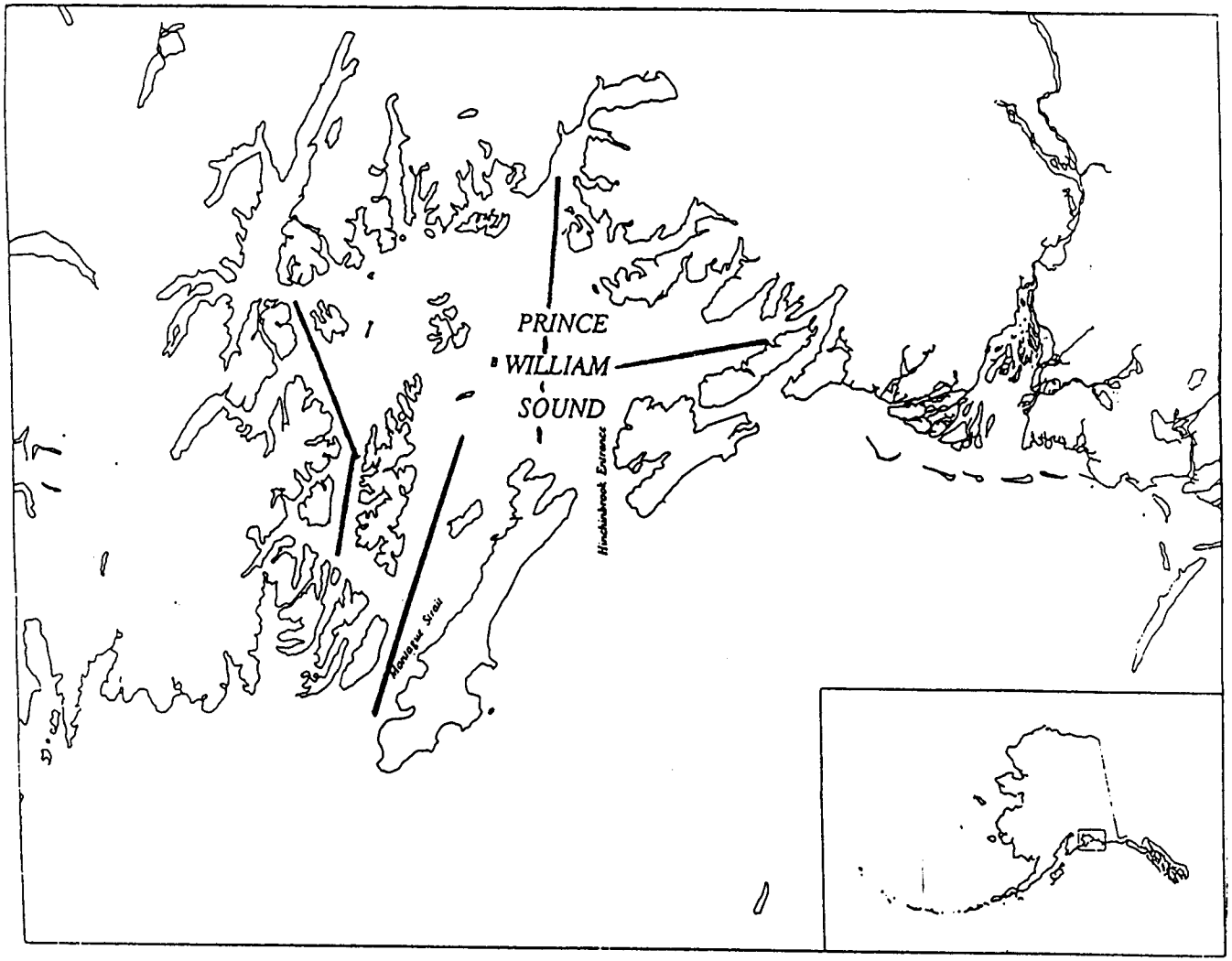

Figure 5. Map of the Broadscale survey of Prince William Sound in 1995 (Legs 7 and 8).

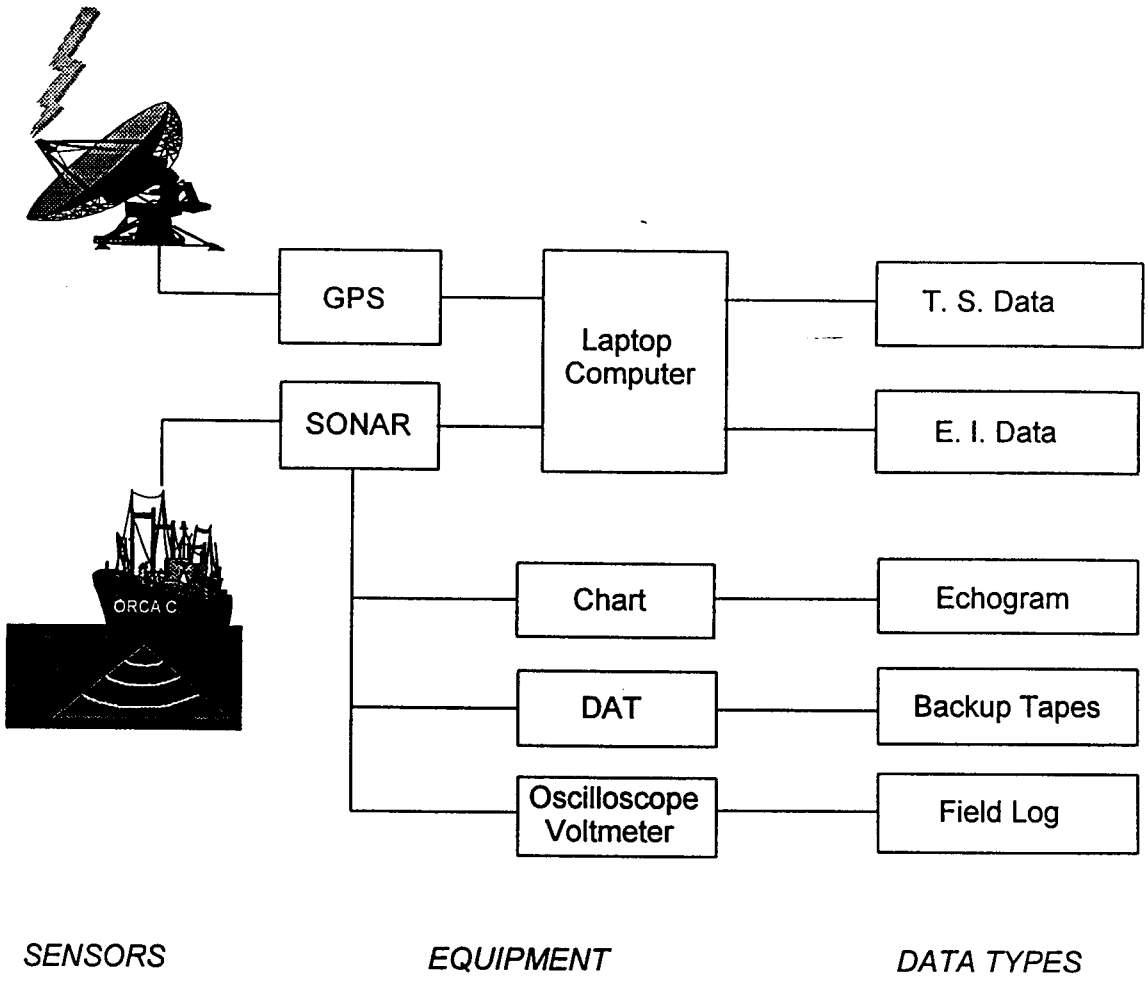

Figure 6. Data acquisition system used in SEA 1994 and 1995 acoustic surveys.

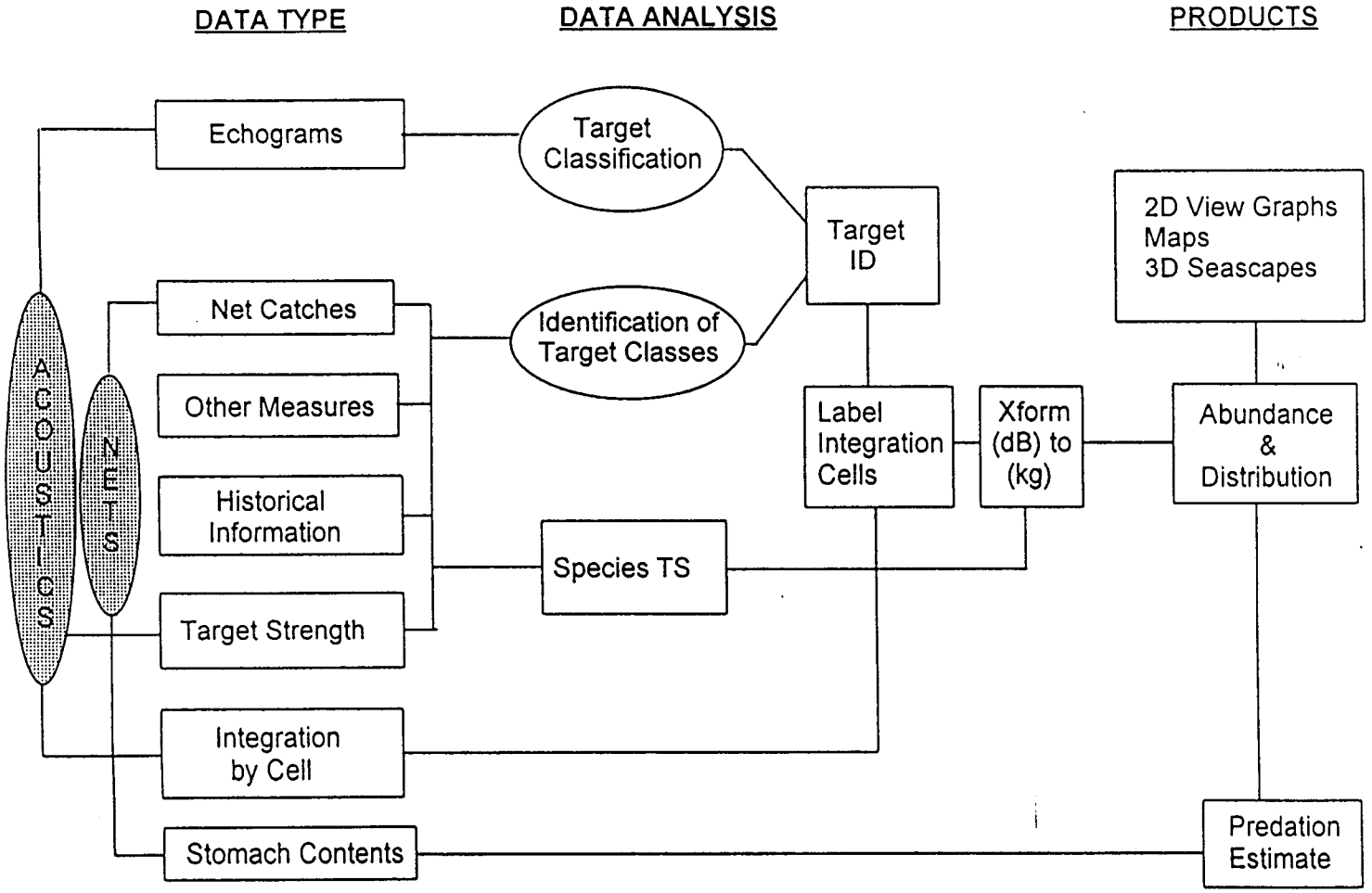

Figure 7. Data processors and analysis system used in SEA 1994 and 1995 acoustics investigations.

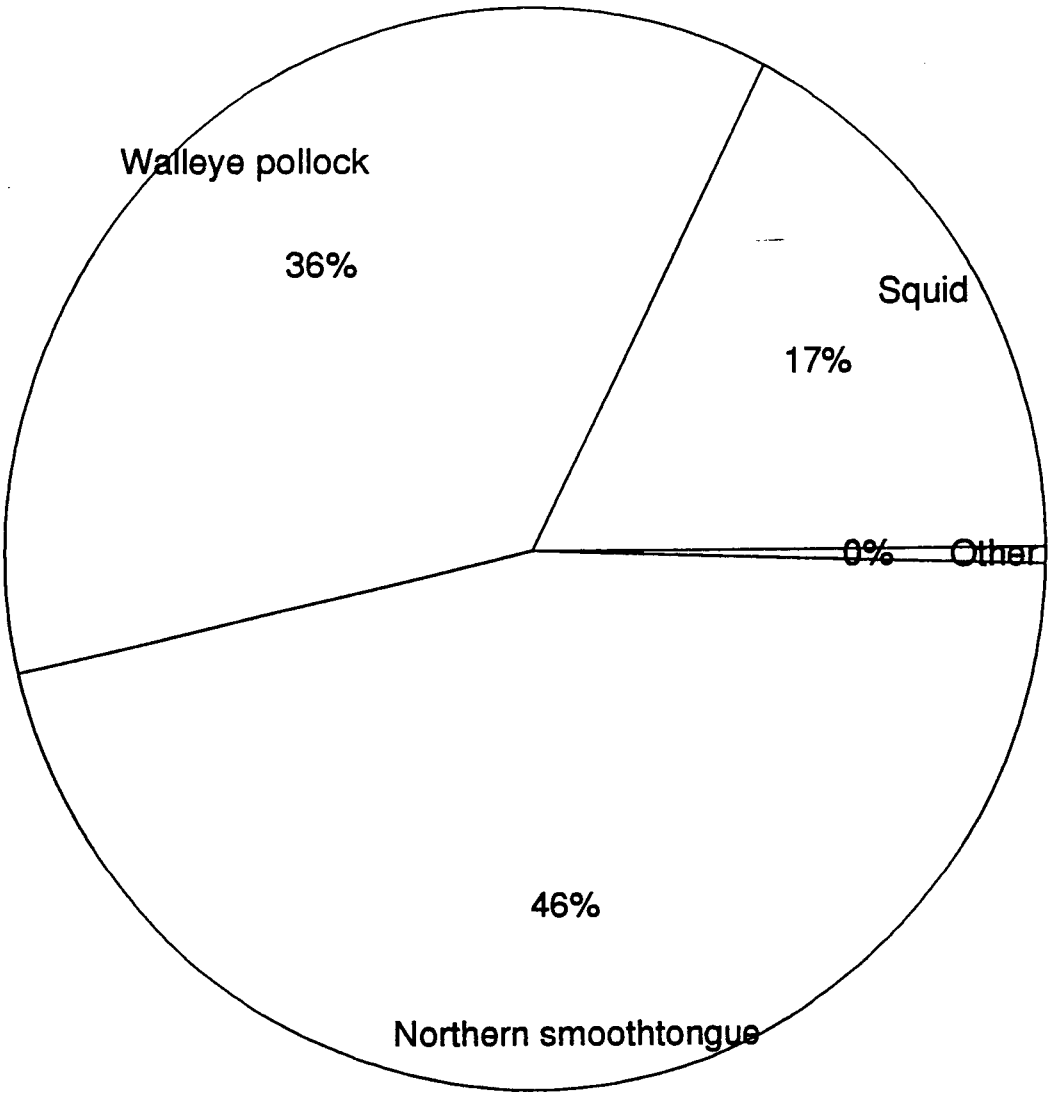

Figure 8. Species composition of night-time midwater trawls in May and June 1994, northwest Prince William Sound.

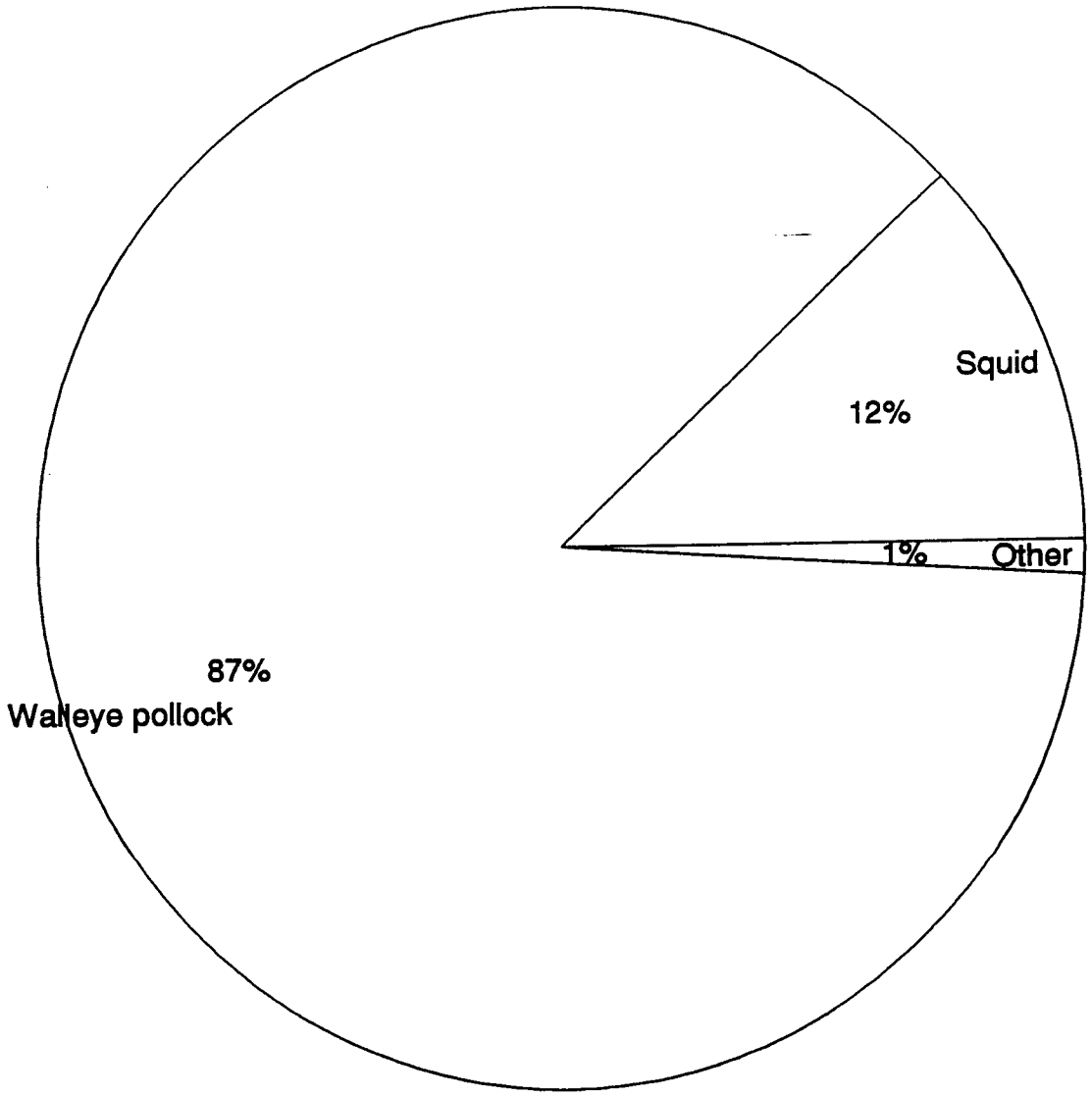

Figure 9. Species composition of night-time midwater trawls in May and June of 1995, northwest Prince William Sound.

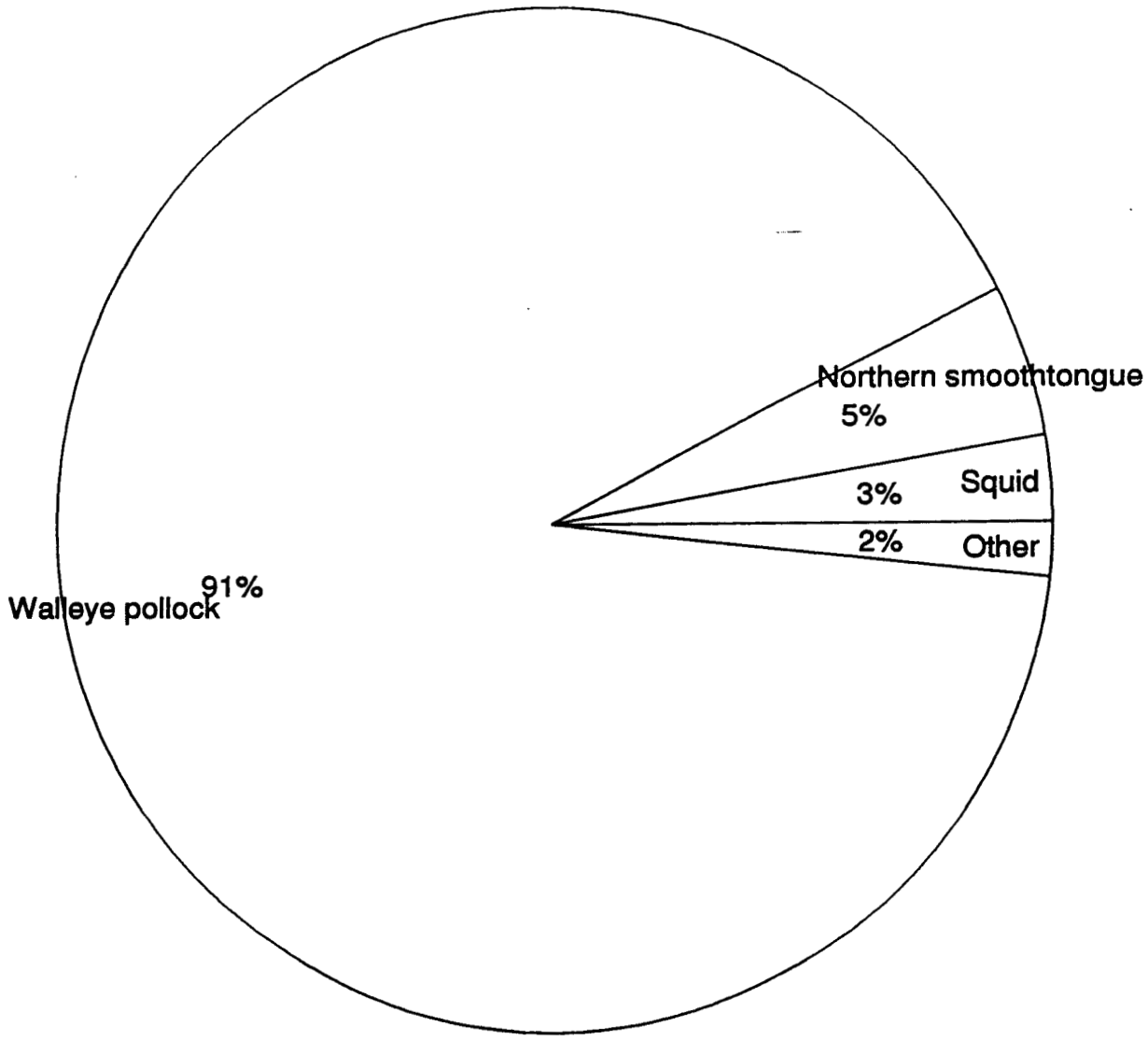

Figure 10. Species composition of day and night-time midwater trawls made in May and June 1995, northwest Prince William Sound.

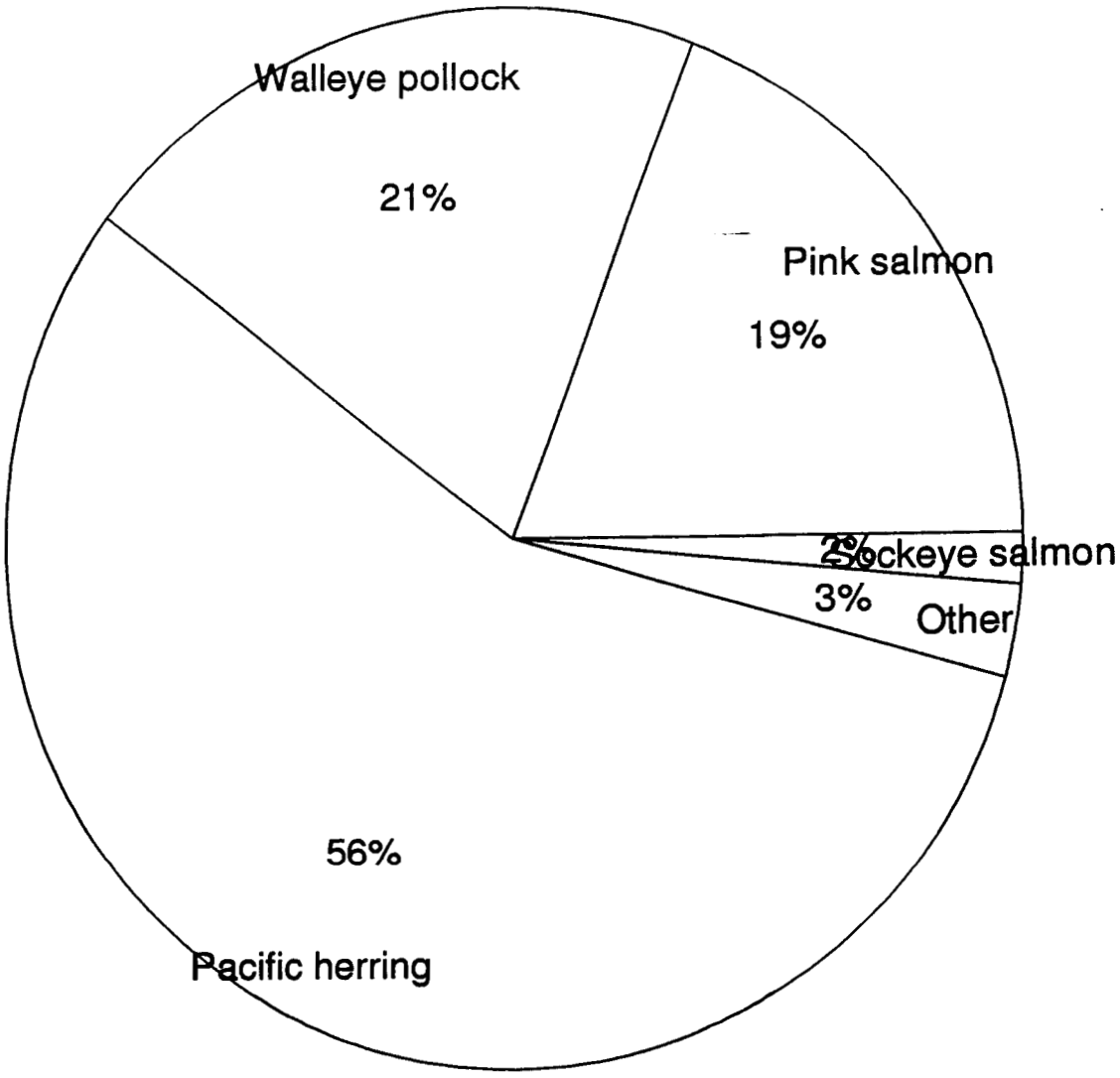

Figure 11. Species composition of purse seine hauls in May and June 1994, northwest Prince William Sound.

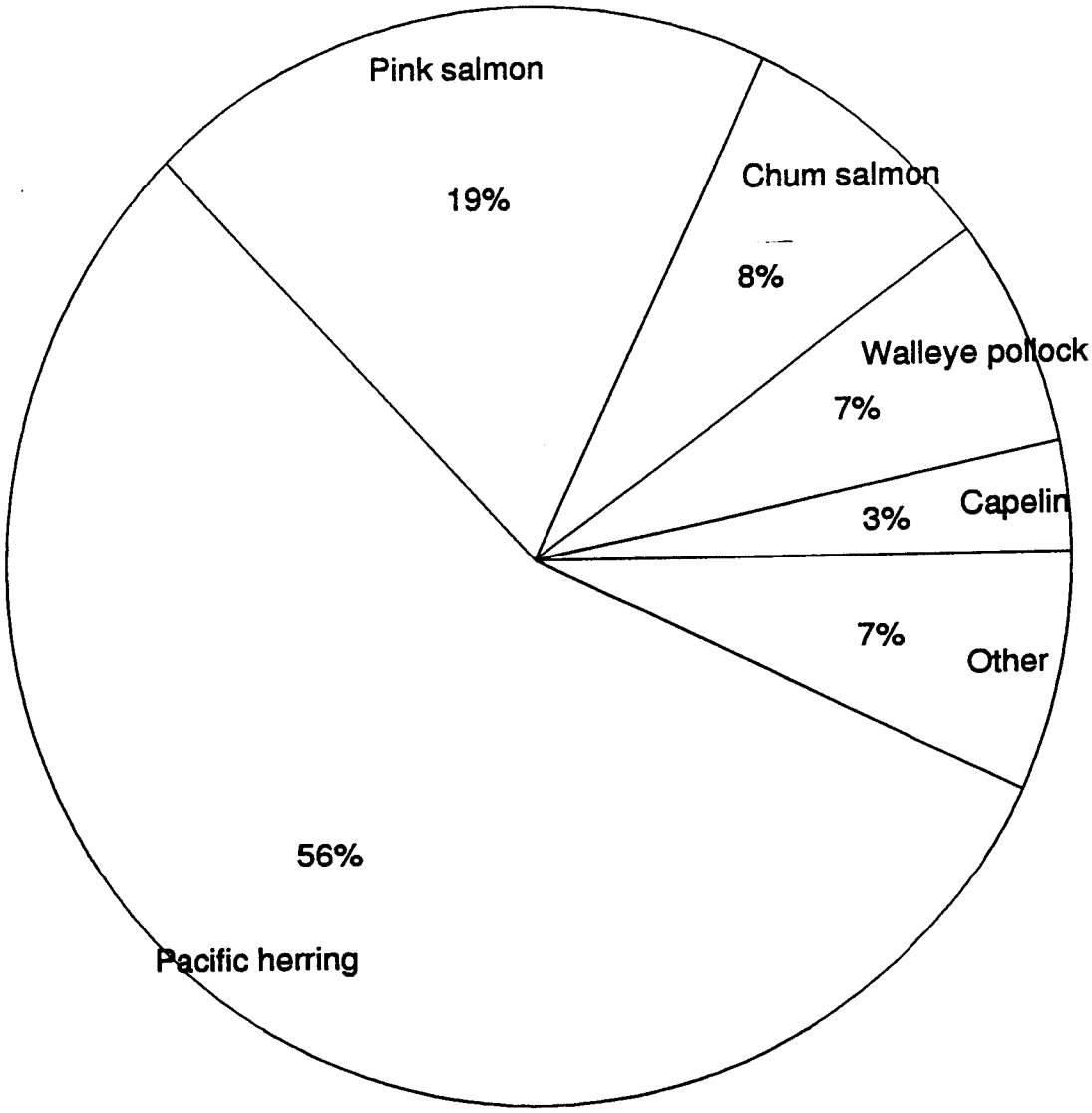

Figure 12. Species composition of purse seine sets made in May and June 1995, northwest Prince William Sound.

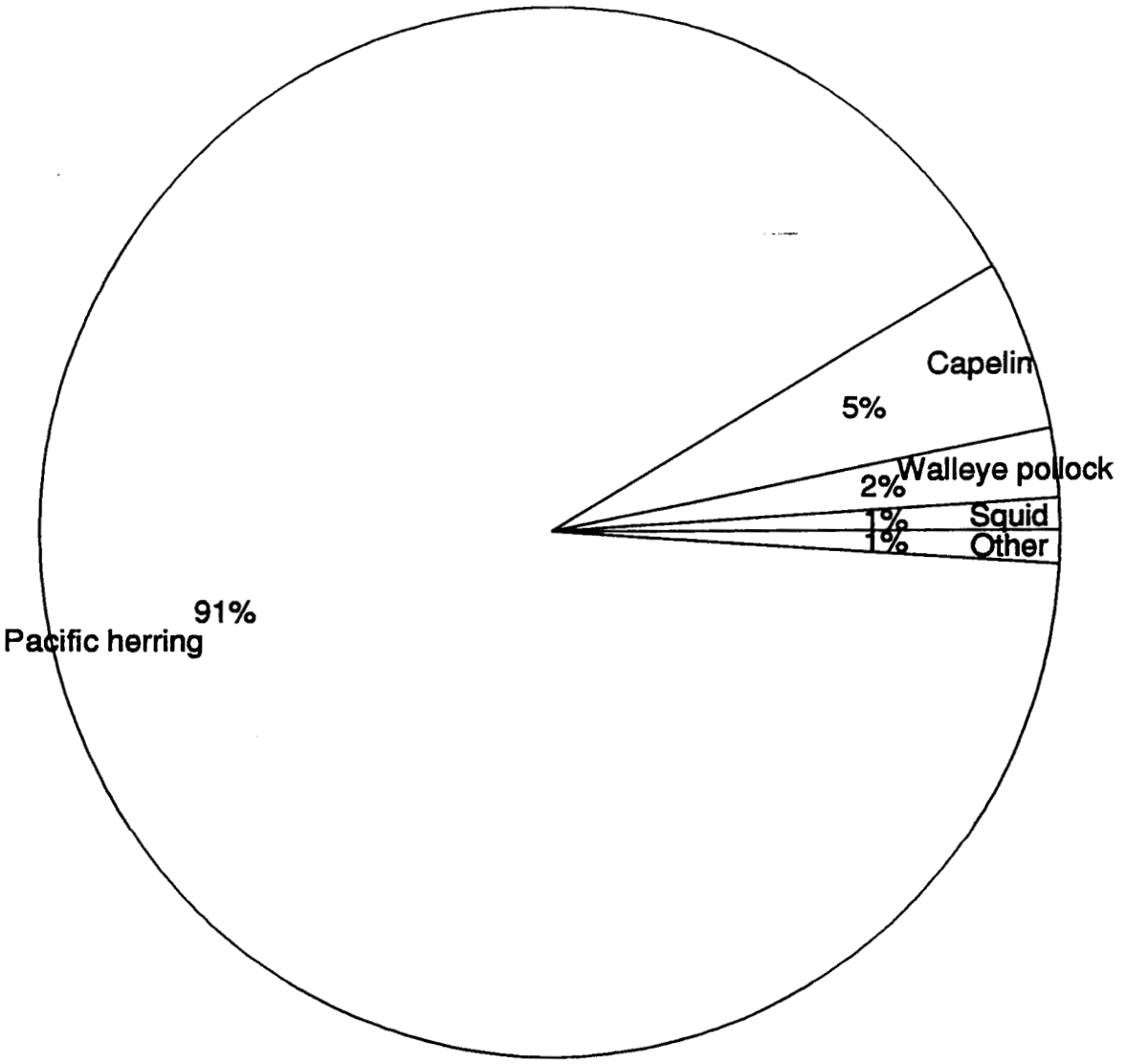

Figure 13. Species composition of pair trawl hauls made in May and June of 1995, northwest Prince William Sound.

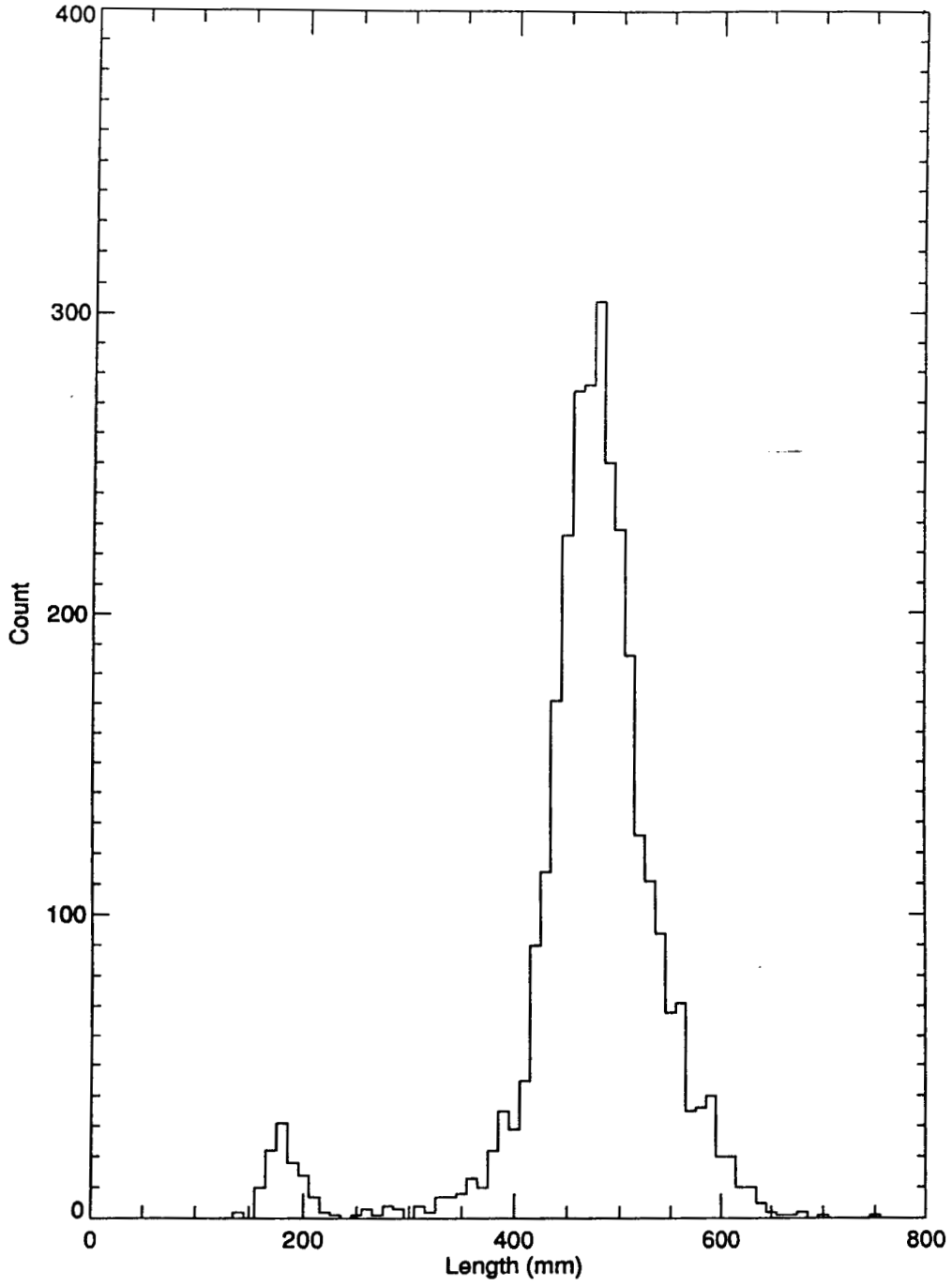

Figure 14. Length frequency of pollock in midwater trawl catch, May - June 1994, northwest Prince William Sound.

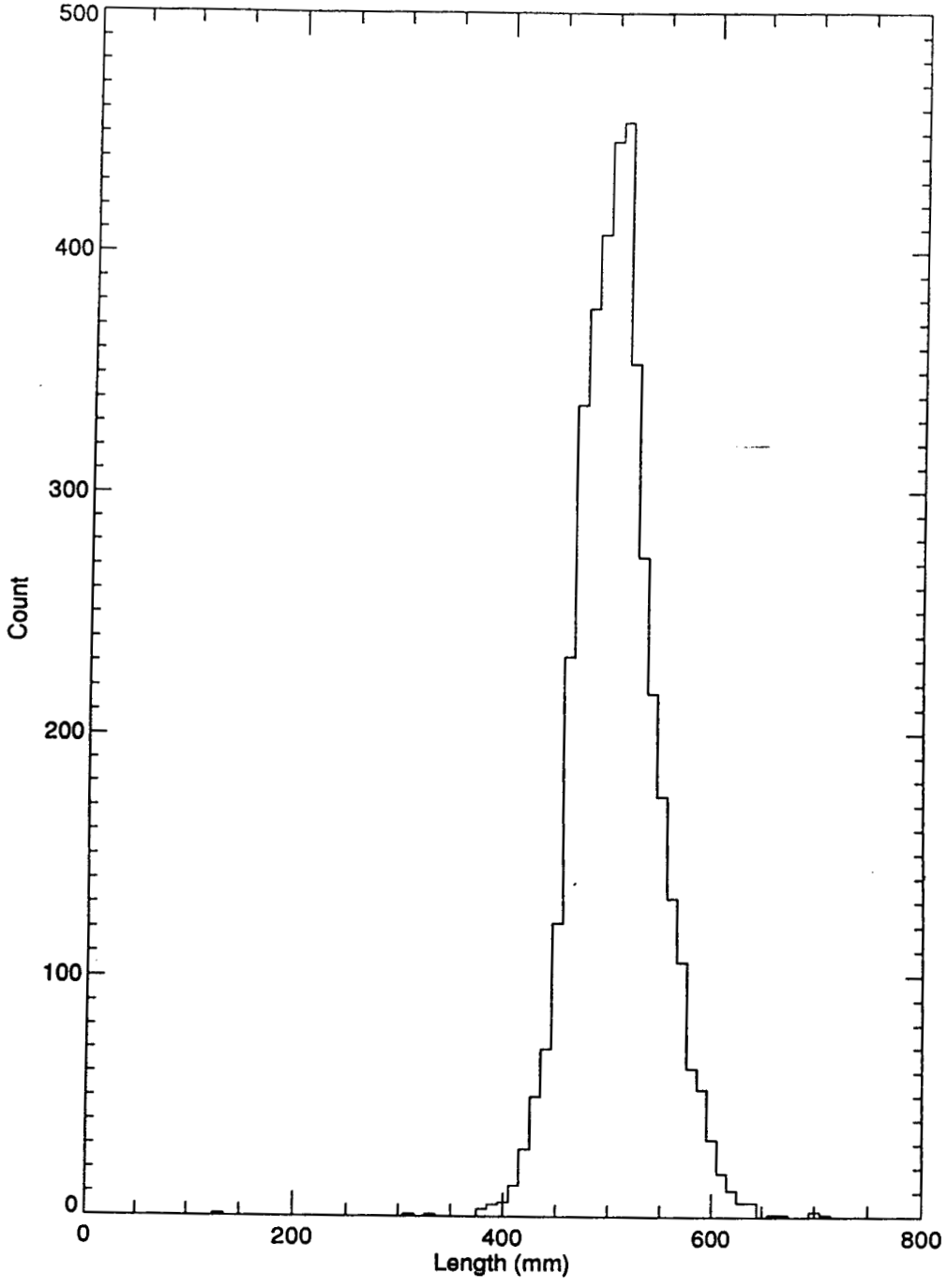

Figure 15. Length frequency of pollock in midwater trawl catch, May-June 1995, northwest Prince William Sound.

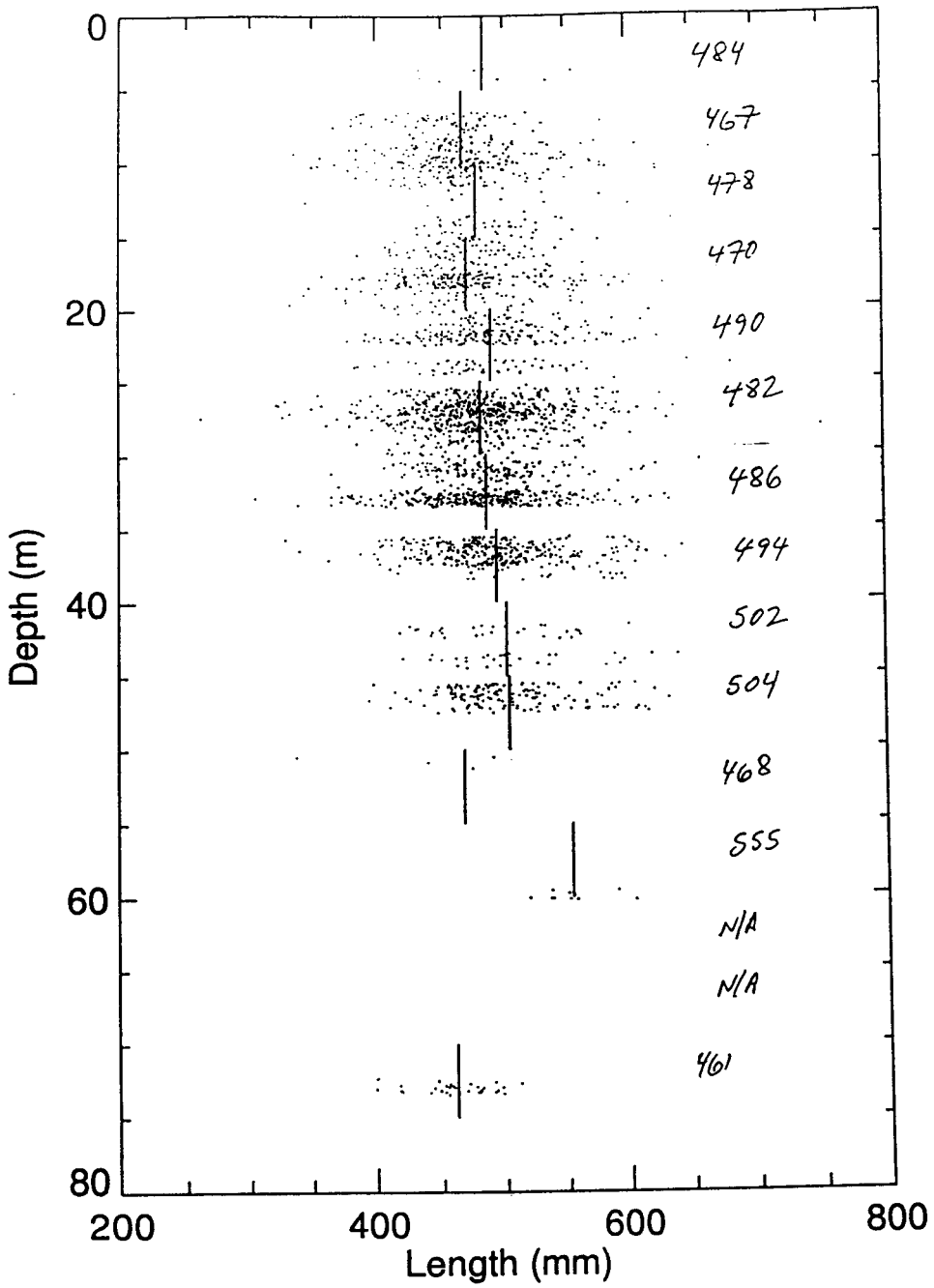

Figure 16. Length of pollock by depth in the midwater trawl catch, May-June 1995, northwest Prince William Sound

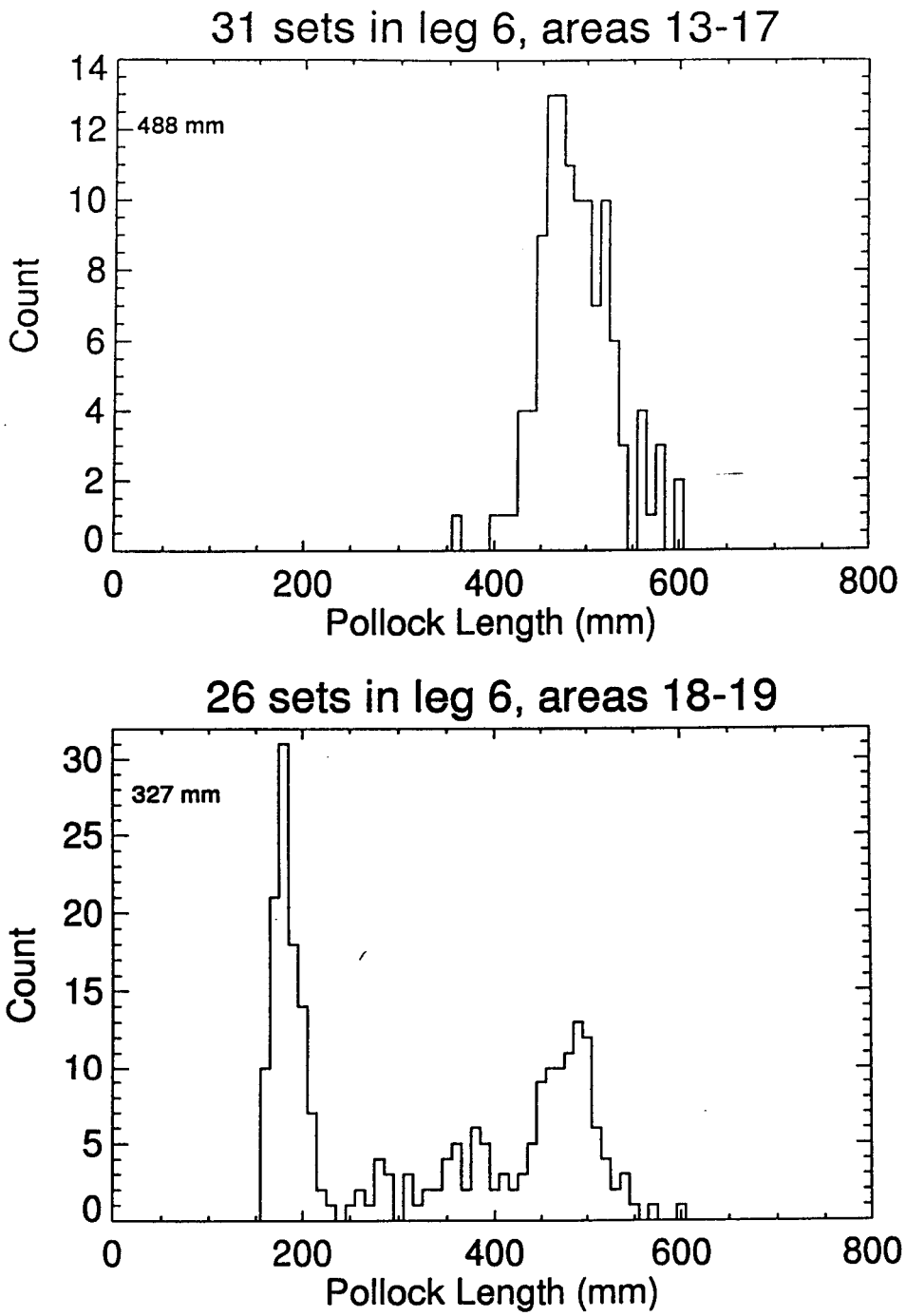

Figure 17a & b. Length frequency of pollock in the midwater trawl catch: (a) northern and central, and (b) southern areas of western Prince William Sound, July 1994.

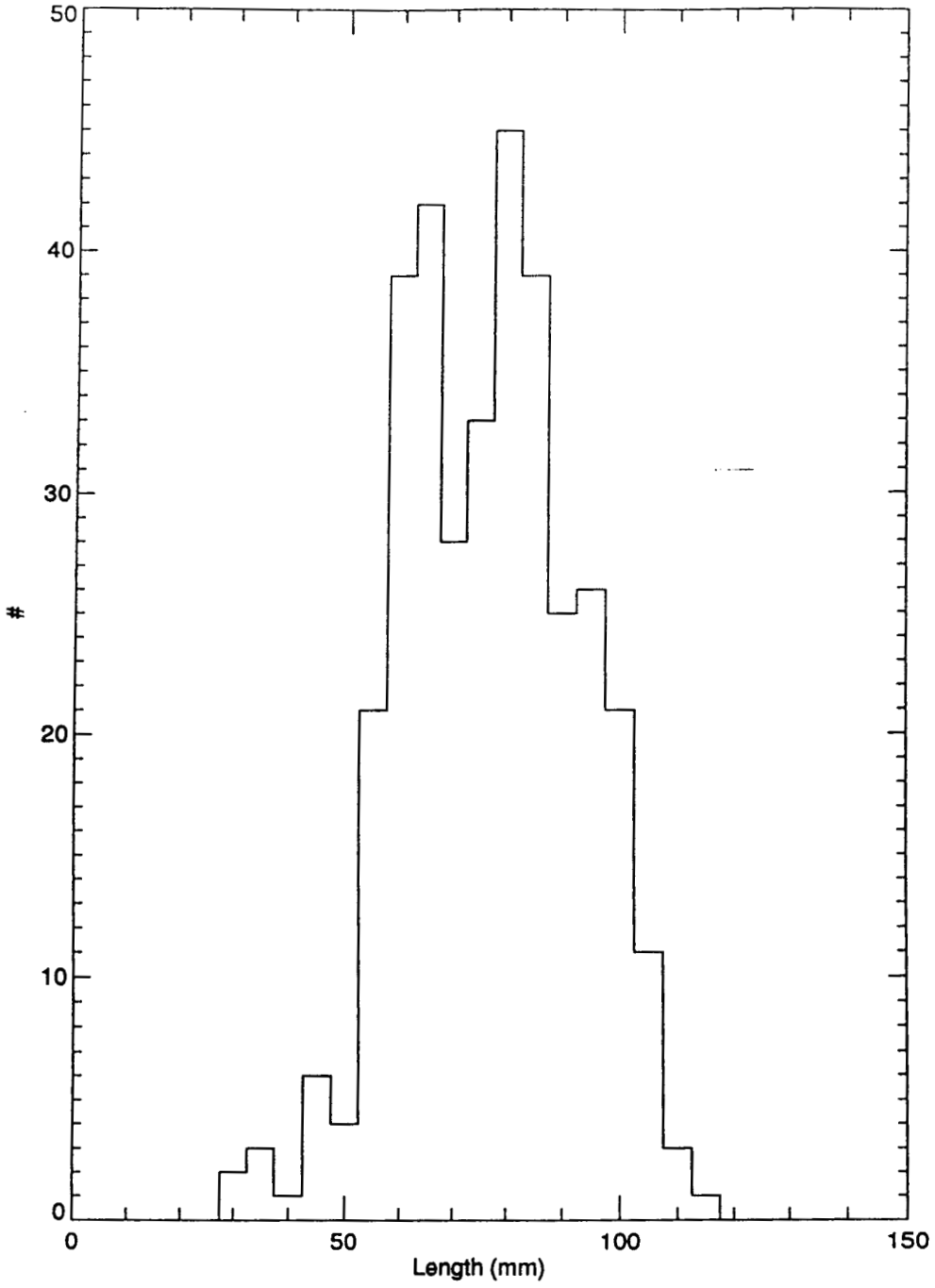

Figure 18. Length frequencies of northern smoothtongue in the midwater trawl catch in May-June 1994, northwest Prince William Sound.

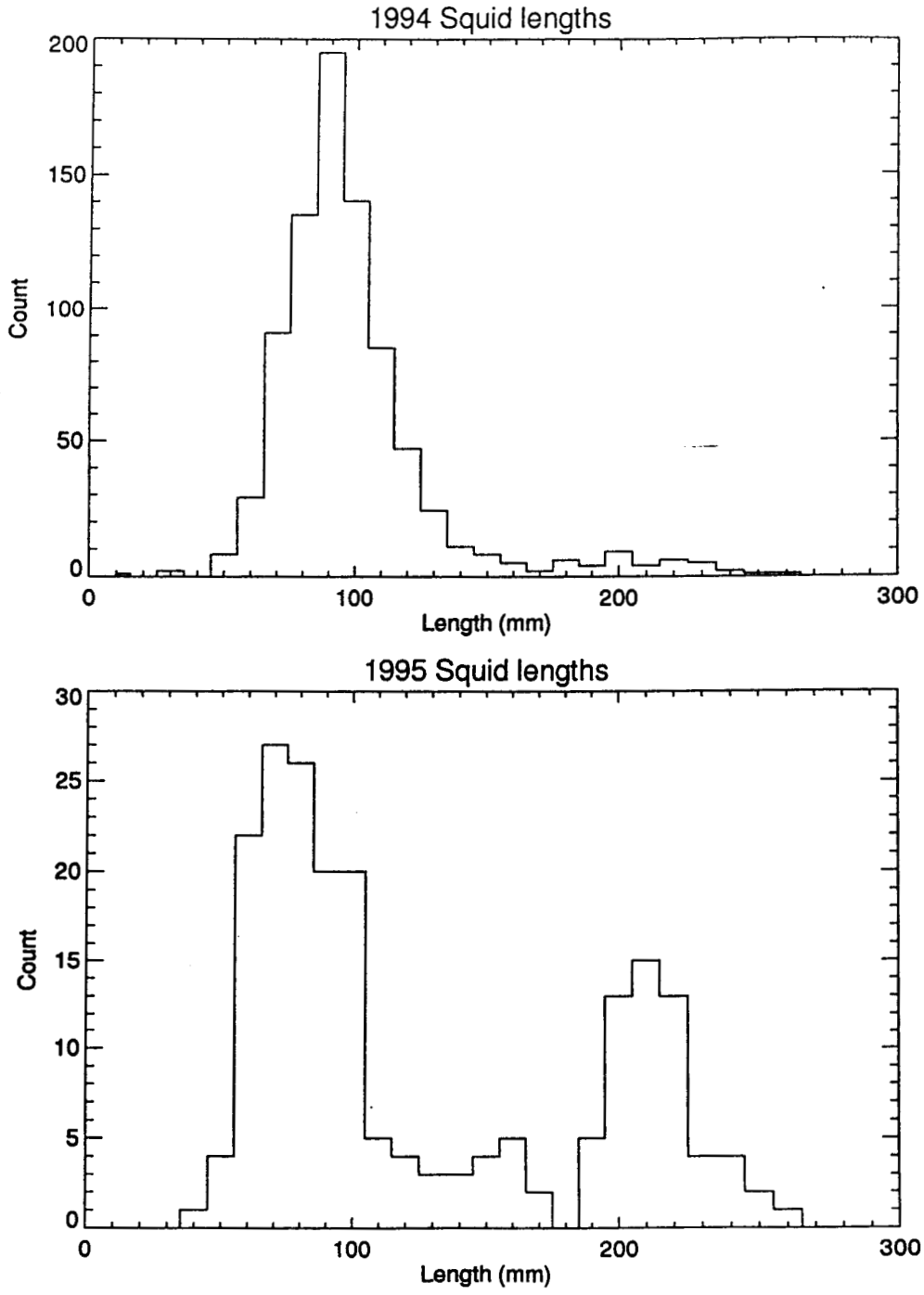

Figure 19. Length frequencies of squid in the midwater trawl catch in May-June 1994(a) and 1995(b), northwest Prince William Sound.

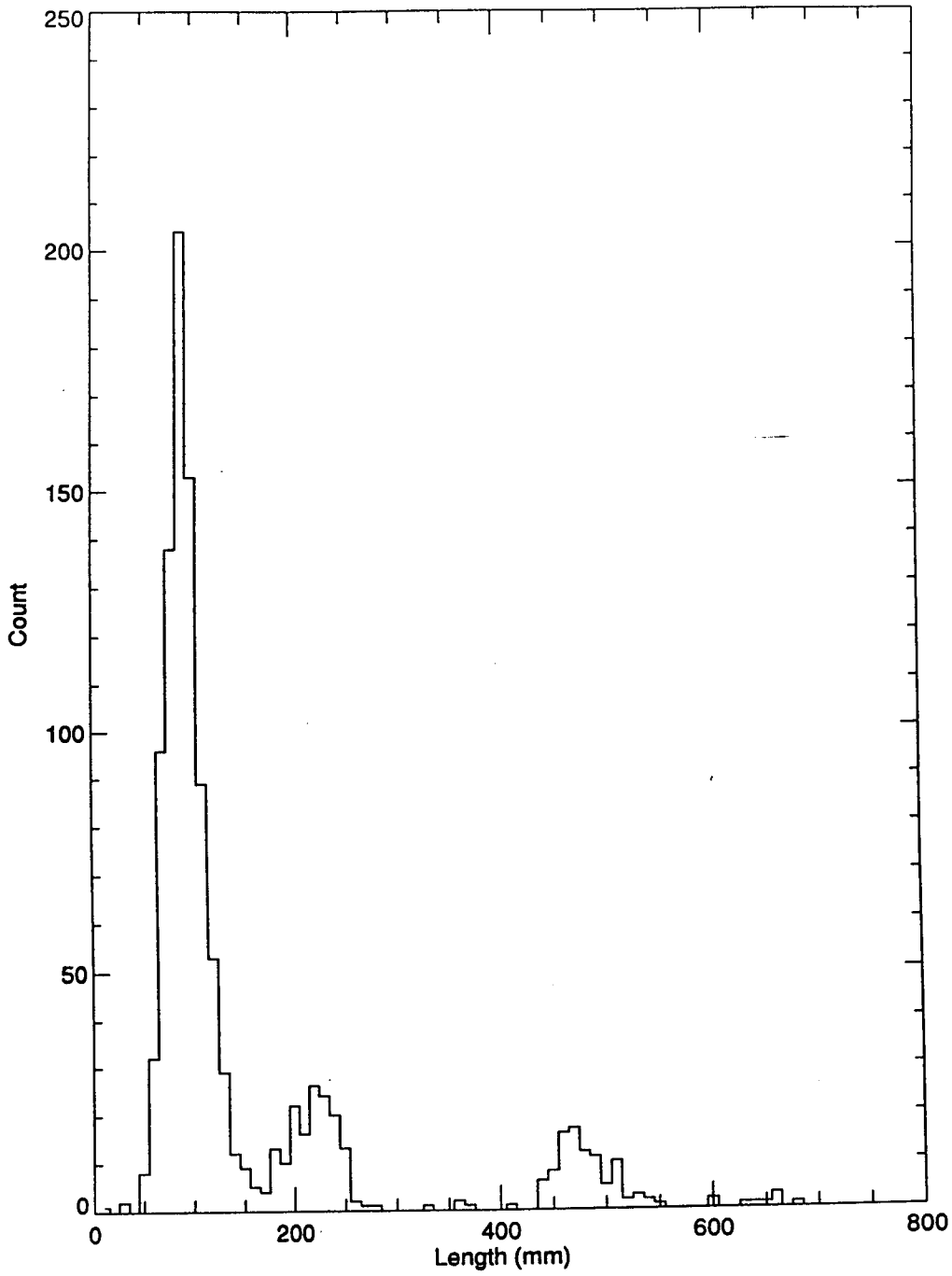

Figure 20. Length frequencies of non-pollock nekton caught by the midwater trawl in May-June 1994, northwest Prince William Sound.

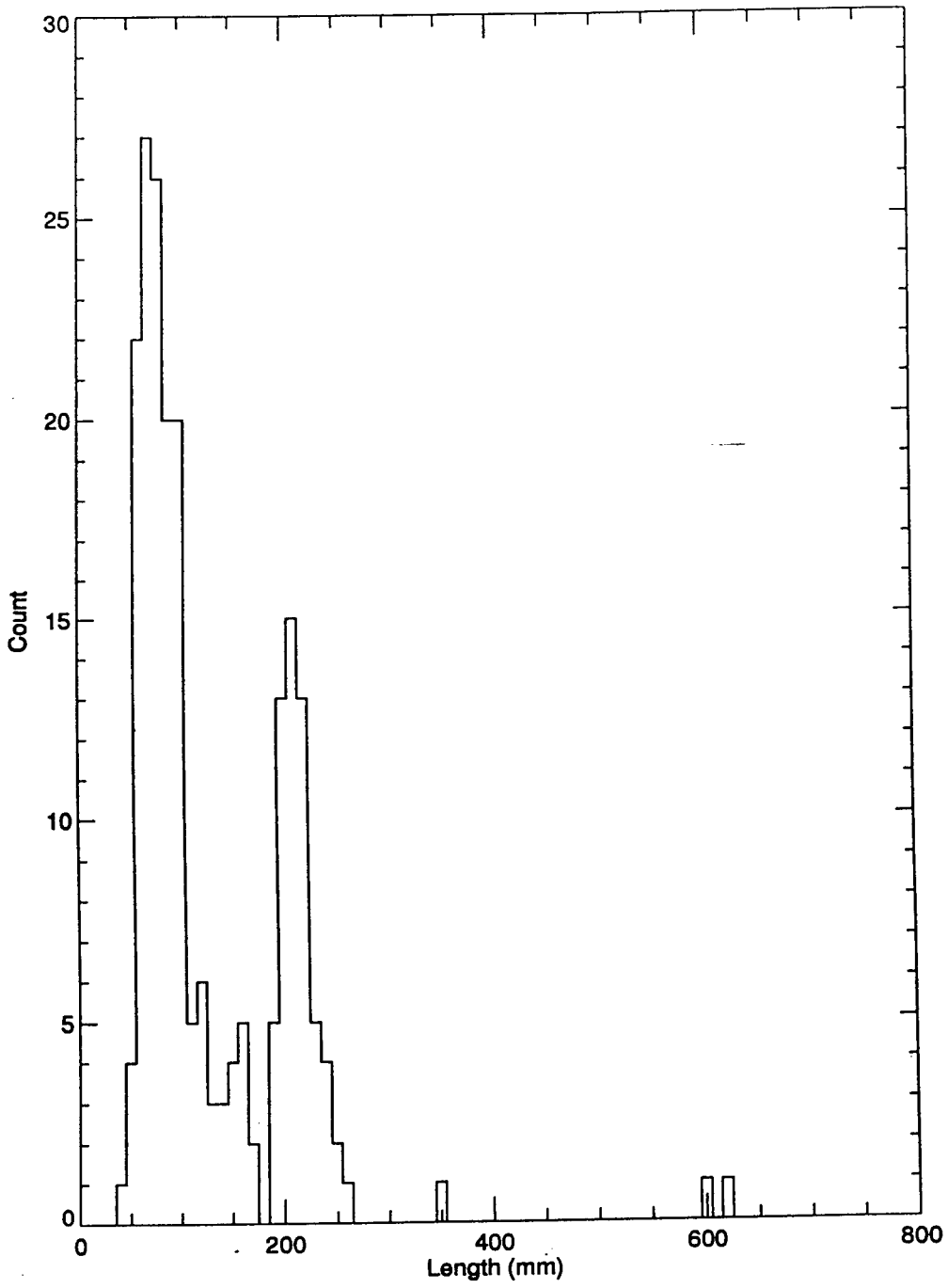

Figure 21. Length frequencies of non-pollock nekton captured by the midwater trawl in May-June 1995, northwest Prince William Sound.

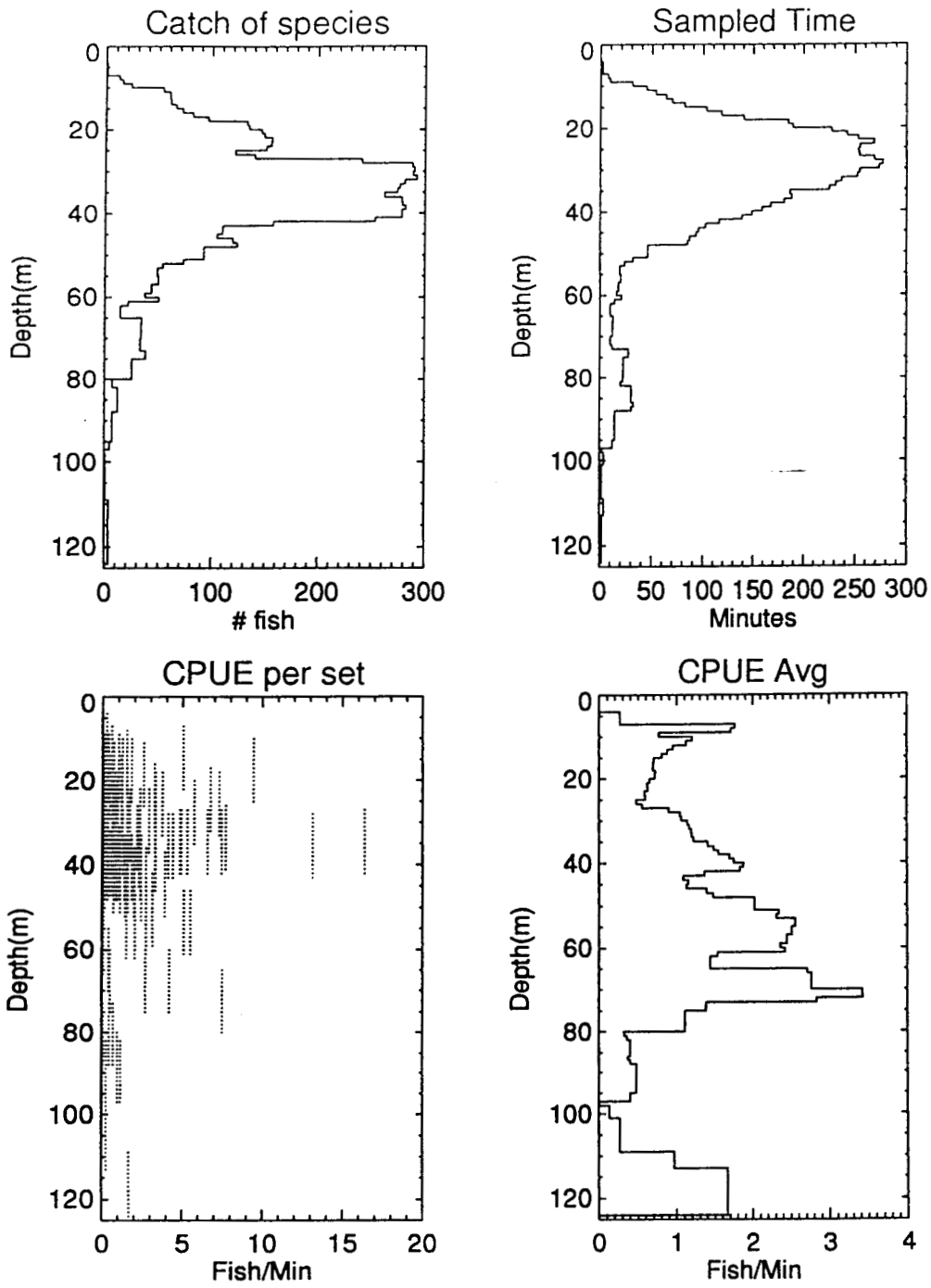

Figure 22. Vertical distribution of adult pollock from midwater trawl catches in 1994, northwest Prince William Sound.

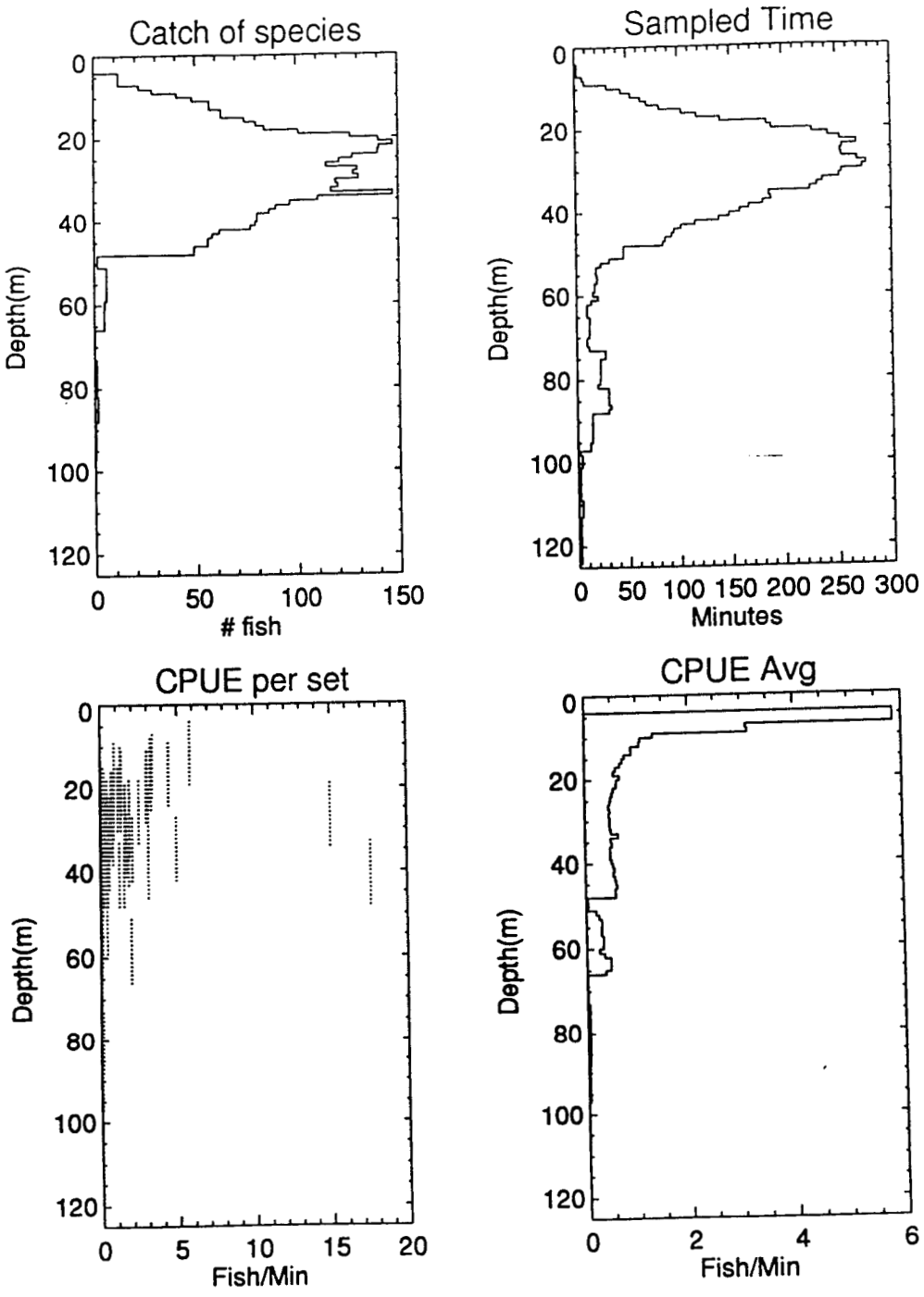

Figure 23. Vertical distribution of squid from midwater trawl catches in 1994, northwest Prince William Sound.

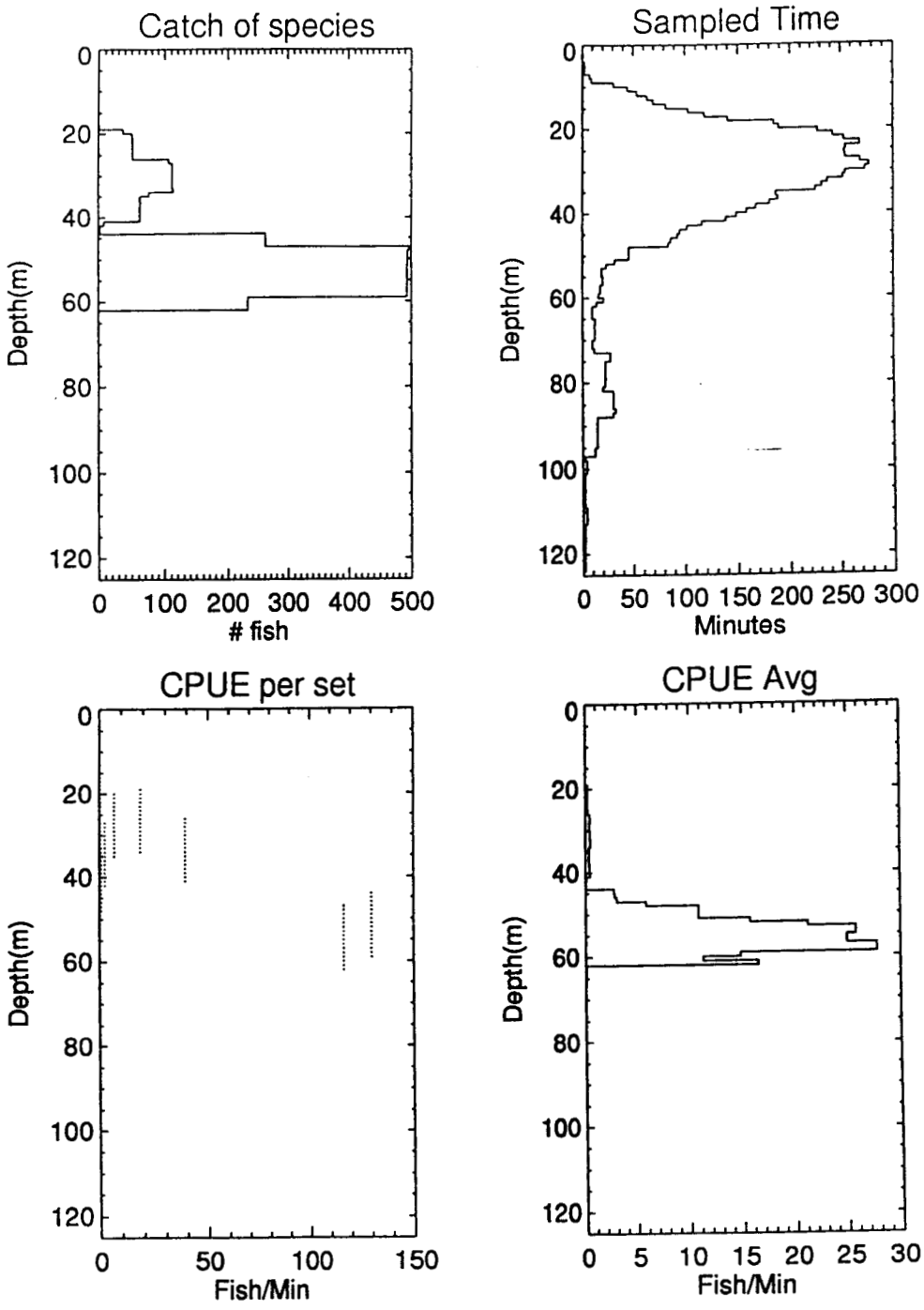

Figure 24. Vertical distribution of northern smoothtongue from midwater trawl catches in 1994, northwest Prince William Sound.

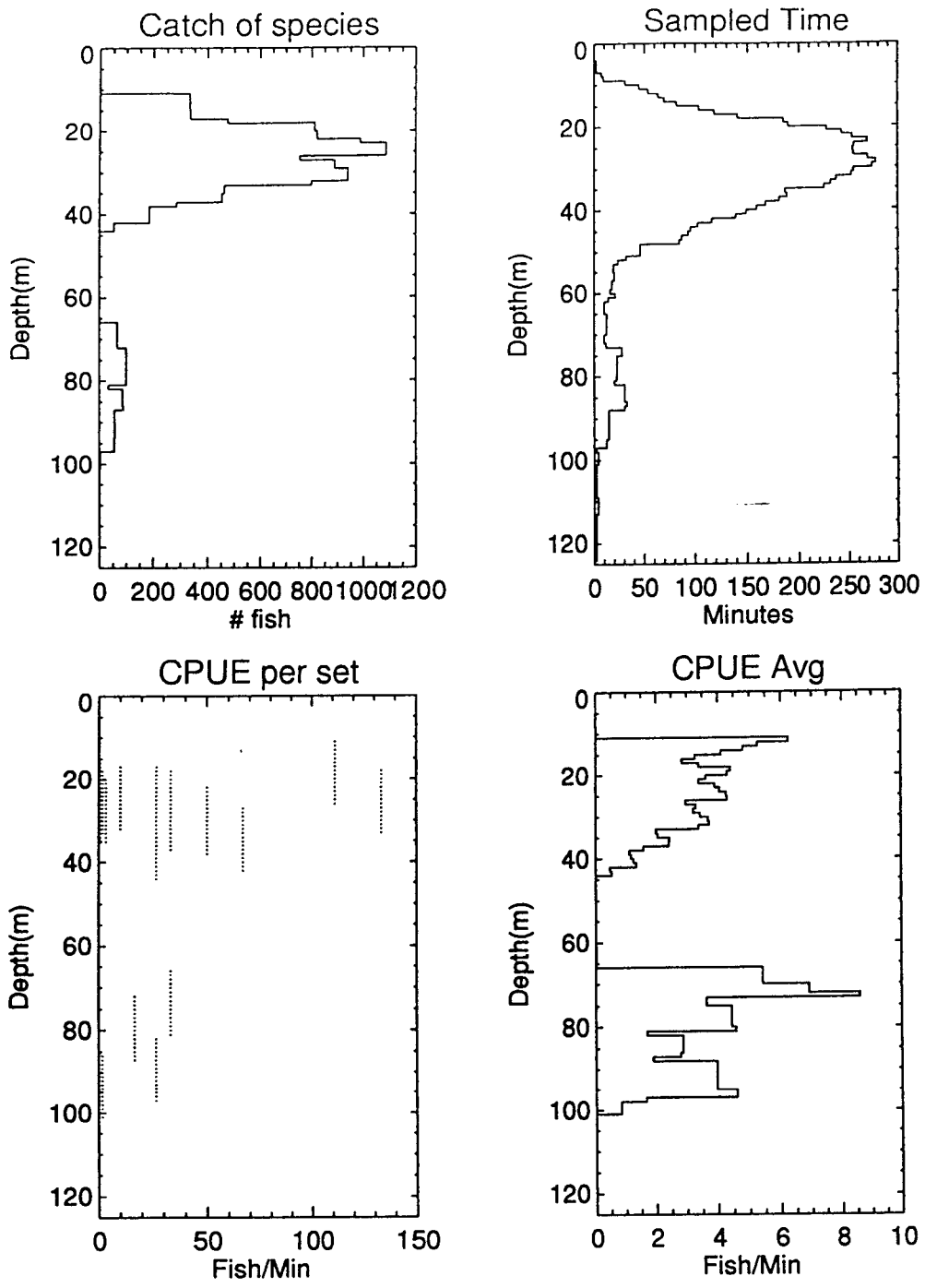

Figure 25. Vertical distribution of juvenile pollock from midwater trawl catches in 1994, northwest Prince William Sound.

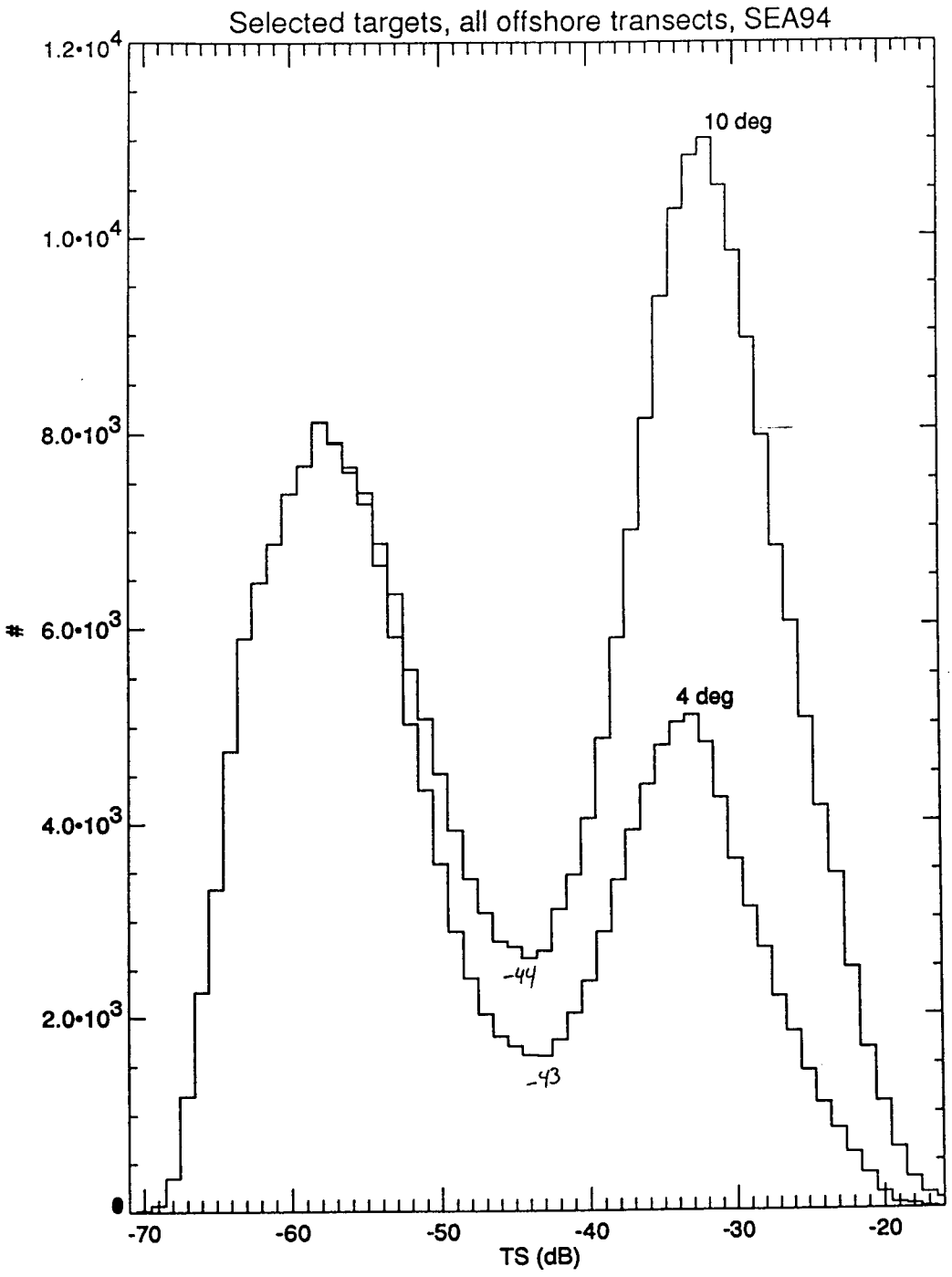

Figure 26. Distributions of acoustic target strengths for offshore surveys in 1995.

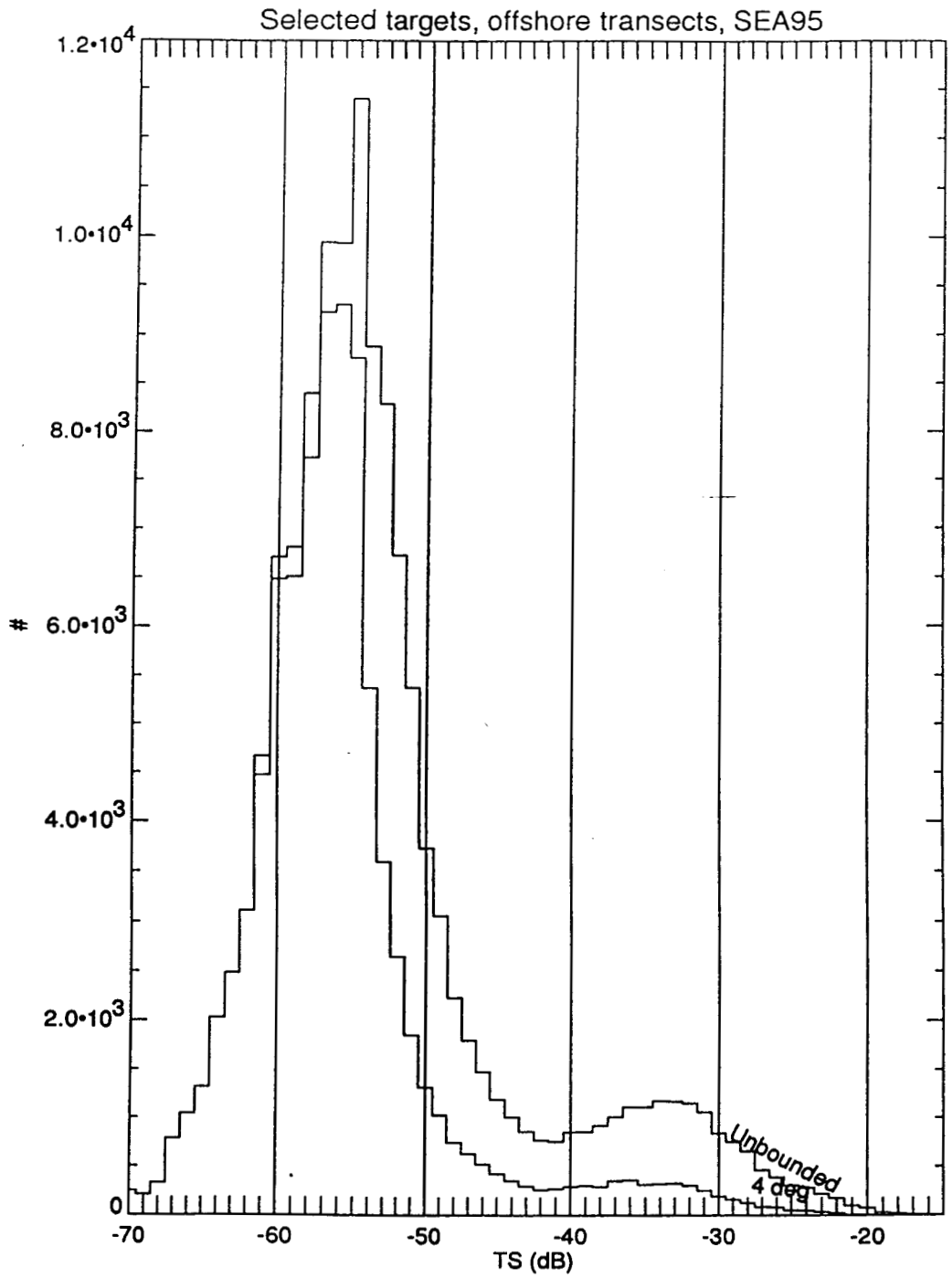

Figure 27. Distributions of acoustic target strengths for offshore surveys in 1995.

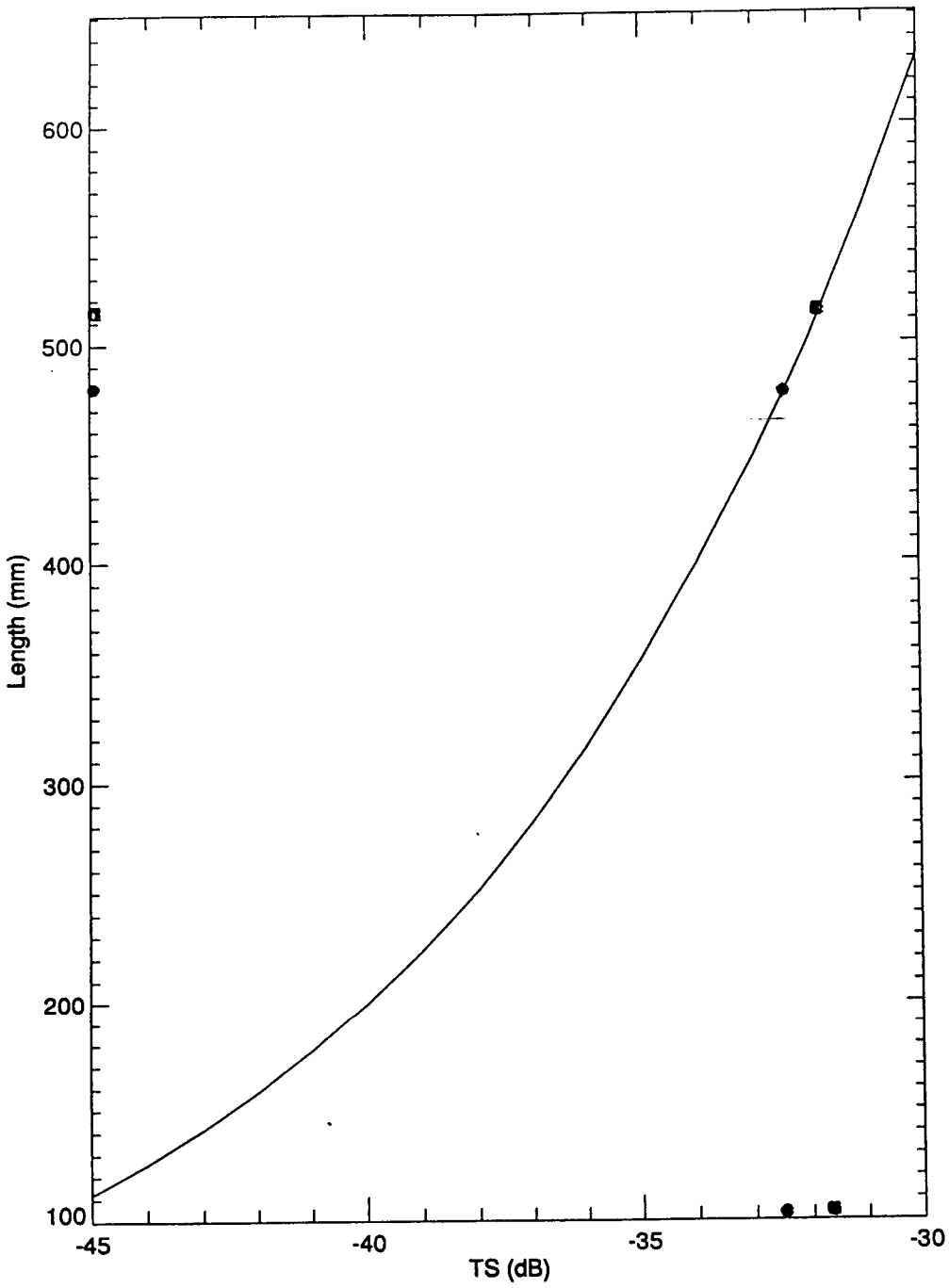

Figure 28. Target strengths of pollock by length: -20 Log L-66 (Traynor and Erenberg 1979). Circles = 1994, Squares = 1995.

