Exxon Valdez Oil Spill
State/Federal Natural Resource Damage Assessment Final Report

Early Marine Salmon Injury Assessment in Prince William Sound

Fish/Shellfish Study Number 4A
Final Report

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Study History: Fish/Shellfish Study Number 4A was initiated as part a detailed study plan in 1989 under Fish/Shellfish Study Number 4 (Early Marine Salmon Injury Assessment in Prince William Sound) and continued through 1991. After providing evidence of reduced growth and fry-to-adult survival, the project effort continued in 1992 under Fish/Shellfish Study Number 4A, in order to focus on quantifying the effect of oil contamination on fry growth and to adequately establish that environmental and oil effects were not confounded.

Abstract: We investigated the effects of the *Exxon Valdez* oil spill and evaluated natural environmental effects on the migration, growth, and survival of juvenile pink salmon during the first two months of marine residence in Prince William Sound using coded-wire tagged juveniles released from hatcheries in 1989-1991. Juveniles from Koernig Hatchery migrated from the nearby moderately-oiled area to the lightly-oiled southern coast of Knight Island in 1989; similar migration was not observed in 1990 and 1991. Growth rates of juveniles released from Koernig Hatchery in 1989 were significantly lower ($P=.034$) near the hatchery than along Knight Island's southern coast, and although lower, not significantly different in 1990 ($P=.103$), and marginally significant in 1991 ($P=.085$). Growth rates of juveniles released from Noerenberg Hatchery in 1989 were significantly lower ($P=.019$) in the moderately-oiled area near Main Bay than near the non-oiled hatchery, but not significantly different in 1990 ($P=.767$) and 1991 ($P=.883$). Exposure to hydrocarbons appeared to reduce the juvenile growth rate by 0.76 to 0.94% body weight day^{-1} in 1989, and was associated with a significantly greater ($P<.05$) frequency of cytochrome P4501A enzyme induction in moderately-oiled areas. Growth rate reduction likely caused a 1.7 to 2.2% reduction in survival to the adult stage among fish reared in oiled areas.

Key Words: Coded-wire tags, *Exxon Valdez* oil spill, growth, hatcheries, hydrocarbon exposure, injury, juvenile pink salmon, marine residence, migration, Oncorhynchus gorbuscha, Prince William Sound, survival.

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EXECUTIVE SUMMARY

This study investigated the effects of the *Exxon Valdez* oil spill (EVOS) on the migration, growth, and survival of juvenile pink salmon during the first two months of their marine residence in Prince William Sound (PWS), Alaska. Coded-wire tagged (CWT) juvenile salmon released from hatcheries in PWS in 1989, 1990, and 1991 were the principal tool used to study migration, growth, and survival. Determining the effect of hydrocarbon exposure on juvenile growth in nature also required an evaluation of natural environmental effects on growth. Stomach contents analysis, statistical analyses, and bioenergetic models were the principal tools used to examine the effects of prey density, prey species composition, temperature, and juvenile salmon density on growth.

A relatively large number of juvenile pink salmon from Armin F. Koernig (AFK) Hatchery migrated out of the moderately-oiled area near the hatchery to the lightly-oiled southern coast of Knight Island in 1989. A similar northward migration of juveniles was not observed in 1990 and 1991. Laboratory studies have demonstrated an avoidance reaction to hydrocarbons in juvenile salmon, but the behavior observed in 1989 cannot be attributed to oil contamination due to our lack of understanding of natural environmental effects on migratory behavior.

The growth of juvenile pink salmon in PWS appeared to be reduced by oil contamination from the EVOS in 1989. Growth rates of juveniles released from AFK Hatchery in 1989 were significantly lower ($P=.034$) in the moderately-oiled area near the hatchery than along the lightly-oiled southern coast of Knight Island. In 1990 and 1991, growth rates of juvenile CWT pink salmon released from the AFK Hatchery were again lower in the oiled area near the hatchery compared with the lightly-oiled southern coast of Knight Island. However, the growth difference was not significant in 1990 ($P=.103$), and marginally significant in 1991 ($P=.085$). The magnitude of the growth difference in 1990 and 1991 was also one half of the growth difference in 1989. Growth rates of juveniles released from Wally H. Noerenberg (WHN) Hatchery in 1989 were significantly lower ($P=.019$) in the moderately-oiled area near Main Bay than in the non-oiled area near WHN Hatchery. Growth rates of juveniles released from WHN Hatchery were not significantly different between oiled and non-oiled areas in 1990 ($P=.767$) and 1991 ($P=.883$).

Exposure to hydrocarbons from the EVOS appeared to reduce the growth rate of juvenile pink salmon by 0.76 to 0.94% body weight day^{-1} in 1989. The observed differences in growth rate do not appear to be caused by measurement or sampling error, or differences in food consumption rate, prey composition, or water temperature. The observed reduction in growth rate was associated with a significantly greater ($P<.05$) frequency of cytochrome P4501A enzyme induction in moderately-oiled areas compared with non-oiled and lightly-oiled areas in 1989. The greater frequency of P4501A enzyme induction in moderately-oiled areas indicates that fish in oil-contaminated habitats expended energy to metabolize and depurate hydrocarbons leaving less energy available for somatic growth. Insufficient data is available at the present time to determine if the level of hydrocarbon exposure was sufficient

to cause the estimated reduction in growth rate attributed to oil contamination. The growth of juvenile CWT pink salmon in 1989 was significantly related ($P=.016$) to survival to the adult stage. The reduction in juvenile growth attributed to oil contamination in 1989 likely caused a 1.7 to 2.2% reduction in survival to the adult stage among fish that reared in oiled areas. The adult pink salmon return to PWS in 1990 was thus lower than if the EVOS had not occurred.

INTRODUCTION

Approximately one month after the *Exxon Valdez* oil spill (EVOS), 518 million juvenile pink salmon were released from four private non-profit hatcheries in Prince William Sound (PWS), Alaska. At about the same time, a nearly equal number of wild pink salmon began to emigrate from numerous streams bordering the Sound. This study focused on the effects of the EVOS on juvenile pink salmon during the first two months of their marine residence, because mortality at this lifestage strongly affects recruitment to the adult stage (Parker 1968; 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). Several studies have documented that hydrocarbon exposure reduces the growth of juvenile pink salmon in the laboratory (Rice et al. 1975; Moles and Rice 1983; Schwartz 1985). Thus, hydrocarbon exposure during the first two months of marine residence may be expected to reduce growth and subsequent survival to the adult stage.

The design of this study was based on a comparison of growth rates of coded-wire tagged juvenile pink salmon that reared in oiled and non-oiled areas of PWS. Coded-wire tags (CWT) are a nearly microscopic magnetic sliver of wire that is inserted in the fishes' snout. A binary code etched onto the wire is used to identify fish from specific CWT groups. During each of the three years of this study (1989-1991), approximately 1 million juvenile CWT pink salmon were released from four hatcheries in PWS. Growth was estimated for CWT juvenile pink salmon recovered during the first two months of marine residence. Several CWT groups of juvenile salmon were released from hatcheries located in oiled and non-oiled areas of the Sound enabling comparison of juvenile growth among fish with different oil-exposure histories.

Determining the effect of hydrocarbon exposure on juvenile growth in nature also required an evaluation of natural environmental effects on growth. Stomach contents analysis, statistical analyses, and bioenergetic models were used to examine the effects of prey density, prey species composition, temperature, and juvenile salmon density on growth. Feeding rate is strongly affected by the density of available prey (Holling 1966). Prey density also determines the net energetic gain from feeding, but the species/size composition of available prey is also important due to differences in energy content (Raymont et al. 1969), visibility (Wright and O'Brien 1982), and handling times of prey (Werner 1974, Vinyard 1982). Temperature affects activity level and thus feeding rate (Panadian and Vivekanandan 1985), but also largely determines both metabolic rate (Brett and Groves 1979) and gastric evacuation rate (Fange and Grove 1979). Fish density may also be a significant factor affecting growth and survival of juvenile salmon (Matthews 1980, Clark and McCarl 1983, McCarl and Rettig 1983, Nickelson 1986, Elmen et al. 1990, Walters and Juanes 1993).

The mortality of juvenile pink salmon during the first two months of marine residence was not directly measured in this study. Movements of fish through the study area and offshore precluded direct estimation of mortality using mark-recapture techniques (Ricker 1975).

Mortality resulting from hydrocarbon exposure was assessed through a comparison of growth in oiled and non-oiled areas and relationships between growth and fry-to-adult survival.

The effect of the EVOS on migration patterns of juvenile pink salmon was examined, because a disruption of migratory behavior during the critical juvenile lifestage may affect survival. Hydrocarbons may affect migratory behavior through damage to olfactory lamellar surfaces (Babcock 1985) or triggering of an avoidance reaction (Rice 1973).

Our ability to estimate the level of exposure of juvenile pink salmon to hydrocarbons from the EVOS was critical to the other components of the study. Induction of cytochrome P4501A enzymes was chosen as the best tool for this purpose. Detection of hydrocarbons through chemical analysis of tissue samples was not used, because fish including pink salmon metabolize hydrocarbons rapidly and accumulation in tissues is seldom detected. Metabolites in the bile indicate hydrocarbon exposure (Roubal et al. 1977, Thomas and Rice 1981), but juvenile pink salmon are too small to collect bile samples. Cytochrome P4501A enzymes are induced approximately 40 hours after initial hydrocarbon exposure and can be detected in microscopic sections of tissues (Kloepper-Sams and Stegeman 1989). The presence of P4501A enyzmes indicates exposure to hydrocarbons, but the source of the hydrocarbons cannot be identified.

This project focused on three goals:

A. Determine the effect of oil contamination from the EVOS on the migratory behavior of juvenile pink salmon in PWS.

B. Determine the effect of oil contamination from the EVOS on juvenile pink salmon growth in oiled nearshore marine nursery areas in PWS.

C. Determine the effect of oil contamination from the EVOS on the fry-to-adult survival of pink salmon that reared in oiled nearshore marine nursery areas in PWS.

OBJECTIVES

(Letters refer to goals described above)

A-1. Compare the distribution of juvenile CWT pink salmon recoveries in PWS between 1989, 1990, and 1991.

B-1. Test for differences in the condition and growth rate of CWT juvenile pink salmon between oiled and non-oiled areas of PWS in 1989, 1990, and 1991.

B-2. Test for differences in weekly growth rates estimates (obtained from otoliths) between oiled and non-oiled areas of PWS in 1989, 1990, and 1991.

B-3. Test for differences in P4501A enzyme induction in juvenile pink salmon tissues between oiled and non-oiled areas of PWS in 1989.

B-4. Test for differences in prey composition and stomach fullness between oiled and non-oiled areas of PWS in 1989, 1990, and 1991.

B-5. Test for differences in ocean temperature between oiled and non-oiled areas of PWS in 1989, 1990, and 1991.

B-6. Assess the relative effects of ocean temperature, prey density, and prey composition on the growth of juvenile pink salmon in PWS.

C-1. Evaluate the relationship between fry growth rate and fry-to-adult survival for CWT pink salmon released in 1989, 1990, and 1991.

C-2. Evaluate the effect of the EVOS on the fry-to-adult survival of pink salmon that reared in oiled nearshore habitats in PWS.

METHODS

Approximately 1 million CWT juvenile pink salmon were released each year from four hatcheries in PWS. The Armin F. Koernig (AFK) Hatchery is located in a moderately-oiled area (GIS Technical Group 1991) in southwest PWS (Figure 1). The Wally H. Noerenberg (WHN) and Cannery Creek (CCH) hatcheries are located in a non-oiled area in northern PWS. And, the Solomon Gulch (SGH) Hatchery is located in a non-oiled area in northeast PWS. Half-length CWTs were injected into the fishes' head using methods described by Sharr and Peltz (1993) and Sharr and Peckham (1993).