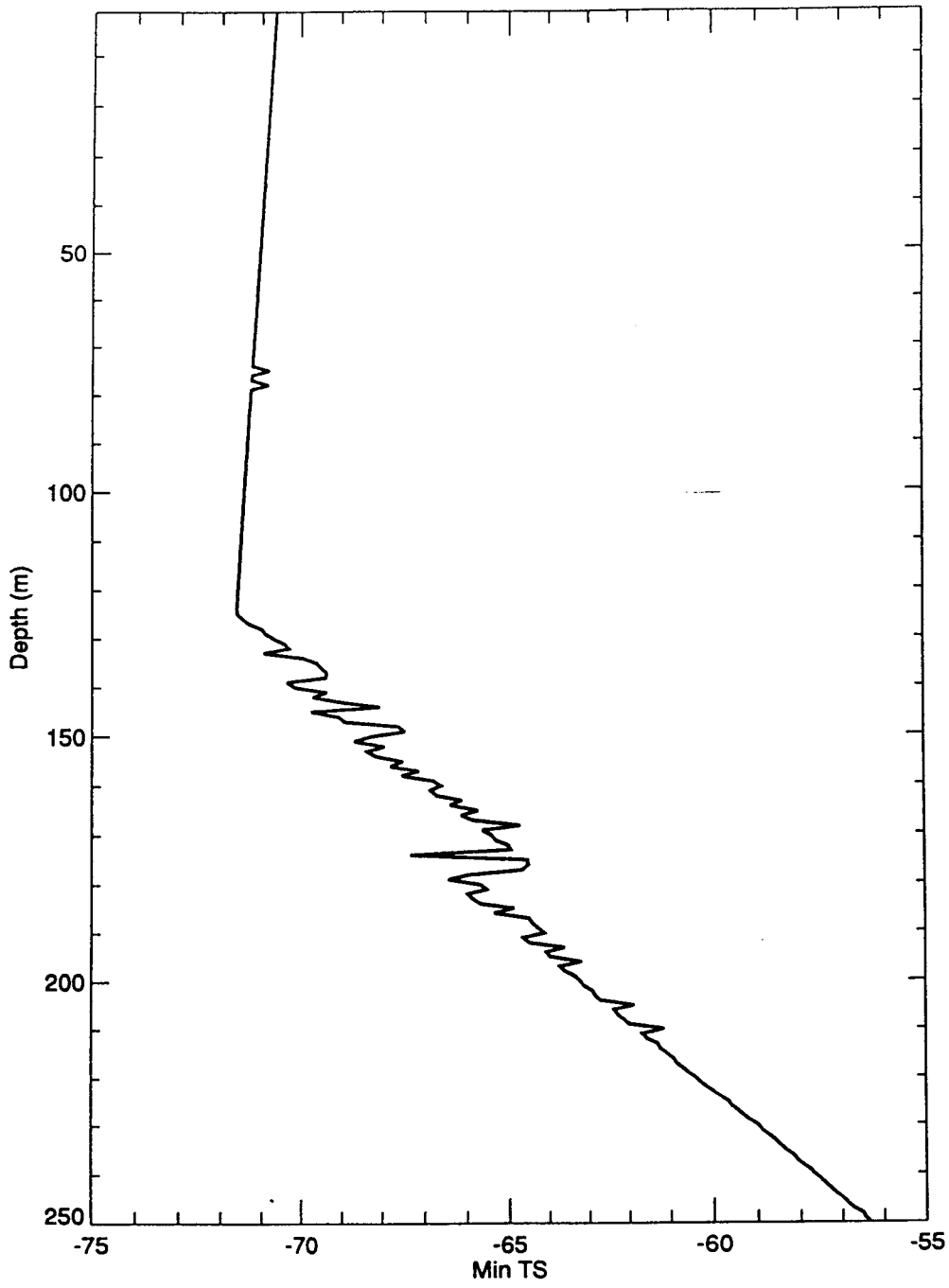

Figure 29. Minimum targets detected by depth in 1994 on offshore surveys, with a 120 kHz echosounder.

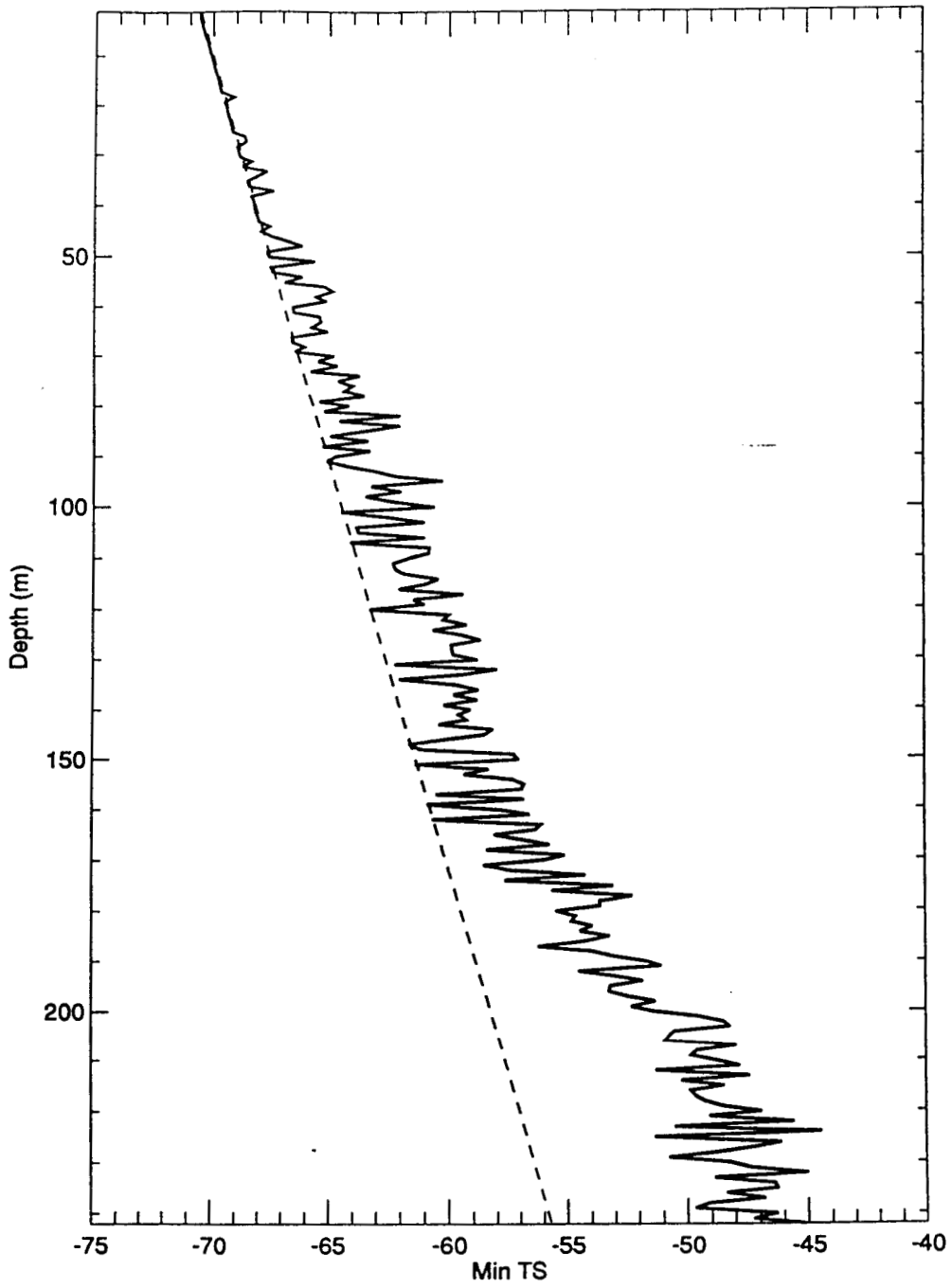

Figure 30. Minimum targets detected by depth in 1995 on offshore surveys, with a 120 kHz echosounder.

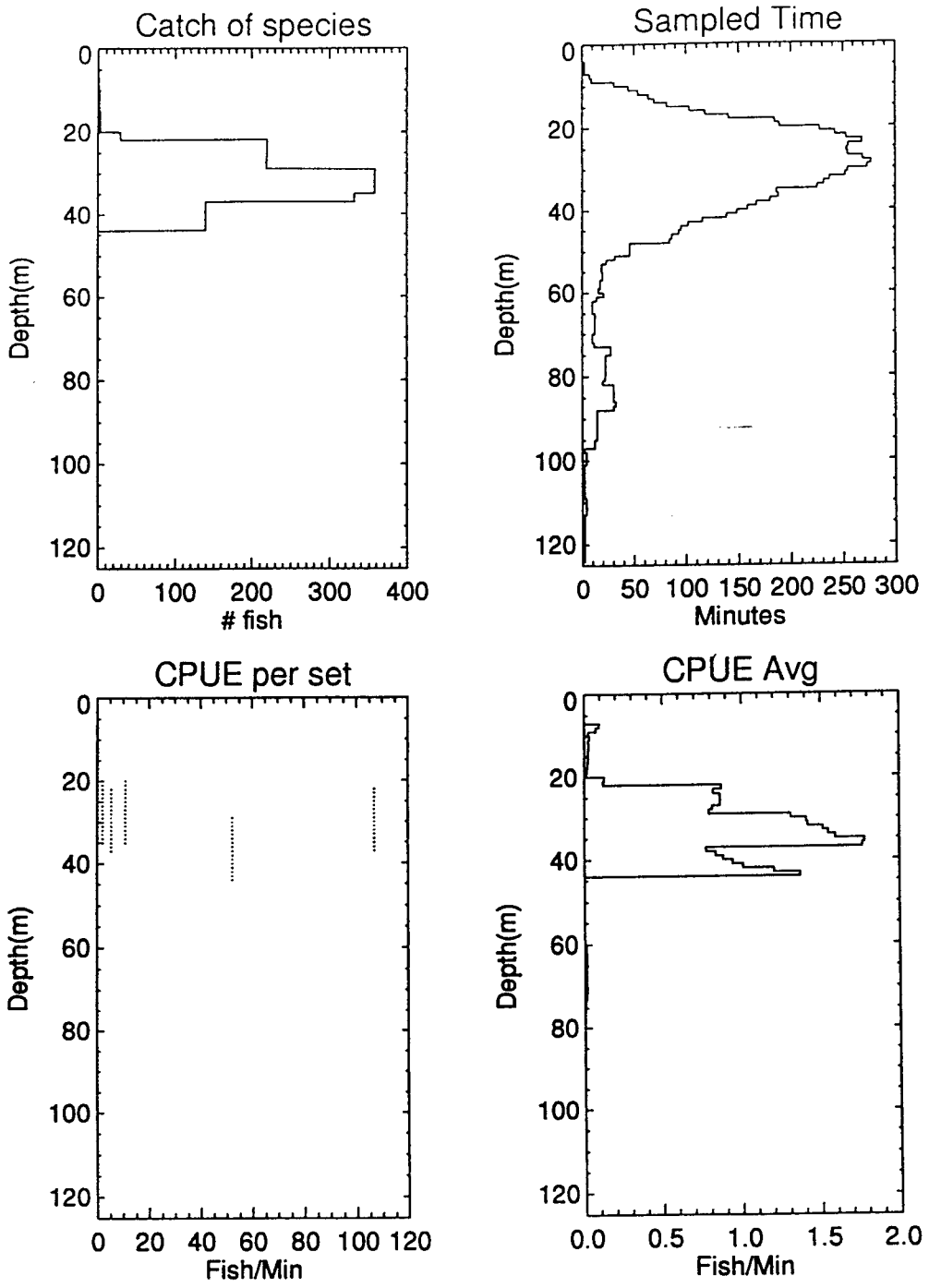

Figure 31. Vertical distribution of Pacific herring from midwater trawl catches in 1994, northwest Prince William Sound.

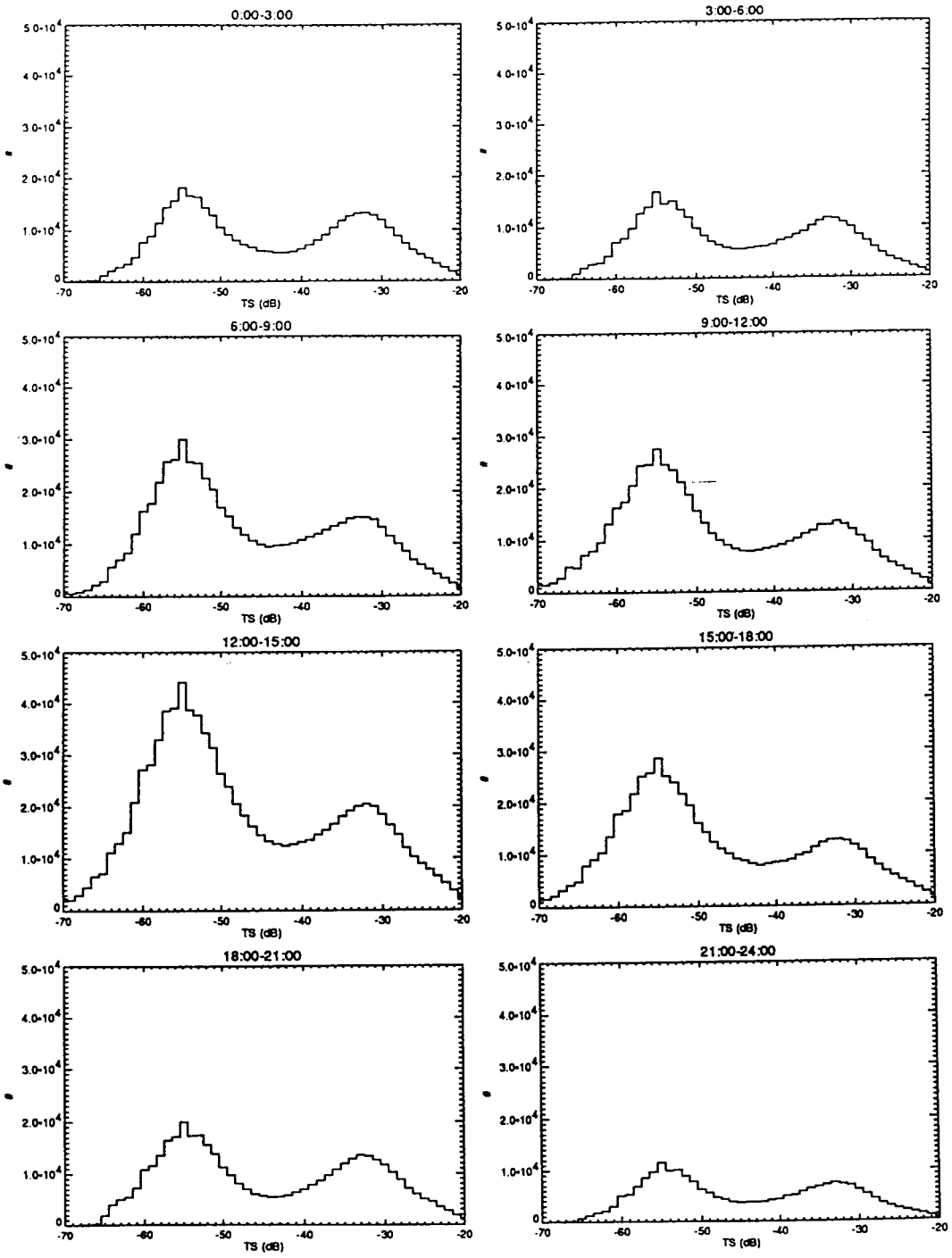

Figure 32. Diel changes in target strength distribution during May-June 1995 surveys, northwest Prince William Sound.

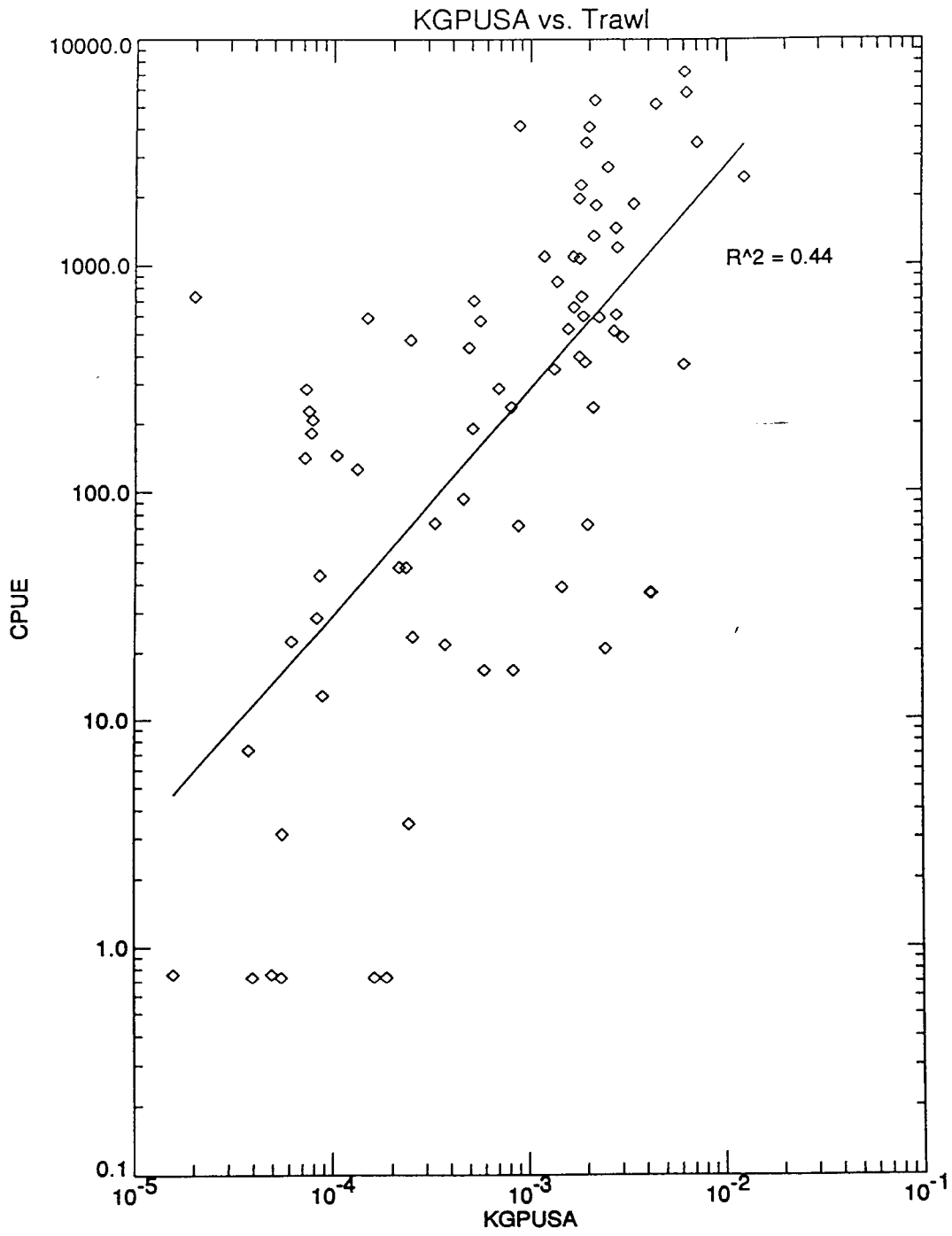

Figure 33. Relationship between echointegration of pollock cells with midwater trawl catches of pollock in 1994, northwest Prince William Sound.

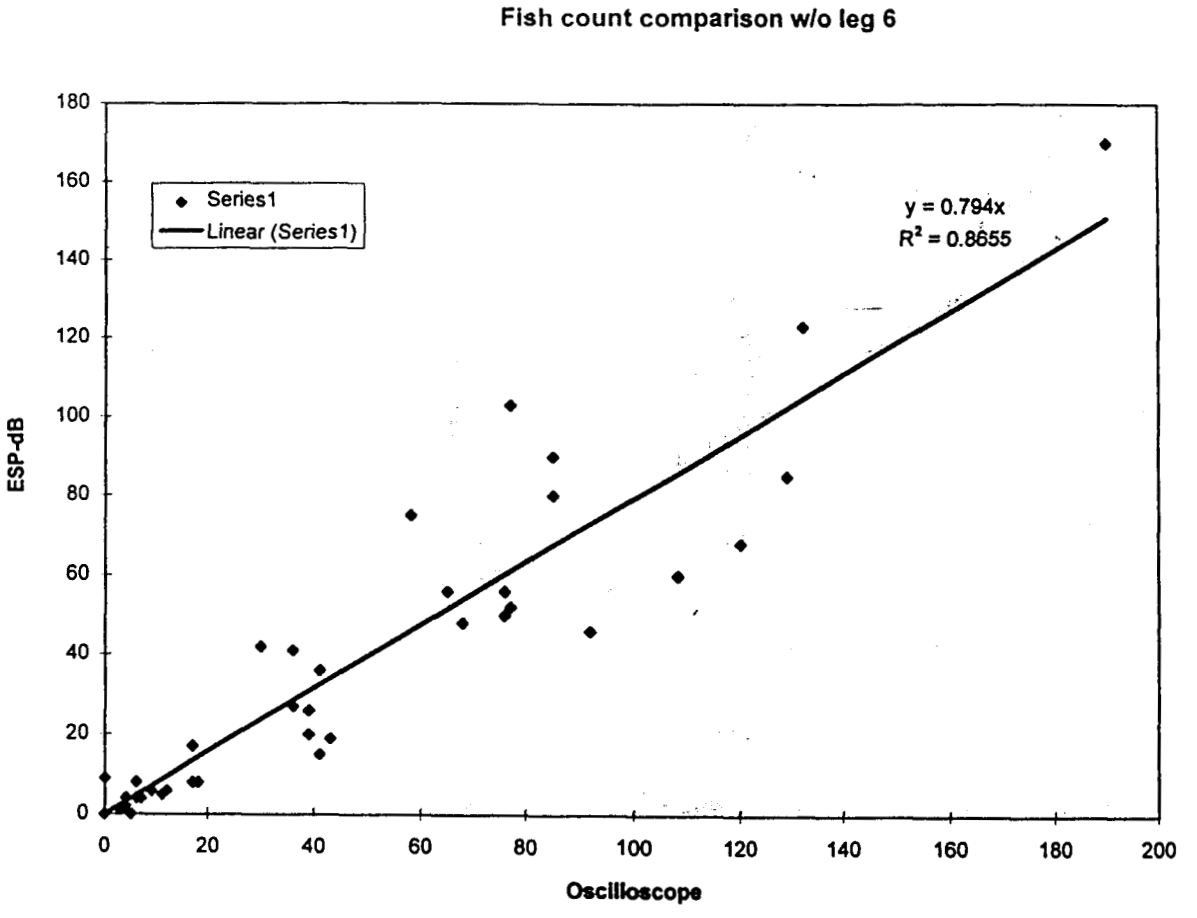

Figure 34. Relationship between manual and auto-counts of pollock on transects from the May-June 1994 surveys of northwest Prince William Sound.

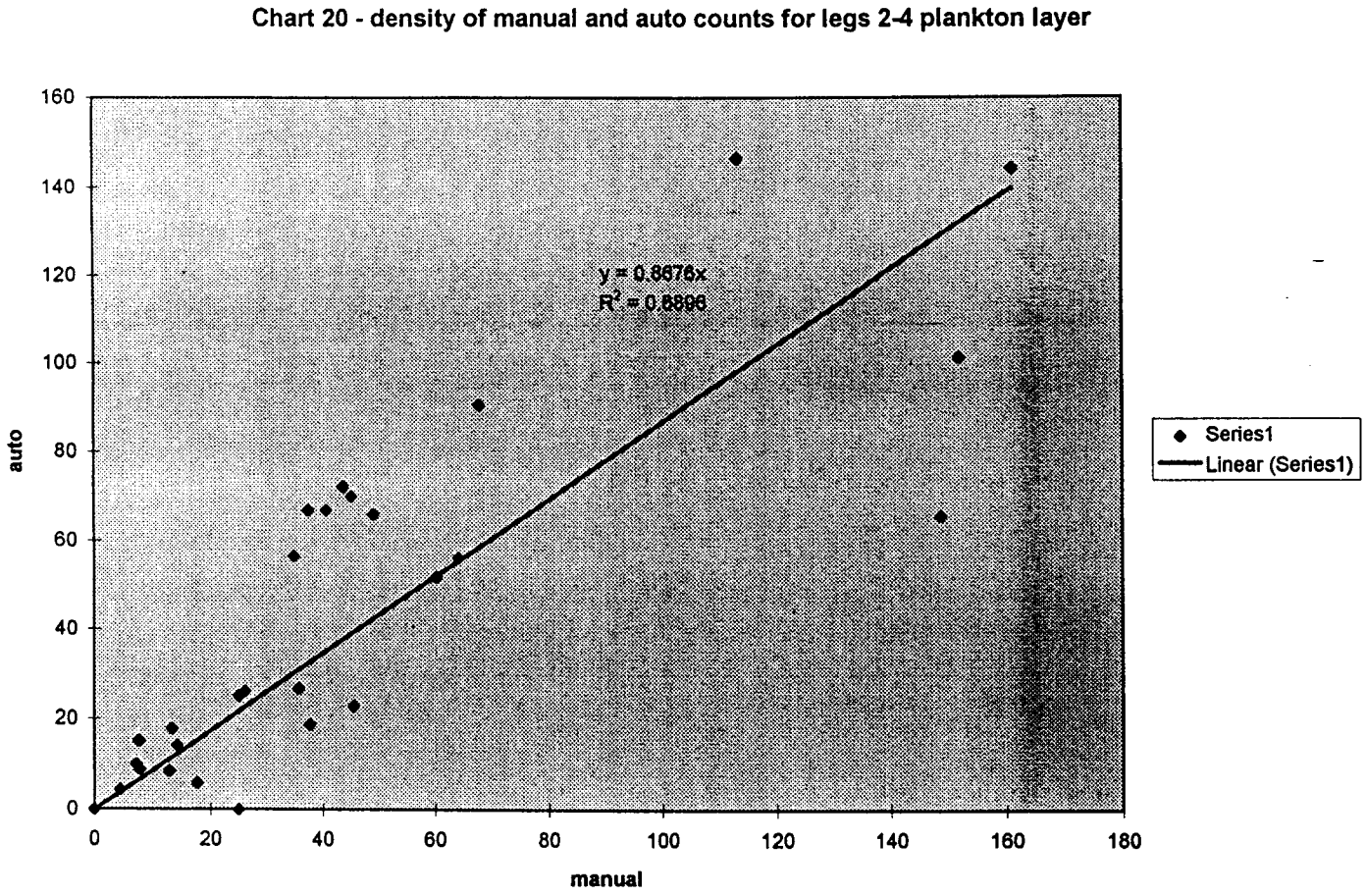

Figure 35. Relationship between manual and auto-counts of pollock in the surface plankton layers. Each point represents a transect conducted in the May-June 1994 survey of northwest Prince William Sound.

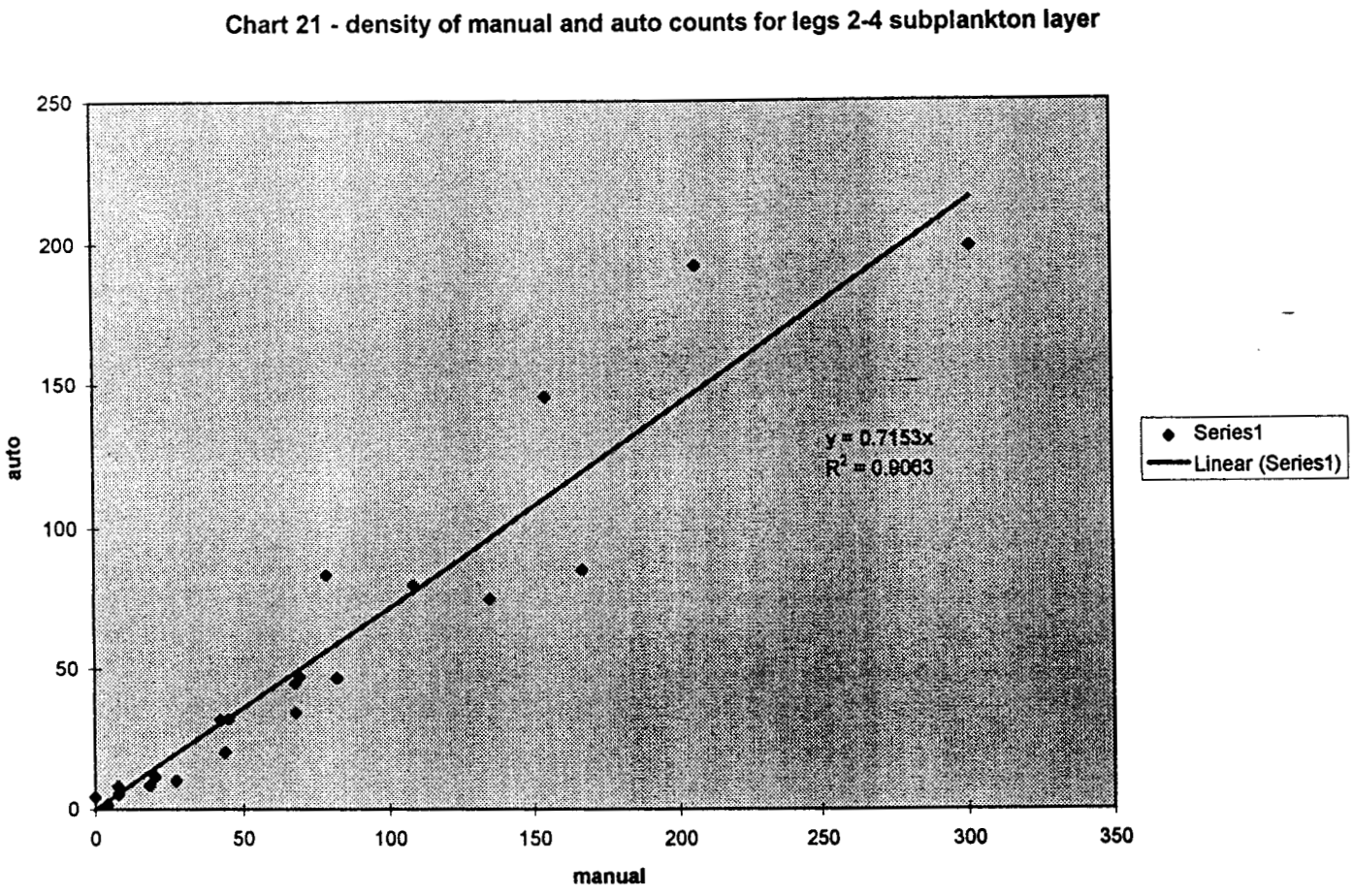

Figure 36. Relationship between manual and auto-counts of pollock beneath the plankton layer. Each point represents a transect conducted in the May-June 1994 survey of northwest Prince William Sound.

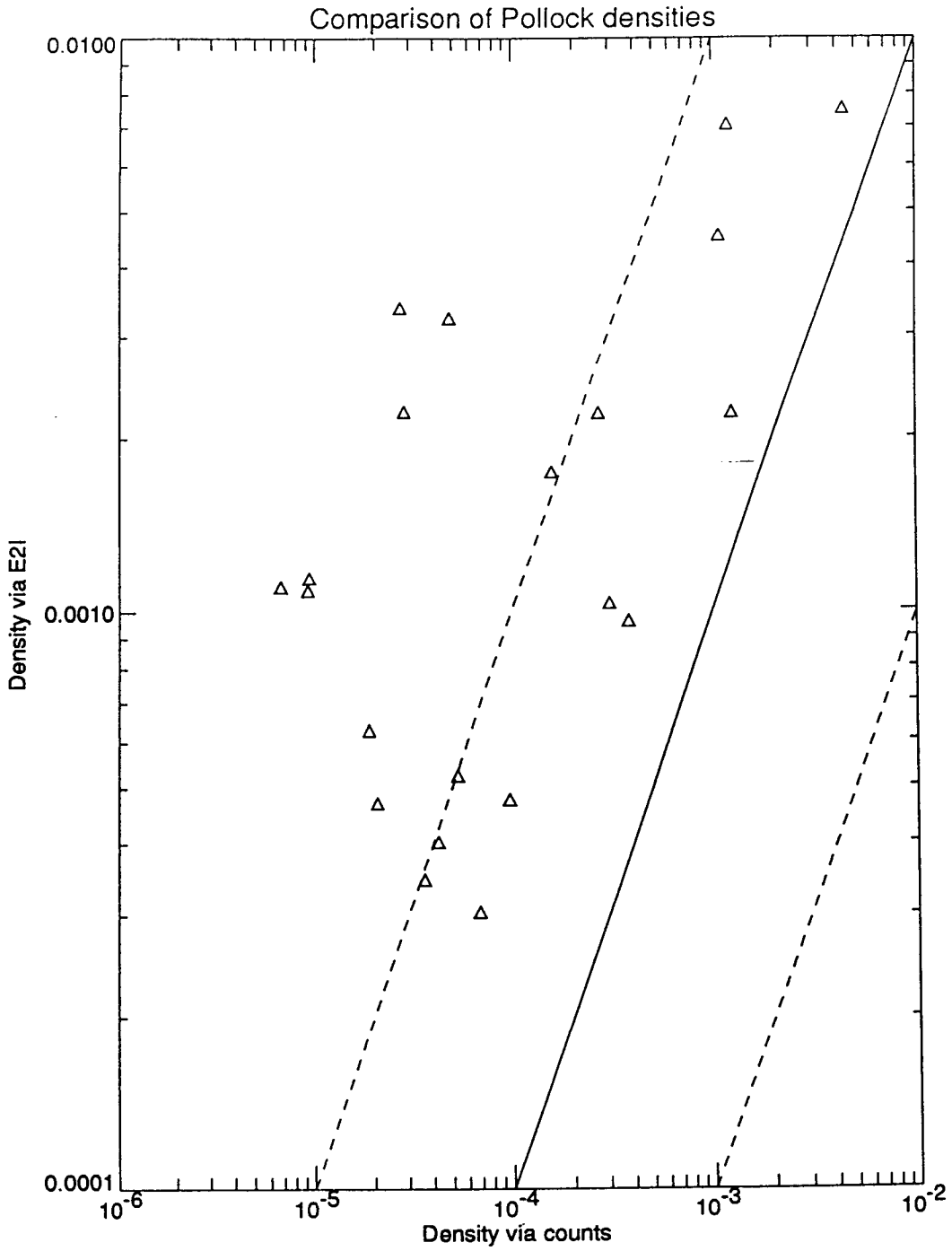

Figure 37. Comparison of the auto-counts of pollock to echointegration for the same cells, May-June 1994, northwest Prince William Sound.

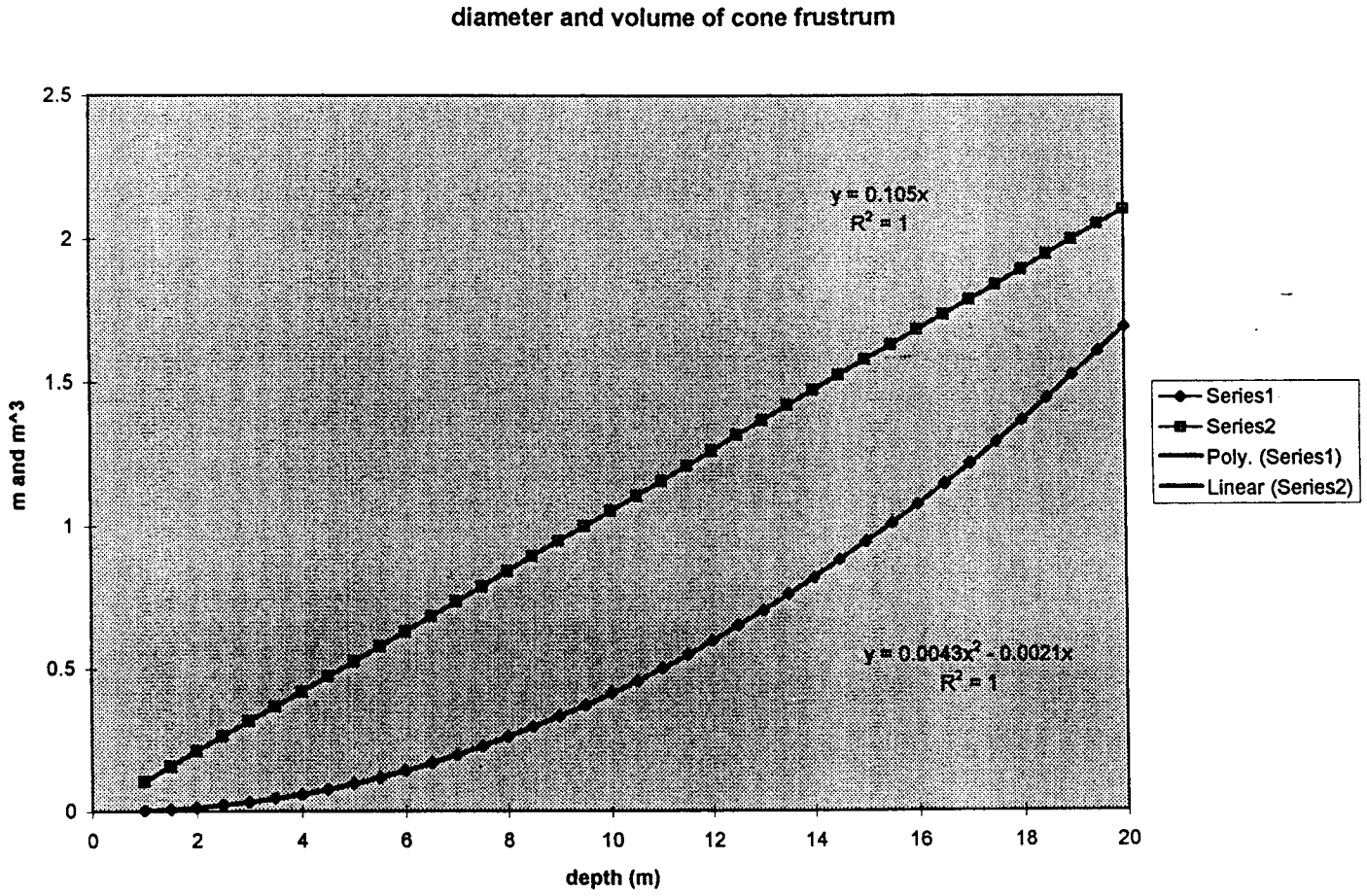

Figure 38. Acoustic sampler volumes and average diameter (of frustrums) by depth assuming a 6° full beam angle.

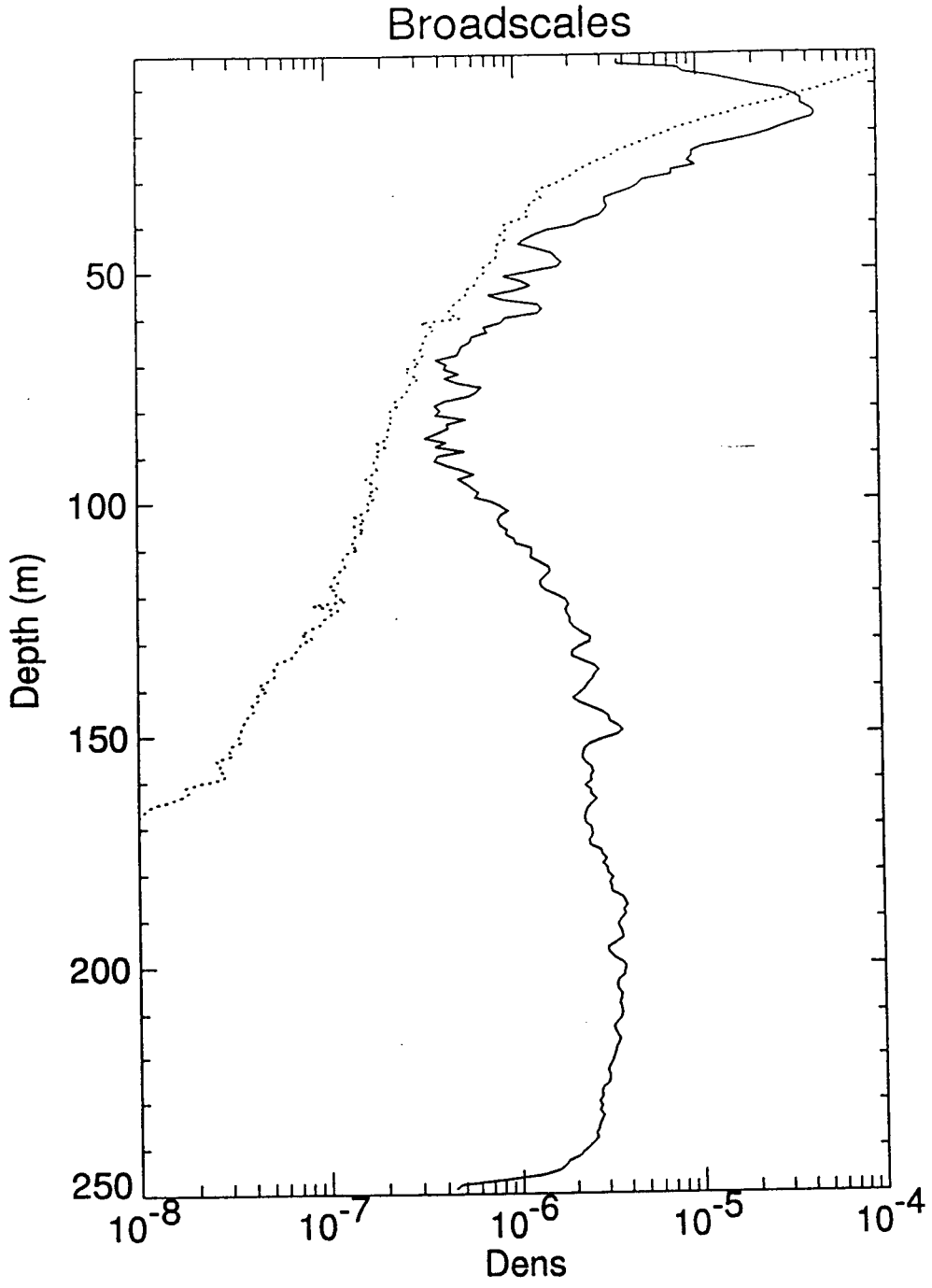

Figure 39. Vertical distribution of pollock and plankton throughout Prince William Sound in May and June, 1996.

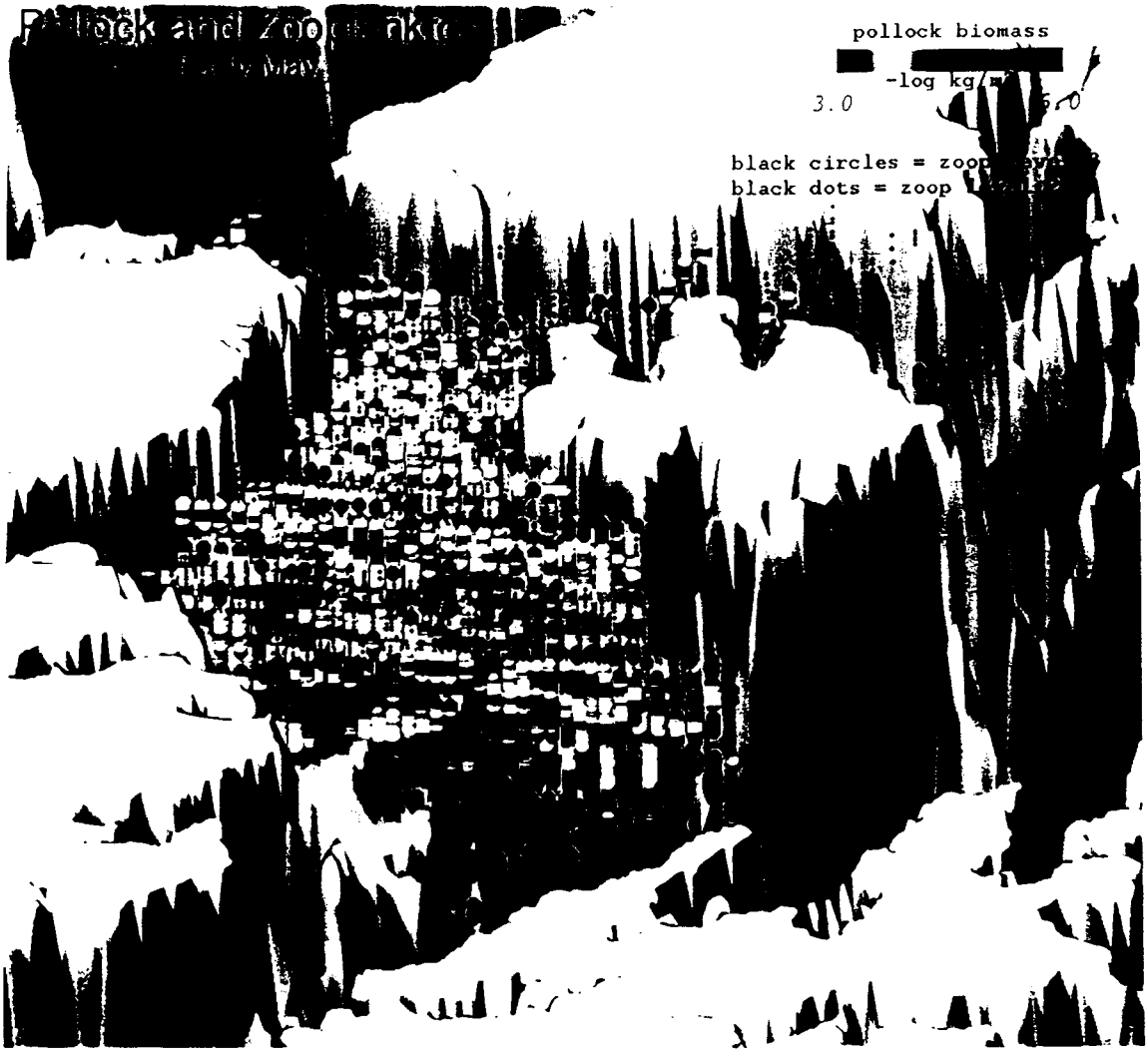

Figure 40. Three dimensional seascape of pollock and plankton patches in northwest Prince William Sound in May 1994.

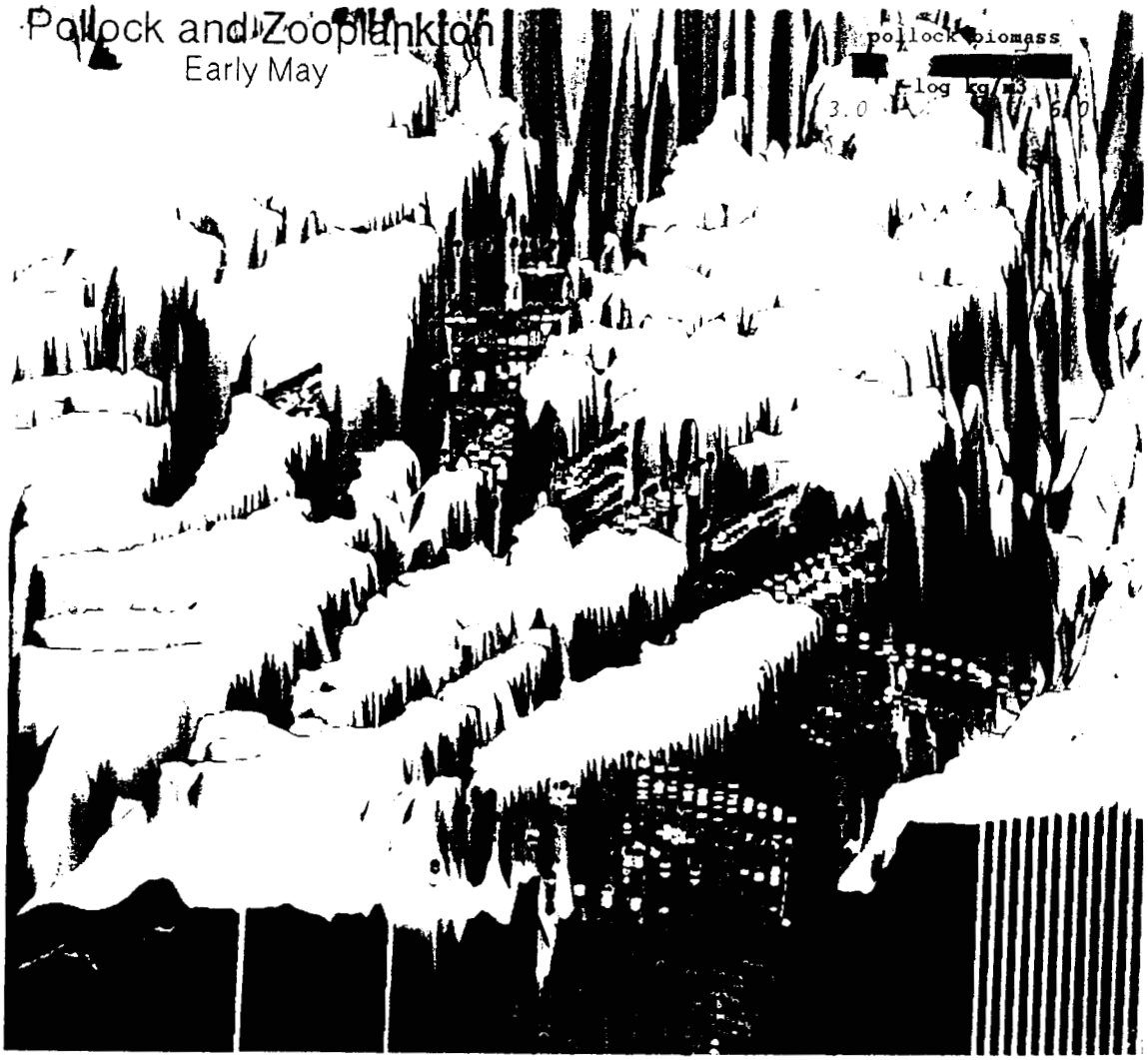

Figure 41. Three dimensional seascape of pollock and plankton patches in southwest Prince William Sound in May 1994.

Figure 42. Three dimensional seascape of pollock and plankton patches in northwest Prince William Sound in July 1994.

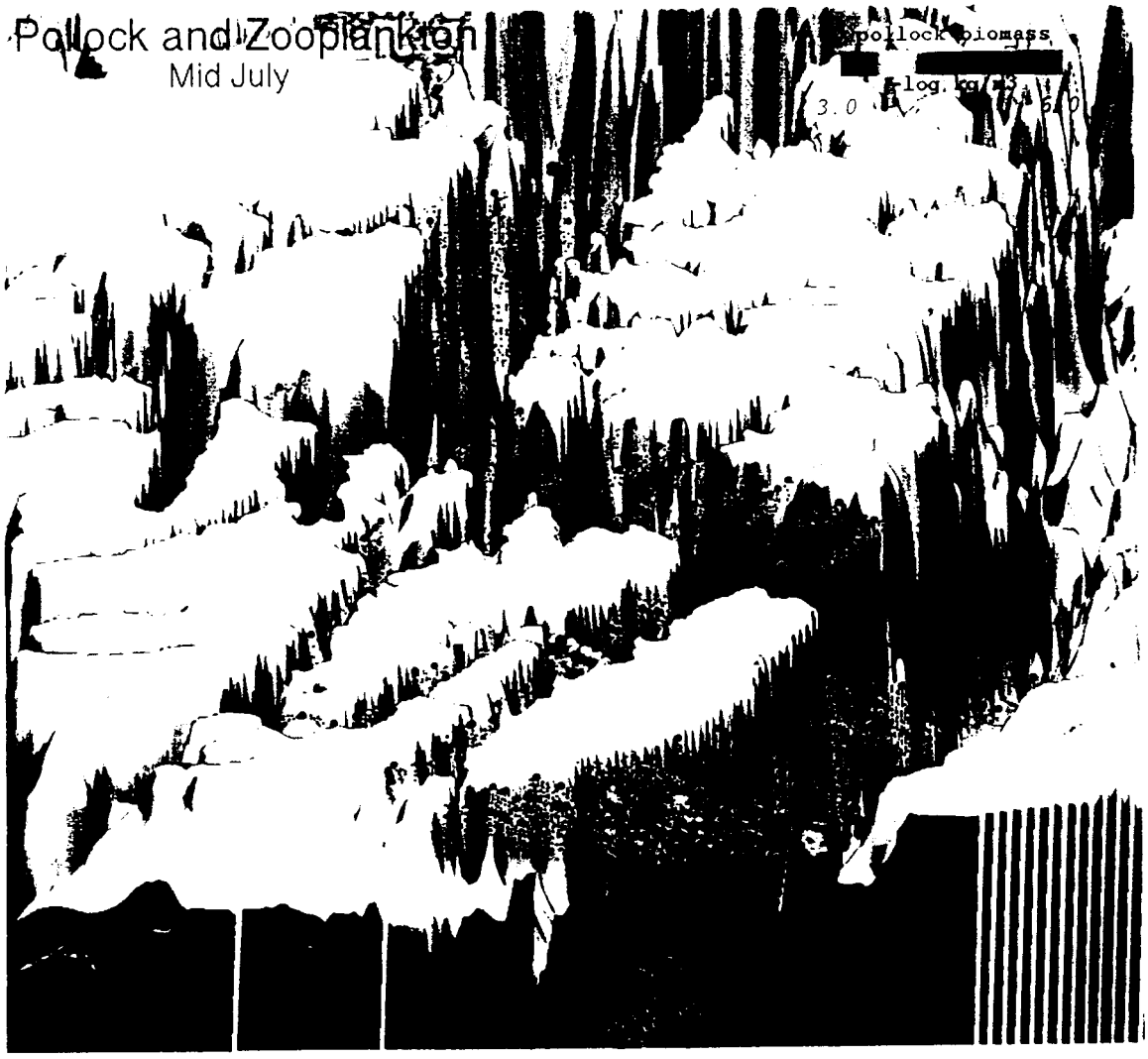

Figure 43. Three dimensional seascape of pollock and plankton patches in southwest Prince William Sound in July 1994.

CHAPTER 3

Winter 1995 estimate of the prespawning biomass of walleye pollock in Prince William Sound, Alaska. G.L. Thomas and T. Brock Stables. Note: This paper is not to be cited without permission from the first author

ABSTRACT

In February-March 1995, a commercial midwater trawl fishery located two concentrations of walleye pollock in southeastern Prince William Sound and harvested over 2,300 mtons before the season was closed. The average size of the Port Bainbridge pollock was 532 mm and bycatch was insignificant (<0.0004 by weight). Subsequent to the fishery, an acoustic-net sampling survey was conducted between February 24 and March 1, 1995 to make a minimum estimate of the walleye pollock biomass remaining in these areas. The first and largest concentration of pollock was found in Port Bainbridge. Port Bainbridge was surveyed twice, on February 25 and March 1. The walleye pollock biomass observed on the first survey was 18,456 +/- 1,951 mtons, and 27,366 +/-7,227 mtons on the second survey. We believe that the increase in biomass on the second survey was due to recruitment of prespawning fish into the Port Bainbridge survey.

The second and smaller concentration of fish was found in south Knight Island passage. This area was also surveyed twice, on February 24 and 28. The walleye pollock biomass observed on the first survey was 1,300+/-487 mtons, and 10,587+/-2,193 mtons on the second survey. The first survey of Knight Island passage was conducted in the vicinity of the previous commercial fishery and was unsuccessful. The second survey extended further south andsuccessfully located fish at the north entrance into Prince of Wales Passage. The biomass estimate of pollock from the second survey (February 28 in south Knight Island Passage and March 1 in Port Bainbridge) was 37,953+/-9,178 mtons. Considering Prince of Wales Passage leads to Port Bainbridge, this southerly movement of pollock may have been part of a larger spawning migration.

In addition to the quantitative surveys of Port Bainbridge and Knight Island, we conducted a reconnaissance survey of the Greater Prince William Sound in an attempt to locate other major concentrations of spawning pollock. No other major concentrations were found, but light to moderate echos suggested that most areas of the Sound contained some pollock.

Given the dynamics of the spawning migration, two surveys are insufficient to estimate the total spawning biomass of pollock. A time series of acoustic surveys in Port Bainbridge that encompass the pollock spawning season are needed to improve our understanding of when the stock has fully recruited to the area. For stock assessment purposes the second survey is most representative of the biomass and this is an underestimate since: (1) low densities of fish were observed throughout the Sound, (2) the fish were probably still recruiting in the Port Bainbridge spawning area because the last survey was still weeks prior to spawning and (3) despite the reconnaissance survey the likelihood of another undetected concentration of spawning pollock is high. This last point is illustrated by our need to conduct two surveys of the Knight Island

Passage before finding the second concentration of fish (ca. 11,000 mtons) that the fishery located just two weeks prior.

INTRODUCTION

Acoustic techniques are widely used throughout the world to assess abundance of pelagic fish stocks, including herring (Thorne 1983a, Thomas 1992). These techniques use calibrated, scientific echosounders to measure the amount of energy reflected from individual and schooled fish. Calibration of echosounders includes measurement of the directivity pattern of the transducer which is used to determine the sample volume of the sound pulse. Measurements of single fish are used to estimate target strength, which when combined with sample volume, converts the echo intensity into fish density and biomass. The echointegration and target strength techniques used to process acoustic data are based on sonar theory (Urick 1975; Ehrenberg and Lytle 1972). Net sampling is routinely conducted to subsample the acoustic targets to verify species, size and obtain other biological information on the ensonified fish (Thomas 1992).

Background

In the spring and summer of 1994, the Sound Ecosystem Assessment (SEA) program funded by the *Exxon- Valdez* oil spill (EVOS) Trustee Council found walleye pollock to be the dominant large fish predator of juvenile fishes in western Prince William Sound. In addition to being a primary predator, SEA discovered the walleye pollock were also the primary competitor of juvenile fishes for the spring bloom of macrozooplankton prey by virtue of their numerical abundance. The spring-summer abundance of pollock queried the question, are there sufficient densities of walleye pollock in the Sound to warrant a wintertime commercial fishery? This question was answered by commercial trawlers in the winter of 1995 when they located pollock schools that occupied near 1 billion cubic meters of the water column, and subsequently harvested over 2,300 mtons in a two week season.

Problem

Due to a shortage of funds and several years of declining budgets, the Alaska Department of Fish and Game has relied on stock assessment by the National Marine Fisheries Service (NMFS), who conducts surveys in the Gulf of Alaska and Bering Sea for groundfish information. Thus, the management of the Sound's pollock stock falls under the guidelines established by NMFS for the Gulf Fishery. This condition will likely remain the same into the future with current state budget projections. The National Marine Fisheries Service, also under the pressure of limited funds, conducted surveys of pollock in Prince William Sound in 1984 and 1990 (Chris Wilson, National Marine Fisheries Service, Seattle, personal communication). Results from these surveys have not been reported.

In 1989 and 1990, bottom trawl surveys were conducted in Prince William Sound to assess impacts of the Exxon Valdez oil spill on commercial species of bottomfish and shellfish. Using

area-swept techniques, this survey estimated that about 9,500 mtons of pollock resided in PWS (Haynes et al. 1991). This biomass was used to manage the 1995 winter fishery in the Sound. This approach is problematic in that: (1) area-swept estimates from bottom trawl surveys are not representative of pollock populations because pollock are primarily pelagic, and (2) the stock assessment data were six years old and not applicable to current harvest management. Risks to management are high enough when in-season sampling is conducted with accepted methods, so the need for stock assessment information is paramount to sustain a viable population for harvesting.

Potential solution

The State would be interested in management of the groundfish fisheries in the Sound if stock assessment information was available. The Science Center is developing the capability to conduct acoustic assessments for a variety of fishes in the region using multifrequency, multibeam echosounding and commercial fishing vessels to subsample the fish targets for biological information. Commercial fishermen have expressed an interest in paying for the assessment through sale of the catch because of the potential economic benefits of increasing information on commercially viable fish stocks. This data could be used by federal and state agencies for management decisions as well.

Justification

Fishery resources in Prince William Sound were damaged by EVOS. Some fish resources that were particularly vulnerable, such as the Pacific herring, are at historically low biomass levels. The large walleye pollock population may have the ability to suppress the speed of recovery of damaged herring stocks. Re-establishing high herring biomass in the Sound is important to the production of most large fish, marine mammal and bird populations because it is prime forage. Commercial harvesting of the Sound's walleye pollock stock may enhance recovery of the herring population via reduced predation. However,the benefits of creating a new fishery in an economically depressed coastal region are problematic.

Description of Project

This survey was a collaborative effort between the Alaska Draggers Association and the PWS Science Center. The Draggers Association provided the trawl and the crew, while the Science Center provided the acoustician, equipment and measurement program to estimate biomass. Search areas were stratified using bottom depth, historical and recent fisheries information, previous survey information, and real time acoustics data. Sonars and echosounders were employed to locate and map schools, as well as to conduct systematic acoustic transects over individual fish concentrations. Subsamples collected by trawls were used to scale the echointegration acoustic data.

MATERIALS AND METHODS

Study Site

Prince William Sound (PWS) is a complex fjord/estuary (Schmidt 1977) located at the northern margin of the Gulf of Alaska (Figure 1). Prince William Sound covers an area of about 8800 km with approximately 3200 km of shoreline (Grant and Higgins 1910). The areas to be searched intensively were south Knight Island Passage and Port Bainbridge (Figure 2).

Acoustic survey

The acoustic survey was conducted between 23 February and 1 March 1995 using a commercial trawler, the F/V Alaskan. The trawler used a commercial search lite sonar to locate school concentrations in suspected areas, was equipped with a GPS-linked, 38-120 kHz dual frequency, dual beam, echosounder to map and estimate the size and density of schools, and was outfitted with a commercial midwater trawl to sample the acoustic targets. Transducers were mounted on a towfin in a down-looking configuration, and towed at about 6 knots at depths of 2-3 m . A Dantrawl Billionark commercial midwater trawl with a mouth opening of 22 by 10 meters was equipped with a net sounder and fished at 1.5 m/sec.

Survey Design - The acoustic survey was a multistage sampling design (Cochran 1977). The first stage used local fishers' knowledge of the walleye pollock schools location and behavior, and data from the recent commercial herring harvest to reduce the search area for concentrations (school groups) in the Sound. We assume that the adult walleye pollock stock overwinter in these areas of the Sound, spawn in mid- to late March, then disperse into the water column to feed initially on the spring macrozooplankton bloom (SEA 1993).

The second stage of the survey design was to search and locate school groups within and between (along transit path) the strata areas suspected of holding pollock schools. Historical information and fishers knowledge played another role in this stage by identifying specific isobaths pollock schools. Hence, walleye pollock schools were located by cruising within specific depth contours using commercial quality search (sweeping) light sonar and down-looking echosounders. The search-light sonar and down-looking sonar were used by the fishers to assess if commercial quantities of pollock were present.

The third stage of the survey was to map and measure the density of the schools found in the search areas. Mapping was conducted by transecting over the schools and recording GPS information. The density was measured along these transects using a dual beam echosounder. The transects were run in a zigzag fashion over the school(s) to map the concentration and then a series of parallel transects were run over the school to estimate density (Figure 3).

The fourth and fifth stages of the sampling were to trawl the acoustically surveyed schools and subsample the catch for biological information, respectively. First , the species composition of the

net catch was used to partition the assessment. Second, the length and weight of the fish were used for target strength analysis.

Acoustic Parameters - Target strength information for herring was derived from average length to target strength (in decibels) per kg fish after Thorne (1983b). Thorne's empirical relationship assumes the following logistical equation: where σ is the mean acoustic backscattering coefficient, W is the mean weight (in kg), l is the mean length (in cm), and a and b are constants.

$$\gamma = \frac{\sigma}{\overline{W}} = a\overline{l}^{-b} \qquad (1)$$

A value for the constant a was obtained from physical data at the site (temperature and salinity) and the literature (speed of sound). A value for the constant b was obtained from a review by Thorne (1983b) using a linear regression of $\log_{10}$l versus 10 log (σ/w), where 10 log (σ/w) is referred to as "target strength per kg." The average length and weight data were compiled from the net catches which were compared to values obtained in the commercial fishery (federal observer, personal communication). These measured data were applied to Thorne's (1983b) empirical relationship to obtain the ratio γ = σ/w and the mean backscatter coefficient (σ).

As a cross check, we generated *in-situ* measurements of target strength from a subset of dual beam acoustic data. We compared our in situ mean backscatter coefficient with Thorne's (1983b) empirical formula (Table 1).

In-situ target strength data were also collected on a 120 kHz standard target (Table 2).

Biomass estimation - Biomass was calculated for each fish concentration found during the large scale survey. The calculation of biomass per unit volume was made using echointegration by single cells jk along transects:

$$\beta_{jk} = \rho_{jk} \cdot \overline{w}_{jk} = \frac{C(ei)_{jk} \cdot P_{jk}}{\frac{\overline{\sigma}_{jk}}{\overline{w}_{jk}}} \qquad (2)$$

where β_{jk} is the biomass in weight per unit volume (cubic meters), ρ_{jk} is the number of scatterers per unit volume (unknown at high densities), w_{jk} is mean weight of the scatterers (also unknown), or for practical purposes by C, the acoustic constant (calibration settings ie., gain etc.), ei_{jk} is the mean of the voltage squared from echo integration (in lieu of numbers), P_{jk} is percentage of cell *jk* within the water column (where bottoms or shorelines are encountered), and σ_{jk} is mean backscattering voltage for the specific targets within cell *jk* per mean weight (dB per kg).

The biomass for a region of surface area (A in square meters) is determined by using a set of line

transects across the region of known fish concentration, along which a total of nrs point estimates of biomass per unit area is obtained. Specifically,

$$B = \frac{\sum_{j=1}^{nrs} \sum_{k=1}^{nst} \beta_{jk}}{nrs} \cdot A \tag{3}$$

where nrs is number of reports (along the line transects), nst is number of one meter depth strata, and A is survey area. Where nst were not one meter bins the appropriate weighting was used to computed means and variances (Seber 1973).

For the biomass estimate, we followed Thorne (1983a). Specifically, we assume that σ_{jk}/w_{jk} is independent of cell *jk*, hence, for all *jk* σ_{jk}/w_{jk} is a constant γ, and γ is given by equation 1. With this assumption, equation 5 simplifies to:

$$\beta_{jk} = \frac{C}{\gamma} \cdot (ei)_{jk} P_{jk} \tag{4}$$

and the biomass B in an area is given as

$$B = \frac{C}{\gamma} \frac{\sum_{j} \sum_{k} \cdot (ei)_{jk} P_{jk}}{nrs} \cdot A \tag{5}$$

When a fish concentration was located, mapped, and surveyed for density, the survey for density was repeated on a later date to estimate error.

RESULTS AND DISCUSSION

Sound Search

The F/V Alaskan surveyed suspected areas of pollock concentration in the eastern and western Sound (Figure 1 and 2). Light sign was observed in several areas from the central to northwestern corner near Wells Passage and to the eastern side from Port Fildalgo to Orca Inlet. However, the only large concentrations were found in the areas that were commercially fished earlier in the month, Port Bainbridge (Figure 4 and 5) and Knight Island Passage (Figure 6). These areas were surveyed twice to evaluate repeatability of the estimate and with a series of 5-16 independent transects to estimate the variability of school density.

Biomass and density was highest, up to 27,366+/-7,227 mtons in Port Bainbridge (Table 3). This

biomass increased between the two surveys by 8,900 mtons. The biomass in the Knight Island Passage area was relatively low in both surveys, 1,300+/-487 mtons, but in the second survey additional coverage of the northern Montague Straight found the school which was believed to be in the Knight Island passage earlier in the month during the fishing season, 10,597+/-2,193 mtons (Figure 7). Figure 8 shows the increase in biomass estimates between surveys.

The total catch from the trawling was estimated a 106,173 kg for 120 minutes of trawling (4.4E-10 cub. m). The catch was over 99% pollock and the mean length and weight were 51 cm and 1.3 kg. Table 4 lists the target strength for the Port Bainbridge pollock, March 1, 1995.

Minimum biomass survey design

The multistage sampling design used for estimating the winter biomass of pollock has been used for estimating fall herring biomass (Thomas et al. 1995). The precision is high, with 95% confidence limits for the four surveys varying from 11% (n=16), 21% (n=9, 26%(n+13) and 37% (n+5). As expected as the number of transects approaches 10 the precision stabilizes. The bonus is the relatively low cost and robustness of this survey design (less than $50K).

The approach is robust since it uses the best information available on fish distributions via experienced fishers to search for the fish to be measured. If the fish change their distribution in the future, and they often do, it will be most likely that fishers will discover the change, and survey search procedures would become immediately adapted. This later step allows for industry participation and develops their responsibility in the assessment process, a theme long overdue in fisheries science.

This survey does not measure the total population size of the Sound's walleye pollock. A survey to assess total population size would require systematic measurements over the entire Sound and adjacent Gulf of Alaska, and then a complementary genetic assessment to establish the presence of different stocks. This survey allows for estimating the bulk of the population available to the fishery, or in a sense the **minimum biomass estimate** of the population. This estimate should be adequate for management and conservation of the fish population. Future improvements to the survey design may be warranted by industry with the desire to improve the exploitation efficiency of the stock on a sustainable basis.

The nesting of quantitative acoustic procedures within semi-quantitative reconnaissance surveys is practical. Albeit commercial fishing sonars and echosounders are not calibrated measurement tools, when coupled with experienced fishers' knowledge, provide a meaningful judgement on the relative distribution of the fish population. The fact that fishers make a living off making real-time estimates of fish density and distribution makes the reconnaissance step robust and believable. Furthermore, when this reconnaissance is coordinated with quantitative sonars to estimate fish density the design approaches proportional allocation of sampling that is most efficient (Cochran 1977). Advantages of the multistage, minimum biomass sampling design are that expenses are low, precision is high and the design adapts to changes in fish distribution.

By assuming that the fishery harvested 2,300 mtons and the biomass of the second survey was minimal of the spawning biomass, 37,963+/-9,420 mtons, the range in exploitation rates is from 5 to 8% (Figure 9). This exploitation rate is conservative but should allow for sustaining a safe harvest of the stock. As confidence in the stock assessment procedures grows, consideration may be warranted for increasing this exploitation rate.

ACKNOWLEDGEMENTS

This project was funded by a team effort from the EVOS Trustee Council and the Alaska Department of Fish and Game through the commercial sale of the experimental catch. We wish to thank Jay Stinson, skipper of the F/V Alaskan, whose interest in the survey effort was the spark that made things work. His volunteering the use of the vessel charter, regardless of remuneration was commendable and set the tone for cooperation between fishermen, state and federal agencies and the Science Center. We also thank the local commercial fishers and processors for their interest and support of this survey and the Prince William Sound Science Center (PWSSC). Finally, the EVOS Trustee Council and staff (Molly McCammon and others) deserves significant thanks because of their long-term commitment to research and development of better methods. We thank all who have supported our efforts to improve conservation of fish populations by monitoring with new technologies.

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Table 1. Parameters of the acoustic equipment used during the fall 1994 herring survey in Prince William Sound.

System	Frequency	Source level	System gain	Transducer	Pulse duration
BioSonics 101	120kHz	225.075dB	-165.264dB	.0010718	0.4 ms
BioSonics 102	200kHz	221.655dB	-155.765dB	.0006515	0.4 ms

	DEPTH INTERVALS						
TS	77-102	102-127	127-152	152-177	177-202	202-227	SUM
-50	0	0	0	0	0	0	0
-48	0	6	0	0	3	0	9
-46	2	6	8	1	10	0	27
-44	0	8	14	2	18	0	42
-42	0	14	19	3	13	1	50
-40	0	4	17	11	20	0	52
-38	0	6	28	13	29	1	77
-36	0	0	9	13	18	0	40
-34	0	2	11	14	17	0	44
-32	0	1	3	18	7	0	29
-30	0	0	13	16	9	1	39
-28	0	0	14	24	5	0	43
-26	0	0	7	22	1	0	30
-24	0	0	0	11	0	0	11
-22	0	0	0	1	0	0	1
-20	0	0	0	1	0	0	1
-18	0	0	0	0	0	0	0
SUM	2	47	143	150	150	3	495

Table 2. TS of fish in the Port Bainbridge school at 38 kHz, 77-102 m range, March 1, 1995.

Table 3. Biomass of pollock and the 95% confidence limits for eight areas surveyed in Prince William Sound in the winter of 1995. Leg 2 biomass is presented as total biomass because it was believed that the fish were still recruiting into the spawning area at the time of the first survey.

Date	Area	Leg	Transects	Biomass (mtons)	95% C.I.
02-24-95	Knight Island Passage	1	5	1,300	487
02-25-95	Port Bainbridg	1	16	18,456	1,951
02-28-95	Knight Island Passage	2	9	10,597	2,193
03-01-95	Port Bainbridge	2	13	27,366	7,227

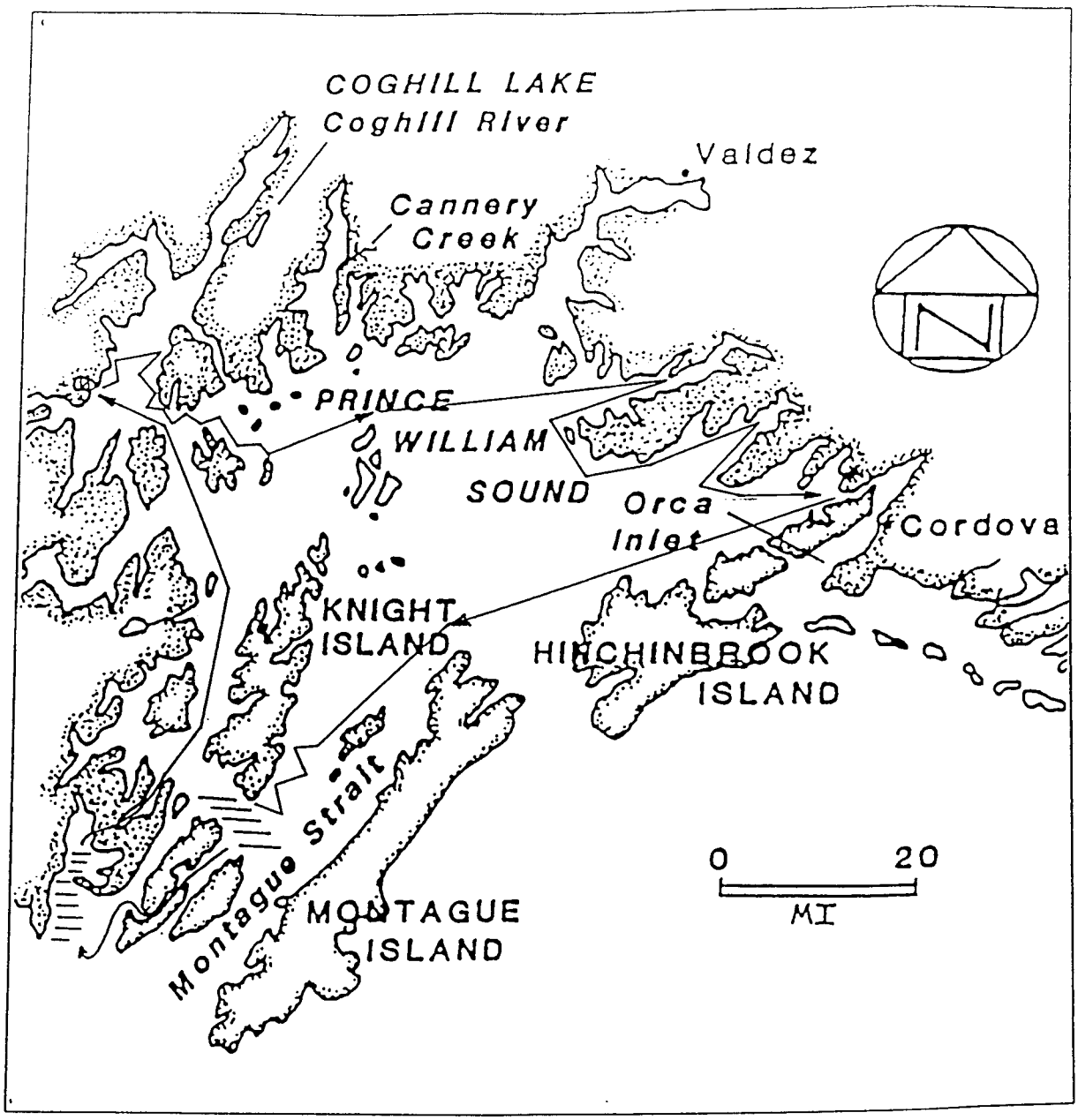


Figure 1. Prince William Sound winter walleye pollock survey Leg 1, February 24-26, 1995.

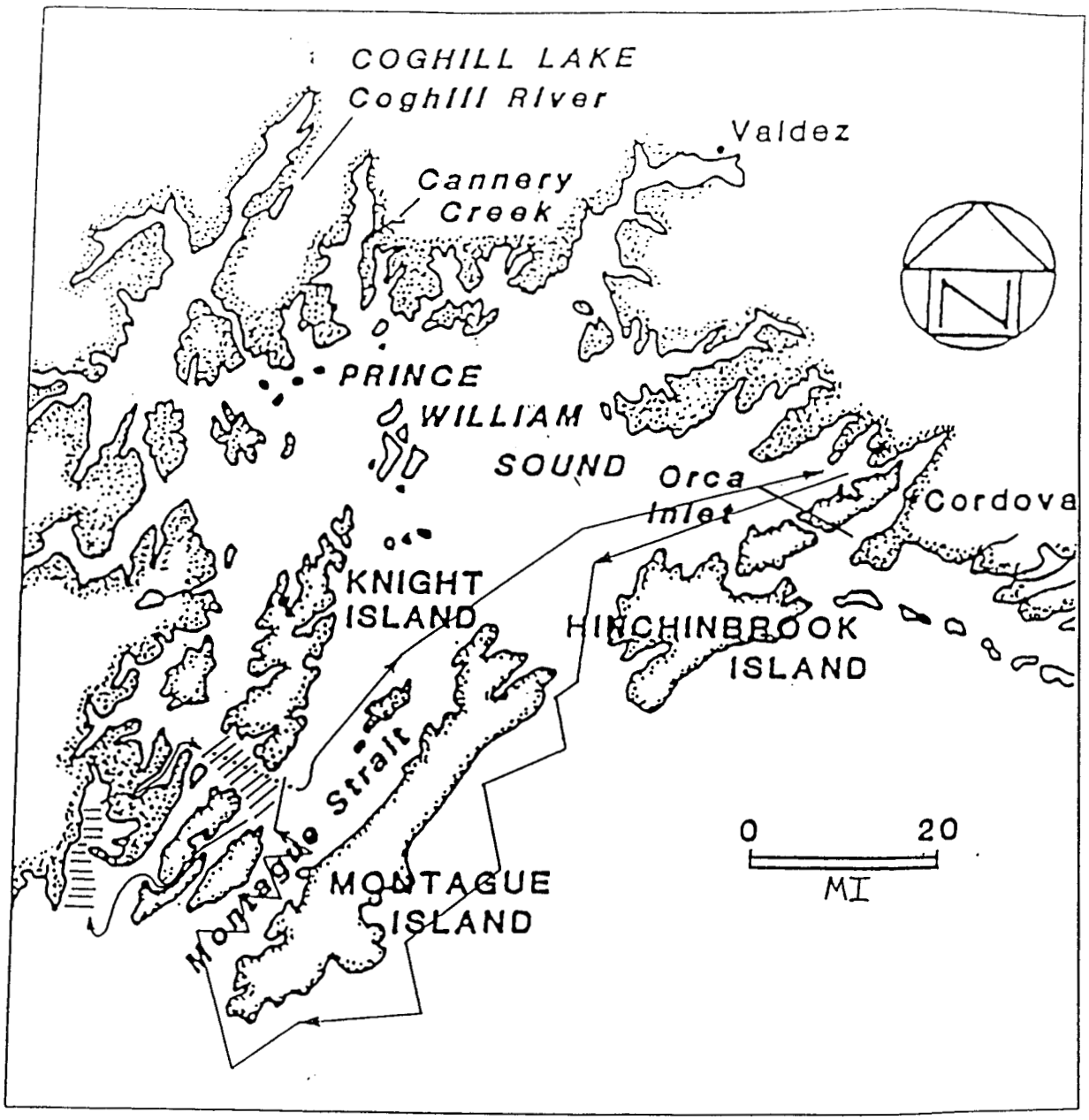
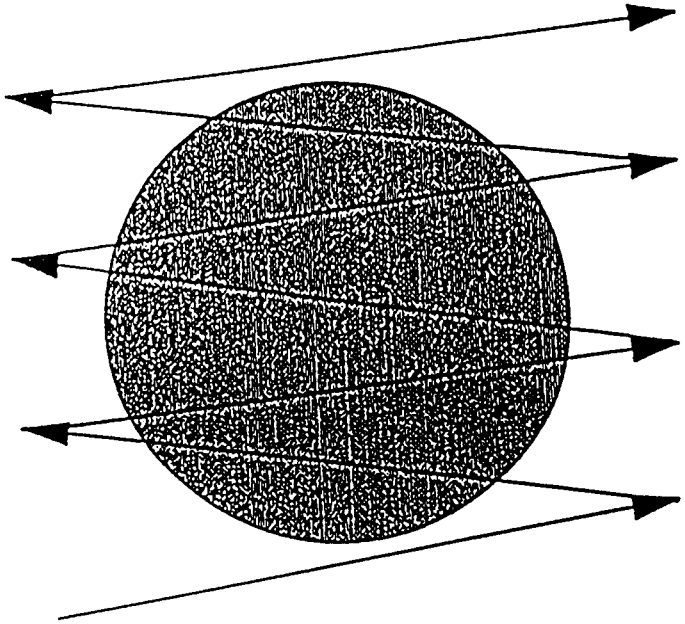
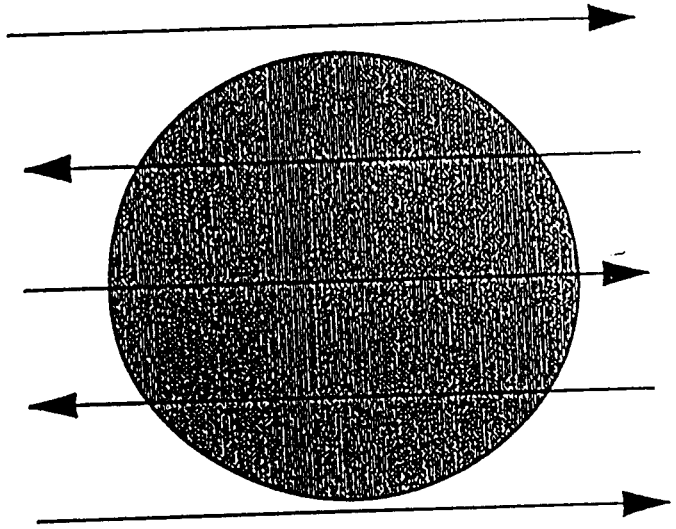


Figure 2. Prince William Sound winter walleye pollock survey Leg 2, Feb. 28 to Mar. 1, 1995.



Transect pattern used to map a known school of walleye pollock, using a dual beam echosounder and GPS.



Transect pattern used to measure the density of the school of walleye pollock.

Figure 3. Transect patterns used to map and measure the density of schools found in the search areas.

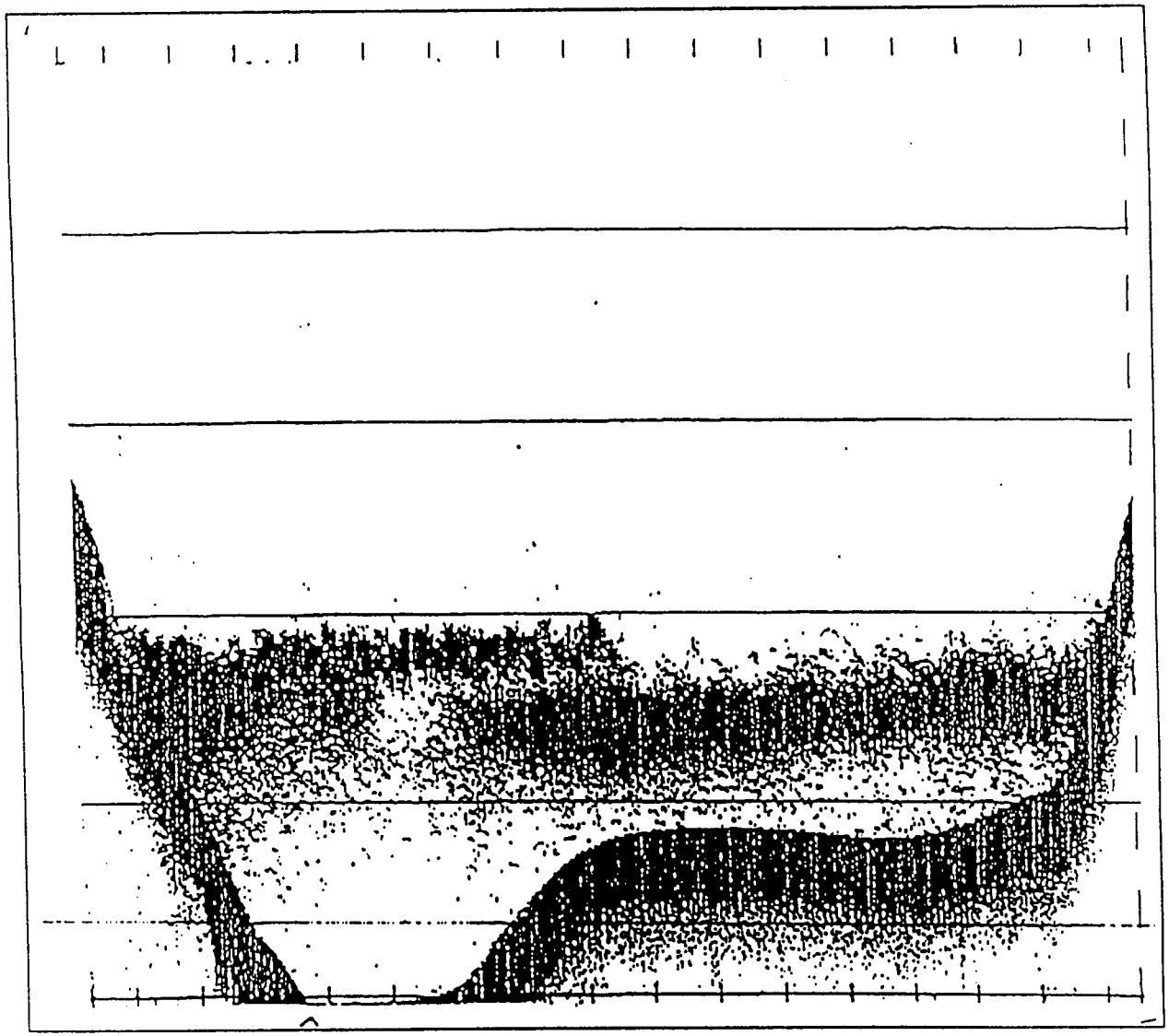


Figure 4. Echogram from Port Bainbridge survey, Leg 1.

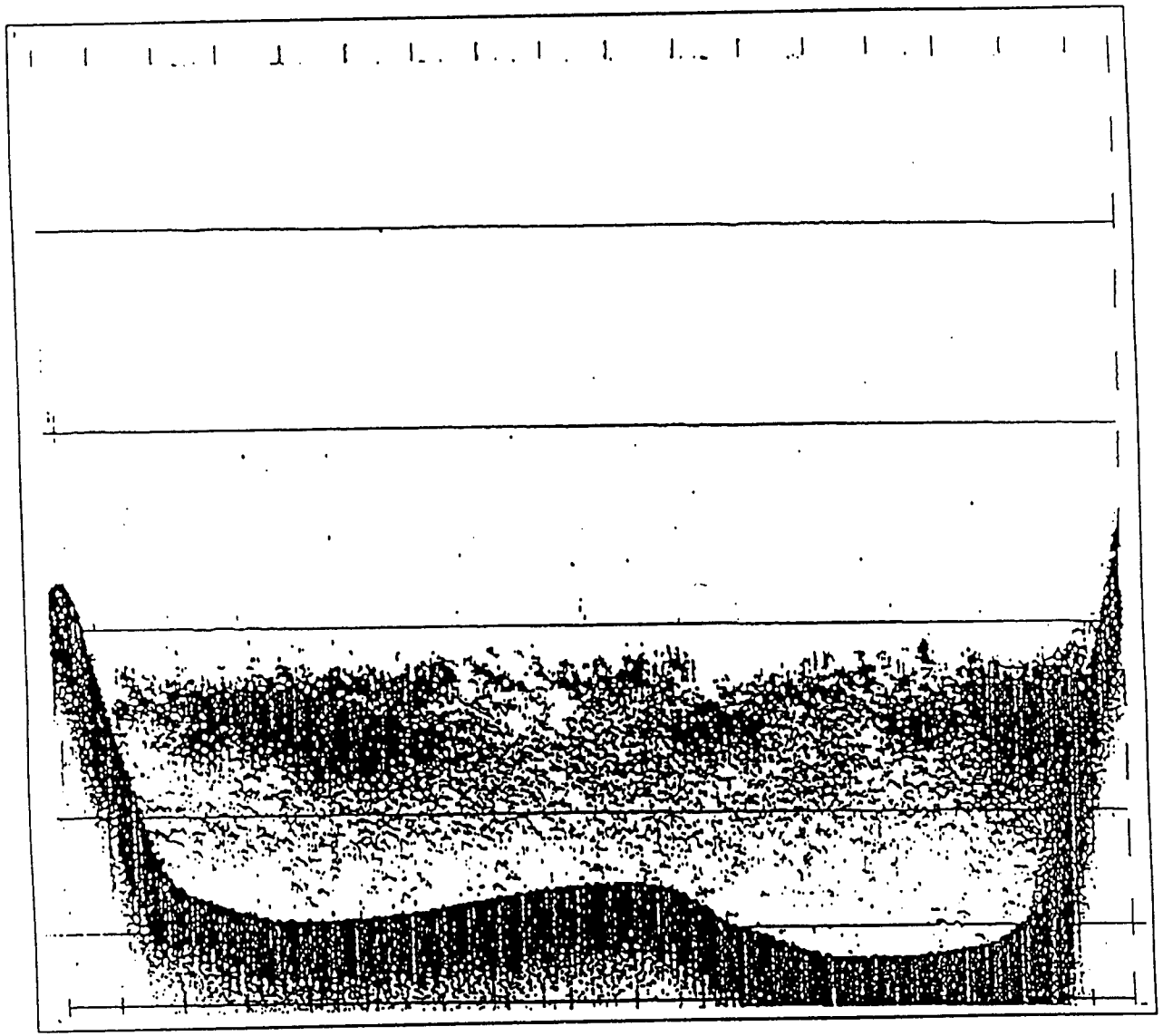


Figure 5. Echogram from Port Bainbridge survey, Leg 1.

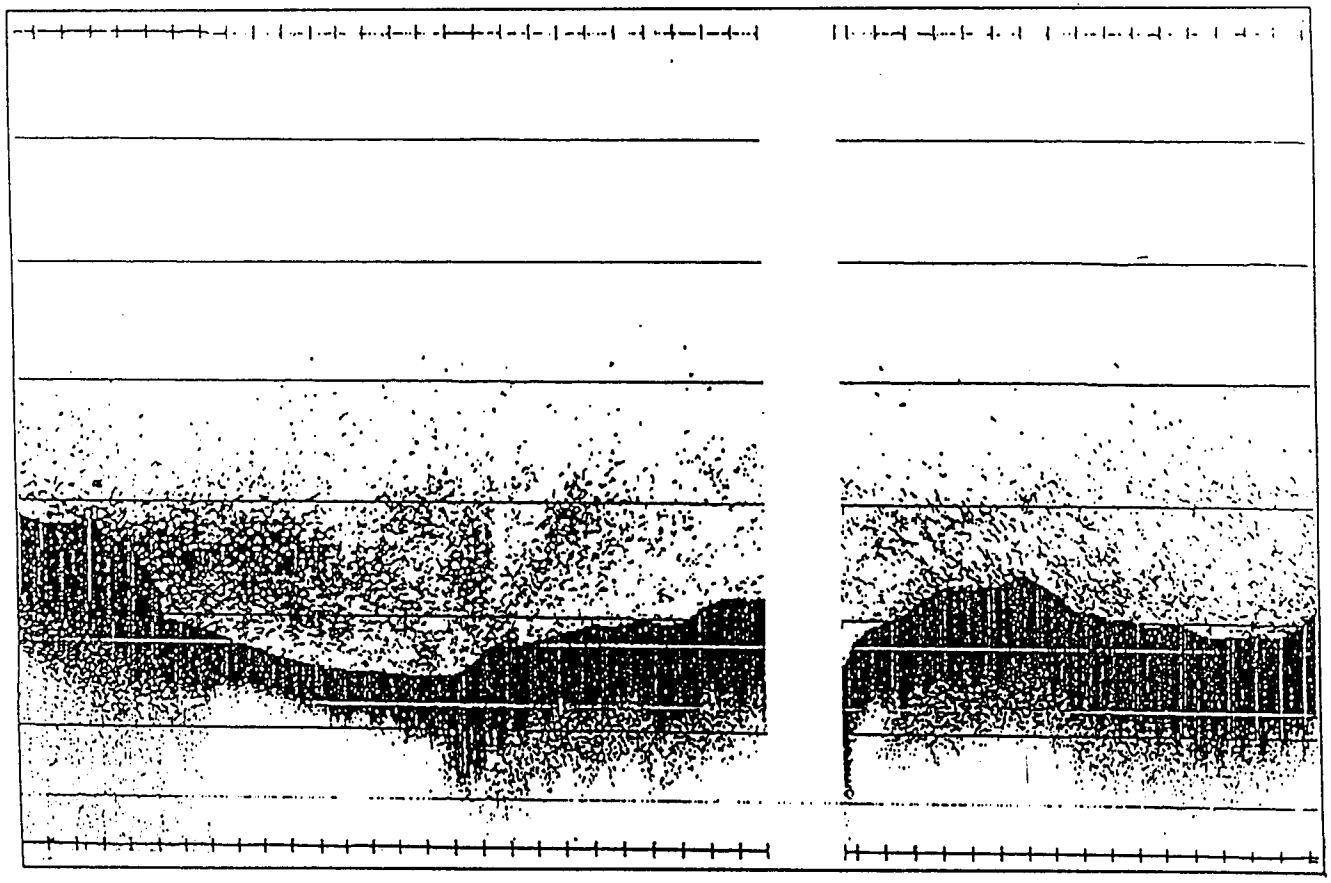


Figure 6. Echogram from Montague Strait/Knight Island Pass survey, Leg 1.

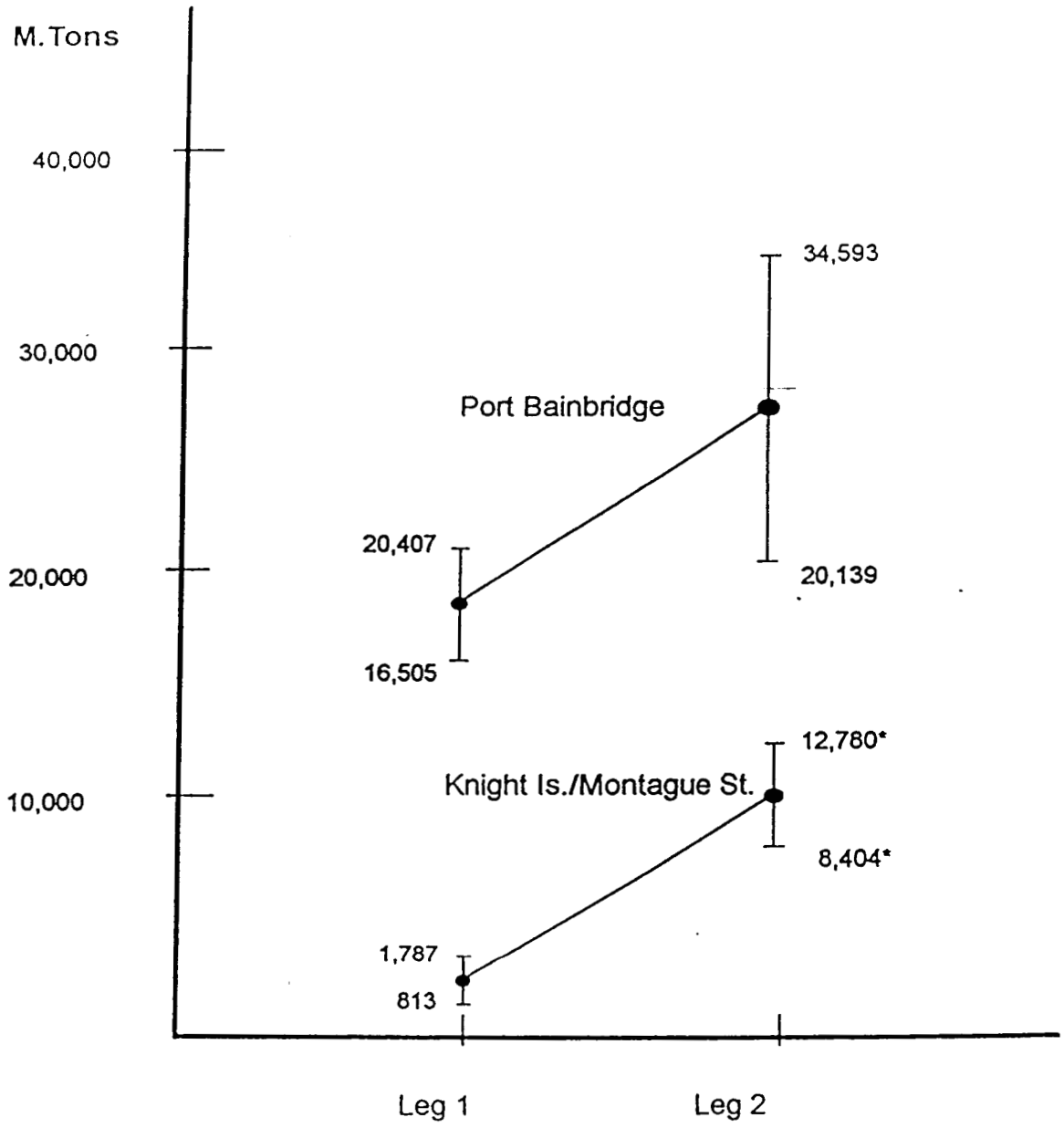


Figure 7. Walleye pollock biomass estimates in Knight Island/Montague Straits and Port Bainbridge survey areas, Legs 1 & 2. *Leg 1 and 2 areas surveyed.

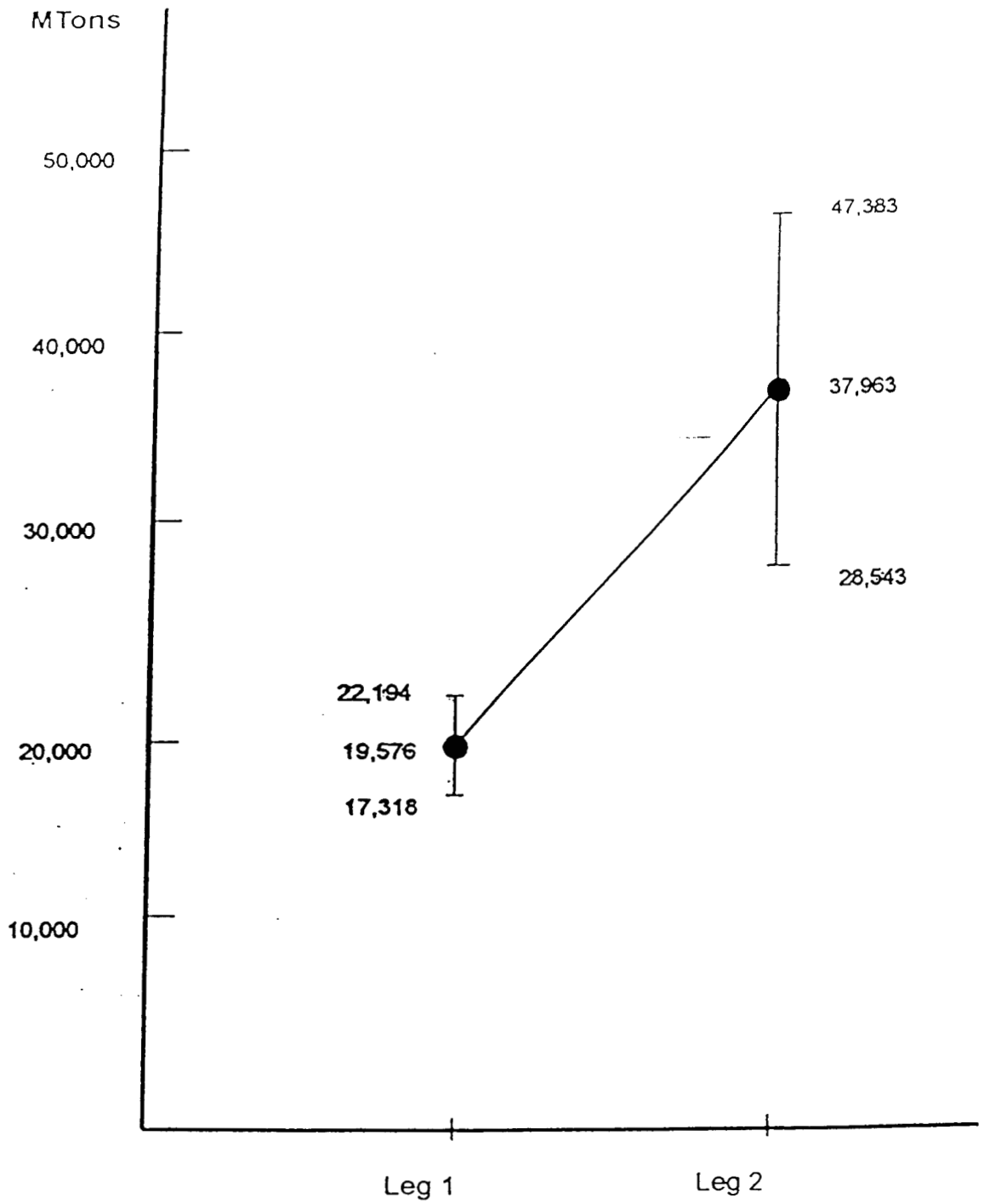


Figure 8. Walleye pollock biomass on repeated surveys, Feb. 26 and March 1, 1995 in PWS.

CHAPTER 4

Acoustic estimates of Pacific herring *Clupea pallisi* biomass in Prince William Sound between the fall of 1993 and winter of 1996. G.L. Thomas, Jay Kirsch (1), Richard E. Thorne (2) and John Wilcock (3). 1- PWS Science Center, 2- BioSonics Inc. Seattle, 3- ADF&G, Cordova.

ABSTRACT

From mid-October to the time of spring spawning, adult herring schools *Clupea pallisi* maintained a uniquely high density and a distinct 20 to 40 meter vertical distribution at night. In contrast, juvenile herring concentrations were observed as 0 to 20 meter surface layers. These behavioral characteristics make herring available to nighttime acoustic stock assessment.

Nighttime acoustic-purse seine surveys were conducted in Prince William Sound in the falls of 1993, 1994 and 1995, springs of 1995 and 1996, and winter of 1996. The surveys used anecdotal and real time acoustic/optical information, and a growing body of knowledge from previous surveys to locate herring concentrations. Survey areas emphasized were the north-central Montague Straits and north-west Orca Sound. Once located, herring concentrations were mapped and their densities measured by running a zig-zag transect course over the concentration. Acoustic data were geotime referenced using GPS. The spring prespawning surveys are aided by aerial overflights by the Alaska Department of Fish and Game to visually spot large herring schools. Extrapolations of density estimates to biomass were limited to the areas where herring were mapped and measured. Where herring concentrations remained in the same area, a series of parallel acoustic transects were conducted using a dual beam scientific sonar to estimate the precision of the fish density estimates and the repeatability of the biomass estimates. Commercial purse seines (herring type) were used to collect species and size composition of the fish targets.

The *in-situ* target strength distribution displayed dominant modes at -43 and -34 dB. The -43 dB mode was appropriate for herring when the target strength discriminator was successful. It was apparent that when the density of herring schools was too large, the discriminator failed because there was a dramatic increase of target strengths within school targets. Overestimation of the target strength was probably due to the discriminator pooling adjacent targets. However, in a few locations there were concentrations of walleye pollock in the water column that produced a valid target strength mode at -32 dB. The vertical distribution of the pollock at night did not overlap with the adult herring schools.

In the 1993-1995 fall surveys, nine concentrations of herring were found that exceeded 1000 metric tons. At least one of these concentrations was found in north-central Montague Straits and the north-west Orca Sound each year. The largest concentrations of herring found in the fall surveys of each year were in the north-central Montague Straits (16,442 metric tons in 1993; 6,526 metric tons in 1994; and 24,291 metric tons in 1995). The largest concentration of herring found during the fall surveys in the north-west Orca Sound was 2,819 metric tons in 1994 and

1995.

In the spring survey of 1995 and winter/spring surveys of 1996, six concentrations of prespawning herring were found that exceeded 1000 metric tons. All of these were located in Zaikof Bay, Rocky Bay and Stockdale Harbor on the north and north-west end of Montague Island. The largest prespawning concentrations seen each year were, 10,480 metric tons in 1994 and 23,203 metric tons in the winter of 1996. The spring 1996 survey commenced after the herring began to spawn and the majority of fish were too nearshore or dispersed to be available to the acoustic survey.

Four of the twelve herring concentrations were surveyed twice at night to determine the repeatability of biomass estimation. In 1993, we estimated a concentration at Applegate Rocks to be 12,875 and 16,442 metric tons on repeated surveys. In the spring of 1995, we estimated a prespawning concentration of herring in Rocky Bay to be 10,480 and 8.05 metric tons on repeated surveys. In the winter of 1996, we estimated a concentration in Zaikof Bay to be 26,309 and 20,097 on repeated surveys. In spring 1996, we estimated a concentration in Stockdale Harbor to be 5,617 and 5,767 metric tons on repeated surveys. A primary source of error in repeating these measurements is to make sure that the repeated survey encompasses the fish concentration. When the fish move, if care is not taken, the first transect can truncate the fish distribution and sometimes the fish move too close to shore to survey. Both of these have lead to underestimation of biomass on repeated surveys. As criteria are established for accepting or rejecting replicates, the accuracy and precision of estimation will improve. Even with this error the estimates only varied by 1% to 13% of the mean.

Another aspect of repeatability is the fall to winter-spring estimates. In 1994, the fall and spring estimates of adult herring biomass were 13,000 metric tons. In 1995, the fall and winter estimates of adult herring biomass were 24 and 23 thousand metric tons. Echointegration-purse seine estimates of adult herring biomass in fall 1993, fall 1994, spring 1995, fall 1995 and winter 1996 were 20, 13, 13, 24 and 23 thousand metric tones, respectively. The observed increase or decrease in the fall estimates of herring biomass is explained by the recruitment of young fish that have grown to sufficient length to join the adult herring schools. The repeatability of the fall and winter/spring measures indicate little over-winter mortality in adults. This is therefore a good time to measure their abundance. This is in contrast to the SEA hypothesis on the importance of overwinter survival of the juvenile herring.

Seasonal changes in the abundance and distribution of adult herring were observed from 1993 to 1996. For decades, commercial fishers have observed the herring to migrate from the Gulf into the north-central Montague Straits in October. In two successive springs, we have seen this fall distribution of herring shift into the Bays on the north side of Montague Island by late winter/early spring. By mid-April, the large concentrations appear to move shallow to spawn and leave the area. Presumably they migrate back to the Gulf where they feed on the shelf until the following fall.

INTRODUCTION

Acoustic techniques are widely-used throughout the world to assess the abundance of pelagic fish stocks, including herring (MacLennan and Simmonds 1992; Thorne 83a,b). Acoustic echointegration and dual beam processing of target strength are based on sonar theory (Urick 1967; Ehrenberg and Lytle 1972). Basically, scientific echosounders are used to measure the amount of energy reflected from fish concentrations, which can be converted to fish density with information on the energy reflected from a single fish (its target strength) and knowledge of the sample volume (transducer directivity). Net sampling is routinely conducted to subsample the acoustic targets to collect biological information (species, size, etc.) on the ensonified fish (Thomas 1992).

Historically, information on the abundance of Pacific herring *Clupea pallisi* in Prince William Sound (the Sound) consists of commercial catch records, aerial estimates of the length of milt patches along beaches and herring spawn deposition surveys (Donaldson et al. 1993; Funk 1994). Single beam acoustic surveys without net sampling support had been used for estimating the biomass in the Sound in the fall from 1980 to 1985 (Merritt et al. 1993). Between 1988-92, the Alaska Department of Fish and Game used spawn deposition surveys in the spring to estimate the biomass of adult herring. A record run of adult herring (134,000 metric tons) was forecast to return in 1993 from the 1992 spawn deposition survey. In 1993, a spawn deposition survey was not conducted.

The spring 1993 aerial survey of the length of milt patches along the beaches suggested that the returning biomass was far less than the expected 100,000+ metric tons. This in-season assessment was used to limit the commercial harvest to about 1,000 metric tons, substantially lower than in 1992 (about 20,000 metric tons). In addition, the herring spawning stock was found to be infected with a potentially lethal virus, viral hemorrhagic septicemia,(VHS) (Meyers et al. 1994). The low biomass of herring and the presence of disease raised serious concerns about continued mortality and the long-term viability of the stock. The collapse of the herring fishery in the spring of 1993 and the absence of a spawn deposition study to estimate herring abundance created the opportunity for a fall acoustic-purse seine survey to estimate biomass.

This paper presents results from six acoustic-purse seine surveys that were conducted in the falls of 1993-6, the winter of 1996 and the springs of 1995-6.

METHODS

Study Site

Prince William Sound is a complex fjord/estuary (Schmidt 1977) located at the northern margin of the Gulf of Alaska (Figure 1). The Sound covers an area of about 8800 km with approximately 3200 km of shoreline (Grant and Higgins 1910).

Acoustic survey

The fall surveys were conducted between mid-October and November using experienced herring fishers on commercial purse seiners for acoustic transecting and to sample the fish targets. The seiners used commercial search-light sonars to locate school concentrations in suspected areas. They were also equipped with GPS linked, dual-beam scientific echosounders to map and estimate the size and density of schools, and were outfitted with commercial purse seines to sample fish concentrations. Transducers were mounted on towfins in a down-looking configuration and towed at about 6 knots at depths of 2 m. The purse seines used ranged from 20 to 60 meters deep with mesh sizes of approximately 3.18 cm stretched.

Survey Design - The acoustic survey was a multistage sampling design (Cochran 1977). The first stage used historical information and local fishers' knowledge of herring behavior to reduce the search area for herring concentrations in the Sound. The areas to be searched intensively were Orca Inlet and Montague Strait (Figure 1). We assume that the adult herring stock over-winters in the Sound and that the stock has completed this migration by the middle of October (DeCino et al. 1994). In general, we attempted to survey during intervals of small tidal exchange to minimize the probability of inclement conditions.

The second stage of the survey design was to search and locate herring school groups within the eastern and western Sound survey areas. This was done with aerial surveys in the spring and with search-light sonars on all surveys. Historical information and fishers' knowledge played another role in this stage by identifying depths where herring schools are found. Hence, herring schools were located by zig-zagging within the 40-100 depth contours using commercial search-light sonar and down-looking echosounders. The search-light sonar and down-looking sonar were used by the fishermen to assess if there were commercial quantities of herring present. When this condition was satisfied, the third stage of the survey was to map and measure the density of herring schools found in the search areas (Northern Hawkins Is., St. Mathews Bay, Knowles-Red Head, Danger Island, Needles, west of Green Island, east of Green Island, Applegate Rocks, Rocky Bay, Zaikof Bay areas).

Mapping was conducted with the geotime-referenced scientific echsounder by running a series of zigzag or parallel transects over the area suspected of containinga fish concentration. The transects were run in a zigzag fashion over the school(s), turning when schools were no longer visible on the echosounder (Figure 2a). In analyzing this data, the zig portion of the transects was considered independent of the zag and two estimates were obtained with precision. In cases where the fish concentration was stable, a systematic series of parallel transects was run to estimate precision (Figure 2b). This survey was repeated, if possible, to develop independent estimates of the biomass of specific fish concentrations.

The fourth and fifth stages of the sampling were to purse seine the acoustically surveyed schools and to subsample the catch for biological information. In general, the species composition of the net catch was used to partition the seine catch subsampling. The length and weight of the fish in the subsample were used to determine target strength using -20 log L -71.3 (MacLennan and

Simmonds 1992) and Thorne (1983).

Acoustic Parameters - Target strength information for herring was derived from the relationship of length to target strength (in dB/kg, Thorne 1983). Thorne's empirical relationship assumes the following logistical equation:

$$\gamma = \frac{\bar{\sigma}}{\bar{W}} = a\bar{l}^{-b} \qquad (7)$$

where σ is the mean acoustic backscattering coefficient, W is the mean weight (in kg), l is the mean length (in cm), and a and b are constants. A value for the constant a was obtained from physical data at the site (temperature and salinity) and the literature (speed of sound). A value for the constant b was obtained from a review by Thorne (1983) using a linear regression of $\log_{10}$l versus 10 log (σ/w), where 10 log (σ/w) is referred to as "target strength per kg." The average herring length and weight data were compiled from samples obtained from the net catches. These measured data were applied to Thorne's (1983) empirical relationship to obtain the ratio $\gamma = \sigma/w$ and the mean backscatter coefficient (σ).

As a cross-check, we generated *in-situ* measurements of target strength from a subset of dual beam acoustic data. We compared our *in -situ* mean backscatter coefficient with Thorne's (1983) empirical formula. We also converted *in-situ* measurements to length using -20 log L -71.3 for comparitive purposes. Parameters of the acoustic equipment used during the fall herring surveys are presented in Table 1.

Biomass estimation - Herring biomass was calculated for each fish concentration found during the large scale survey. The calculation of biomass per unit volume was made using echointegration by single cells *jk* along transects:

$$\beta_{jk} = \rho_{jk} \cdot \bar{w}_{jk} = \frac{C(ei)_{jk} \cdot P_{jk}}{\frac{\bar{\sigma}_{jk}}{\bar{w}_{jk}}} \qquad (8)$$

where β_{jk} is the biomass in weight per unit volume (cubic meters), ρ_{jk} is the number of scatterers per unit volume (unknown at high densities), w_{jk} is mean weight of the scatterers (also unknown), or for practical purposes by C, the acoustic constant (calibration settings ie., gain etc.), ei_{jk} is the mean of the voltage squared from echo integration (in lieu of numbers), P_{jk} is percentage of cell *jk* within the water column (where bottoms or shorelines are encountered) , and σ_{jk} is mean backscattering voltage for the specific targets within cell *jk* per mean weight (dB per kg).

The biomass for a region of surface area (A in square meters) is determined by using a set of line transects across the region of known fish concentration, along which a total of nrs point estimates of biomass per unit area is obtained. Specifically,

$$B = \frac{\sum_{j=1}^{nrs} \sum_{k=1}^{nst} \beta_{jk}}{nrs} \cdot A \qquad (9)$$

where nrs is number of reports (along the line transects), nst is number of one meter depth strata, and A is survey area. Where nst were not one meter bins, the appropriate weighting was used to compute means and variances (Seber 1973).

For the herring biomass estimate, we followed Thorne (1983). Specifically, we assume that σ_{jk}/w_{jk} is independent of cell jk, hence, for all jk σ_{jk}/w_{jk} is a constant γ, and γ is given by equation 1. With this assumption, equation 2 simplifies to: April 27, 1996

$$\beta_{jk} = \frac{C}{\gamma} \cdot (ei)_{jk} P_{jk} \qquad (10)$$

and the herring biomass B in an area is given as

$$B = \frac{C}{\gamma} \frac{\sum_{j} \sum_{k} \cdot (ei)_{jk} P_{jk}}{nrs} \cdot A \qquad (11)$$

When a fish concentration was located, mapped and surveyed to estimate the mean and variance of the fish density, and it was relatively stationary, the survey was repeated to determine error in biomass estimation.

RESULTS AND DISCUSSION

Behavior

From mid-October to early spring, adult herring schools display a unique diel migratory behavior. During the day they are found in dense schools in deep water (Figure 3), and during the night they are found in dense schools layered between 20 and 40 meters in the water column (Figure 4). This behavior breaks down as the adult herring approach spawning. In contrast, from fall to early spring the juvenile herring are found layered between 0 and 20 meters in the water column, nearer to the shoreline and at lower densities than the adults.

Target strength

In general, the *in-situ* target strength distribution displayed modes between -43 and -40 dB but sometimes >-34 dB (Figure 5). The -43 dB mode was appropriate for herring when the target strength discriminator was successful (MacLennan and Simmonds 1992). However, the herring schools were almost always too dense to estimate single fish target strength and these observed modes suggest that the target strength discriminator had failed. Often, there was a gradient of increasing target strengths towards the center of dense school targets which suggested that the discriminator was pooling adjacent targets.

Although the purse seine catches directed at adult herring schools indicated almost pure herring, there were a few areas (primarily in the Green Island area) that walleye pollock were observed to co-occur with the herring. This was also the area where most often the highest biomass of herring was found. In these areas, the walleye pollock were observed as single targets within the water column below 40 meters. There was a corresponding -32 dB mode in the target strength distribution. The vertical distribution of these pollock at night did not overlap with the adult herring schools (Figure 6).

Because of the uncertainty over the representation and accuracy of the *in-situ* target strength measures, the mode of the *in-situ* measurements was only used where other information were unavailable for analyses, such as when net catch data were unavailable to determine the size of fish in the survey area and when the within-school densities were not high. Where purse seine catch data were available to identify the targets, the target strength information was derived from average length of the catch.

Population Trends

Large aggregations of herring were consistently observed during late fall and early winter in the northern Montague Strait area between 1988 and 1992 (Donaldson et al. 1993). From 1993 to the present, this distribution has prevailed. In the past, it has been assumed that these herring represent between 50 and 75% of the herring biomass in the Sound (Gaudett 1984; Funk 1994).

In the fall of 1993, the survey was aided by the fact that two weeks earlier a commercial fishery had taken place which located the herring concentrations. In 1994 there was no fishery, therefore two purse seiners were used to search the Orca Sound and Montague Straits areas of the Sound over a period of 14 days. In the fall and winter of 1995, the primary objective of the survey was to map juvenile herring (which also serves to map adults). A group of five vessels was used for over 20 days throughout the Sound, Port Bainbridge, and Resurrection Bay. Thus, there was increasing coverage of the Sound for determining population distribution from 1993 to 1996. Furthermore, aerial surveys were conducted in the spring to locate herring concentrations, although these often focused on the herring after they began spawning. These surveys show that the bulk of the adult herring population has over-wintered in the northern Montague Straights over the past 3 years.

Biomass estimation

Four of the twelve herring concentrations observed over the three years on six surveys were surveyed twice at night to determine the repeatability of biomass estimation. In 1993, we estimated a concentration at Applegate Rocks to be 12,875 and 16,442 metric tons on repeated surveys. In the spring of 1995, we estimated a prespawning concentration of herring in Rocky Bay to be 10,480 and 8.05 metric tons on repeated surveys. In the winter of 1996, we estimated a concentration in Zaikof Bay to be 26,309 and 20,097 on repeated surveys. In spring 1996, we estimated a concentration in Stockdale Harbor to be 5,617 and 5,767 metric tons on repeated surveys. A primary source of error in repeating these measurements is to make sure that the repeated survey encompasses the fish concentration. When the fish move, if care is not taken, the first transect can truncate the fish distribution and sometimes the fish move too close to shore to survey. Both of these have lead to underestimation of biomass on repeated surveys. With more experience, distributional criteria can be established for accepting or rejecting replicates as valid for estimation. Even with this error the estimates only varied by 1% to 13% of the mean.

Another aspect of repeatability is the fall to winter-spring estimates. In 1994, the fall and spring estimates of adult herring biomass were 13,000 metric tons. In 1995, the fall and winter estimates of adult herring biomass were 24 and 23 thousand metric tons. Echointegration-purse seine estimates of adult herring biomass in fall 1993, fall 1994, spring 1995, fall 1995 and winter 1996 were 20, 13, 13, 24 and 23 thousand metric tones, respectively (Figure 7). The observed increase or decrease in the fall estimates of herring biomass is explained by the recruitment of young fish that have grown to sufficient length to join the adult herring schools. The repeatability of the fall and winter/spring measures indicate little over-winter mortality in adults. Therefore, this is a good time to measure their abundance. This is in contrast to the SEA hypothesis on the importance of overwinter survival of the juvenile herring.

Seasonal changes in the abundance and distribution of adult herring were observed from 1993 to 1996. For decades, commercial fishers have observed the herring to migrate from the Gulf into the north-central Montague Straits in October. In two successive springs, we have seen this fall distribution of herring shift into the Bays on the north side of Montague Island by late winter/early spring. By mid-April, the large concentrations appear to move shallow to spawn and leave the area. Presumably they migrate back to the Gulf where they feed on the shelf until the following fall.

Survey Design

The nesting of quantitative acoustic procedures within semi-quantitative reconnaissance surveys is practical. Albeit commercial fishing sonars and echosounders are not calibrated measurement tools, when coupled with experienced fishers' knowledge, they provide a meaningful judgement on the relative distribution of the fish population. The fact that fishers make a living from these real-time estimates of fish density and distribution makes the reconnaissance step robust and believable. Furthermore, when this reconnaissance is coordinated with quantitative sonars to estimate fish density the design approaches proportional allocation of sampling, which is most

efficient (Cochran 1977). Advantages of the multistage, minimum biomass sampling design are that expenses are low, precision is high and the design adapts to changes in the fish distribution.

Acoustic-purse seine estimates of fall-winter herring biomass provide a practical approach for stock assessment. The precision is high with 95% confidence limits on the density estimates of schools typically under 20%, and the repeatability of the biomass estimate varying less than 20%. Although this level of error is very low relative to other assessment techniques, it should get even smaller as acceptance criteria are developed.

Although the acoustic survey does not measure the total population size (presumptuous for any assessment technique) the approach is robust since it uses the historical and recent fisheries information, and experienced fishers to search and locate fish concentrations. The use of commercial fishers is advantageous since it is in their best interests not to miss fish concentrations that contribute to the population estimate that determines the harvest levels. Geotime linkage of the acoustic data insures its independenceas well.

Acoustic surveys to assess total population size would require systematic measurements over the entire Sound and adjacent Gulf of Alaska, and complementary genetic assessment to establish the presence of different stocks. Given the contagiousness of the herring, such a design is impractical and unnecessary. The present survey design estimates the bulk of the population available to the fishery with minimal extrapolation. In a sense it is a **minimum biomass estimate** of the exploitable population.

ACKNOWLEDGEMENTS

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Urick, Robert J. 1967. Principles of underwater sound. McGraw Hill. New York. 384 pp.

Table 1. Parameters of the acoustic equipment used during the fall 1994 herring survey in Prince William Sound.

SYSTEM	FREQUENCY	SOURCE LEVEL	SYSTEM GAIN	TRANSDUCER DIRECTIVITY	PULSE DURATION
BioS. 101	120 kHz	-225.075 dB	-165.264 dB	.0010718	0.4 ms
BioS. 102	200 kHz	-221.655 dB	-155.756 dB	.0006515	0.4 ms

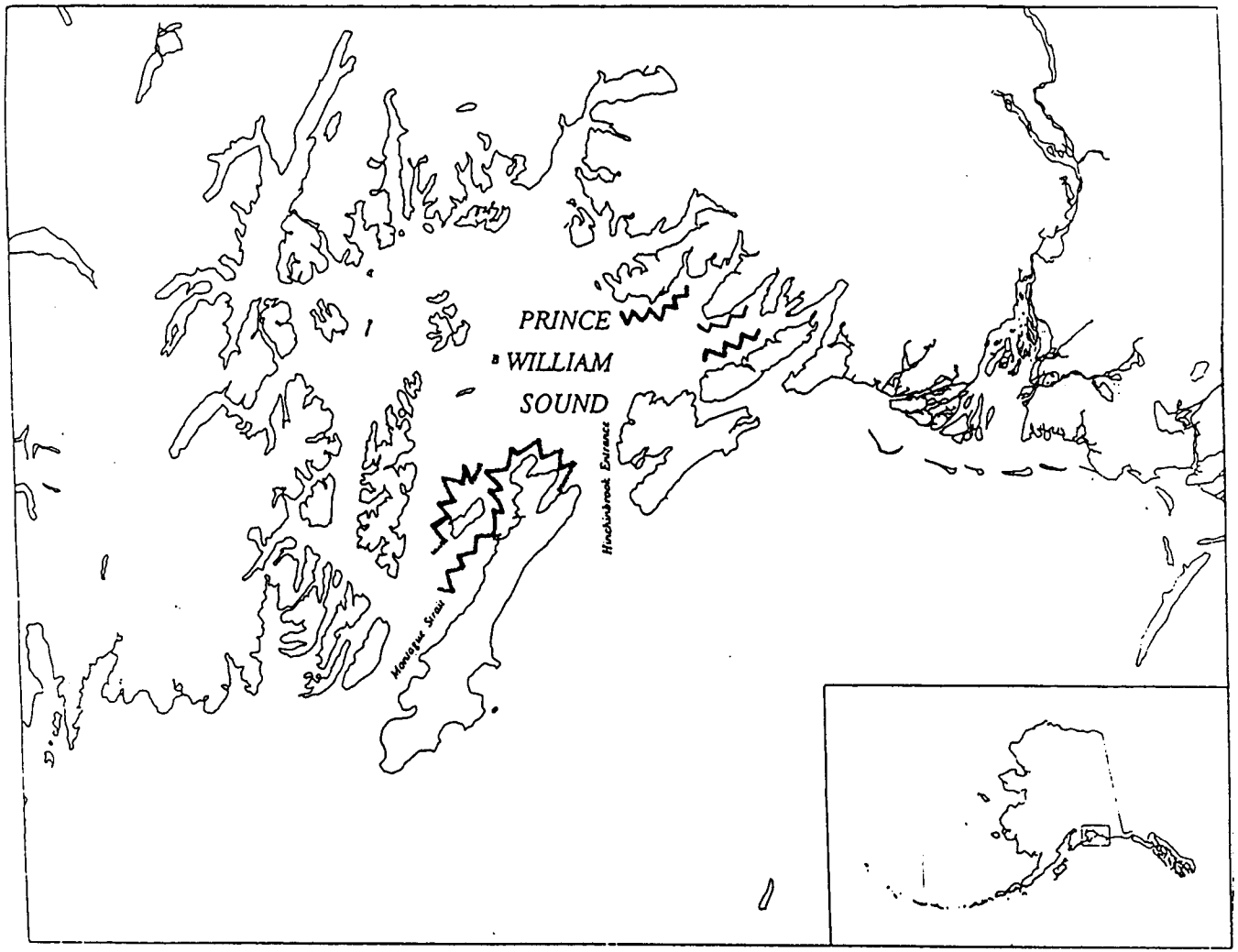

Figure 1. Map of Prince William Sound showing principal survey areas for adult herring between 1993-1996.

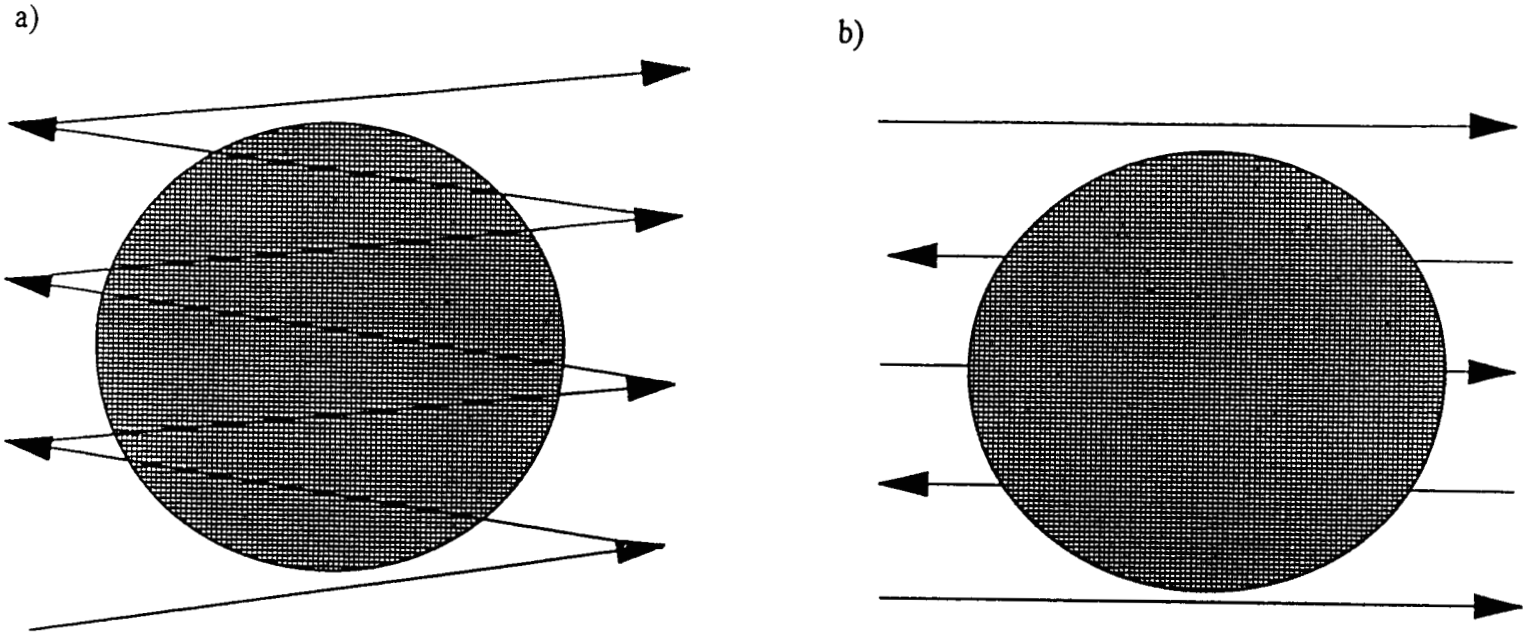

Figure 2. Transect patterns used to map and measure the density of schools found in the search areas. Figure a) Transect pattern used to map a hypothetical concentration of herring, using a dual beam echosounder and GPS. Figure b) Transect patterns used to repeat the measurements of the density of a hypothetical herring concentration when it was stable.

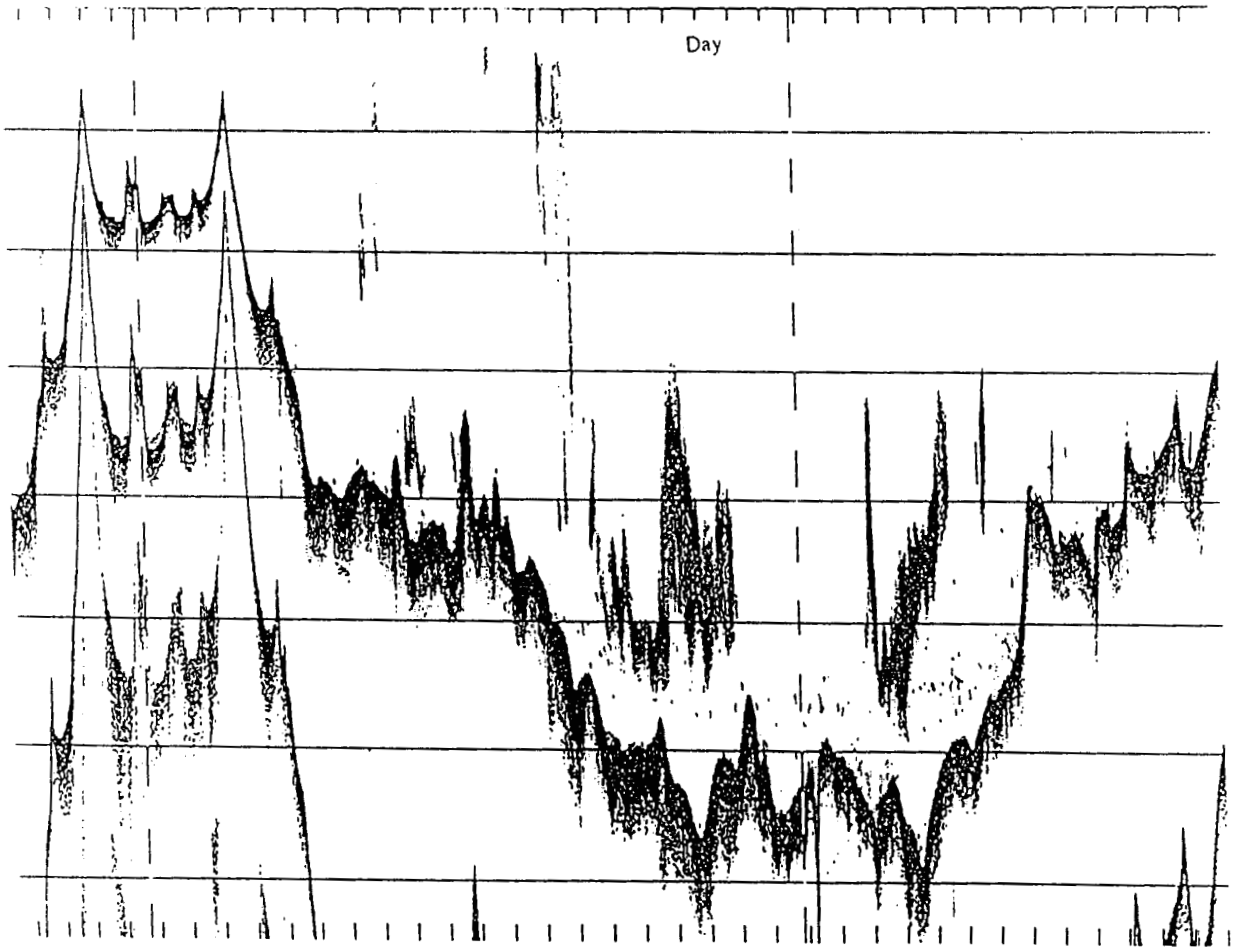

Figure 3. Echogram showing a typical day-time distribution of adult herring in the fall in Prince William Sound.

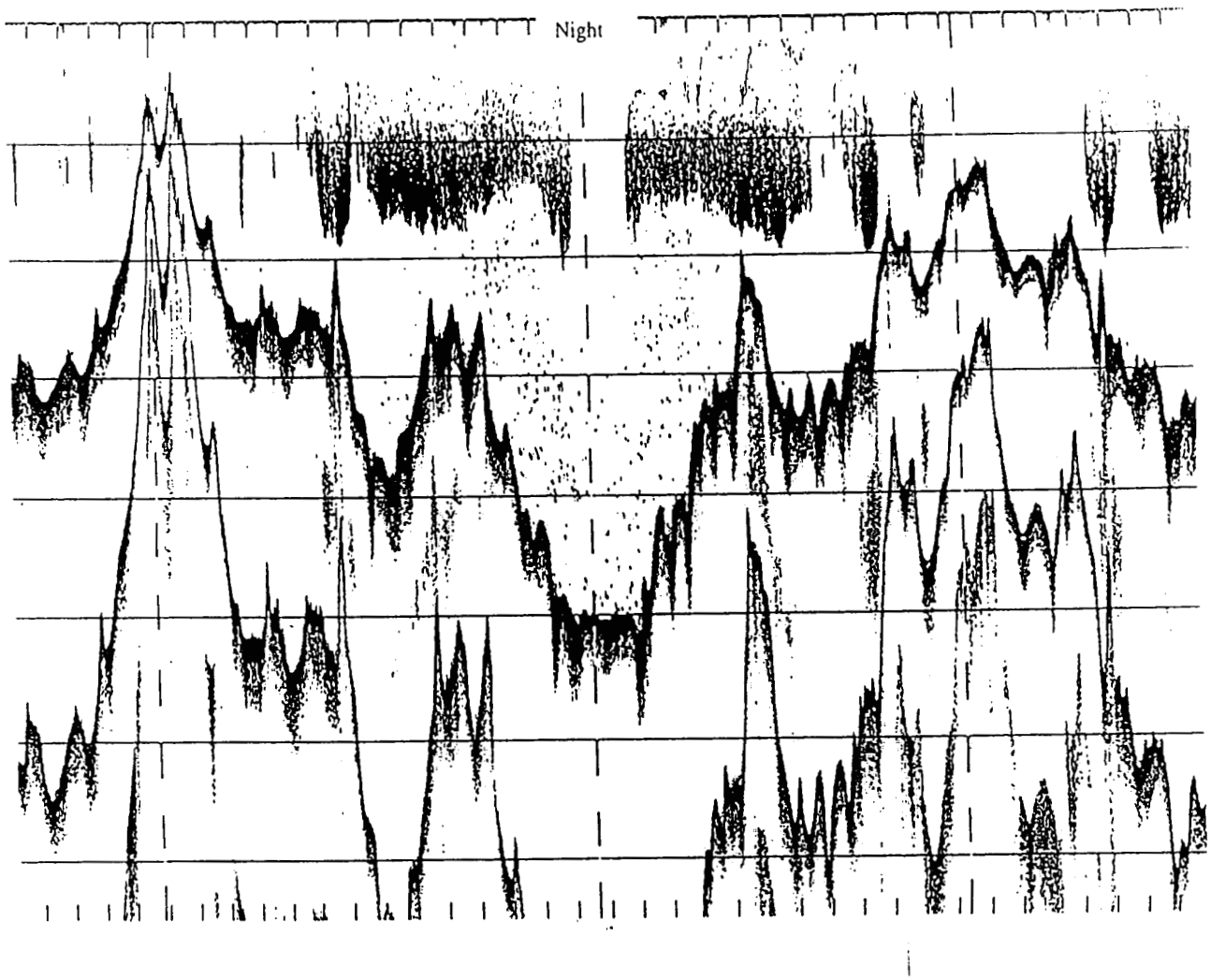

Figure 4. Echogram showing a typical night-time vertical distribution of herring during the fall in Prince William Sound.

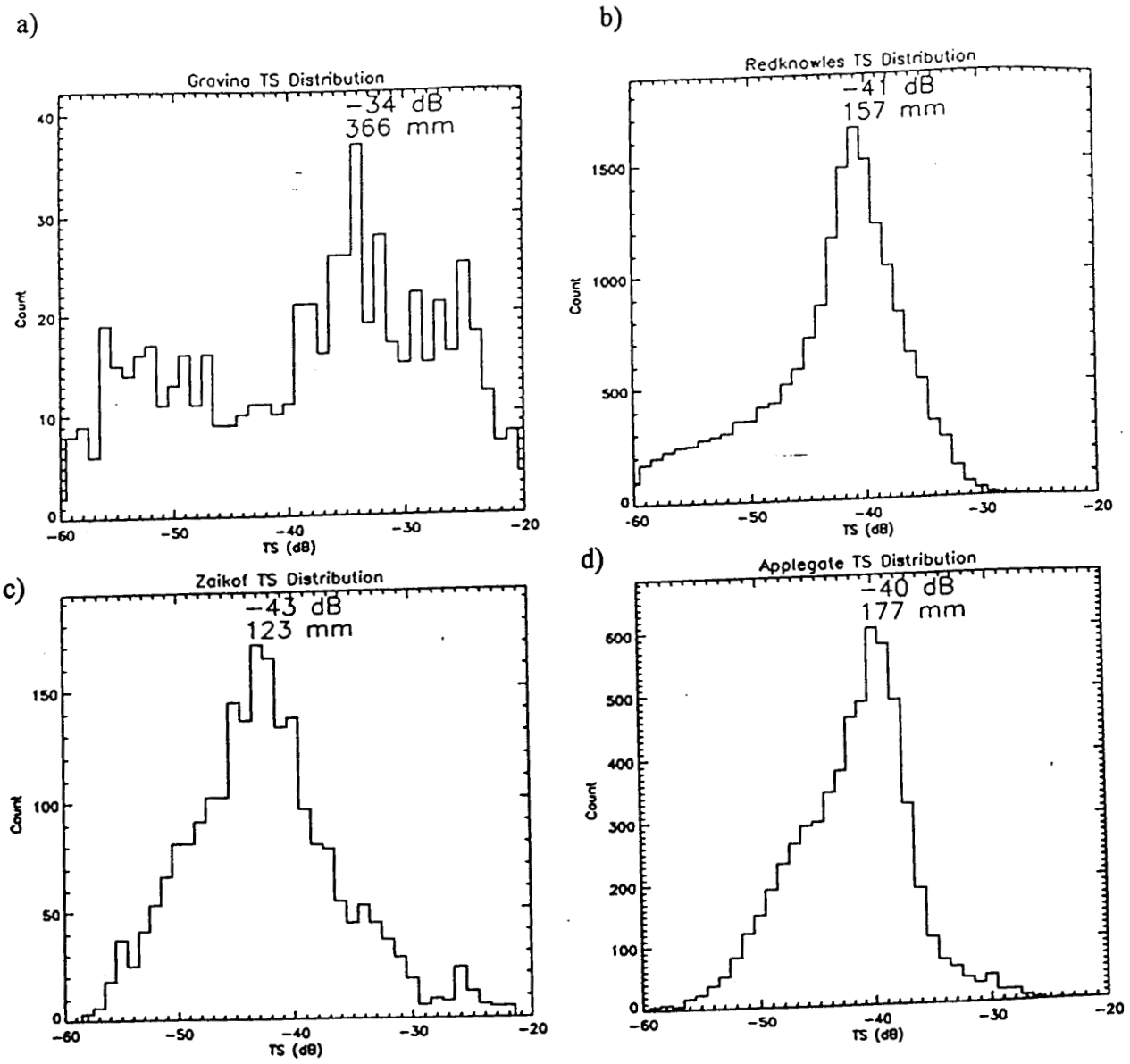

Figure 5. In-situ target strength measurements of herring in Prince William Sound. Figure a) The highest density school was observed at Gravina Point.

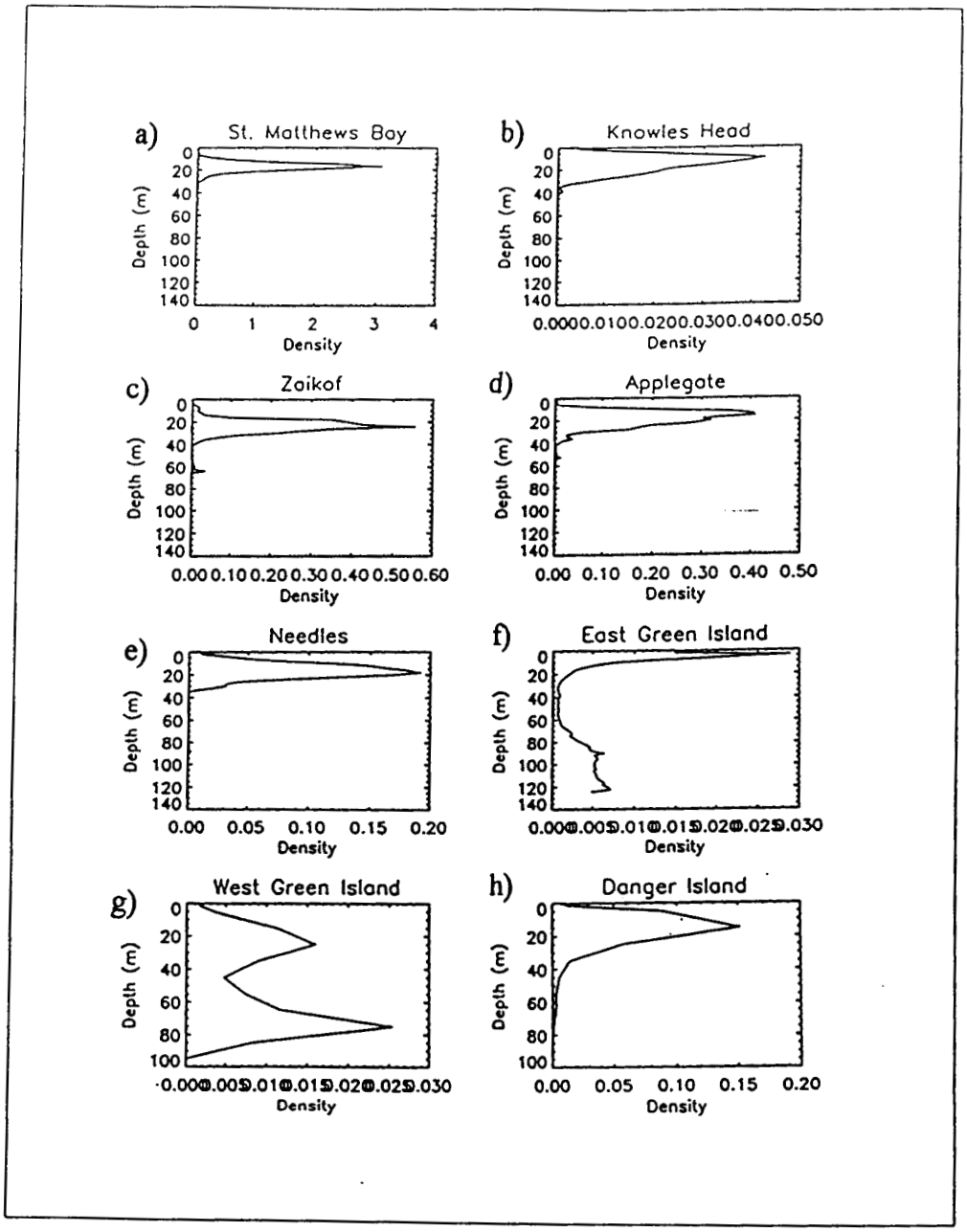

Figure 6. Vertical distribution of adult herring at eight locations in the fall of 1994. Figures (f) and (g), east Green Island and west Green Island respectively, are where large concentrations of pollock were seen below 40 meters.

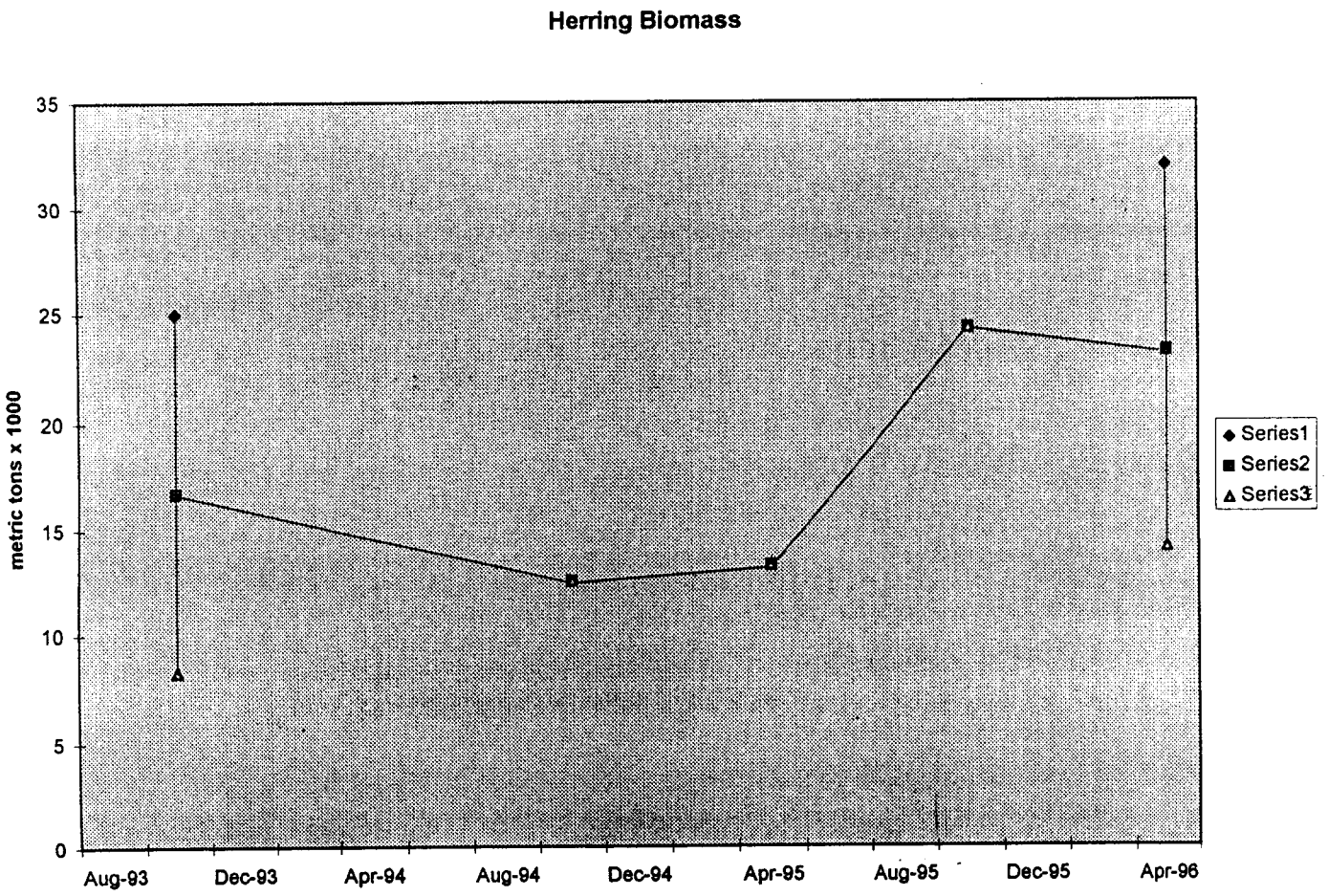

Figure 7. Herring biomass measured on the fall 1993 to winter 1996 surveys in Prince William Sound.

Exxon Valdez Oil Spill
Restoration Project Annual Report

Investigations of Disease Factors Affecting Declines of Pacific Herring Populations in Prince William Sound

Sections:

I. Field Survey of Diseases in Prince William Sound Herring
II. Laboratory Challenge of Pacific Herring With and Without Stressors
III. Survival, Performance and Reproduction in Pacific Herring

Restoration Project 95320S
Annual Report

This annual report has been prepared for peer review as part of the *Exxon Valdez* Oil Spill Trustee Council restoration program for the purpose of assessing project progress. Peer review comments have not been addressed in this annual report.

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April 1996

Exxon Valdez Oil Spill
Restoration Project Annual Report

Investigations of Disease Factors Affecting Declines of Pacific Herring Populations in Prince William Sound

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April 1996

Investigations of Disease Factors Affecting Declines
of Pacific Herring Populations in Prince William Sound

Restoration Project 95320S
Annual Report

Study History: In 1993 there was a sudden and unexplained disappearance of approximately 60% (80K tons) of spawning herring in Prince William Sound, and the following year another 30K tons disappeared. An emergency project (94320 S) was authorized under emergency conditions in April 1994, and was not a part of a work plan. This emergency project was funded to investigate the circumstances surrounding the massive disappearance and it found that VHS, a viral disease heretofore unreported from Pacific herring was present in a number of surviving herring which also exhibited external ulcers and cutaneous hemorrhaging. In 1994 VHSV was present in less than 6% of the fish examined, however the prevalence of *Ichthyophonus hoferi*, a fungal pathogen of fish increased from 5% to 29%. As a result of these findings, the Alaska Department of Fish and Game (ADF&G) issued an RFP to study these declines in herring and to determine the involvement of the two above mentioned diseases. A detailed work plan was written as three components: 1) Field surveys (University of California, Davis); 2) Controlled laboratory infections (University of Washington, Seattle) and Physiological studies (Simon Fraser University, British Columbia). The three components of the study were designed to interact and supply information to each other in order to answer the questions regarding infection, pathogenicity and long-term recovery prospects of Prince William Sound herring.

Abstract: Field studies begun in 1993-1994 with the examination of herring from Prince William Sound (PWS) continued in 1995 in both Sitka Sound (SS) and PWS. Severe focal skin reddening or ulcers were >7 times more prevalent in spawning fish from PWS than from SS in 1995, less than its prevalence in 1994. The prevalence of *I. hoferi* was the same in PWS in 1995 as in 1994 and did not differ from SS in 1995. No VHSV was isolated from spawning fish from PWS or SS in 1995 but was isolated from >6% of pre-spawning PWS fish. External examinations concluded that spawning PWS herring were healthier in 1995 than in 1994, but those examined from SS in 1995 were in worse condition. Laboratory studies commenced to determine if VHSV and *Ichthyophonus* were pathogenic for Pacific herring by exposing laboratory reared specific pathogen-free (SPF) juvenile fish to pure cultures of both. Despite rapid death following exposure, they did not demonstrate the ulcers and extensive cutaneous hemorrhaging frequently observed in wild herring. *I. hoferi* was cultured for transmission and pathogenicity studies. Studies with SPF fish continue to determine the pathogenicity of this organism and its effect on herring recovery in PWS.

Key Words: *Clupea pallasi*, *Exxon Valdez*, hematology, herring, *Ichthyophonus*, morbidity, mortality, Prince William Sound, Viral Hemorrhagic Septicemia (VHS).

Project Data: (will be addressed in the final report)

Citation:

Marty, G.D., D.E. Hinton, R.M. Kocan, M.L. Landolt, J.R. Winton, C.J. Kennedy, and A.P. Farrell. 1996. Investigations of disease factors affecting declines of Pacific herring populations in Prince William Sound, *Exxon Valdez* Oil Spill Restoration Project Annual Report (Restoration Project 95320S), Alaska Department of Fish and Game, Habitat and Restoration Division, Anchorage, Alaska.

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Executive Summary

Overview of Disease Sections I, II, & III.

Restoration project 95320-S consists of three separate but interrelated components which are headed by Dr. Gary Marty of the University of California, Davis.(Component I), Dr. Richard Kocan, University of Washington (Component II) and Dr. Christopher Kennedy of Simon Fraser University, British Columbia (Component III). In depth summaries of each component can be found at the beginning of each section of this Annual Report.

Component I involves field surveys of morbidity and mortality in herring in Prince William Sound (PWS) and Sitka Sound (SS). Data generated from these studies gives managers the ability to track disease effects and recovery, thus enabling them to make more informed management decisions. This information also gives other investigators data on how natural infections and epizootic behave under natural conditions, thus allowing them to design meaningful controlled pathogenicity studies.

This study began in 1993-1994 with the examination of herring from PWS only. In 1995 herring were examined from Sitka and Prince William Sound and it was found that severe focal skin reddening or ulcers were more 7 times more prevalent in spawning fish from PWS than from SS. However, the prevalence of these conditions in PWS in 1995 was less than that found in 1994. The prevalence of I. hoferi was the same in PWS in 1995 as in 1994 and was not different from what was seen in SS in 1995. No VHSV was isolated from spawning fish from PWS or SS in 1995 but it was isolated from >6% of pre spawning fish from PWS. It was concluded that based on external examination, spawning PWS herring were healthier in 1995 than in 1994, but were in worse condition than spawning fish examined from SS in 1995. (A more detailed summary of this study will appear as an addendum).

Component II is establishing the pathogenicity of VHSV & *Ichthyophonus hoferi* in wild and laboratory-reared pathogen-free herring under controlled conditions, as well as documenting the influence of stress factors on the course and outcome of diseases in herring. This is accomplished by infecting fish with known levels of pathogens and comparing the data with that obtained from PWS and other habitat utilized by Pacific herring. By superimposing physical, chemical and biological stresses on fish infected with various pathogens, it is possible to determine the role played by stressors on the course of disease in both immune and non-immune individuals.

This project began in 1995 and focused mainly on the growth and husbandry of Pacific herring in the laboratory. It was necessary to establish a pathogen-free population of herring in order to evaluate the pathogenicity of individual organisms. The year was also dedicated to establishing *Ichthyophonus* cultures in the laboratory and evaluating methods for its cultivation and transfer to wild herring. Both of these objectives were met, and studies began on the exposure of these fish to various concentrations of VHSV as well as *I. hoferi*. Koch's Postulates were fulfilled, establishing with certainty that VHSV was a primary pathogen of juvenile herring and that it was capable of causing massive mortality in these young fish. Studies on juvenile wild fish were also undertaken and it was found that wild herring in Puget Sound were already infected with VHSV by the time they were 5 months old, and that capture and confinement of these fish resulted in massive epizootics killing 60% of the population. The survivors however, were solidly immune to re infection by VHSV, even at concentrations of virus in excess of 100 times the minimum lethal dose. *I. hoferi*, on the other hand, showed equivocal results when injected into wild herring of all ages, but was definitely lethal to pathogen-free juvenile herring.

Component III uses blood and tissues obtained from fish examined in Components I and II. These samples are used to determine hematological parameters for healthy and diseased herring as well as the status of physiological changes observed in infected fish relative to their uninfected counterparts. Measurements such as hematocrit (red blood cell levels), leucocrit (white blood cell levels), enzymes related to tissue damage and reproductive hormones are being investigated to determine if they could be used as biomarkers for identifying disease conditions in the field.

Because Component III depends heavily on Component II for specific pathogen-free herring, its major activity began late in 1995 when experiments were initiated with SPF herring which had reached 6 months of age. However, during the Summer of 1995 samples were collected from wild 5-month-old herring that had just suffered an epizootic of VHS as well as wild herring that had been fed *Ichthyophonus*. These blood samples showed that fish became extremely anemic, and the anemia persisted for several months after mortality ceased and virus could no longer be isolated from surviving individuals. Since none of the fish showed any signs of infection by *Ichthyophonus*, it was concluded that the observed response was to the VHSV. In addition, changes in differential white blood cell counts and elevated leucocrits (white blood cell volume) following the epizootics, indicated that the fish were responding with a cellular immune response. Most of the fish surviving the epizootic were observed to have bright green livers. Although it is not know if this condition is related to the VHS virus or some other parasite resent in the wild fish, it was presumably due to stasis of bile flow.

Exxon Valdez Oil Spill
Restoration Project Annual Report

Investigations of Disease Factors Affecting Declines of Pacific Herring Populations in Prince William Sound

Section I: Causes of Morbidity in Pacific Herring from Sitka Sound and Prince William Sound, Alaska, during Spring 1995

Restoration Project 95320S
Section I - Field Component
Annual Report

This annual report has been prepared for peer review as part of the *Exxon Valdez* Oil Spill Trustee Council restoration program for the purpose of assessing project progress. Peer review comments have not been addressed in this annual report.

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Investigations of Disease Factors Affecting Declines of
Pacific Herring Populations in Prince William Sound

Section I: Causes of Morbidity in Pacific Herring from Sitka Sound
and Prince William Sound, Alaska, during Spring 1995

Restoration Project 95320S
Annual Report

Study History: The project effort was initiated under Restoration Project 94320S. An annual report was issued in 1995 by Marty, G.D., E.F. Freiberg, T.R. Meyers, J. Wilcock, C.R. Davis, T.B. Farver, and D.E. Hinton, under the title *Ichthyophonus hoferi*, viral hemorrhagic septicemia virus, and other causes of morbidity in Pacific herring spawning in Prince William Sound in 1994. The project effort was continued under Restoration Project 95320S, Disease Impacts on PWS Herring Populations, the subject of this annual report.

Abstract: Pacific herring (*Clupea pallasi*) populations in Prince William Sound declined from an estimated 9.9×10^7 kg in 1992 to about 1.5×10^7 kg in 1994. Based on complete bacteriology, virology, hematology, and histopathology, viral hemorrhagic septicemia virus and the fungus *Ichthyophonus hoferi* contributed most to population decline in 1994. Study was expanded in 1995 to include examination of prespawning fish and fish from a reference site, Sitka Sound. In 1995, moderate or severe focal skin reddening or ulcers were more prevalent in spawning fish from Prince William Sound (2.8%) than in spawning fish from Sitka Sound (1.3%), but lesion prevalence at both sites was less than in spawning fish from Prince William Sound in 1994 (8.4%). *Ichthyophonus* prevalence in Prince William Sound spawning fish in 1995 (29%) was the same as in 1994 and no different from Sitka Sound in 1995 (26%). At both sites in 1995, prevalence of *Ichthyophonus* among all fish was higher in 7-year-old fish than in 2- and 3-year-old fish (Prince William Sound, 35% vs. 9.6%; Sitka Sound, 31% vs. 22%). Also, viral hemorrhagic septicemia virus was not isolated from any spawning fish in Prince William Sound or Sitka Sound, but VHSV was isolated from 6.2% of prespawning fish from Prince William Sound.

Key Words: *Clupea pallasi*, disease, *Exxon Valdez* oil spill, histopathology, *Ichthyophonus hoferi*, morbidity, Pacific herring, plasma chemistries, Prince William Sound, Sitka Sound, viral hemorrhagic septicemia virus (VHSV).

Citation:

Marty, G.D., C.R. Davis, T.R. Meyers, J. Wilcock, E.F. Freiberg, and D.E. Hinton. 1996. Investigations of disease factors affecting declines of Pacific Herring populations in Prince William Sound. Section I: Causes of morbidity in Pacific herring from Sitka Sound and Prince William Sound, Alaska, during spring 1995, *Exxon Valdez* Oil Spill Restoration Project Annual Report (Restoration Project 95320S), University of California, Davis, California.

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Executive Summary

Introduction

Before the 1993 spawning season, 120,000 tons of Pacific herring (*Clupea pallasi*) were forecast to arrive on the spawning grounds of Prince William Sound (PWS); only 20,000 tons appeared. Viral hemorrhagic septicemia virus (VHSV), but no other significant pathogens, was isolated from those herring. In 1994, when only 17,000 tons of an expected 27,000 tons of herring appeared on the spawning grounds, this study was initiated to investigate the cause of herring population decline. Given the history of VHSV isolation in 1993, the study was designed to investigate the role of infectious disease in the PWS Pacific herring population, with primary emphasis on the role of VHSV. Study of spawning fish in PWS in 1994 revealed two significant diseases: 1) 5% of the fish had VHSV, and the virus was commonly associated with external lesions described by biologists and fishers in 1993; and 2) 29% of the fish were infected with the fungus *Ichthyophonus hoferi*, and the fungus was associated with severe internal lesions (external lesions were less common).

We concluded that disease was significantly contributing to population decline, but background disease prevalence and the role of reproductive stage were unknown. Therefore, field study was expanded in 1995 to include prespawning samples and samples from a reference site, Sitka Sound (SS). Like PWS, the Pacific herring population in SS has a dominant 1988 year class, but the SS population continues to support commercial and subsistence fishing. Although a recently closed pulp mill that contributed an unknown amount of pollution into SS, there was no history of a large oil spill. Prince William Sound Pacific herring fisheries were severely curtailed in 1993, and were never opened in 1994, 1995, or 1996. This section reports the findings from field disease studies in 1995. Laboratory study was initiated to explore details of VHSV and *Ichthyophonus* infections under controlled conditions; results from the laboratory component are reported in sections II and III.

Objectives

Field study had three objectives: 1) determine the relation among VHSV, *Ichthyophonus*, macroscopic and microscopic lesions, plasma chemistries, and immune status; 2) determine the role of reproductive stage on the general health of herring [Are lesions and VHSV more severe during a given reproductive stage? Does a history of previous oil exposure correlate with prevalence and severity of disease?]; and 3) determine the impact of disease on population size and structure of herring [Are fish of a particular year class more likely to be diseased than other year classes?].

Methods

To determine disease status, Pacific herring were sampled in 3 groups: 1) 240 spawning fish from SS (March 25-29, 1995); 2) 80 immature or prespawning fish from PWS (April 11,

1995); and 3) 180 fish with ripe gonads (spawning) from PWS (April 24-27, 1995). Because of administrative delays in the Request for Proposal process used for this project, funding was approved too late to obtain prespawning samples as proposed in SS. All 500 fish were subjected to complete necropsy, which included weight, standard length, and scoring of external lesions. Samples were taken for histopathology (12 organs), virus isolation (head kidney and spleen), age determination (from scales), and plasma chemistry analysis (total protein, albumin, osmolality, cholesterol, glucose, total bilirubin, 5 enzymes, and 5 electrolytes). Also, white blood cell differential counts were made from blood smears, and smears were examined for the only other known herring virus, viral erythrocytic necrosis (all were negative). For histopathology, tissues were coded for blind study, and lesions were ranked on a four-point scale as none (0), mild (1), moderate (2), or severe (3). In all fish with severe external lesions, kidney was cultured for bacteria (all were negative). As a measure of immune function, an assay for Pacific herring immunoglobulin (IgM) was developed and used on plasma samples from 476 fish. To determine significance, results were analyzed statistically. Parasites or other pathogens that were not statistically associated with lesions or alterations in blood values were not considered pathologically significant.

Results

Moderate or severe focal skin reddening or ulcers were more prevalent in spawning fish from PWS (2.8%) than in spawning fish from SS (1.3%), but prevalence of these lesions in 1995 was less than in spawning fish from PWS in 1994 (8.4%). For internal lesions, *Ichthyophonus* prevalence in PWS spawning fish (29%) was the same as in 1994 and no different from the *Ichthyophonus* prevalence in spawning fish from SS (26%). At both sites in 1995, prevalence of *Ichthyophonus* among all fish was higher in 7-year-old fish than in 2- and 3-year-old fish (PWS, 35% vs. 9.6%; SS, 31% vs. 22%), but age differences were significant only for PWS fish. Viral hemorrhagic septicemia virus was not isolated from any spawning fish in PWS or SS, but VHSV was isolated from 6.2% of prespawning fish from PWS.

Prevalence of 4 subtle inflammatory lesions was significantly greater (chi-square test, $P \leq 0.05$) in spawning fish from PWS than SS: 1) white blood cells around vessels in skeletal muscle (PWS = 77%, SS =65 %); 2) foci of white blood cells in the liver (PWS = 81%, SS = 49%); 3) foci of white blood cells in the heart (PWS = 32%, SS = 24%); and 4) foci of white blood cells in the stomach (PWS = 13%, SS = 1.7%).

Parasite prevalence in spawning fish from PWS and SS was significantly different for 3 parasites in 1995: 1) testicular coccidian *Eimeria sardinae* (PWS = 85%, SS = 66%); 2) renal intraductal protozoan (PWS = 11%, SS = 3.8%); and 3) branchial *Epitheliocystis*, (PWS = 15%, SS = 25%). Differences in prevalence of other common parasites were not significantly different: 1) peritoneal larval Anisakidae (PWS = 100%, SS = 99%); 2) unclassified intestinal coccidian (PWS = 95%, SS = 91%); 3) hepatic coccidian *Goussia clupearum* (PWS = 73%, SS = 71%); 4) gall bladder myxosporean *Ceratomyxa auerbachi* (PWS = 39%, SS = 32%); 4) renal intraductal

myxosporean *Ortholinea orientalis* (PWS = 29%, SS = 20%); 6) gastric trematodes (PWS = 12%, SS = 10%); and 7) branchial monogenetic trematodes (PWS and SS = 11%).

Discussion

After the first year of study in 1994, we considered whether the oil spill could have been linked to disease outbreak 4 years later. Fish that were hatched or were yearlings in 1989 at the time of the spill (1988 and 1989 year classes) might have incurred permanent damage to their ability to fight disease (i.e., irreversible immunosuppression), so we examined the association of lesions with age. Several lesions were significantly associated with age (e.g., pigmented macrophage aggregates), but nearly all these lesions were more severe in older fish (i.e., fish hatched before 1988). Also, among VHSV, *Ichthyophonus*, and 10 other common parasites, none were more prevalent in the 1988 and 1989 year classes than in the entire sampled population. Therefore, the weight of evidence suggested that the disease outbreak in PWS was not a result of permanent immune suppression caused by hydrocarbon exposure when fish were larvae or yearlings. In 1995, prevalence of *Ichthyophonus* in PWS was significantly higher in the 1988 year class. However, because *Ichthyophonus* prevalence was also relatively higher in the 1988 year class in SS, increased *Ichthyophonus* prevalence in these fish was more likely a function of advanced age and not directly related to previous oil exposure.

None of the diseases in Pacific herring in PWS were unique. In our previous studies of Pacific herring in PWS and Auke Bay, Alaska (1989-1993), *Ichthyophonus* was present, but its prevalence was never more than 15%. Published studies of acute population declines in Atlantic herring (*Clupea harengus*) reported that *Ichthyophonus* was the primary cause. In those studies, *Ichthyophonus* prevalence was ≥25%. The first studies of VHSV in Pacific herring in PWS (1993) were done on samples pooled from several fish, so prevalence could not be determined. Since then, VHSV has been isolated from Pacific herring sampled elsewhere in Alaska, British Columbia, and Washington. Only one other report of disease-associated population decline of Pacific herring has been published; the cause was not determined, but clinical and pathological findings were more similar to our findings associated with VHSV than with *Ichthyophonus*. We established that VHSV and *Ichthyophonus* were associated with several lesions in 1994, and their role as a primary invaders has been confirmed on disease-free fish reared in the laboratory (see section II, this report).

The weight of evidence implicates VHSV as the major cause of disease and mortality in PWS Pacific herring in 1993. By 1994, both VHSV and *Ichthyophonus* were important. And in 1995, the role of VHSV was decreasing (e.g., no virus was isolated from spawning fish) while *Ichthyophonus* continued to be a major cause of disease. Based on external examination, spawning PWS Pacific herring were in better condition in 1995 than in 1994, but PWS spawning fish were in worse condition than SS spawning fish. By comparison, *Ichthyophonus* prevalence was similar among all three sample groups. The significance of VHSV in prespawning PWS samples is unknown, but prespawning samples were collected from PWS and SS in 1996 to better understand the dynamics of VHSV before spawning. For *Ichthyophonus*, high prevalence in

spawning fish is reason for concern for both the PWS and SS populations. However, because *Ichthyophonus* cases were concentrated among 7-year-old fish, and 2- and 3-year-old fish were infected at historically endemic levels, the *Ichthyophonus* outbreak may also be subsiding. If younger fish remain relatively free of *Ichthyophonus*, disease is less likely to impair their continued recruitment into the fishery in 1996 and 1997. As with VHSV, the second year of sampling in SS is critical for determining the role of *Ichthyophonus* in population size in PWS and SS. Also, ongoing laboratory studies with *Ichthyophonus* and VHSV (University of Washington and Simon Fraser University) will begin to determine methods of transmission, routes of infection, incubation period, and other variables that might provide clues to how these diseases can be managed in the future.

Conclusions

Disease was probably the primary force driving population decline in 1993, 1994, and 1995, but its role in 1995 was primarily limited to older fish. Preliminary evidence from analysis of fall 1995 samples indicates that the proportion of 7-year-olds in the Pacific herring populations of both PWS and SS decreased by up to 80% between spring and fall samples. This provides evidence that the high rate of *Ichthyophonus* infection in older fish was significant. No other variables—food availability, predation, water temperature, currents, or recruitment—were needed to explain the significant decline in the 1988 year class. These other variables may be more important during population recovery, but if young fish cannot escape disease transmission, disease will continue to limit recovery. Pacific herring populations in PWS were not healthy in 1994 or 1995, and until Pacific herring populations recover, continued study of herring disease is recommended.

Introduction

When the *Exxon Valdez* oil spill occurred in March, 1989, the biomass of spawning Pacific herring in PWS was the highest in 20 years of reliable estimates (about 10×10^7 kg; Figure 1), and the population remained near record levels through 1992. Pacific herring in PWS first spawn when 3 or 4 years old. They rarely live more than 12 years, and abundant year classes recruit into the fishery about once every 4 years. In 1993, recruitment from the 1988 year class was expected to be excellent; therefore, fisheries biologists predicted a record spawning biomass of 11×10^7 kg before the spawning season (Figure 1). However, when the 1993 spawning season commenced, only 17% of the expected biomass appeared, fish were lethargic, and many had external hemorrhages. Hence, PWS Pacific herring fisheries were severely curtailed in 1993, and were never opened in 1994, 1995, or 1996. In PWS, Pacific herring normally support 5 commercial fisheries, with an average annual ex-vessel value of $8.3 million. Roe fisheries, the most valuable, are harvested in April just before spawning.

Toxicants such as crude oil cause more severe damage in younger fish, particularly larvae (McKim 1985); therefore long-term effects of the oil spill were thought most likely to occur in the

1988 and 1989 year classes which entered the spawning population in 1992 and 1993. Indeed, preliminary study of 4-year-old PWS Pacific herring in 1992 revealed less reproductive success in fish spawning in previously oiled sites than in unoiled sites, and fish with poor reproductive success had more severe microscopic lesions (Kocan et al. In Press). In 1993, the North American strain of viral hemorrhagic septicemia virus (VHSV) was isolated from pooled samples of Pacific herring from PWS, but no other significant pathogens were isolated (Meyers et al. 1994). Because VHSV had not previously been isolated from Pacific herring, its role in population decline could not be determined. By 1994, spawning biomass declined to the lowest level ($1.5x10^7$ kg) recorded in 20 years of reliable estimates.

This study was initiated in 1994 to determine the cause of morbidity in PWS Pacific herring. Study included thorough necropsy, virology, bacteriology, hematology, and histopathology linked to traditional age-weight-length analysis. Our primary hypothesis was that VHSV was the most important cause of mortality, but the study was designed to diagnose other potential pathogens. We confirmed that VHSV was a significant cause of morbidity, and we also found that the fungus, *Ichthyophonus hoferi*, was important. Ten other parasites each affected more than 10% of the sampled population, but their role in population decline probably was minimal. Also, prevalence of most parasites was independent of age. We concluded that disease was significantly contributing to population decline, but background disease prevalence and the role of reproductive stage were unknown.

Study was expanded in 1995 to include prespawning samples and samples from a reference site (SS). The Pacific herring population in SS supports commercial and subsistence fishing, and there is no history of a large oil spill. In PWS in 1995, VHSV was a less important pathogen, but *Ichthyophonus* continued to be significant. We also found that *Ichthyophonus* was a significant pathogen in SS. Laboratory study was initiated to explore details of VHSV and *Ichthyophonus* infections under controlled conditions. This section reports the findings from field disease studies in 1995; results from the laboratory component are reported in sections II and III.

Methods

Necropsy

Pacific herring were captured in 2 different sites. At the reference site (SS), 240 fish in spawning condition were captured by purse seine or cast net (March 25 - 29, 1995), transported to a heated garage in Sitka, and subjected to complete necropsy. In Prince William Sound (PWS), fish in 2 different spawning stages were captured and subjected to complete necropsy: 1) 80 fish with immature or unripe gonads (prespawning) were sampled from Zaikof Bay on Montague Island on April 11,1995, on board the *Auklet*; and 2) 180 fish with ripe gonads (spawning) were sampled from Rocky Bay on Montague Island from April 24 through 27, 1995, on board the *R/V Montague*. All PWS fish were captured using purse seines. Because of

administrative delays in the Request for Proposal process used for this project, funding was approved too late to obtain prespawning samples as proposed in SS.

Each fish was assigned a necropsy number, 95HER-1 through 95HER-500, in order of necropsy. After capture in SS, fish were held in plastic containers filled with about 60 L of seawater (7 fish per container) for no more than 4 hours before necropsy. After capture in PWS, fish were held in plastic containers filled with about 300 L of seawater for no more than 3.5 hours before necropsy. In groups of 2, herring were anesthetized in tricaine methane sulfonate (Finquel®), weighed and measured (standard length), and a scale was removed for age determination. Several diagnostic procedures were done on each fish:

1) external lesions were scored as none (0), mild (1), moderate (2), or severe (3). After lesions were scored, a summary "external lesion score" was determined for each fish. The external lesion score was the most severe score for fin base reddening, caudal fin reddening, focal skin reddening, or diffuse skin reddening. External lesions "iris reddening" and "caudal fin fraying" were not used for determination of external lesion score. Gonadal fullness was estimated and scored as 3 (75-100% full), 2 (50-74% full), 1 (25-49% full), or 0 (0-25% full).

2) about 1.5 mL of blood was drawn from the caudal vein into 3-mL syringes that contained 0.1 mL of lithium heparin (1,000 IU/mL); a capillary tube was filled and centrifuged (5500 × *g* for 5 min) for determination of packed cell volume (PCV), a blood smear was made and air-dried, and remaining blood was centrifuged (13,600 × *g* for 5 min) and plasma was chilled on ice for analysis by Med Veterinary Laboratory, Concord, California, within 72 h of sampling. A 100-µL plasma aliquot from each fish was frozen separately for IgM analysis; details of assay development are described elsewhere (p. I-10 below). Too little plasma was collected from 24 fish for IgM analysis; plasma chemistry analysis was given highest priority for analysis.

Osmolality was analyzed on a Micro Osmometer Model 3MO-plus from Advanced Instruments (Norwood, MA) using 20 µL of sample. All other analyses were done using about 200 µL of sample in a Monarch-plus analyzer from Instrumentation Laboratories (IL®) that was calibrated and run at a stabilized 25° C. Plasma was analyzed for total protein (biuret method), albumin (bromocresol green method), and CO_2 (enzymatic method); IL® substrates were used to analyze calcium, cholesterol, glucose, phosphorus, total bilirubin, alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and creatine phosphokinase (CPK); Sigma® substrates were used to analyze gamma glutamyltransferase (GGT); ion selective electrodes were used to analyze sodium, potassium, and chloride.

Blood smears were sent to the laboratory of Chris Kennedy, Simon Fraser University, where they were stained with Diff-Quik (Dade Diagnostics, Inc., Aquada, Puerto Rico) and 30 1000×-fields were examined for cytoplasmic inclusions of viral

erythrocytic necrosis (VEN; all were negative). Also, differential leukocyte counts were done by counting approximately 100 white blood cells in randomly selected fields (mean = 48 fields per slide).

3) for virus isolation, head kidney and spleen from each fish were pooled in a plastic bag and shipped on ice to the Alaska Department of Fish and Game Fish Pathology Laboratory in Juneau, Alaska; skin lesions, if present, were sampled and bagged separately for individual virus assay. Propagation of 1 cell line (EPC), media formulation, and tissue preparation for cell line inoculation was as described by Meyers et al. (1994). Propagation of a second cell line (PHE, a herring cell line) was the same except that the media used did not contain tryptose phosphate broth.

4) for histopathology, samples of gill, liver, gonad, spleen, trunk kidney, gastrointestinal tract, heart, skin, skeletal muscle, and brain were fixed in 10% neutral buffered formalin;

5) bacterial isolation was attempted from herring with severe external lesions; kidney tissues were aseptically inoculated onto trypticase soy agar (TSA) and marine agar and plates were incubated at 23° C for at least 5 days (all were negative);

6) a touch preparation of kidney was air-dried, stained with Stat Stain® (American Histology Reagent Company, Lodi, CA), and examined for pansporoblasts of the myxosporean *Ortholinea orientalis*; extent of infestation was scored as for external lesions;

7) liver and gonads were weighed;

8) herring worms (Anisakidae) in the peritoneal cavity were counted;

9) archived samples (frozen at −80° C) from each fish included liver (0.1 - 0.2 g, in 1.5-mL plastic vials), and a wedge of epaxial skeletal muscle from just anterior to the dorsal fin (also in a 1.5-mL plastic vials);

10) from 5 PWS fish with gross lesions consistent with *Ichthyophonus* infection, affected organs were minced with a clean razor blade, transferred to tissue culture media at 4°C, and delivered to Dr. Richard Kocan at the University of Washington for further study.

We had proposed to split the 180-fish PWS spawning sample into 160 randomly selected fish and 20 fish selected based on gross lesions. Because only 3 fish were found with sufficiently severe lesions to meet the special selection criteria, 177 spawning fish were selected at random from PWS. At both SS and PWS, nearly all fish in the spawning sample had gonads in spawning condition. Some of the fish in SS were actively spawning when captured, whereas fish in Rocky bay, PWS, did not start active spawning until the last day of sampling. Among fish in the PWS prespawning sample, 44 of 80 (55%) were reproductively immature. The proposal called for sampling only mature fish, but to maintain a random selection process, immature fish were also

necropsied. Samples from these immature fish were useful for determining the distribution of disease in young Pacific herring.

Histopathology

Tissues from 500 herring were sent to the Aquatic Toxicology Laboratory, University of California, Davis, and randomly assigned a histopathology number (95H5-1 through 95H5-500) for blind study. Pieces of skin/skeletal muscle and gill were postfixed in Bouin's for 24 h and then returned to 10% neutral buffered formalin. Tissues were processed routinely into paraffin, sectioned at 5 μm, and stained with hematoxylin and eosin. Tissues from each organ were read in ascending numerical order using the random histopathology number. In most cases, all tissues from one organ were read before tissues from the next organ were started. Lesions were scored using a four-point scale as none (0), mild (1), moderate (2), or severe (3). For quality control, autolysis and artifact in each organ were scored on the same four-point scale. Ranking of lesions was often based on the number of structures (e.g., *Ichthyophonus* resting spores) per 100× field; the 100× field was examined through a 10× objective lens and a 10× ocular lens on an Olympus binocular light microscope. After all organs were examined and lesions scored, data were rearranged by necropsy number and basic statistics (e.g., prevalence in SS vs. PWS) were calculated.

IgM detection in Plasma: ELISA Development and Assay

In April 1995, prespawning Pacific herring from PWS were caught in purse seines and held in 300 L salt water for a maximum of 2 hours. Fish were anesthetized in tricaine methane sulfonate (Finquel®) and blood was collected from the caudal vein into heparinized syringes. Samples from 100 fish were pooled into 50-mL plastic tubes and centrifuged at 3000 rpm for 10 minutes. Plasma was harvested and held at −20° C for transport to the laboratory, then stored at −70° C until processed.

Heparinized pooled plasma was thawed and centrifuged at 4° C at 10,000 *g* for 30 minutes to remove precipitated fibrin. The supernatant was placed in Spectra-por 2 molecularporous membrane tubing and suspended in 4 L of 5 mM Tris dialysis buffer, pH 7.4 at 4° C. The dialysis solution was changed every 12 hours for 4 days. The precipitate (euglobulin fraction) was collected via centrifugation at 4° C. The pellet was washed twice in 5 mM Tris buffer and resuspended in 0.1 M Tris HCL plus 1 M NaCl, pH 8.6. The solution was desalted (Sephadex PD10 column). Protein content was determined via the Lowry (Folin:Ciocalteu) method.

The euglobulin fraction was analyzed by sodium dodecyl-sulfonate polyacrylamide gel electrophoresis (SDS-PAGE) under reduced and nonreduced conditions. Proteins were electrophoretically transferred to polyvinylidene difluoride (PVDF) membranes (Immobilon-P, Millipore) and stained with Coomassie blue. Membranes were also incubated with specific polyclonal rabbit anti-herring euglobulin antibodies (described below). Internal sequence analysis

was carried out on the heavy chain portion after SDS-PAGE and electrophoretic transfer to PVDF membranes (Protein Structure Laboratory, UC Davis).

The euglobulin fraction was deglycosylated using a modification of a previously described protocol (Mattes and Steiner 1978). Euglobulin (2.5 mg) was eluted in 0.04 M sodium acetate buffer, pH 5.4, 0.1 M NaCl using a Sephadex PD-10 column. An equal volume of 20 mM sodium periodate was added to the eluent and incubated at room temperature for one hour. Glycerol was added to 50% total volume and the mixture was dialyzed, using Spectra-por 2 tubing, against 4 L PBS, pH 7.4, overnight at 4° C. The tubing content was centrifuged at 14,000 *g* for 2 minutes. The supernatant was collected, divided into aliquots and stored at −20 or −70 ° C. Protein concentration was determined via Lowry (Folin-Ciocalteau) method.

A single male New Zealand White rabbit received an initial inoculation of 400 µg deglycosylated euglobulin in complete Freund's adjuvant. A second inoculation of 400 µg deglycosylated euglobulin in incomplete Freund's adjuvant was administered 4 weeks later. Titered by ELISA, rabbit antibodies to the herring euglobulin were detected at a dilution of 1:51,200 in rabbit serum collected 10 days after the second inoculation. Polyclonal rabbit immunoglobulin was purified by methods previously described (Mckinney and Parkinson 1987). A portion of the purified antibody was biotinylated with NHS-LC-Biotin according to manufacturer instructions (Pierce).

The ELISA was optimized using serial dilutions of rabbit-anti herring immunoglobulin run on wells coated with 1 or 4 µg/mL euglobulin and streptavidin at 1:1000 or 1:2000. With an optimal rabbit immunoglobulin dilution of 1:2500 (1.2 µg/mL) and streptavidin dilution of 1:1000, serial dilutions of euglobulin were run against several dilutions of biotinylated antibody. From these data, the linear portion of the standard curve was determined and a single concentration (2.5 µg/mL) of euglobulin was then run against serial dilutions of biotinylated antibody to determine the optimal dilution.