Juvenile CWT pink salmon were recovered using beach and purse seines deployed from a 6 m long aluminum skiff in six areas of PWS (Figure 1). The level of oil contamination in each sampling area in 1989 was assessed from a simple visual examination of shoreline oiling maps prepared by the GIS Technical Group (1991). The level of oil contamination in the six sampling areas was designated as follows: area 1 - non oiled, areas 2 through 4 - moderately oiled, and areas 5 and 6 - lightly oiled (Figure 1). Each year sampling began during the third week of May and extended until about the beginning of July. A 40 m long beach seine and 70 m long purse seine were used to capture the fish. Juvenile salmon were generally captured in areas where the fish were very abundant, because large numbers of fish needed to be recovered to obtain adequate samples of CWT fish. After the fish were captured, they were transferred to a floating net pen where they were held during processing. The number of fish captured in each set of the net was estimated volumetrically. Coded-wire tagged fish were isolated by passing the entire catch through a CWT detector (Northwest Marine Technology). Each CWT fish was placed in an individual pre-weighed glass vial and frozen. Lengths and weights of CWT fish were measured later on shore where accuracies of 0.01 g and 0.5 mm were obtained. In 1989 and 1990, samples of untagged fry (n=60) were collected and

preserved in 10% formaldehyde at sites where CWT fry were recovered. In 1991, samples of untagged fry were preserved in 70% ethanol for preservation of otoliths. Water temperature at 1 m depth was measured with a thermistor or mercury thermometer at all sites where CWT juvenile salmon were recovered. In 1989 and 1990, CWTs were extracted and interrogated at the Alaska Department of Fish and Game (ADFG) CWT Laboratory in Juneau. In 1991, CWTs were extracted and interrogated as they were recovered in the field. Methods developed by the ADFG CWT Laboratory for extracting and interrogating coded-wire tags were employed each year. Coded-wire tagged juvenile pink salmon were also recovered by National Marine Fisheries Service investigators in 1989 and 1990.

Results with statistical significance less than 0.05 are also reported and discussed. Probability levels for statistical tests were considered significant at $P < .05$, marginally significant at $.05 < P < .10$, and not significant at $P > .10$.

Objective A-1:

Numbers of CWT juvenile pink salmon recovered at different sites were summed for each hatchery. Maps were prepared indicating the number of CWT recoveries from each hatchery at various sites in each year. A qualitative comparison of migration patterns in different years was made by simple visual evaluation of the maps from 1989, 1990 and 1991. An analysis of migration rate was not conducted, because sampling was not initiated until after most tag-code groups had been released from the hatcheries. Thus, the arrival of each tag-code group in each area could not be adequately documented. An analysis of catch per unit of effort (CPUE) was not conducted, because the catchability of the fish (Ricker 1975) varied considerably depending on weather and fish size. CPUE also varied considerably as the skill of the crew improved over time.

Objective B-1:

An exponential model was used to estimate growth rates (G_i) of individual CWT juvenile pink salmon, i.e.

$$G_i = \frac{\ln(W_c) - \ln(\overline{W_r})}{t_c - t_r} \qquad (1)$$

where W_c is the weight of individual 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 of release for a specific tag-code group (Ricker 1975). The weight of individual fish at release was not known. In 1989, the exact date of release for individual fish was also not known, because specific tag-code groups were released over a period several days. In 1990 and 1991, specific tag-code groups were released on a single day, so the date of release for individual fish was

known. Comparison of growth rates of CWT juvenile pink salmon between oiled and non-oiled areas involved the following two assumptions: (1) fish of different sizes within a CWT group were distributed randomly to oiled and non-oiled areas after release, and (2) fish released on different days within a CWT group were distributed randomly to oiled and non-oiled areas.

Analysis of variance was used to test for differences in growth rate among tag-code groups, treatment groups, recovery areas, and months (May and June). Recovery site was used as the sample unit in the analysis (Hurlbert 1984). Mean growth rates were estimated for each tag code and treatment group recovered during May and June (1989-1991) at 47 sites in oiled and non-oiled areas of PWS. Three treatment groups receiving different feeding regimes at the hatcheries were included in the analysis. An early-fed group was composed of individuals released during high zooplankton abundance after 1-2 weeks of feeding in net pens. An unfed group was released during high zooplankton abundance after only 2-5 days of feeding. A late fed group was released during declining zooplankton abundance and increasing temperatures after 1-2 weeks of feeding. A split-plot design was used to test for differences in mean growth rate among tag-code and treatment groups in 1989, 1990, and 1991 (Hurlbert 1984). This approach was taken to determine whether growth rates were different among tag-code or treatment groups that were reared differently or released at different times. Growth rate may differ among groups of juvenile pink salmon that enter the ocean at different times during the spring (Mortenson et al. 1991). A split-plot design was used to test for differences in growth of juvenile CWT salmon in the early-fed group released from the AFK Hatchery and recovered in moderately- and lightly-oiled areas in May and June, 1989. For this group, CWT juvenile pink salmon were recovered from a sufficient number of sites in each area during both May and June to avoid missing cells in the analysis (Appendix III). A random design was used to test for differences in mean growth rate between oiled and non-oiled areas in 1990 and 1991, because the number of sites repeatedly sampled in each area during each month was not adequate for a split plot design.

Differences in the condition of CWT juvenile pink salmon between oiled and non-oiled areas were examined in 1989, 1990, and 1991. The relationship between body weight (W) and length (L) was described by

$$W = aL^b \qquad (2)$$

where a is the condition factor and b is the slope of the linear-transformed model (Ricker 1975). Analysis of covariance was used to test for differences in the intercept and slope of the linear-transformed model between oiled and non-oiled areas. The slope of the regression (b) was used as a measure of the condition of juvenile CWT pink salmon in different areas. Site was used as the sample unit in the analysis.

Objective B-2:

Otolith microstructures were analyzed to determine if otoliths could be used to obtain accurate estimates of age and weekly growth rates of juvenile pink salmon. Development of these techniques was important to the study because weekly growth estimates would greatly improve evaluation of environmental and oil effects on growth. Thin sections of the otoliths were prepared using methods developed by Volk et. al. (1984). A computer image analysis system (Biosonics, Seattle, WA) was used to collect data from the otoliths. Measurements were taken primarily from left sagittal otoliths, but right sagittal otoliths were used when the left otolith was in poor condition. A reference line was drawn from the otolith rostrum through the center of the primordia. On left and right saggital otoliths, primary radius lines were drawn from the center of the primordial mass to the outer edge at 30° and 330° to the reference line, respectively. On each otolith, the distances to the marine check and outer edge were measured along the primary radius line. The marine check was visually identified as a dark band. Increments laid down before the marine check were typically much less distinct than those laid down after the fish entered saltwater. Otolith increments were visually counted from the marine check to the outer edge.