To determine IgM concentrations in plasma samples, 96-well immunoassay plates (Falcon®, Pro-bind™) were coated with 50 µL/well rabbit anti-herring euglobulin antibody diluted 1:2500 (1µg/mL) in 50 mM bicarbonate/carbonate buffer, pH 9.6 and incubated overnight at 4° C or 2 hours at 37° C. Following incubation, plates were washed 5 times with Tween TBS (TTBS: 50 mM Tris, pH 8.0, 1 mM EDTA, 150 mM NaCl and 1 mL Tween-20 per L) and shaken vigorously to remove excess fluid. Wells were blocked with 5% nonfat dry milk in TTBS and incubated at 37° C for 60 minutes. Plates were then washed 5 times. Fifty (50) µL of test sample were added to each well and incubated at 37° C for 60 minutes. Plates were again washed 5 times, followed by addition of 50 µL/well of biotinylated rabbit anti-herring euglobulin antibody diluted 1:800 in blocking buffer. After a 60 minute incubation at 37° C, the plates were again washed, followed by addition of 50 µL/well peroxidase-streptavidin diluted 1:1000 in blocking buffer. After a 30 minute incubation at 37° C, plates were washed 9 times. A TMB substrate solution (100 µL/well) was added and incubated 20 to 40 minutes at 37°C. The reaction was stopped with addition of 50 µL/well 1 M H_2SO_4. Each plate contained reference blanks and

standard curves in triplicate. The standard curve was constructed using serial dilutions of euglobulin of known protein concentration. Plates were read at 450 nm on a Ceres 900 Hdi plate reader (Bio-Tek) and interpreted with Kineti Calc™ version 2.12 software using end point curvilinear regression.

The purified euglobulin fraction consisted primarily of a protein with a molecular weight of approximately 800 kD. Under reducing conditions, this protein broke down into a 70,000 kD heavy chain and 3 light chains in the 20-23 kD range (characterization in progress). Amino acid sequencing from the N-terminal end was not possible due to blocking; however, internal sequences had significant sequence homology with Atlantic salmon (*Salmo salar*), rainbow trout (*Oncorhynchus mykiss*), tarpon (*Elops* sp.), and sheep Ig heavy chain constant regions.

The intraplate coefficient of variation for the ELISA (same plate, same day, same sample: 5 standard dilutions run in triplicate on a total of 60 plates) ranged from 0.26% to 36.31% (mean = 10.90%). The interplate coefficient of variation (different plate, different day, same sample: 9 samples run in triplicate on 3 different days) ranged from 3.66% to 32.60% (mean = 16.09%). The lower limit of detection for the ELISA was 0.039 µg IgM/mL. The test can be performed in 4-6 hours.

Statistical Analysis

Analysis involved two major hypotheses: 1) fish with lesions were different from fish without lesions; and 2) fish from PWS were different from SS. Because unripe prespawning fish were not captured in Sitka, and prespawning samples from PWS had many immature fish not present in spawning samples, statistical analysis usually focused on comparing spawning fish from SS (n = 240) and PWS (n = 180). In most cases, lesions with a score of none (0) were used as controls for determining significance of lesions. The association of categorical variables (e.g., none, mild, moderate, and severe) with continuous variables (e.g., CPK values) was determined using one-way analysis of variance (one-way ANOVA). For example, the CPK values for fish with a liver *Ichthyophonus* score of zero were compared to livers with mild, moderate, and severe *Ichthyophonus*. When necessary, categories were combined to ensure that each group had at least 8 fish. Also, some values were ln transformed before analysis; % data were arcsine square root transformed before analysis. In most cases, data were retransformed to the geometric mean and the first-order Taylor series was used to estimate the standard error of retransformed geometric means. Category-specific means and standard errors were calculated for each continuous variable and compared using Tukey's Studentized range method. Levene's test was used to evaluate the homogeneity of variance assumption for the ANOVA. Analyses were run separately for spawning fish from SS and PWS.

Analysis of variance with two grouping factors was used to evaluate the relationship between continuous variables (e.g., CPK) and independent variables site, gender, and site-gender interactions. Analyses were run separately for spawning fish from SS and PWS.

The association between 2 selected categorical variables (e.g., caudal fin fraying versus scores for hepatic focal necrosis) was evaluated using chi-square methods for categorical data analysis; comparisons were considered valid only if individual expected cell frequencies were >1. Odds ratios were calculated for standard (2×2) 2-way contingency tables only. Data from SS and PWS were combined for this analysis.

To measure the strength of the linear relationship between 2 continuous variables, the correlation coefficient r was calculated separately for spawning fish from PWS and SS. Multiple regression analysis was used in 1994 to examine the relationships between selected dependent variables (e.g., plasma albumin) and associated variables (e.g., focal skin reddening, splenic congestion, and VHSV). However, determining significance of potentially important interactions requires large numbers of fish; therefore, additional multiple regression analyses are not a part of this annual report. Instead, we anticipate that multiple regressions will be used frequently in the final report (with n ≈ 2,000) to characterize more fully variations in plasma chemistry values.

To determine if certain age classes of fish were more likely to be infected by certain parasites, the association of fish age with common parasites was evaluated using the chi-square test for homogeneity. Fish were separated by site. Fish from SS were grouped into three categories for analysis: 2 or 3 years old, 4 to 6 years old, or ≥7 years old. Fish from PWS were grouped into 4 categories for analysis: 2 or 3 years old, 4 to 6 years old, 7 years old, or ≥8 years old. Regardless of severity of infestation, fish with a given parasite were classified as positive, and fish without the parasite were classified as negative.

For all analyses, comparisons were considered significant when $P \leq 0.05$ and highly significant when $P \leq 0.01$. Use of the term "prevalence" refers to the sample prevalence.

Results

Note on the contents the results section in this report: The annual report for project 94320-S (Marty et al. 1995) contains detailed descriptions and micrographs of most of the significant lesions in Pacific herring. Those descriptions are not repeated in this report, but will be included in the final synthesis report. This report concentrates on significant differences in lesions, necropsy findings, and plasma chemistry values in samples from 1995. Variables considered included spawning stage, site of capture, and year of capture, with special emphasis on organisms and lesions likely to result in population level effects.

Revisions from 1994 Study (94320S)

In the 1994 annual synthesis report, the intestinal trematode category incorrectly included intestinal cestodes. Also, the testicular coccidian *Eimeria sardinae* was not scored. Slides were re-examined and scores corrected. Among 211 intestines examined, corrected scores for intestinal trematodes include 204 none, 6 mild, and 0 moderate or severe (overall prevalence = 2.9%); corrected scores for intestinal cestodes include 205 none, 1 mild, 4 moderate, and 0 severe

(overall prevalence = 2.4%). Among 102 testes that were reexamined, coccidians were scored as 44 none, 52 mild, 6 moderate, and 0 severe (overall prevalence in males = 57%). Statistical analysis was not redone for this report, but will be part of the final synthesis report.

Necropsy Findings and External Gross Lesions - 1995 Samples

Difference in mean age of the 3 sample groups were highly significant (Table 1). Prespawning samples from PWS included 55% immature fish, whereas immature fish were rare in spawning samples from PWS and SS. Spawning fish from PWS had relatively more 7-yr-olds than did the SS samples, so mean age of spawning PWS fish was greater that SS fish (Table 1). Significant differences in other morphometric necropsy variables were consistent with age differences between the sample groups. Hold time was significantly longer for fish from SS because of the extra time it took to transport SS fish to a garage before necropsy (necropsy was done on a ship at the capture site in PWS). Hold time never exceeded 4.1 hr at either site. Fin base reddening was significantly associated with increased hold time and plasma CO_2, particularly in PWS samples.

Prevalence of external lesions was greater in spawning fish from PWS than in spawning fish from SS. The summary external lesion score was moderate or severe in 46 of 180 (26%) of PWS fish but only 16 of 240 (6.7%) SS fish. Further, focal skin reddening in spawning fish was moderate or severe in 2.8% of spawning PWS fish but only 1.1% of SS fish. By comparison, 8.3% of spawning fish from PWS in 1994 had moderate or severe focal skin reddening.

The relation between external lesions and plasma chemistry values was often highly significant, but significant variables in SS and PWS fish were never the same (Table 2). However, a few plasma chemistry values were consistently related to external lesions when spawning samples from PWS in 1994 were compared to spawning samples from SS in 1995; examples include: 1) increased caudal fin fraying vs. increased osmolality and calcium; and 2) increased iris reddening vs. increased calcium, osmolality, potassium, and phosphorus.

External lesions, especially caudal fin fraying, were significantly associated with several gross and microscopic lesions (Table 3). Interestingly, increased focal skin reddening was not associated with any external lesions but was significantly associated with several *Ichthyophonus* scores and with the intestinal coccidian (*Goussia* sp?). As scores for external lesions increased, scores for associated lesions usually increased. Two exceptions were (1) lower hepatic lipidosis scores were were associated with higher scores for caudal fin fraying, and (2) lower scores for the intestinal coccidian (*Goussia* sp?) were associated with higher scores for diffuse skin reddening. Also, scores for diffuse skin reddening were significantly lower when gonads were full; that is, scores for diffuse skin reddening tended to increase as spawning progressed.

Microscopic Lesions - 1995 Samples

Ichthyophonus

Overall prevalence of *Ichthyophonus* was the same in spawning samples from PWS in 1994 (29%, 62 of 212) and 1995 (29%, 52 of 180), and prevalence of *Ichthyophonus* in 1995 SS samples (26%, 62 of 240) was not significantly different. In 1994, *Ichthyophonus* prevalence among age groups was not significantly different, but in 1995, *Ichthyophonus* was significantly more frequent in the 1988 year class (35%) than in the 1992 and 1993 year classes (9.6%); therefore, the 1988 year class made up a disproportionally high proportion of the *Ichthyophonus* in PWS in 1995 (Figure 2). Interestingly, the 1988 year class also made up a disproportionally high proportion of the *Ichthyophonus* cases in SS in 1995 (Figure 3), but difference were not significant.

All organs contained *Ichthyophonus* (Table 2), and the multinucleate resting spore stage was the most common form. Morphology of *Ichthyophonus* and the host reaction were similar to those reported in infections in Atlantic herring (*Clupea harengus*) (Daniel 1933, Sindermann 1970). Scoring, histologic features, and differential diagnoses were nearly the same as reported last year (Marty et al. 1995). The 1 exception was scoring of *Ichthyophonus* in the stomach, where 2 scores were given: 1) ICH - the standard *Ichthyophonus* as described in 1994 required that organisms were in the sections before a score was given; and 2) ICH+ - includes all standard ICH scores, but also includes scores for stomachs that contained characteristic ~200-µm-diameter rims of inactive fibroblasts and mature collagen, but no *Ichthyophonus* organisms. Differences between ICH and ICH+ scores in SS were highly significant (10.4 vs. 18.8%, chi-square test, 2×2 contingency table), whereas differences between ICH and ICH+ scores in PWS spawning fish were not significant (15.6 vs. 18.9%).

Although the overall *Ichthyophonus* prevalence in spawning fish was 29% in PWS and 26% in SS, in no single organ was *Ichthyophonus* prevalence > 22% in PWS or > 17% in SS (Figure 4). As in 1994, the skin and skeletal muscle had the highest prevalence of mild *Ichthyophonus* cases, whereas cases in the heart and kidney were more likely to be severe (Figure 4). A sum-*Ichthyophonus* (sumICH) score was calculated for each fish by adding the individual *Ichthyophonus* scores from all 10 organs for that particular fish as described in the 1994 report (Marty et al. 1995). In 1995, the highest sumICH scores were 23 for PWS and 19 for SS.

Association of *Ichthyophonus* scores with plasma chemistries was variable (Table 2). In 1994, increased AST and CPK values were significantly associated with increased *Ichthyophonus* scores in every organ (univariate ANOVA). In 1995, the association of *Ichthyophonus* scores with AST and CPK was less distinct: AST and CPK significantly increased with most SS *Ichthyophonus* scores, but only AST significantly increased with most PWS *Ichthyophonus* scores. Increased IgM levels were significantly associated with increased *Ichthyophonus* scores in all organs analyzed, and differences were highly significant.

VHSV

Only 5 fish were positive for VHSV, all from prespawning samples in PWS. None of the 180 spawning samples from PWS or the 240 spawning samples from SS were positive for VHSV. The 5 positive fish included a 3-yr-old immature female, 3- and 4-yr-old mature females, a 4-yr-old mature male, and a 7-yr-old mature male. Interestingly, mature fish comprised only 45% of the prespawning samples but were 80% of the VHSV+ cases among prespawning samples. All 5 VHSV+ cases were cultured on the EPC cell line, whereas only one case was also positive using the PHE cell line. Among external lesions, all 5 VHSV+ fish had mild to severe caudal fin fraying, but only 2 fish had caudal fin reddening (fish #s 241 and 288), only 1 fish had fin base reddening (mild in fish #288), only 1 fish had focal skin reddening (mild in fish #241), and no VHSV+ fish had diffuse skin reddening. Hence, 3 VHSV+ fish had no external lesions other than mild caudal fin fraying.

Because only 5 fish were VHSV+, statistical analysis against plasma chemistries was not done. However, true mean IgM values for VHSV+ fish (416 mg/mL) were less than true mean IgM values for other PWS prespawning fish (460 mg/mL), and considerably less than true mean values for spawning fish from SS (719 mg/mL) and PWS (975 mg/mL). Also, lactate levels–an indication of metabolic acidosis–were greater in VHSV+ fish than in other PWS prespawning fish (92 vs. 61 mmol/dL), even though CO_2 levels–an indication of respiratory acidosis–were less in VHSV+ fish than in other PWS prespawning fish (5.2 vs. 6.2 mmol/L).

Gender-associated Lesions

Site differences in oocyte morphology were minimal (Table 4), but comparisons were made difficult by the severity of artifact in ripe eggs. Unripe eggs in prespawning fish sectioned well, whereas ripe eggs often were lost during processing or sectioning; hence, more unripe than ripe eggs were retained on each section and available to be counted. Ruptured and atretic follicle were rare in ovaries from all samples groups (Table 4).

Gonads had several differences in lesion prevalence. As in 1994, lesions more frequent in ovaries included hyalinization of vessel walls and pigmented macrophage aggregates, and prevalence of both lesions in 1995 (about 40%; Table 2) was slightly less than in 1994 (about 60%). In 1994, granulomatous inflammation was more common in testes than in ovaries, but neither occurred at greater than 9% prevalence. By comparison, granulomatous inflammation in 1995 was more common than in 1994, more common in ovaries than testes, prevalence was greater in fish from PWS than SS, and prevalence among males was greater in prespawning mature fish than in spawning fish (Table 2). As in 1994, *Ichthyophonus* was rare in either gonad (Table 2, Figure 4).

Gender differences spawning fish were significant for several nongonadal lesions (Table 5). Prevalence of *Ichthyophonus* in heart, liver, and skeletal muscle was significantly greater in females than males. Gall bladder myxosporeans (*Ceratomyxa auerbachi*) were significantly more

frequent in females (as they were in 1994). Females were more likely than males to have hepatic lipidoses or severe depletion of pancreatic zymogen granules, but females were less likely to have hepatic glycogen depletion. Livers were not stained specifically for glycogen or lipid, so some lipid vacuoles in females might have been mistakenly classified as glycogen. Males had a significantly greater frequency of vacuolated proximal renal tubular epithelial cells.

In addition to these lesions, females were heavier, longer, and had greater gonad and liver weights than males (Table 7). Gender differences were significant for several plasma chemistries and hematology variables (Table 7). Females had significantly lower albumin, calcium, cholesterol, CO_2, glucose, packed cell volume, potassium, sodium, % thrombocytes, and total protein. Females had significantly higher ALP and % thrombocytes. Gender differences were not significant for other plasma chemistries.

Iris reddening

Most lesions significantly associated with iris reddening were more prevalent in fish with mild or moderate iris reddening than in fish with no iris reddening (Table 5). For example, caudal fin fraying and renal congestion were significantly more frequent in fish with mild or moderate iris reddening. By comparison, splenic congestion was more likely in fish with no iris reddening (Table 5).

Intraperitoneal Herring Worms (Anisakidae)

All 240 Pacific herring sampled from PWS contained larval parasites of the family Anisakidae within their peritoneal cavities, and 236 of 238 examined from SS had intraperitoneal Anisakidae. No attempt was made to differentiate species (e.g., *Anisakis* vs. *Contracecum*), and parasite morphology and inflammatory response were consistent with previous descriptions (Hauck and May 1977). Unlike in 1994, gender differences in numbers of herring worms were were not significant. But as in 1994, increasing severity of several lesions were significantly associated with increased numbers of herring worms (Table 6). For example, increased numbers of herring worms were associated with increased severity of gastric eosinophilic leukocytes, gastric serositis, and gastric trematodes. By comparison, decreased numbers of herring worms were associated with increased severity of hepatic pigmented macrophage aggregates and focal, intimal, hyperplasia of intestinal arterioles. Middle-aged fish tended to have more peritoneal Anisakidae, and age differences were significant.

Other Lesions and Potential Pathogens

No significant bacterial pathogens were isolated, and none of the blood smears had evidence of VEN. Spawning Pacific herring had 11 other common parasites, most of which were

associated with few lesions. These parasites, roughly in descending order of prevalence (spawning fish), included:

1) intestinal coccidian *Goussia*? sp. (PWS = 95%, SS = 91%);
2) hepatic coccidian *Goussia* [*Eimeria*] *clupearum* (PWS = 73%, SS = 71%);
3) testicular coccidian *Eimeria sardinae* (PWS = 85%, SS = 66%);
4) gall bladder myxosporean *Ceratomyxa auerbachi* (PWS = 39%, SS = 32%)
5) renal intraductal myxosporean *Ortholinea orientalis* (PWS = 29%, SS = 20%);
6) branchial *Epitheliocystis* (PWS = 15%, SS = 25%);
7) renal intraductal protozoan (PWS = 11%, SS = 3.8%);
8) gastric trematodes (PWS = 12%, SS = 10%);
9) branchial monogenetic trematodes (PWS and SS = 11%);
10) intestinal trematodes, e.g., *Lecithaster gibbosus* (PWS = 8.9%, SS = 2.1%); and
11) intestinal cestodes, e.g., *Nybelinia surmenicola* (PWS = 3.3%, SS = 2.5%).

Site differences in parasite prevalence were statistically significant (chi-square test) only for the testicular coccidian, renal intraductal protozoan, and branchial *Epitheliocystis*. Branchial ciliated protozoa (e.g., *Trichodina*) were rare in 1995 spawning samples from both PWS (1.3%) and SS (1.1%) compared to spawning samples from PWS in 1994 (12%).

Intestinal coccidians were common in small numbers throughout the intestine, including the intestinal cecae. In 1994, only 1% of the fish had moderate infestation (i.e., >15 organisms per 400× field), and infestation was not associated with alterations in plasma chemistry values. By comparison, in PWS in 1995, 12% of the spawning fish and 31% of the prespawning fish had moderate infestations (Table 2); prevalence of moderate infestations in SS in 1995 was only 2%. Severity of intestinal coccidians in 1995 was significantly related to greater CPK and AST values in PWS fish but not in SS fish.

Morphologic features and distribution of the hepatic coccidian were very similar to descriptions of *Goussia clupearum* in Atlantic and Pacific herring (Morrison and Hawkins 1984, Marty et al. 1995). Despite the relatively large volume of hepatic parenchyma displaced by the parasites in severe cases, inflammation was minimal. Increased lesion scores were significantly related to decreased age (Table 2), and for SS fish only, increased lesion scores were associated with decreased plasma glucose levels.

As in 1994, diagnosis of the renal intraductal myxosporean *Ortholinea orientalis* was less sensitive by histopathology. This difference was particularly obvious in spawning fish from PWS, where the touch preparation prevalence (28%) was significantly higher than the prevalence determined by histopathology (7%). Overall prevalence was significantly greater in spawning fish from PWS in 1995 than in 1994 (29 vs. 19%, chi-square test). Also, as histopathology scores for *Ortholinea orientalis* in spawning PWS fish increased, values for PCV, neutrophils, and basophils significantly increased (Table 2). Plasma chemistry values for other methods of diagnosis and for SS were either not significant or significant trends were nonlinear.

Prevalence of an unidentified protozoan (or myxosporean?) in the archinephric duct (kidney) was significantly higher in spawning fish from PWS than SS, but prevalence in PWS spawning fish in 1994 and 1995 was not significantly different. Infestation was significantly related to changes in plasma chemistries in SS but not in PWS (Table 2).

The gall bladder sometimes contained large numbers of the myxosporean *Ceratomyxa auerbachi*. Prevalence among 1995 spawning fish from PWS and SS were not significantly different, but 1995 prevalence in PWS (39%) was significantly greater than 1994 prevalence (19%). The gall bladder is small (3 - 5 mm in diameter) and difficult to include in all sections, but a higher frequency of gall bladders were examined in 1995 (97%) than in 1994 (66%). Hence, the increased prevalence in 1995 may have resulted from better sampling in 1995. Severe infestations sometimes had mild mononuclear inflammation in the lamina propria, but infestations were not significantly associated with changes in plasma chemistries.

Prevalence of 4 subtle inflammatory lesions was significantly greater (chi-square test, $P \leq 0.05$) in spawning fish from PWS than SS: 1) perivascular leukocytes in skeletal muscle (PWS = 77%, SS =65 %); 2) focal parenchymal leukocytes in the liver (PWS = 81%, SS = 49%); 3) focal parenchymal leukocytes in the heart (PWS = 32%, SS = 24%); and 4) foci of white blood cells in the submucosa and muscularis of the stomach (PWS = 13%, SS = 1.7%).

Age-associated Changes

The most consistent age-related change, as in 1994, was increased severity of pigmented macrophage aggregates in older fish. Indeed, age-related changes were significant in all organs in which pigmented macrophage aggregates were scored: exocrine pancreas, liver, ovary, spleen, and trunk kidney (Table 2). External lesion scores that significantly increased with age included fin base reddening (PWS only) and iris reddening (SS only). Microscopic lesion scores that were significantly related to increased age included (Table 2): 1) gill lamellar telangiectasis; 2) renal tubular epithelial vacuolation (SS only); 3) hepatic pericholangial leukocytes (PWS only); 4) hepatocellular lipidosis (SS only); 5) perivascular leukocytes in skeletal muscle connective tissue (SS only); 6) focal, intimal arteriolar hyperplasia in intestinal cecal vessels; and 7) splenic ellipsoid hyalinization (both sites). Microscopic lesion scores that were significantly related to decreased age included: 1) focal parenchymal leukocytes in the heart (PWS only); 2) intestinal cecal Anisakidae (SS only); 3) interstitial renal congestion (SS only); and 4) splenic vascular congestion (SS only).

Increased severity scores for several parasites were significantly related to decreased age among spawning fish in 1995 (ANOVA): 1) intestinal trematodes (PWS only; prevalence was too low in SS for statistical analysis); 2) hepatic coccidian *Goussia clupearum* (both sites); 3) branchial *Epitheliocystis* (PWS only); and 4) testicular coccidian *Eimeria sardinae* (both sites). By comparison, severity scores for common parasites *Ichthyophonus* and *Ortholinea orientalis* were not significantly related to age.

Comparing age-related prevalence of common parasites using the chi-square test for homogeneity sometimes produced results markedly different than those produced through one-way ANOVA of severity scores. The best example is with the hepatic coccidian *Eimeria sardinae*, in which decreasing age was significantly related to severity score (ANOVA), but prevalence was unrelated to age (chi-square test, $P > 0.40$). Histopathology prevalence for the renal intraductal myxosporean *Ortholinea orientalis* was significantly associated with age in SS but not PWS (chi-square test), but age-related differences were not significant for either site using ANOVA. Overall prevalences of *Ichthyophonus* (Figure 3) and branchial *Epitheliocystis* (Figure 5) were significantly associated with age in PWS but not SS. Prevalence of the testicular coccidian *Eimeria sardinae* was significantly associated with age at both sites and with both analysis techniques (Figure 6), where prevalence was generally highest in the youngest mature fish but was lower in immature fish and 7-year-old fish. In 1994, the gall bladder myxosporean *Ceratomyxa auerbachi* was significantly more common in older fish and the renal intraductal protozoan (or myxosporean?) was significantly more common in younger fish; however, neither parasite had significant age-related differences in prevalence in 1995 (both sites).

Several values for plasma chemistries, weights, and length were significantly related to age (Table 8). Values that significantly increased with age at both sites included IgM, all weights (body, gonad, and liver), and length. Only CPK significantly decreased with age at both sites. Hematology values did not consistently change with age at both sites, although among spawning fish from PWS, % lymphocytes decreased with age as % neutrophils increased.

Leukocyte Differential Counts

Although interpretation of leukocyte differential counts is limited without knowledge of the total white blood cell count, the values provide useful information for generating hypotheses that can be further examined with laboratory study, particularly in Section III of this project (C. Kennedy, Simon Fraser University). Several lesions were significantly related to changes in the frequency of various leukocytes (Table 2). For example, increased frequency of neutrophils was significantly related to *Ichthyophonus* scores in PWS samples from the brain, heart, intestine, liver, spleen, stomach (only the ICH+ lesion score), and skin/skeletal muscle. By comparison, in SS samples, neutrophil frequency was increased with *Ichthyophonus* only in skin/skeletal muscle, but basophil frequency was increased with *Ichthyophonus* scores in gill, liver, and stomach (both ICH and ICH+ lesion scores). Basophil frequency was never significantly increased with *Ichthyophonus* in PWS samples. Increased frequency of neutrophils was significantly related to increased scores for branchial hematopoietic cells in PWS but not SS samples (Table 2).

Lymphocyte frequency significantly increased with scores for several lesions in in PWS but not in SS (Table 2); examples include: 1) hepatic coccidian *Eimeria sardinae*; 2) intestinal foreign body granuloma; 3) intestinal mesenteric steatitis; and 4) focal parenchymal leukocytes in the liver. Note that the frequency of lymphocytes was significantly less in samples from SS than in samples from PWS, regardless of gender (Table 7). Splenic granulomatous inflammation was the only lesion for which lymphocyte frequency significantly increased in SS samples. Lymphocyte

frequency significantly decreased for both PWS and SS samples for only 2 lesions: interstitial renal congestion and pancreatic zymogen granule depletion.

Significant changes in thrombocyte frequency were uncommon, and when they occurred, they were usually matched by an opposite change in frequency of another white blood cell. For example, in SS samples with intestinal steatitis, thrombocyte frequency significantly decreased while frequency of lymphocytes, neutrophils, basophils, and eosinophils significantly increased. Eosinophil frequency did significantly change for any lesions other than intestinal steatitis. Only 3 monocytes were identified among all 500 smears examined; therefore, statistical analysis was not useful for comparing monocyte frequency.

Plasma chemistries

As hold time increased, increases in plasma potassium were highly significant at both sites (Table 9). The increase in plasma CO_2 with increased hold time was more highly correlated in PWS than in SS (Table 9), but CO_2 in SS samples was significantly higher than PWS samples, regardless of hold time (Table 7). Increased plasma lactate and decreased plasma glucose were significantly correlated with hold time for samples from SS but not PWS, and SS lactate levels were significantly higher than PWS lactate levels regardless of hold time (Tables 7 and 9). Changes in several other plasma chemistries were not as significant in relation to hold time ($|r| < 0.25$). A complicating factor was that SS fish had commenced spawning whereas PWS fish had not. Therefore, many of the marginally significant changes might have been related to spawning condition rather than hold time.

Among enzymes, AST and CPK values were most variable, and differences in several lesion scores could be discerned on the basis of AST and CPK (Table 1). Values for AST were significantly greater in samples from SS than from PWS (Table 7). Variability of ALP was intermediate, but several lesions were significantly related to increased ALP values (e.g., intestinal trematodes and gastric *Ichthyophonus*, both in PWS samples; Table 2). Values for ALP were significantly greater in females than males, and significantly greater in samples from SS than from PWS (Table 7). Variability of ALT and GGT was minimal and measured values, except for one fish, were never greater than 18 U/L.

Albumin and total protein were unusually low when compared to published values for other species (McDonald and Milligan 1992), but 1995 values were comparable to 1994 values, except that 1995 values for albumin were higher than in 1994. Both albumin and total protein were significantly higher in males than females, but IgM levels were not significantly different by gender (Table 7). Albumin and total protein were significantly higher in SS than in PWS, but IgM levels in samples from PWS were significantly higher than samples from SS (Table 7).

Annual Trends in Spawning Biomass and Pathogen Prevalence

Sample prevalence of *Ichthyophonus* in this study was 1.5 to 10 times that of years before 1994 (Table 10). When only scores for liver, kidney, and spleen are considered, *Ichthyophonus* prevalence in 1994 and 1995 (23 and 24%) was no longer significantly different from sample prevalence in 1989 and 1990 (13 and 15%), but was significantly higher than all other samples (chi-square test, 2×2 contingency tables). By comparison, prevalence of *Goussia clupearum* has remained fairly constant between 41 and 63% for most years, but 1995 prevalence in SS and PWS was the highest recorded. Based only on histopathology, the prevalence of *Ortholinea orientalis* prevalence seemed to be higher in 1991 than in 1994 or 1995 (Table 9). The slight increase in *Ortholinea orientalis* prevalence in spawning fish from PWS in this study (28%) was probably at least partly due to increased efficiency of diagnosis when touch preparations were examined; prevalence data before 1994 were derived from histopathology only. Prevalence of VHSV was lower in spawning fish from PWS in 1995 than in 1994, but prevalence was not determined before 1994 because appropriate tissues were not examined.

Discussion

Note on the contents the discussion section in this report: The annual report for project 94320-S (Marty et al. 1995) contains detailed discussion, including historical perspective, on most of the significant lesions and plasma chemistry changes in Pacific herring. That discussion is not repeated in this annual report, but will be included in the final synthesis report. This report concentrates on significant differences in lesions, necropsy findings, and plasma chemistry values in samples from 1995. Variables, considered included spawning stage, site of capture, and year of capture, with special emphasis on organisms and lesions likely to result in population level effects.

Ichthyophonus hoferi

In Pacific herring from PWS, the large increase in prevalence of *Ichthyophonus* in 1994 was not associated with an unusual population decline between 1994 and 1995 (Figure 1). However, *Ichthyophonus* seems to have been the cause of unexpected high mortality of 7-year-old fish in both PWS and SS during the summer of 1995. The main difference in *Ichthyophonus* epidemiology between 1994 and spring 1995 was the age distribution of the fungus. In 1994, all age groups were infected in equal proportions. But in spring 1995, prevalence of *Ichthyophonus* was significantly greater in older fish. Preliminary evidence from fall 1995 samples indicates that the population of 7-year-olds (1988 year class) in both SS and PWS was significantly lower than in spring 1995, and the prevalence of *Ichthyophonus* in the remaining fish was only about 60% of spring levels. Because no other pathogens were significantly more common in older fish, *Ichthyophonus* seems the most likely cause of differential mortality in these older fish. It may be that *Ichthyophonus* takes several months to years to cause mortality after a Pacific herring is infected, and mortality may require interaction with other variables such as ageing, predation, or other parasites.

In Atlantic herring populations as recently as 1991, major population decline along the Scandinavian coasts was attributed to *Ichthyophonus* (Lang 1992). Because *Ichthyophonus* seems to cause mortality more readily in Atlantic herring than in Pacific herring, the role of *Ichthyophonus* in population decline may be less in Pacific herring than in Atlantic herring. To clarify details of the pathogenesis, continued study is proposed as part of the laboratory component of this project, to include infectivity studies with *Ichthyophonus* cultured from the Atlantic ocean.

Among fish infected with *Ichthyophonus* in PWS and SS, the general pattern and distribution of lesions were very similar except in the stomach. The stomachs of fish from SS were much more likely to contain empty foreign-body type granulomas (i.e., no organisms in the granulomas; scored as ICH+). Although the stomach ICH+ prevalence was identical for PWS and SS (19%), prevalence of stomachs with *Ichthyophonus* was 16% in PWS but only 10% in SS. This may indicate that SS fish were better able to mount an effective inflammatory reaction against *Ichthyophonus*. Alternatively, the granulomas may have been caused by another organism or foreign substance not affecting PWS fish.

Ichthyophonus infections in both sites in 1995 were significantly related to increased IgM levels–consistent with the chronic nature of the disease. Further definition of the IgM response would require development of an ELISA specific for anti-*Ichthyophonus* antibodies. Infection with *Ichthyophonus* in 1995 was less commonly related to changes in plasma CPK than in 1994, particularly in PWS samples, although AST was about equally effective both years. Both enzymes were consistently good markers of *Ichthyophonus* in 1995 samples from SS. The role of CPK in *Ichthyophonus* infections will be further defined by laboratory determination of CPK isozymes as part of this project (96162; section III, biochemistry and physiology) during the next fiscal year.

VHSV

The lack of VHSV in spawning samples from both PWS and SS clearly demonstrates that Alaskan populations of Pacific herring can spawn without expressing significant quantities of VHSV. Also, the role of VHSV in PWS population decline is decreasing–consistent with the relatively stable population size from 1994 to 1995. Significance of the 5 prespawning VHSV isolates is unknown. Because prespawning fish were difficult to catch, the sample may not have been representative of the entire population. Schools of fish observed on sonar were mostly too deep for the purse seine, so the net seemed to only "skim" fish off the top of the school. Alternatively, it may be that a small fraction of Pacific herring normally express VHSV just before final gonadal maturation. Unpublished observations of VHSV isolates from other Alaskan waters by T.R. Meyers support this hypothesis. Analysis of prespawning samples captured in 1996 should provide more evidence for the role of VHSV in prespawning samples. [In PWS prespawning 1996 samples, 50% were sampled at night, after the fish rose towards the surface; hence, the samples seemed more representative of the schools from which they were caught.]

The biggest difference between VHSV-infected fish in 1995 and 1994 was the lack of external lesions in VHSV+ fish in 1995. Among spawning fish with VHSV in 1994, 9 of 11 had mild to severe fin base reddening, but in 1995 prespawning samples, only 1 of 5 had mild fin base reddening and none were moderate or severe. It may be that VHSV commonly causes external lesions only during spawning, a period of great physiologic stress; at other times, external signs of disease may be minimal.

The reason for increased prevalence of several subtle inflammatory lesions (e.g., focal parenchymal leukocytes in the liver) in fish from PWS than from SS is unknown. These foci may be remnants of VHSV-induced damage. For example, hepatic focal necrosis was associated with VHSV in 1994, and leukocytes would normally be part of the healing process in recovered fish. Alternatively, these foci of leukocytes may be normal in wild fish, and decreased frequency of leukocytes in SS may have reflected their active spawning status (i.e., a result of stress-induced leukocytopenia during spawning). As evidence, the frequency of neutrophils and basophils in blood smears from SS significantly decreased as gonadal fullness decreased (Table 2). Confirmation of one of these hypotheses probably will require examination of fish in various stages of the disease, including recovery, after known exposure to VHSV in the laboratory.

External Lesions and Iris Reddening

External lesions are too nonspecific to be consistently related to any single cause. As evidence, in 1994 the external lesion fin base reddening was significantly associated with VHSV, and no external lesions were significantly associated with *Ichthyophonus*. But in 1995, VHSV+ fish had few external lesions, whereas focal skin reddening was significantly associated with *Ichthyophonus* infection. The lists of internal lesions associated with various external lesions (Table 3) provide evidence that external lesions were useful indicators of poor health, but their use for more specific diagnoses seems limited.

Focal skin reddening and diffuse skin reddening are distinct in their pathogenesis. In 1994, both lesions were clumped into focal skin reddening. In 1995, the lesions were separated. Although diffuse skin reddening was relatively uncommon in 1995 samples, affecting about half as many fish as focal skin reddening, the lesions significantly associated with diffuse skin reddening were different from focal skin reddening in all but one case. Focal skin reddening was most often associated with *Ichthyophonus*, whereas diffuse skin reddening was more a marker of spawning stage and infection with coccidians. Removal of diffuse skin reddening from the focal skin reddening score may have been all that was needed to show that *Ichthyophonus* was significantly related to severity of focal skin reddening. The second year of study at both sites (1996) will test the validity of these associations.

As in 1994, mild iris reddening seems to be the normal condition in Pacific herring in spawning condition. Of 10 significantly associated lesions in 1995, 7 were more severe when iris reddening was scored as none than when iris reddening was scored as mild or moderate. There were too few moderate cases to determine if moderate lesions might also be significantly different

from mild lesions. Many of the lesions associated with changes in iris reddening were closely associated with the cardiovascular system.

Other Potential Pathogens

The best candidate for significant pathogens among the other common parasites was the unidentified intestinal coccidian (*Goussia*? sp.), particularly in fish from PWS. Both herring worms (Anisakidae) and the intestinal coccidian occurred in more than 90% of the fish, but only the intestinal coccidian was significantly related to increased plasma chemistries (e.g., CPK and AST) as lesion scores increased. Numbers of herring worms increased with severity of several lesions, but the inflammatory reaction was usually minimal. Fish with moderate cases of the intestinal coccidian were more likely to have severe depletion of pancreatic zymogen granules, indicating that affected fish were in relatively poor condition. However, the causal relation between the intestinal coccidian and poor condition is unknown. Do intestinal coccidians cause poor condition, or are they allowed to multiply when fish are in poor condition? Answering these questions would require controlled laboratory study. Preliminary evidence from fall 1995 samples provides evidence that severity of infestation with the intestinal coccidian decreased over the summer; overall prevalence dropped to about 30% and no fish had moderate lesions. Lack of inflammation against these parasites is further evidence that their contribution to population decline is minimal. As a secondary candidate for significant pathogen, infection with the renal intraductal myxosporean *Ortholinea orientalis* also was related to changes in plasma chemistries, and prevalence in 1995 was slightly greater than in 1994.

Age- and Gender-associated Lesions

Age-related differences in parasite prevalence changed from 1994 to 1995. In 1994, the gall bladder myxosporean *Ceratomyxa auerbachi* was more common in older fish, and the renal intraductal protozoan (or myxosporean) was more common in younger fish, but no parasites were significantly more common in the dominant 1988 year class. By 1995, the gall bladder myxosporean and renal intraductal protozoan were no longer associated with age, but *Ichthyophonus* and the testicular coccidian *Eimeria sardinae* were significantly related to age. *Ichthyophonus* was more common in older fish. However, because the trend in SS was towards higher *Ichthyophonus* prevalence in older fish, age-related differences in *Ichthyophonus* were attributed to ageing changes rather than to latent effects from the oil spill.

Plasma Chemistries

Plasma chemistries were highly sensitive markers of several lesions, reproductive status, and other variables (e.g., age and weight). Because several plasma chemistry changes are significantly related to the process of spawning, many of the significant difference attributed to site of capture probably resulted from slight differences in spawning stage of fish from SS (some had started spawning) and PWS (none had started spawning before sampling). Samples from both sites in 1996 included actively spawning fish and spawned out fish, so site-related differences

in plasma chemistries might not be as distinct in 1996. The significant site-related differences in lactate and CO_2 provided information that was used to alter fish holding techniques as early as fall 1995 samples. Fish for sampling are now held in about 300 L of seawater (in large totes used by fish processors) to allow for maximum movement while fish are held from capture to necropsy. The effect of this change on lactate and CO_2 levels will be included in next year's report.

Conclusions

Disease was probably the primary force driving Pacific herring population decline since 1993. No other variables–food availability, predation, water temperature, currents, or recruitment–were needed to explain this significant decline, although these variables may have contributed to conditions that were favorable for initiation of a disease epidemic. This conclusion is based on integration of results from this project, literature review including Meyers et al. (1994), plus information from biologists, fishers, and preliminary laboratory study where both VHSV and *Ichthyophonus* killed Pacific herring in the absence of other diseases. Among the 2 significant diseases, VHSV was most important in 1993. By 1994, *Ichthyophonus* prevalence had increased, possibly as a result of VHSV-induced immunosuppression, so that *Ichthyophonus* and VHSV were of about equal importance. By 1995, VHSV prevalence had decreased to where it was less important as a cause of mortality. If *Ichthyophonus* prevalence continues to decrease, as indicated by fall 1995 samples, and young herring in PWS remain relatively free of disease, then disease should no longer limit population recovery. Continued monitoring of disease in PWS and SS will increase our knowledge of how disease interacts with pelagic schooling fish like Pacific herring, and further study will serve to document population recovery or, alternatively, identify reasons that recovery fails to occur.

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Table 1. Mean values for continuous necropsy variables in Pacific herring sampled from Sitka Sound (SS) and Prince William Sound (PWS) in March and April, 1995. Note that all spawning fish from both sites were sexually mature, but only 45% (36 of 80) of the PWS "prespawning" fish were sexually mature. Also, gonads from all fish were in ripe, but some fish from Sitka Sound were partly or completely spawned out, whereas no fish from Prince William Sound had spawned before being sampled. Group means within each variable were compared using one-way analysis of variance and Tukey's multiple comparison procedure; groups with the same letter are not significantly different ($P \leq 0.05$). Comparisons in which Levene's test for equality of variance was significant ($P \leq 0.05$) are marked (*).

Variable	Site	Spawning status	# examined	Mean	±SE	ANOVA *P* value
Age (yrs)	PWS	prespawning	79	3.0^{A}	0.2	<0.001*
	PWS	spawning	180	6.6^{C}	0.1	
	SS	spawning	239	5.1^{B}	0.1	
standard length (mm)	PWS	prespawning	80	170.0^{A}	3.1	<0.001*
	PWS	spawning	180	219.4^{C}	1.3	
	SS	spawning	240	201.8^{B}	1.2	
body weight (g)	PWS	prespawning	80	62.0^{A}	4.5	<0.001*
	PWS	spawning	180	142.4^{C}	2.8	
	SS	spawning	240	122.2^{B}	2.6	
liver weight (g)	PWS	prespawning	80	0.7^{A}	0.1	<0.001*
	PWS	spawning	166	1.1^{C}	0	
	SS	spawning	237	1.0^{B}	0	
ovary weight (g)	PWS	prespawning	23	16.4^{A}	3.4	<0.001*
	PWS	spawning	85	35.8^{C}	3.9	
	SS	spawning	92	26.8^{B}	2.8	
testis weight (g)	PWS	prespawning	13	13.8^{A}	3.8	<0.001*
	PWS	spawning	94	28.1^{C}	2.9	
	SS	spawning	147	19.3^{B}	1.6	
hold time (min)	PWS	prespawning	80	94.5^{A}	4.5	<0.001
	PWS	spawning	180	95.9^{A}	3.1	
	SS	spawning	240	151.7^{B}	2.9	

Variable	Site	Spawning status	# examined	Mean	±SE	ANOVA *P* value
IgM (mg/mL)[a]	PWS	prespawning	57	413.6[A]	51.2	<0.001*
	PWS	spawning	177	814.8[C]	72.0	
	SS	spawning	239	637.8[B]	40.0	
packed cell volume (%)[b]	PWS	prespawning	80	35.3[A]	1.3	<0.001*
	PWS	spawning	179	45.3[B]	0.6	
	SS	spawning	223	44.7[B]	0.8	
peritoneal cavity - number of herring worms (Anisakidae)	PWS	prespawning	80	13.5	1.0	NS[c]
	PWS	spawning	180	14.5	0.7	
	SS	spawning	238	13.4	0.5	
gills - number of 0.5-mm-diameter white foci	PWS	prespawning	76	0.6	0.3	NS
	PWS	spawning	179	0.5	0.1	
	SS	spawning	232	0.5	0.1	

[a]Values for IgM were compared after natural log transformation; values listed here are retransformed to the geometric means and the first-order Taylor series approximation of the standard error.

[b]Values for packed cell volume were arcsine square root transformed for statistical analysis; mean values listed here are retransformed, and the first-order Taylor series was used as an approximation of the standard error of the mean.

[c]NS = not significant.

Organ - lesion or tissue type	Spawning status - site	n	Lesion Score Prevalence %=0	%=1	%=2	%=3	%>0	Significant trends (*P*-value)
fin base reddening	prespawning-PWS	79	78.5	16.5	3.8	1.3	21.5	ND
	spawning-PWS	180	46.7	41.7	8.9	2.8	53.3	↑- age (0.004), hold time (<0.001), CO_2 (0.001), bilirubin (0.027), neutrophils (0.005) ↓- phosphorus (0.002), cholesterol (0.014), total protein (0.001), albumin (0.003) NT- potassium (0.045)
	spawning-SS	240	63.3	32.1	3.3	1.3	36.7	NT- hold time (0.020), calcium (0.025), CO_2 (0.030)
iris reddening	prespawning-PWS	79	72.2	27.8	0.0	0.0	27.8	ND
	spawning-PWS	180	30.0	67.8	2.2	0.0	70.0	↓- ALP (0.031), PCV (0.030)
	spawning-SS	240	30.8	68.3	0.8	0.0	69.2	↑- age (0.001), sodium (<0.001*), potassium (0.001*), phosphorus (<0.001*), calcium (<0.001), lactate (<0.001*), osmolality (<0.001) ↓- glucose (0.012)
skin reddening, diffuse	prespawning-PWS	80	97.5	2.5	0.0	0.0	2.5	ND
	spawning-PWS	180	88.9	8.9	1.7	0.6	11.1	↑- hold time (<0.001), potassium (0.006), CO_2 (0.017) ↓- phosphorus (0.014)
	spawning-SS	240	92.1	7.1	0.8	0.0	7.9	↑- total protein (0.018), PCV (0.002)
skin reddening, focal	prespawning-PWS	80	68.8	23.8	0.0	7.5	31.3	ND
	spawning-PWS	180	79.4	17.8	1.7	1.1	20.6	↑- $\log_e$CPK (0.003), $\log_e$AST (0.006*)
	spawning-SS	240	71.3	27.5	0.8	0.4	28.8	none

Table 2. Lesion severity (% of fish classified in each lesion score) and lesion prevalence (% of sample having lesion score >0) in Pacific herring sampled from Sitka Sound (SS), Alaska, during March 1995, or from Prince William Sound (PWS) during April 1995. Lesions were scored as none (0), mild (1), moderate (2), or severe (3). Note that all spawning fish from both sites were sexually mature, but only 45% (36 of 80) of the PWS "prespawning" fish were sexually mature. Also, gonads from all spawning fish were ripe. Some spawning fish from Sitka Sound were partly or completely spawned out, whereas no fish from Prince William Sound had spawned before being sampled. Age, hold time, and blood values were compared for groups of spawning fish based on lesion scores using one-way analysis of variance and Tukey's multiple comparison procedure. Significant trends ($P \leq 0.05$) were based on rank order of mean responses for fish groups classified by lesion scores. Compared to fish with the lowest lesion score, mean response for the fish group with the highest lesion score was significantly higher (↑), lower (↓), or there was no significant trend (NT) in the rank order. For comparisons in which Levene's test for equality of variance was significant (*), only ANOVA comparisons with $P \leq 0.010$ are shown.

Organ - lesion or tissue type	Spawning status - site	n	Lesion Score Prevalence %=0	%=1	%=2	%=3	%>0	Significant trends (*P*-value)
External gross lesions:								
caudal fin fraying	prespawning-PWS	80	12.5	73.8	12.5	1.3	87.5	ND[a]
	spawning-PWS	180	5.6	57.2	33.3	3.9	94.4	↓- glucose (0.019), cholesterol (0.008) NT- PCV (0.027), IgM (0.014)
	spawning-SS	240	10.4	82.5	7.1	0.0	89.6	↑- sodium (0.002), calcium (<0.001*), osmolality (0.028)
caudal fin reddening	prespawning-PWS	80	51.3	45.0	3.8	0.0	48.8	ND
	spawning-PWS	180	35.6	46.1	18.3	0.0	64.4	↓-cholesterol (0.006), lactate (0.031), total protein (0.011), albumin (0.005)
	spawning-SS	240	47.9	50.4	1.7	0.0	52.1	none

[a]ND = not done

Organ - lesion or tissue type	Spawning status - site	n	Lesion Score Prevalence %=0	%=1	%=2	%=3	%>0	Significant trends (*P*-value)
Other Gross findings:								
gonad fullness	prespawning-PWS	36	0.0	0.0	2.8	97.2	100	ND
	spawning-PWS	176	0.0	0.6	0.0	99.4	100	ND
	spawning-SS	238	13.0	7.1	15.5	64.3	87.0	↑- chloride (<0.001*), glucose (<0.001), total protein (0.001), PCV (0.001) ↓- sodium (0.002), phosphorus (0.001), osmolality (0.002*), neutrophils (<0.001) NT- age (0.048), ALP (0.024), basophils (0.041), log_eAST (0.037)
Brain microscopic lesions:								
Ichthyophonus	prespawning-PWS	80	97.5	1.3	1.3	0.0	2.5	ND
	spawning-PWS	180	88.9	11.1	0.0	0.0	11.1	↑- neutrophils (0.003*), log_eAST (0.036), IgM (<0.001)
	spawning-SS	240	92.5	7.1	0.4	0.0	7.5	↑- ALP (0.029), total protein (0.024), log_eCPK (0.005), log_eAST (<0.001), IgM (<0.001)
meningeal eosinophilic granular leukocytes	prespawning-PWS	80	11.3	67.5	21.3	0.0	88.8	ND
	spawning-PWS	180	11.1	61.7	26.7	0.6	88.9	NT- potassium (0.003), PCV (0.047)
	spawning-SS	240	13.8	57.9	26.3	2.1	86.3	↑- osmolality (0.002*) ↓- log_eCPK (0.011) NT- PCV (0.044)
meningoencephalitis	prespawning-PWS	80	95.0	3.8	1.3	0.0	5.0	ND
	spawning-PWS	180	92.8	7.2	0.0	0.0	7.2	none
	spawning-SS	240	96.7	3.3	0.0	0.0	3.3	↑- ALP (0.054), CO_2 (0.044), PCV (0.051), IgM (0.036)

Organ - lesion or tissue type	Spawning status - site	n	Lesion Score Prevalence %=0	%=1	%=2	%=3	%>0	Significant trends (*P*-value)
Gall bladder microscopic lesions:								
myxosporean (*Ceratomyxa auerbachi*)	prespawning-PWS	72	81.9	13.9	4.2	0.0	18.1	ND
	spawning-PWS	175	60.6	30.3	9.1	0.0	39.4	NT- total protein (0.039)
	spawning-SS	216	67.6	23.6	8.3	0.5	32.4	none
Gill microscopic lesions:								
ciliated protozoa (e.g., *Trichodina* spp.)	prespawning-PWS	80	98.8	1.3	0.0	0.0	1.3	ND
	spawning-PWS	180	98.9	1.1	0.0	0.0	1.1	ND
	spawning-SS	240	98.8	1.3	0.0	0.0	1.3	ND
Epitheliocystis	prespawning-PWS	80	67.5	32.5	0.0	0.0	32.5	ND
	spawning-PWS	180	85.0	14.4	0.6	0.0	15.0	↓- age (0.023)
	spawning-SS	240	75.0	22.9	2.1	0.0	25.0	↑- $\log_e$CPK (0.026), $\log_e$AST (0.032)
foreign body granuloma	prespawning-PWS	80	92.5	6.3	1.3	0.0	7.5	ND
	spawning-PWS	180	94.4	5.6	0.0	0.0	5.6	none
	spawning-SS	240	94.2	5.8	0.0	0.0	5.8	none
gill arch inflammation or hematopoiesis	prespawning-PWS	80	0.0	98.8	1.3	0.0	100.0	ND
	spawning-PWS	180	0.0	95.0	5.0	0.0	100.0	↑- neutrophils (0.006), IgM (0.005)
	spawning-SS	240	0.8	93.8	5.4	0.0	99.2	↓- cholesterol (0.016), PCV (0.050)

Organ - lesion or tissue type	Spawning status - site	n	Lesion Score Prevalence %=0	%=1	%=2	%=3	%>0	Significant trends (*P*-value)
Ichthyophonus	prespawning-PWS	80	96.3	1.3	0.0	2.5	3.8	ND
	spawning-PWS	180	85.6	10.6	3.3	0.6	14.4	↑- basophils (0.003*), $\log_e$AST (0.020), IgM (<0.001) ↓- CO_2 (0.023)
	spawning-SS	240	89.2	7.9	2.9	0.0	10.8	↑- total protein (0.054), basophils (0.025), $\log_e$CPK (0.045), $\log_e$AST (<0.001), IgM (<0.001) ↓- glucose (0.014)
lamellar hyperplasia	prespawning-PWS	80	100.0	0.0	0.0	0.0	0.0	ND
	spawning-PWS	180	99.4	0.6	0.0	0.0	0.6	ND
	spawning-SS	240	99.6	0.4	0.0	0.0	0.4	ND
lamellar telangiectasis	prespawning-PWS	80	93.8	6.3	0.0	0.0	6.3	ND
	spawning-PWS	180	77.2	21.7	1.1	0.0	22.8	↑- age (0.022) ↓- lactate (0.001*)
	spawning-SS	240	84.6	12.9	2.5	0.0	15.4	↑- glucose (0.030)
monogenetic trematodes (e.g., *Gyrodactylus* spp.)	prespawning-PWS	80	98.8	1.3	0.0	0.0	1.3	ND
	spawning-PWS	180	89.4	10.6	0.0	0.0	10.6	↓- potassium (0.039)
	spawning-SS	240	89.2	10.8	0.0	0.0	10.8	↑- IgM (0.020)

Organ - lesion or tissue type	Spawning status - site	n	Lesion Score Prevalence %=0	%=1	%=2	%=3	%>0	Significant trends (*P*-value)
Gonad - female microscopic lesions:								
eosinophilic granular leukocytes	immature prespawning-PWS	25	8.0	24.0	16.0	52.0	92.0	ND
	mature prespawning-PWS	24	54.2	37.5	0.0	8.3	45.8	ND
	spawning-PWS	85	40.0	43.5	8.2	8.2	60.0	↓- cholesterol (0.020), total protein (0.035), albumin (0.021), PCV (0.050)
	spawning-SS	94	42.6	40.4	6.4	10.6	57.4	none
granulomatous inflammation	immature prespawning-PWS	25	8.0	68.0	12.0	12.0	92.0	ND
	mature prespawning-PWS	24	25.0	70.8	4.2	0.0	75.0	ND
	spawning-PWS	85	31.8	65.9	2.4	0.0	68.2	↓- neutrophils (0.003*)
	spawning-SS	93	43.0	53.8	1.1	1.1	55.9	↓- bilirubin (0.020)
hyalinization of vessel walls	immature prespawning-PWS	25	96.0	4.0	0.0	0.0	4.0	ND
	mature prespawning-PWS	24	87.5	8.3	4.2	0.0	12.5	ND
	spawning-PWS	85	58.8	31.8	9.4	0.0	41.2	↓- ALP (0.039) NT- age (0.009)
	spawning-SS	94	53.2	38.3	8.5	0.0	46.8	NT- age (0.008*)

Organ - lesion or tissue type	Spawning status - site	n	Lesion Score Prevalence %=0	%=1	%=2	%=3	%>0	Significant trends (*P*-value)
Ichthyophonus	immature prespawning-PWS	24	95.8	4.2	0.0	0.0	4.2	ND
	mature prespawning-PWS	23	100.0	0.0	0.0	0.0	0.0	ND
	spawning-PWS	84	97.6	2.4	0.0	0.0	2.4	ND
	spawning-SS	93	100.0	0.0	0.0	0.0	0.0	ND
macrophage aggregates (pigmented)	immature prespawning-PWS	25	76.0	16.0	4.0	4.0	24.0	ND
	mature prespawning-PWS	24	87.5	12.5	0.0	0.0	12.5	ND
	spawning-PWS	85	60.0	40.0	0.0	0.0	40.0	none
	spawning-SS	94	73.4	26.6	0.0	0.0	26.6	↑- age (0.001*), osmolality (0.010*) ↓- $\log_e$CPK (0.030)
Gonad - male microscopic lesions:								
Eimeria sardinae	immature prespawning-PWS	15	93.3	6.7	0.0	0.0	6.7	ND
	mature prespawning-PWS	12	16.7	66.7	8.3	8.3	83.3	ND
	spawning-PWS	94	14.9	68.1	14.9	2.1	85.1	↑- lymphocytes (0.021) ↓- age (<0.001) NT- thrombocytes (0.034), neutrophils (0.022)
	spawning-SS	145	34.5	60.0	5.5	0.0	65.5	↓- age (<0.001), lactate (0.026), total protein (0.013), albumin (0.001) NT- ALP (0.040), sodium (0.036), phosphorus (0.028), calcium (0.032), cholesterol (0.013)

Organ - lesion or tissue type	Spawning status - site	n	Lesion Score Prevalence %=0	%=1	%=2	%=3	%>0	Significant trends (*P*-value)
eosinophilic granular leukocytes	immature prespawning-PWS	15	6.7	20.0	0.0	73.3	93.3	ND
	mature prespawning-PWS	12	50.0	50.0	0.0	0.0	50.0	ND
	spawning-PWS	94	84.0	13.8	0.0	2.1	16.0	↑- calcium (0.022), lymphocytes (0.041)
	spawning-SS	146	59.6	26.7	4.8	8.9	40.4	none
granulomatous inflammation	immature prespawning-PWS	14	57.1	21.4	14.3	7.1	42.9	ND
	mature prespawning-PWS	12	75.0	25.0	0.0	0.0	25.0	ND
	spawning-PWS	94	88.3	11.7	0.0	0.0	11.7	none
	spawning-SS	146	95.9	4.1	0.0	0.0	4.1	ND
hyalinization of vessel walls	prespawning-PWS							
	- immature	15	100.0	0.0	0.0	0.0	0.0	ND
	- mature	12	100.0	0.0	0.0	0.0	0.0	ND
	spawning-PWS	94	100.0	0.0	0.0	0.0	0.0	ND
	spawning-SS	145	100.0	0.0	0.0	0.0	0.0	ND
Ichthyophonus	prespawning-PWS							
	- immature	15	100.0	0.0	0.0	0.0	0.0	ND
	- mature	12	100.0	0.0	0.0	0.0	0.0	ND
	spawning-PWS	94	100.0	0.0	0.0	0.0	0.0	ND
	spawning-SS	146	97.3	2.7	0.0	0.0	2.7	ND

Organ - lesion or tissue type	Spawning status - site	n	Lesion Score Prevalence %=0	%=1	%=2	%=3	%>0	Significant trends (*P*-value)
macrophage aggregates (pigmented)	immature prespawning-PWS	15	93.3	6.7	0.0	0.0	6.7	ND
	mature prespawning-PWS	12	100.0	0.0	0.0	0.0	0.0	ND
	spawning-PWS	94	100.0	0.0	0.0	0.0	0.0	ND
	spawning-SS	146	99.3	0.7	0.0	0.0	0.7	ND
spermatocyte numbers (3 = abundant)	immature prespawning-PWS	14	100.0	0.0	0.0	0.0	0.0	ND
	mature prespawning-PWS	12	0.0	0.0	0.0	100.0	100.0	ND
	spawning-PWS	94	0.0	0.0	1.1	98.9	100.0	ND
	spawning-SS	146	1.4	6.2	17.8	74.7	98.6	↑- glucose (<0.001) ↓- sodium (0.001*), phosphorus (0.013), osmolality (0.005*), neutrophils (<0.001) NT- ALP (0.014), chloride (0.048)
Heart microscopic lesions:								
atrial phagocyte hypertrophy	prespawning-PWS	80	100.0	0.0	0.0	0.0	0.0	ND
	spawning-PWS	179	100.0	0.0	0.0	0.0	0.0	ND
	spawning-SS	240	100.0	0.0	0.0	0.0	0.0	ND
epicarditis	prespawning-PWS	80	38.8	61.3	0.0	0.0	61.3	ND
	spawning-PWS	179	34.6	60.9	3.9	0.6	65.4	↑- IgM (0.021) ↓- glucose (0.023), cholesterol (0.030)
	spawning-SS	240	43.8	53.8	2.1	0.4	56.3	↓- cholesterol (0.013)

Organ - lesion or tissue type	Spawning status - site	n	Lesion Score Prevalence %=0	%=1	%=2	%=3	%>0	Significant trends (*P*-value)
Ichthyophonus	prespawning-PWS	80	93.8	1.3	0.0	5.0	6.3	ND
	spawning-PWS	179	77.7	7.3	7.8	7.3	22.3	↑- neutrophils (0.001*), IgM (<0.001)
	spawning-SS	240	82.9	6.7	4.6	5.8	17.1	↑- $\log_e$CPK (0.007), $\log_e$AST (<0.001), IgM (<0.001) NT- PCV (0.035), thrombocytes (0.031), lymphocytes (0.011)
leukocytes, focal, parenchymal	prespawning-PWS	80	68.8	31.3	0.0	0.0	31.3	ND
	spawning-PWS	179	67.6	31.8	0.6	0.0	32.4	↓- age (0.017), $\log_e$ALT (0.031)
	spawning-SS	240	76.3	23.8	0.0	0.0	23.8	↓- lactate (0.025), neutrophils (0.012)
mineralization, myocardial	prespawning-PWS	80	100.0	0.0	0.0	0.0	0.0	ND
	spawning-PWS	179	100.0	0.0	0.0	0.0	0.0	ND
	spawning-SS	240	100.0	0.0	0.0	0.0	0.0	ND
thrombosis	prespawning-PWS	80	98.8	1.3	0.0	0.0	1.3	ND
	spawning-PWS	179	92.2	7.8	0.0	0.0	7.8	↑- lactate (0.047)
	spawning-SS	240	95.4	3.8	0.8	0.0	4.6	none
Intestine and intestinal cecae, microscopic lesions:								
Anisakidae	prespawning-PWS	80	17.5	58.8	11.3	12.5	82.5	ND
	spawning-PWS	180	17.2	54.4	18.3	10.0	82.8	none
	spawning-SS	240	22.1	52.1	19.2	6.7	77.9	↓- age (<0.000*) NT- glucose (0.043), thrombocytes (0.051)

Organ - lesion or tissue type	Spawning status - site	n	Lesion Score Prevalence %=0	%=1	%=2	%=3	%>0	Significant trends (*P*-value)
arteriolar hyperplasia, focal, intimal	prespawning-PWS	80	72.5	27.5	0.0	0.0	27.5	ND
	spawning-PWS	180	48.3	45.6	6.1	0.0	51.7	↓- phosphorus (0.022)
	spawning-SS	240	57.1	41.7	1.3	0.0	42.9	↑- age (0.053) ↓- bilirubin (0.040)
cestodes	prespawning-PWS	80	86.3	3.8	10.0	0.0	13.8	ND
	spawning-PWS	180	96.7	2.8	0.6	0.0	3.3	ND
	spawning-SS	240	97.5	1.7	0.8	0.0	2.5	ND
coccidian, intraepithelial (*Goussia*? sp.)	prespawning-PWS	80	3.8	65.0	31.3	0.0	96.3	ND
	spawning-PWS	180	5.0	82.8	12.2	0.0	95.0	↑- $\log_e$CPK (0.037), $\log_e$ALT (0.021)
	spawning-SS	240	8.8	89.6	1.7	0.0	91.3	none
eosinophilic granular leukocytes, submucosal	prespawning-PWS	80	0.0	92.5	7.5	0.0	100.0	ND
	spawning-PWS	180	1.7	96.1	2.2	0.0	98.3	ND
	spawning-SS	240	2.1	95.0	2.9	0.0	97.9	ND
foreign body granuloma	prespawning-PWS	80	72.5	27.5	0.0	0.0	27.5	ND
	spawning-PWS	180	55.6	43.9	0.6	0.0	44.4	↑- neutrophils (0.047) ↓- PCV (0.052), lymphocytes (0.025)
	spawning-SS	240	65.0	35.0	0.0	0.0	35.0	↑- neutrophils (0.003)

Organ - lesion or tissue type	Spawning status - site	n	Lesion Score Prevalence %=0	%=1	%=2	%=3	%>0	Significant trends (*P*-value)
Ichthyophonus	prespawning-PWS	80	93.8	3.8	2.5	0.0	6.3	ND
	spawning-PWS	180	88.3	11.7	0.0	0.0	11.7	↑- glucose (0.031), total protein (0.037), neutrophils (0.009*), IgM (<0.001)
	spawning-SS	240	91.7	8.3	0.0	0.0	8.3	↑- $\log_e$CPK (0.029), $\log_e$AST (0.004), IgM (0.002) ↓- calcium (0.040), lactate (0.031)
steatitis	prespawning-PWS	80	5.0	88.8	6.3	0.0	95.0	ND
	spawning-PWS	180	0.0	94.4	5.6	0.0	100.0	↑- potassium (0.024) ↓- ALP (0.034)
	spawning-SS	240	0.0	94.6	5.4	0.0	100.0	↑- lymphocytes (0.012), neutrophils (0.045), basophils (0.040), eosinophils (0.020), $\log_e$CPK (0.010), $\log_e$AST (0.001), IgM (0.001) ↓- cholesterol (0.030), thrombocytes (<0.001)
trematodes (e.g., *Lecithaster gibbosus*), cecal	prespawning-PWS	80	81.3	18.8	0.0	0.0	18.8	ND
	spawning-PWS	180	91.1	8.3	0.6	0.0	8.9	↑- ALP (0.026), lactate (0.038) ↓- age (0.001)
	spawning-SS	240	97.9	2.1	0.0	0.0	2.1	ND

Organ - lesion or tissue type	Spawning status - site	n	Lesion Score Prevalence %=0	%=1	%=2	%=3	%>0	Significant trends (*P*-value)
Kidney (trunk) microscopic lesions:								
congestion, interstitial, vascular	prespawning-PWS	79	93.7	5.1	1.3	0.0	6.3	ND
	spawning-PWS	180	91.1	8.3	0.6	0.0	8.9	↑- phosphorus (<0.001*), $\log_e$CPK (0.001), $\log_e$AST (<0.001), $\log_e$ALT (0.015) ↓- chloride (0.002*), glucose (<0.001), cholesterol (0.045), lymphocytes (0.018)
	spawning-SS	240	79.6	20.0	0.0	0.4	20.4	↑- phosphorus (0.009) ↓- age (0.014), chloride (0.006), glucose (0.009), lymphocytes (0.014)
granulomatous inflammation	prespawning-PWS	79	83.5	16.5	0.0	0.0	16.5	ND
	spawning-PWS	180	77.8	20.0	1.7	0.6	22.2	none
	spawning-SS	240	82.1	15.8	2.1	0.0	17.9	↑- cholesterol (0.004), total protein (0.009), albumin (0.051)
hematopoietic cells (relative area)	prespawning-PWS	79	3.8	73.4	22.8	0.0	96.2	ND
	spawning-PWS	180	2.8	78.3	18.3	0.6	97.2	↑- neutrophils (0.001*)
	spawning-SS	240	9.6	76.7	13.8	0.0	90.4	↑- IgM (<0.001) ↓- hold time (0.023)
Ichthyophonus	prespawning-PWS	79	94.9	1.3	0.0	3.8	5.1	ND
	spawning-PWS	180	81.1	8.3	6.1	4.4	18.9	↑- IgM (<0.001) NT- lymphocytes (0.035), neutrophils (0.004*)
	spawning-SS	240	82.9	7.5	5.4	4.2	17.1	↑- $\log_e$CPK (0.013), $\log_e$AST (<0.001), IgM (<0.000) ↓- PCV (0.003) NT- total protein (0.002*)

Organ - lesion or tissue type	Spawning status - site	n	Lesion Score Prevalence %=0	%=1	%=2	%=3	%>0	Significant trends (*P*-value)
intratubular mineral, with associated tubular hyperplasia	prespawning-PWS	79	97.5	2.5	0.0	0.0	2.5	ND
	spawning-PWS	180	97.2	2.8	0.0	0.0	2.8	ND
	spawning-SS	240	97.1	2.1	0.8	0.0	2.9	ND
intraductal protozoan	prespawning-PWS	79	92.4	7.6	0.0	0.0	7.6	ND
	spawning-PWS	180	88.9	10.6	0.6	0.0	11.1	none
	spawning-SS	240	96.3	3.8	0.0	0.0	3.8	↑- glucose (0.051), $\log_e$ALT (0.047) ↓- neutrophils (0.018)
macrophage aggregates, pigmented	prespawning-PWS	79	10.1	57.0	29.1	3.8	89.9	ND
	spawning-PWS	180	1.1	28.9	48.3	21.7	98.9	↑- age (<0.001*), neutrophils (0.001)
	spawning-SS	240	5.8	43.3	41.3	9.6	94.2	↑- age (<0.001*), glucose (0.035) ↓- $\log_e$CPK (<0.001), $\log_e$AST (0.001) NT- lactate (0.047), total protein (0.034), albumin (0.049), IgM (<0.001)
Ortholinea orientalis (intraductal myxosporean), histopathology	prespawning-PWS	79	86.1	10.1	0.0	3.8	13.9	ND
	spawning-PWS	180	92.8	4.4	2.8	0.0	7.2	↑- PCV (0.048), neutrophils (0.049), basophils (0.036) ↓- potassium (0.020)
	spawning-SS	240	96.3	2.5	1.3	0.0	3.8	none
Ortholinea orientalis (intraductal myxosporean), kidney touch preparation	prespawning-PWS	79	75.9	8.9	3.8	11.4	24.1	ND
	spawning-PWS	180	72.2	16.7	4.4	6.7	27.8	↓- total protein (0.019), albumin (0.019) NT- neutrophils (0.030), $\log_e$ALT (0.042)
	spawning-SS	240	81.3	13.3	2.5	2.9	18.8	NT- potassium (0.026)

Organ - lesion or tissue type	Spawning status - site	n	Lesion Score Prevalence %=0	%=1	%=2	%=3	%>0	Significant trends (*P*-value)
Ortholinea orientalis (intraductal myxosporean); sum of both techniques	prespawning-PWS	80	71.3	11.3	2.5	15.0	28.8	ND
	spawning-PWS	180	71.1	15.6	5.6	7.8	28.9	NT- cholesterol (0.026), albumin (0.039)
	spawning-SS	240	79.6	14.2	2.9	3.3	20.4	NT- ALP (0.040), bilirubin (0.037), basophils (0.009)
tubular dilation (of lumen)	prespawning-PWS	79	93.7	5.1	1.3	0.0	6.3	ND
	spawning-PWS	180	96.7	3.3	0.0	0.0	3.3	ND
	spawning-SS	240	89.6	9.2	1.3	0.0	10.4	↑- hold time (0.024), sodium (0.002), potassium (0.002), phosphorus (0.002), neutrophils (0.001), $\log_e$ALT (0.049) ↓- chloride (0.003*), PCV (0.010)
tubular epithelial vacuolation	prespawning-PWS	79	88.6	11.4	0.0	0.0	11.4	ND
	spawning-PWS	180	73.9	26.1	0.0	0.0	26.1	↑- glucose (0.020), cholesterol (0.011) ↓- phosphorus (<0.001)
	spawning-SS	240	59.6	39.2	1.3	0.0	40.4	↑- age (0.046)
Liver microscopic lesions:								
cholangitis or biliary hyperplasia	prespawning-PWS	80	97.5	1.3	1.3	0.0	2.5	ND
	spawning-PWS	180	92.2	7.8	0.0	0.0	7.8	↑- hold time (0.005)
	spawning-SS	240	92.9	7.1	0.0	0.0	7.1	↑- calcium (0.047), cholesterol (0.028)
coccidiosis (*Goussia* [*Eimeria*] *clupearum*)	prespawning-PWS	80	32.5	27.5	18.8	21.3	67.5	ND
	spawning-PWS	180	29.4	40.6	18.9	11.1	70.6	↓- age (0.002) NT- total protein (0.028)
	spawning-SS	240	27.5	41.3	15.8	15.4	72.5	↓- age (0.040), glucose (0.007) NT- cholesterol (0.052), neutrophils (0.054)

Organ - lesion or tissue type	Spawning status - site	n	Lesion Score Prevalence %=0	%=1	%=2	%=3	%>0	Significant trends (*P*-value)
eosinophilic granular leukocytes, perivascular	prespawning-PWS	80	23.8	73.8	2.5	0.0	76.3	ND
	spawning-PWS	180	6.7	74.4	18.9	0.0	93.3	none
	spawning-SS	240	7.5	80.0	12.5	0.0	92.5	↑- ALP (0.005), IgM (0.003*) ↓- hold time (0.010), potassium (0.041) NT- calcium (0.021), lymphocytes (0.028), $\log_e$ALT (0.034)
glycogen depletion, hepatocellular	prespawning-PWS	80	0.0	0.0	0.0	100.0	100.0	ND
	spawning-PWS	180	0.0	0.0	3.3	96.7	100.0	ND
	spawning-SS	240	0.0	0.0	2.5	97.5	100.0	ND
granulomatous inflammation	prespawning-PWS	80	76.3	21.3	2.5	0.0	23.8	ND
	spawning-PWS	180	58.3	38.9	1.7	1.1	41.7	↑- ALP (0.005), total protein (0.036) ↓- CO_2 (0.047)
	spawning-SS	240	66.3	31.7	1.7	0.4	33.8	↑- total protein (0.027), IgM (0.013)
Ichthyophonus	prespawning-PWS	80	90.0	6.3	1.3	2.5	10.0	ND
	spawning-PWS	180	78.9	11.1	7.2	2.8	21.1	↑- neutrophils (0.011), $\log_e$AST (0.026), IgM (<0.001) NT- phosphorus (0.025), bilirubin (0.032)
	spawning-SS	240	83.3	9.2	4.2	3.3	16.7	↑- basophils (0.010), $\log_e$AST (0.004), IgM (<0.001) ↓- thrombocytes (0.013)

Organ - lesion or tissue type	Spawning status - site	n	Lesion Score Prevalence %=0	%=1	%=2	%=3	%>0	Significant trends (*P*-value)
leukocytes, focal, parenchymal	prespawning-PWS	80	25.0	72.5	2.5	0.0	75.0	ND
	spawning-PWS	180	18.9	78.9	2.2	0.0	81.1	↑- chloride (0.001*), calcium (<0.001), glucose (<0.001*), cholesterol (0.001), lactate (<0.001), total protein (<0.001), albumin (<0.001), osmolality (0.005), PCV (0.004), lymphocytes (<0.001) ↓- thrombocytes (0.008), $\log_e$AST (0.008)
	spawning-SS	240	51.3	48.3	0.0	0.4	48.8	none
leukocytes, pericholangial	prespawning-PWS	80	76.3	22.5	1.3	0.0	23.8	ND
	spawning-PWS	180	68.9	29.4	1.7	0.0	31.1	↑- age (0.023) ↓- potassium (0.008)
	spawning-SS	240	71.3	27.1	1.7	0.0	28.8	none
lipidosis, hepatocellular	prespawning-PWS	80	86.3	10.0	2.5	1.3	13.8	ND
	spawning-PWS	180	93.3	6.7	0.0	0.0	6.7	↑- total protein (0.009), albumin (0.012) ↓- neutrophils (0.035)
	spawning-SS	240	60.0	29.2	9.6	1.3	40.0	↑- age (<0.001), hold time (<0.001), ALP (0.027), sodium (<0.001), potassium (<0.001*), phosphorus (<0.001*), calcium (0.026), lactate (<0.001), osmolality (<0.001) ↓- thrombocytes (0.038), IgM (0.031)

Organ - lesion or tissue type	Spawning status - site	n	Lesion Score Prevalence %=0	%=1	%=2	%=3	%>0	Significant trends (*P*-value)
macrophage aggregates, pigmented	prespawning-PWS	80	6.3	61.3	25.0	7.5	93.8	ND
	spawning-PWS	180	0.0	33.3	38.3	28.3	100.0	↑- age (<0.001), neutrophils (0.004) NT- glucose (0.032), cholesterol (0.004)
	spawning-SS	240	2.5	52.1	29.6	15.8	97.5	↑- age (<0.001*), neutrophils (0.016), IgM (<0.001) NT- glucose (<0.001), total protein (0.012), $\log_e$CPK (0.011), $\log_e$AST (<0.001)
necrosis, hepatocellular, focal	prespawning-PWS	80	100.0	0.0	0.0	0.0	0.0	ND
	spawning-PWS	180	100.0	0.0	0.0	0.0	0.0	ND
	spawning-SS	240	99.2	0.8	0.0	0.0	0.8	ND
necrosis, hepatocellular, single cell	prespawning-PWS	80	93.8	6.3	0.0	0.0	6.3	ND
	spawning-PWS	180	96.7	2.2	0.6	0.6	3.3	ND
	spawning-SS	240	97.5	2.5	0.0	0.0	2.5	ND
Pancreas, exocrine, microscopic lesions:								
macrophage aggregates, pigmented	prespawning-PWS	80	71.3	28.8	0.0	0.0	28.8	ND
	spawning-PWS	180	38.9	60.6	0.6	0.0	61.1	↑- age (0.002*), PCV (0.017), IgM (0.019)
	spawning-SS	240	44.6	55.4	0.0	0.0	55.4	↑- age (0.000) ↓- $\log_e$CPK (0.017), $\log_e$AST (0.001)

Organ - lesion or tissue type	Spawning status - site	n	Lesion Score Prevalence %=0	%=1	%=2	%=3	%>0	Significant trends (*P*-value)
zymogen granule depletion	prespawning-PWS	80	0.0	3.8	57.5	38.8	100.0	ND
	spawning-PWS	180	0.0	0.6	46.1	53.3	100.0	↑- phosphorus (<0.001), thrombocytes (<0.001), log_eAST (0.004*) ↓- chloride (0.009), calcium (0.006), glucose (<0.001), cholesterol (<0.001), lactate (0.001), total protein (<0.001), albumin (<0.001), osmolality (0.003), PCV (0.043), lymphocytes (<0.001*)
	spawning-SS	240	0.0	0.4	35.4	64.2	100.0	↑- neutrophils (0.038) ↓- chloride (0.001), glucose (<0.001), cholesterol (0.002), total protein (0.002), lymphocytes (0.044)
Skin and skeletal muscle, microscopic lesions:								
arteriolar hyperplasia, focal, intimal	prespawning-PWS	80	63.8	36.3	0.0	0.0	36.3	ND
	spawning-PWS	180	52.2	47.8	0.0	0.0	47.8	↑- log_eAST (0.034) ↓- PCV (0.007)
	spawning-SS	240	53.3	46.7	0.0	0.0	46.7	↑- lactate (0.024), albumin (0.041), neutrophils (0.024) ↓- thrombocytes (0.034)
Ichthyophonus	prespawning-PWS	80	92.5	2.5	2.5	2.5	7.5	ND
	spawning-PWS	180	82.8	11.1	5.0	1.1	17.2	↑- neutrophils (0.007), lymphocytes (0.026), IgM (<0.001)
	spawning-SS	240	84.2	11.7	3.8	0.4	15.8	↑- log_eAST (<0.001), IgM (<0.001) NT- log_eCPK (0.031)

Organ - lesion or tissue type	Spawning status - site	n	Lesion Score Prevalence %=0	%=1	%=2	%=3	%>0	Significant trends (*P*-value)
leukocytes, perivascular	prespawning-PWS	80	36.3	63.8	0.0	0.0	63.8	ND
	spawning-PWS	180	22.8	77.2	0.0	0.0	77.2	↑- sodium (0.017) ↓- cholesterol (<0.001), total protein (0.050)
	spawning-SS	240	34.6	65.4	0.0	0.0	65.4	↑- age (0.001)
myodegeneration or myonecrosis	prespawning-PWS	80	98.8	0.0	1.3	0.0	1.3	ND
	spawning-PWS	180	95.0	3.3	1.7	0.0	5.0	↑- $\log_e$CPK (0.003) ↓- potassium (0.051), glucose (0.018)
	spawning-SS	240	96.3	3.3	0.4	0.0	3.8	↑- $\log_e$ALT (0.016)
myositis	prespawning-PWS	80	85.0	12.5	1.3	1.3	15.0	ND
	spawning-PWS	180	92.2	7.2	0.6	0.0	7.8	↑- $\log_e$CPK (0.050), $\log_e$AST (0.030) ↓- sodium (<0.001*), osmolality (0.015)
	spawning-SS	240	96.7	3.3	0.0	0.0	3.3	↑- basophils (0.004*) ↓- thrombocytes (0.010)
Spleen microscopic lesions:								
arteriolar hyperplasia, focal, intimal	prespawning-PWS	79	83.5	11.4	0.0	0.0	11.4	ND
	spawning-PWS	180	81.7	17.8	0.0	0.0	17.8	none
	spawning-SS	235	82.6	17.4	0.0	0.0	17.4	↓- hold time (0.001), potassium (0.048)

Organ - lesion or tissue type	Spawning status - site	n	Lesion Score Prevalence %=0	%=1	%=2	%=3	%>0	Significant trends (*P*-value)
congestion, vascular	prespawning-PWS	79	1.3	24.1	21.5	53.2	98.7	ND
	spawning-PWS	180	2.8	71.7	18.3	7.2	97.2	↓- albumin (0.023), osmolality (0.036), IgM (0.026) NT- hold time (0.015), chloride (0.017), CO_2 (0.022), glucose (0.051), lactate (0.018), $\log_e$AST (0.042)
	spawning-SS	235	23.0	58.7	13.2	5.1	77.0	↓- age (0.004*), sodium (<0.001), phosphorus (<0.001), calcium (<0.001*), lactate (<0.001), osmolality (0.001) NT- potassium (0.024)
ellipsoid hyalinization or hypertrophy	prespawning-PWS	79	24.1	72.2	3.8	0.0	75.9	ND
	spawning-PWS	180	2.2	75.6	22.2	0.0	97.8	↑- age (0.018), ALP (0.046) ↓- $\log_e$CPK (0.001), $\log_e$AST (0.010)
	spawning-SS	235	5.5	82.1	12.3	0.0	94.5	↑- age (0.002*), chloride (0.016)
granulomatous inflammation	prespawning-PWS	79	97.5	2.5	0.0	0.0	2.5	ND
	spawning-PWS	180	92.8	6.1	0.6	0.6	7.2	↑- neutrophils (0.038), basophils (0.015) ↓- calcium (0.024), glucose (0.021), lactate (0.013), lymphocytes (0.006)
	spawning-SS	235	94.9	4.3	0.4	0.4	5.1	↑- lymphocytes (0.031) ↓- hold time (0.007*), calcium (0.013)

Organ - lesion or tissue type	Spawning status - site	n	Lesion Score Prevalence %=0	%=1	%=2	%=3	%>0	Significant trends (*P*-value)
Ichthyophonus	prespawning-PWS	79	92.4	3.8	2.5	1.3	7.6	ND
	spawning-PWS	180	82.2	7.2	7.2	3.3	17.8	↑- neutrophils (<0.001*), log_eAST (0.005), IgM (<0.001) NT- hold time (0.037), CO_2 (0.032), log_eCPK (0.018)
	spawning-SS	235	85.1	8.5	3.8	2.6	14.9	↑- log_eAST (0.001), IgM (<0.001) NT- total protein (0.017)
macrophage aggregates, pigmented	prespawning-PWS	79	15.2	40.5	32.9	11.4	84.8	ND
	spawning-PWS	180	0.6	15.6	48.3	35.6	99.4	↑- age (<0.001), neutrophils (0.018), IgM (0.043) ↓- log_eCPK (0.013) NT- glucose (0.045), cholesterol (0.054), osmolality (0.048)
	spawning-SS	235	14.0	30.6	31.9	23.4	86.0	↑- age (<0.001), glucose (0.044), neutrophils (0.010), IgM (<0.001) ↓- log_eCPK (0.003), log_eAST (<0.001) NT- osmolality (0.017)
Stomach microscopic lesions:								
eosinophilic granular leukocytes, submucosal	prespawning-PWS	80	0.0	76.3	23.8	0.0	100.0	ND
	spawning-PWS	180	0.0	76.1	23.9	0.0	100.0	↓- glucose (0.012), cholesterol (0.001), total protein (0.007), albumin (0.017)
	spawning-SS	240	0.4	88.3	11.3	0.0	99.6	↑- log_eALT (0.034), IgM (0.016) ↓- neutrophils (0.045)
foreign body granuloma	prespawning-PWS	80	88.8	11.3	0.0	0.0	11.3	ND
	spawning-PWS	180	85.0	15.0	0.0	0.0	15.0	none
	spawning-SS	240	85.4	14.2	0.4	0.0	14.6	none

Organ - lesion or tissue type	Spawning status - site	n	Lesion Score Prevalence %=0	%=1	%=2	%=3	%>0	Significant trends (*P*-value)
Ichthyophonus (includes only cases with organisms)	prespawning-PWS	80	95.0	3.8	1.3	0.0	5.0	ND
	spawning-PWS	180	84.4	11.7	3.9	0.0	15.6	↑- total protein (0.008), albumin (0.042), IgM (<0.001) ↓- hold time (0.048), CO_2 (0.033)
	spawning-SS	240	89.6	8.8	1.7	0.0	10.4	↑- ALP (0.041), basophils (0.016), $\log_e$AST (0.019), IgM (0.007)
Ichthyophonus+ (includes cases with characteristic inflammation, but no organisms)	prespawning-PWS	80	93.8	5.0	1.3	0.0	6.3	ND
	spawning-PWS	180	81.1	15.0	3.9	0.0	18.9	↑- total protein (0.040), neutrophils (0.024), IgM (<0.001) ↓- hold time (0.035), CO_2 (0.051)
	spawning-SS	240	81.3	17.1	1.7	0.0	18.8	↑- basophils (0.019), $\log_e$AST (0.035), IgM (0.007)
leukocytes, focal, parenchymal	prespawning-PWS	80	98.8	1.3	0.0	0.0	1.3	ND
	spawning-PWS	180	86.7	12.2	1.1	0.0	13.3	↑- PCV (0.007) ↓- CO_2 (0.040)
	spawning-SS	240	98.3	1.7	0.0	0.0	1.7	ND
serositis	prespawning-PWS	80	47.5	52.5	0.0	0.0	52.5	ND
	spawning-PWS	180	42.2	57.8	0.0	0.0	57.8	none
	spawning-SS	240	45.4	54.6	0.0	0.0	54.6	↓- PCV (0.053), lymphocytes (0.053)
trematodes, intraluminal (e.g., Hemiuridae)	prespawning-PWS	80	83.8	16.3	0.0	0.0	16.3	ND
	spawning-PWS	180	88.3	11.7	0.0	0.0	11.7	none
	spawning-SS	240	89.6	10.0	0.4	0.0	10.4	↑- glucose (0.038) ↓- age (0.027), neutrophils (0.008)

Table 3. Other lesions associated with external lesions in Pacific herring sampled from Prince William Sound and Sitka Sound, Alaska, during spawning, 1995. Chi-square test for association (n = 420 for most comparisons). For lesions with minimum expected cell frequency <1 (*), only chi-square tests with $P \leq 0.010$ are included. Trends in the associated lesion scores were classified in comparison to an increase in the given external lesion score. As the external lesion score increased, the associated lesion score either increased (↑), decreased (↓), or changes in the associated lesion score were not linear (NL; e.g., as scores for the external lesion increased, associated lesion scores initially increased and then later decreased). Lesions not listed were not significant.

	↑ caudal fin fraying		↑ caudal fin reddening		↑ fin base reddening		↑ focal skin reddening		↑ diffuse skin reddening	
Associated lesion	Trend	*P*-value	Trend	*P*-value	Trend	*P*-value	Trend	*P*-value	Trend	*P*-value
caudal fin fraying			↑	<0.001	↑	<0.001*				
caudal fin reddening	↑	<0.001			↑	<0.001*			↑	<0.001
fin base reddening	↑	<0.001*	↑	<0.001*					↑	<0.001*
diffuse skin reddening			↑	<0.001	↑	<0.001*				
iris reddening	NL	0.014								
eggs in stomach	NL	0.005	NL	0.021						
brain *Ichthyophonus*	↑	0.016					↑	0.004*		
branchial *Ichthyophonus*	↑	0.022					↑	0.006*		
cardiac epicarditis									↑	0.034
cardiac *Ichthyophonus*							↑	<0.001*		
cardiac thrombosis			NL	0.032						

	↑ caudal fin fraying		↑ caudal fin reddening		↑ fin base reddening		↑ focal skin reddening		↑ diffuse skin reddening	
Associated lesion	Trend	*P*-value	Trend	*P*-value	Trend	*P*-value	Trend	*P*-value	Trend	*P*-value
gastric focal parenchymal leukocytes	↑	0.007	↑	0.001						
gastric trematodiasis									↓	0.022
gastric serositis					↑	0.008	↑	0.036		
gastritis, submucosal			↑	0.007						
gonadal fullness	↑	0.049							↓	0.005
gonadal granulomatous inflammation									↑	0.028
gonadal pigmented macrophage aggregates			↑	0.030						
hepatic coccidiosis (*Goussia clupearum*)	NL	0.045							NL	0.008
hepatic eosinophilic granular leukocytes	↑	0.053	↑	0.019						
hepatic focal/multifocal parenchymal leukocytes	NL	0.032								
hepatic *Ichthyophonus*			↑	0.127			↑	<0.001*		
hepatic lipidosis	↓	0.011								

	↑ caudal fin fraying		↑ caudal fin reddening		↑ fin base reddening		↑ focal skin reddening		↑ diffuse skin reddening	
Associated lesion	Trend	*P*-value	Trend	*P*-value	Trend	*P*-value	Trend	*P*-value	Trend	*P*-value
hepatic pericholangial leukocytes	↑	0.023	NL	0.017						
intestinal Anisakidae			↑	0.036						
intestinal coccidian (*Goussia*? sp.)							NL	<0.001*	↓	0.023
intestinal foreign body granulomas					↑	0.007				
intestinal mesenteric steatitis	↑	0.009								
renal congestion			NL	0.039						
renal hematopoietic cells	↑	0.020								
renal *Ichthyophonus*							↑	0.001*		
renal pigmented macrophage aggregates	NL	0.001								
splenic *Ichthyophonus*	↑	0.046								
skeletal muscle, perivascular leukocytes	↑	0.031					↑	0.030		
testicular coccidian (*Eimeria sardinae*)									NL	0.045

Table 4. Oocyte morphology in mature female Pacific herring sampled from Sitka Sound (SS) and Prince William Sound (PWS) in March and April, 1995. Note that all spawning fish from both sites were sexually mature, but only 45% (36 of 80) of the PWS "prespawning" fish were sexually mature.

Variable	Spawning status-site	# of fish	Mean #	±SE
oocyte atresia - mature follicles	prespawning-PWS	24	0.0	0.5
	spawning-PWS	85	0.1	0.7
	spawning-SS	94	0.0	0.0
ruptured follicles	prespawning-PWS	24	0.0	0.0
	spawning-PWS	85	0.0	0.5
	spawning-SS	94	0.0	0.0
yolked oocytes	prespawning-PWS	24	66.2	5.4
	spawning-PWS	85	14.9	4.5
	spawning-SS	94	13.6	4.4
nonyolked oocytes	prespawning-PWS	24	66.8	5.2
	spawning-PWS	85	87.1	8.3
	spawning-SS	94	116.6	8.7
% yolked oocytes	prespawning-PWS	24	49.9	4.0
	spawning-PWS	85	15.7	3.6
	spawning-SS	94	15.8	4.6

Table 5. Lesion frequency (%) within variables of gender and iris reddening in Pacific herring sampled from Prince William Sound and Sitka Sound, Alaska, during spawning, 1995. Lesions were scored as none (0), mild (1), moderate (2), or severe (3). Chi-square test for homogeneity. Lesions not listed were not significant. For some lesions, sum of individual frequencies within a category is different from 100% due to rounding differences.