Validation studies were conducted before otolith measurements were used to estimate age or weekly growth rates. The analysis tested the following two assumptions: (1) otolith increments are produced daily and (2) otolith growth is proportional to fish growth (Campana and Neilson 1985). Campana (1990) determined that the relationship between fish size and otolith size may vary with somatic growth rate, resulting in relatively large otoliths in slow growing fish. Analysis of covariance was used to test for differences in the relationship between natural logarithm of otolith radius (um) and fish body weight (g) and otolith increment count and age (release to recapture), respectively, between slow and fast growing fish. Slow and fast growing fish were defined as those exhibiting growth rates above and below the overall mean growth. Only otoliths from CWT fish collected in 1990 and 1991 were used in the analysis, because the age from date of release was known for these fish.

Objective B-3:

Induction of cytochrome P4501A monooxygenases was used to estimate the degree of exposure of juvenile pink salmon to hydrocarbons in oiled and non-oiled areas of PWS in 1989 (Stegeman 1992). Cytochrome P4501A is an enzyme induced when fish are exposed to hydrocarbons in the environment (Stegeman and Lech 1991). Untagged juvenile pink salmon collected at sites where CWT fish were captured were used in the analysis (Appendix I). All samples were preserved in a buffered 10% formaldehyde solution. Individual fish corresponding in size to the dominant CWT group in the catch at each site were selected for analysis. Immunohistochemical methods were used to detect induction of P4501A enyzmes in various fish tissues (Smolowitz et al. 1989, Stegeman et al. 1991). Each fish was embedded in paraffin and thin sectioned. A monoclonal antibody that binds to P4501A monooxygenases was applied and detected by staining. Sample analysis involved a visual assessment of stain

distribution and intensity in various tissues. The sample size obtained for each tissue was determined by the number of cases in which the tissue was visible in thin sections.

Fisher's Exact Test (Kendall and Stuart 1979) was used to test for differences in the frequency of occurrence of P4501A staining in various tissues between two time periods and between oiled and non-oiled areas. Individual fish were used as the sample unit in the analysis. It was assumed that the samples obtained for analysis were randomly selected among sites and time periods. For each tissue type, a minimum sample size of ten (minimum expected value of five) was needed from each sampling area to conduct the analysis. If less than ten samples were obtained in any sampling area, the tissue was not included in the analysis. Adequate sample sizes were obtained for eleven tissues (Appendix I).

Fisher's Exact Test was also used to test for differences in the frequency of occurrence of P4501A staining in various tissues between two time periods. The induction of P4501A monooxygenases declines over time after initial exposure to hydrocarbons (Kloepper-Sams and Stegeman 1989). A Fisher Exact Test for differences in the frequency of occurrence of P4501A staining before and after June 10, 1989 along the southern coast of Knight Island was conducted. June tenth was chosen because adequate samples were obtained both before and after this date in the area along the southern coast of Knight Island. It was not possible to test for differences in P4501A induction between time periods in any other areas due to lack of sufficient samples.

Objective B-4:

Stomach contents analysis was used to determine whether prey composition or stomach fullness was different between oiled and non-oiled areas in 1989, 1990, and 1991. Samples of untagged juvenile pink salmon ($n=10$) were preserved at oiled and non-oiled sites where CWT fish were captured. Prey organisms in each stomach were enumerated in the following categories: large calanoid copepods (>2.5 mm), small calanoid copepods (<2.5 mm), harpacticoid copepods, and 'other prey'. This approach was taken because the feeding rate of juvenile pink salmon is reduced when only small prey organisms are available (Parsons and LeBrasseur 1973). Prey biomass in each category was estimated by the product of prey abundance and average prey wet weight. Total stomach contents weight was estimated by the sum of prey biomass in all categories. Stomach fullness was estimated by total stomach contents weight as a proportion of fish body weight.

Analysis of variance was used to test for differences in stomach fullness and prey biomass in each category after the data was rank transformed (Conover and Iman 1981). A completely random two-way factorial design was used. Dependent variables in the model included sampling area (oil, non-oil) and date (May, June). Preliminary data analyses indicated that there were no significant differences in stomach fullness or prey biomass in each category between morning and evening (before or after 2 p.m.). Site was used as the sample unit in the analysis of variance. The analysis is based on the assumption that the sites were randomly sampled within each stratum.

Objective B-5:

Analysis of covariance was used to test for differences in ocean temperature between the oiled and non-oiled areas where juvenile salmon from the AFK and WHN hatcheries were recovered in 1989, 1990, and 1991. The analysis was conducted to determine whether differences in temperature between oiled and non-oiled areas may have caused differences in growth rate. The independent variable in the analysis of covariance was sampling area (oil, non-oil) with time as a covariate. Least-squares mean ocean temperature was estimated for each sampling area.

Objective B-6:

Analysis of covariance was used to estimate the relative effects of number of fry released, zooplankton abundance, and ocean temperature on the growth of juvenile CWT pink salmon. The dependent variable in the model was the mean growth of juvenile CWT pink salmon by sampling area and recovery date. Only growth rates for the 'early fed' and 'late fed' treatment groups released from PWS hatcheries in 1990 and 1991 were included in the analysis. It was assumed that oil contamination did not affect the growth of juvenile pink salmon in 1990 and 1991. Carls et al. (in press b) did not detect induction of P4501A enzymes in juvenile pink salmon in 1990. The growth rates of fish released in 1989 were not included because environmental (temperature, food abundance, etc.) and oil effects may have been confounded. The growth rates of fish from the 'unfed' treatment group were not included because environmental and rearing effects may have been confounded. Rearing effects which may affect growth include the duration of net pen rearing and size at release. The independent variables in the model were (1) the total number of fry released from each hatchery in each treatment group (discrete variable), (2) the mean zooplankton settled volume for the period from release to recovery, and (3) the mean ocean temperature for the period from release to recovery. Three classes were established for number of fry released: (1) fry release less than 50 million, (2) fry release greater than 50 million and less than 150 million, and (3) fry release greater than 150 million. Least-squares mean growth rate was estimated for each class.

The ocean temperature and zooplankton data used in the analysis were collected by the staff of the Prince William Sound Aquaculture Corporation. Ocean temperatures were measured and zooplankton samples were collected twice each week at two stations located in the bay and passage adjacent to each hatchery. Only data from stations located in the passages adjacent to each hatchery were used in this analysis. Ocean temperature was measured with a mercury thermometer at 1 m depth. Zooplankton settled volume was estimated from replicate 20 m vertical tows (n=2) taken with a 0.5 m diameter ring net (mesh size = 243 um). Zooplankton settled volume was estimated after the samples were allowed to settle in a graduated cylinder for 24 hours. Zooplankton species were not identified or enumerated.

Zooplankton samples were collected in May, 1991 to determine if zooplankton volume measured at the PWSAC hatcheries was representative of zooplankton biomass in areas where juvenile pink salmon were sampled. Zooplankton biomass was estimated from replicate 20 m vertical tows (n=2) taken with a 0.5 m diameter ring net (mesh size = 243 um) at 27 stations in PWS. Samples were preserved in a 10% buffered formaldehyde solution for later analysis in the laboratory. Zooplankton species composition was estimated from replicate subsamples taken from a known volume with a Stemple pipette. The number of replicate subsamples taken was varied depending upon the density of animals to obtain at least 100 individuals from each important species. Zooplankton species were identified to the lowest possible taxonomic level. Copepod stages were identified for all major copepod species. The biomass of each taxonomic group was estimated from the product of abundance and average weight. The average wet weight of each copepod species and stage were taken from Coyle et al. (1990). Settled volume was estimated for each zooplankton sample using the methods described above. Regression analysis was used to estimate the relationship between zooplankton settled volume and zooplankton biomass (wet weight). Zooplankton settled volume measured at PWSAC hatcheries was converted to wet weight biomass using the regression equation. Analysis of variance was used to test for differences in zooplankton biomass between PWSAC hatcheries and areas where juvenile pink salmon were captured.

A bioenergetics model was constructed to estimate the growth and feeding rates of juvenile pink salmon at maximum ration between 4 and 14 °C. This was the approximate range of temperatures observed during May and June in PWS in 1989. A relationship between temperature and growth at maximum ration was used to estimate growth (Mortenson and Savikko 1993). Food consumption was estimated by a simple mass balance equation:

$$I = \frac{G + R}{A} \qquad (3)$$

where I = food consumption (cal day^{-1}), R = total metabolism (cal day^{-1}), and A = assimilation coefficient (Brett and Groves 1979). An assimilation coefficient of 0.85 was used (Ware 1975). Energy content was assumed to be 5400 cal g^{-1} dry weight (Griffiths and Dillinger 1991).

Total metabolism was assumed to be approximately equal to the sum of standard metabolism, active metabolism, and feeding 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). Brett and Glass (1973) provided parameter estimates for power functions relating active metabolism to body weight at three temperatures. This data was used to estimate the active metabolism of a 1 g pink salmon at

5.3 and 15.0°C. Metabolic rates at other temperatures were estimated assuming a linear relationship between temperature and metabolic rate. An oxycalorific equivalent of 3.25 cal mg^{-1} O^2 was used to convert oxygen consumption to calories (Brett and Groves 1979). 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 was added to active metabolism after an initial estimate of food consumption. Food consumption including feeding metabolism was then estimated again using equation 3.

The time required for a 1 g pink salmon to obtain maximum daily ration at specific prey densities was estimated to evaluate whether prey density limited juvenile salmon growth in 1989. The approximate range of prey densities measured in PWS in 1989 was used in this analysis. Holling (1966) developed a model to estimate the feeding rate of invertebrates in relation to prey density, i.e.,

$$I = \frac{\gamma pU}{1 + \gamma pUh} \qquad (4)$$

where I 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 (1) was 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) were used to estimate a regression equation relating reactive distance to fish length and prey length, i.e.,

$$d_r = 0.29 L_f^{1.1} + 3.3 L_p \qquad (5)$$

($r=.98$, $p<.005$) where d_r is the reactive distance (cm), L_f is total fish length (cm) and L_p is prey length (mm). Given d_r, the cross-sectional area of the reactive field (γ) is πd_r^2. Bailey et al. (1979) 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} was assumed. 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 could 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. The inverse feeding rate (I^{-1}) was used to estimate the time required for a fish to obtain maximum daily ration.

Objective C-1:

Analysis of covariance was used to evaluate the relationship between mean juvenile pink salmon growth and survival to adult for CWT groups released from hatcheries in 1989, 1990, and 1991. The dependent variable in the model was the survival of each of the tag-code groups released from PWS hatcheries (Sharr and Peltz 1993, Sharr and Peckham 1993). The independent variable was release year with the mean growth rate of all the juvenile pink salmon recovered in each respective tag-code group as a covariate. Only tag-code groups with at least ten recoveries of juvenile pink salmon were included in the analysis.

Objective C-2:

The effect of the EVOS on the survival of pink salmon that reared in oiled nearshore habitats in 1989 was assessed using the regression equation obtained under objective C-1 and the mean growth rates of juvenile salmon in oiled and non-oiled areas of PWS. Expected survival was predicted from the regression equation obtained under objective C-1 using mean growth rates in oiled and non-oiled areas as the independent variable. Analysis of covariance was used to adjust mean growth rate in oiled and non-oiled areas for differences in ocean temperature. Mean growth by site and date was the dependent variable in the analysis of covariance and mean temperature from fry release to recapture was the covariate. Differences between the point estimates of survival in oiled and non-oiled areas were used to assess the effect of oil contamination on survival.

RESULTS

Objective A-1:

A relatively large number of juvenile pink salmon from AFK Hatchery migrated out of the moderately-oiled area near the hatchery to the lightly-oiled southern coast of Knight Island in 1989. One hundred and ten CWT juvenile pink salmon from AFK Hatchery were recovered along the southern coast of Knight Island in 1989 (Figure 2). Only 13 and 43 CWT fish from AFK Hatchery were recovered in this same area in 1990 and 1991, respectively (Figures 3 and 4). The relatively high catch of juvenile CWT pink salmon in this area in 1991 resulted from greater sampling effort on relatively few fish. This was done to obtain samples of juvenile CWT salmon for growth analyses. Visual observations also indicated a much greater abundance of juvenile salmon along the lightly-oiled southern coast of Knight Island in 1989 compared with 1990 and 1991. There were no apparent interannual differences in the migration of juvenile CWT pink salmon from WHN Hatchery that appeared to be related to oil contamination.