Variable and lesion	Lesion score	Frequency		χ^2 *P*-value	Odds ratio[a]	95% Confidence interval for odds ratio
Gender		Female (n ≈ 179)	Male (n ≈ 240)			
brain eosinophilic granular leukocytes	0	13	13	0.003		
	1	68	53		ND[b]	
	2+3	20	34			
cardiac *Ichthyophonus*	0	75	85	0.001		
	1	10	5		ND	
	2	10	3			
	3	4	8			
epicarditis	0	30	48	0.001		
	1	66	50		ND	
	2+3	4	3			
gall bladder myxosporeans (*Ceratomyxa auerbachi*)	0	54	72	<0.001		
	1	31	23		ND	
	2+3	14	5			
gonadal granulomas (or focal granulomatous inflammation)	0	37	93	<0.001		
	1	63	7		22	12, 39

Variable and lesion	Lesion score	Frequency		χ^2 *P*-value	Odds ratio[a]	95% Confidence interval for odds ratio
Gender (continued)		Female (n ≈ 179)	Male (n ≈ 240)			
gonadal eosinophilic granular leukocytes	0	41	69	<0.001		
	1	42	22		ND	
	2	7	3			
	3	9	6			
gonadal pigmented macrophage aggregates	0	67	100	<0.001		
	1	33	0		118	16, 859
gonadal hyalinized vessel walls	0	56	100	<0.001		
	1	35	0		ND	
	2	9	0			
hepatic glycogen depletion	2	7	0	<0.001		
	3	93	100		0	none
hepatic *Ichthyophonus*	0	77	85	0.012		
	1	16	6		ND	
	2	5	6			
	3	3	3			
hepatic lipidosis	0	68	79	0.016		
	1	22	18		ND	
	2+3	9	4			
intestinal foreign body granuloma	0	51	68	0.001		
	1+2	49	32		2.0	1.3, 3.0

Variable and lesion	Lesion score	Frequency		χ^2 *P*-value	Odds ratio[a]	95% Confidence interval for odds ratio
Gender (continued)		Female (n ≈ 179)	Male (n ≈ 240)			
pancreatic zymogen granule depletion	1+2	24	53	<0.001		
	3	76	48		3.5	2.3, 5.4
renal proximal tubular epithelial vacuolation	0	74	60	0.002		
	1+2	26	40		0.51	0.33, 0.78
skeletal muscle *Ichthyophonus*	0	80	86	0.053		
	1	16	8		ND	
	2+3	4	6			
splenic ellipsoid hyalinization	0	4	4	0.002		
	1	72	85		ND	
	2	24	11			

Variable and lesion	Lesion score	Frequency		χ^2 *P*-value	Odds ratio[a]	95% Confidence interval for odds ratio
Iris reddening		Mild/Moderate (n ≈ 290)	None (n ≈ 128)			
caudal fin fraying	0	6	14	0.014		
	1	75	65		ND	
	2+3	20	21			
gastric eosinophilic granular leukocytes	0+1	80	90	0.018		
	2	20	10		2.1	1.1, 4.1
gonadal hyalinized vessel walls	0	78	89	0.023		
	1	18	9		ND	
	2	4	2			
hepatic pigmented macrophage aggregates	0+1	41	55	0.042		
	2	36	28		ND	
	3	23	17			
hepatic lipidosis	0	71	83	0.028		
	1	23	13		ND	
	2+3	7	5			
intestinal mesenteric Anisakidae	0	21	17	0.014		
	1	57	45		ND	
	2	16	26			
	3	7	12			
renal congestion	0	82	90	0.046		
	1+2+3	18	10		1.9	1.0, 3.7

Variable and lesion	Lesion score	Frequency		χ^2 *P*-value	Qdds ratio[a]	95% Confidence interval for odds ratio
Iris reddening (continued)		Mild/ Moderate (n ≈ 290)	None (n ≈ 128)			
renal intratubular mineral	0	98	95	0.033		
	1+2	2	5		0.30	0.094, 0.97
skeletal muscle arteriolar hyperplasia, focal, intimal	0	50	60	0.047		
	1	50	40		1.5	1.0, 2.3
splenic congestion	0	18	6	<0.001		
	1	67	59		ND	
	2	13	21			
	3	3	13			

[a]Odds ratio is defined as the ratio of the odds of a fish being at one level of a condition (e.g., having a scorable lesion) as opposed to being at another level of a condition (e.g. having no lesion) for one category of a variable (e.g., female) to the corresponding odds for the other category of the variable (e.g. male). For example, females were 118 times more likely to have pigmented gonadal macrophage aggregates than were males, fish with mild/moderate iris reddening were 1.9 times more likely to have renal congestion than were fish with no iris reddening.

[b]ND = not done; odds ratios were not calculated for lesions with more than 2 groups.

Table 6. Number of intraperitoneal herring worms (Anisakidae) in categories based on age or lesion scores in Pacific herring sampled from Prince William Sound (PWS) and Sitka Sound (SS), Alaska, during spawning, 1995. One-way analysis of variance (ANOVA) and Tukey's multiple-comparison procedure. Means were ln transformed for statistical analysis; values shown are geometric means and first-order Taylor series approximation of standard errors. If Levene's test for equality of variances was significant (*), only comparisons with $P \leq 0.010$ are listed. Within rows, geometric means with a superscript in common were not significantly different ($P > 0.05$); lesions not shown were not significant.

Variable - site	Category based on lesion score									*P*-value for ANOVA
	A			B			C			
	Mean	SE	n	Mean	SE	n	Mean	SE	n	
	age = 2, 3			age = 4, 5, 6			age = 7			
age - PWS	$12.4^{A,B}$	4.0	19	15.2^{A}	3.6	31	10.8^{B}	1.4	105	0.044
age - SS	12.7^{A}	1.4	92	16.1^{A}	3.6	36	8.9^{B}	1.0	104	<0.001
gastric eosinophilic granular leukocytes - PWS	mild			moderate			NA[a]			
	11.1^{A}	1.3	137	13.9^{A}	2.6	43				0.052
gastric serositis - PWS	none			mild			NA			
	10.3^{B}	1.6	76	12.9^{A}	1.6	104				0.027
gastric trematodes - PWS	none			mild			NA			
	11.3^{B}	1.2	159	15.3^{A}	4.4	21				0.050
hepatic pigmented macrophage aggregates - SS	none/mild			moderate			severe			
	12.3^{A}	1.3	129	10.0^{A}	1.6	70	10.2^{A}	2.2	37	0.048
hepatic lipidosis - SS	none			mild/moderate/severe			NA			
	12.0^{A}	1.2	141	10.1^{B}	1.3	95				0.039

Variable - site	Category based on lesion score									*P*-value for ANOVA
	A			B			C			
	Mean	SE	n	Mean	SE	n	Mean	SE	n	
intestinal arteriolar hyperplasia, focal, intimal - SS	12.3[A]	none 1.3	134	9.9[B]	mild/moderate 1.3	102		NA		0.009
intestinal mesenteric Anisakidae - PWS	8.2[C]	none 1.5	31	11.4[B,C]	mild 1.6	98	13.5[A,B]	moderate 2.5	33	
- SS	8.6[C]	1.5	52	10.3[C]	1.0	122	14.8[B]	2.5	46	
intestinal mesenteric Anisakidae -PWS (cont.)	19.9[A]	severe 4.8	18							PWS <0.001
- SS (cont.)	23.5[A]	4.7	16							SS <0.001
meningitis - SS	11.0[B]	none 0.9	229	21.3[A]	mild 9.6	7		NA		0.006
renal congestion - SS	10.7[B]	none 1.0	188	13.6[A]	mild/severe 2.1	48		NA		0.019
skeletal muscle, perivascular leukocytes - PWS	9.1[B]	none 2.0	41	12.7[A]	mild 1.3	139		NA		0.005

[a]NA = not applicable (i.e., only 2 categories were used)

Table 7. Mean plasma chemistry and hematology values in males and females sampled from Sitka Sound and Prince William Sound, Alaska during spawning in 1995. Analysis of variance. Note that gonads from all fish were in spawning condition; some fish from Sitka Sound were partly or completely spawned out, whereas no fish from Prince William Sound had spawned before being sampled.

		Sitka Sound				Prince William Sound						
		Males		Females		Males		Females		Significance[a]		
Variable	age	mean	SE	mean	SE	mean	SE	mean	SE	site	sex	site*sex
Age	all[b]	5.0	0.2	5.3	0.2	6.5	0.2	6.8	0.2	***	NS	NS
	7[c]	7.0	0.0	7.0	0.0	7.0	0.0	7.0	0.0	NS	NS	NS
Length (mm)	all	200.1	1.5	204.5	1.9	216.4	1.8	222.7	1.7	***	***	NS
	7	215.0	1.2	217.0	1.5	222.4	1.4	226.7	1.3	***	*	NS
Body weight (g)	all	116.7	3.2	130.6	4.3	135.0	3.9	150.4	3.8	***	***	NS
	7	149.9	3.4	157.2	4.2	146.5	3.4	159.8	3.8	NS	**	NS
Gonad weight (g)	all	19.2	1.1	26.7	1.8	28.1	1.1	35.8	1.2	***	***	NS
	7	27.6	1.8	33.2	2.7	32.2	1.0	38.5	1.3	**	***	NS
Liver weight (g)	all	0.8	0.0	1.2	0.0	1.0	0.0	1.2	0.0	***	***	*
	7	1.1	0.0	1.4	0.0	1.1	0.0	1.3	0.0	**	***	*
Hold time (min)	all	154.0	3.7	148.1	4.6	96.9	4.6	95.0	4.2	***	NS	NS
	7	147.4	5.7	145.6	6.4	95.1	6.6	98.5	5.5	***	NS	NS
SumICH	all	1.7	0.4	1.9	0.4	2.1	0.5	2.5	0.5	NS	NS	NS
	7	1.8	0.6	2.1	0.6	2.9	0.8	2.9	0.7	NS	NS	NS
PCV (%)[d]	all	45.4	0.5	43.5	0.7	46.2	0.5	44.4	0.4	NS	***	NS
	7	45.7	0.7	43.8	1.0	46.6	0.6	44.4	0.5	NS	**	NS
Albumin (g/dL)	all	1.1	0.0	0.9	0.0	1.0	0.0	0.9	0.0	*	***	NS
	7	1.1	0.0	0.9	0.0	1.0	0.0	0.9	0.0	*	***	NS

		Sitka Sound				Prince William Sound						
		Males		Females		Males		Females		Significance[a]		
Variable	age	mean	SE	mean	SE	mean	SE	mean	SE	site	sex	site*sex
$\log_e$ IgM (mg/mL)[e]	all	751.0	29.2	668.8	39.5	999.7	64.5	947.3	78.3	***	NS	NS
	7	831.4	48.5	753.1	59.5	1198.1	99.5	1095.3	112.3	***	NS	NS
Total protein (g/dL)	all	2.5	0.0	2.3	0.1	2.5	0.0	2.1	0.1	*	***	NS
	7	2.8	0.1	2.4	0.1	2.6	0.1	2.2	0.1	*	***	NS
ALP (U/L)	all	52.0	1.2	58.4	1.9	46.6	1.1	50.1	1.4	***	***	NS
	7	53.7	1.6	58.8	2.7	47.3	1.5	51.1	1.8	**	*	NS
ALT (U/L)	all	4.2	0.2	4.7	0.3	4.3	0.2	4.6	0.5	NS	NS	NS
	7	4.0	0.4	4.1	0.5	4.6	0.3	3.9	0.4	NS	NS	NS
AST (U/L)	all	568.9	22.3	717.2	42.8	279.6	24.3	288.7	39.2	***	*	*
	7	493.9	36.7	644.9	57.7	291.6	33.2	257.9	48.0	***	NS	NS
$\log_e$ CPK (U/L)[e]	all	1074.3	81.2	1260.5	174.7	1285.1	130.2	1487.8	276.3	NS	NS	NS
	7	878.9	119.1	1141.1	281.3	1283.0	169.2	1068.0	197.6	NS	NS	NS
GGT (U/L)	all	0.9	0.1	0.7	0.1	1.2	0.1	0.8	0.1	NS	*	NS
	7	1.0	0.1	0.6	0.1	1.5	0.2	0.7	0.1	*	***	NS
Calcium (mg/dL)	all	12.2	0.1	12.0	0.2	11.5	0.1	11.2	0.1	***	*	NS
	7	12.4	0.2	11.8	0.3	11.6	0.1	11.3	0.1	***	*	NS
Chloride (mmol/L)	all	143.2	1.3	141.5	1.3	135.8	3.4	140.3	3.4	NS	NS	NS
	7	144.8	2.6	139.6	1.7	128.9	4.7	143.1	4.1	NS	NS	**
Cholesterol (mg/dL)	all	273.5	5.7	200.5	5.1	289.8	7.6	217.1	7.0	*	***	NS
	7	286.2	10.1	201.5	7.3	297.8	10.5	227.8	8.6	*	***	NS

		Sitka Sound				Prince William Sound						
		Males		Females		Males		Females		Significance[a]		
Variable	age	mean	SE	mean	SE	mean	SE	mean	SE	site	sex	site*sex
$\log_e CO_2$ (mmol/L)[e]	all	13.2	0.3	13.6	0.8	8.4	0.3	7.9	0.3	***	NS	NS
	7	13.7	0.5	13.4	1.1	8.3	0.3	8.2	0.3	***	NS	NS
Glucose (mg/dL)	all	105.8	3.5	91.6	3.4	129.9	4.1	91.3	2.6	***	***	***
	7	124.2	5.3	101.2	4.6	136.9	5.2	95.6	3.2	NS	***	*
Lactate (mmol/dL)	all	96.0	3.8	93.3	4.5	73.8	3.2	68.1	2.7	***	NS	NS
	7	95.4	6.2	97.5	6.8	77.6	4.1	72.4	3.4	***	NS	NS
Osmolality	all	423.4	3.5	420.3	5.0	418.5	1.5	410.6	1.6	*	NS	NS
(mOsm/kg)	7	425.8	5.1	425.6	5.8	419.3	1.8	413.9	1.8	*	NS	NS
Phosphorus	all	7.4	0.2	7.4	0.3	4.8	0.2	5.6	0.1	***	*	NS
(mg/dL)	7	7.6	0.4	7.7	0.4	4.8	0.2	5.5	0.2	***	NS	NS
Potassium (mmol/L)	all	2.4	0.1	2.4	0.1	1.7	0.1	1.8	0.1	***	NS	NS
	7	2.2	0.1	2.3	0.1	1.7	0.1	1.9	0.1	***	NS	NS
Sodium (mmol/L)	all	209.7	1.2	207.7	1.6	191.1	0.6	184.3	2.2	***	**	NS
	7	209.3	2.0	207.6	1.8	192.0	0.8	183.5	3.4	***	*	NS
Total bilirubin	all	0.1	0.0	0.1	0.0	0.1	0.0	0.1	0.0	**	NS	NS
(mg/dL)	7	0.1	0.0	0.1	0.0	0.1	0.0	0.1	0.0	NS	NS	*
Basophils (%)	all	1.0	0.1	0.8	0.1	0.5	0.1	0.4	0.1	***	NS	NS
	7	1.0	0.2	0.7	0.1	0.4	0.1	0.6	0.1	***	NS	NS
Eosinophils (%)	all	0.6	0.1	0.4	0.1	0.5	0.1	0.6	0.1	NS	NS	NS
	7	0.6	0.2	0.4	0.1	0.4	0.1	0.6	0.1	NS	NS	NS

		Sitka Sound				Prince William Sound						
		Males		Females		Males		Females		Significance[a]		
Variable	age	mean	SE	mean	SE	mean	SE	mean	SE	site	sex	site*sex
Lymphocytes (%)	all	15.9	0.8	16.0	0.9	27.4	1.2	21.0	1.0	***	**	***
	7	15.5	1.3	16.1	1.2	25.8	1.5	19.9	1.2	***	NS	**
Monocytes (%)	all	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	NS	NS	NS
	7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	NS	NS	NS
Neutrophils (%)	all	9.4	0.7	6.7	0.5	9.1	0.7	7.7	0.7	NS	**	NS
	7	10.4	1.2	7.4	0.8	9.4	1.0	8.3	0.9	NS	*	NS
Thrombocytes (%)	all	73.1	1.0	76.1	1.0	62.4	1.3	70.3	1.2	***	***	*
	7	72.5	1.7	75.4	1.4	63.9	1.6	70.6	1.7	***	**	NS

[a]Significance is designated as $P > 0.05$ (NS), $P \leq 0.05$ (*), $P \leq 0.01$ (**), or $P \leq 0.001$ (***).

[b]Sample size of all ages varies slightly for some variables, but usually was as follows: Sitka Sound males (n = 146), Sitka Sound females (n = 94), Prince William Sound males (n = 94), Prince William Sound females (n = 86).

[c]Sample size of 7-yr-olds varies slightly for some variables, but usually was as follows: Sitka Sound males (n = 56), Sitka Sound females (n = 50), Prince William Sound males (n = 50), Prince William Sound females (n = 55).

[d]All % values were arcsin square root transformed for analysis; however, true means and standard errors of actual % values are reported here.

[e]Values for CO_2 and CPK were ln transformed for analysis of variance, but true means and standard errors of actual % values are reported here.

Table 8. Significantly different values (ANOVA, $P \leq 0.05$) based on age (yrs). Pacific herring were sampled during spawning in Prince William Sound (PWS) and Sitka Sound (SS), Alaska, 1995. Note that gonads from all fish were in spawning condition; some fish from Sitka Sound were partly or completely spawned out, whereas no fish from Prince William Sound had spawned before being sampled. For comparisons in which Levene's test for equality of variance was significant (*), only comparisons with $P \leq 0.010$ are shown. Plasma chemistries, hematology variables, and weights and lengths not shown were not significant.

	Age = 2, 3 (PWS, n = 19) (SS, n = 93)		Age = 4, 5, 6 (PWS, n = 31) (SS, n = 37)		Age = 7 (PWS, n = 105) (SS, n = 106)		
Variable	Mean	SE	Mean	SE	Mean	SE	*P*-value
Plasma chemistry							
albumin (g/dL) - SS	0.93	0.02	1.02	0.03	1.05	0.02	0.002
AST[a] (U/L) - SS	634.6	63.5	547.3	80.7	495.2	49.6	0.003
calcium (mmol/L) - SS	11.87	0.20	12.79	0.27	12.09	0.18	0.038
cholesterol (mg/dL) - SS	233.2	6.2	266.1	11.9	245.9	7.5	0.053
CPK[a] (mmol/L) - PWS	1449.5	598.2	835.5	313.1	746.2	139.7	0.026
CPK[a] (mmol/L) - SS	967.8	169.7	702.7	199.6	638.4	113.2	0.004
glucose (mg/dL) - SS	86.5	3.8	98.2	6.8	113.3	3.7	<0.001
IgM[a] (mmol/L) - PWS	422.4	102.8	686.1	113.5	967.8	106.4	<0.001
IgM[a] (mmol/L) - SS	552.8	56.4	669.8	97.5	709.8	65.5	0.001
osmolality (mOsm/kg) - SS	413.3	5.2	433.0	7.4	425.7	3.8	0.041
total protein (g/dL) - SS	2.27	0.05	2.51	0.10	2.60	0.05	<0.001

	Age = 2, 3 (PWS, n = 19) (SS, n = 93)		Age = 4, 5, 6 (PWS, n = 31) (SS, n = 37)		Age = 7 (PWS, n = 105) (SS, n = 106)		
Variable	Mean	SE	Mean	SE	Mean	SE	*P*-value
Hematology							
lymphocytes[b] (%) - PWS	33.1	4.6	27.0	4.3	21.9	1.9	<0.001
neutrophils[b] (%) - PWS	2.83	1.4	6.71	1.9	7.96	1.2	<0.001
Weight and Length							
body weight (g) - PWS	75.5	2.7	120.8	3.5	153.5	2.7	<0.001*
body weight (g) - SS	85.8	1.8	119.3	5.3	153.3	2.7	<0.001*
gonad weight (g) - PWS	12.6	0.8	25.5	1.4	35.5	0.9	<0.001*
gonad weight (g) - SS	12.9	0.7	21.6	2.3	30.2	1.6	<0.001*
liver weight (g) - PWS	0.55	0.03	0.89	0.05	1.19	0.03	<0.001*
liver weight (g) - SS	0.65	0.03	0.86	0.05	1.25	0.03	<0.001*
length (mm) - PWS	183.2	1.6	210.5	1.5	224.6	1.0	<0.001
length (mm) - SS	185.1	1.2	201.1	2.4	215.9	1.0	<0.001*

[a]Values were ln transformed for statistical analysis; values shown are geometric means and first-order Taylor series approximation of standard errors.

[b]Percent values were arcsine square root transformed for statistical analysis; values shown are re-transformed means and first-order Taylor series approximation of standard errors.

Table 9. Linear correlations (r) of age (yr), body weight and gonad weight (g), standard length (mm), hold time (min), sum-*Ichthyophonus* (sumICH) scores, albumin (g/dL), $\log_e$IgM, and blood values in Pacific herring sampled from Prince William Sound (PWS) and Sitka Sound (SS), Alaska, during spawning, 1995. Note that gonads from all fish were in spawning condition; some fish from Sitka Sound were partly or completely spawned out, whereas no fish from Prince William Sound had spawned before being sampled. Significant correlations ($P < 0.05$) are denoted (*); sample size varies from 170 to 180 for PWS and 220 to 240 for SS. Values for PCV and white blood cells were arcsine square root transformed for analysis.

Variable - site	Age	Body weight	Length	Gonad weight	Hold time	sumICH	Albumin	$\log_e$IgM
Body weight - PWS	0.717*							
Body weight - SS	0.796*							
Length - PWS	0.795*	0.897						
Length - SS	0.786*	0.906*						
Gonad weight - PWS	0.601*	0.833*	0.779*					
Gonad weight - SS	0.510*	0.832*	0.659*					
Hold time - PWS	-0.008	0.002	-0.008	0.059				
Hold time - SS	-0.145*	-0.090	-0.100	-0.023				
SumICH - PWS	0.088	0.011	0.038	-0.055	-0.096			
SumICH - SS	0.029	-0.008	0.030	-0.000	-0.007			
Albumin - PWS	0.134	0.277*	0.198*	0.156*	-0.299*	0.063		
Albumin - SS	0.236*	0.274*	0.264*	0.116	-0.054	0.082		
IgM - PWS	0.264*	0.210*	0.262*	0.140	-0.167*	0.367*	0.362*	
IgM - SS	0.227*	0.174*	0.206*	0.081	-0.116	0.335*	0.247*	
Liver weight - PWS	0.656*	0.766*	0.769*	0.675	-0.008	0.151	0.138	0.106
Liver weight - SS	0.732*	0.832*	0.782*	0.640*	-0.162*	0.084	0.189*	0.142*

Variable - site	Age	Body weight	Length	Gonad weight	Hold time	sumICH	Albumin	$\log_e$IgM
PCV - PWS	-0.067	-0.051	-0.065	-0.086	-0.262*	-0.068	0.386*	0.195*
PCV - SS	0.108	0.239*	0.155*	0.258*	-0.061	-0.166*	0.250*	-0.051
Total protein - PWS	0.053	0.201*	0.115	0.092	-0.356*	0.132	0.924*	0.361*
Total protein - SS	0.281*	0.424*	0.356*	0.357*	-0.050	0.132*	0.692*	0.221*
$\log_e$ AST - PWS	-0.062	-0.202*	-0.166*	-0.226*	-0.099	0.197*	-0.174*	0.067
$\log_e$ AST - SS	-0.208*	-0.209*	-0.206*	-0.151*	0.141	0.282*	0.021	0.081
ALP - PWS	0.082	0.202*	0.183*	0.218*	-0.239*	0.079	0.331*	0.196*
ALP - SS	0.106	0.115	0.087	-0.008	-0.038	0.117	0.412*	0.136*
$\log_e$ ALT - PWS	-0.022	-0.075	-0.044	-0.040	-0.007	0.089	-0.067	0.146
$\log_e$ ALT - SS	-0.120	-0.135*	-0.158*	-0.078	0.169*	-0.082	0.004	-0.083
$\log_e$ CPK - PWS	-0.126	-0.2404	-0.205*	-0.256*	-0.213*	0.110	-0.101	0.003
$\log_e$ CPK - SS	-0.170*	-0.221*	-0.201*	-0.176*	-0.002	0.183*	0.009	-0.039
GGT - PWS	0.198*	0.146*	0.176*	0.072	-0.101	0.031	0.076	0.215*
GGT - SS	0.009	0.015	0.011	-0.030	-0.138*	0.159*	0.154*	0.217*
Calcium -PWS	-0.033	0.056	-0.043	-0.013	-0.036	0.064	0.521*	0.241*
Calcium - SS	0.051	0.091	0.099	0.104	0.209*	0.027	0.298*	-0.024
Chloride -PWS	-0.034	0.034	0.024	0.053	0.046	-0.086	0.110	-0.046
Chloride -SS	0.064	0.178*	0.163*	0.232	-0.135*	-0.018	0.042	0.043
Cholesterol -PWS	0.050	0.175*	0.079	0.074	-0.230*	-0.080	0.814*	0.182*
Cholesterol -SS	0.118	0.204*	0.162*	0.089	-0.047	-0.079	0.565*	0.179*
CO_2 - PWS	0.005	0.003	0.039	0.015	0.566*	-0.130	-0.229*	-0.041
CO_2 - SS	0.004	-0.008	0.019	-0.059	0.227*	0.024	0.168*	0.035

Variable - site	Age	Body weight	Length	Gonad weight	Hold time	sumICH	Albumin	$\log_e$IgM
Glucose -PWS	0.014	0.188*	0.095	0.079	-0.107	0.041	0.625*	0.105
Glucose - SS	0.306*	0.466*	0.371*	0.441*	-0.278*	-0.123	0.292*	0.142*
Lactate - PWS	0.000	0.178*	0.055	0.171*	0.144	0.037	0.345*	0.070
Lactate - SS	0.066	0.138*	0.117	0.133*	0.270*	-0.055	0.286*	-0.022
Osmolality - PWS	0.061	0.169*	0.143	0.137	-0.070	-0.015	0.381*	0.163*
Osmolality - SS	0.127	0.050	0.143*	-0.098	0.192*	-0.003	-0.009	-0.026
Phosphorus -PWS	-0.021	-0.070	-0.039	-0.039	-0.471*	0.084	0.032	0.144
Phosphorus -SS	0.060	-0.021	0.033	-0.139*	0.180*	-0.036	0.144*	-0.020
Potassium -PWS	-0.101	0.008	-0.032	0.048	0.661*	-0.070	-0.212*	-0.199*
Potassium -SS	-0.148*	-0.179*	-0.159*	-0.161*	0.608*	-0.035	-0.107	-0.121
Sodium - PWS	-0.003	-0.049	-0.020	0.044	0.151*	0.057	-0.063	-0.023
Sodium - SS	0.033	-0.037	0.094	-0.166*	0.275*	-0.013	-0.007	-0.078
Total bilirubin - PWS	-0.006	-0.004	0.017	0.059	0.117	-0.146*	0.088	-0.150*
Total bilirubin - SS	-0.136*	-0.143*	-0.048	-0.125	0.260*	-0.064	-0.077	-0.020
Thrombocytes - PWS	0.152	0.185	0.192*	0.206*	-0.104	-0.114	-0.134	-0.016
Thrombocytes - SS	-0.102	-0.000	-0.055	0.101	-0.065	-0.105	0.043	0.035
Lymphocytes - PWS	-0.337*	-0.240*	-0.304*	-0.272*	0.150*	-0.046	0.171*	-0.199*
Lymphocytes - SS	0.039	0.083	0.068	0.119	-0.075	0.060	-0.075	-0.115
Neutrophils - PWS	0.328*	0.142	0.226*	0.140	-0.044	0.244*	0.012	0.330*
Neutrophils - SS	0.111	-0.113	-0.016	-0.325*	0.144*	0.044	0.020	0.073
Basophils - PWS	-0.015	-0.099	-0.053	-0.161*	-0.039	0.165*	-0.108	0.034
Basophils - SS	0.014	0.028	0.036	0.047	0.152*	0.122	0.100	0.160*

Variable - site	Age	Body weight	Length	Gonad weight	Hold time	sumICH	Albumin	$\log_e$IgM
Eosinophils - PWS	-0.058	-0.079	-0.033	-0.028	0.051	-0.054	-0.047	0.071
Eosinophils - SS	-0.046	-0.112	-0.117	-0.087	0.159*	0.080	-0.098	0.012
Monocytes - PWS	0.020	0.029	0.045	0.001	-0.033	-0.050	0.098	-0.027
Monocytes - SS	-0.071	-0.042	-0.045	-0.058	0.040	-0.027	0.029	-0.114
# Anisakidae - PWS	-0.102	-0.145	-0.075	-0.166*	0.073	-0.002	-0.205*	-0.007
# Anisakidae - SS	-0.247*	-0.178*	-0.200*	-0.126	0.047	-0.103	-0.035	0.006

Table 10. Sample prevalence (%) of parasites and virus in adult Pacific herring in Prince William Sound, Alaska, 1989-1995.

Sample Date	n	*Goussia clupearum*	*Ichthyophonus hoferi*[a]	*Ortholinea orientalis*[b]	Viral hemorrhagic septicemia virus
1989 April[c]	40	63	13	TNE[d]	TNE
1990 October[c]	99	60	15	6.1	TNE
1991 April[c]	59	54	5.1	17	TNE
1991 October[c]	48	54	2.1	15	TNE
1992 April[e]	105	53	5.7	3.1	TNE
1993 April[f]	79	41	5.1	4.3	2 of 3 5-fish pools
1994 April	212	61	24 (29)	5.7 (19)	4.7
1995 April (spawning)	180	73	23 (29)	7.2 (29)	0.0

[a]Prevalence in liver, kidney, and spleen for all samples except April 1989, where only liver and spleen were examined. Note that more organs were examined in 1994 and 1995, and those results are in parentheses.

[b]Prevalence values for *Ortholinea orientalis* are for histopathology. Note that touch preparations of kidney were examined in 1994 and 1995, and those results are included in the overall prevalence values in parentheses.

[c]unpubl. data from G.D. Marty, M. S. Okihiro, and D. E. Hinton

[d]TNE = Tissue not examined

[e](Kocan et al. In Press)

[f](Meyers et al. 1994) and unpubl. data from T.R. Meyers

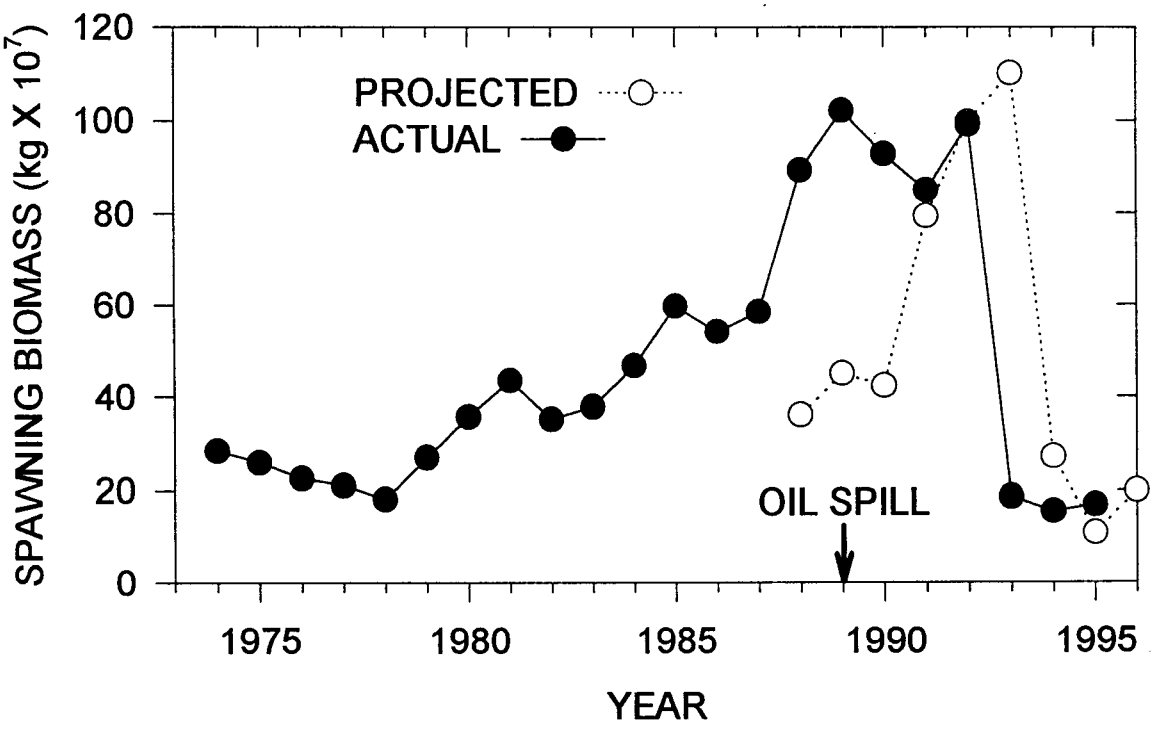

Figure 1. Biomass estimates of mature Pacific herring in Prince William Sound, Alaska. Unexploited spawning biomass projected in the year before spawning (PROJECTED) and calculated after spawning (ACTUAL) using the age-structure assessment model. Estimates were made by Fritz Funk, Alaska Department of Fish and Games, Juneau, Alaska; unpubl. data.

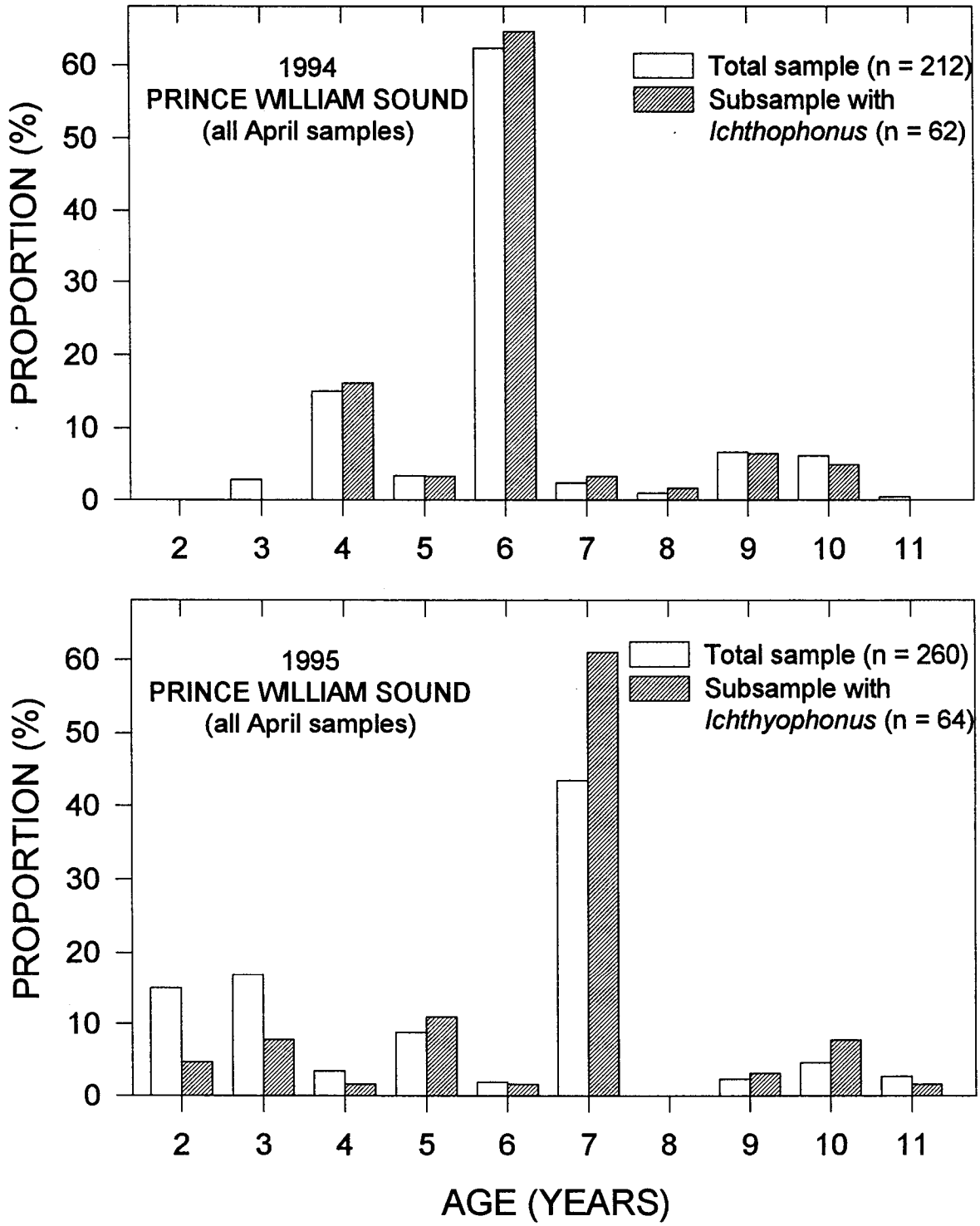

Figure 2. Age distribution of spawning Pacific herring in Prince William Sound, Alaska, that had *Ichthyophonus* compared with the age distribution of fish that were examined for *Ichthyophonus*. Top - Pacific herring sampled during April, 1994. Bottom - Pacific herring sampled during April, 1995.

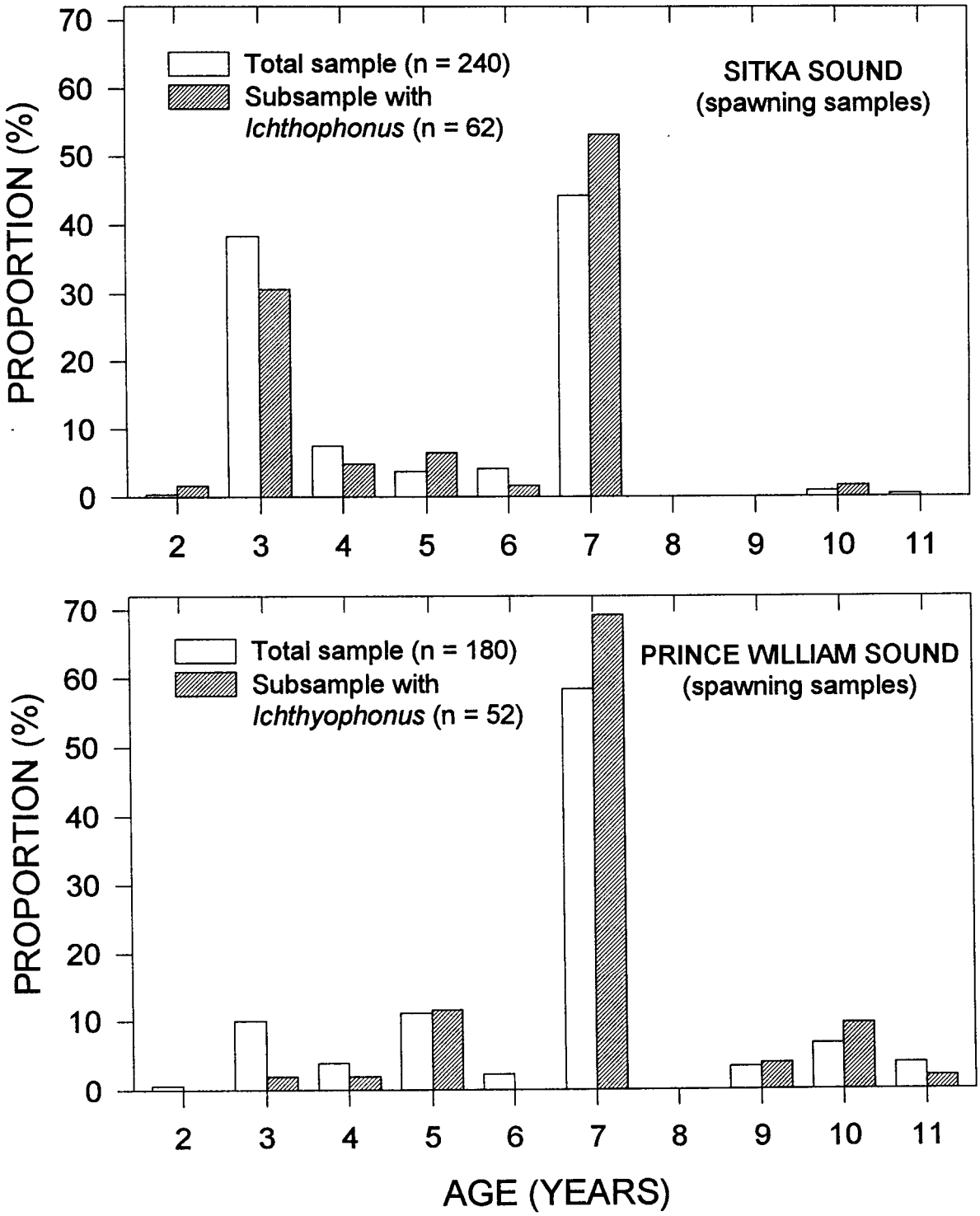

Figure 3. Age distribution of spawning Pacific herring that had *Ichthyophonus* compared with the age distribution of fish that were examined for *Ichthyophonus*. Top - Pacific herring sampled from Sitka Sound, Alaska, during March, 1995. Bottom - Pacific herring sampled from Prince William Sound, Alaska, during April, 1995.

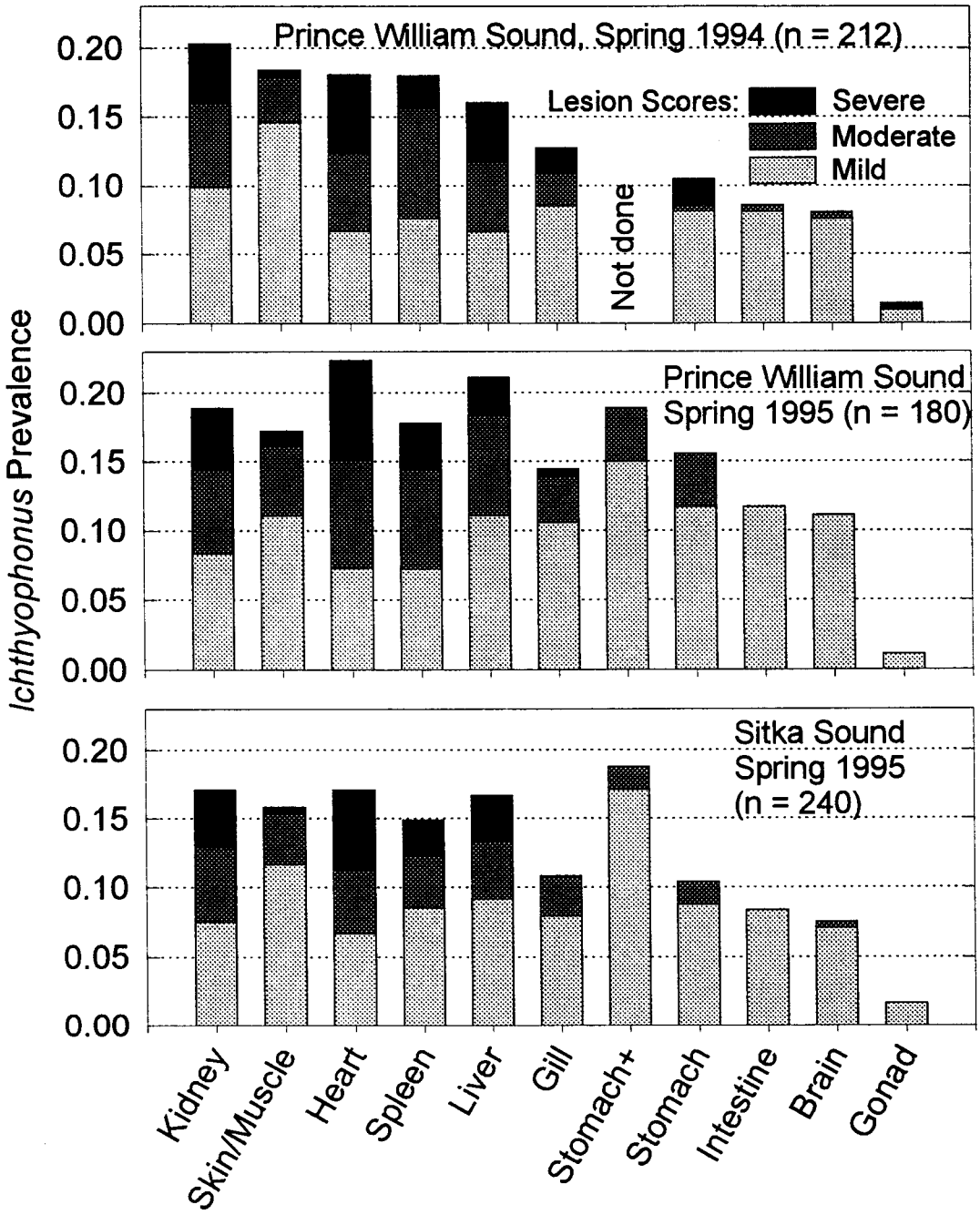

Figure 4. Sample prevalence of *Ichthyophonus* lesion scores in various organs of mature Pacific herring sampled from Prince William Sound in 1994 and 1995, and Sitka Sound in 1995. Lesions were scored as none (0), mild (1), moderate (2), or severe (3).

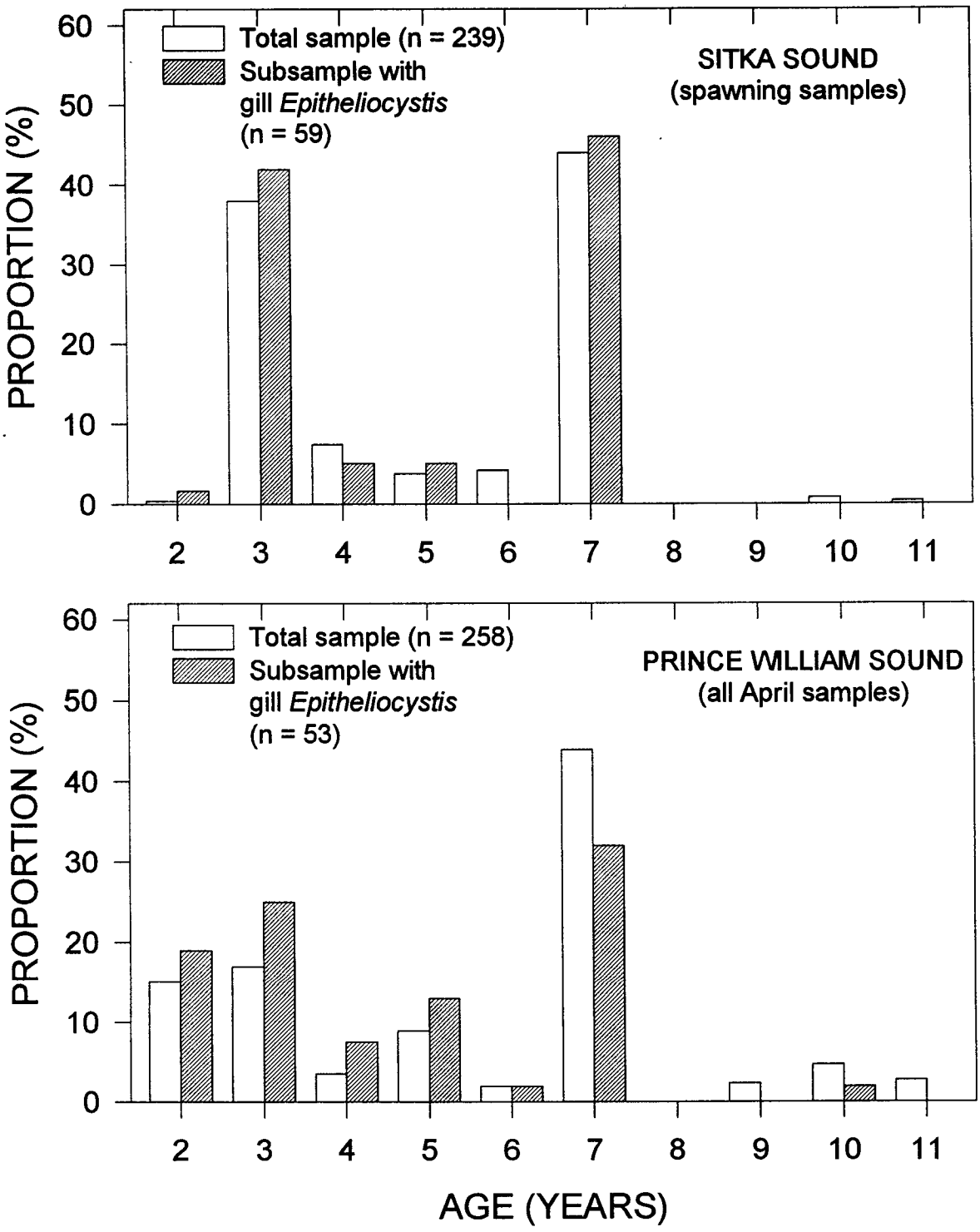

Figure 5. Age distribution of spawning Pacific herring in 1995 that had branchial *Epitheliocystis* compared with the age distribution of fish that were examined for branchial *Epitheliocystis*. Top - Samples from Sitka Sound, Alaska. Bottom - Samples from Prince William Sound, Alaska.

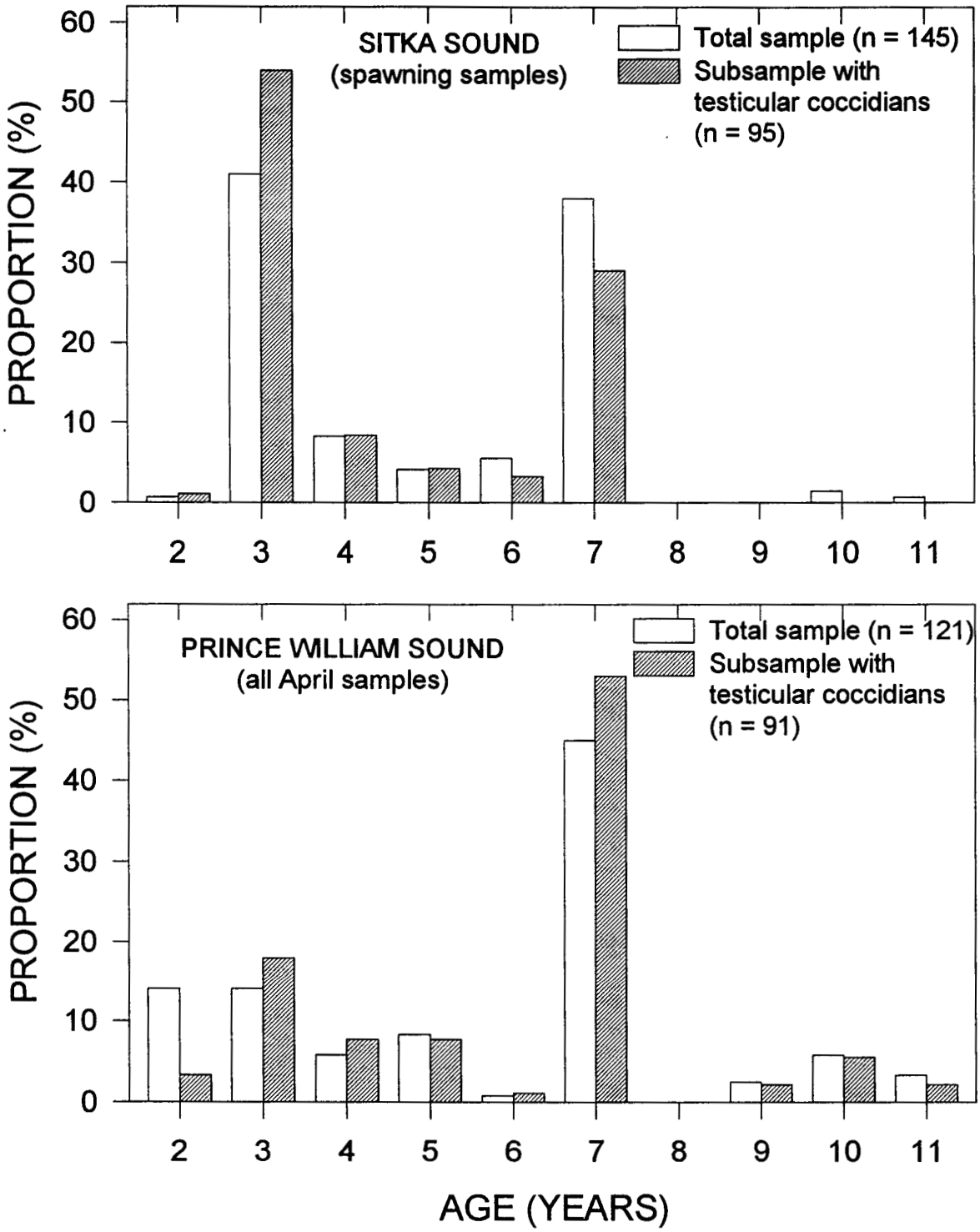

Figure 6. Age distribution of male Pacific herring in 1995 that had testicular coccidians (*Eimeria sardinae*) compared with the age distribution of fish that were examined for testicular coccidians. Top - Samples from Sitka Sound, Alaska. Bottom - Samples from Prince William Sound, Alaska.

Exxon Valdez Oil Spill
Restoration Project Annual Report

Investigations of Disease Factors Affecting Declines of Pacific Herring Populations in Prince William Sound

Section II. Laboratory Challenge of Pacific Herring With and Without Stressors

Restoration Project 95320S
Annual Report

This annual report has been prepared for peer review as part of the *Exxon Valdez* Oil Spill Trustee Council restoration program for the purpose of assessing project progress. Peer review comments have not been addressed in this annual report.

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April 1996

Investigations of Disease Factors Affecting Declines
of Pacific Herring Populations in Prince William Sound

Section II. Laboratory Challenge of Pacific Herring With and Without Stressors

Restoration Project 95320S
Annual Report

Study History: Restoration Project 95320S was initiated in response to an RFP issued by Alaska Department of Fish and Game and was intended to determine the relationship of diseases on the declines in Prince William Sound herring populations since 1993. A three component work plan was prepared: 1) Field component (University of California, Davis); 2) Controlled infection component (University of Washington, Seattle) and 3) Physiology component (Simon Fraser University, B.C.). The report covers the period of March through September, 1995; however, the study is ongoing.

Abstract: Viral hemorrhagic septicemia virus (VHSV) was conclusively shown to be capable of causing disease and extensive mortality in nonimmune juvenile Pacific herring. SPF herring began dying 7 days post-exposure with peak mortality occurring on days 10-11. No mortality was observed in SPF herring exposed to VHSV concentrations of $< 2 \times 10^2$ PFU$*$ml^{-1} and no virus could be isolated from their tissues. Virus was first detected in tissues of experimentally infected SPF herring 48 h post-exposure and peaked at 96 h. Fish began shedding new virus 48 h post-exposure with maximum shedding occurring on days 4-5 post exposure, just prior to peak mortality. Histopathologic examination of moribund fish 2 to 8 days post-exposure revealed: 1) multifocal coagulative necrosis of liver hepatocytes, 2) diffuse necrosis of the kidney interstitial hematopoietic tissues, and 3) diffuse necrosis of the spleen, epidermis and subcutis. No virus was isolated from any wild herring at the time of capture. However, 2-3 weeks post-capture approximately 60% of the 0-year herring died with massive hemorrhages of the skin, fins and mouth. Plaque assays revealed > 90% of the dead fish had $> 1 \times 10^6$ PFU$*$gm^{-1} tissue. Ninety percent of the live fish sampled from the same tanks carried slightly lower titers of virus from 2 to 14 days post-capture, then virus titers declined until they were undetectable by 4 weeks post-capture. Mortality was significantly less (< 10%) in older fish. Surviving herring exposed to $1X10^3$ to $1X10^6$ PFU$*$ml^{-1} for 1 hour 6-8 weeks post-capture exhibited no mortalities in any age class and no virus could be isolated from tissues of these fish 10 days post-exposure. Laboratory-reared SPF herring injected IP with ca 1,000 *Ichthyophonus* spores began dying by 11 days post exposure and had visible lesions on the heart, liver and spleen. Skin lesions (spores and small holes) were detectable by 36 days, as were spores in the musculature under the skin. By 56 days post exposure 90% of the fish were dead. Infected tissues from these herring were cultured then injected IP into coastrange sculpins (*Cottus aleuticus*), all of which became infected and/or died by 14 days post exposure. Infected sculpin tissues were fed to other sculpins which also became infected and cultured positive for *Ichthyophonus*. No control sculpins were found to be naturally infected. Three year classes of wild herring (0-year, 1+ and 3+) were captured from Puget Sound between June 1995 and February 1997 and examined for the presence of *Ichthyophonus* by gross examination and in vitro culture of heart, liver and spleen. External skin lesions were observed in 6%, 5% and 8% of the three groups respectively while 6% 23% and 52% of each group cultured positive for *Ichthyophonus*. There was no significant difference in mortality between the infected and uninfected individuals within age classes.

Key Words: *Clupea pallasi*, *Exxon Valdez* oil spill, *Ichthyophonus*, morbidity, mortality, Prince William Sound, Viral Hemorrhagic Septicemia Virus (VHSV).

Citation:

Kocan, R.M., M.L. Landolt, and J.R. Winton. 1996. Investigations of disease factors affecting declines of Pacific herring populations in Prince William Sound. Section II. Laboratory challenge of Pacific herring with and without stressors, Restoration Project Annual Report (Restoration Project 95320S), University of Washington, Seattle, Washington.

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Executive Summary

Introduction

In 1993, four years after the Exxon Valdez oil spill in Prince William Sound, there was a dramatic and unexpected loss of Pacific herring. The spawning biomass declined from an expected 120K tons to less than 30K tons. This decline continued into 1994 when less than 20K tons of spawning adults returned to Prince William Sound. A study designed to evaluate the health of the returning adults in 1993 and 1994 revealed that there was an unexpected appearance of the agent which causes viral hemorrhagic septicemia (VHS) in 1993 and a significant increase in prevalence of Ichthyophonus hoferi, as suspected pathogen of herring in 1994. Because of these findings a disease study was initiated with the objective of clarifying the role of these pathogens in the decline of Pacific herring in Prince William Sound.

Objectives

Laboratory rearing of Pacific herring

- Obtain and hatch herring eggs
- Rear herring larvae for experimental use

Verification of disease-free laboratory herring

- Histopathology
- In vitro culture of *Ichthyophonus*
- In vitro culture of VHSV

Challenge without stressors

- Challenge lab-reared herring with VHSV
- Challenge lab-reared herring with *Ichthyophonus*
- Assay experimental fish for VHSV & *Ichthyophonus*

Continuing '95 projects ('95 -> '96)

Methods

Specific Pathogen-free (SPF) herring: Pacific herring were artificially spawned and their eggs incubated in filtered UV-sterilized seawater. The newly hatched larvae were initially fed oyster trochophores and rotifers, followed by brine shrimp nauplei treated with Selco@ and finally frozen adult brine shrimp, commercial trout chow and frozen krill. The growth rate and disease status of the larvae and juveniles was monitored daily, as was the water quality in which they were rearing. When the fish reached 90 days old, they were used for controlled disease studies.

Challenge without stressors: Pathogen-free 5-month-old herring were challenged with both VHS virus (VHSV) and *I. hoferi* to determine the pathogenicity of these organisms in the absence of physical or chemical stressors. Increasing concentrations of VHSV ($7X10^2$; $7X10^4$; $7X10^6$ PFU) was added to aquarium water for 1 hour, after which the water was turned on and the fish allowed to develop the disease without further experimental exposure.

I. hoferi was injected intraperitoneally into herring, rainbow trout and Japanese medaka to determine its host range and growth characteristics.

Naturally infected herring: Wild 0-year Puget Sound herring were netted from herring balls from August through October and brought into the laboratory for study. Fish were initially housed at about 200 fish per 70 gallon tank. Subsequent catches were distributed into tanks in densities ranging from 5 fish per tank to 85 fish per tank in order to study the effects of density as well as capture stress. These fish were used for both VHS and *Ichthyophonus* studies.

Fish-to-fish transmission: Wild 0-year herring which survived an epizootic of VHS but still showing signs of disease (eg. hemorrhaging) were introduced into tanks of SPF herring. The SPF fish were observed for signs of disease for three weeks, dead fish were collected for viral assay, and at the end of the study all fish were assayed for the presence of VHSV.

Density as a stressor: Wild 0-year herring were distributed into 70 gallon tanks at densities ranging from 5 fish/tank to 85 fish/tank to determine if density has an effect on the natural epizootics observed in these fish after they were brought into the laboratory. Fish were monitored for mortality and dead fish assayed for VHSV. At the termination of the study all survivors were also assayed for virus.

VHSV in rainbow trout with & without pristane
The rainbow trout (*Oncorhynchus mykiss*) was examined as a possible surrogate laboratory species for studying the effect of VHS. In conjunction with studies on infectivity and pathogenicity, some fish were exposed to pristane, a branched alkane known to have immunosuppressant effects in mammals. The rationale being that crude oil contains numerous molecules other than polycyclic aromatic hydrocarbons that may affect the health of aquatic organisms following an oil spill such as occurred in 1989 in Prince William Sound. Pristane is a naturally occurring compound in some marine organisms as well as a component of crude oil. Considering these relationships, it was felt that a preliminary look at its effects on the immune system of fish was in order.

Four groups of 30 fish each were used to examine the infectivity and pathogenicity of VHS virus and the influence of pristane on the course of infection. The fish were 6-months-old with mean lengths and weights at the start of the study of 13.7 mm and 99.1 gm. The exposed groups were exposed to 1X10^6 PFU$*$ml^{-1} for one hour, while the controls consisted of unexposed controls, culture medium alone (MEM) and pristane plus MEM.

Results

Specific Pathogen-free (SPF) herring: The mean growth rate for SPF herring for the first 90 days was 0.33 mm* d-1. By 6 months post-hatch they were 4.5-6.0 cm in length. Periodic histologic examination of tissues as well as in vitro culture of specific organs revealed neither VHSV or I. hoferi. No other pathogens could be identified and the fish were considered to be "pathogen-free" and suitable for use in controlled disease studies.

Challenge without stressors: SPF herring proved to be highly susceptible to VHSV when infected via water-borne exposure. By two weeks post exposure 100% (29/29) mortality occurred in fish exposed to $7X10^6$ PFU, 95% mortality in those exposed to $7X10^6$ PFU (18/19) and 62% (16/26) exposed to $7X10^2$ PFU. Fish dying during this period had > 6 logs of virus / gm tissue regardless of the level of initial exposure. Eight survivors of the

low dose were below detection limits while one was just above the detection limit (eg. 2.9 PFU). The single survivor of the medium dose had just 4.8 PFU per gram tissue.

This study fulfilled Koch's Postulates for VHSV as a pathogen of Pacific herring, indicating that it can cause mass mortality in susceptible populations of this species.

Naturally infected herring: Upon initial capture wild 0-year herring showed no signs of infection by VHSV (0/30; 0/30). By the second week in captivity in densities of 200 fish / 70 gallon tank, massive mortalities had occurred, reaching over 50% by week three. The disease prevalence in both live and dead individuals was 100% at this time, but by 4 weeks in captivity the number of individuals with detectable infections had returned to "0". When these fish were challenged with VHSV at $3X10^6$ PFU for one hour they were solidly immune, showing no sighs of mortality or disease.

When wild herring were injected with *Ichthyophonus* they developed active infections, but because both exposed and unexposed fish died at the same rate, it was not possible to show a cause-and-effect relationship between herring mortality and infection by *Ichthyophonus*. Studies are planned to infect SPF herring with this organism to eliminate the complication of multiple natural infections which might cloud the issue of *Ichthyophonus*' capability to cause mortality in this species. Neither the rainbow trout nor Japanese medaka were susceptible to infection by injection of *Ichthyophonus*. Other species (English sole, fresh water sculpin, tide pool sculpin) are being examined to determine the host range of this organism.

Fish-to-fish transmission: Individual wild herring with signs of VHS were capable of transmitting the disease via water to pathogen-free herring. Mortality was 27% in the SPF fish by week 3 with 27% of the survivors testing positive for the virus by the third week.

Density as a stressor: There appeared to be a density related mortality in wild herring captured in September, but not in those captured in October, 1995. The VHSV infection rate was the same regardless of tank density (11% - 17%). The intensity of infection (PFU/gm tissue) was somewhat higher in the October fish, but both groups averaged over 5 PFU/gm tissue. The titer per gram tissue did however, show a distinct decrease from 8 PFU/gm (log10) during the first week in captivity to 0 PFU by the fourth week.

VHSV in rainbow trout with & without pristane
After 3 weeks, mortality was observed only in the VHSV exposed groups with no significant difference between pristane and non-pristane treated groups. Pristane alone had no effect on survival. Fish dying during the first two weeks following exposure were uniformly positive for VHSV, while those dying during the third week were negative even though they showed signs of disease and severe anemia. This is similar to what was observed in wild Pacific herring which experienced an epizootic of VHS following capture and confinement.

Discussion & Conclusions

Pacific herring can be artificially spawned and reared in the laboratory under disease-free conditions and for long enough periods to conduct meaningful studies on herring pathogens. Although they are not as easy to rear under culture conditions as some other species, the techniques for growing this species are well established and they can be cultured successfully if adequate manpower and facilities are dedicated to the effort. Trying to unravel the pathogenesis of specific organisms in wild species in virtually impossible because of the complication of superimposed and uncontrolled infections by other

organisms. SPF herring have allowed us to study one organism at a time (one variable) as well as multiple organisms all under our control. This will allow us to confirm the pathogenicity of both VHSV and *Ichthyophonus hoferi* and establish their role in the massive losses of herring which occurred in Prince William Sound in 1993-1994.

VHS is capable of causing mass mortality in both wild and laboratory reared pathogen-free Pacific herring. The organism appears to be transmitted via water from infected to susceptible fish with an incubation period ranging from 4 to 10 days. Once infected, the virus is amplified in herring tissues and can reach levels of $> 10^6$ PFU / gram tissue. Survival of an epizootic appears to confer a solid immunity to individual fish, even when challenged with extremely high levels of virus.

Wild herring appear to be carrying VHSV by the time they are 5-months-old and are very susceptible to epizootics of VHS when captured or crowded in captivity. They also appear to become solidly immune once they recover from an infection.

Objectives

Laboratory rearing of Pacific herring

Obtain and hatch herring eggs

Rear herring larvae for experimental use

Verification of disease-free laboratory herring

Histopathology

In vitro culture of *Ichthyophonus*

In vitro culture of VHSV

Challenge without stressors

Challenge lab-reared herring with VHSV

Challenge lab-reared herring with *Ichthyophonus*

Assay experimental fish for VHSV & *Ichthyophonus*

Methods

Laboratory rearing of Pacific herring

Obtain and hatch herring eggs

Herring eggs were obtained from Prince William Sound (PWS) in April and from Puget Sound (PS) in May, 1995. Actively spawning herring were captured by gill net, the eggs removed from 8 to 10 females, pooled and broadcast onto a 300 cm^2 piece of nytex netting at a density of 3-5 eggs * cm^{-2}. Once the adherent eggs were securely stuck to the netting, milt from 3-5 males was pooled in sterile seawater and poured over the eggs. Fertilization and transport was in pathogen-free seawater made from aged tap water and sea salts. In the laboratory, each nytex net containing approximately 1,200 eggs was placed into a 70 gal flow-through tank supplied with filtered natural seawater from Admiralty Inlet (Puget Sound) and sterilized with ultraviolet (u.v.) light. Eggs remained in the tanks throughout incubation, hatching and subsequent rearing.

Rear herring larvae for experimental use

Pacific herring larvae were continuously maintained on filtered-u.v. sterilized natural seawater. Larvae were initially fed trochophores (oyster), rotifers (*Brachionus* sp) and *Artemia* larvae. The rotifers and brine shrimp were treated with Super Selco@ for 8 hours prior to feeding in order to maintain adequate levels of omega-3 fatty acids in the larval diet. Trochophores were discontinued after two weeks and rotifers after 12 weeks. At three

months the fry were introduced to frozen brine shrimp and commercial trout chow (1-2 mm) and have been continuously maintained on these food items. During the first 90 days post-hatch, larval growth data was collected every 5-7 days, then monthly thereafter. The entire collection was catalogued and deposited in the University of Washington larval fish collection for future study and teaching purposes.

Verification of disease-free laboratory herring

Histopathology

When the fish were 6-months-old, a subset of 25 randomly selected individuals was taken from 8 different tanks and were fixed in 10% neutral buffered formalin for histologic examination. The organs were examined primarily for the presence of *I. hoferi* and pathologic damage associated with VHSV infection. However, other common herring pathogens were also looked for.

In vitro culture of *Ichthyophonus*

Heart, liver, kidney and spleen were removed from 50 SPF herring at 6-months-old, examined microscopically (wet prep) and cultured in MEM-10. Cultures were microscopically examined weekly for 3 months for the presence of *I. hoferi*. Control cultures were prepared from fish experimentally infected by I.P. injection with spores previously cultured from PWS herring tissues.

In vitro culture of VHSV

A random sample of 6-month-old SPF herring were assayed for the presence of VHSV by culturing the homogenized viscera in the presence of EPC cells and examining the cultures for the presence of plaques. Positive controls consisted of cultures infected with a known amount of virus. Infections were quantitated by comparing the number of plaques produced from each fish.

Disease challenge without stressors

Challenge lab-reared herring with VHS

SPF herring fry were divided into triplicate groups of 10 fish each and exposed for 3 h to increasing concentrations of VHS virus ($6.7X10^2$; $6.7X\ 10^4$ & $6.7X\ 10^6$ $pfu*ml^{-1}$ plus negative controls). After 1 h the water was turned on and the fish were maintained in flowing filtered seawater for 2 weeks, during which time they were observed for visible hemorrhage, mortality, and behavioral changes. As fish died their condition was recorded and they were frozen at -70°C until assayed for virus. The study was terminated after 21 days.

Challenge lab-reared herring with *Ichthyophonus*

Wild herring, rainbow trout and Japanese medaka (*Orizias latipes*) were exposed to up to 10,000 *Ichthyophonus* cells by either intraperitoneal injection (I.P.) or orally (per os).

SPF herring were also exposed to *I. hoferi* by injecting 1,000 spores intraperitoneally into 10 80-90 mm 7-month-old fish. Controls consisted of 10 fish injected with only culture medium (MEM) and 10 fish injected with 1,000 spores of the North Atlantic strain of *I. hoferi* which had been in culture for ~ 2 years. Each group was maintained in a 70 g flow-

through tank and observed for mortality and abnormal behavior. Dead fish were removed daily and preserved in 10% Formalin for later evaluation. After 6 weeks the study was terminated and the remaining fish either fixed in 10% Formalin for histopathology or cultured for the presence of *I. hoferi* .

VHSV in rainbow trout with & without pristane

VHSV in rainbow trout with & without pristane

The rainbow trout (*Oncorhynchus mykiss*) was examined as a possible surrogate laboratory species for studying the effect of VHS. In conjunction with studies on infectivity and pathogenicity, some fish were exposed to pristane, a branched alkane known to have immunosuppressant effects in mammals. The rationale being that crude oil contains numerous molecules other than polycyclic aromatic hydrocarbons that may affect the health of aquatic organisms following an oil spill such as occurred in 1989 in Prince William Sound. Pristane is a naturally occurring compound in some marine organisms as well as a component of crude oil. Considering these relationships, it was felt that a preliminary look at its effects on the immune system of fish was in order.

Four groups of 30 fish each were used to examine the infectivity and pathogenicity of VHS virus and the influence of pristane on the course of these infections. The fish were 6-months-old with mean lengths and weights at the start of the study of 13.7 mm and 99.1 gm. The exposed groups were exposed to 1X10^6 PFU$*$ml^{-1} for one hour, while the unexposed groups consisted of unexposed controls, culture medium alone (MEM) and pristane plus MEM.

Survey of wild PS herring for VHSV infection

Survey of wild PS herring for VHSV infection

Wild 5-to-6-month-old herring were netted from "herring balls" in Discovery Bay (WA), returned to the laboratory in tanks gassed with pure O_2 and distributed into 70 gal flowing, unfiltered seawater tanks at densities of 100-200 fish per tank. A subset of 60 fish was immediately removed and frozen at -70°C for later virus assay. The remainder of the fish were maintained for 30 days, during which they were observed for mortality, external lesions and behavioral aberrations. Dead fish were collected several times per day and frozen at -70°C until assayed for virus.

Hematologic and biochemical evaluation of wild VHS survivors

A sub sample of fish surviving beyond 30 days was samples for hematologic and biochemical changes associated with VHSV infection (See Section III: Kennedy-Simon Fraser University).

Determine VHSV transmission rate for wild herring

Three weeks after capture, wild herring surviving an epizootic of VHS were placed into tanks containing SPF herring to determine if they were capable of transmitting the virus via water. Each of 3 tanks contained 10 SPF herring and either 1 or 3 wild VHS survivors and controls consisted of three tanks containing only SPF herring.

Results

Laboratory rearing of Pacific herring

Obtain and hatch herring eggs

Eggs obtained from PWS hatched 14-16 d post fertilization and began actively feeding on rotifers (*Brachionus* sp) and *Artemia* nauplei one week later. They survived for 30 days but died when a valve was inadvertently opened and stagnant seawater containing hydrogen sulfide (H_2S) entered the tanks. The problem was corrected and PS eggs hatched normally one month later and are still surviving at this writing (February 1996).

Rear herring larvae for experimental use

The mean growth rate for the first three months post hatching was 0.33 mm * day^{-1} (Figure 1). By 6 months post-hatch the fish reached 4.5-6.0 cm in length and by 8 months were 85 - 100 cm. Water temperature increased 2°C, from 11.2 to 13.2°C between early June and August (Figure 2).

Verification of disease-free laboratory herring

Histopathology

No evidence of VHSV or *Ichthyophonus* infection was detected by histologic examination of tissues from 6-month-old larvae. An unexplained cartilage growth was observed on the lip of about 10% of the fry after 3 months, but did not affect growth or feeding behavior. The growth was surgically removed from a subset of 10 anesthetized fish with no apparent long-term affect on the fish's survival or growth. These fish have survived for >30 d and appear indistinguishable form unaffected cohorts.

In vitro culture of *Ichthyophonus*

No *Ichthyophonus* was detected in tissues of > 60 6-month-old SPF fish cultured in MEM-10 tissue culture medium for up to 90 days. Infected herring could be detected by culture in every case but only 70-80% of the experimentally infected fish could be detected by examining wet-preps using a limited amount of tissue.

In vitro culture of VHSV

No VHS virus was detected in assays carried out on >90 SPF fry at 6 months of age. Idiopathic mortality in SPF fish was relatively low (< 1%) during the first 6 months post hatching, and those that did die showed no signs of VHSV or other recognized herring pathogens.

Disease challenge without stressors

Challenge lab-reared herring with VHSV

Mortalities began 4 days post exposure and continued until the study was terminated at 14 days. During the first week mortalities were clearly dose related (Figure 3a) while during the second week the remaining fish died (Figure 3b), presumably as a result of being

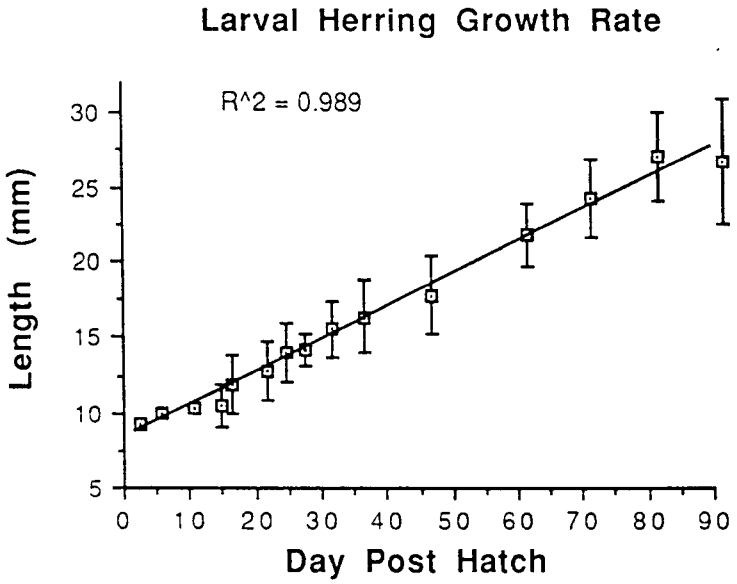

Figure 1. Three month growth data for Pacific herring larvae reared in captivity in filtered - U.V. sterilized natural seawater

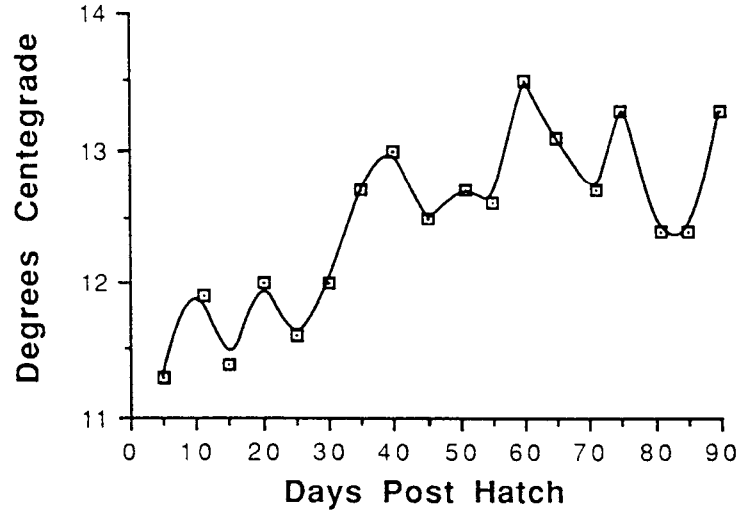

Figure 2. Temperature range of herring larval seawater from May 27 through August 20, 1995

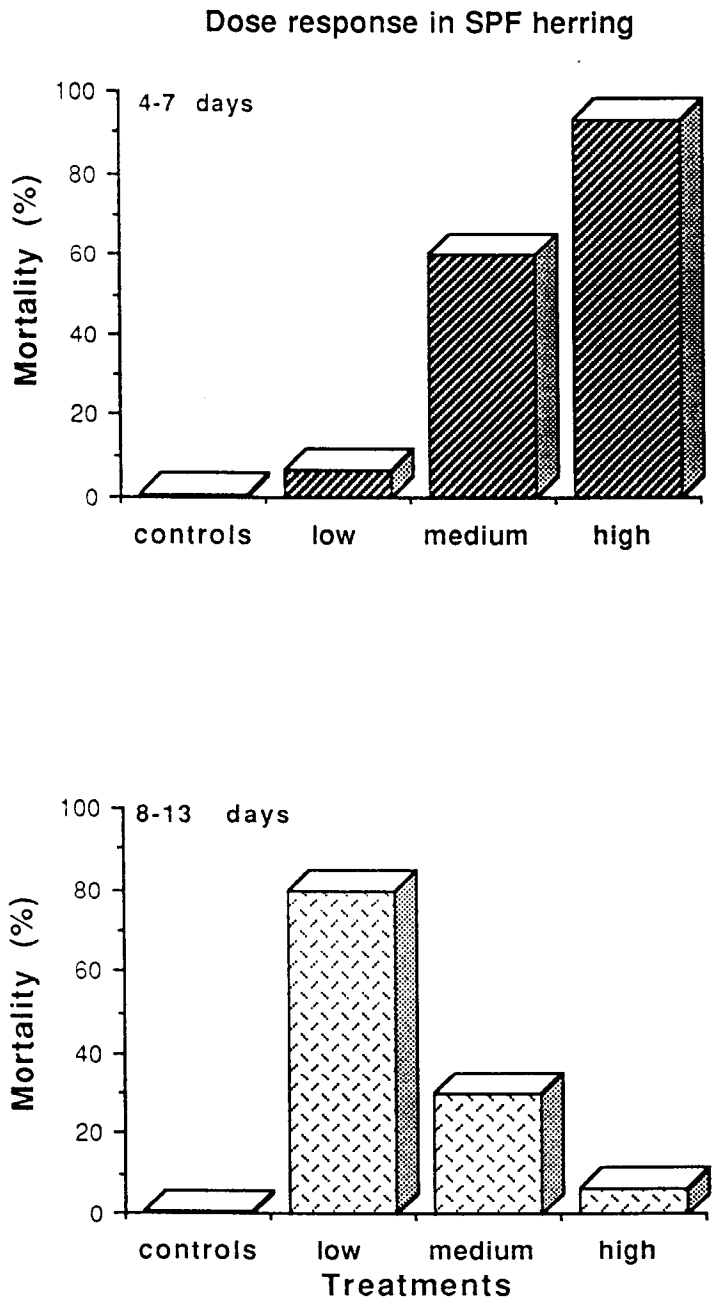

Figure 3. Mortality dose response for VHS virus in specific pathogen-free (SPF) laboratory reared herring one week following exposure (top panel), and secondary mortality (bottom panel) resulting from exposure to virus shed by herring dying following initial exposure.

exposed to virus shed by fish dying during the first week. Virus assays of dead fish showed that all fish from the high and medium level exposures were infected at the time of death, while only 1 of 2 dead fish from the low exposure groups was infected (Table I). Among the surviving fish at the end of the study, only one from the medium exposure group was infected, and at a level 100 times lower than that found in the dead fish (Table II).

Challenge of herring with *Ichthyophonus*

Herring was the only species that could be infected, and then only by I.P. injection of cultured cells. (Table III). The heart, spleen, liver and kidney were the most frequently infected organs and *I.hoferi* cells could be seen grossly or under 40X magnification on the surface of the organs (Figure 4). It appeared that following death of the host, the *I. hoferi* cells began to grow "hyphae" which contained microscopic "spores" at their tips. Individuals examined immediately after death contained primarily large resting spores.

SPF herring injected with the PWS strain of I. hoferi exhibited an 80% mortality rate by 6 weeks post exposure. The viscera contained grossly visible spores of I.hoferi in 50% of the fish. Tissues are presently being processed for histopathologic confirmation of infection.

Table I. Number of SPF herring infected with VHSV at the time of death. Individual fish assayed following dissection (head & tail removed), homogenization, and dilution.

Challenge Group ($PFU*ml^{-1}$)	tank number	# infected / total examined	mean log10 $PFU*gm^{-1}$
Low	7	7/9	6.0[a]
($7X10^2$)	35	9/9	6.7
	12	0/8	--
Medium	5	10/10	6.1
($7X10^4$)	20	9/9	6.1
High	9	9/9	6.2
($7X10^6$)	14	10/10	6.1
	37	10/10	6.1

(a) Lower detection limit = 2.6 $PFU * gm^{-1}$

Table II. Number of surviving SPF herring infected with VHSV 14 days post exposure. Two 4-fish pools (when available) were assayed following dissection (head & tail removed), homogenization, and dilution.

Challenge Group ($PFU*ml^{-1}$)	tank number	# of fish examined	mean log10 $PFU*ml^{-1}$
Controls	2	8	BDL[a]
(uninfected)	28	8	BDL
	33	8	BDL
Low	7	8	BDL
($7X10^2$)	12	1	2.9
	35	0	--
Medium ($7X10^4$)	20	1	4.8
High ($7X10^6$)		No survivors	

(a) Below detection limit (2.6 $PFU * ml^{-1}$)

Table III. Experimental Exposure of Pacific herring, rainbow trout and Japanese medaka with cultured *Ichthyophonus* cells

Species	Expt. #	Route of exposure	Incubation period	Infected (%)
Herring	1	I.P.	25d	4/5 (80)
	2	I.P.	18d	4/4 (100)
	3	per os	18d	0/8 (0)
	4	per os	21d	0/20 (0)
Rainbow	1	I.P.	61d	0/6 (0)
trout		per os	61d	1/6 (17)
	2	I.P.	28d	0/4 (0)
	3	I.P.	42d	0/20 (0)
		per os	42d	0/20 (0)
	4	I.P.		0/4 (0)
Medaka	1	I.P.	71d	0/2 (0)
	2	I.P.	137d	0/3 (0)
		per os	157d	0/3 (0)
	3	per os	60d	0/6 (0)
	4	per os	20d	0/3 (0)

VHSV in rainbow trout with & without pristane

VHSV in rainbow trout with & without pristane

After 3 weeks, mortality was observed only in the VHSV exposed groups with no significant difference between pristane and non-pristane treated groups (Table IV). Pristane alone had no effect on survival. Fish dying during the first two weeks following exposure were uniformly positive for VHSV, while those dying during the third week were negative even though they showed signs of disease and severe anemia (See Section III; Physiology). This is similar to what was observed in wild Pacific herring which experienced an epizootic of VHS following capture and confinement.

Table IV. Mortality and infection in rainbow trout exposed to VHSV and pristane

	N	morts	%	VHSV +/-
Controls	30	0	0	-
MEM (blank)	30	0	0	-
VHSV	30	12	40	+(a)
Pristane + VHSV	30	7	23	+(a)

(a) week 1 & 2 morts positive for VHSV; week 3 morts negative for VHSV but showed severe anemia

Tasks carried over: FY95 -> FY96

Survey of wild PS herring for VHSV infection

No VHS virus was detected in >60 fish sampled immediately after capture. However, heavy mortality began during the first week after capture and continued for about 30 days post capture. By the second week in captivity virus was isolated from 100% (10/10) of the fish sampled (Table IV). Mortality began to wain by the third week but fish continued to exhibit external signs of hemorrhaging and were lethargic. Both dead and surviving fish exhibited obvious hemorrhaging around the jaw, eyes, skin and fins. During week three post capture some hemorrhaging fish were shown to be capable of transmitting the VHSV to SPF fish, which also died with skin hemorrhages (see Task 95/96-2).

Table V. VHSV isolated from 5-month-old wild herring (no treatment)

Status	time in captivity (days)	number examined	% infected
Pre-crisis	0	30	0
Crisis (die-off)	10-21	10	100
Post-crisis (mortality ends)	>30	30	0

Hematologic and biochemical evaluation of wild VHS survivors

Blood samples were collected for hematology and biochemical evaluation (see Section III: Kennedy -Simon Fraser University).

Determine VHSV transmission rate for wild herring

SPF fish exposed to 3 wild fish suffered both mortality and infection by VHSV 3 weeks post exposure, while those exposed to only one wild fish exhibited neither mortality nor infection by VHSV (Table V).

Table VI. Mortality in SPF herring exposed to wild herring which had survived an epizootic of VHS.(a)

	% mortality	% VHS positive dead fish	% VHS positive survivors
Controls	0	--(b)	0
1 Wild fish(c)	0	--	0
3 Wild fish(c)	26.7	87.5	27.2

(a) N = 30; (3 tanks / treatment) X (10 fish / tank)
(b) No mortalities
(c) Wild fish selected during week 4 of epizootic

Determine the effect of stressors on VHS in Pacific herring.

Density was considered a factor for the transmission of VHS virus to susceptible herring. To determine the effect of density, 0-year herring (5-6 months old) were captured by dip net, transported to the Marrowstone Island laboratory and immediately distributed into triplicate 50 gal tanks at 5, 10, 25, 45 and 65 fish * $tank^{-1}$. Significant mortality occurred during the first week of confinement and continued for about 2 weeks, after which mortalities declined and fish numbers per tank remained stable after 4 weeks (Figure 4). Dead fish were collected twice daily, frozen at -70°C and assayed for VHS virus. Infection rates for all groups ranged from 10% -17%, with no obvious density effect. Survivors showing signs of hemorrhage were used for natural transmission studies to SPF herring.

Between August and September wild herring increased 50% in weight and 7.2% in length (1.81 gm and 5.7 mm respectively). The older fish did not exhibit the same density dependant mortality seen in the first group (Figure 5) but did show an obvious decline in virus titer (Figure 6). No virus could be detected in the fish by the fourth week post-capture.

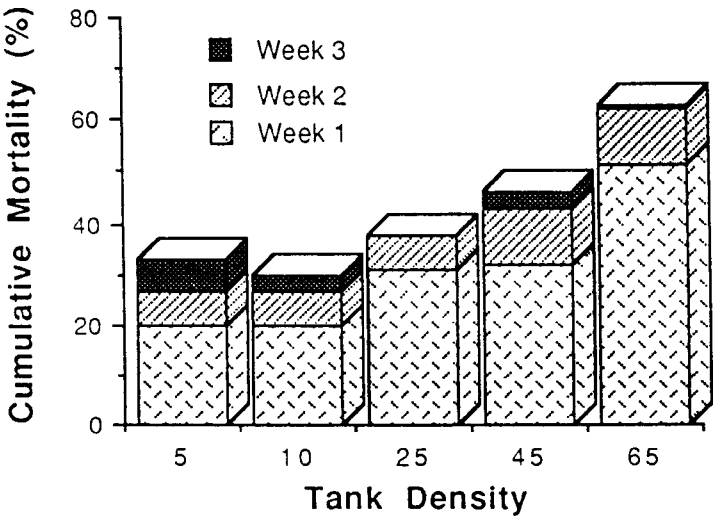

Figure 4. Mortality in wild 0-year herring captured 15 August 1995 from Pt. Wilson, WA. Mean wgt = 3.6 +/- 0.74 gm; mean length = 78.9 +/- 4.71 mm.

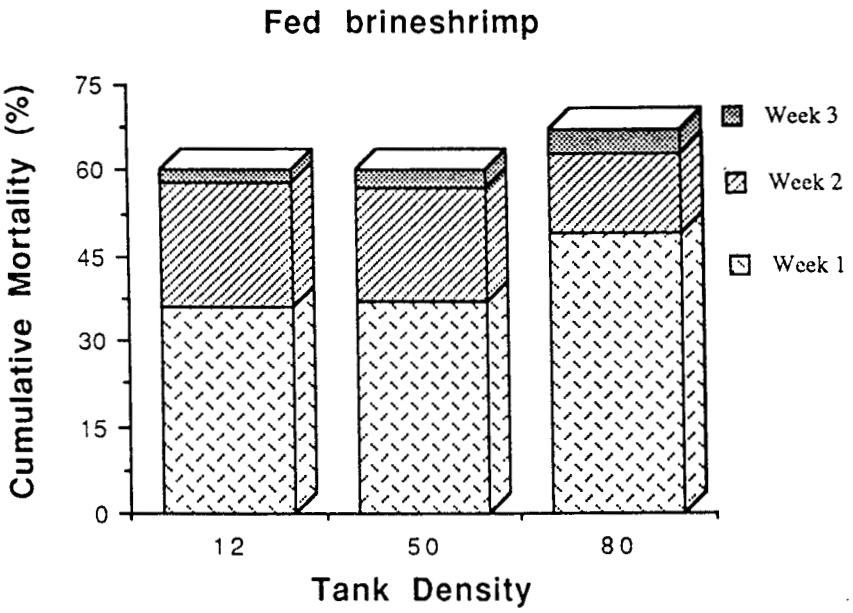

Figure 5. Density dependant mortality in wild 0-year herring captured 13 September 1995 from Discovery Bay, WA. Wgt = 5.41 +/- 0.65 gm; Length = 84.6 +/- 3.54 mm.

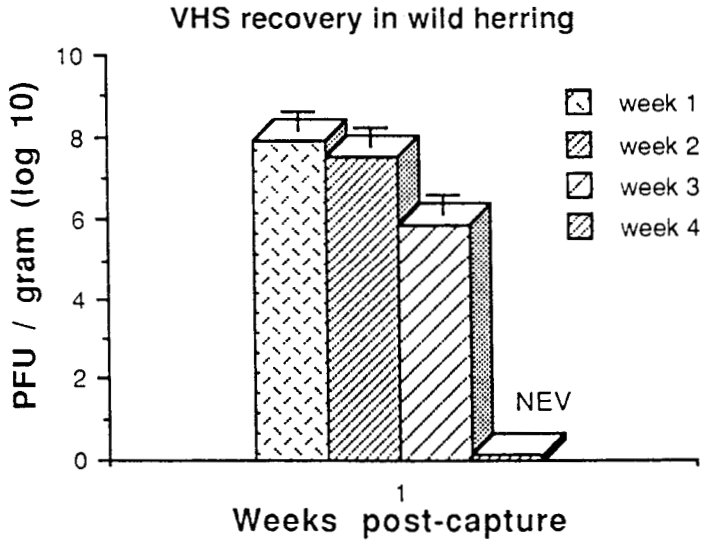

Figure 6. VHS virus tissue concentration in wild 0-year herring during a 3-week epizootic following capture (herring source; WDF, Discovery Bay; 13 Sept. '95).

Determine pathogenicity of *Ichthyophonus* for herring

At this point the natural route of transmission for *I. hoferi* in Pacific herring is not known. Oral transmission has not been successful using organisms isolated from herring tissues or cultured cells, but transmission by I.P. injection has been successful. The stage of this organism that is released from the herring host and the infective stage are not known at this time. It appears that the tissue phase of the *Ichthyophonus* organism is not the natural infective stage for herring, but rather a second biological host is required for rapid and efficient transmission of the agent.

Conclusions:

1. Pacific herring can be successfully reared disease-free in the laboratory in sufficient numbers to be used for experimental purposes.

2. Transmission of VHS virus is direct from fish-to-fish via water.

3. Non-immune juvenile Pacific herring are extremely sensitive to the VHS virus.

4. 0-year Puget Sound herring are infected with VHS virus before they are 6-months-old.

5. Artificial crowding of wild herring produces a VHS epizootic which can result in > 60% mortality.

6. Rainbow trout become infected with VHSV and exhibit morbidity and mortality similar to that seen in juvenile Pacific herring.

7. The natural route of infection of *Ichthyophonus* is not known, but the pathogenicity of the tissue stages has been demonstrated by artificial transmission via injection.