Objective B-1:

Growth rates of juvenile CWT pink salmon were significantly different among tag-code groups released from WHN Hatchery ($P<.001$) in 1989; and WHN ($P<.001$), AFK ($P=.030$), and CCH ($P<.001$) hatcheries in 1990. Growth rates were marginally significantly different among tag-code groups released from WHN Hatchery ($P=.070$) in 1991. Multiple comparison tests indicated that, in general, tag codes applied in 1990 and 1991 could be combined within treatment groups (early fed, unfed, and late fed) employed at the hatcheries. Combining tag codes within treatment groups in 1990 and 1991 also allowed direct comparison with results from 1989 when only one tag code was used for each treatment group. Growth rates were significantly different among treatment groups released from WHN Hatchery ($P=.002$) in 1989; AFK ($P<.001$), WHN ($P=.013$), and CCH ($P=.047$) hatcheries in 1990, and AFK hatchery ($P<.001$) in 1991 (Table 1). The number of tag recoveries in each treatment group and time period were examined to determine in which groups adequate sample sizes were available to test for differences between oiled and non-oiled areas. The early-fed groups released from AFK and WHN hatcheries consistently comprised the largest proportion of the recoveries for any single group in each year (39% and 24% in 1989, 35% and 17% in 1990, and 42% and 32% in 1991, respectively). The majority of the early-fed CWT recoveries from the WHN and AFK hatcheries occurred in areas 1 and 2 and areas 4 and 5, respectively (Appendix II). As a result, subsequent growth rate analyses were restricted to the early-fed groups released from these two hatcheries. For the WHN Hatchery, growth rates were compared between the non-oiled area near the hatchery (area 1) and the moderately-oiled area near Main Bay (area 2). For the AFK Hatchery, growth rates were compared between the lightly-oiled area along the southern coast of Knight Island (area 5) and the moderately-oiled area near the AFK hatchery (area 4; Figure 5).

Two growth analyses were conducted for the juvenile CWT pink salmon released from AFK Hatchery in 1989. Samples of juvenile CWT salmon from the early-fed group were obtained at five sites in the moderately-oiled area near the AFK Hatchery during both May and June (Appendix III). Initially, all sites were included in a split-plot analysis of variance. Results from this analysis indicated a significant ($P=.033$) oil-by-time interaction (Table 2). Growth of juvenile CWT salmon was significantly lower in the oiled area near AFK Hatchery than along the lightly-oiled southern coast of Knight Island in both May and June (Table 3). Growth increased ($P=.071$) from May to June in the lightly-oiled area along the southern coast of Knight Island, but did not increase ($P=.126$) from May to June in the moderately-oiled area near AFK Hatchery. A second split-plot analysis of variance was conducted with the Squirrel Bay site removed. Mean growth at this site was considerably lower than at other sites in the moderately-oiled area near AFK Hatchery, and only one CWT juvenile pink salmon was recovered at this site in June (Appendix III). Results from the second analysis also indicated that the growth of juvenile CWT pink salmon released from AFK Hatchery was significantly lower ($P=.034$) in the moderately-oiled area near the hatchery than along the lightly-oiled southern coast of Knight Island (Figure 6; Table 2). The oil-by-time interaction was marginally significant ($P=.069$). Comparison of growth rates between areas

in May and June indicated no difference (P=.394) in May. However, growth rates in June were significantly lower (P=.011) in the oiled area near AFK Hatchery compared with the lightly-oiled southern coast of Knight Island (Table 3). The results from the second analysis excluding the Squirrel Bay site provide a more conservative estimate of the difference in growth between these two areas.

In 1990 and 1991, growth rates of juvenile CWT pink salmon released from the AFK Hatchery were again lower in the oiled area near the hatchery compared with the lightly-oiled southern coast of Knight Island (Figure 7; Table 4). The growth difference was not significant in 1990 (P=.103), and marginally significant in 1991 (P=.085). The magnitude of the growth difference in 1990 and 1991 was also one half of the more conservative estimate of the growth difference in 1989.

Growth rates of juvenile CWT pink salmon released from the WHN Hatchery in 1989 were significantly lower (P=.019) in the oiled area near Main Bay than in the non-oiled area near the WHN Hatchery (Figure 7; Table 4). Growth rates of juvenile pink salmon released from the WHN Hatchery were not significantly different between oiled and non-oiled areas in 1990 (P=.767) and 1991 (P=.883).

The condition of juvenile CWT pink salmon released from AFK and WHN hatcheries was not significantly different between oiled and non-oiled areas in 1989 and 1990. In 1991, the condition of juvenile pink salmon released from the WHN Hatchery was significantly greater (P=.009) in the non-oiled area near the hatchery than in previously oiled area near Main Bay (Table 4).

Objective B-2:

Analysis of covariance indicated that otolith radius length was significantly related to fish body weight (P<.001), and that the slope and intercept of the regression was not significantly different between slow and fast growing fish (Figure 8; Table 5). Analysis of covariance also indicated that otolith increment count was significantly related to fish age (P<.001); however, the slope of the regression was significantly different (P=.014) between slow and fast growing fish (Figure 8; Table 5). The difference between the regression slopes indicated that fewer increments than days were produced in slow growing fish, and more increments than days were produced in fast growing fish. The significant relationship between increment count and growth rate indicates that otoliths cannot be used to accurately estimate age or weekly growth rates of juvenile pink salmon.

Objective B-3:

The frequency of occurrence of P4501A enzyme induction closely coincided with the degree of oil contamination observed in each sampling area. The sizes of fish selected for analysis, and the sample sizes obtained by tissue type are listed in Appendix I. Sufficient samples were available for eleven tissue types. The frequency of occurrence of P4501A staining was

significantly greater in gill pillar cells and cecal epithelium before than after June 10, 1989 along the southern coast of Knight Island (Table 6). This result indicates that the level of exposure to hydrocarbons declined over time in this sampling area. This response is expected for fish that migrated from the moderately-oiled area near the AFK Hatchery into the lightly-oiled area along the southern coast of Knight Island. Subsequent comparisons of the frequency of occurrence of staining between oiled and non-oiled areas were limited to the time period before June 10, 1989. The frequency of occurrence of P4501A staining in gill pillar cells and gill epithelium was significantly greater ($P < .001$) in the moderately-oiled area near Main Bay than in the non-oiled area near the WHN Hatchery (Table 7). The frequency of occurrence of P4501A staining in gill pillar cells was significantly greater ($P = .015$) in the moderately-oiled area near AFK Hatchery than in the lightly-oiled area along the southern coast of Knight Island. The frequency of occurrence of staining was also greater in gill epithelium near the AFK Hatchery, but the difference was marginally significant ($P = .068$).

Objective B-4:

The percent of total stomach contents weight comprised of large copepods and harpacticoid copepods was significantly greater ($P < .05$) in May than June during 1989 in all sampling areas (Table 8). In 1990, large copepods comprised a significantly greater ($P = .040$) proportion of stomach contents weight during May than June in areas where fish from the WHN Hatchery were recovered. Harpacticoid copepods comprised a significantly greater ($P < .001$) proportion of stomach contents weight during May than June in all areas sampled in 1989 and 1990.

Prey composition and stomach fullness were not significantly different between the two areas where fish from the WHN Hatchery were recovered in 1989 (Table 9). Small copepods comprised a significantly greater ($P = .005$) proportion of the diet in the moderately-oiled area near AFK Hatchery in 1989 than in the lightly-oiled area along the southern coast of Knight Island. In 1990, large copepods and harpacticoid copepods comprised a significantly greater proportion ($P < .05$) of the diet in the moderately-oiled area near AFK Hatchery than in the lightly-oiled area along the southern coast of Knight Island. In 1990, stomach fullness was also greater in the non-oiled area near the WHN Hatchery than in the moderately-oiled area near Main Bay, but the difference was only marginally significant ($P = .061$). In 1991, there were no differences in stomach fullness or prey composition in areas where fish from the WHN Hatchery were captured. Due to small sample sizes, no statistical comparison of stomach fullness or prey composition was possible between the two areas where fish from the AFK Hatchery were recovered in 1991.

Objective B-5:

Analysis of covariance indicated that ocean temperatures were generally warmer along the lightly-oiled southern coast of Knight Island than in the oiled area near the AFK Hatchery. The mean difference in temperature was .9°C ($P = .075$) in 1989, 2.4°C ($P < .001$) in 1990,

and .7°C (P=.290) in 1991 (Figures 9-11, Table 10). There were no significant differences in ocean temperature between the non-oiled area near WHN Hatchery and the oiled area near Main Bay in 1989, 1990, or 1991 (Figure 9-11; Table 10).

Objective B-6:

Analysis of variance indicated that zooplankton biomass in 1991 was not significantly different between areas near each hatchery where juvenile pink salmon were sampled and stations in the passages adjacent to AFK (P=.825), WHN (P=.373), and CCH (P=.508) hatcheries (Figure 12). Analysis of covariance indicated that the growth of juvenile CWT pink salmon was significantly related (P=.050) to mean ocean temperature measured in the passages adjacent to each hatchery (Table 11). The number of fry released and zooplankton settled volume were not significantly related (P>.05) to mean growth (Table 11).

The bioenergetics model estimated a gross growth conversion efficiency of 23% at all temperatures (Table 12). This value is within the normal range for juvenile fish (Ivlev 1945; Winberg 1960; Brett and Groves 1979). Estimated times required to obtain maximum daily ration indicated that food abundance does not limit the growth of juvenile pink salmon feeding exclusively on large copepods (*Neocalanus plumchrus*). However, the growth of juvenile pink salmon feeding exclusively on small copepods (*Pseudocalanus spp.*) may be limited by feeding rate at temperatures above 10° C or prey densities below 0.10 g m^{-3} (Table 13). This conclusion is based on the assumption that fry feed continuously during daylight (20 hours per day) if necessary to obtain maximum daily ration and that only one size group of copepods is taken. Field observations indicate that juvenile pink salmon feed continuously during daylight hours (Simenstad et al. 1980); although, more than one size group of prey is typically taken (Tables 8 and 9).

The results from the bioenergetic model indicated that the growth of juvenile pink salmon likely was not affected by small-scale differences in prey density between oiled and non-oiled areas of PWS during May, 1989. Pink salmon diets in May 1989 were composed of large copepods (40-52%), small copepods (21-27%), and various other invertebrates (Table 8). The biomass of large calanoid copepods ranged between 0.10 and 1.20 g m^{-3} (Wertheimer et al. 1993) and water temperatures were generally less than 10° C (Figure 9). Under these conditions, a maximum of 10 to 12 hours of feeding time would be required to obtain maximum daily ration. The feeding times required to obtain maximum daily ration do not decrease at higher prey density indicating that growth is not limited by feeding rate at these levels of prey density and prey composition. The proportion of the diet comprised of large calanoid copepods appears to be critical in determining whether growth is limited by feeding rate.

Growth may have been affected by small-scale differences in prey density between oiled and non-oiled areas in June, 1989. At that time, zooplankton biomass decreased to less than 0.10 g m^{-3}, water temperatures exceeded 10° C, and the proportion of the diet comprised of large

large calanoid copepods decreased to between 1 and 24 % (Table 8). Under these conditions, it is likely that the growth of juvenile pink salmon is limited by feeding rate (Table 13).

Objective C-1:

Analysis of covariance indicated that the mean growth rate of juvenile CWT pink salmon in each tag-code group was significantly related ($P < .001$) to survival to adult. However, the slopes of the growth-survival relationship were different among years ($P = .001$). Regression analysis was used to estimate the parameters of the growth-survival relationship for each year separately. The mean growth rate of juvenile CWT pink salmon in each tag-code group was significantly related ($P = .016$) to survival to adult in 1989 (Figure 13). The slope of the regression indicated that a change in growth rate of 1% body weight per day resulted in a change in survival rate of 2.3%. The mean growth rate of juvenile CWT pink salmon in each tag-code group was not significantly related to survival to adult in 1990 ($P = .527$) and 1991 ($P = .774$; Figure 13).

Objective C-2:

An analysis of covariance was conducted to adjust the mean growth rates of juvenile CWT pink salmon in 1989 for the marginally significant difference ($P = .075$) in ocean temperature between the oiled area near AFK Hatchery and the lightly-oiled area along the southern coast of Knight Island. The results from the analysis indicated that growth was significantly different ($P = .010$) between the two areas, but that ocean temperature was not significantly related ($P = .356$) to growth (Figure 14). The mean growth rates obtained from the analysis of variance under objective B-1 were used to predict survival to adult for juveniles that reared in oiled and non-oiled areas using the regression equation for fish released in 1989 obtained under objective C-1. The differences in predicted survival between oiled and non-oiled areas were 2.2% and 1.7% for juvenile CWT pink salmon released from the WHN and AFK hatcheries, respectively, in 1989 (Table 14).