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Exxon Valdez Oil Spill
Restoration Project Annual Report

Investigations of Disease Factors Affecting Declines of Pacific Herring Populations in Prince William Sound

Section III: Survival, Performance and Reproduction in the Pacific Herring, *Clupea harengus pallasi*: Effects of Environmental Contamination, Viral Hemorrhagic Septicemia Virus and *Ichthyophonus hoferi*.

Restoration Project 95320S
Annual Report

This annual report has been prepared for peer review as part of the *Exxon Valdez* Oil Spill Trustee Council restoration program for the purpose of assessing project progress. Peer review comments have not been addressed in this annual report.

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April 1996

Investigations of Disease Factors Affecting Declines of Pacific Herring Populations in Prince William Sound

Section III: Survival, Performance and Reproduction in the Pacific Herring, *Clupea harengus pallasi*: Effects of Environmental Contamination, Viral Hemorrhagic Septicemia Virus and *Ichthyophonus hoferi*.

Restoration Project 95320S
Annual Report

Study History: The project effort was initiated under Restoration Project 95320S in response to a request for proposals to investigate disease factors affecting Pacific herring decline in Prince William Sound. The proposal was a joint effort of the University of Washington, Simon Fraser University and University of California at Davis.

Abstract: It has been demonstrated that exposure of fish to the stressors viral hemorrhagic septicemia virus (VHSV), the hydrocarbon pristane and *Ichthyophonus hoferi* (ITP) can significantly alter their hematological and immunological status, specifically hematocrit, leucocrit and differential white blood cell counts. Multiple stressors (VHSV and pristane) appear to act synergistically in their effects on fish. Baseline levels of hematological and immunological parameters in herring which have survived a natural epizootic of VHSV are reported. Higher fish stocking densities can modulate the immunological status in fish following a natural epizootic of VHSV, results which have important management implications. No effects of ITP on these parameters were seen in herring, 3 or 9 weeks following exposure. These preliminary results may, in part, begin to explain the trends in prevalence of VHSV and ITP in herring caught in Prince William Sound following the *Exxon Valdez* oil spill.

Key Words: *Clupea harengus pallasi*, *Exxon Valdez* oil spill, hematology, herring, *Ichthyophonus hoferi,* immunology, oil, Viral Hemorrhagic Septicemia Virus (VHSV).

Citation:

Kennedy, C.J., and A.P. Farrell. 1996. Investigations of disease factors affecting declines of Pacific herring populations in Prince William Sound. Section III: Survival, performance and reproduction in the Pacific herring, *Clupea harengus pallasi*: Effects of environmental contamination, viral hemorrhagic septicemia virus and *Ichthyophonus hoferi*, *Exxon Valdez* Oil Spill Restoration Project Annual Report (Restoration Project 95320S), Simon Fraser University, Burnaby, British Columbia, Canada.

Introduction

In 1993, less than half of the expected 5 year old 1988 year class of Pacific herring, *Clupea harengus pallasi*, returned to spawn in Prince William Sound. Approximately 15 to 43% of the returning fish were observed to have external lesions including ulcerations and hemorrhaging beneath the skin. Meyers et al. (1993) reported isolation of a rhabdovirus, identified as the North American strain of viral hemorrhagic septicemia virus (VHSV), by serum neutralization and cDNA probe methods. VHSV has now been isolated from herring over a wide geographical area spanning the USA/Canada International boundary. It has been suggested that VHSV may be indigenous to Pacific herring throughout Alaska and possibly the Pacific Northwest (Meyers et al. 1993). Therefore, the role of VHSV in the population decline of the herring populations in Prince William Sound remains unclear. One suggestion is that mortality may occur during these epizootics from progressive ulcerating skin lesions resulting in possible osmoregulatory failure and/or entry points for other pathogens (Meyers et al. 1993). These authors suggest that the virus may manifest its effects following stress from various factors including viral erythrocytic necrosis virus (VENV), spawning, commercial fishing or nutritional deficiency through lack of forage. More recent studies have indicated that VHSV was present in about 5% of herring tested in 1994, but lesions associated with *Ichthyophonus hoferi* (ITP) infection were present in about 29% of herring sampled and is suggested as the major cause of herring morbidity between the 1992 and 1993 spawning seasons (Marty et al. 1994).

It is possible that stress due to anthropogenic contamination, i.e. the *Exxon Valdez* oil spill, either directly through water and sediment exposure or indirectly *via* the food chain affected fish health or performance leading to the observed high mortalities and infection rates in surviving fish. Other studies have shown that stress from exposure to polycyclic aromatic hydrocarbons (PAHs), toxic compounds found in crude oil, reduce reproductive capacity and impair immunological responses of fish, resulting in reduced survival or fitness (Garrett, 1993). It has been shown that VHSV expression in carrier fish appears to be enhanced under stress of exposure to oil (Meyers, unpublished report). Furthermore, it is suggested that even if VHSV is not the primary pathogen, the high level of ITP incidence is indicative of a much weaker immune system in the herring. In addition, the extent of ITP infection and tissues infected (heart, skeletal muscle and brain) suggest life threatening effects (Freiberg and Farver, 1995;Marty et al., 1994). At this time, field and laboratory data confirm that a) oil exposure in 1989 could affect juvenile survival; b) VHSV prevalence was high in 1993; c) ITP prevalence was high in 1994; d) females from previously oiled sites produced fewer live larvae.

Objectives

Given the present information base, it is not clear whether VHSV, ITP , or oil exposure, or some combination of these stressors contributed to a decline in herring survival, performance or reproductive fitness. Moreover, survivors of oil exposure, VHSV or ITP infection may continue to experience a reduction in

fitness which may have consequences for continued longterm survival and reproduction. The effects of environmental modulators such as stocking density on the responses of herring to these stressors has important implications to herring management strategies.

The longterm objectives of this section of the project are to document cause-effect and interactive relationships for oil, VHSV and ITP on herring survival, performance and reproduction and to establish the effects of abiotic modifiers such as density and temperature on herring responses to these stressors. The overall hypothesis being tested in this project is: 'The exposure of herring to VHSV, ITP or oil or combinations of these parameters reduces herring fitness in one or more of the following categories: 1) immunology, 2) biochemistry, 3) performance, and 4) reproduction.'

The specific objectives for 1995 were as follows:

1) To develop and build an exposure system for the delivery of oil to herring holding aquaria. To build a swim tunnel apparatus for examining the swimming performance of juvenile herring.

2) To supply analytical support for Section I (the field component) of this research project. Blood smears from 500 herring sampled from Prince William Sound were to be analyzed for differential white blood cell counts and erythrocytes scored for inclusion bodies. Plasma samples from these fish were to be analyzed to determine the relative proportions of creatine phosphokinase (CPK) isozymes present.

3) Disease-free young of the year from PWS raised by Dr. Kocan's group were not available for experiments for Section III in 1995 due to their small size. Testing with these fish has begun in 1996. Therefore, test sampling and analysis of several hematological, immunological and biochemical parameters were performed in wild caught Pacific herring from Washington State and a surrogate species, the rainbow trout, before testing in 1996 on disease-free Prince William Sound herring began.

Methods

Objective 1: Apparatus development.

Dosing of herring with oil is scheduled and proceeding in 1996. The dosing apparatus or 'oil generators' which had been selected for this research project are those developed by Carls et al. (unpublished). Essentially, this apparatus consists of a 15 cm diameter X 80 cm tall polyvinyl chloride plastic cylinder containing either rock or ceramic beads. Water upwells through the cylinder and flows into the bottom of the individual treatment tank. Appropriate levels of hydrocarbons are generated through the apparatus as indicated by hydrocarbon analysis documented by Carls et al. (unpublished) using Alaska North Slope Crude oil. A trap inside the generator prevents slick overflow.

Experiments on the effects of stressors on herring swimming performance have been started in 1996. The swim-test apparatus assembled is described in Nikl and Farrell (1993). Briefly, the apparatus consists of a 2,470-L ovoid, fiberglass raceway tank equipped with two variable-output propulsion motors. Two test chambers are used to house the fish inside the raceway. A series of straightening vanes, screens and contraction cones were placed upstream of the chambers to correct for rotational disturbances, smoothing the velocity profile within the enclosed cylindrical testing chambers. Water velocity is controlled by regulating voltage output to the propulsion motors. A portable current meter was used to determine water velocity within the test chambers at various voltages.

Objective 2: Field study support.

Blood smears from 500 Pacific herring sampled March and April 1995 in Prince William Sound were received from Dr. G. Marty of the University of California at Davis. Smears were stained with Diff-Quik (Dade Diagnostics, Inc., Aquada, Puerto Rico), using the recommended protocol on the product package. Smears were examined microscopically at 1000X oil immersion magnification. Approximately 100 white blood cells were counted from the randomly selected fields. The number of fields examined varied with the smear, however, on average, 48 fields per slide were counted. The number of red blood cells in each field were not counted but were similar: with approximately 150-175 red blood cells per field. White blood cells were differentiated into six cell types; thrombocytes, lymphocytes, neutrophils, basophils, eosinophils and monocytes. Identification of each type was based on morphology and staining characteristics (Ainsworth 1992: Sherburne 1973). Red blood cells form each smear were examined for viral erythrocytic necrosis (VEN) .

Due the strong statistical relationship between CPK and lesions in herring (Marty unpublished), frozen plasma samples from 100 Pacific herring sampled March and April 1995 in Prince William sound were received from Dr. G. Marty for CPK analysis. Plasma samples were unfrozen and kept at 0°C. Electrophoretic analysis of plasma was performed according to Sigma Chemical Co. (Mississauga, Ont.) to identify and calculate the relative proportions of CPK1, CPK2, CPK3, brain, cardiac and skeletal muscle isozymes, respectively.

Objective 3: Herring and surrogate species testing and technique development

Disease-free young of the year from PWS were not available in 1995 for fitness experiments due to their small size. At 5-6 g these juveniles will be suitable for sublethal toxicological testing and disease challenges in 1996. Although not scheduled for 1995, several preliminary experiments were performed.

1) Hematological and immunological status of herring following ITP exposure

0-year herring were captured and transported to the Marrowstone Island laboratory where they underwent a natural VHSV epizootic (see Section II-Dr.

Kocan for details on fish capture and holding). Fish were then dosed with both low and high doses of ITP spores *per os* as described in Section II (laboratory study-Dr. Kocan). Fish were sampled 3 and 9 weeks following ITP exposure. Blood was removed by severing the caudal peduncle. Hematocrit (% volume of packed red blood cells), leucocrit (% volume of packed white blood cells) and differential white blood cell counts were examined. Methods for these measurements are summarized in Stolen et al. (1992).

2)Rainbow trout challenge with VHSV and pristane

Previous studies have successfully used rainbow trout of this size range in determining the sublethal toxicity of several natural wood products and antisapstain chemicals (Johansen et al. 1995; Kennedy et al. 1995). In this section, rainbow trout were used as a surrogate species to determine the effects of pristane (a hydrocarbon and immunosuppresive agent in mammalian systems), VHSV and a combination of these factors on trout immunocompetence. Trout were exposed to pristane, VHSV or combination according to procedures outlined in Section II (Laboratory studies-Dr. Kocan). Fish were sampled 21 days following exposure. Blood was removed by severing the caudal peduncle. Several immunological indicators including hematocrit (% volume of packed red blood cells), leucocrit (% volume of packed white blood cells), differential white blood cell counts (as described earlier), phagocyte activity using the nitroblue tetrazolium assay and glass adherent phagocytes and lysozyme assay using the lysoplate method were examined. Methods for these measurements are summarized in Stolen et al. (1992).

3) Density effects on hematological and immunological status of herring following a VHSV epizootic

0-year herring were captured and transported to the Marrowstone Island laboratory and distributed into triplicate tanks at varying densities as described in Section II (laboratory studies-Dr. Kocan). Significant mortalities occurred during the first week of confinement (VHSV epizootic) and continued for about 2 weeks, after which mortalities ceased and fish numbers in each tank remained stable. Fish were sampled 8 weeks following the start of the experiment and blood sampled by severing the caudal peduncle. Several immunological indicators including hematocrit (% volume of packed red blood cells), leucocrit (% volume of packed white blood cells), differential white blood cell counts (as described earlier), phagocyte activity using the nitroblue tetrazolium assay and glass adherent phagocytes and lysozyme assay using the lysoplate method were examined (Stolen et al. 1992).

Statistical analysis

Values are reported as means ± standard error. All data were analyzed by analysis of variance (ANOVA) and were considered significant at $p<0.05$. Percent data were arcsin transformed before statistical analysis.

Results

Objective 1: Apparatus development.

A schematic of the dosing apparatus is shown in Figure 1. Fifteen of the units have been built for the oil exposure studies. A schematic of the swim raceway apparatus is shown in Figure 2. One unit has been built for the swimming performance studies.

Objective 2: Field study support.

Statistical analysis and reporting of differential white blood cell counts and presence of viral erythrocytic necrosis are given in Section I (Field studies-Dr. Marty) of this annual report. CPK analysis proved unsuccessful because of the improper handling (several thaw and freeze episodes) of field plasma samples during blood chemistry analysis at the University of California laboratory (Section I of the project).

Objective 3: Experiments with herring and rainbow trout

1) Hematological and immunological status of herring following ITP exposure

Hematocrit and leucocrit values for herring treated with varying doses of ITP spores for 3 or 9 weeks can be seen in Table 1. There were no significant differences for leucocrit, hematocrit or white blood cell differential counts between control fish and those treated with low or high oral doses of ITP spores, 3 or 9 weeks post-exposure. Observation of blood cells indicated that large intracytoplasmic, eosinophilic-staining inclusion bodies were found in the erythrocytes of 70.4% of herring exposed to ITP. These inclusion bodies resemble those associated with viral erythrocytic necrosis virus (VEN). The red blood cells are degenerate as displayed by cytoplasmic vacuolization, karyorrhexis and nuclear pyknosis. These cells are generally round with a moderate degree of basophilia, immature cells and mitotic figures. These observations are consistent with those of researchers who have demonstrated the presence of VEN by transmission electron microscopy. Further investigation needs to be done to determine if VEN is present, and to what extent it may be affecting the herring populations ability to combat VHSV or ITP. It is likely that several pathogens are simultaneously contributing to the increased mortality, especially if the population is immunocompromised.

Figure 1. Schematic diagram of an 'Oil Generator' designed after Carls et al. (unpublished) for dosing of herring to oil in single and multiple stressor experiments.

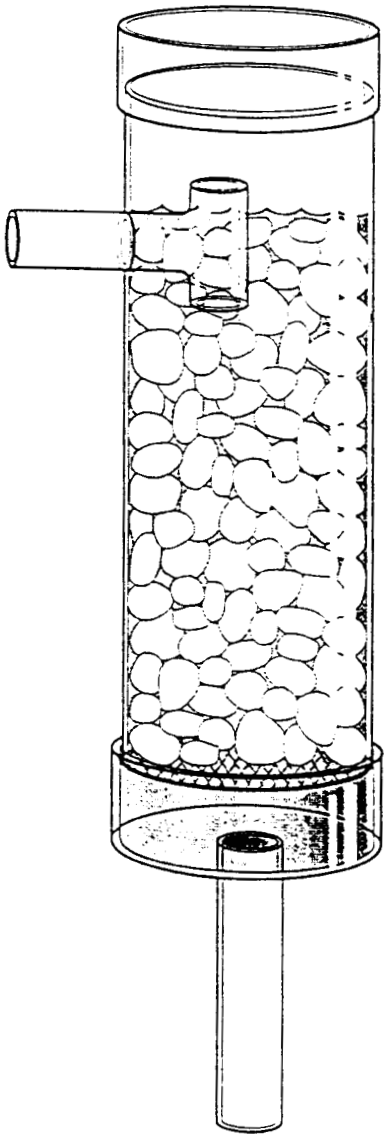

Figure 2. Schematic diagram of the swimming raceway to be used in physiological fitness testing of herring following exposure to single or multiple stressors.

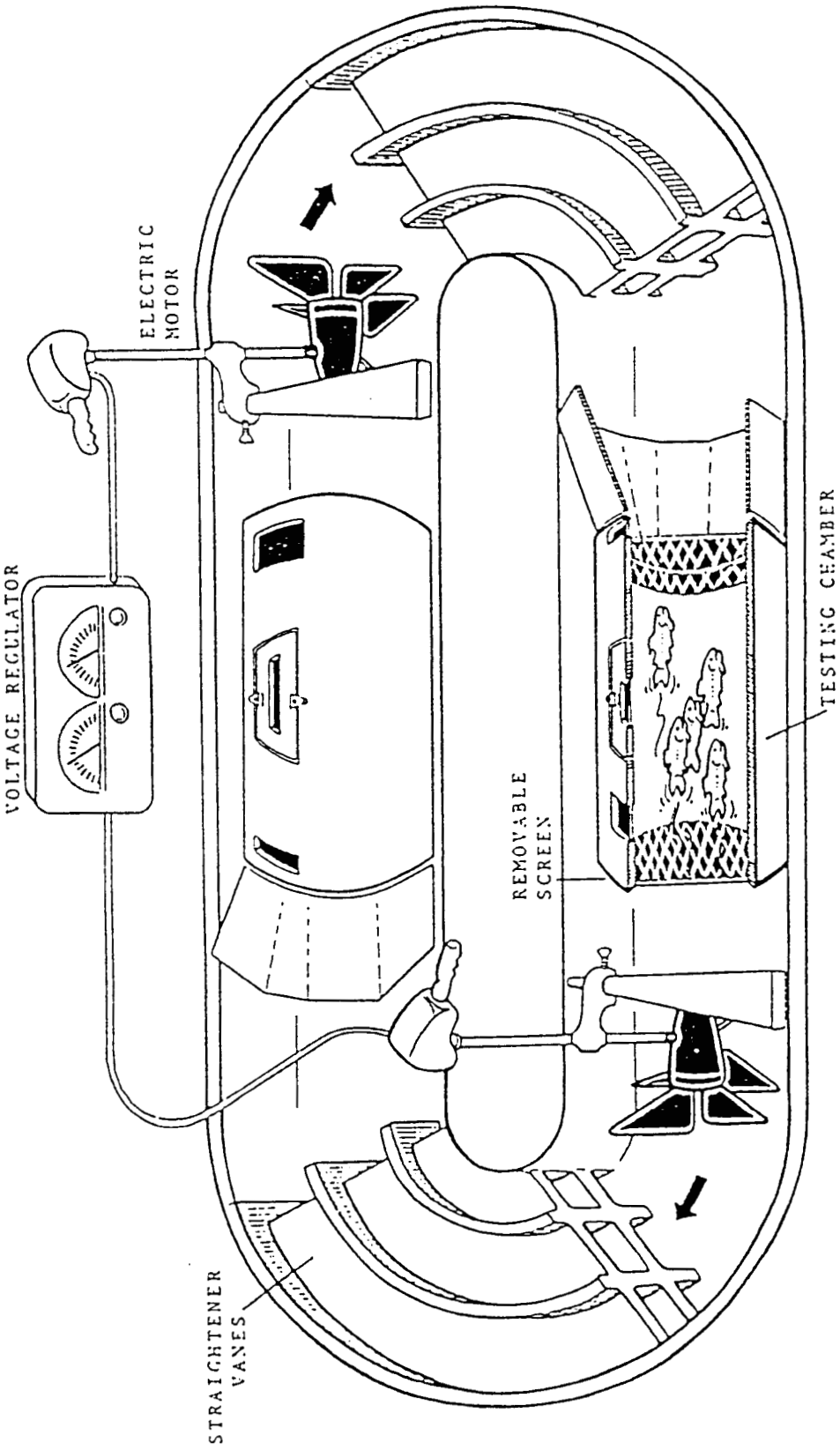

Table 1. The effects of ITP exposure on hematological parameters in wild Pacific herring which had undergone a natural epizootic of VHSV. Samples were taken from fish at 3 and 9 weeks following ITP exposure. Values are means±standard errors. There were no significant differences in measured parameters between ITP-exposed and control fish at a significance level of $p<0.05$.

ITP concentration	Duration (weeks post exposure)	Hematocrit (%)	Leucocrit (%)
0	3	17.1±3.1	0.29±0.10
0	9	24.5±4.9	0.44±0.10
Low	3	14.1±1.8	0.48±0.19
Low	9	24.5±3.0	0.35±0.09
High	3	22.2±3.0	0.51±0.20
High	9	36.1±2.7	0.44±0.11

Table 2. The effects of ITP exposure on differential white blood cell counts in wild Pacific herring which had undergone a natural epizootic of VHSV. Samples were taken from fish at 3 weeks following ITP exposure. Values are means±standard errors. There were no significant differences in cell counts between control and exposed groups at a significance level of $p<0.05$.

ITP Conc.	Lymphocytes (%)	Macrophages (%)	Neutrophils (%)	Thrombocytes (%)	Monocytes (%)	Eosinophils (%)
Control	64.1±4.3	1.8±0.5	6.7±1.9	26.7±5.2	0.3±0.2	0.4±0.4
Low	74.6±2.9	0.8±0.3	1.9±0.5	22.0±3.2	0.7±0.4	0.1±0.1
High	65.1±5.2	0.1±0.1	6.3±2.3	28.0±5.4	0.0±0.0	0.0±0.0

2)Rainbow trout challenge with VHSV and pristane

Hematological and immunological parameters in trout exposed to pristane, VHSV or a combination of both factors are shown in Table 2. Trout exposed to pristane only showed significant reductions in leucocrit values when compared to control fish. Trout exposed to VHSV only showed significant reduced values in both hematocrit and leucocrit values when compared to control fish. When trout were exposed to both pristane and VHSV, significant reductions were

seen in both hematocrit and leucocrit values. VHSV and pristane, when administered together, appeared to act synergistically in their effects on these parameters. No statistically significant effect was seen in lysozyme activity with any treatment. White blood cell differential counts indicated that trout exposed to pristane and VHSV had a leucopenia with a concurrent neutrophilia and lymphocytopenia (Table 4).

Table 3. The effects of pristane and VHSV exposure on hematological and immunological parameters in juvenile rainbow trout. Values are means±standard errors. The * denotes a significant difference between fish exposed to Minimum Essential Medium (MEM, a balanced saline solution as control) and either VHSV or pristane, or combination, at a significance level of $p < 0.05$.

Treatment	Hematocrit (%)	Leucocrit (%)	Lysozyme Activity (Units/ml plasma)
MEM	45.2±1.8	1.26±0.10	9.34±2.18
Pristane	41.8±0.8	0.66±0.10 *	7.66±1.93
VHSV	25.3±6.7 *	0.65±0.34 *	11.49±1.87
VHSV/Pristane	22.4±4.9 *	0.32±0.01 *	11.81±1.9

Table 4. The effects of pristane and VHSV exposure on differential white blood cell counts in juvenile rainbow trout. Values are means±standard errors. The * denotes a significant differences between control and exposed fish at a significance level of $p<0.05$.

Treatment	Lymphocytes (%)	Macrophages (%)	Neutrophils (%)	Thrombocytes (%)	Monocytes (%)
MEM	90.4±1.4	2.5±1.0	4.1±1.0	0.5±0.3	2.5±0.9
PRIS/ MEM	88.9±1.7	2.6±0.7	6.4±1.5	1.2±0.5	0.9±0.4
PRIS/ VHS	53.6±9.2 *	6.3±1.6	29.2±8.8 *	0.5±0.4	1.3±0.6
VHS	88.2±2.2	3.4±0.7	7.1±1.7	0.0±0.0	1.3±0.4

3) Density effects on hematological and immunological status of herring following a VHSV epizootic

Hematocrit and leucocrit values for herring which underwent a natural VHSV epizootic under various density conditions can be seen in Table 5. Significant reductions in hematocrit were seen in fish at fish kept at densities of 80 fish per tank compared to fish at a density of 12 or 50 per tank. Significant reductions in leucocrit were seen in fish kept at densities of 50 and 80 fish per tank compared to fish at a density of 12 per tank. Lysozyme activity appeared to be unaffected by fish stocking density. These values represent baseline data for fish which had undergone a natural epizootic of VHSV and cannot be compared to preinfected fish where no data is available. Phagocytic activity data have not been analyzed statistically and will be reported in the next annual report. 100% of fish in these experiments showed large intracytoplasmic, eosinophilic-staining inclusion bodies in the erythrocytes as seen in the herring exposed to ITP. Again, no conclusions regarding this observation can be made and needs to be investigated further.

Table 5. The effects of fish stocking density on hematological and immunological parameters in wild Pacific herring which had undergone a natural epizootic of VHSV. Values are means±standard errors. The * denotes a significant difference from fish stocked at 12/tank at a significance level of $p < 0.05$.

Stocking Density (Fish per tank)	Hematocrit (%)	Leucocrit (%)	Lysozyme Activity (Units/ml plasma)
12	44.1±2.6	3.2±1.1	4.3±6.0
50	39.7±4.9	1.5±0.1 *	10.4±6.0
80	28.0±3.9 *	1.3±0.1 *	12.2±6.2

Table 6. The effects of fish stocking density on differential white blood cell counts in wild Pacific herring which had undergone a natural epizootic of VHSV. There were no significant differences in differential white blood cell counts between fish stocked at higher densities compared to the lowest density at a significance level of $p<0.05$.

Stocking Density (Fish/tank)	Lymphocytes (%)	Neutrophils (%)	Macrophages (%)	Monocytes (%)	Thrombocytes (%)	Eosino. (%)
12	59.9±6.3	18.5±6.1	1.1±0.5	0.1±0.1	19.8±3.8	0.0±0.0
50	42.3±4.3	9.4±2.7	0.7±0.5	0.0±0	47.9±4.7	0.1±0.1
80	35.±4.0	1.0±0.0	0.1±0.1	0.0±0.0	59.4±4.1	0.0±0.0

Discussion

The results for research in this section of this annual report are preliminary and supportive of research which has begun in 1996. No experiments with disease-free Prince William Sound juvenile herring were planned or performed due to the small size of herring at this stage. However, preliminary studies were performed with rainbow trout and wild caught Pacific herring from Washington State to assess the feasibility of planned herring experiments taking place in 1996.

The majority of the work performed in 1995 was allocated to apparatus development and assembly. Both oil generators and swim raceway are built and functioning and have been transported to the Marrowstone Island Field Station in order to begin experiments with PWS herring raised by Dr. Kocan. The probability of success of 1996-98 planned experiments has been increased dramatically by the successful raising of disease-free PWS juvenile herring at Marrowstone Island by Dr. Kocan.

Support services for Section I of the research (field studies) were successful. Statistical analysis and conclusions regarding the results of the differential white blood cell counts, examination for viral erythrocytic necrosis and creatine phosphokinase isozymes in field sampled herring are discussed in Section I (field study-Dr. Marty) of this annual report.

Results utilizing wild caught Pacific herring and rainbow trout have yielded important information for the success of the research in 1996-1998. The techniques utilized for the measure of hematological and immunological parameters have proven successful for use in herring and will be used for the experiments in 1996-1998. The preliminary study with rainbow trout

indicated that exposure of fish to the alkane pristane, a known immunosuppressant in mammalian systems, causes significant alterations in immune system parameters. Exposure of trout to VHSV also altered components of the immune system in trout which may have implications for secondary infections in surviving fish. Pristane and VHSV exposure together appeared to act synergistically on the immune system. Results also indicate that abiotic factors may modify the hematological and immunological responses of herring to stressors such as VHSV. ITP exposure *via* the oral route did not cause any significant alterations in herring hematological or immunological parameters. It is unclear at this time whether fish were infected with ITP by the experimental exposure route. Moreover, effects of ITP infection may occur later in the sampling period and so conclusions regarding the effects of ITP are premature. The information obtained from these studies indicates that the research approach in this project using multiple stressors in combination (oil, VHSV and ITP) may yield important results regarding cause and effect relationships between the stressors and Pacific herring decline in PWS.

Conclusions

The preliminary studies performed in 1995 provide significant support to the proposed research plan. It has been demonstrated that exposure of juvenile trout to the stressors VHSV and pristane, a hydrocarbon, can alter their hematological and immunological status. This is an important first step in evaluating any link between oil exposure and altered immunocompetence in fish. Furthermore, two of the stressors combined acted synergistically on some of the measured parameters in trout. Baseline levels of hematological and immunological parameters in herring which have survived a natural epizootic of VHSV are reported. Higher fish stocking densities can modulate the immunological status in fish following such an epizootic, results which have important management implications. No effects of ITP on these parameters were seen in herring, 3 or 9 weeks following exposure. These preliminary results may, in part, begin to explain the trends in prevalence of VHSV and ITP in herring caught in PWS following the oil spill.

Acknowledgments

The authors wish to acknowledge the work of Dr. Richard Kocan (author of Section II of this report) for his efforts at obtaining a SPF stock of PWS herring and wild Washington State herring which are essential for the successful completion of this project. We would like to also thank him for the maintenance of fish stocks and exposure of herring and trout to pristane, VHSV and ITP.

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Exxon Valdez Oil Spill
Restoration Project Annual Report

Juvenile Herring Growth and Habitats

Restoration Project 95320T
Annual Report

This annual report has been prepared for peer review as part of the *Exxon Valdez* Oil Spill Trustee Council restoration program for the purpose of assessing project progress. Peer review comments have not been addressed in this annual report.

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April 1996

Juvenile Herring Growth and Habitats

Restoration Project 95320T
Annual Report

Study History: Restoration Project 95320T was initiated as a core project of the Sound Ecosystem Assessment (SEA; PWSFERPG 1993), an integrated, multi-investigator ecosystems study of Prince William Sound (PWS). SEA was initiated because the lack of knowledge of the ecological processes affecting pink salmon and herring confounded the identification of damage caused by the *Exxon Valdez* oil spill. The PWS herring population crashed in 1993 apparently due to a viral infection (VHSV). Viral infection by this agent occurs more frequently in fish exposed to oil. Local residents, frustrated by the loss of valuable fisheries and the inability to accurately identify the causes, strongly voiced support for research. They formed a group, appealed to the EVOS Trustee Council, and as a result of their effort SEA was created in 1994. Research on juvenile herring began in April 1995. This study differs from traditional herring fisheries research because of the ecological context within which these species are examined. Models are used to link physical processes to distribution of larval herring and food production in retention areas, evaluations of nursery habitat within retention areas, and overwinter survival of young herring. A sensitivity analysis, examining variations in life history parameters at different stages of development and the resulting changes in survival and recruitment, suggested that the larval and juvenile phases are critical to stock recovery and should be the focus of the SEA herring program. It is the survival of those life stages which will ultimately determine recruitment and the rate of recovery of the herring population in PWS.

Abstract: We focused on the distribution, feeding and condition of ages 0-2 herring (juvenile) in order to characterize their habitat. Herring were collected incidentally during net sampling for pink salmon in 1994 and 1995. Aerial surveys were conducted to analyze broad scale herring distribution in 1995. Juvenile herring were more widely distributed than adults. Preliminary results suggested that four major areas have high densities of juveniles: 1) eastern, 2) central, 3) southwestern Prince William Sound, and 4) bays in the outer Kenai Peninsula. Juveniles were abundant and available to net sampling during April, June through early August, and October. Age-0 herring migrated or moved to nearshore waters in late summer. Distribution and movements of juvenile herring in May, September and through the winter months are poorly understood. Herring were found in bays or shallow shelves with tidal mixing (less than 200 m from shore and 50 m in depth). In 1996, the distribution data and habitat characteristics will be compared with stomach contents and condition indices to evaluate differences between areas affecting survival. Historic and traditional knowledge will be compiled with the 1996 sampling data. This preliminary work enabled us to design a biologically and statistically rigorous survey and sampling plan for FY96.

Key Words: *Clupea pallasi*, Pacific herring, juvenile, habitat, Prince William Sound, distribution.

Citation: Norcross, B. L., E. D. Brown, K. D. E. Stokesbury, and M. Frandsen. 1996. Juvenile Herring Growth and Habitats, *Exxon Valdez* Oil Spill Restoration Project Annual Report (Restoration Project 95320T), University of Alaska Fairbanks, Institute of Marine Science, Fairbanks, Alaska.

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INTRODUCTION

Pacific herring (*Clupea pallasi*) is a key species in the marine ecosystem of Prince William Sound (PWS). The health of the apex predator community may depend on the magnitude of herring recruitment and the condition of individual fish. The PWS herring population decline has had significant negative impacts on the commercial and subsistence fisheries. PWS herring are presently listed by the EVOS Trustee Council as damaged and not recovering. Direct restoration of this species is not practical. However, understanding and monitoring the recovery of this species is important in order to improve stock assessment for management of commercial fisheries and to guide restoration of the injured species that feed upon them.

Determining biological and physical parameters influencing early herring life history stages in PWS is important for understanding recruiting processes affecting population recovery. Previous exposure to oil, severe ocean conditions, limited food availability, and density-dependent mortality may have been the cause of the recent disease event and 1993 crash of the PWS herring population (Brown et al. 1995; Pearson et al. 1993; Brown et al. 1996). In 1993 the PWS herring population was at an all time high, several of the dominant year classes had been exposed to oil, the heavily impacted 1989 year class were recruiting as four-year-olds, food was scarce, and sea surface temperatures were relatively cold during the two previous years (Brown et al. submitted). In 1995, the biomass of herring in PWS reached a historic low and further losses of juvenile herring could compromise recovery. A recent herring recruitment model suggests that survival rates and the probability of individuals being retained in the population are higher in distinct spawning areas (Campbell and Graham 1991). Management of herring in Maine now includes spawning closures at various time and places along the Maine coast based on larval transport and survival data (Campbell and Graham 1991). Our research will provide information on the processes of survival of early life history stages of herring in PWS that can be used by managers to facilitate the recovery of the population.

The member/vagrant theory hypothesizes that a sufficient number of individuals must be retained within a spatial area, defined by the restrictions of sexual reproduction (i.e. being in the right place at the right time), for the population to continue over time (Sinclair 1988). Individuals that are retained produce the next generation while individuals that are not become vagrants, and do not contribute to the next generation of that population, although they may become founders of new populations. Larval distribution is a critical aspect of retention, however, larval research can be expensive and time-consuming. Initially, we plan to use the SEA ocean dynamics model to simulate larval drift and compare that simulation to larval distribution data collected in 1989 (Norcross et al. submitted). We will attempt to determine nursery grounds based on physical and biological characteristics, i.e. develop conceptual habitat models of herring nursery or retention areas. This technique of formulating conceptual models of nursery areas has been applied to demersal fish and is

currently being used by the principal investigator for flatfish nursery areas around Kodiak Island (Norcross et al., 1995). We hope to focus future studies on index areas in order to understand processes affecting losses within these areas and larval drift.

In 1995, we proposed to: (1) identify nursery areas for juvenile herring; (2) characterize those areas according to physical and biological parameters, and (3) start a time series to develop indices of relative abundance and condition of herring.

Preliminary results addressing the FY95 objectives enabled the formation of a statistically rigorous survey design for 1996 to examine juvenile herring distribution and habitats. This also allowed us to focus the general FY95 objectives into concise objectives dealing with the specific characteristics of the PWS herring stock in FY96. Herring were collected as ancillary data from net sampling for salmon conducted April - July 1994 and 1995, primarily on the western side of PWS. Additionally, aerial surveys were conducted specifically to analyze broad scale herring distribution in 1995. Broadscale temporal and spatial patterns of distribution have been analyzed and small scale measurements from the acoustic data will be analyzed this winter. After locating the herring, we began to compile the data sets needed to characterize the nursery areas through measurements of the associated physical (temperature, salinity, depth, bathymetry, distance from shore, currents, and fluctuations in light levels) and biological parameters (zooplankton and predator densities). Once we have described juvenile herring distribution patterns using acoustic, aerial and net sampling survey data, we will examine the temporal consistence of these patterns (Fargo et al. 1990). We will examine regional, seasonal, and differences in patterns and relate these patterns to changes in physical and biological parameters. From these data, key areas of herring recruitment can be determined and an index of relative abundance can be developed. To have confidence in this index, it will be necessary to analyze the distribution and abundance of herring in PWS for several years to interpret similarities and differences among years and sub-populations. Knowing fluctuations in year-class strength prior to recruitment to the fishery, using a fishery-independent method, would be extremely valuable to management agencies and the fishing profession. The Principal Investigator, using a similar technique, demonstrated significant differences in annual recruitment patterns of a demersal species, summer flounder (*Paralichthys dentatus*), at a level that affected the fishery in four years (Norcross and Wyanski 1994).

Definitions:

Juvenile herring are considered to be ages 0-2.

Nearshore/offshore to be determined from the oceanography of PWS, from a pilot study across fronts and through vertical structures within fronts.

Nine Regions for Distribution Analysis (Figure 1):

1. Southeast Area: includes Orca Inlet, north side of Hawkins Island, Sheep and Simpson Bays, and Port Gravina.

2. Northeast Area: includes Port Fidalgo, Tatitlek Narrows, Blight Island, Valdez Arm and Port.

3. North Shore: includes Glacier Island and waters west of Pt. Freemantle, Long, Wells, and Unakwik Bays west to Kinniklik, Eaglek Bay west to Ragged Pt.

4. Northwest Area: includes Esther Island, Port Wells, Wells Pass., Perry and Culross Islands, Port Nellie Juan, and Knight Island Passage to Pt. Nowell.

5. North Central Sound: Naked Island and Smith Islands; northeast side of Knight Island; pelagic region east of Naked Island to Johnstone Point on northern Hinchinbrook Island.

6. South Central Sound: Montague and Green Islands and Montague Straits including east side of Latouche.

7. Hinchinbrook Entrance: eastern shore of Montague Island and Port Etches on Hinchinbrook.

8. Southwest Area: south Knight Island Passage from Pt. Nowell and including islands, bays and passes to Port Bainbridge; the western boundary is Cape Puget.

9. Outer Kenai: Bays and outer coast from Cape Puget west to Nuka Pt. including Resurrection and Aialik Bays.

Herring work in relation to SEA hypotheses

Under Lake/River Program

Determine the trajectory of free-drifting larvae and their distribution as age-0 juvenile herring using a larval drift simulation. Determine differences in zooplankton concentrations between areas due to variations in zooplankton production and transport to nursery areas.

Under Predator/Prey Program

This hypothesis was developed primarily for pink salmon. Since herring are annual residents of PWS and the surrounding area and predation varies according to life stage, distribution of herring and predators, we will construct hypotheses on predation processes once primary habitats have been located and characterized. We will derive a proxy measure for mortality, most likely due to predation, through the overwintering survival model. The

assumption is that herring under a certain energetic content are more susceptible to predation.

Under Herring Overwintering Program

Determine how the condition of juvenile herring entering their first winter and winter ocean conditions affect survival, measured by changes in relative abundance of those fish.

OBJECTIVES

In the original study plan for FY95, the following **initial objectives** were listed:

1. Begin to determine relative spatial and temporal abundance, growth of and primary predators on juvenile herring in eastern and western regions of PWS.

2. Begin to compare seasonal differences in the parameters listed in objective one.

3. Relate seasonal and annual differences in juvenile herring population parameters to relative abundance of prey resources and predators (including birds) sampled at the same time and locations. Confirm primary predators with information on stomach contents or behavioral observations where possible.

4. Determine the physical characteristics of habitats where herring aggregations are found including temperature, depth, salinity, distance from shore, time of day and tide cycle at capture, and current profile at that time and place.

5. Begin work on a predictive model of age-0, -1, and -2 herring location knowing the location and relative abundance of embryos at the natal habitats, the information gained through this and other studies, and the ocean circulation within the sound for any given year.

We were able to restate our first year **research questions** more clearly once we began to examine and understand the information available on juvenile herring:

1) Does distribution of embryos and ocean circulation affect the distribution of juvenile herring?

2) What are the physical (temperature, salinity, current structure, depth, distance from shore) and biological (trophic structure) characteristics of juvenile herring habitat?

3) Are there differences in distribution and condition (length and weight at age, energetic, stable isotopes) of age-0 and older juvenile herring in relation to habitat?

We proposed to evaluate the nursery habitat for juvenile herring in order to understand processes affecting survival and recruitment. In order to do this, we relied on other projects within and outside of SEA to provide us with the data sets needed to examine the idea of "optimal versus suboptimal" habitats. Some of the data sets needed were not available until the end of the fiscal year and many others are still being formulated. Our first year was more information gathering than analysis and once we understood the data available within the predetermined sampling confines, we were ready to **restate our objectives** and more clearly define our research direction:

1) Determine diel vertical distribution of juvenile herring.

2) Determine horizontal distribution of juvenile herring by regions of PWS and Outer Kenai.

3) Determine nearshore/offshore distribution of juvenile herring.

4) Determine temporal distribution of juvenile herring, i.e., where herring are throughout summer season.

5) Use above information to develop field study plan for 1996.

METHODS

Data Compilation

A large part of our efforts have been and will continue to be devoted to compiling data sets from other projects funded by EVOS. We have compiled the net sampling data from 1994-1995 SEA pink salmon (Willette, 95320-A & E) and 1994-1996 APEX forage fish databases (Haldorson). Data from net sampling includes age, weight, length (AWL), condition, energetic content (Paul, 95320-U) and isotopes (carbon nitrogen ratios and delta C-13 values; Kline, 95320-I). We will also rely on other projects for collections of habitat characteristics including zooplankton species composition and concentrations (Cooney, 95320-H), CTD, acoustic Doppler and other oceanographic parameters describing regions and sites (Vaughan and Salmon, 95320-M), and density of competitors such as age-0 and -1 pollock (Thomas, 95320-N; Haldorson, APEX). Finally, we have developed databases for broadscale distribution information on herring, other schooling fish, and foraging birds from our 1995 summer aerial surveys. The compilation of these data sets allows us to conduct analyses on several spatial and temporal scales. Currently, there is a three month lag between our data collection and the beginning of analysis. There is a six to ten month lag between data collection, our receiving the data, and preparing for analysis of other SEA and APEX projects. These time lags must be considered in project planning and allowances must be made by the funding agencies. Multidisciplinary efforts of this scale require more time than single investigator efforts.

Net Sampling

In 1994 and 1995, researchers sampled predators and prey of pink salmon, including both juvenile and adult herring, in the western corridors of PWS. Sampling gear included mid-water trawls, anchovy seines, gillnets, hoop traps and small tow nets. Diel surveys (24 hr sampling) were conducted during the months of April through September from Esther Island to the passages in southwestern PWS in 1994. During the months of April and May 1995 sampling effort focused around Esther Island, and at three isolated locations where herring typically occur (Port Gravina, Orca Inlet, and Zaikof Bay). Seine catches of herring under 150 mm (fork length) and over 150 mm were extracted from the R-base database where catches were archived. Catch data were sorted by numbers of herring collected in each size category, for each month, to examine horizontal and seasonal distribution. The distance from shore of each fish collection (measured using the ship's radar) was plotted to determine the nearshore/offshore distribution. Information on the diel migration of herring was not obtainable from the catch data. Acoustic data sets will be used in the future to assess the three dimensional distribution over 24 hr as well as seasonal time scales.

Aerial Surveys

Broad scale aerial surveys covered PWS and Outer Kenai from Hinchinbrook Entrance to Nuka Point. A survey of the entire area required 3-6 hours of observations daily for 5 days using a Cessna 185 float plane at an altitude of 305 m (1000 ft). When lower altitudes were flown, due to a low cloud ceiling, it was noted in the data base. A hand held GPS connected to a lap top computer with a flight log program recorded latitude, longitude, and time of day on a 2 second interval. At the beginning of each flight the pilot, weather, water visibility, wind, wind direction, tide stage, and other notes concerning the survey were recorded in the log program. Information such as fish schools, approximate surface area of schools, foraging birds or mammals, or other oceanographic features were also recorded on the computer log program. Criteria developed to characterize fish schools closely followed that used by the Alaska Department of Fish and Game for the past twenty years (Lebida and Whitmore 1985; Brady 1987). In 1995, we tried to eliminate tidal effects on the distribution of fish schools by flying during the flood tide whenever possible. Fish school shape was described as round (characteristic shape for herring; Misund 1993), oblong, U-shaped, irregular or streak (jellyfish often from long white "streaks"). Suspected species composition (generally herring, capelin or sandlance for fish schools and kittiwakes or glacous-winged gulls for birds) and any validation (visually identification or fish collection) of school was recorded. Bird behavior was recorded as foraging, resting on water, resting on shore, aggregated tightly on water over school, traveling or flying in a "broad area search". Fish schools sighted during the surveys were counted and roughly categorized by size or approximate surface area of school:

small surface area $< = 50\ m^2$

medium surface area $> = 50\ m^2$ but $< = 450\ m^2$

large surface area > 450 m^2 with an estimate noted

In 1995, the surface area of schools was estimated by categorizing school size. Surface area estimates of schools were made with a sighting tube constructed of PVC pipe with a grid drawn on mylar on the end. The tube is 216 mm and can be calibrated for ground distance covered by reference line (X) for any survey altitude, when length of the grid reference line (L), focal length of the tube (F), and survey altitude (A) are known, by using the equation:

$$X = A ({}^{L} / {}_{F})$$ (Bering Sea Herring Operation Plan: 1981; Brady 1987).

The use of the grid is particularly important for estimating the size of large schools. For elliptical shaped schools, maximum length and maximum width will provide a rough estimate of surface area; for irregularly shaped schools (U-shaped, long wavy bands, etc.) measured length and width of separate sections were combined to calculate a total estimate. Ground reference points of known or easily measurable surface areas, such as a helipad, should be used to train the eye to the scale on the sighting tube grid for any specific altitude flown prior to each survey series.

RESULTS

Prince William Sound was divided into nine regions and all the data was categorized into these regions (Figure 1). Net sampling in 1994 and 1995 and aerial surveys in 1995 revealed spatial and temporal aspects of juvenile herring distribution. Some of the aerial data from May and early June of 1995 is currently being digitized because the log program was not available at the time of recording. Data from acoustic surveys to accompany the net sampling data were not available for incorporation in this report, as they are presently being analyzed. From these preliminary analysis we designed a biologically focused, statistically rigorous survey for the 1996 field season. The following results are preliminary.

Horizontal

In 1995, herring, especially juveniles 0-2 years of age, were found over a wide area based on aerial observations (Figure 2-4). Although juvenile herring were broadly distributed, there seem to be areas where greater numbers are concentrated: 1) Port Gravina in eastern PWS, 2) northern Montague Island and Green Island, 3) southwestern PWS, including Whale and Jackpot Bays and Port Bainbridge, and 4) Resurrection and Aialik Bays in the outer Kenai Peninsula. Orca Bay and Inlet including Middle Ground Shoal may also be an important area and may encompass Port Gravina as the primary eastern PWS rearing area. Visual plots of net catches for 1994 and 1995 provided little information about the horizontal distribution of herring because net sampling was restricted to limited parts of the sound (Willette 95320-A&E; Figures 5-20). However, in areas where net sampling and aerial surveys were both conducted (Figure 2, 19 and 20), observations were generally in agreement.

Temporal

Juvenile herring were most abundant in net catches from June and least abundant in April during the springs and summers of 1994 and 1995 (Figure 21). Prior to 1995, no surveys conducted during other times of the year. From the air, herring appeared to be most abundant in late June through early August (Figures 2-4). The net catch results indicate a seasonal flux of juvenile herring to nearshore waters in PWS starting in May.

Nearshore/Offshore

In 1994 and 1995, there were no nearshore descriptions of hydrography and bathymetry, only distance from shore and depths were recorded. Juvenile herring were most abundant within 200 m of shore in depths less than 50 m based on net collections (Figure 22). Catches were log transformed to reveal trends in the data. From the air, fish schools were generally observed nearshore (Figure 23) and more herring were found inside bays than in passes (Figures 2-4). Large concentrations of herring schools were observed off Green Island, near northern Montague Island. Herring were observed over the extensive shallow shelves (less than 50m in depth) in that region.

In late July to early August, age-0 herring were observed in nearshore waters particularly near Green Island, northern Montague and Snug Corner Cove inside Port Fidalgo.

Diel Vertical

Diel vertical migration of herring was not well documented in the data sets available. Surface schools of small age-0 and -1 herring were observed from the floats of the survey plane in tight schools which would contract and expand in response to visual stimulus. In deeper water, the surface schools would dive in response to the aircraft and foraging gulls at the surface. The acoustic data (Thomas, 95320-N) from areas over a 24 hr period will reveal more detail and will be available in the near future.

1996 Survey Design

Based on results from cruises in 1994 and 1995 and anecdotal information from historic fisheries and ADFG surveys, we have designed a survey for FY96. This survey will focus on collecting data to meet the goals and objectives identified by the Herring Work Group of SEA.

Field Survey Design

Broadscale aerial surveys will continue during the summer when surface schools are visible. When conducting aerial surveys, we will following the same methodology described in this report. The difference between 1996 and 1995 surveys will be the coordination with vessels. We will conduct aerial surveys simultaneously with acoustic surveys and net sampling. In a pilot study, we will use a compact airborne spectrographic imagery system to more accurately assess school surface area and compare this information to trained observers (CASI; Nakashima and Borstad, 1993; Funk et al., in press). This instrument is becoming increasingly cost-effective to use and may become an important tool for long term monitoring

of not only juvenile herring, but other forage fish visible from aircraft (G. Borstad, Seattle, WA, personal communication).

Surveys conducted from vessels will have two basic components: 1) broadscale reconnaissance, and 2) diel investigations at specified sites. Both components will be conducted in each of the nine regions we have identified (Figure 1). During the FY96 field season (Fall 1995 through summer of 1996), we will conduct 10-12 reconnaissance surveys and visit 9-11 diel sites within the nine regions. In 1996, we will complete an analysis of the broadscale distribution data that will allow us to identify critical regions. For the 1997 season, we will reduce the numbers of reconnaissance and diel surveys to 6 focusing on the critical regions. Our goal is to conduct one reconnaissance and one diel survey in each region identified for study. Each set of a reconnaissance and a diel survey will take two days to complete.

During broadscale reconnaissance surveys, the objective is obtain regional scale relative abundance indices of juvenile herring and other fish species using fishing and scientific acoustics. The species making up the acoustic targets will be identified using net collections. Reconnaissance surveys will be conducted over a 12 hr period. Surface migrations of pelagic fishes at night has been well documented (Mais 1974). In PWS, adult herring are more easily enumerated using acoustics at night (DeCino et al). During the fall and spring surveys, the entire reconnaissance survey period is dark. During the summer surveys, the 12 hr survey period will encompass sunset, dark of night and sunrise. The reconnaissance surveys consist of an acoustics vessel, a seiner, trawler and fish processing vessel. During the 12 hours, the acoustic vessel will be mounted with a horizontal scan, vertical (20 degree, 50 KHz), and Biosonics 70KHz Digital and 200 KHz Analog sonars (Thomas, 95320-N) and driven in a series of onshore and offshore zig zags. The vessels will proceed from a maximum offshore distance of one nautical mile and approximately 200 m depth to a minimum onshore depth of 10 m. The seiner and trawler will be dispatched to sample aggregations or layers of fish for verification of acoustic targets. A maximum random subsamples of 1000 fish of each species collected will be measured (fork length) and used to translate acoustic targets into individuals per meter cubed. A random subsample of 450 herring from each herring aggregation will be collected for measurements of weight and age in addition to the length measurements. GPS coordinates will be logged on laptop computer software and schools or acoustic layers observed on the horizontal scan or vertical sonars will be recorded while maintaining a vessel speed of approximate 9-11 knots. When a concentration of targets that look like herring are observed, the vessel will slow to 4-6 knots and the acoustic sonars will be deployed. This design will survey a much larger area as acoustic measurements of target density will only be collected in the presence of targets. In areas surveyed using the horizontal scan, but without data from the Biosonics gear, density of the target species is assumed to be near zero with a threshold density established by measurements taken with the scientific sonar. Both data sets, from the horizontal scan or Biosonics gear will be used in the broadscale distribution analysis. For statistical treatment of potentially dependent transects collected in a zig zag manner, zigs will be separated from zags and treated as replicates (for a more detailed description of treatment of acoustic data see

Thomas, 95320-N). The result will be two matching sets of parallel offshore and onshore transects.

At the diel sites, identified as juvenile herring aggregation areas within a region, an 12-18 hr survey will be broken into four 2-4 hr sampling periods. Diel sites will be approximately 10 to 20 km^2 in surface area. A site will consist of an entire bay or section of shoreline along an island or in a pass. The first sampling period will begin at sunset (1800 hours). The second period will occur during the early evening (2200 hours). The third period will occur during the late night (0200 hours). The fourth period will begin at sunrise (0800 hours). A light meter will be deployed on shore or on the crow's nest of the acoustics vessel to measure light level for 5 minute intervals. The vessel configuration for diel surveys will consist of an acoustic, a seiner, a nearshore trawler, a nearshore oceanography and a fish processing vessel.

The acoustics vessel will be outfitted with the two frequencies used for the reconnaissance survey as well as a 420 KHz vertical system. During each 2 hr sampling period, a series of parallel acoustic transects will be conducted perpendicular to shore. The minimum number of transects needed for statistical significance for biomass estimates or relative abundance is 12 (Thomas, 95320-N). A single nearshore transect parallel and close to shore will also be conducted to provide more detail for the description of nearshore distribution using primarily the side looking mounted sonar. The methods for processing this data are currently being worked out by Dick Thorne, Biosonics, Jay Kirsch, Prince William Sound Science Center (PWSSC; 95320-N) and Ken Coyle (APEX Predators), UAF. The chief scientist aboard the acoustic vessel will identify fish schools for target verification and dispatch the seiner or trawler to sample them.

The seiner will be dispatched one half hour after the start of each 2 hr sampling period to sample the upper 30 m of the water column. The seiner will use two 200 m long anchovy seines with stretched mesh size of 25 mm, one 35 m and one 20 m deep. The trawler will sample deeper layers. The trawler will use with a 400 mesh eastern trawl with approximately 300 kg, 1.52 X 2.13 m Nor' Eastern Astoria V trawl doors. Headrope and footrope lengths of 21.34 and 28.96 m respectively. The estimated fishing height and width of the net are 2.74 and 12.20 m respectively. This trawl has 10.16 cm mesh in the wings and body, 8.89 cm in the intermediate and cod end, and a 3.18 cm cod end liner. A net sounder will be attached to the head rope of the trawl to provide information on fishing depth and size of opening of the trawl. The trawler will also have a 3 1.0 mm NIOs (Tucker trawl) with cable, double messenger system, and codends to validate smaller targets. Trawls will be towed a distance of 1.85 km (1 nautical mile) measured with the ship's GPS. The trawler will keep a constant speed of 4.6 km per hour (2.5 knots). Start time for fishing will be recorded when the net is set at a specified length of cable and depth. Stop time will be recorded as soon as cable retrieval has begun. Samples will be collected according to protocols outlined in a cruise plan.

The nearshore oceanography vessel will follow an independent set of protocols during the 18 hr diel. They will define nearshore versus offshore areas using oceanographic descriptions, define physical boundaries of the nursery areas, and assess the vertical structure of the water column through the tide cycle. Detailed methodology for nearshore oceanography sampling is being developed cooperatively with Vaughan and Gay, SEA Oceanography (95320-M). This vessel will also be used to collect discrete zooplankton samples. The equipment on board will include 2 Seabird CTDs, an Acoustic Doppler Current Profiler (ADCP), an Optical Plankton Counter (OPC) with continuous measuring CTD both mounted on an aquashuttle for towing, and 2 .3 mm mesh vertical plankton nets. The ADCP will be mounted to the side of the vessel. The vessel will follow transects designed to meet the sample objectives at about 11-15 km per hour (6-8 knots) using the ADCP and OPC. After each set of continuous transects, the vessel will go to a series of discrete sites for CTD and zooplankton casts. In addition to visiting each diel site and collecting descriptive habitat data, the oceanography vessel will sample four sites of similar dimensions to the diel sites to provide information on habitats void of herring.

Finally, the fish processing vessel will be dispatched to a fixed location and set up for the processing of samples from the two fishing vessels. During reconnaissance surveys, the processing vessel will head to a location well ahead of the reconnaissance fleet and anchor. Half way through the reconnaissance survey, catches from the fishing boats will be deliver to the processing vessel. The remainder of the samples will be delivered at the end of the reconnaissance. All vessels will converge at the diel location following the end of the reconnaissance, anchor and rest. At the end of each 2 hr sampling period during the diel survey, the catch from each fishing vessel will be delivered to the processing vessel. Samples will be processed according to the protocols listed in the cruise plan.

Planned Data Analyses

There are three categories of analysis planned for the data collected during this survey (Figure 24):

1) analysis of broadscale (nine regions PWS to Outer Kenai) spatial and temporal distributions using aerial and acoustic data.

2) habitat analysis, comparing biological and relative abundance indices of juvenile herring with biological and physical habitat characteristics from each diel site. The analysis will be conducted on three time scales: 24 hr, seasonally, and annually.

3) diet and feeding analysis.

Horizontal broadscale distribution

The acoustic/net collection data will provide mean values of several biological indices on different spatial scales (km^2, diel sites, regions). These indices include average density (g/m^3 and individual/m^3) of acoustic herring targets (verified by net sampling as juveniles herring during reconnaissance surveys), size at age, and condition indices (Fulton's index

and energetic content; Paul, 95320-U), and surface area of schools spotted from summer aerial surveys. Position, numbers and sizes of schools will continue to be recorded to calculate the aerial extent of herring within each sample region. We will use power analysis to estimate appropriate sample size and examine the problems of autocorrelation (randomizing the data being one possible solution). We will then apply ANOVA procedures to detect significant differences in the biological indices between regions and regression analysis.

The aerial survey data (CASI and trained observers) will provide spatial distribution information of schooling fish, primarily herring. These two techniques will be compared to determine their precision and accuracy. It will then be possible to divided these observations into a nested quadrat design, randomize the data on different spatial scales and apply quadrat techniques to interpret these data (Gunderson 1993). Using quadrat analysis it will be possible to describe the observed spatial distributions statistically and compare them to random (Poisson), contagious (negative binomial) or regular distributions. It will also be possible to determine mean surface area of fish schools on different temporal scales, such as, tidal phase, day time, seasonal, and spatial scales, such as, km, diel sites (bays verses passes), regions, and PWS. Examining these spatial distributions on temporal and seasonal scales will lead to the underlying physical and biological parameters influencing these distributions. Once these distributions and the parameters influencing them are determined it will be possible to estimate their effect on herring survival and recruitment into the PWS stock.

Habitat analysis

For this analysis we will use a series of multivariate techniques in an iterative process using data collected at the diel sites. We will follow a nested design using three time scales starting with 1) annual means for sites, 2) followed by monthly or seasonal means, and 3) finishing with means within a 24 hr time period. In this manner, important processes affecting biological production operating within each of these time scales will be identified. Multivariate procedures may include principal component analysis to reveal selectivity patterns, clustering, ordination procedures, canonical correlation analysis and ending with multiple regressions. Biological indices used as dependent variables will include size and age, feeding analysis, condition indices, carbon nitrogen ratios, delta C-13 values, and relative abundance from acoustic and aerial surveys. Independent variables include zooplankton concentrations (all time scales), CTD data (all time scales), surface area to flow ratios for nursery areas (12 months), current profiles and tidal flows (all time scales), regional oceanographic descriptions (12 months), characterization of vertical structure in water column (12 months), relative abundance of competitors (age 0-1 pollock, all time scales), and abundance of marine mammals and sea birds (all time scales).

Diet and feeding analysis

Data on juvenile herring feeding behavior will be collected on several spatial and temporal scales. Juvenile herring during each diel sample will be dissected and their stomach contents will be identified. Thus it will be possible to determine if vertical migration occurs primarily for feeding and how much daily energy is acquired. It will also be possible to

correlate this data with the age-weight-length, oceanographic and isotope data and condition indices to compare spatial (diel sites, regions) and temporal (seasonal and annual) variations.

We will work with other investigators in the herring working group of SEA to use the results of these analyses to develop the model products (see summary of model components in discussion). These results will provide critical input for those models.

DISCUSSION

We were able to determine the 1995 summer broadscale horizontal distribution of juvenile herring in PWS through the results of the aerial survey. This data set provided limited information on juvenile herring as net collections and acoustic surveys were not conducted simultaneously and the 1995 field surveys focused on salmon, which required a different sampling protocol. Visual plots from net catches (Figures 5-20) were deceiving since net surveys were concentrated in the western corridor and three discrete sites around the Esther hatchery in 1995. Because the net sampling of 1994 and 1995 occurred largely in passes and in the mouths of bays, it may be that many of juveniles farther in the bays were not sampled. Analysis of the acoustic data from 1994 and 1995 (collected simultaneously to net catches) will reveal more detail about relative abundance and vertically distribution in the limited areas herring were sampled.

Juveniles were seasonally abundant during the months of April, and June through to early August in 1994 and 1995. Age-0 herring were observed by aerial survey to recruit to nearshore waters in late July and early August, presumably just after metamorphosis (based on trawl surveys from 1989; Norcross et al. submitted). Presently the overwintering distributions of juvenile herring in PWS are unknown, however, our surveys in October 1995 and March 1996 will begin to describe these distributions.

A detailed analysis of the physical characteristics of each area, especially oceanographic qualities (salinity, temperature, bathymetry, current profiles, mixing), will help us describe juvenile herring nearshore habitats. Preliminary observations indicate that herring tend to rear in areas of significant tidal mixing where food is entrained, but which provide adequate shelter over shallow shelves (S. Gay, 95320-M, personal communication).

Juvenile and adult herring appear to undergo significant diel migrations cued by light levels (Evans et al. 1975). In the summer, with high light levels most of the day, herring may spend their day avoiding predators and their relatively short lived periods of dark feeding on zooplankton. In Zaikof Bay on northern Montague Island, juvenile herring were observed on the bottom or schooled in tight balls close to the surface. At dusk, herring seem to spread out over a wider area to feed. During daylight in the summer, we observed a school spreading out to feed until the school received a visual stimulus, such as shadow from our airplane or diving bird, caused them to reform a tight school and flee. Analysis of

acoustic data as well as better designed surveys will enable us to document this vertical aspect of juvenile herring distribution and determine its underlying biological significance.

We were able to develop a survey design targeted to answer questions posed by SEA about the early life history of juvenile herring. This was largely based on the aerial survey data and data provided by the first two years of related projects in SEA. We have also been able to develop a SEA herring research direction because of the formation of working groups within SEA. A Herring Working Group has developed a conceptual herring recruitment model which will guide our next 3 years of research.

A Pacific herring recruitment model is being developed by integrating various submodels, each of which focuses on an early life history stage. We hypothesize that, like other clupeids, year-class strength of Pacific herring in Prince William Sound (PWS) is determined during its early life history (Blaxter and Hunter, 1982; Stocker et al. 1985). All field and laboratory experiments for all involved components of SEA in FY96-97 will relate to one or more of these submodels. Two major SEA hypotheses are the focus of these submodels and will be linked within the overall herring recruitment model. The Lake/River hypothesis applies to transport and distribution of herring at the larval stage. We will use the Ocean Dynamics Model (Mooers and Wang, 95320-J) to conduct simulations of larval drift to predict the distribution of age-0 herring within PWS. We expect to be able to examine various drift patterns in response to simulated lake (i.e. retention), river (i.e. rapid movement through the sound), and combinations of varying amounts of "lake" and "river" in accordance with the recent evolution of the lake/river hypothesis. The Herring Overwinter Survival Model is based on the hypothesis that survival of herring through their first winter is key to survival and ultimate year-class strength of juvenile herring and is dependent upon the condition juvenile herring and overwinter ocean conditions. We will approach this hypothesis by examining the distribution and condition of herring in the fall, throughout the winter and again in the spring. We hypothesize that there will be a decline in condition indices over the winter. We further hypothesize that differences in the fall condition indices of juvenile herring between areas is related to geographic location and the physical and biological conditions that characterize these locations. The Summer Habitat Model will compare the relative abundance and condition of juvenile herring between locations in which habitat characteristics have been documented to determine "optimal" versus "suboptimal" nurseries. The Summer Habitat Model will link with the Overwintering Survival Model and together they will produce a Herring Recruitment Model for the first two years of the life history of PWS herring.

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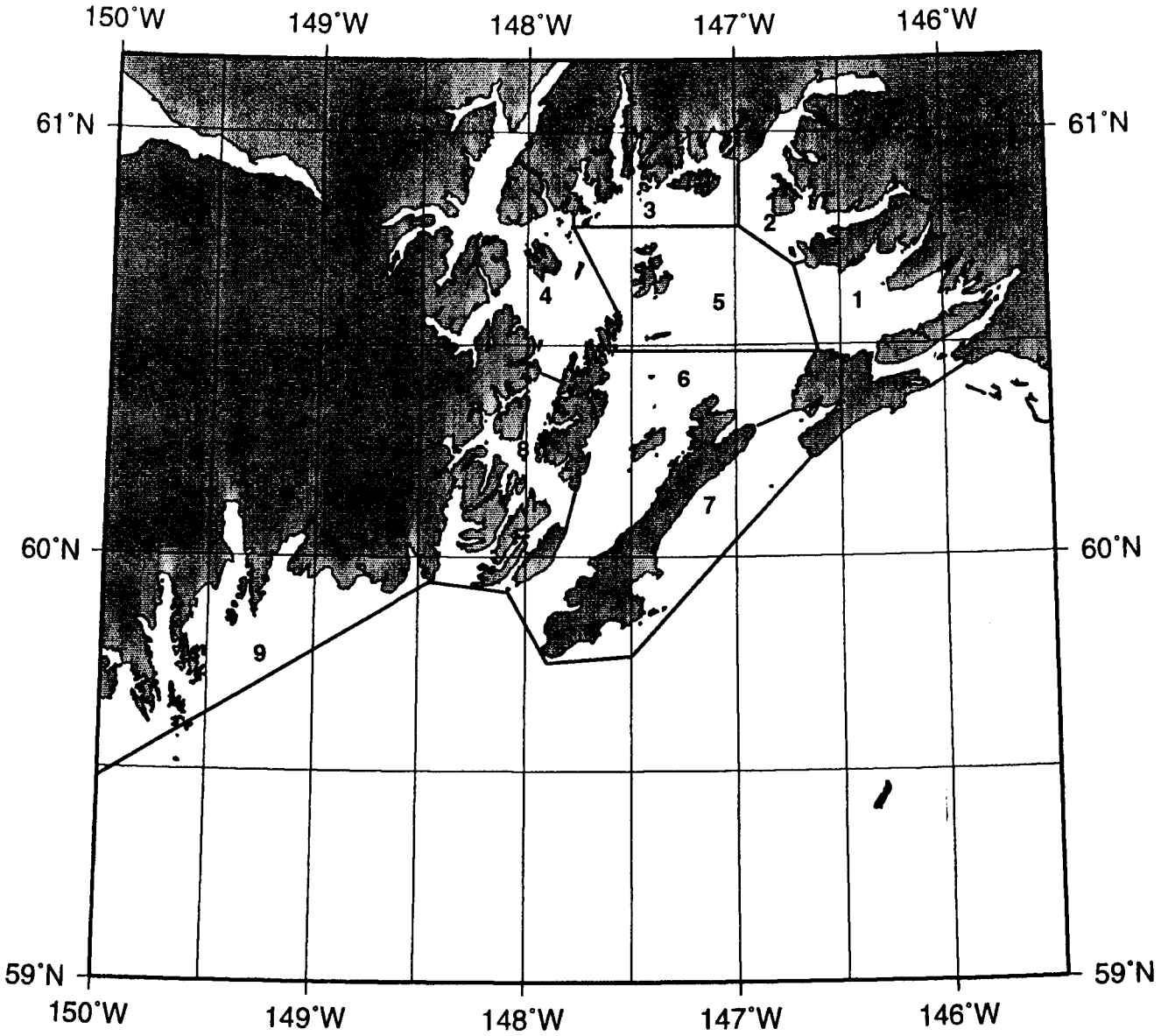

Figure 1. Regional divisions of Prince William Sound for the analysis of broadscale Pacific herring distribution.

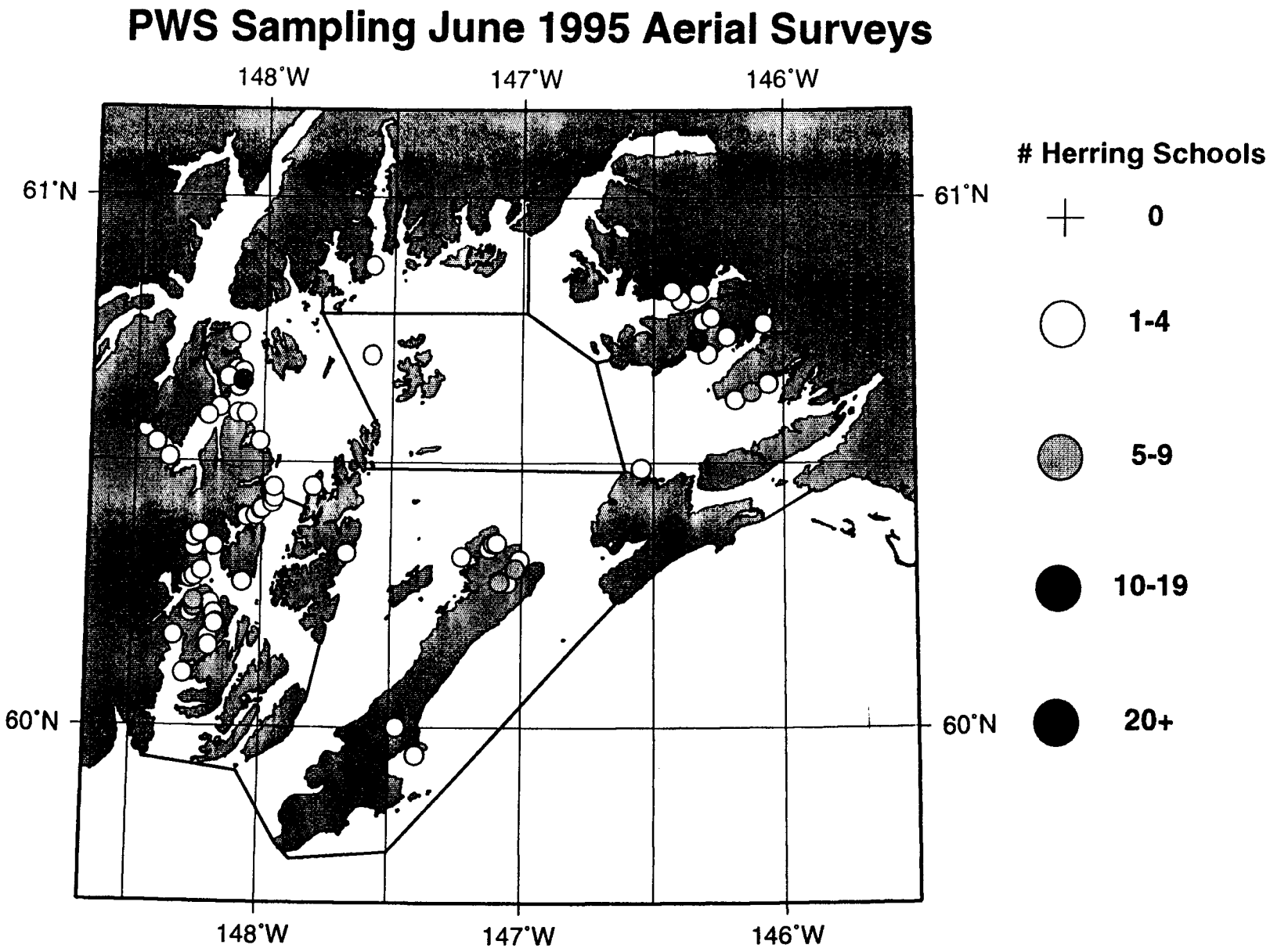

Figure 2. Distribution of Pacific herring schools in Prince William Sound in June, 1995.

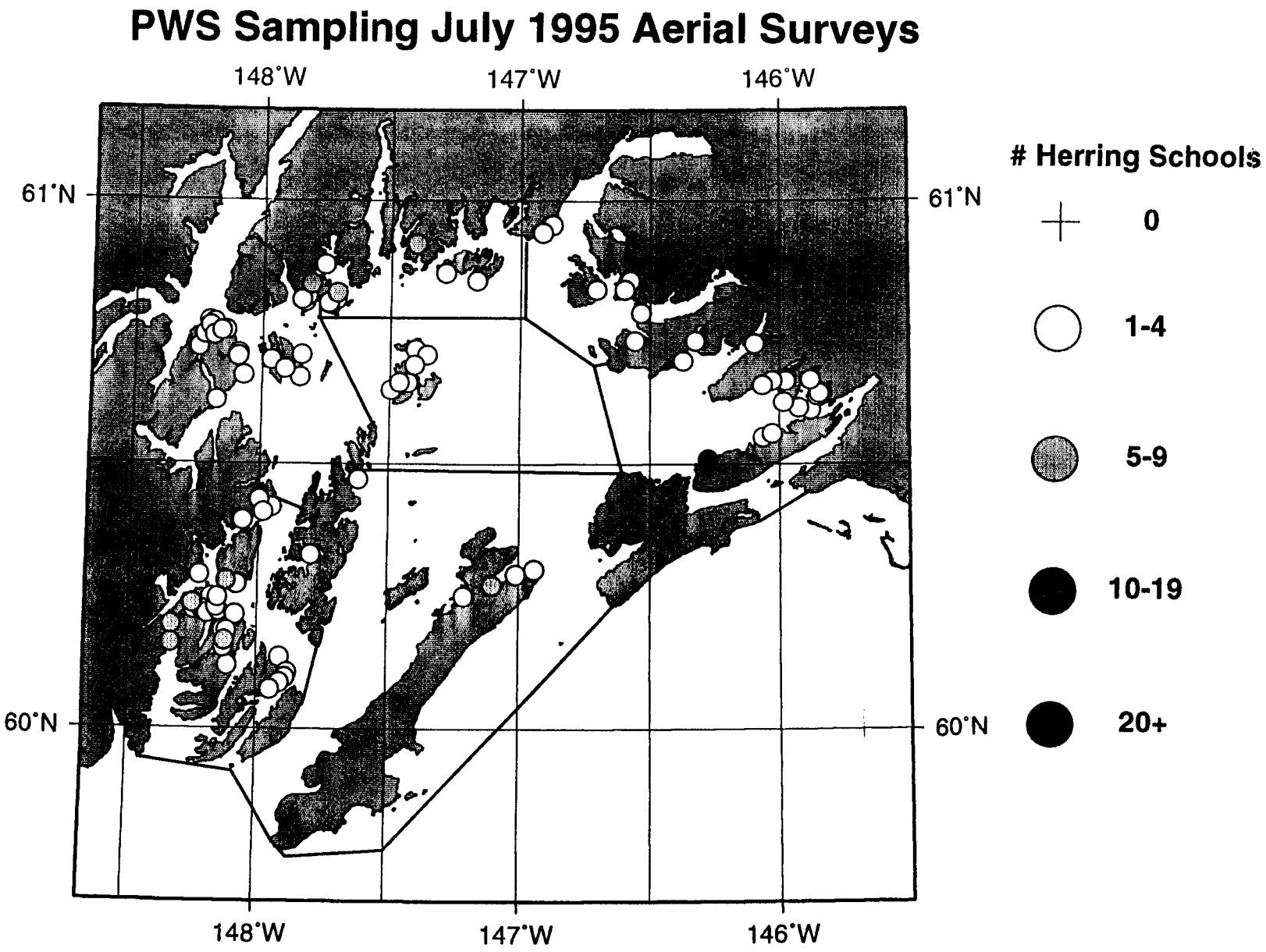

Figure 3. Distribution of Pacific herring schools in Prince William Sound in July, 1995.

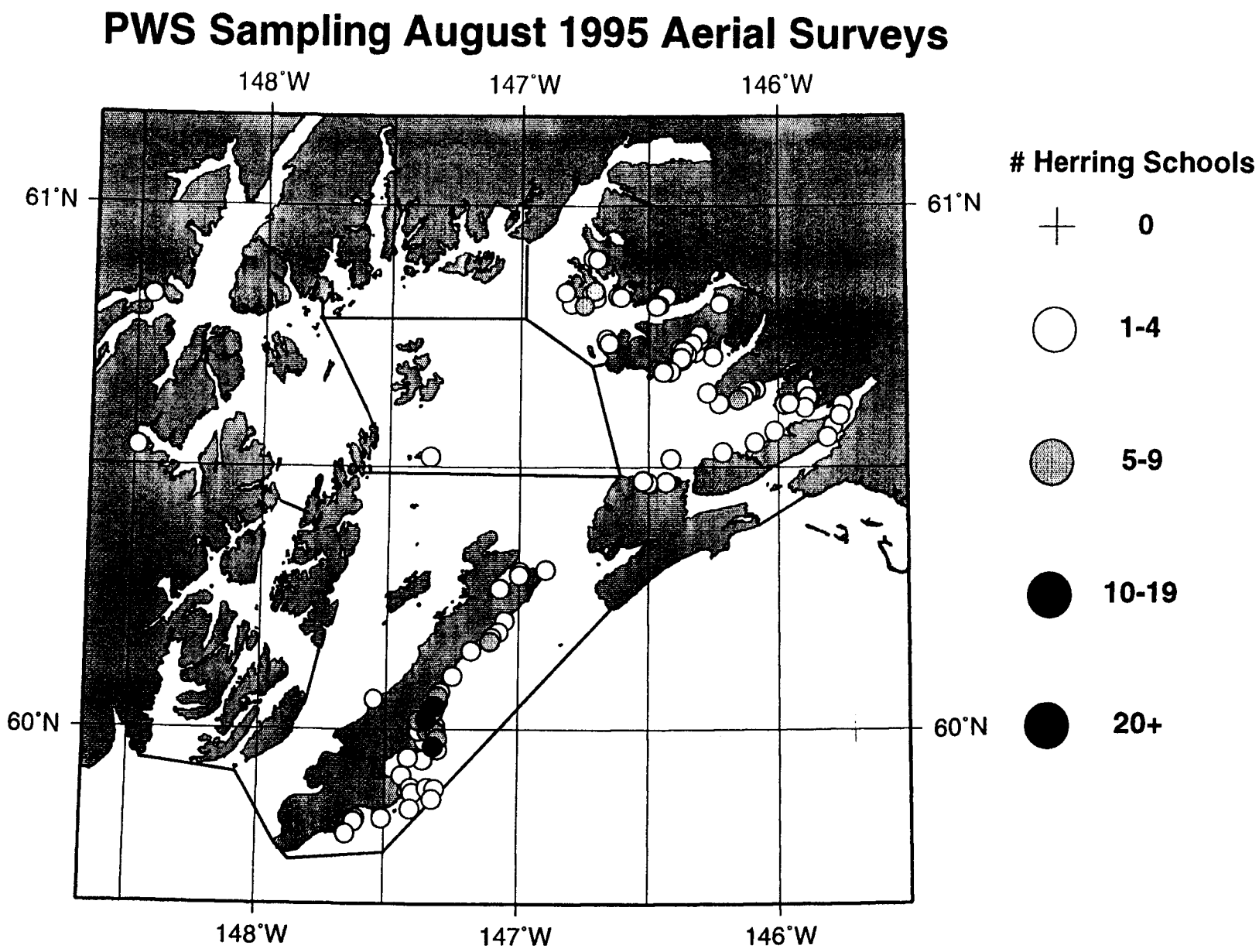

Figure 4. Distribution of Pacific herring schools in Prince William Sound in August, 1995.

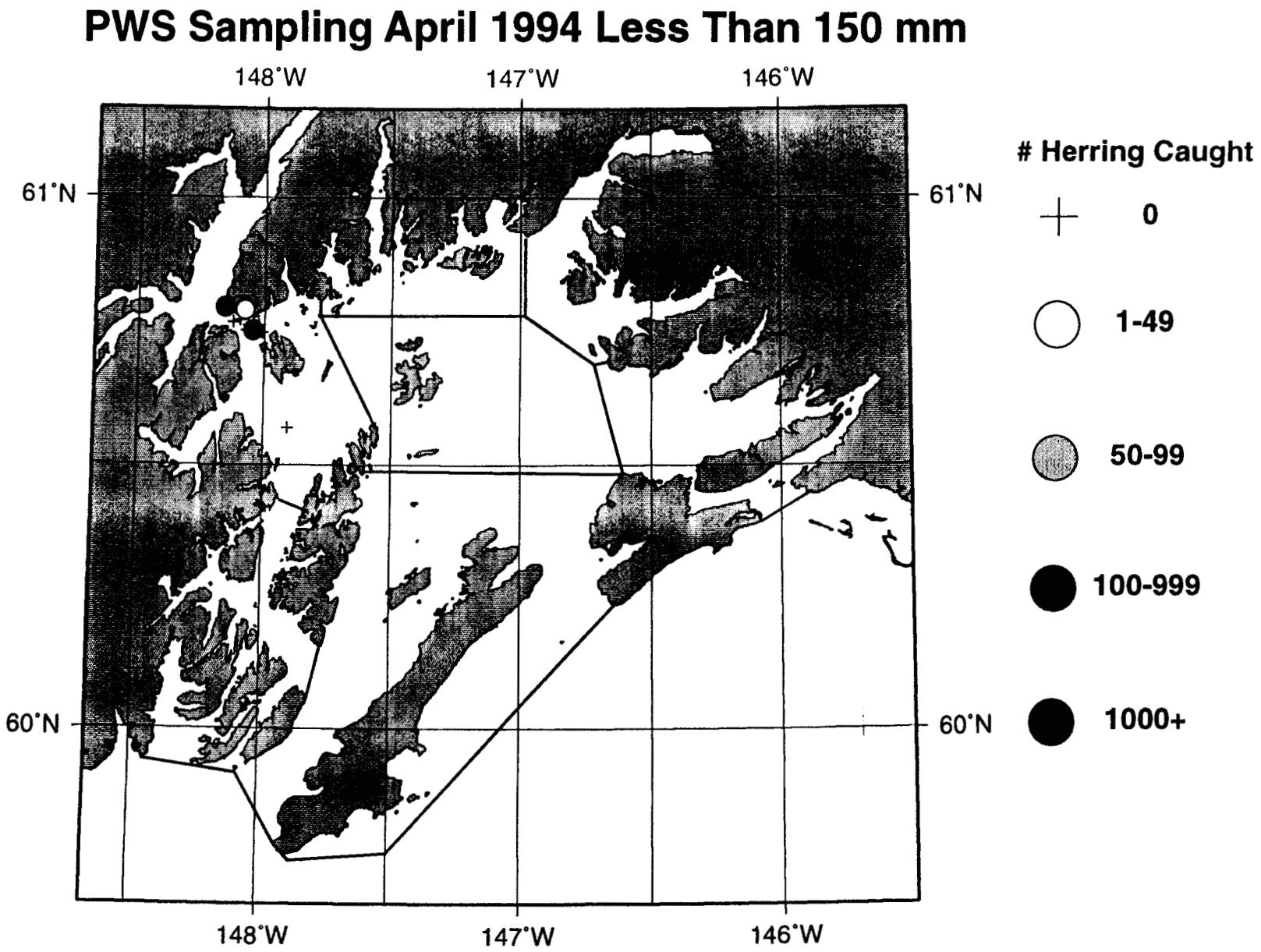

Figure 5. Net catches of Pacific herring less than 150 mm fork length on the west side of Prince William Sound in April, 1994.

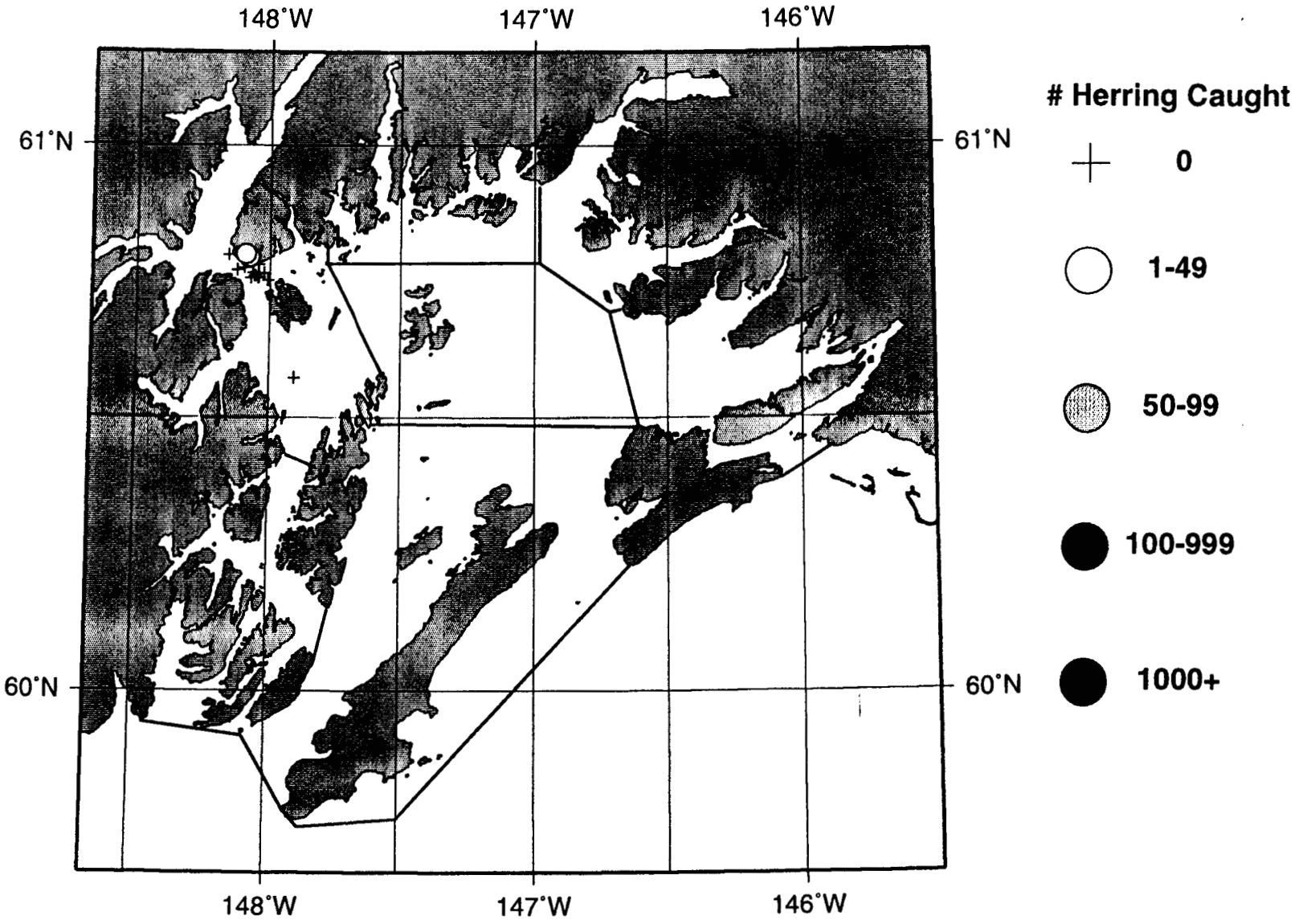

Figure 6. Net catches of Pacific herring greater than or equal to 150 mm fork length on the west side of Prince William Sound in April, 1994.

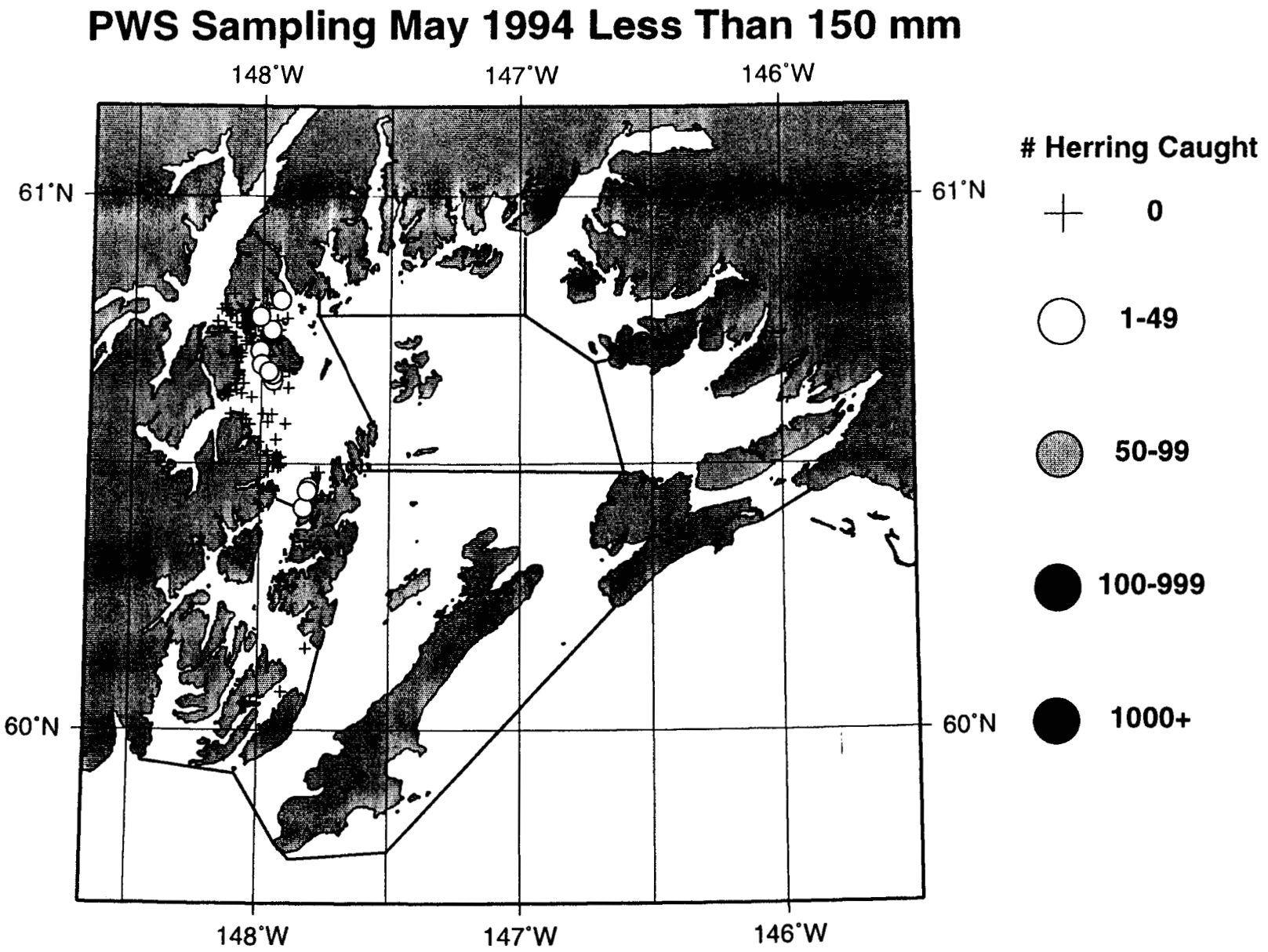

Figure 7. Net catches of Pacific herring less than 150 mm fork length on the west side of Prince William Sound in May, 1994.

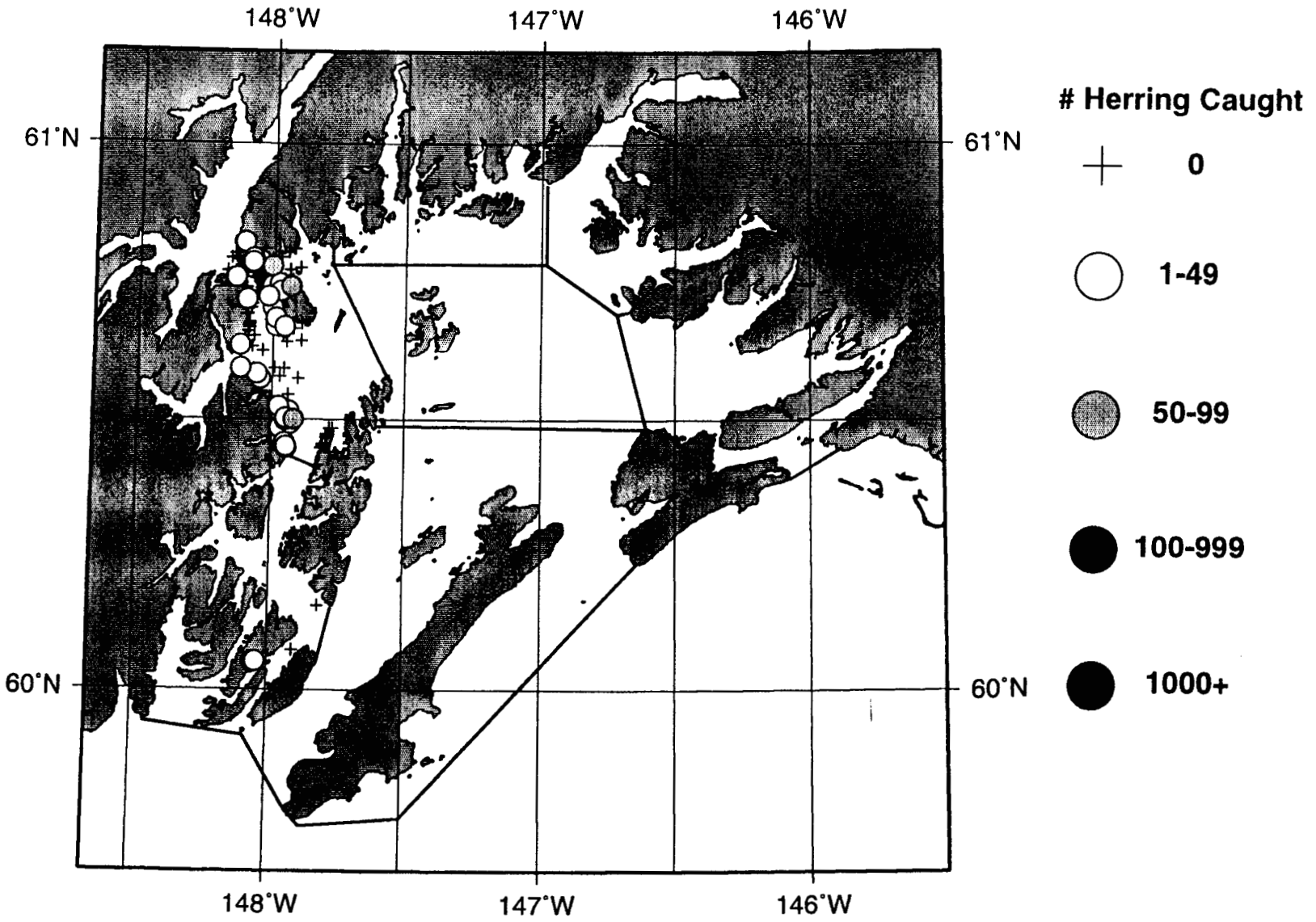

Figure 8. Net catches of Pacific herring greater than or equal to 150 mm fork length on the west side of Prince William Sound in May, 1994.

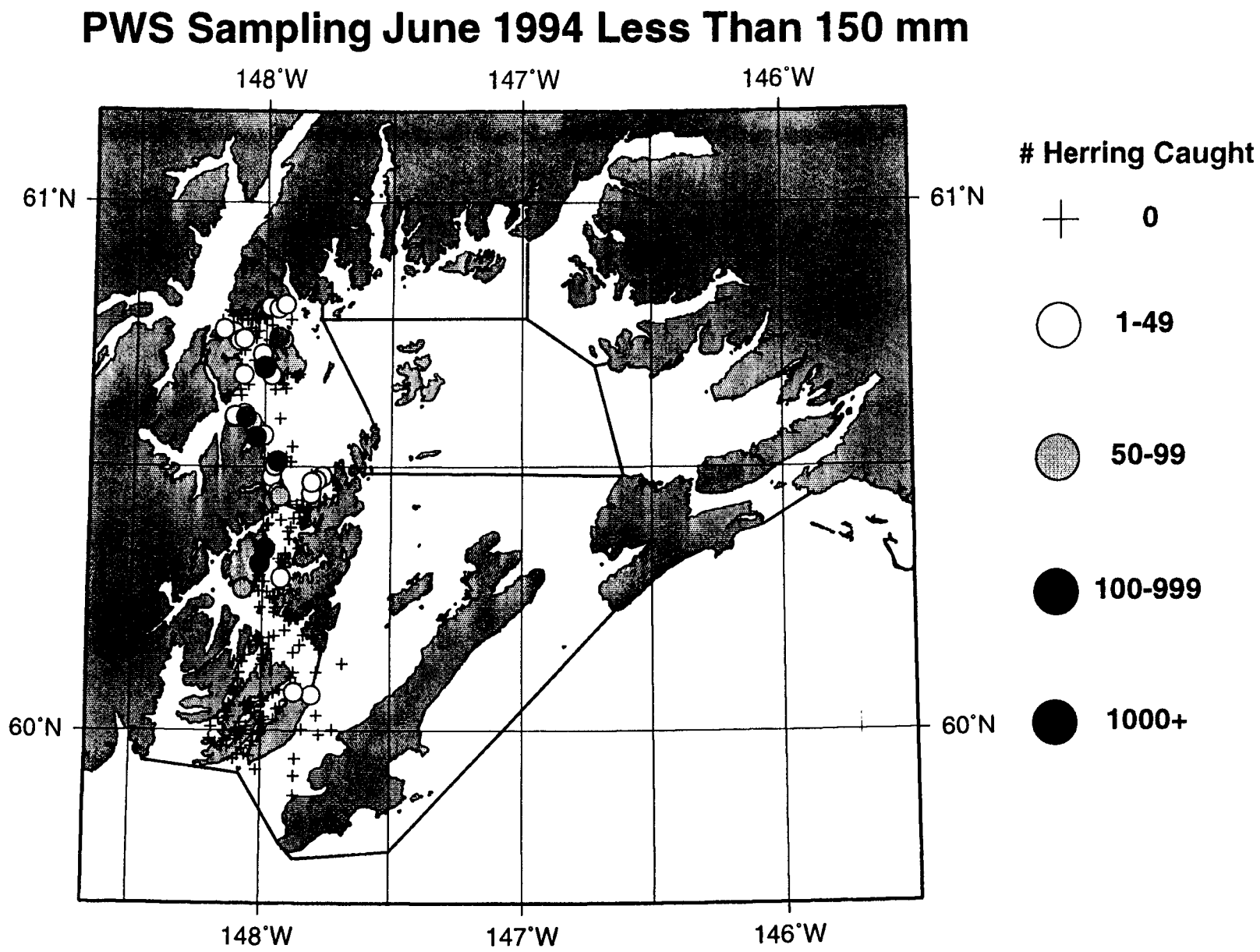

Figure 9. Net catches of Pacific herring less than 150 mm fork length on the west side of Prince William Sound in June, 1994.

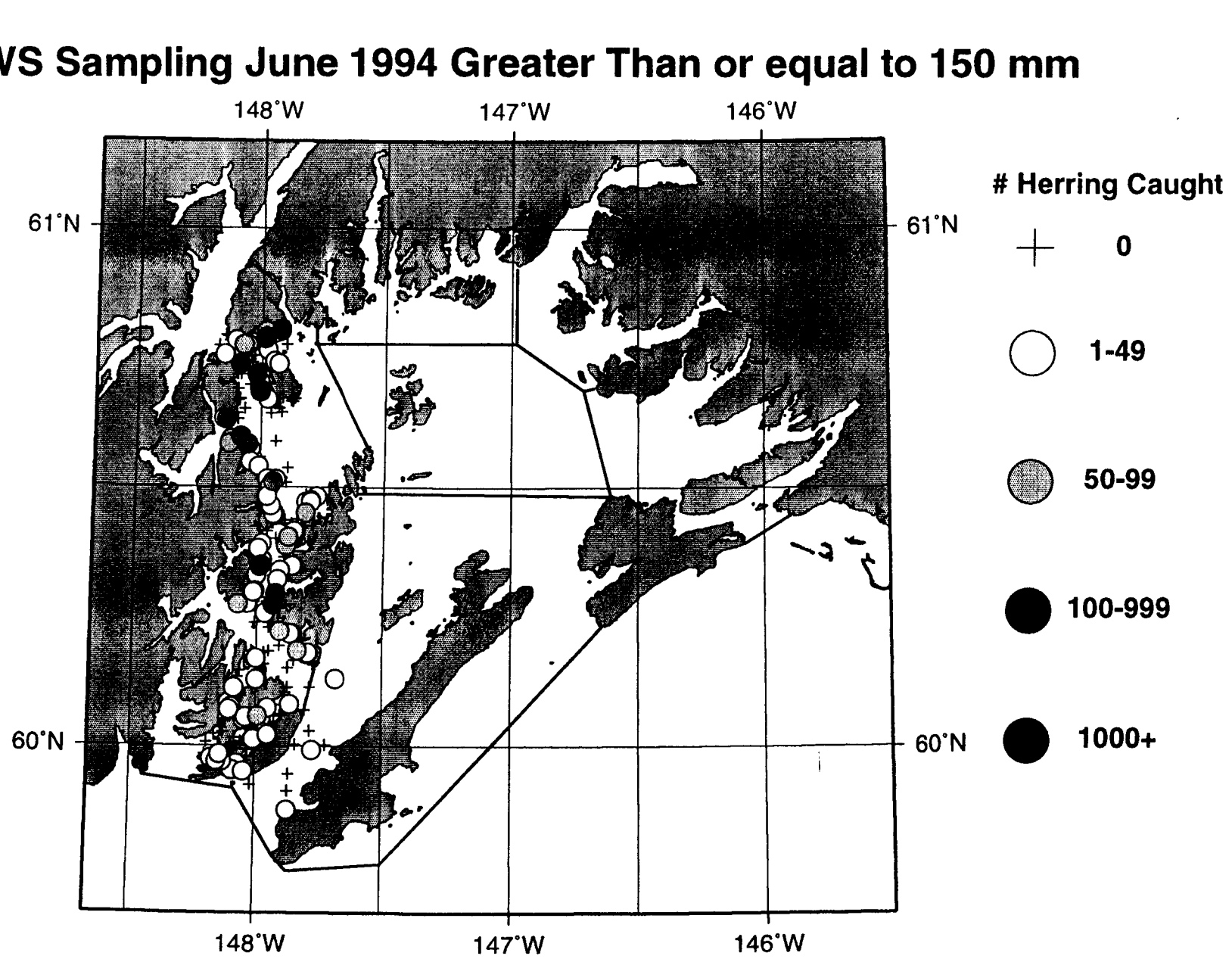

Figure 10. Net catches of Pacific herring greater than or equal to 150 mm fork length on the west side of Prince William Sound in June, 1994.

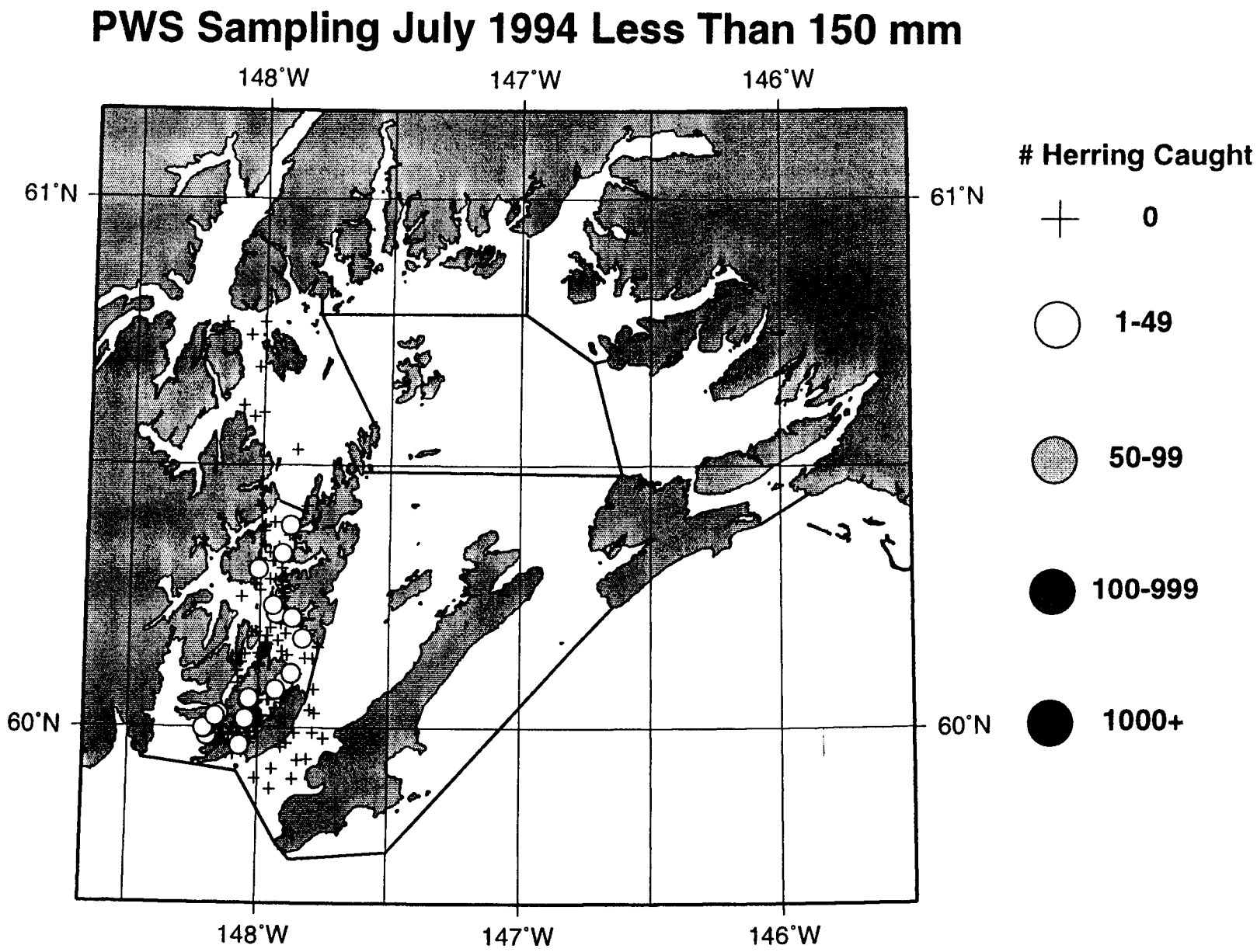

Figure 11. Net catches of Pacific herring less than 150 mm fork length on the west side of Prince William Sound in July, 1994.

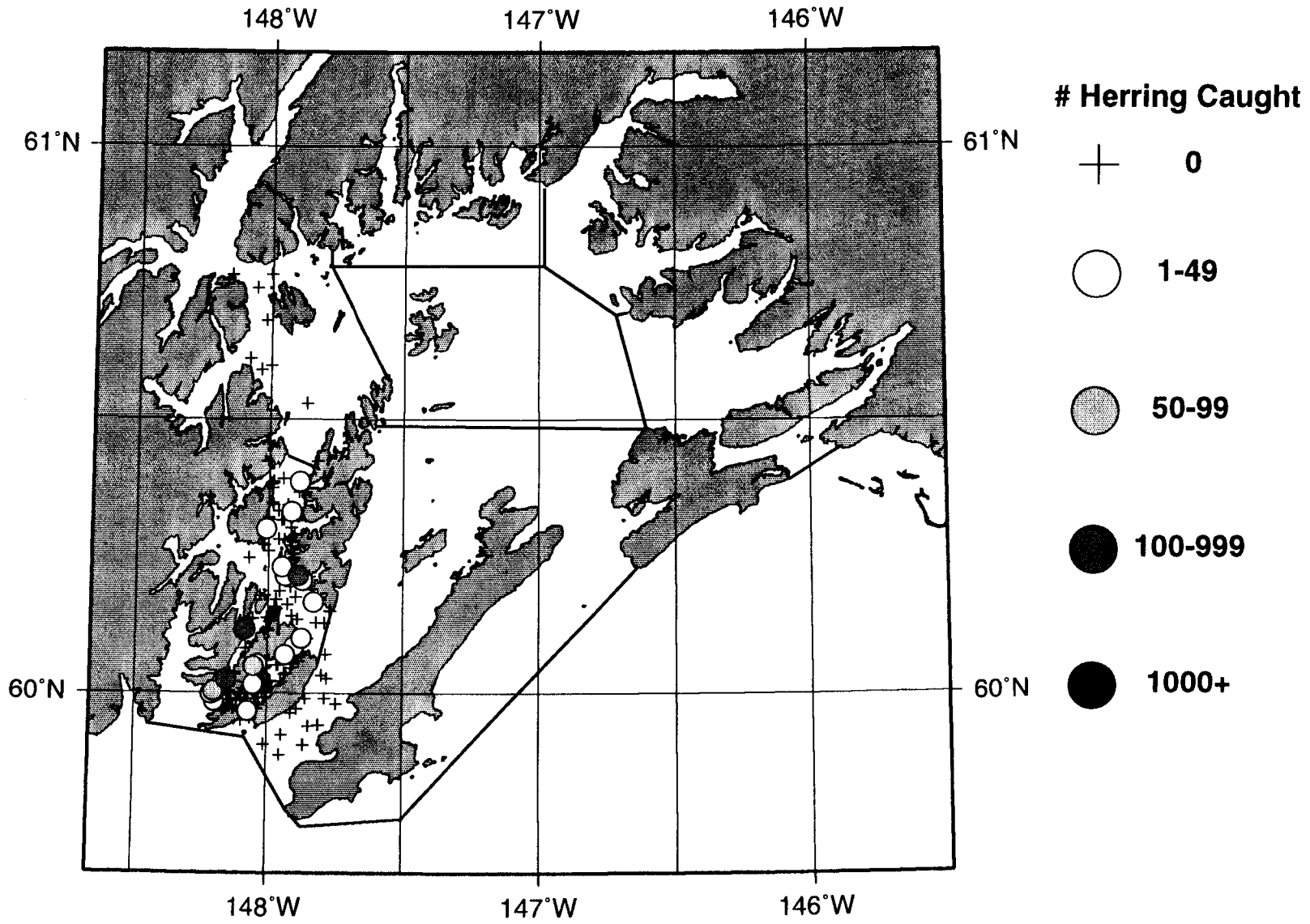

Figure 12. Net catches of Pacific herring greater than or equal to 150 mm fork length on the west side of Prince William Sound in July, 1994.

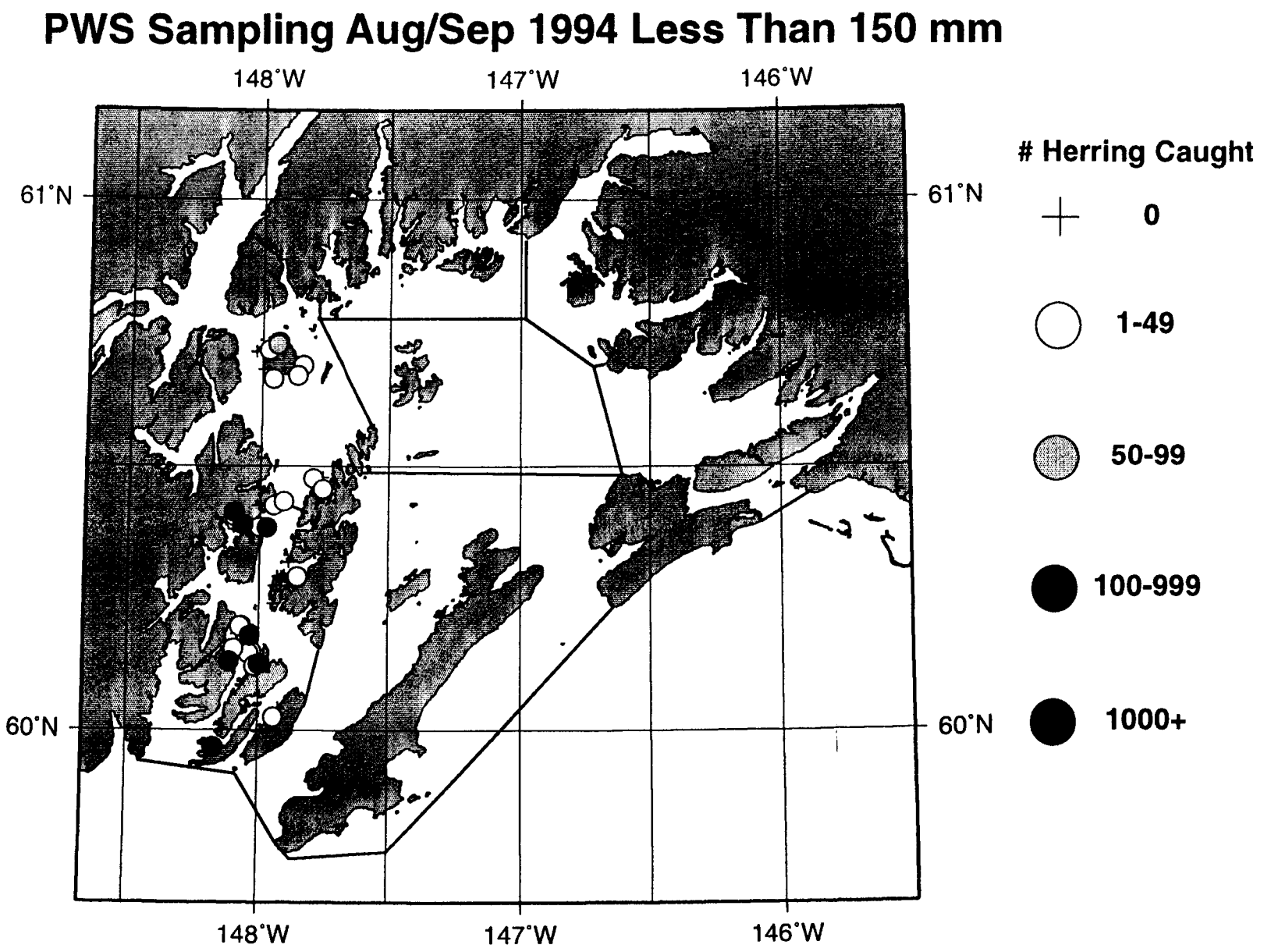

Figure 13. Net catches of Pacific herring less than 150 mm fork length on the west side of Prince William Sound in May, August through September, 1994.

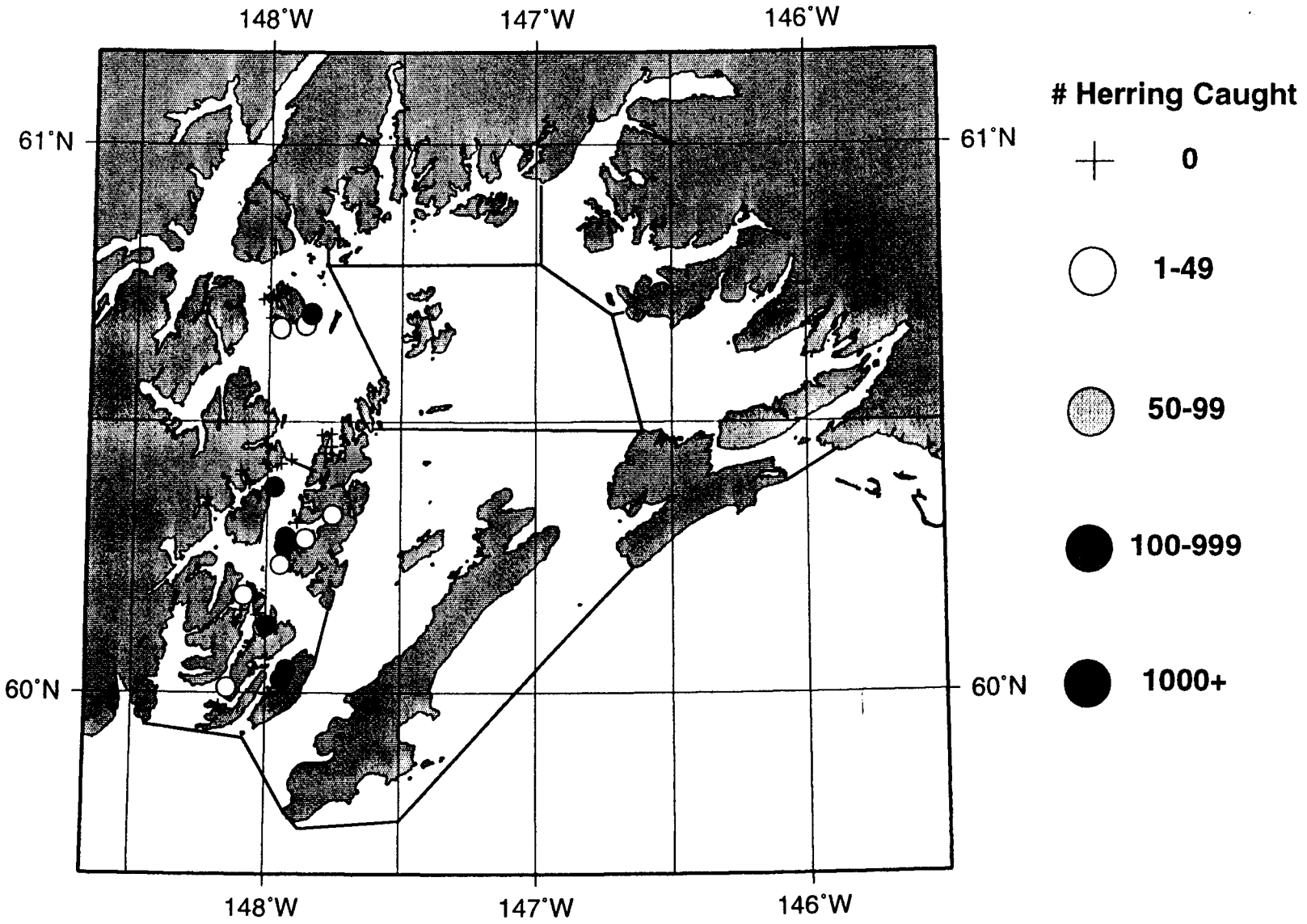

Figure 14. Net catches of Pacific herring greater than or equal to 150 mm fork length on the west side of Prince William Sound in August through September, 1994.

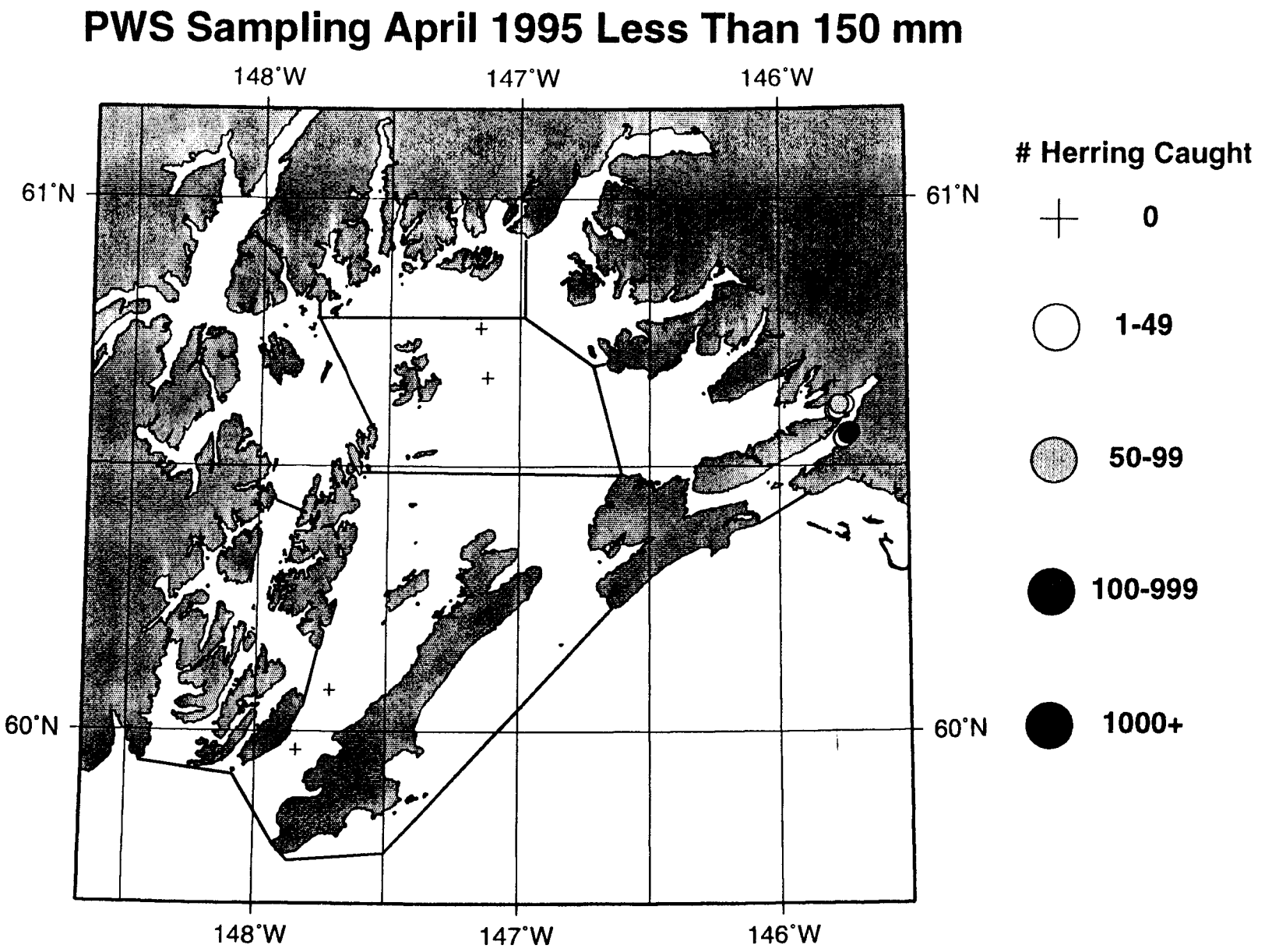

Figure 15. Net catches of Pacific herring less than 150 mm fork length on the west side and other selected sites within Prince William Sound in April, 1995.

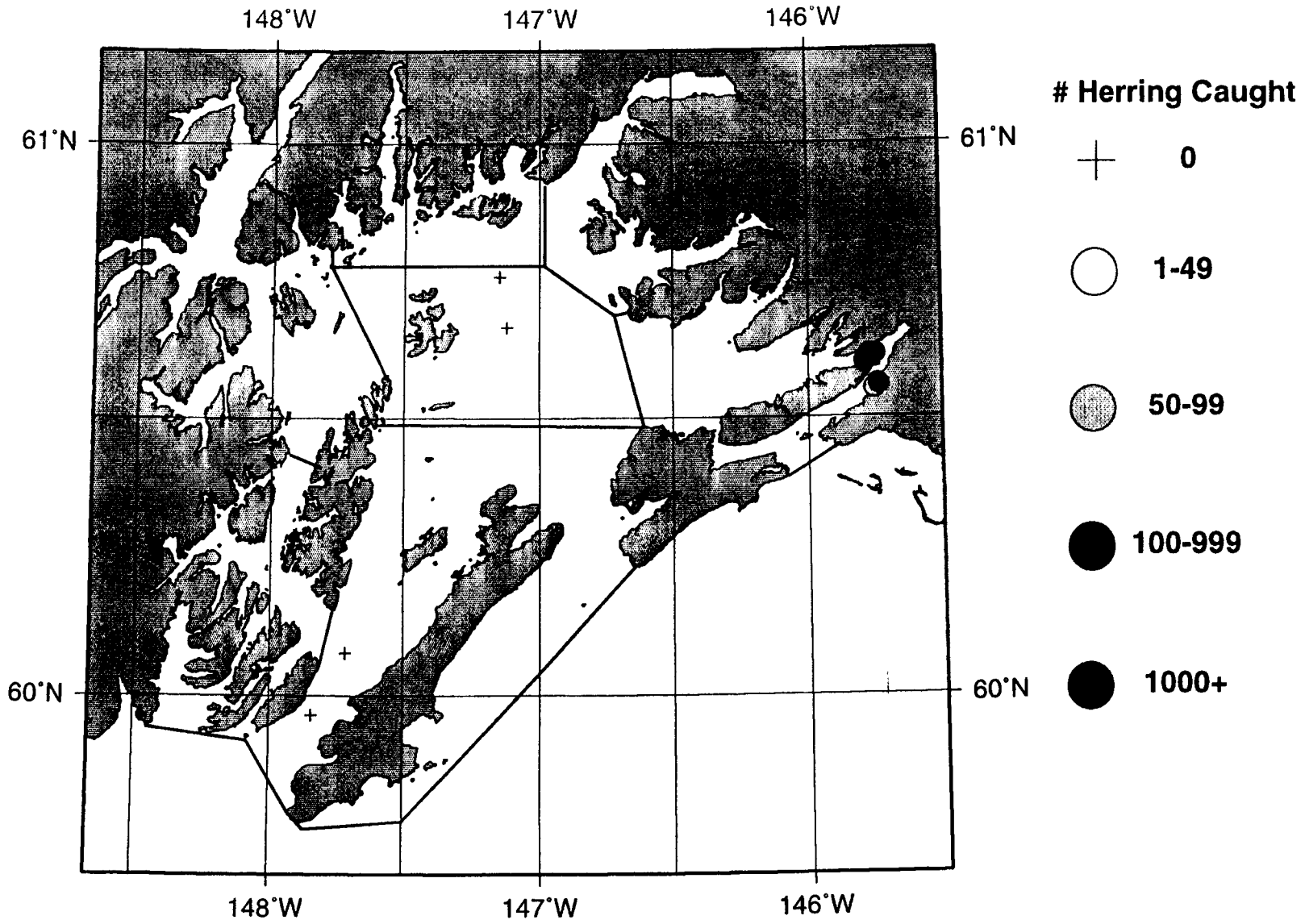

Figure 16. Net catches of Pacific herring greater than or equal to 150 mm fork length on the west side and other selected sites within Prince William Sound in April, 1995.

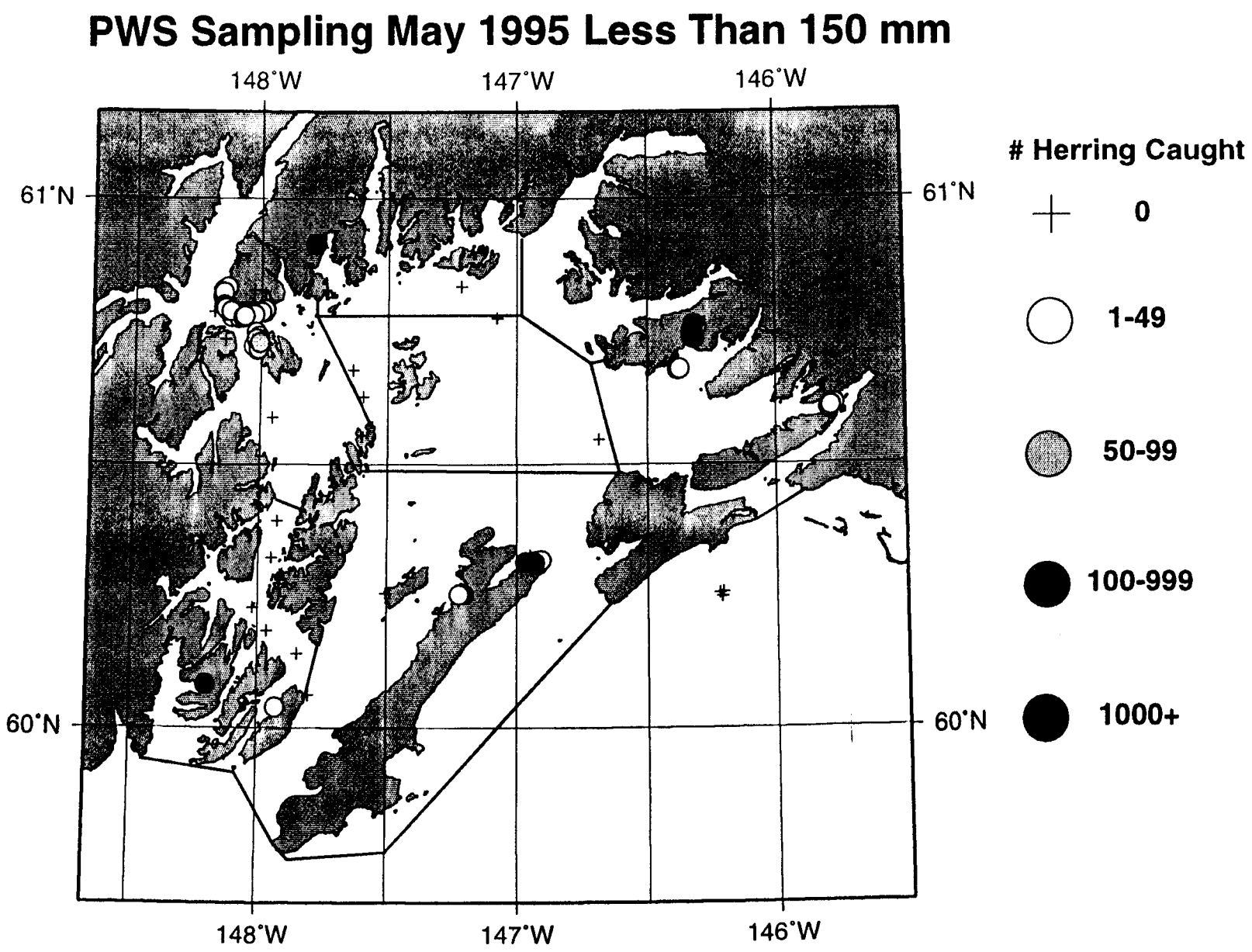

Figure 17. Net catches of Pacific herring less than 150 mm fork length on the west side and other selected sites within Prince William Sound in May, 1995.

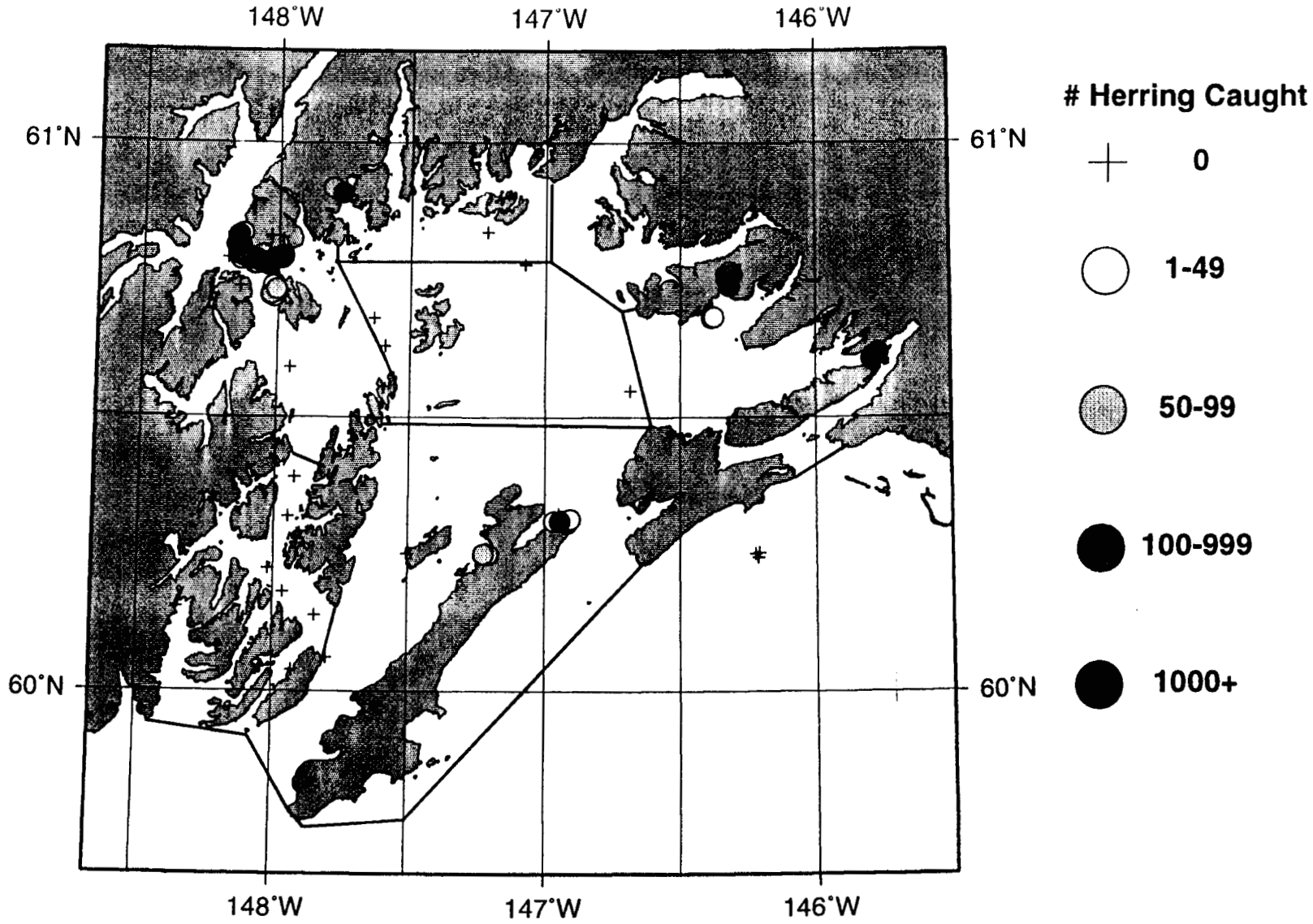

Figure 18. Net catches of Pacific herring greater than or equal to 150 mm fork length on the west side and other selected sites within Prince William Sound in May, 1995.

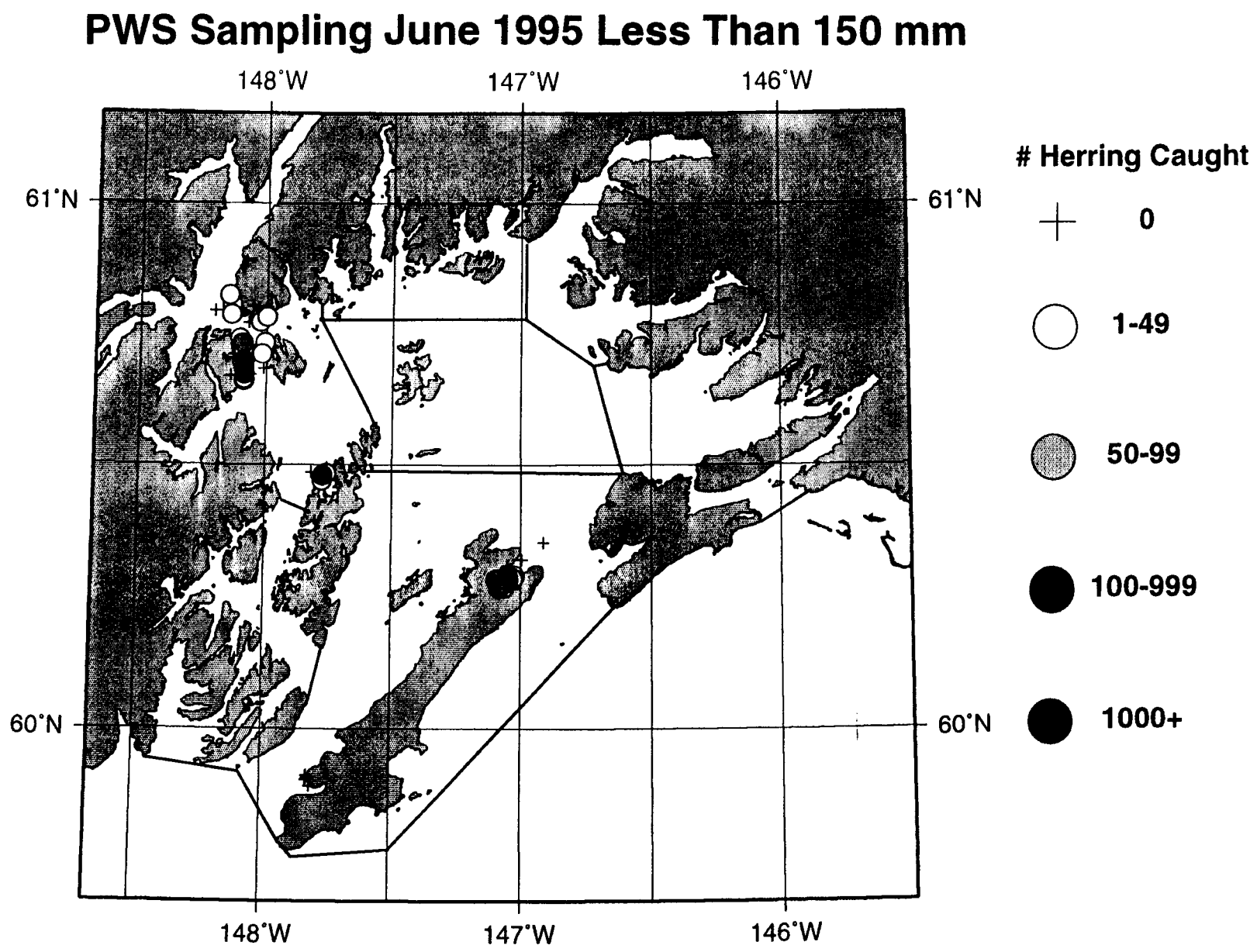

Figure 19. Net catches of Pacific herring less than 150 mm fork length on the west side and other selected sites within Prince William Sound in June, 1995.

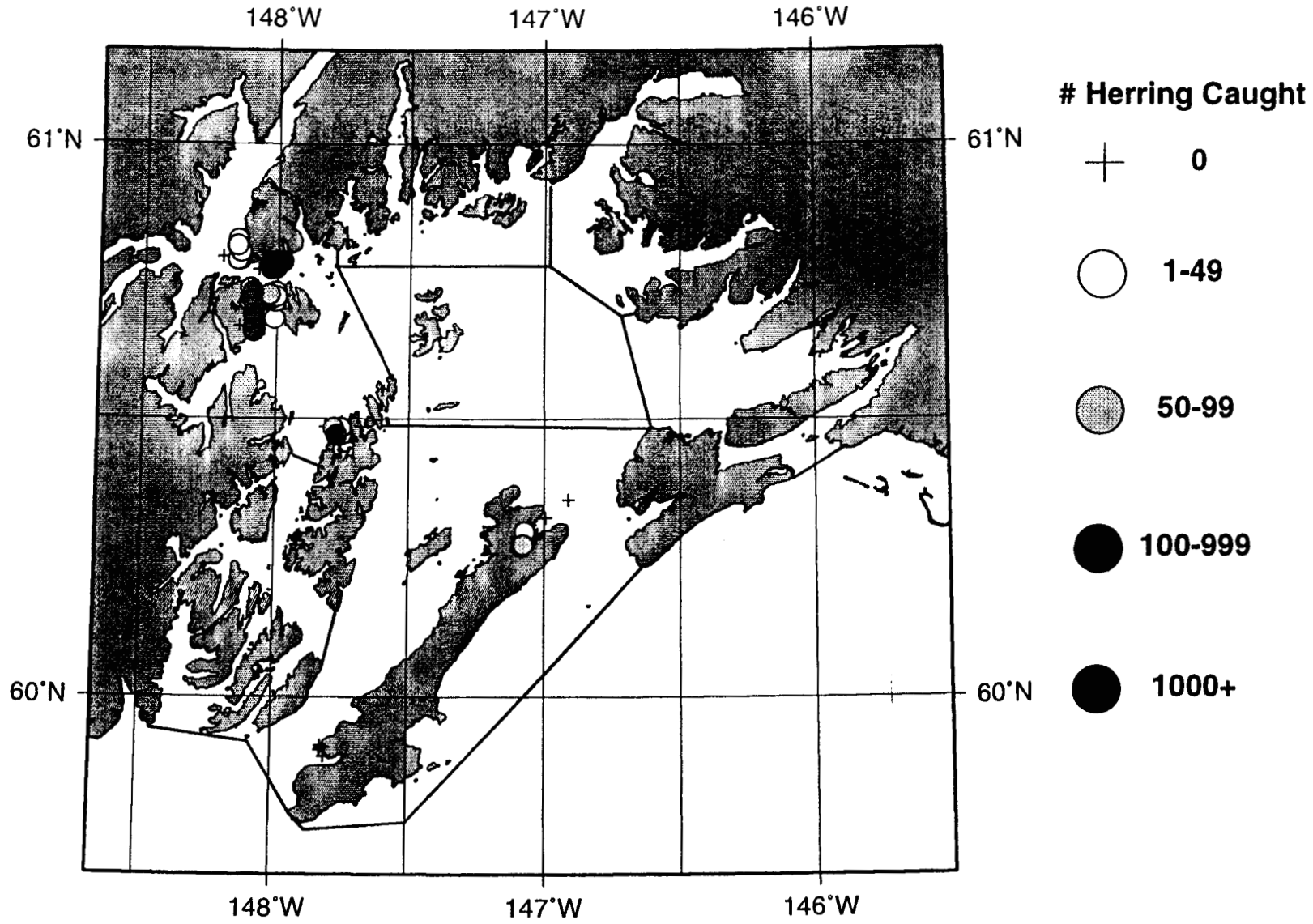

Figure 20. Net catches of Pacific herring greater than or equal to 150 mm fork length on the west side and other selected sites within Prince William Sound in June, 1995.

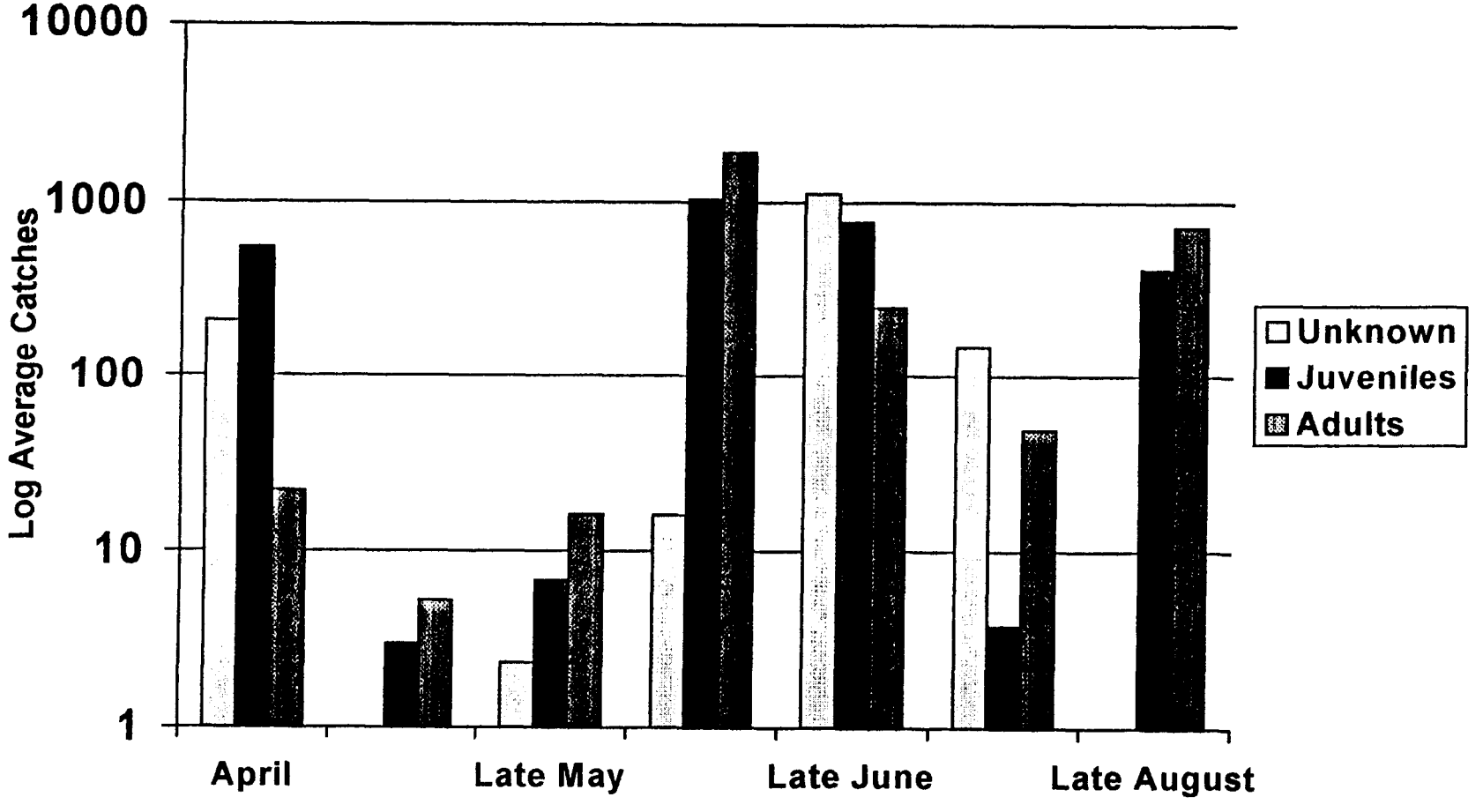

Figure 21. Seasonal distribution of log transformed net catches of Pacific herring less than and greater than 150 mm fork length, juveniles and adults respectively, collected primarily in western Prince William Sound in 1994. Herring that did not have length measurements were classified as unknown.

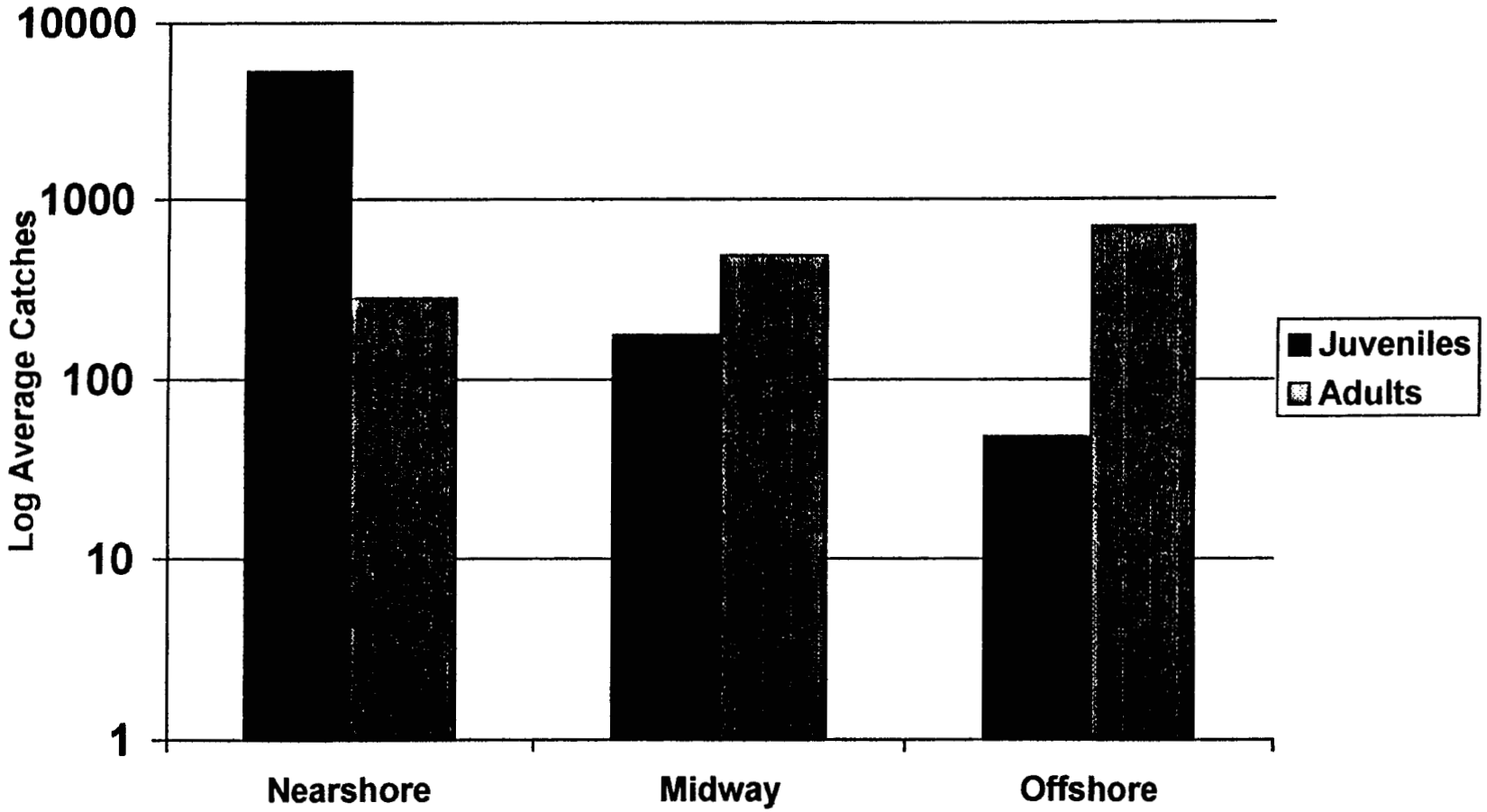

Figure 22. Distance from shore (m) measured by radar of log transformed net catches with juvenile Pacific herring less than 150 mm fork length and Pacific herring over 150 mm fork length in 1995. Nearshore was less than 150 m, midway was 151-300 m and offshore was over 300 m.

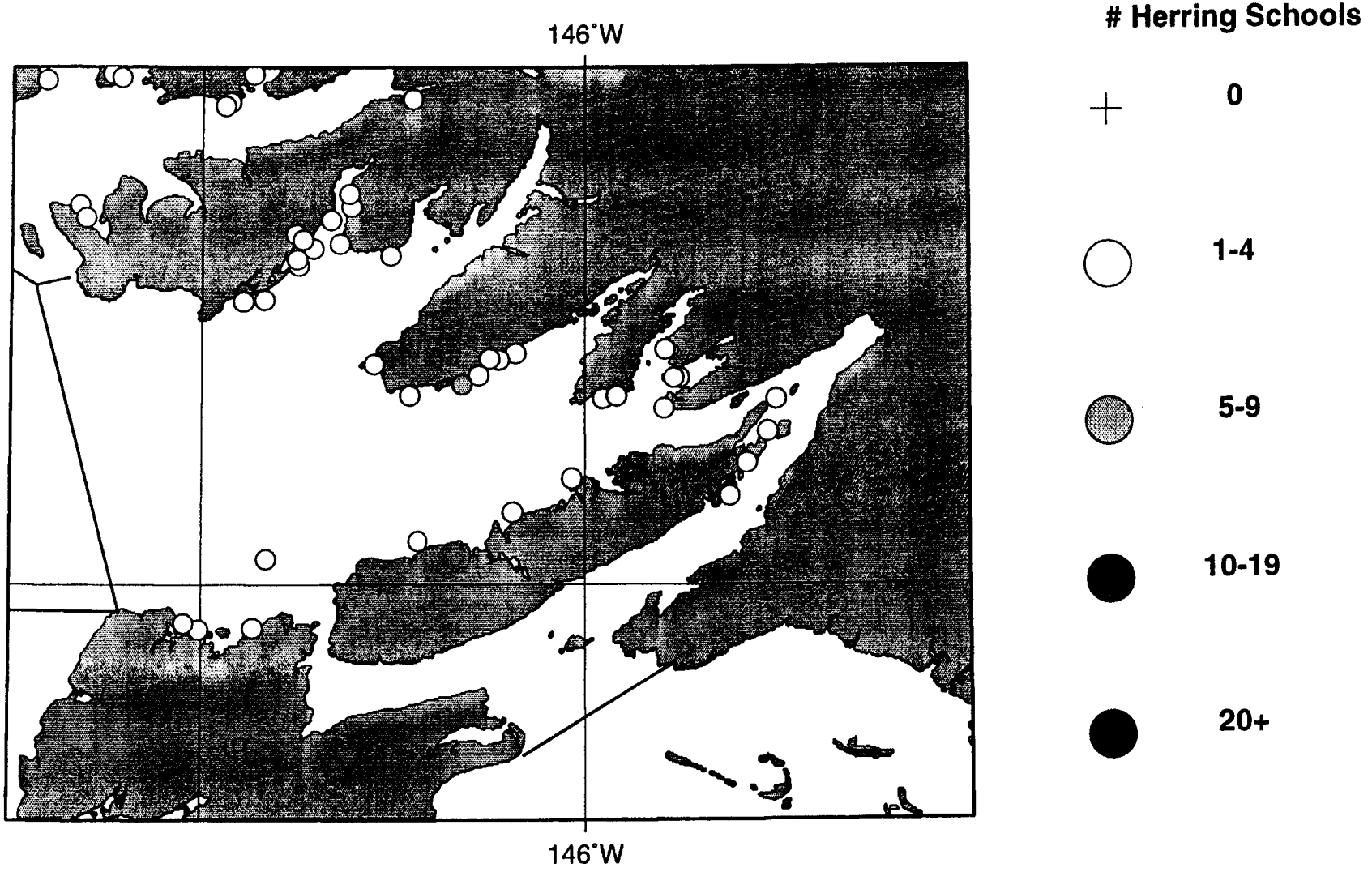

Figure 23. Distribution of Pacific herring schools within the eastern region (1) of Prince William Sound in August, 1995.

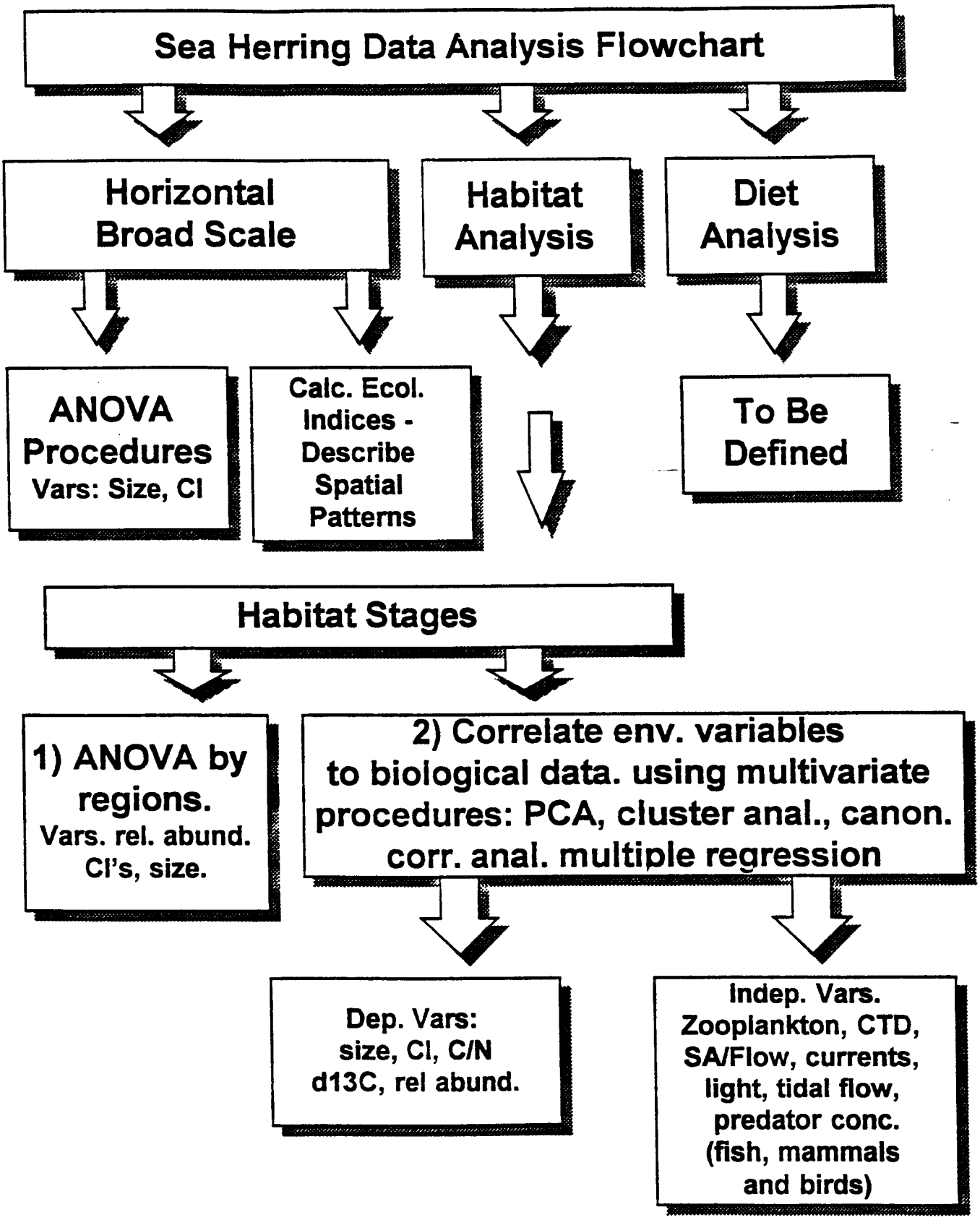

Figure 24. Planned analyses of Paciic herring distribution, population and condition indices, stomach contents, and physical and biological habitat characteristics from data collected in 1995 and 1996.

Chapter 13

95320U Energetics of Pollock, Herring and Pink Salmon

Project Number and Title

SEA 95320U - Energetics of herring, pollock and pink salmon.

Principle Investigator

Dr. A. J. Paul, University of Alaska, Seward Marine Center, P.O. Box 730, Seward, AK 99664 (Phone: 907-224-5261)

Study History

SEA is a hypothesis driven ecosystem study designed to obtain an understanding of the mechanisms that influence levels of production for pink salmon and herring in Prince William Sound by investigating their early life stages. SEA research focuses on the flow of energy and material (carbon or nitrogen) through the food web. Tracking this flow provides insight into links between primary and secondary production and species interactions. Food and predation are key forces that operate within the context of environmental parameters like temperature and transport. This component of SEA provides basic data base information on somatic energy content of key species so that interactions within and between species can be quantified. Energetic data flows to the SEA models which will be used to predict levels of fish production. This project was initiated in April 1995, thus this report constitutes the first year of activity. The first year was planned to be exploratory since none of the proposed measurements had ever been made before. Future directions of this component will be guided by the lessons learned in year one and by the SEA and EVOS planning process.

Abstract

The *Exxon Valdez* oil spill may have altered the trophic structure of the plankton feeding fish community by injuring intertidal spawning species. This project has started to describe the interannual over-winter somatic energy cycle of juvenile *Clupea harengus pallasi*. This information was not previously available and is needed to describe feeding success and to determine if the over-winter period is important in regulating recruitment of age 0 herring. Samples throughout Prince William Sound from two sites in the fall of 1994, seven sites in the spring of 1995 and ten sites in the fall of 1996 showed that there were large geographical differences in the nutritional status of recruiting herring. This is also true for fish within a school. The energy profile of over-wintering herring showed many of them were food limited.

Laboratory studies showed that juvenile herring use about 0.68 $KJ.g^{-1}$ wet wt per month when fasting and succumb to starvation when their somatic energy content is 3.5 to 3.0 $KJ.g^{-1}$ wet wt. These results suggest that fish with less than 5.5 $KJ.g^{-1}$ wet wt can not rely on stored energy reserves alone to survive the winter. Samples of juvenile herring that were collected from ten sites in the fall of 1995 showed that age 0 herring entering the winter of 1995-1996 generally had energy contents of less than 6 $KJ.g^{-1}$ wet wt. This initial survey suggests that many age 0 herring entered the over-wintering phase of 1995 in poor nutritional status and unless they could acquire significant energy through winter feeding, many of them would not survive. Analogous spring sampling will be done in 1996 to compare the energetics of fall fish and those that survived the winter. Similar sampling

is scheduled for two more years so that the interannual variation in somatic energy storage can be described.

This project has started to examine herring ovarian energy indices relative to weight and age to understand the populations' egg production potential which is the first step in the recruitment process. In 1995, sampling was done in three areas - the northeast, northwest and southwest portions of Prince William Sound. Ovaries will be sampled again in 1996 for comparative purposes as there is no previous information on the variability in the amount of energy allocated to egg production.

This project measures fall and spring somatic energy content of juvenile pollock (*Theragra chalcogramma*) to compare their nutritional status to that of competitors such as juvenile herring and pink salmon fry. This energetic profile will aid in the understanding of how pollock compete with these two injured fish species. Pollock are a major prey of many seabird species injured by the oil spill and our energetic measures will be useful in estimating bird energy intake. The first samples were collected from eleven sites in the fall of 1995 and are now being processed. Extensive sampling is scheduled again in the spring of 1996.

Measurements of the spring somatic energy content of pink salmon fry (*Oncorhynchys gorbuscha*) were made from four 1995 collections. We found that energy content of fry was related to their relative abundance, but not to body length. In 1996, energetic measurements of wild and hatchery pink salmon fry will be carried out so comparisons of their nutritional status versus geography and origin can be made. These measurements are part of the SEA effort to understand the role energy intake plays in the recruitment process of this injured species.

The information gathered by this energetics project is being related to SEA zooplankton surveys, prey selection studies and trophic isotopic studies through the SEA modeling effort.

Key Words: *Clupea harengus pallasi,* herring, energetics, ovary, *Oncorhynchys gorbuscha,* pink salmon fry, somatic energy, *Theragra chalcogramma,* pollock.

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Executive Summary

During its first year this project examined somatic energy content of pink salmon fry, age 0 and 1 herring (spring and late fall), age 0 pollock (spring and late fall), herring ovaries just prior to spawning, and adult herring bodies during fall. These parameters are key measures for SEA models which predict levels of fish production and species interactions. The key results for the species under study include the following.

Pink salmon fry: 1) There is considerable geographical variation in the energy content of pink salmon fry. 2) The relative abundance of the fry affects the energy content of the fry. 3) Fry allocate energy to increases in length preferentially. These energetic profiles will be used to understand transfer of energy from secondary producers, effects of intra-specific competition (including hatchery and wild fry), and consumption of fry by predators.

Age 0 and 1 herring: 1) There is considerable geographical variation in the fall energy content of different schools of herring recruits. 2) From the fall sampling it appears that recruits with insufficient energy reserves to survive a winter by fasting are common. 3) Herring recruits store energy for a winter fast and as their length increases, so does energy content at a much higher level than seen in pink salmon fry. 4) Based on our current, and incomplete, knowledge of the energy needs for age 0 herring during the over-winter period, it appears that many individuals are undernourished. These measures of energy storage and use will provide insight into the potential of individuals to survive winter. It will also help understand the effect of "river and lake" conditions, and be used to estimate consumption of herring by predators.

Age 0 pollock: Pollock exhibit the same growth mode as pink salmon fry, preferentially increasing in length. These samples are being processed now and no data is available for this report. The energetic profile for age 0 pollock will aid in the understanding of how pollock compete with the two injured species pink salmon fry and herring recruits.

Herring ovaries: The initial samples illustrate that ovary energy is strongly related to fish weight and there is not a strong geographical influence on this parameter. The amount of energy allocated

to ovaries will be correlated with egg production through EVOS models.

Adult herring whole bodies: SEA measures secondary production by a variety of methods. Adult herring are users of secondary production and their somatic energy content at the end of the feeding season reflects secondary productivity. The somatic energy values provide a separate measure by which the SEA secondary productivity estimates can be evaluated. The first samples were collected in the fall of 1995 and not enough data exists to comment on the outcome of this aspect of the study.

All energetic measures address SEA hypotheses and provide critical measurements for SEA models.

Introduction

During the first field season this project was a feasibility level study and it focused on somatic energy content of three species of pelagic fish in the EVOS region. These types of measurements have never been made before in Alaskan waters. We started to explore over-winter survival of juvenile herring and herring reproductive energetics. A portion of the effort examined somatic energy in age 0 pollock during the fall and spring, which are trophic analogs with herring, so the nutritional status of these forage species can be compared. Historically, herring and pollock have been among the most abundant pelagic forage fishes in south central Alaska. After the *Exxon Valdez* oil spill, the herring population of Prince William Sound has been exhibiting reduced abundance, disease and spawning anomalies that may be related to pollution. This research effort will help identify the role of food in delimiting survival of recruiting herring. The study of herring energetics will be one input into the SEA secondary production model.

This project is also looking at the energetics of pink salmon fry, including both wild and hatchery fish. This aspect of the project will allow for a better understanding of the trophic interactions of small pollock, herring and pink salmon that co-occur in time and space. The salmon work will also improve our understanding of the trophic interactions between wild and hatchery salmon fry.

Typically high latitude fishes store energy during spring and summer feeding and throughout the winter reallocate energy to maintenance and reproduction (Smith *et al.*, 1990). Thus, seasonal tissue samples must be taken to account for the temporal variation in energy content. Age 0 and 1 year old herring store energy during the summer feeding season and either fast or feed at low rates during the winter. If they have insufficient energy stores to maintain normal schooling activities until the spring zooplankton bloom, then high mortalities might occur. Low energy storage might be due to low zooplankton standing stocks or to competition for food resources.

Objectives

The objectives of this project for year one were:

1. Describe the interannual somatic energy content of ages 0 and 1 herring relative to geographical location.
2. Examine fall and spring energy stores of juvenile herring from several sites in Prince William Sound and model energy usage to describe the role nutritional status plays in over-winter

survival and feeding requirements.

3. Describe the spawning energetics of herring. Measure ovarian energy relative to weight, length, age and spawning site in female herring at three sites.
4. Measure fall energy content of adult herring from three sites.
5. Measure fall and spring somatic energy content of juvenile pollock and make comparisons of their nutritional status to geographical location and that of juvenile herring and pink salmon fry.
6. Measure the spring somatic energy content of pink salmon fry to see if this methodology can be used to compare their nutritional status versus geography, secondary production, competitor abundance and origin.
7. Relate the analysis of all the above objectives to SEA zooplankton surveys, prey selection studies and trophic isotopic studies through the SEA modeling effort and multi-authored journal papers.

Methods

The methods applied to the energy cycles were similar to those used by the investigator in previous bioenergetic studies (Harris *et al.*, 1986; Paul *et al.*, 1993, Smith *et al.*, 1988; Smith *et al.*, 1990). All fish lengths in 320U were standard length (SL) measured to the nearest mm. All whole fish or ovary weights were taken to the nearest 0.1 g. All calorimetric samples were weighed to the 0.0001 g level.

Herring Ovarian Energetics

Adult herring were collected just prior to and after spawning by ADF&G, and frozen in seawater. During the spring of 1995, ovary energetic samples were collected from three sites (see collection log table at end of Methods, Table 1). In the laboratory, measurements taken on the females were standard length, wet weight, age (aging was done by E. Brown's component of SEA), and condition factor [CF = g wet wt x 100/(cm standard length)^3]. Sample sizes and locations are given in Table 1. Males in the sample were discarded without any measurement. Wet weight of the whole female fish and the ovary was measured to the nearest 0.1 g. Small subsamples of ovary were removed, weighed to the nearest 0.1 g, and dried for energy measurement. After freeze drying, test tissues were placed in a convection oven at 60°C until they reach a constant weight. Individual tissue wet and dry weight values were used to calculate the moisture content. Dried tissues were ground in a mill and measurements of caloric content made by bomb calorimetry. Ovarian energy measures were coordinated with fecundity estimates carried out using standard methods. Energetic estimates of ovaries after spawning were obtained from the post spawning collections.

Fall Whole Body Energy of Adult Herring

During the fall of 1995, collections of adult herring were made from three sites (Table 1). Fish were frozen in seawater aboard ship and kept frozen for processing. Fish of both sexes were analyzed for standard length, wet weight, age (aging was done by E. Brown's component of SEA), condition factor [CF = g wet wt x 100/(cm standard length)^3], and whole body energy content using the above methods. After taking weight, standard length, sex, and removing scales for aging, the still frozen bodies were ground. The tissue was then made into a paste in a mortar. A 30 g subsample was then freeze dried, oven dried and 0.5 g burned in the calorimeter.

Spring and Fall Age 0 and Age 1 Herring Body Energy

Juvenile herring samples were taken from several geographical areas in Prince William Sound (see Table 1 for sites and sample sizes). During 1994, two fall samples were collected and stored, frozen, in seawater until funding of this project (Table 1). During the spring of 1995 there were six collections of herring recruits for energetics and in the fall, ten more collections (Table 1). Fish were analyzed for standard length, wet weight, age (aging was done by E. Brown's component of SEA), condition factor [CF = g wet wt x 100/(cm standard length)^3], and whole body energy content using standard calorimetric methods. Carbon and nitrogen ratios of selected fish were measured by SEA 320-I.

Whole body energy profiles for starving age 0 herring were measured in the laboratory during the winter months of 1995 and 1996 (Harris *et al.*, 1986; Smith *et al.*, 1986). In the first experiment, age 0 herring were starved to death to get the energetic profile of fish that starve during the winter fast. In that experiment there were 33 fish, and the water temperature ranged from 6 to 4°C. In the next observation, fasting fish were collected in time series so crude estimates of daily energy use could be made. In that experiment there were 22 fish collected at time zero, 22 fish at day 27, and 22 fish at day 45, and the water temperature ranged from 7 to 5°C. Whole body energy content was measured for each individual using the standard methods. During the winter of 1996-1997, juvenile herring will be captured every two months from over-wintering areas somewhere near Cordova to supplement lab energy use estimates with real world measures. The somatic energy data set will be used by the SEA modeling component to create a model describing the over-winter energy needs of juvenile herring and the likelihood of individuals to survive the winter.

Somatic Energy of Pink Salmon Fry

Collections were made from four sites to see how useful somatic energetics would be to the SEA pink salmon fry studies. The relative abundance of fry was determined (Willette component of SEA), fish collected (see Table 1 for sample sizes, dates, locations) and frozen in seawater for analysis. Fish were analyzed for standard length, wet weight, condition factor [CF = g wet wt x 100/(cm standard length)^3], and whole body energy content using standard calorimetric methods.

Somatic Energy of Age 0 Pollock

Pollock under 100 mm SL were collected at seven sites (see Table 1 for sample sizes, dates, locations) and frozen in seawater for analysis. Fish were analyzed for standard length, wet weight, condition factor [CF = g wet wt x 100/(cm standard length)^3], and whole body energy content using standard calorimetric methods.

Table 1. Collections of specimens for energetic measurements for SEA 320U during year one. (Key: HO = Herring ovary; AHWB = Adult herring whole body; JHWB = Juvenile herring whole body; PSFWB = Pinks salmon fry whole body; A0PWB= Age 0 pollock whole body.)

Tissue Type	Date (month/day/year)	Location in PWS	Number of Fish
HO	4/4/95	Ester Island	35
HO	4/14/95	Port Fidalgo	29
HO	4/17/95	Montague Island	46
AHWB	10/25/95	Green Island	100
AHWB	11/1/95	Knowles Head	100
AHWB	11/3/95	Jack Bay	100
JHWB	10/26/94	Orca Inlet	92
JHWB	10/29/94	Port Gravina	100
JHWB	5/17/95	Ester Island	36
JHWB	5/5/95	Parry Island	108
JHWB	5/2/95	Montague Island	57
JHWB	5/27/95	Port Gravina	101
JHWB	5/7/95	Hogg Bay	63
JHWB	4/30/95	Orca Inlet	101
JHWP	5/14/95	Eaglek Bay	100
JHWB	11/5/95	Eaglek Bay	100
JHWB	11/3/95	Jack Bay	100
JHWB	10/20/95	Zaikof Bay	59
JHWB	11/7/95	Sawmill Bay	100
JHWB	10/25/95	Green Island	100
JHWB	10/19/95	Whale Bay	97
JHWB	11/1/95	Knowles Head	98
JHWB	10/16/95	Simpson Bay	100
JHWB	11/3/95	Snug Cornor Cove	100
JHWB	11/8/95	Hogg Bay	42
PSFWB	5/4/95	Ester Island	92

Tissue Type	Date (month/day/year)	Location in PWS	Number of Fish
PSFWB	5/31/95	Ester Island	62
PSFWB	5/27/95	Port Gravina	42
PSFWB	6/2/95	Perry Island	42
A0PWB	10/16/95	Simpson Bay	100
A0PWB	10/20/95	Zaikof Bay	100
A0PWB	10/18/95	Whale Bay	100
A0PWB	11/1/95	Knowles Head	100
A0PWB	11/5/95	Eaglek Bay	100
A0PWB	11/7/95	Sawmill Bay	100
A0PWB	11/7/95	Hogg Bay	100

Results

Herring Ovarian Energetics

Ovary energy was measured from herring collected at three sites. Figure 1 shows the mean (and sd) for the values. The allocation of energy to ovaries is related to feeding conditions (Hay *et al.*, 1988). There was a strong correlation between fish weight and energy content of the ovary (Fig. 1, lower panel). However, there was no marked differences in weight specific energy content with geographical location of the collection (Fig. 1, middle panel) once fish weight was factored into the analysis (Fig. 1, upper panel versus middle panel). Post spawning samples demonstrated that almost all the energy stored in the ovary prior to spawning is released with spawning. Collections will be made for the last time in the spring of 1996 to obtain insight into the interannual variation in energy allocated to ovaries.

Fall Whole Body Energy of Adult Herring

The data for the first year's sampling of this parameter is not yet processed.

Spring and Fall Age 0 and Age 1 Herring Body Energy

Two samples of herring recruits were available in the SEA archives from the fall of 1994. One from Port Gravina had an average energy content of 7.0 $KJ.g^{-1}$ wet wt; while a concurrent sample from Orca Inlet had an average of 4.6 $KJ.g^{-1}$ wet wt (Fig. 2, upper panel). These samples demonstrated that there is a strong geographical difference in the energy content of recruiting herring. In spring 1995, the age 0 and 1 herring that survived the winter had average energy contents generally between 4 and 6.5 $KJ.g^{-1}$ wet wt (Fig. 2, middle panel). Those fish entering the winter of 1995-1996 typically had a similar energetic content as the fish surviving the previous winter, 4.0-6.5 $KJ.g^{-1}$ wet wt (Fig. 2, lower panel) suggesting that many of them may not survive. Spring sampling in 1996 will identify the energy content of survivors.

In the laboratory, experiments with 0 age herring have demonstrated that fish starved to death have somatic energy contents of 3 to 3.5 $KJ.g^{-1}$ wet wt. Fasting fish use about 0.66 $KJ.g^{-1}$ wet wt a month at 6.6°C. Under those conditions, a recruit facing a 90 day fast would need to have an energy content >5.5 $KJ.g^{-1}$ wet wt to survive. Many of the fish in the fall of 1995 sampling had lesser amounts of energy suggesting that growth and energy storage in many age 0 herring is limited by food availability. Future thermal refinements to the laboratory energy use model, and winter time sequence sampling of fish from the field, are planned to improve the bioenergetic energy use model. The bioenergetic model will assess the potential of individuals to survive the winter.

Somatic Energy of Pink Salmon Fry

In 1995, the work with somatic energy content of fry was experimental to see if it would be a useful tool to quantify feeding success of fish relative to their abundance, location and origin. Collections were made from three sites. At the Ester Island site there was a high abundance of hatchery fry, at Perry Island fry occurred in more moderate abundance and in Port Gravina there were far fewer wild fry (abundance data from Willette component of SEA). At the site where high numbers of fry competed for prey, the average energy content was 3.2 $KJ.g^{-1}$ wet wt versus 3.6 and 4.4 $KJ.g^{-1}$ wet wt for the areas with decreasing intraspecific competition (Fig. 3). These differences are statistically significant. Concurrent data sets of temperature and prey abundance are being analyzed to enhance the interpretation of these results, but the feasibility study does show that measuring energy content helps identify the effects of feeding success.

Somatic Energy of Age 0 Pollock

Samples from seven sites in the SEA study area were collected in the fall of 1995. All the samples have been processed, however, the data will not be analyzed in time for this report. The data will provide insight into how recruiting herring and age 0 pollock compete for food resources and their relative success.

Discussion

The energetics portion of SEA was initiated in mid-April 1995 so it is just completing its first year of operation. The first year was a feasibility effort since none of the parameters measured had been measured before. The examination of somatic energy content of age 0 herring, and their competitors, age 1 herring and age 0 pollock, shows promise for understanding the level of competition between these pelagic analogs. Coupled with SEA models and APEX and SEA stomach analysis, these prey competition interactions will be quantified. The somatic energy measures should identify individuals that have not stored enough energy to survive the winter period. The SEA herring recruit bioenergetic model, combined with wide scale geographical sampling of age 0 herring, will help predict post-larval year class recruitment potential during the first year of life.

The energy analysis of adult herring ovaries in the spring will be correlated with EVOS herring egg deposition models (**Haldorson *et al.*, 19xx**) and SEA primary-secondary production models. These energy measures will possibly turn out to be inexpensive proxies for hind-casting annual secondary productivity.

Conclusions

Somatic energy measurements are a valuable tool for identifying the transfer of energy through the food web to SEA target species, pink salmon fry and herring. They measure subtle differences that are unobservable from length wet weight measures and quantify gross differences. Quantifying energy transfers is critical to building SEA models and the energetics component of SEA provides these input values. Energetics measures are being used to simulate and test SEA hypothesis lake-river and over-wintering, and the production and competition models. Additionally, the energetic data set allows for trophic comparisons of pelagic analogs like pollock and herring, and transfer of energy to other animals such as APEX birds.

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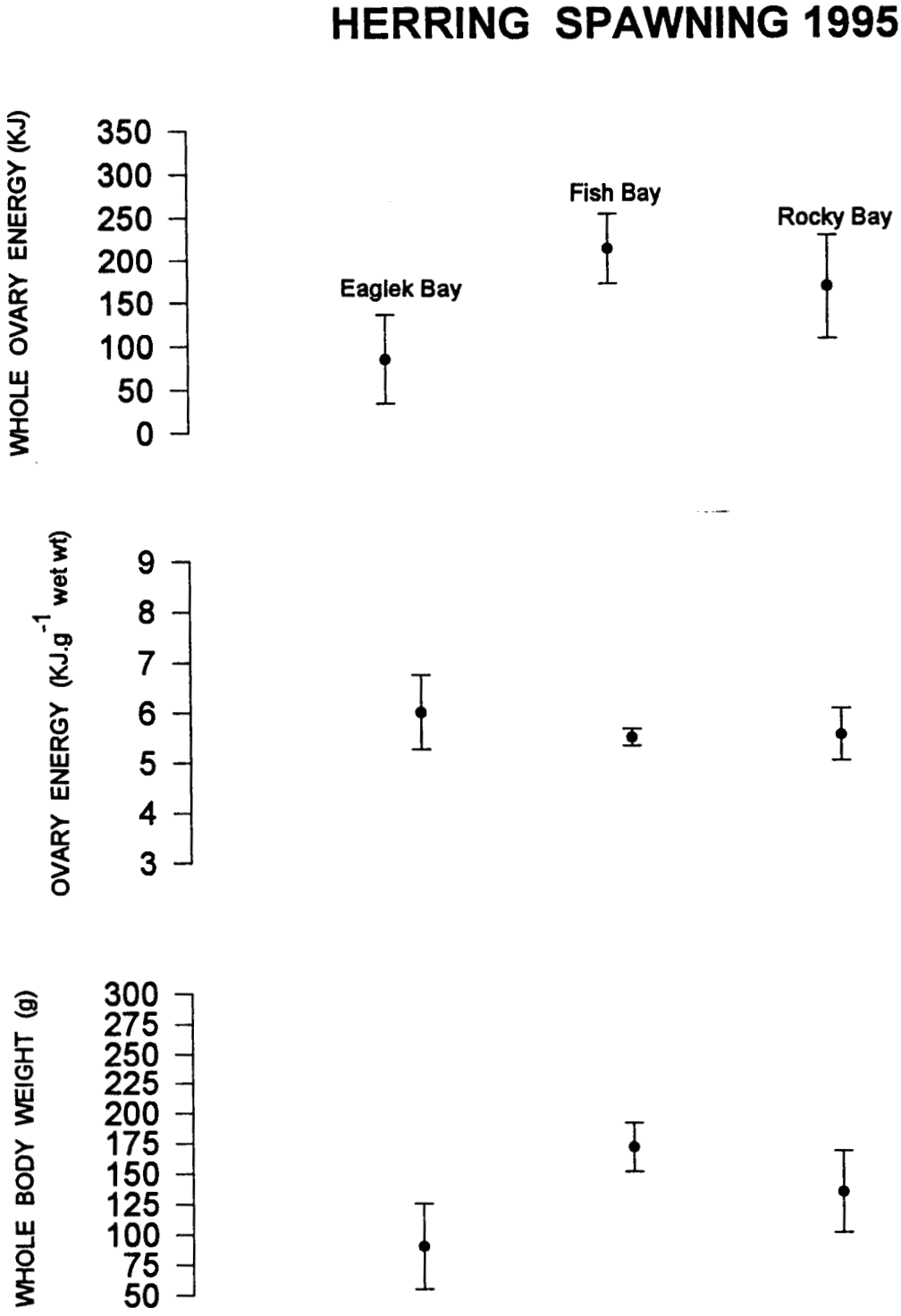

Figure 1. Herring ovary energetics in the spring of 1995. Data = mean, sd.

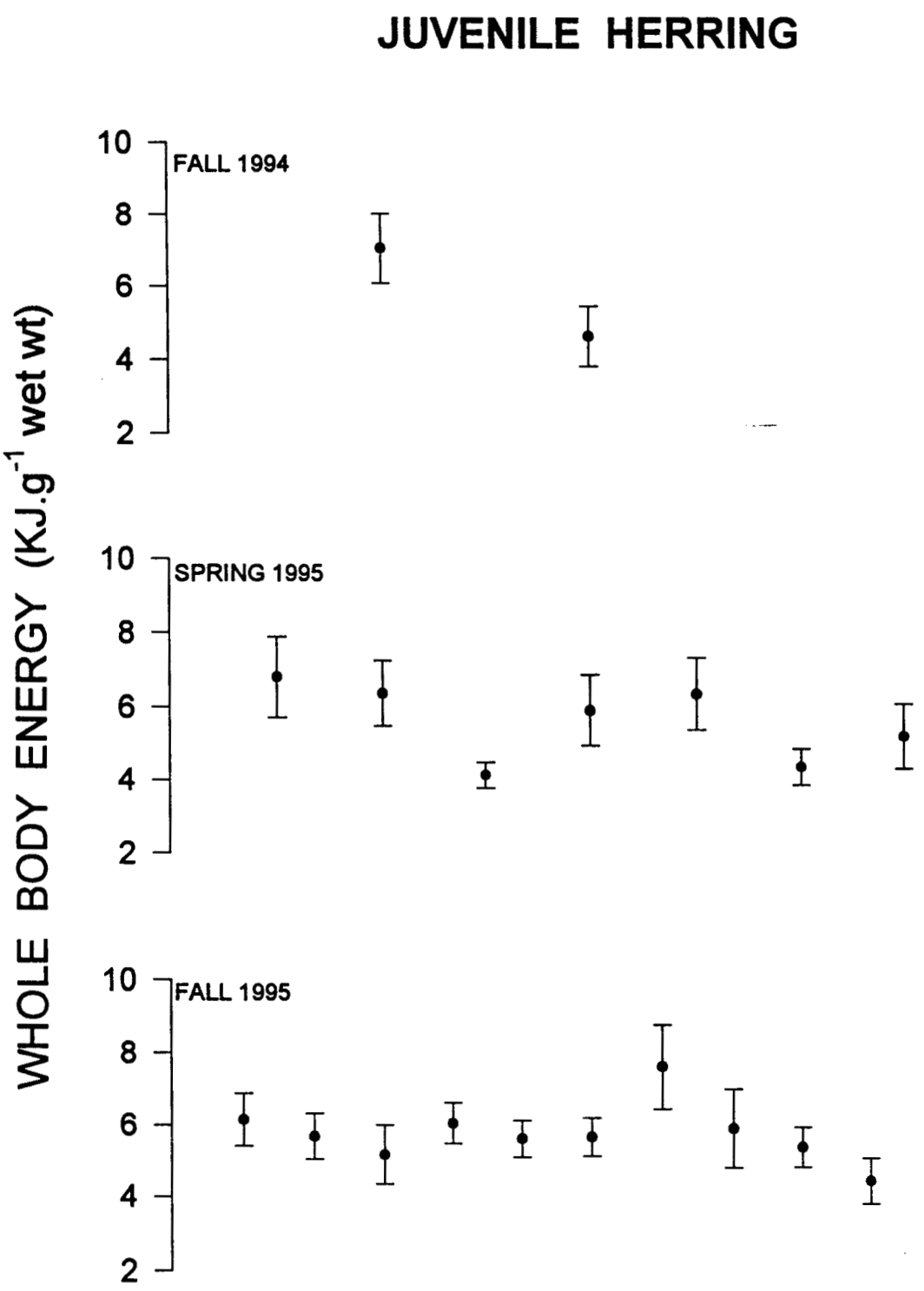

Figure 2. Somatic energy content (mean and sd) of juvenile herring. Each data set represents fish from a different geographical area.

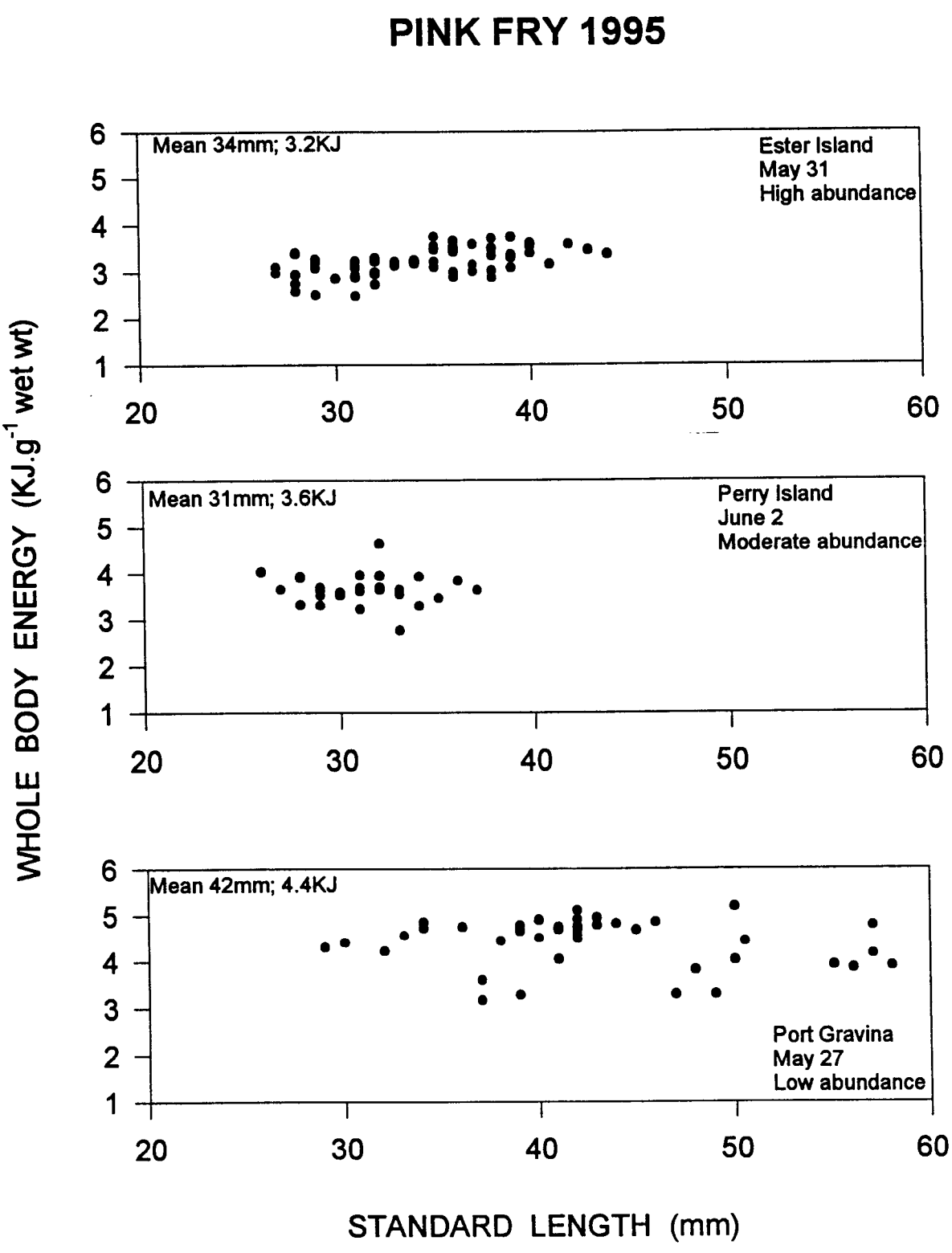

Figure 3. Whole body energy of pink salmon fry in three areas of Prince William Sound.

Exxon Valdez Oil Spill
Restoration Project Final Report

Sound Ecosystem Assessment (SEA): Estimating Local Avian Predation Rates on Hatchery-Released Fry

Restoration Report 95320-Y
Final Report

not final report

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April 1996

Sound Ecosystem Assessment (SEA):
Estimating Local Avian Predation Rates on Hatchery-Released Fry

Restoration Report 95320-Y
Final Report

Study History: Restoration Project 95320-Y began in 1995, and received funds for analysis and close-out in 1996. Project 95320-Y was part of the Sound Ecosystem Assessment program. This is the final report on activities conducted by this project.

Abstract: We estimated the mortality of hatchery-raised pink (Oncorhynchus gorbuscha) and chum (Oncorhynchus keta) salmon fry as a result of seabird predation near a Prince William Sound salmon hatchery. Field counts of seabirds and observations of feeding rates for several plunge-diving seabirds were obtained from a skiff during April, May, and June 1995. Several 100 birds of seven piscivorous species aggregated in front of the hatchery. Aggregations were largest in May and declined by early June. Consumption rates were determined from focal animal sampling and by calculation of energetic demand. Most of the per capita consumption rates based on behavioral data were lower than those calculated from energetic considerations. The use of energetic models and estimated fry movement rates provided a range of 2.7 to 5.9 million juvenile salmon (1.1% to 2.4% of released fry) consumed during the study period. Sixty-four percent of this range was attributed to differences between the energetics models; the remainder to the assumptions about fry movement rates. Early marine mortality of salmon fry resulting from seabird predation may increase in years of higher seabird abundance; however, mortality may be reduced by releasing fry later in the season when bird numbers have declined.

Gulls recorded during aerial surveys were significantly associated with spawning herring and with hatchery sites, but not with linear miles of herring spawn. The correlation of bird aggregations with the presence of small fish (spawning herring and hatchery locations) indicates that concentrations of forage fish have an important influence on the distribution of birds at sea during this time of the year. Bird numbers in all samples (boat counts, volunteer data, and aerial surveys) increased in May when fry were released. By June, boat counts and aerial surveys showed a decline in bird numbers suggesting that fry released from the hatcheries in April and May face considerably more risk of predation from birds than do fry released in June.

Key Words: salmon hatcheries, predation, pink salmon, chum salmon, seabirds, Arctic Tern, Black-legged Kittiwake, Mew Gull, Bonaparte's Gull, Marbled Murrelet, Common Merganser, Red-breasted Merganser, *Exxon Valdez*, Prince William Sound

Citation: Scheel, D., K. R. Hough. Sound Ecosystem Assessment (SEA): Estimating Local Avian Predation Rates on Hatchery-Released Fry. *Exxon Valdez* Oil Spill Restoration Project final report (Restoration Project 95320-Y). Prince William Sound Science Center, Cordova, AK.

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EXECUTIVE SUMMARY

The 'predator-prey relationship' hypothesis of SEA asserted that loss to predation is the most important variable element of pink salmon (Oncorhynchus gorbuscha) fry survival, and that the intensity of this predation depends on the availability of alternative prey such as macrozooplankton. This hypothesis is particularly relevant to predicting and managing the recovery of pink salmon populations from EVOS, and to the role of hatcheries in restoring these populations.

The objectives described in the 1995 Detailed Project Description were to record the size and composition of seabird foraging aggregations near one hatchery during the fry release period; to measure consumption rates for plunge-diving birds using focal-animal sampling; to collect data from volunteer observers at each hatchery site on the abundance of birds; and to estimate the magnitude of local predation on hatchery-released fry immediately following release, using the count data from this study and estimates of consumption rates from energetics models published in the literature.

We counted up to several hundred birds a day from a skiff during April, May, and June 1995 in front of Wally Noerenberg Hatchery. Aggregations were largest early in the study period and declined by early June. The ten most common species of birds present in these counts included seven piscivores; but 50% of all birds consisted of just the two most abundant species: Black-legged Kittiwakes (Rissa tridactyla) and Marbled Murrelets (Brachyramphus marmoratus).

Consumption rates were determined from focal animal sampling and by calculating the energetic demand of all piscivorous birds counted. We used three models of seabird energetics (Nagy 1987, Birt-Friesen et al. 1989). The models we chose to predict energetic demands were derived from measurements of field metabolic rates of seabirds. Differences between the data used to develop the allometric equations resulted in variation in the predicted field metabolic rates: Nagy's (1987) model included some tropical birds, while Birt-Friesen et al. (1989) presented models restricted to cold-water seabirds. Values predicted by Nagy (1987) were below those predicted by Birt-Friesen et al. (1989), presumably because warm-water species typically have lower metabolic rates than arctic and subarctic birds (Ellis 1984).

Most of the per capita consumption rates based on behavioral data were lower than those calculated from energetic considerations. The use of energetic models and estimated fry movement rates provided a range of 2.7-5.9 million juvenile pink and chum (Oncorhynchus keta) salmon (1.1% to 2.4% of released fry) consumed during the study period. Sixty-four percent of this range was attributed to differences between the energetics models; the remainder to the assumptions about fry movement rates. Early marine mortality of salmon fry resulting from seabird predation may increase in years of higher seabird abundance. This mortality might be reduced by releasing fry later in the season when bird numbers have declined.

An analysis of the distribution of white seabirds (largely gulls) based on five aerial surveys over western Prince William Sound indicated that gulls were associated with spawning herring and with hatchery sites. We suggest that the timing of salmon release (or outmigration) relative to that of spawning migrations by herring and other forage fish determines the magnitude of bird aggregations that feed on salmon fry. Further data is needed to confirm this.

INTRODUCTION

The 'predator-prey relationship' hypothesis of SEA asserted that loss to predation is the most important variable element of pink salmon fry survival, and that the intensity of this predation depends on the availability of alternative prey such as macrozooplankton. This hypothesis is particularly relevant to predicting and managing the recovery of pink salmon populations from EVOS, and to the role of hatcheries in restoring these populations. Field observations in 1994 suggest that in some cases, predation on young fish by birds may be as important as predation by larger fishes. One SEA project, Pink Salmon & Herring Predation (320-E), is designed to evaluate the significance of fish predation on 0-age class fishes. The goal of this project (95320-Y) is to estimate the localized intensity of bird predation on hatchery-released pink salmon fry.

Chapter one reports data on the size and composition of seabird aggregations in front of Wally Noerenberg Hatchery during the spring of 1995, and calculates the magnitude of predation by these birds on salmon fry. This chapter is presented as a draft manuscript to be submitted for publication (Scheel, D., K. R. Hough. Salmon fry predation by seabirds near an Alaskan hatchery). Chapter two presents additional data from this study that was not appropriate in the draft manuscript, including survey data on bird aggregations at the other four salmon hatcheries that operate in Prince William Sound, and an analysis of the spatial distribution of gulls based on aerial survey data.

OBJECTIVES

The objectives for this study are listed below. Our primary methods and results for objectives (1), (2), and (4) are reported in Ch. 1, and are not repeated elsewhere in the report. Supporting material and the complete results for objective (3) are included in Ch 2. As described in the 1995 Detailed Project Description, the objectives were to:

1) Record size and composition of seabird foraging aggregations near one hatchery-release site immediately pre-release, during release, and immediately post-release of salmon fry.

2) Sample behavior at foraging aggregations to measure dive and capture rates for avian foragers that bring prey to the surface before consuming them (e.g. gulls and terns).

3) Organize volunteer observers at each hatchery release site in the Sound to record the qualitative abundance and composition of foraging aggregations near hatcheries during pre-release, release, and post-release periods.

4) Using data from this proposal and literature values for consumption rates, estimate the extent of local predation on hatchery-released fry immediately following release.

CHAPTER 1: Salmon fry predation by seabirds near an Alaskan hatchery

SALMON FRY PREDATION BY SEABIRDS NEAR AN ALASKAN HATCHERY

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ABSTRACT

We estimated the mortality of hatchery-raised pink (Oncorhynchus gorbuscha) and chum (Oncorhynchus keta) salmon fry from seabird predation near a salmon hatchery in Lake Bay, Prince William Sound. Field counts of seabirds and observations of feeding rates for several plunge-diving seabirds were obtained during salmon fry releases between April and June 1995. Several hundred birds of seven piscivorous species aggregated in front of the hatchery. Aggregations were largest in May and declined by early June. Consumption rates were determined from focal animal sampling and by calculation of energetic demand. Most of the per capita consumption rates based on behavioral data were lower than those calculated from energetic considerations. From energetic models and fry movement rates, we estimated that 2.7 to 5.9 million juvenile salmon (1.1% to 2.4% of released fry) were consumed during the study period. Differences between the energetics models account for sixty-four percent of this range; assumptions about fry movement rates account for the remainder. Early marine mortality of salmon fry resulting from seabird predation may increase in years of higher seabird abundance; however, mortality may be reduced by releasing fry later in the season when bird numbers have declined.

INTRODUCTION

Schools of small fish are a concentrated food source, and often attract predators. Seabirds aggregate over and feed on schooling fish in the marine environment (Brown 1980, Hoffman et al. 1981, Duffy 1983, 1989), and in coastal waters such as rivers, streams (Wood 1985, Ruggerone 1986, Wood 1987a, 1987b, Kålås et al. 1993) and shallow-water estuaries (less than five meters deep. Bayer 1986, Kålås et al. 1993). Seabirds can consume 20% or more of local fish production (Furness & Monaghan 1987) and thus may be a substantial source of mortality. Studies of salmon production lost to seabirds have focused on chinook (Oncorhynchus tshawytscha), sockeye (Oncorhynchus nerka), and coho (Oncorhynchus kisutch) salmon (e.g. Ruggerone 1986; Wood 1987a, 1987b) in North America; and on Atlantic salmon (Salmo salar), Brown trout (Salmo trutta), and Arctic char (Salvelinus alpinus) in Europe (Kålås et al. 1993). Estimates of losses to seabird predators in these studies range between 1% and 65% of hatchery and/or wild salmon production.

The Sound Ecosystem Assessment program, currently taking place in Prince William Sound, Alaska, is attempting to identify major predators on juvenile pink salmon (Oncorhynchus gorbuscha) and to account for the early marine mortality of these fish. Predators of salmon fry are of particular interest because of the commercial value of salmon and because salmon production is supplemented by a large hatchery program. Wally Noerenberg Hatchery in northwestern Prince William Sound releases pink and chum (Oncorhynchus keta) salmon into a deep-water (to 70 m) estuary. In 1994, thousands of seabirds were observed in front of this hatchery (pers. comm. M. Willette, T. Cooney). This observation prompted our study.

We counted seabirds aggregated at the Wally Noerenberg hatchery and calculated seabird consumption of salmon fry based on models of seabird energetic demand. These were compared to calculations based on the capture rates of focal animals. We show that in 1995, a few hundred birds were present per day at this hatchery. These birds probably consumed less than 2.5 percent of hatchery production during the period of fry release. Seabirds were reportedly more abundant

in front of this hatchery in 1994 than during our study. Mortality from seabird predation may be higher in years when birds are more abundant.

METHODS

Study site

Seabirds were counted in April-June 1995, in front of the Wally Noerenberg Hatchery, located at 60°48'N, 148°05'W on Lake Bay, Esther Island in Prince William Sound. The Sound is located in southcentral Alaska on the northern edge of the Gulf of Alaska (Fig. 1). Prince William Sound is a large, deep (to 476 fathoms), tidal estuarine system comprised of mountainous islands, protected embayments, and glacial fjords.

Wally Noerenberg Hatchery is owned and operated by the Prince William Sound Aquaculture Corporation. Between 29 April and 15 June 1995, the hatchery released into Lake Bay 169.4 million pink salmon fry at an average release weight of 0.29 grams and 72.3 million chum salmon fry at 0.88 grams (Prince William Sound Aquaculture Corporation). Dense schools of fry were visible nearshore throughout the entire study period following the first release. Fry were observed initially in Lake Bay, and within a day or two were also seen throughout Quillian Bay, at Esther Point, and along the west shore of Esther Island.

Bird counts

During the periods of 25 April to 18 May and 3-7 June 1995, we counted birds once or twice daily in Lake Bay, Quillian Bay, and immediately in front of these bays in Wells Passage (Fig. 1). Two observers in a small open skiff counted all birds seen over the water. We ran the boat approximately 50 meters from shore along both sides of each bay and birds were counted on both sides of the boat from the adjacent shore out to the middle of the bay. In area 14, that was open to Wells Passage, birds were counted to 100 meters on either side of the boat. In this count area, to survey further offshore, we made two transects: the first was 50 meters from the shore, and the second was approximately 250 meters off shore. Distances were measured using a Leitz optical rangefinder. At times, weather prohibited counting the entire study area. Our analyses include only days when all fourteen count areas were surveyed (N=21). Birds were identified to species where possible or to the finest taxonomic resolution discernable in the field. The majority of birds were identified to species.

Consumption of fry

Only the most abundant piscivorous seabirds (Table 1) were included in estimates of consumption of fry. Plunge-diving birds (Haney & Stone 1988) were classified as piscivores if fish were listed as a primary component of their diet (Ehrlich et. al 1988) and they were observed feeding on fry; pursuit-diving birds were considered to be piscivores if fish were listed as a primary component of their diet and they were observed in mixed-species foraging flocks near salmon fry.

Per capita consumption was calculated using two methods. For three plunge-diving species, consumption was calculated based on fry capture rates observed during focal animal sampling. For these species as well as four others, consumption rates were also calculated using energetics models, and the energy content of fry. We compared the results of these two methods.

Behavioral data

Focal animal sampling was conducted on three species: Black-legged Kittiwakes, Bonaparte's Gulls, and Arctic Terns. These plunge-diving piscivores were observed during feeding bouts in mixed-species flocks. An individual active bird in the flock was chosen and followed by binoculars for a period of three to five minutes. Sampling ended after five minutes or when the bird flew out of view. If the bird could not be observed for at least three minutes, the sample was discarded. Samples were not adjusted for the proportion of time an individual bird spent in non-foraging activities (e.g. resting). A successful capture was recorded if we saw a small fish in the beak when the bird emerged from a plunge dive.

Capture rates were calculated for each of the three species as the average number of successful captures per minute. Estimates of the time per day spent in active feeding were made from the reported behavior of radio-tagged Kittiwakes (Irons 1992). We are aware of no other literature on the daily active foraging times of terns or gulls, and so used our calculation for kittiwakes as the foraging time for all species. Daily per capita consumption was estimated for each species as the capture rate times the daily time spent actively feeding.

Energetic models

Energetic modeling provides an alternative way to calculate daily consumption of fry by seabirds. We used mass-metabolic rate regression equations available in the literature (Table 2) to calculate field metabolic rates (FMRs) for the common piscivores in our study area. These models provided a range of predicted FMRs (Fig. 2). We compared measured FMRs of Black-legged Kittiwakes (Gabrielsen et al. 1987) and Arctic Terns (Uttley et al. 1994) to the model predictions. Measured FMRs for the other species were not available.

Average release weights for each pen of pink and chum salmon fry were obtained from the Prince William Sound Aquaculture Corporation and growth rate of pink salmon fry was obtained from M. Willette (Alaska Department of Fish & Game, pers. comm.). Growth was measured from recaptured coded-wire tagged fry and calculated to be 4.2% body weight per day for fry released from Wally Noerenberg Hatchery during 1995. Pink and chum fry were found in mixed schools in front of the hatchery and were likely feeding on the same prey. We therefore assumed that the measured growth rate of pink salmon fry applied to chum salmon fry as well. We calculated the weight of each cohort of fry, starting with the measured release weight on the day of release, for each day until 15 June 1995. The mass of the average fry in front of the hatchery changed depending on fry growth, release of new fry and loss of fry to movement or predation. To estimate the mass of the average fry on a given day, we assumed that fry from each release cohort left the area at a fixed rate per day. The average mass of fry remaining was then calculated from known release weights, dates, and growth rates. We used two different estimates of fry departure rates: a low movement rate of 2.5% departure per day and a high rate of 50% departure per day (Fig. 3). Higher movement rates resulted in less growth before fry left the area, and hence lower mass per fry on average.

The energy content of pink salmon fry captured on May 31 at Esther Point in Wells Passage (Fig. 1) was measured using bomb calorimetry ($\bar{x}$=3.21 kilojoules per gram wet weight, N=62. A.J. Paul, unpublished data). We recognize that the energetic content may differ between pink and chum fry and may change throughout the season. However, to simplify our calculations,

we used this value as a reasonable approximation of salmon energy content for the purposes of this paper. We used this value as the energy content of pink and chum salmon throughout the study period. For each cohort of fry, the energy content per fry was calculated for each day as the weight of the fry times 3.21 kJ/g. Finally, we calculated the energy content of the average fry in front of the hatchery as the energy content for each cohort of fry releases weighted by the abundance of fry from that cohort assuming low or high movement rates (Fig. 3).

Seabird daily per capita consumption rates were calculated as the predicted FMRs for each species divided by the salmon fry energy content. Per capita consumption was multiplied by the number of seabirds present to estimate total consumption of fry each day. These were summed to calculate cumulative consumption over the study period. We assumed birds ate sufficient fry to meet their energy demands. No corrections were made for weight gain or loss by the birds, for less than 100% salmon in the diet, or for less than 100% digestion efficiency.

RESULTS

Numbers of birds

Sixty-five species of birds were observed during boat counts in this study. However, ten species collectively made up 93% of all counts (Table 1). Of these, seven were piscivores. Overall, piscivorous birds increased in the count area during the period of fry releases (Fig. 4). Black-legged Kittiwakes arrived and dispersed quickly, apparently in response to each individual release, although this relationship was not significant (Kruskal-Wallis ANOVA, $df=2$, $T=4.6$, $p=0.098$). Marbled Murrelet numbers began to increase about one week following the start of releases and continued to increase throughout the release period. A notable exception to this trend was the decrease in Marbled Murrelet numbers beginning 11 May, which corresponded to a ten-day period (8-17 May) in which there was only one fry release (13 May). Murrelet numbers began to rise again following the 13 May release (Fig. 4). Groups of Bonaparte's Gulls and Arctic Terns fed in the area for several days and then moved on. Red-breasted Mergansers increased gradually through mid-May and declined by early June. Common mergansers were abundant initially, but declined beginning in early May. Mew gulls were present in small numbers throughout.

Observed feeding rates

Black-legged Kittiwakes, Arctic Terns, and Bonaparte's Gulls were observed in mixed-species foraging flocks consuming fry at rates from 60-100 fry per hour (Table 3). Multiplied by the estimated 2.3 hours of foraging per day, this yielded estimates of daily per capita consumption from 150 fry per day for Bonaparte's gulls to 230 fry per day for Black-legged Kittiwakes. Compared to daily per capita consumption rates based on a 'standard' average fry (from May 10, mass = 0.53 g), these estimates were lower than estimates from the energetic models of Birt-Friesen et al. (1989). They were lower than estimates from Nagy (1987) for Kittiwakes and Bonaparte's Gulls, but slightly higher for Arctic Terns (Fig. 5).

Cumulative mortality to fry

Cumulative mortality to fry reflected differences in the energetics models (Fig. 6). Using Nagy's (1987) model, we arrived at a total mortality from birds of 2.7 to 3.6 million fry (for low and high fry movement respectively). Birt-Friesen et al.'s (1989) models gave estimates of 3.8 to

5.9 million fry (all cold-water seabirds, low fry movement and cold-water flapping seabirds, high fry movement respectively).

During 1995, 241.7 million pink and chum fry were released into Lake Bay. Our estimates of mortality from fish-eating birds represent between 1.1 and 2.4 percent of the total release. Our assumptions about the movement rates of fry account for 36% of this range in estimates, while differences between the energetic models account for the remaining 64% (Fig. 6).

DISCUSSION

We estimated total fry consumed by birds using three models of seabird energetics (Nagy 1987, Birt-Friesen et al. 1989). These results were compared to a daily consumption rate estimated from focal-animal samples on actively foraging birds. Using these methods, we were able to provide a range of estimates, from low values predicted by the behavioral sampling and the energetic model of Nagy (1987) to a high value predicted by Birt-Friesen et al. (1989).

The models we chose to predict energetic demands were derived from measurements of field metabolic rates of seabirds. Other models of seabird energetics were available, but predicted basal metabolic rates (Ellis 1984, Diamond et al. 1993, Gabrielsen 1994). To avoid estimating a conversion between basal and field metabolic rates, we restricted our analyses to models that were based on FMRs. Nonetheless, more than half of the difference between our low and our high estimates was attributed to differences between the three energetic models.

Differences between the data used to develop the FMR allometric equations likely explain why the models vary. Nagy's (1987) model included 33% warm-water birds, 13% non-designated species and only 53% cold-water species (following Birt-Friesen et al. 1989, N=15 samples from 10 species). In contrast, Birt-Friesen et al. (1989) presented models restricted to cold-water seabirds. Values predicted by Nagy (1987) were below those predicted by Birt-Friesen et al. (1989), presumably because Nagy included warm-water species, which typically have lower metabolic rates than arctic and subarctic birds (Ellis 1984).

We presented two models from Birt-Friesen et al. (1989): one for all seabirds in cold-water regions (N=16 samples from 16 species), the second for flapping seabirds in cold-water (N=8 samples from 8 species). The latter model predicts higher FMRs, because flapping is energetically expensive. While the first model is based on a larger sample size and is presumably more general, it includes data from a variety of species not typical of our study area. The model for cold-water flapping seabirds includes Black-legged Kittiwakes, two murre species, two petrel species, Black Guillemots, Least Auklets, and Northern Gannets. These species are similar to the birds aggregated in front of Wally Noerenberg Hatchery during this study. Although the cold-water model for flapping seabirds encompasses a small range of body sizes (Montevecchi et al. 1992), it includes species that span a greater range in body size than the seven piscivores in our study. We therefore this model is most appropriate for our purposes.

There are several possible reasons why estimates from the behavioral data differ from those of the energetics models. First, we applied a literature value for Black-legged Kittiwakes foraging time to Arctic Terns and Bonaparte's Gulls because of a lack of published values for the latter two species. It is not known how this estimate compares to true values for these birds. Second, we did not correct for the fact that bird diets may include items other than salmon fry. We saw birds that appeared to capture and consume a prey item without having fish visible in

their beaks. These birds may have been consuming smaller prey such as amphipods. Birds may also have been selectively preying on larger fry in a school (but see Kålås et al. 1993 for a report of birds selectively taking smaller, not larger, fry). If birds derived a portion of their diet from zooplankton or selectively chose larger salmon fry, fewer fry would be required to meet their energetic demands. Third, FMR allometric equations were based on values obtained during the breeding season, primarily during chick-rearing (Nagy 1987, Birt-Friesen et al. 1989). Breeding birds may have higher energy demands than non-breeders (Gabrielsen & Mehlum 1989, Baird 1990; Gabrielsen 1994). Although our study overlapped the early breeding season for some species (Isleib & Kessel 1973), most birds were not rearing chicks and thus may have had lower energetic demands than predicted by the FMR equations. The magnitude of these biases are unknown. Fourth, we assumed a digestive efficiency of 100% in our calculations. Measures of digestive efficiency for seabirds range from 69% (White-chinned Petrel chicks fed light fish, Jackson 1986) to 82% (for piscivorous birds, Ricklefs 1974 in Furness & Monaghan 1987). If digestive efficiency was 80%, our estimated consumption from energetic models would increase by twenty-five percent. Fifth, foraging birds may turn over at a site, so that counts of the birds in the area at any one time would underestimate the number of birds feeding there in the course of a day. We were unable to estimate the magnitude of this bias.

The behavioral estimates overlapped the energetic estimates at the lower end only. Two of the known biases in our models suggest that the energetics calculations would overestimate consumption, which may explain why energetic estimates were higher than those from focal animal sampling. However, the measured FMRs for both Black-legged Kittiwakes (Gabrielsen et al. 1987) and Arctic Terns (Uttley et al. 1994) fell towards the higher estimates of FMR (Fig. 2), indicating that our calculations of energetic demand were reasonable.

We estimated a cumulative mortality to fry of 1.1-2.4%. We were unable to partition this mortality between pink and chum salmon fry. This estimate falls toward the low end of estimates of salmon losses to bird predators: Wood (1987b) found loss rates to birds as high as 65%. However, several authors (Ruggerone 1986, Wood 1987a, Kålås et al. 1993) report rates in the range of 1-10%, rates very close to our estimates. A similar study on fish predators at hatcheries (Collis & Beaty 1995) found that Northern Squawfish aggregated to feed at salmon release sites, but total consumption was not calculated due to difficulties of estimating predator numbers. As part of the Sound Ecosystem Assessment program, estimates of fish predation rates on juvenile pink salmon are being made by other researchers (G. Thomas, M. Willette) using fisheries acoustics and examination of stomach contents. Their results along with ours will allow a comparison of the relative magnitude of fish and avian predation on pink salmon in Prince William Sound.

We were told by residents at the hatchery that there were relatively few birds present the year of our study, and that in previous years, 'thousands' of birds were attracted to fry releases and fed in the areas we were surveying. Losses of salmon fry to seabirds would clearly be greater in years when many more birds were present, which would be consistent with comparable or higher predation rates reported from other studies of seabirds aggregating to feed on salmon (Ruggerone 1986, Wood 1987a, 1987b, Kålås et al. 1993). The general decline in the numbers of all piscivorous birds at the hatchery by early June may be due to the constraints on seabird foraging

as birds enter the nesting season. This suggests that releasing fry later in the year would reduce losses to avian predators.

ACKNOWLEDGEMENTS

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Table 1: Ten species of birds accounted for 93% of counts in the hatchery area. The body masses of the seven piscivorous species (P) are given.

Common name	Species	Diet	BM (kg)	Total count	Prop.
All species				7333	1.00
Black-legged Kittiwake	Rissa tridactyla	P	0.383[a]	1957	0.27
Marbled Murrelet	Brachyramphus marmoratus	P	0.205[b]	1851	0.25
Glaucous-winged Gull	Larus glaucescens			793	0.11
Arctic Tern	Sterna paradisaea	P	0.101[c]	499	0.07
Red-breasted Merganser	M. serrator	P	0.794[d]	413	0.06
Common Merganser	Mergus merganser	P	1.134[d]	355	0.05
Barrow's Goldeneye	Bucephala islandica			319	0.04
Harlequin Duck	Histrionicus histrionicus			265	0.04
Bonaparte's Gull	L. philadelphia	P	0.205[e]	211	0.03
Mew Gull	L. canus	P	0.430[f]	178	0.02

[a] Gabrielsen et al. 1987; [b] Kuletz et al. 1995; [c] Uttley et al. 1994; [d] Palmer 1976; [e] Terres 1980; [f] Ellis 1984.

Table 2: Parameters for the regression y = mx + b, where y is the log of field metabolic rate and x is the mass of the bird.

Source	Intercept (SE)	Slope (SE)	Mass range	Unit	N
Nagy 1987[1]	0.904 (0.187)	0.704 (0.061)	420-9440	g	15
Birt-Friesen 1989[2]					
cold-water, all seabirds	3.13 (0.03)	0.646 (0.040)	0.043-13.0	kg	16
cold-water, flapping flight	3.24 (0.05)	0.727 (0.039)	0.083-3.210	kg	8

[1] Nagy (1987) reports mass as grams rather than kilograms, and we retain his usage in this table.
[2] This author reports regressions for cold-water seabirds, and cold water seabirds using flapping flight.

Table 3: Observed feeding rates.

Species	Sample time (N)	Fry/hr	Foraging hrs/day
Black-legged Kittiwake	0:42 (9)	98.2	2.3[1]
Bonaparte's Gull	0:20 (4)	66.0	2.3
Arctic Tern	0:31 (7)	65.2	2.3

[1] Hours per day spent foraging was calculated as the time per foraging trip spent in search behavior (e.g. localized circling, plunging; 60-80 min/trip. Irons 1992) times the number of foraging trips per day. We assumed birds that made two foraging trips per day (Irons 1992) and spent 70 minutes per trip actively searching and feeding. This estimate was used for all species.

FIGURE CAPTIONS

Fig. 1: Study location. The small inset indicates the location of Prince William Sound in Alaska; the larger inset shows Esther Island relative to Prince William Sound. In the main map, Wally Noerenberg Hatchery is indicated by a star. Numbered polygons represent the count areas surveyed from a small skiff. Polygons 1-7 collectively form Lake Bay, 9-13 form Quillian Bay, and 8 and 14 form the Wells Passage count area.

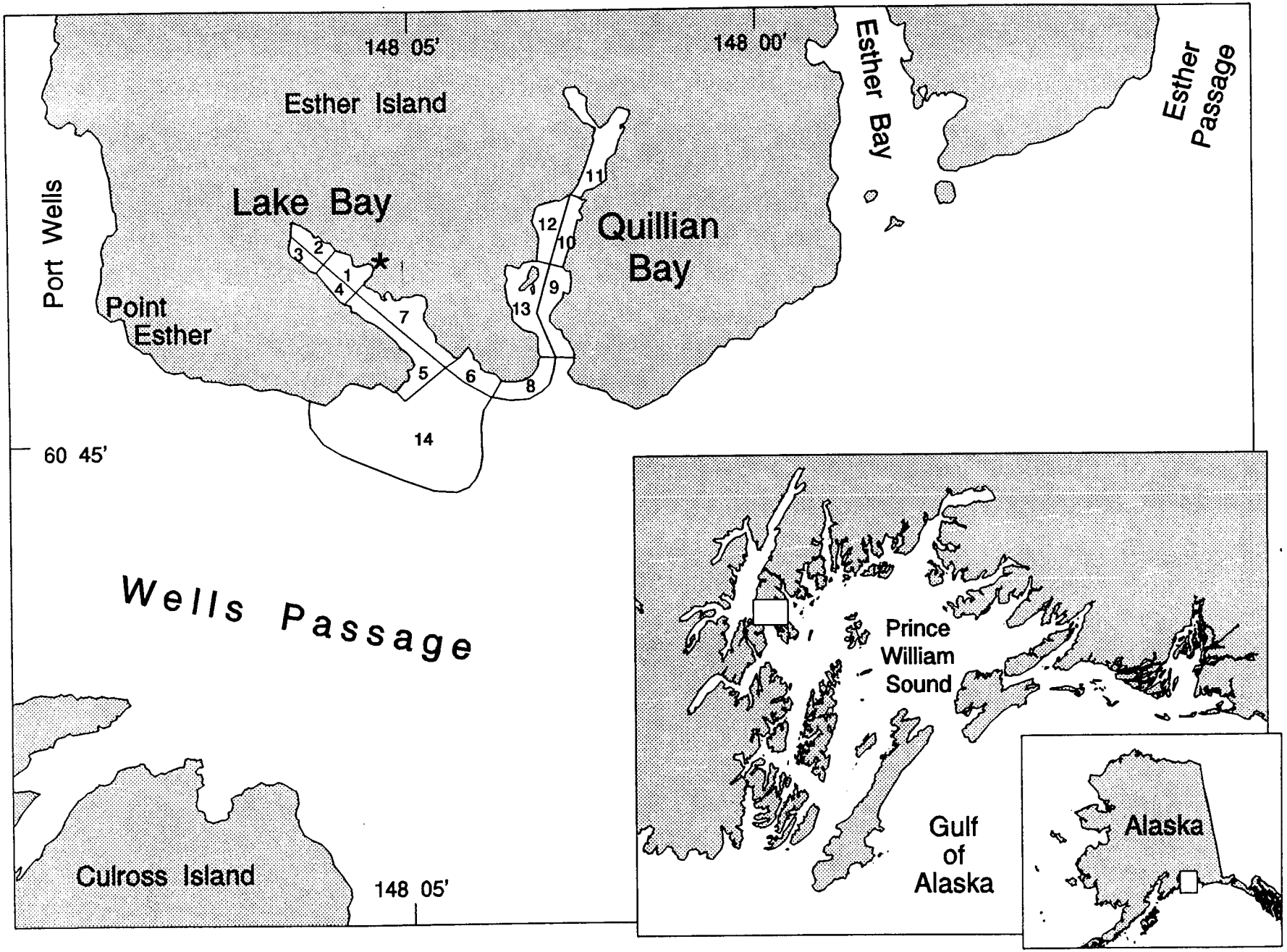
Fig. 2: Comparison of some published energetic models for seabirds. The mass regression equations and parameters are given in Table 3. Measured field metabolic rates (FMR) for Black-legged Kittiwakes (FMR equals the average of on nest and off nest measurements, Gabrielsen et al. 1987) and Arctic Terns (Uttley et al. 1994) are also plotted for comparison to predicted values. See text for a discussion of differences between the models. The species are plotted based on the body masses in Table 1 and are: Arctic Tern (ARTE), Marbled Murrelet (MAMU), Bonaparte's Gull (BOGU), Black-legged Kittiwake (BLKI), Mew Gull (MEGU), Red-breasted Merganser (RBME), and Common Merganser (COME).

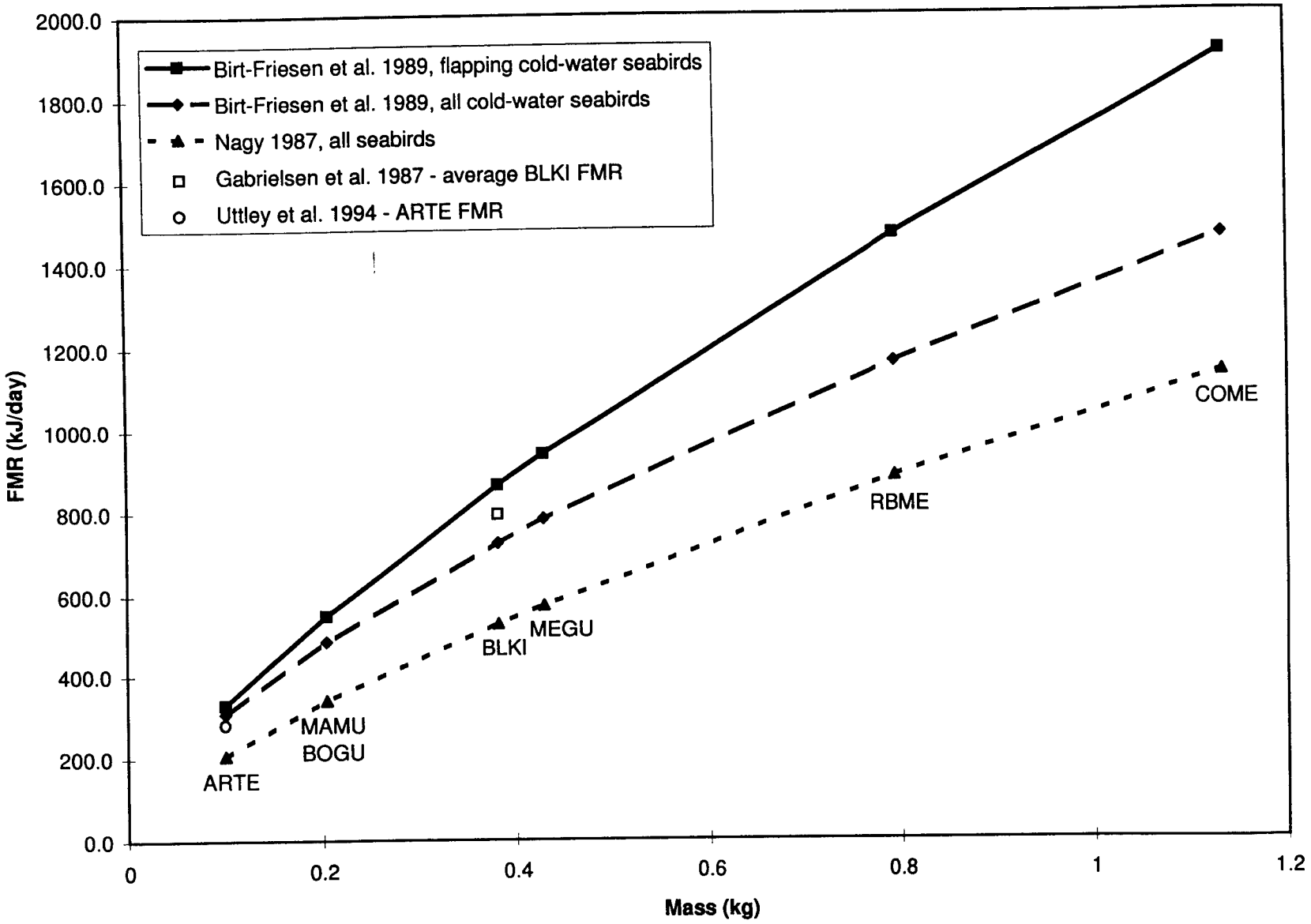
Fig. 3: Numbers of fry remaining in front of the hatchery under two estimates of movement rates. We assumed that either 2.5% (low movement) or 50% (high movement) of the fry in the area leave each day. The high movement scenario was equivalent to assuming an average movement rate of two kilometers per day. Note that while this parameter had a large effect on the number of fry remaining in the area (left axis, boxes), the effect on the energy content of the average fry (right axis, no boxes) was much smaller.

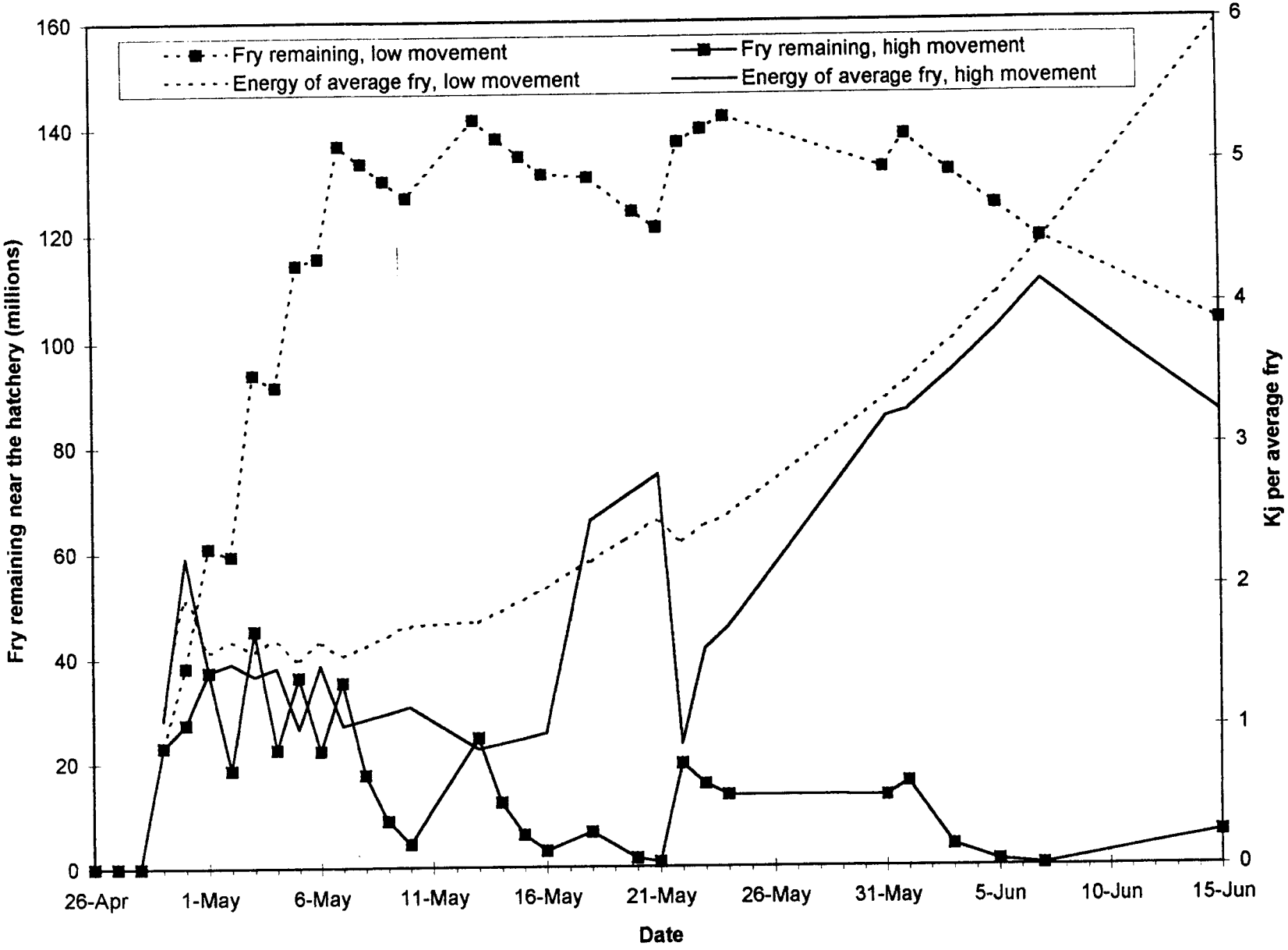
Fig. 4: The numbers of Marbled Murrelets, Black-legged Kittiwakes, and total piscivores counted in the study area. Salmon fry release dates and numbers are shown for comparison. Note that zero on the right axis has been raised to visually separate the fry release line from the bird counts. Piscivores increased in the area in response to fry releases, and decreased when intervals between releases were longer.

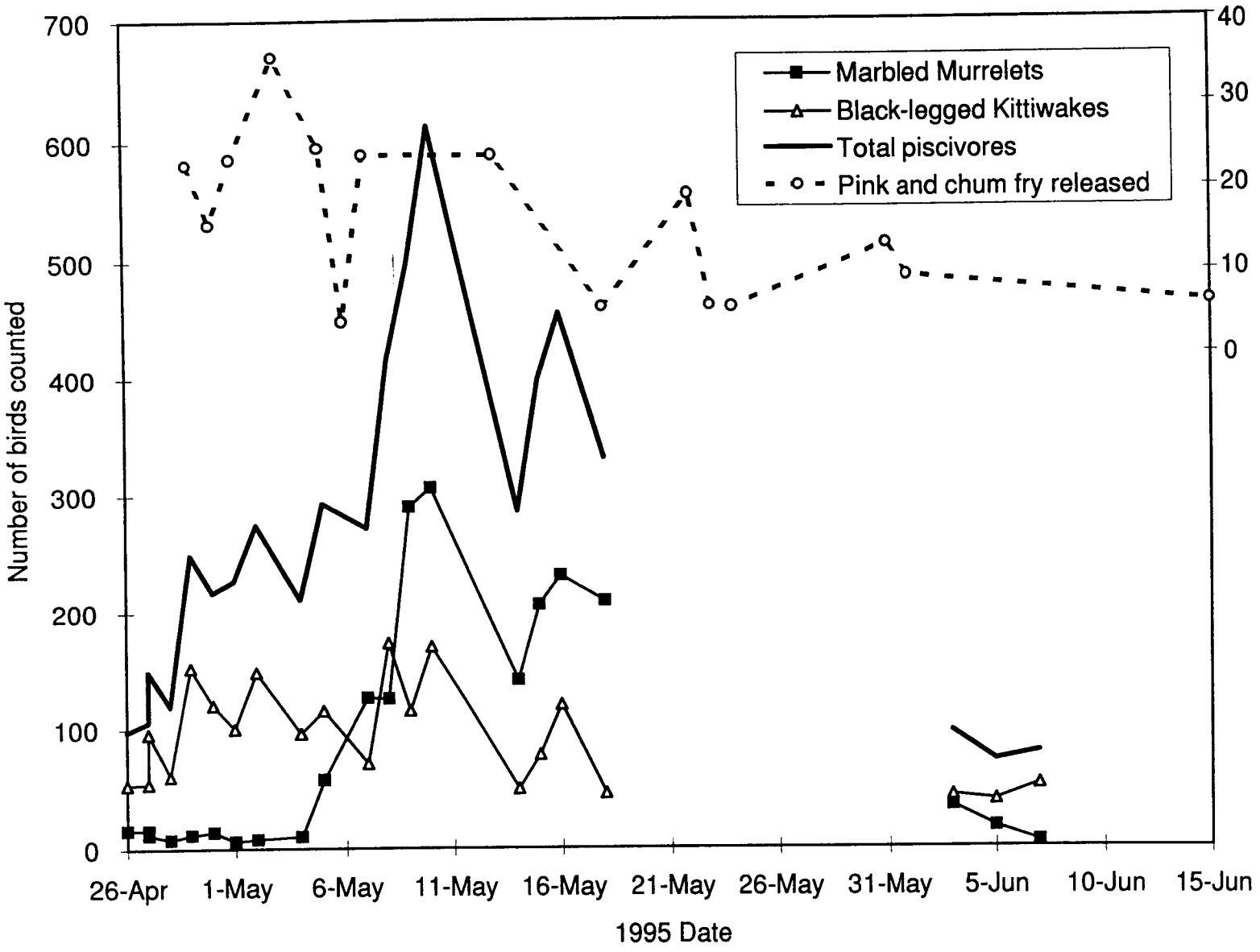
Fig. 5: Comparison of per capita consumption based on observed feeding rates and energetic models.

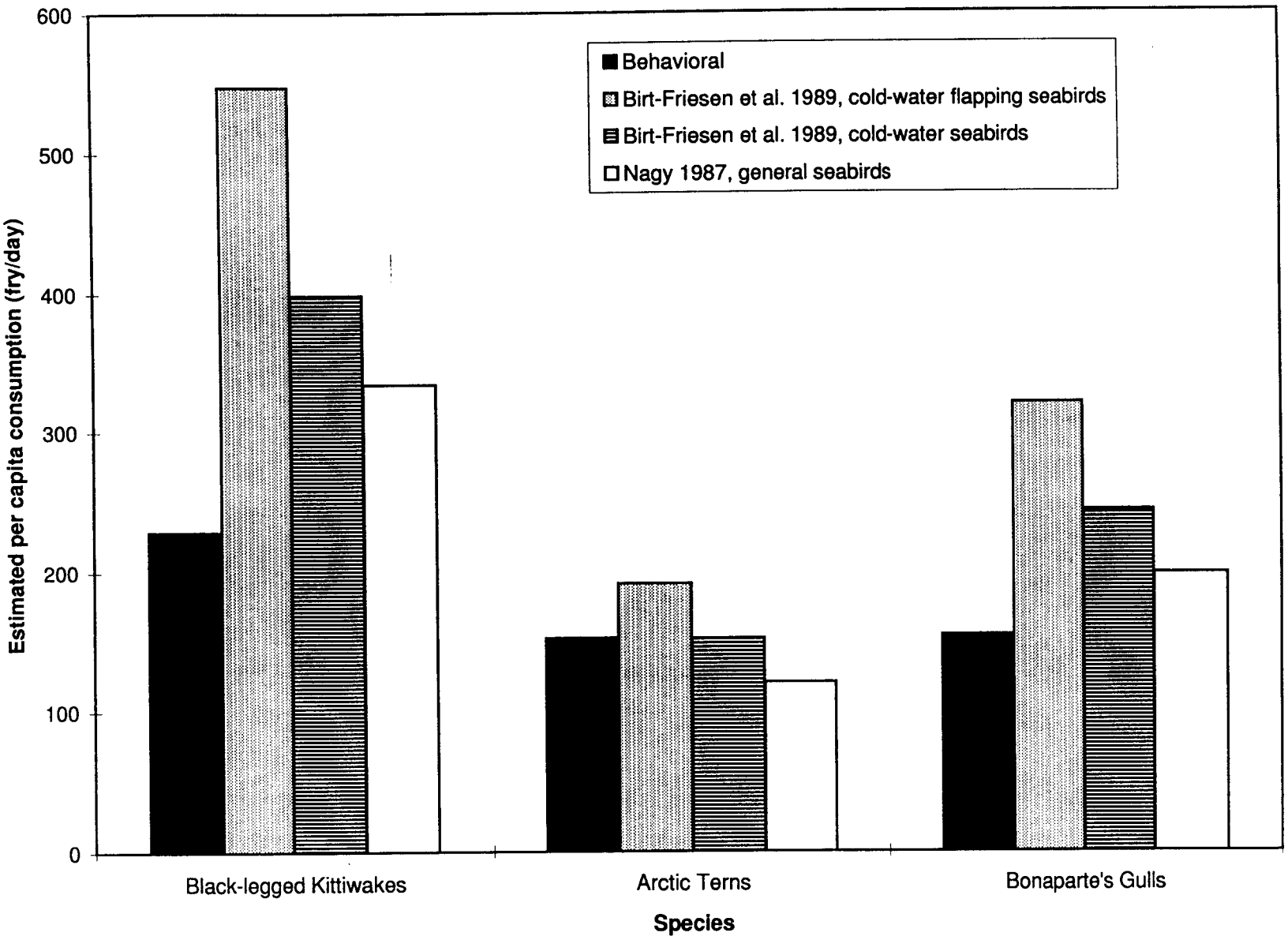
Fig. 6: Cumulative consumption of pink and chum fry by seven species of birds from the first day of fry release to 7 June 1995, the last day that birds were counted. Consumption was based on the three energetics models listed along the x-axis, and calculated assuming both high and low fry movement rates. See text for details.

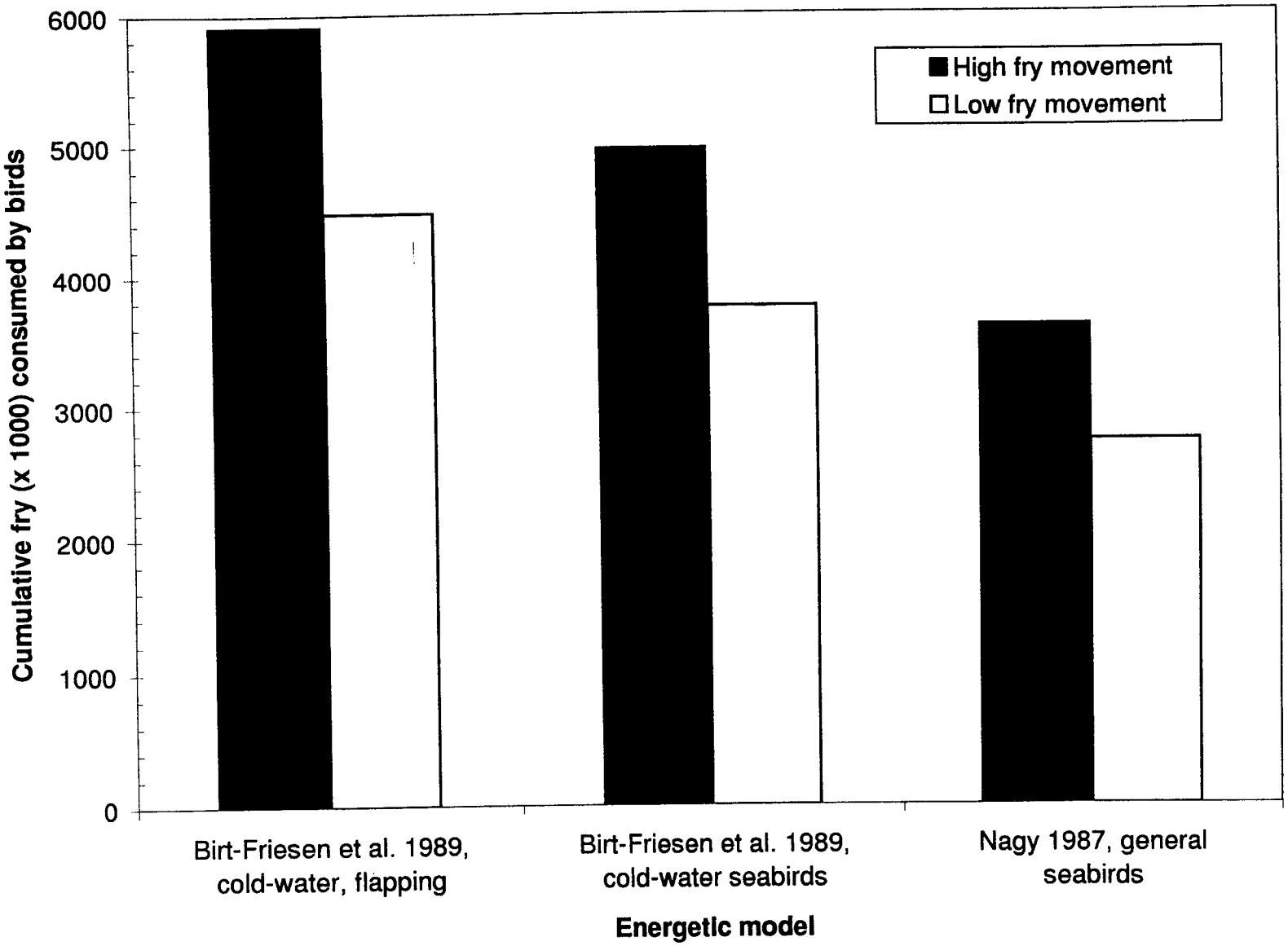

148 05'
148 00'
Esther Island
Esther Bay
Esther Passage
Port Wells
Lake Bay
Quillian Bay
Point Esther
1
2
3
4
5
6
7
8
9
10
11
12
13
14
60 45'
Wells Passage
Culross Island
148 05'
Prince William Sound
Gulf of Alaska
Alaska


Birt-Friesen et al. 1989, flapping cold-water seabirds
Birt-Friesen et al. 1989, all cold-water seabirds
Nagy 1987, all seabirds
Gabrielsen et al. 1987 - average BLKI FMR
Uttley et al. 1994 - ARTE FMR
FMR (kJ/day)
2000.0
1800.0
1600.0
1400.0
1200.0
1000.0
800.0
600.0
400.0
200.0
0.0
0
0.2
0.4
0.6
0.8
1
1.2
Mass (kg)
ARTE
MAMU
BOGU
BLKI
MEGU
RBME
COME


Fry remaining, low movement
Fry remaining, high movement
Energy of average fry, low movement
Energy of average fry, high movement
Fry remaining near the hatchery (millions)
Kj per average fry
Date
0
20
40
60
80
100
120
140
160
0
1
2
3
4
5
6
26-Apr
1-May
6-May
11-May
16-May
21-May
26-May
31-May
5-Jun
10-Jun
15-Jun


Marbled Murrelets
Black-legged Kittiwakes
Total piscivores
Pink and chum fry released
Number of birds counted
Fry released (millions)
1995 Date
0
100
200
300
400
500
600
700
0
10
20
30
40
26-Apr
1-May
6-May
11-May
16-May
21-May
26-May
31-May
5-Jun
10-Jun
15-Jun


600
500
400
300
200
100
0
Estimated per capita consumption (fry/day)
Behavioral
Birt-Friesen et al. 1989, cold-water flapping seabirds
Birt-Friesen et al. 1989, cold-water seabirds
Nagy 1987, general seabirds
Black-legged Kittiwakes
Arctic Terns
Bonaparte's Gulls
Species


6000
5000
4000
3000
2000
1000
0
Cumulative fry (x 1000) consumed by birds
High fry movement
Low fry movement
Birt-Friesen et al. 1989, cold-water, flapping
Birt-Friesen et al. 1989, cold-water seabirds
Nagy 1987, general seabirds
Energetic model

CHAPTER 2: Incidental species, other hatcheries, and the spatial distribution of gulls.

Methods and Results for Objectives (1) Bird aggregations, (2) Behavioral sampling, and (4) Consumption are reported in Ch. 1. Some further material for these objectives are reported here, as are the Methods and Results for objective (3) Volunteer data. Except where otherwise noted, all data was collected near Wally Noerenberg Hatchery on Esther Island.

METHODS

1) Size and composition of aggregations: All birds in Lake and Quillian Bays (Esther Is.) were counted from a small boat (as described in Ch. 1). Bird numbers were always low enough (less than 500 of any species) that precise counts could be made from the skiff without the use of photographic counting.

Birds were also censused over broader areas from aerial surveys using high-resolution video photography. Five successful survey flights were conducted, on 24 April, 1, 8, 16 May, and 3 June 1995. Two other SEA projects conducted additional flights using similar methods and including some of the same areas (projects 95320-Q, Avian predation on herring spawn [M.A. Bishop], and 95320-T, Juvenile herring distribution [E. Brown]). However, as of this report, data from these studies were not yet available for combined analyses, and data reported here are from this study only. Hatcheries and other parts of Prince William Sound were surveyed from a small plane equipped with a global positioning system (GPS) linked high resolution video camera. Surveys were flown at an altitude of 180 meters (600 feet). Single white birds could readily be seen and counted from the videotape filmed at this altitude. During each survey, two observers recorded all birds observed on either side of the plane out to a visual angle of about 30°. The video camera recorded a transect 200 meters wide directly under the plane while observers counted to either side of the plane to about 500 meters. Birds appearing on film were counted during playback from a small television screen and their positions were mapped. It was not possible to identify birds on videotape to species, nor to reliably see dark birds. The tapes were therefore analyzed as a sample of white birds, largely gulls.

GPS-recorded flight paths and the locations of bird flocks were entered into the geographic information system Arc/Info. Information on the distribution of fish was obtained from Prince William Sound Aquaculture Corporation (release dates, numbers, and sizes of fry reared at each hatchery) and the Alaska Department of Fish & Game (estimated tons of herring gathered for spawning at beaches, linear miles of spawn deposited). A 20 km X 20 km grid was overlaid on the study area (Fig. 1) and the linear miles of survey flight, total number of birds, cumulative hatchery fry released, and total tons of herring seen at beaches over the 7 days preceding each flight were calculated for each grid cell. ANOVAs were used to evaluate the correspondence of bird numbers per linear kilometer of survey to each of the other variables.

2) Behavior: Focal-animal sampling was conducted on individual birds in foraging flocks in or at the mouths of Lake and Quillian Bays (as described in Ch. 1).

3) Volunteer organization: Five hatcheries operate in Prince William Sound: Wally Noerenberg Hatchery on Esther Is., Lake Bay; Armin F. Koernig Hatchery on Evans Island, Sawmill Bay; Main Bay Hatchery in Main Bay; Cannery Creek Hatchery in Unakwik Inlet on Cannery Creek; and Solomon Gulch Hatchery in Valdez Arm on Solomon Gulch. Each hatchery was visited to recruit one or several volunteer staff to count and identify birds. Volunteers at each location were provided with Bushnell 8X40 binoculars, a Field Guide to the Birds of North America (National Geographic Society 1987), and a guide to local species of gulls. At each hatchery, an area was designated that could be easily observed from a point on shore. Each area included about 500 meters of shoreline and birds were counted out to 200 meters, although the dimensions of the areas varied according to the local geography. Data forms and instructions were provided. Volunteers were asked to record the birds in the count area as regularly as possible, preferably daily from April at least through the last release of fry from the hatchery. Discussions with hatchery managers and site visits by D. Scheel suggested that at least some of the volunteers did not reliably distinguish different species of birds. We therefore restricted our analyses to considering all gull species as a single group and all non-gull species as a second group.

4) Calculating consumption rates:
Detailed methods for estimating the cumulative consumption of fry by birds at the Wally Noerenberg Hatchery are given in Ch. 1.

RESULTS

Most results for Objectives (1), (2), and (4) are reported in Ch 1. Some further material for these objectives is included here, as are the results for objective (3).

1) Size and composition of aggregations
Bird censuses: Birds were surveyed on a total of 23 days. Thirty-six counts of birds were made in Lake Bay; 30 included portions of Wells Passage at the mouth of the bay (Ch. 1, Fig. 1); 24 surveys included Quillian Bay. Sixty-five bird species were represented in a total of 8,746 birds counted (Table 1). Of these, two thirds were counted in Lake Bay and one third in Quillian. Results of bird counts regarding the ten most common species of birds in the count area are reported in Ch. 1.

Aerial surveys: Five aerial surveys were flown during which bird aggregations were counted and mapped (Fig. 2-6). Total birds per kilometer of flight was higher in grid cells that contained schooling herring (ANOVA, $F=7.79$, $p = 0.007$) and in grid cells containing a hatchery (ANOVA, $F=22.16$, $p < 0.001$). In these surveys, seabird numbers declined from April through June, which was reflected in a negative correlation with the date (ANOVA, $F=6.70$, $p = 0.013$).

2) Behavior:
A total of 30 focal animal samples were attempted on three species of plunge-diving seabirds in mixed-species flocks: Black-legged Kittiwakes, $N=15$, Arctic Terns, $N=10$, and Bonaparte's Gulls, $N=5$. Of these, the birds in eight samples (total duration 38 minutes) could

not be seen closely enough to observe whether fish were caught in their beaks following capture attempts. These samples were discarded from further consideration. Of the remaining 22 samples, two were discarded because the birds were not observed for at least three minutes. The remaining 20 samples were used to estimate feeding rates and daily consumption of these three species (Ch. 1).

3) Volunteer data:

Volunteers at four hatcheries made a total of 189 counts of birds (Table 2). Gulls, terns and other fish-eating birds made up most of the counts at Armin F. Koernig Hatchery (Evans Island), and Main Bay Hatchery. At Cannery Creek and Solomon Gulch, ducks and other birds were almost as common as gulls, terns and other piscivores (Table 2). At each hatchery except Main Bay, the numbers of gulls and terns increased following the first release of fry (Table 2; Fig. 7). At Main Bay, counts dipped in the week following the first fry release, but showed an overall increase from April to June (Fig. 7).

4) Calculating consumption rates: See Ch. 1 for complete results.

DISCUSSION

This report presents two main results. The first, discussed in detail in Ch. 1, is an estimate of consumption of pink and chum salmon fry in front of Wally Noerenberg Hatchery. The second is an analysis of the distribution of white seabirds (largely gulls) based on five aerial surveys over western Prince William Sound (Figs. 1-6). Gulls were associated with spawning herring and with hatchery sites.

Large numbers of gulls were present at the north end of Montague Island (Fig. 2-3), one of the major herring spawning beaches in Prince William Sound. Boat counts of birds aggregating where spawn was present indicated that most of these were Glaucous-wing Gulls gorging on spawn. Plunge-diving species that commonly feed on small fish were rare (pers. comm., M.A. Bishop. See reports of 94- and 95320-Q, Avian predation on herring spawn). However, gull numbers during all aerial surveys were not correlated with linear miles of herring spawn, but were correlated with the recent presence of schools of herring aggregated to spawn. The correlation of 'white' bird aggregations with the presence of small fish (spawning herring and hatchery locations) indicates that concentrations of forage fish have an important influence on the distribution of birds at sea during this time of the year. The variation in bird numbers that are attracted to hatchery releases each year (see Ch. 1) may be explained if birds aggregate at the best feeding sites: when available, these are the spawning migrations of herring, sandlance or other fish. When the spawning of these forage fish does not coincide with fry release or outmigration, then larger numbers of birds may be attracted to salmon fry at hatcheries and in streams. Additional data is required to test this idea; it is possible that this pattern might be documented under studies conducted by the Apex Predator Experiment group (APEX).

Gull abundances in the aerial surveys declined over the course of the study. The avian counts recorded at Wally Noerenberg Hatchery also declined in late May and early June (Ch. 1). Gulls therefore appear to leave the open shorelines of the Sound or become less visible towards the end of May. At this time, these birds are beginning their breeding seasons (Isleib & Kessel

1973) and may spend less time foraging and more time at colonies. It is also possible that gulls shift their activities further up into inlets and fjords.

LITERATURE CITED

Isleib ME, Kessel B (1973) Birds of the North Gulf Coast - Prince William Sound region, Alaska. University of Alaska Press, Fairbanks

National Geographic Society (1987) Field Guide to the birds of North America. 2nd ed. The National Geographic Society, Washington, D.C.

Table 1: Bird species list from Esther Island hatchery surveys, 1995. "Where else seen" column reflects birds surveyed outside of Lake and Quillian Bays. "Total Occurrences" reflect total number of birds observed in each bay.

	Common Name	Species	Where Else Seen	Total occurance in: Lake Bay	Quillian Bay
COLO	Common Loon	*Gavia immer*		0	7
PALO	Pacific Loon	*Gavia pacifica*		3	1
RTLO	Red-throated Loon	*Gavia stellata*	Granite Bay	0	0
RNGR	Red-necked Grebe	*Podiceps grisegena*	Granite Bay, Esther Bay	2	3
HOGR	Horned Grebe	*Podiceps auritus*	Granite Bay, Esther Bay	0	3
DCCO	Double-Crested Cormorant	*Phalacrocorax auritus*	Esther Rocks, Egg Rocks	1	0
PECO	Pelagic Cormorant	*Phalacrocorax pelagicus*	Esther Rocks, Egg Rocks, Granite Bay	9	17
GTBH	Great Blue Heron	*Ardea herodias*	Esther Bay	2	0
SACR	Sandhill Crane	*Grus canadensis*	Wells Passage, migrating in April	0	0
TRSW	Trumpeter Swan	*Cygnus buccinator*	Wells Passage	0	0
CAGO	Canada Goose	*Branta canadensis*	Esther Bay, Wells & Esther Passages	0	7
MALL	Mallard	*Anas platyrhynchos*		12	45
GWTE	Green-winged Teal	*Anas crecca*		35	24
AMWI	American Wigeon	*Anas americana*		14	0
NOPI	Northern Pintail	*Anas acuta*		22	0
NOSH	Northern Shoveler	*Anas clypeata*	Forbidden Beach	0	0
	Scaup sp. (Greater or Lesser)	*Aythya spp.*	Esther Bay.	0	0
WWSC	White-winged Scoter	*Melanitta fusca*	Wells Passage, Forbidden Beach, Port Wells	0	0
SUSC	Surf Scoter	*Melanitta perspicillata*	Wells Passage, Port Wells	0	0
HADU	Harlequin Duck	*Histrionicus hitrionicus*	Esther & Egg Rocks, Esther & Granite Bay	340	23
OLDS	Oldsquaw	*Clangula hyemalis*		0	8
BAGO	Barrow's Goldeneye	*Bucephala islandica*	Esther & Granite Bay; Forbidden Beach	225	306
COGO	Common Goldeneys	*Bucephala clangula*		0	1
BUFF	Bufflehead	*Bucephala albeola*		80	3
COME	Common Merganser	*Mergus merganser*	Esther & Granite Bay; Forbidden Beach	431	104
RBME	Red-breasted Merganser	*Mergus serrator*	Esther & Granite Bay; Forbidden Beach	88	350
BLOY	Black Oystercatcher	*Haematopus bachmani*	Esther & Granite Bay; Esther & Egg Rocks	0	0
WHIM	Whimbrel	*Numenius phaeopus*		0	3
GRYE	Greater Yellowlegs	*Tringa melanoleuca*		0	2
SPSA	Spotted Sandpiper	*Actitis macularia*		0	3
WATA	Wandering Tattler	*Heteroscelus incanus*		3	1
DOSP	Unidentified Dowicher	*Limnodromus spp.*		2	2
LESA	Least Sandpiper	*Calidris minutilla*	At the hatchery	3	0
BASA	Baird's Sandpiper	*Calidris bairdii*	At the hatchery	1	0
PAJA	Parasitic Jaeger	*Stercorarius parasiticus*	Forbidden Beach	0	0
BOGU	Bonaparte's Gull	*Larus philadelphia*	Forbidden Beach	406	2
MEGU	Mew Gull	*Larus canus*	Esther & Granite Bay; Forbidden Beach	189	59

	Common Name	Species	Where Else Seen	Total occurance in: Lake Bay	Quillian Bay
HEGU	Herring Gull	*Larus argentatus*		14	0
GWGU	Glaucous-winged Gull	*Larus gaucescens*	Everywhere	1120	126
BLKI	Black-legged Kittiwake	*Rissa tridactyla*	Everywhere	1608	599
ARTE	Arctic Tern	*Sterna paradisaea*	Granite Bay	397	50
PIGU	Pigeon Guillemot	*Cepphus columba*	Esther & Granite Bay; Forbidden Beach	44	41
MAMU	Marbled Murrelet	*Brachyramphus marmoratus*	Everywhere	673	1029
KIMU	Kittlitz's Murrelet	*Brachyramphus brevirostris*	Possible sighting, Barry Arm.	0	0
BAEA	Bald Eagle	*Haliaeetus leucocephalus*	Esther & Granite Bay; Forbidden Beach	68	15
NOHA	Northern Harrier	*Circus cyaneus*		0	1
RUHU	Rufous Hummingbird	*Selasphorus rufus*	At the hatchery	1	0
BEKI	Belted Kingfisher	*Ceryle alcyon*		3	0
STJA	Steller's Jay	*Cyanocitta stelleri*	Wooded shores	No count	No count
NWCR	Northwestern Crow	*Corvus caurinus*	Wooded shores, intertidal	No count	No count
CORA	Common Raven	*Corus corax*	Wooded shores	No count	No count
RCKI	Ruby-crowned Kinglet	*Regulus calendula*	At the hatchery	No count	No count
GCTH	Grey-cheeked Thrush	*Catharus minimus*	By call only	Heard	Heard
HETH	Hermit Thrush	*Catharus guttatus*	By call only	Heard	Heard
VATH	Varied Thrush	*Ixoreus naevius*	Wooded shores, by call	No count	No count
AMRO	American Robin	*Turdus migratorius*		0	1
WAPI	Water Pipit	*Anthus spinoletta*		4	0
AMDI	American Dipper	*Cinclus mexicanus*	By streams	2	1
OCWA	Orange-crowned Warbler	*Vermivora celata*	Shoreline shrubs, by call	No count	No count
WIWA	Wilson's Warbler	*Wilsonia pusilla*	Shoreline shrubs, by call	No count	No count
SASP	Savannah Sparrow	*Passerculus sandwichensis*	At the hatchery	6	0
DEJU	Dark-eyed Junco	*Junco hyemalis*	Wooded shores, by call	No count	No count
FOSP	Fox Sparrow	*Passerella iliaca*	At the hatchery	3	0
PISI	Pine Siskin	*Carduelis pinus*	Wooded shores, by call	No count	No count
CORE	Common Redpoll	*Carduelis flammea*	Wooded shores	No count	No count
SEOT	Sea Otter	*Enhydra lutris*	Everywhere	19	12
RIOT	River Otter	*lutra canadensis*	Wooded shores, Lake & Quillian; net pens	7	5
	Mink	*Mustela vison*	Wooded shores, Lake & Quillian; net pens	2	1
STSL	Steller's Sea Lion	*Eumetopias jubatus*	Well's Passage, Forbidden Beach	9	5
HASE	Harbor Seal	*Phoca vitulina*	Granite Bay	9	13
DAPO	Dall's Porpoise	*Phocoenoides dalli*	Well's Passage	No count	No count
HAPO	Harbor Porpoise	*Phocoena phocoena*	Well's Passage	No count	No count
	Minke, Humpback, and Killer whales also reported inside Lake Bay.			No count	No count
	Black Bear		Granite & Quillian Bay	No count	No count

Table 2: Volunteer Bird Count Data

Hatcheries	Earliest Fry Release Date	Total Days[1]	Total Gulls & Terns[2]	Total Fish-eating Birds[3]	Other s[4]
Armin F. Koernig	25-Apr-95	33	534	180	300
Cannery Creek	29-Apr-95	30	992	37	1036
Main Bay	30-Apr-95	99	1363	373	1110
Solomon Gulch	2-May-95	27	85	21	82
Totals		189	2974	611	2528

[1] Total days data was collected by volunteers at hatcheries listed above.
[2] Species of gull consisted of: Bonaparte's, Mew, Herring, Glaucous-winged, Glaucous, Black-legged Kittiwake, Sabine's and unknown Gulls. Tern species were: Caspian's, Arctic, Aleutian, and Common Terns.
[3] Species list for fish-eating birds: Pelagic and unidentified Cormorant, Great Blue Heron, Common, Red-breasted, and unidentified Merganser, Common, and unidentified Murre, Pigeon Guillemot, Marbled and unidentified Murrelet and unidentified Brachyramphus.
[4] Other bird species included in data: Common and Red-throated Loon, Common, Barrow's and unidentified Goldeneyes, Bufflehead, Bald Eagle, Belted Kingfisher, Black Oystercatcher, Mallard, American Wigeon, Leach's Storm-petrel, Northern Pintail, Canada Goose, Green-winged Teal, Lesser and Greater Yellowlegs, , Whimbrel, Greater Scaup, Gadwall, Solitary and Least Sandpiper, unidentified Dabbling Duck, Black, White-winged and Surf Scoters, Common Raven, unidentified Grebe, Wandering Tattler, Sandhill Crane, and Northwestern Crow.

FIGURE CAPTIONS

Figure 1: Twenty kilometer by twenty kilometer grid overlaid on Prince William Sound for analyses of aerial survey data.

Figure 2: Locations of birds visible from the air (gulls and large ducks) on 24 April 1995 (prior to release of hatchery-reared fry). The flight path is indicated by the solid black line. The number of birds is indicated by the size of the spot marker. Bird locations were recorded by a computer-linked global positioning system. Birds were counted by observers and the numbers were verified by counting from high-resolution videotape.

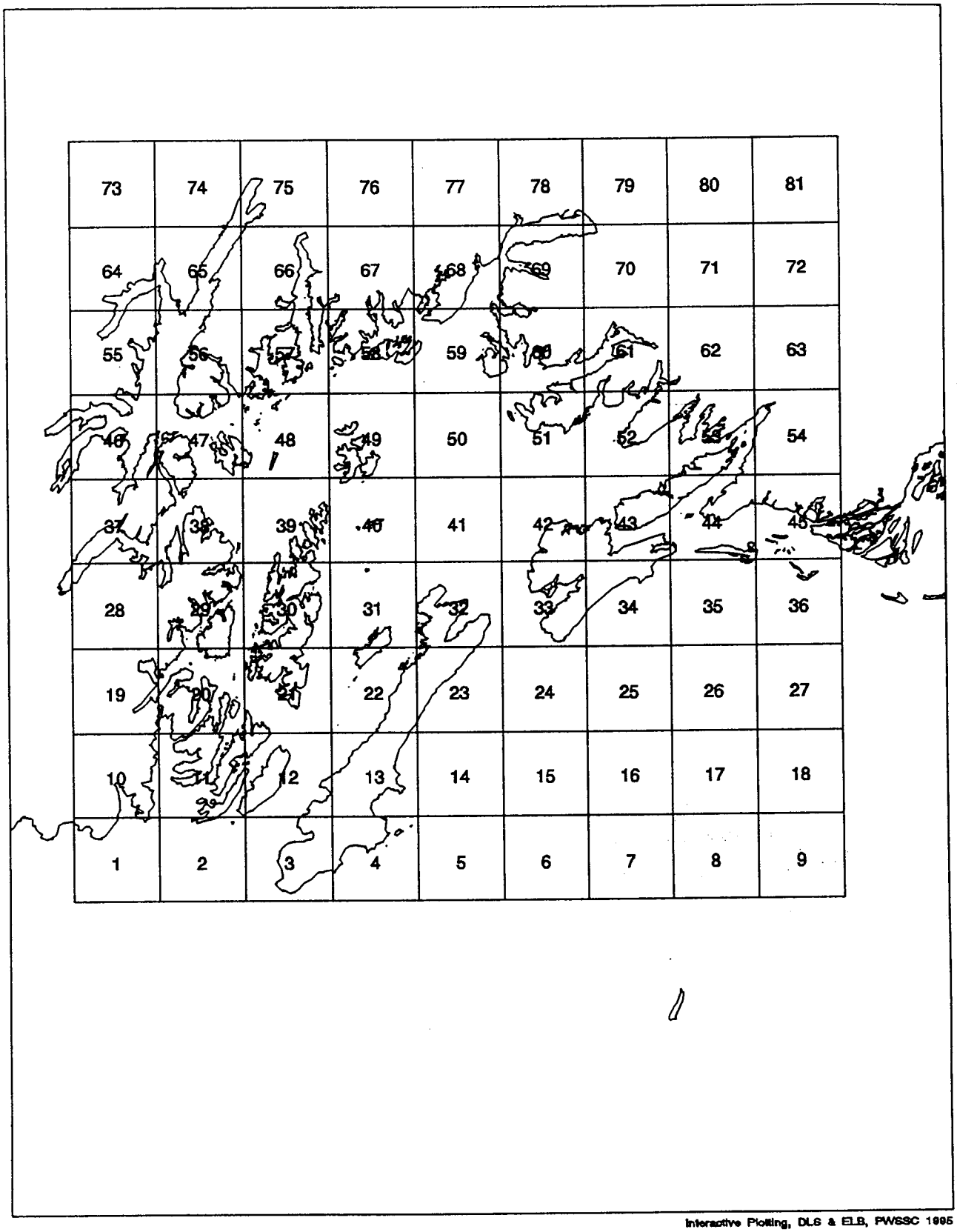
Figure 3: Locations of birds, 1 May 1995 (after fry releases had begun). Details as in Figure 2.

Figure 4: Locations of birds, 8 May 1995. Details as in Figure 2.

Figure 5: Locations of birds, 16 May 1995. Details as in Figure 2.

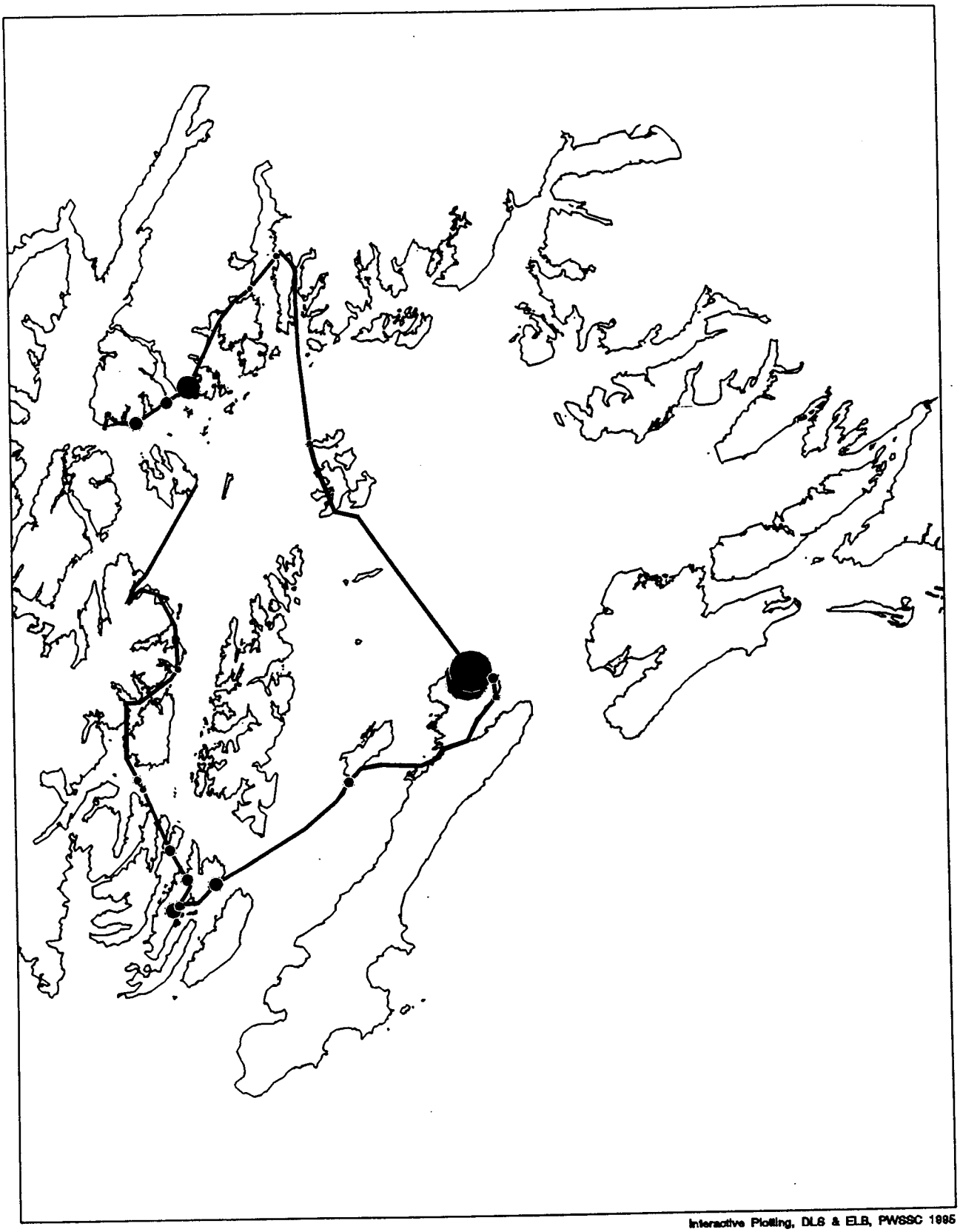
Figure 6: Locations of birds, 3 June 1995. Details as in Figure 2.

Figure 7: The number of gulls and terns counted by volunteers in front of four hatcheries in Prince William Sound. The hatcheries are Armin F. Koernig (AFK), Cannery Creek (CCH), Main Bay (Main), and Solomon Gulch (SGH). Numbers presented are averages over all counts in a given calendar week. Numbers were generally low prior to fry releases (compare Table 2), however, increases in counts did not closely correspond with release periods.



Analysis grid and grid numbers. Each cell is 20 by 20 kilometers.

FIG. 2



24-Apr-95; Smallest point indicates five birds

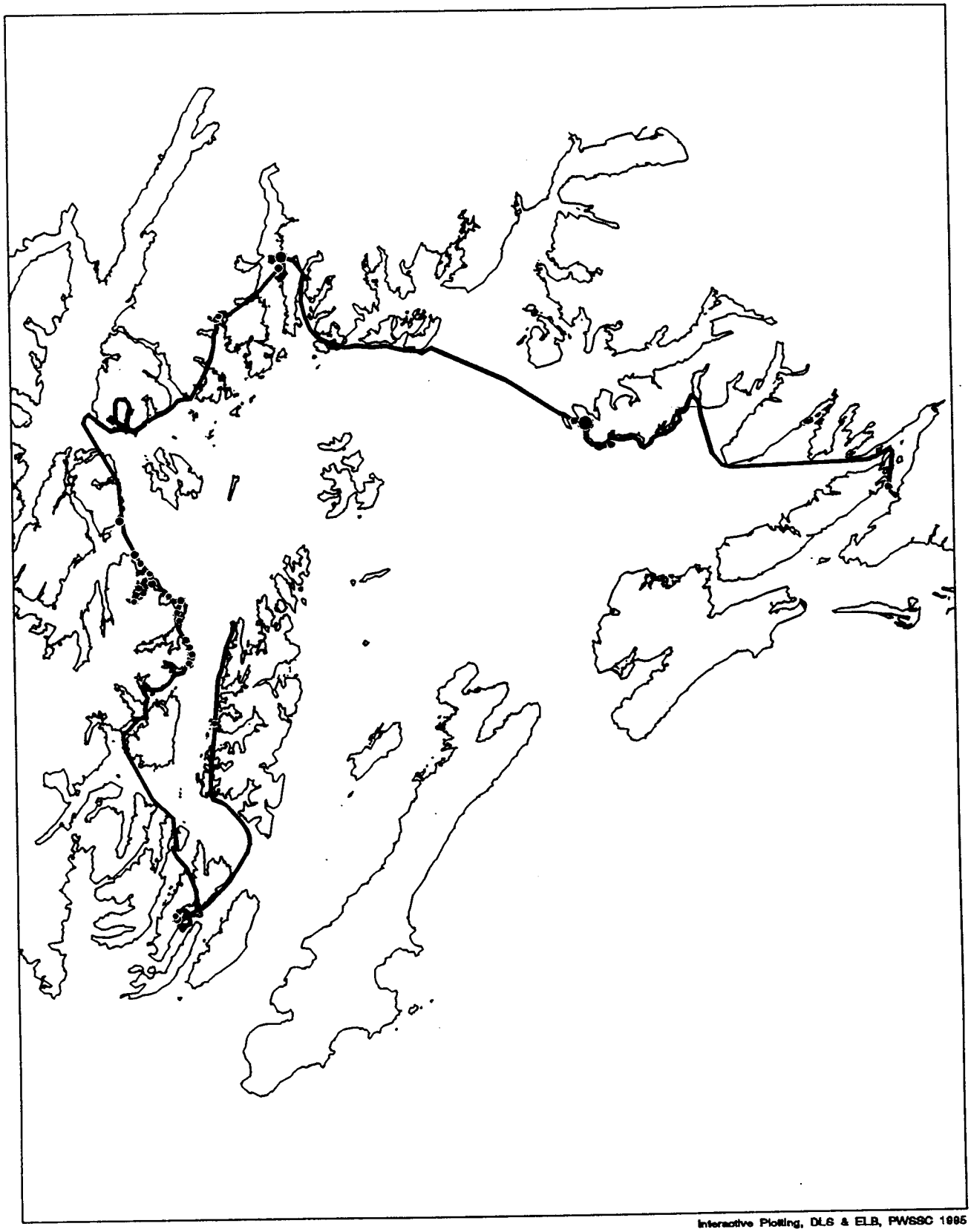
01-May-95; Smallest point indicates five birds.

FIG. 4

08-May-95; Smallest point indicates five birds.

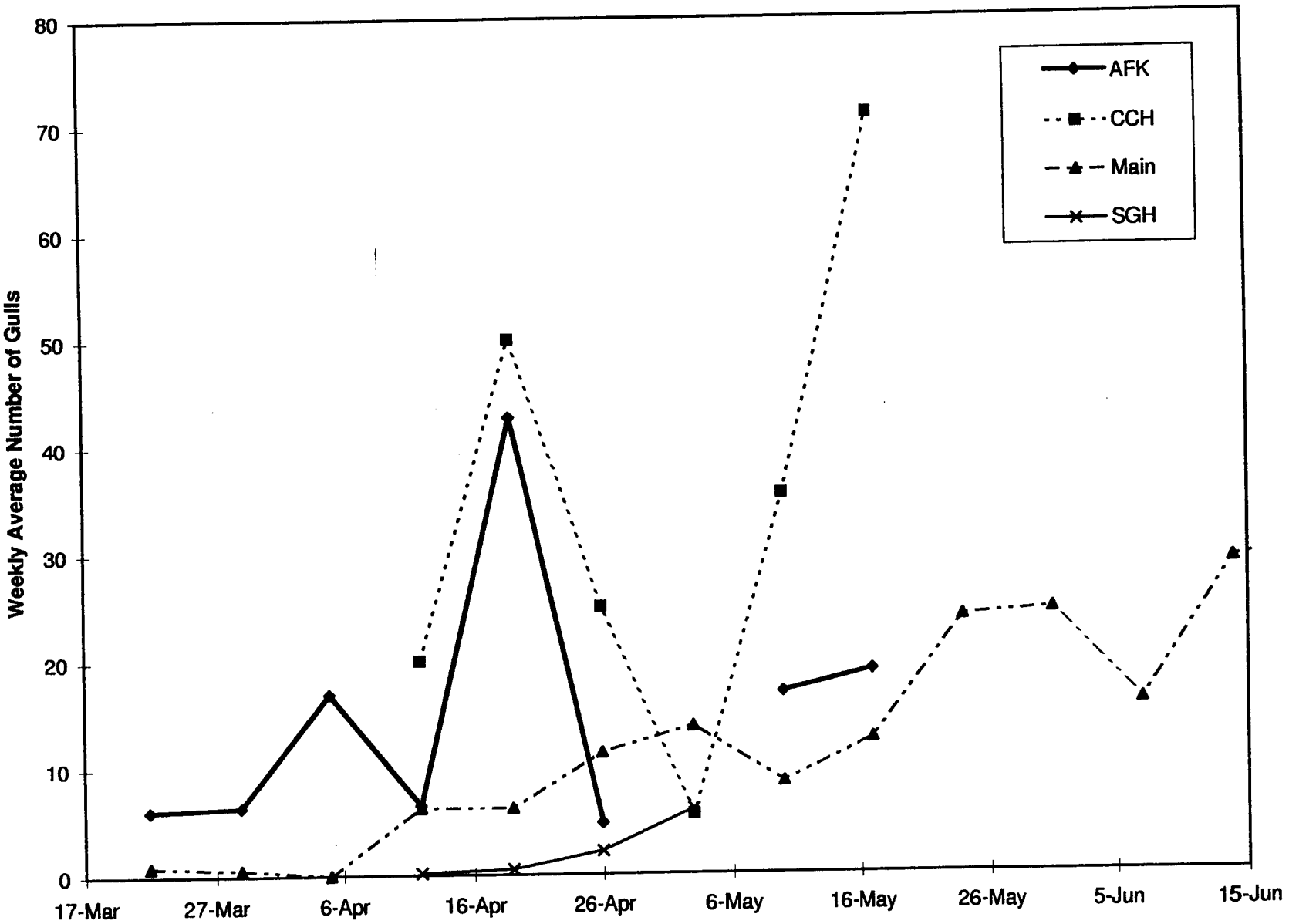
16-May-95; Smallest point indicates five birds.

FIG. 6



03-June-95; Smallest point indicates five birds.

FIG. 7


Weekly Average Number of Gulls
80
70
60
50
40
30
20
10
0
17-Mar
27-Mar
6-Apr
16-Apr
26-Apr
6-May
16-May
26-May
5-Jun
15-Jun
AFK
CCH
Main
SGH

CONCLUSIONS

This project is one part of the Sound Ecosystem Assessment program's effort to identify major predators on pink salmon fry, the magnitude of predation, and the variability in predation over time and space. Seabirds consumed 1.1-2.4% of fry released from Wally Noerenberg Hatchery in 1995. These results provide a good starting point for comparison of the magnitude of predation by birds to that of the other major predators (e.g. walleye pollock and squid). Data on fish that prey on pink salmon fry are provided by the SEA project, Juvenile Salmon and Herring Integration. We suggest that the timing of salmon release (or outmigration) relative to that of spawning migrations by herring and other forage fish may determine the magnitude of bird aggregations that feed on salmon fry.

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