DISCUSSION

Goal A: Determine the effect of oil contamination from the EVOS on the migratory behavior of juvenile pink salmon in PWS.

Although a greater number of juveniles from AFK Hatchery migrated to the lightly-oiled southern coast of Knight Island in 1989 compared with 1990 and 1991, no definitive conclusions can be drawn regarding the cause of these movements. Laboratory experiments have established that juvenile pink salmon will avoid sublethal concentrations of Prudhoe Bay crude oil in seawater. The avoidance thresholds of juvenile pink salmon ranged from 16.0 to 1.6 mg L^{-1} in June and August, respectively (Rice 1973). Non-avoidance thresholds ranged from 8.8 to 0.75 mg L^{-1} in June and August, respectively (Rice 1973). These thresholds represent the levels for discrimination and not the concentrations actually avoided by motivated migratory juvenile pink salmon (Rice 1973). Hydrocarbon concentrations measured

in Sawmill Bay within two weeks after the pink salmon release were substantially below the avoidance and non-avoidance thresholds (Short and Rounds 1993). However, a greater frequency of occurrence of P4501A staining in juvenile pink salmon captured near the AFK Hatchery indicated that these fish were exposed to hydrocarbons (Table 7). Insufficient data is available at the present time to determine if juvenile pink salmon migrating from the AFK Hatchery in 1989 encountered hydrocarbon concentrations sufficient to cause an avoidance reaction.

The migratory behavior of juvenile pink salmon released from AFK Hatchery in 1989 was likely also affected by natural environmental conditions. However, the effect of natural environmental conditions on juvenile salmon migratory behavior is poorly understood. Simenstad et al. (1980) concluded that juvenile pink and chum salmon emigrated rapidly from Hood Canal when food abundance was low. No data is available regarding relative food abundances at AFK Hatchery compared with the southern coast of Knight Island in 1989; however, stomach fullness in 1989 was not different between these two areas (Table 9). Hurley and Woodall (1968) concluded that juvenile pink salmon less than 60 mm in length prefer relatively warm temperatures (12.0-13.5°C). Cooler ocean temperatures near AFK Hatchery compared with the southern coast of Knight Island may have caused juvenile pink salmon to migrate northward in 1989, but ocean temperatures were also cooler near AFK Hatchery in 1990 and 1991. Insufficient data is available to determine the relative effects of oil contamination and natural environmental conditions on the migratory behavior of juvenile pink salmon in 1989.

Goal B: Determine the effect of oil contamination from the EVOS on juvenile pink salmon growth in nearshore marine nursery areas in PWS.

Exposure to hydrocarbons from the EVOS appeared to reduce the growth of juvenile pink salmon by 0.76 to 0.94% body weight (BW) day^{-1} in 1989 (Table 14). The following alternative hypotheses must be evaluated to establish whether these observed differences in growth rate were likely caused by oil contamination from the EVOS:

(1) the differences in mean growth rate were caused by measurement or sampling error;

(2) the differences in mean growth rate were caused by differences in food consumption rate or prey composition;

(3) the differences in mean growth rate were caused by differences in ocean temperature and its affect on metabolic rate;

(4) the differences in mean growth rate were not associated with evidence of differential exposure to hydrocarbons from the EVOS; and

(5) the estimated reduction in growth rate attributed to oil contamination is not associated with a level of hydrocarbon exposure sufficient to cause the estimated response.

(1) growth differences caused by measurement or sampling error

It cannot be determined if the pattern of mean growth rates observed in 1989 is due to error in the estimation of growth rates of individual fish. The early-fed tag-code groups were released from the WHN and AFK hatcheries over a period of 8 and 5 days, respectively, in 1989. Because release date was not known for individual fish, mean release date for each tag-code group was used to estimate growth. Thus, this growth analysis is based on the assumption that fish released from the hatcheries on different days were distributed randomly between moderately-oiled, and lightly- and non-oiled areas. Body weight at release was also not known for individual fish, so the mean body weight at release for each tag-code group was used to estimate growth. An analysis of the errors caused by this uncertainty in release date and size at release indicates that a non-random distribution of fish with different dates of release and body weights at release could have caused the observed differences in growth rate between moderately-oiled, and lightly- and non-oiled areas in 1989. However, juveniles released from the WHN and AFK hatcheries both exhibited a reduction in growth rate slightly less than 1% BW day^{-1} in moderately-oiled areas compared with non-oiled and lightly-oiled areas in 1989. Untagged juvenile pink salmon also exhibited a reduction in growth of about 1.4 % BW day^{-1} (Wertheimer and Celewycz 1995). The similar apparent growth response of untagged juveniles as well as fish from both WHN and AFK hatcheries indicates that a non-random distribution of fish with different release dates and sizes at release probably was not the cause of the observed difference in growth between areas.

The growth estimates obtained from equation 1 using mean body weight at release and mean date of release are not actual growth rates for individual fish. Consider the distributions of $(\ln W_c - \ln W_r)$ and $(\ln W_c - \ln W_r)$, where W_c and W_r are defined as in equation 1, and W_r is the weight at release of individual fish. Any disparity in variance between them should be considered when interpreting a statistical test based on the former that is meant to make inference regarding the latter. Specifically, an analysis of variance based on a distribution with a smaller variance might indicate significant differences where in fact there are none. It can be shown that the difference in variance depends upon the relative magnitudes of the variance of $\ln W_r$ and $\ln W_c$ and the covariance of $\ln W_r$ and $\ln W_c$. Assuming equal variances of $\ln W_r$ and $\ln W_c$, the difference in the variances of $(\ln W_c - \ln W_r)$ and $(\ln W_c - \ln W_r)$ depends on the magnitude of the covariance of $\ln W_r$ and $\ln W_c$. It is then true that for a correlation greater than 0.5, the variance based on W_r will actually be greater than that based on W_r, resulting in a conservative statistical test. In the present study, otolith analysis was used to back calculate the weight at release of individual (W_r) juvenile CWT pink salmon. $\mathrm{Ln}W_r$ was significantly correlated with $\ln W_c$ in three age groups with correlations of 0.77(n=180), 0.66(n=231), and 0.80(n=21), all above 0.5. This suggests that our tests based on W_r are in fact conservative tests of the true growth differences.

The observed differences in growth do not appear to be caused by sampling error. Recovery site was used as the sample unit in this growth analysis. The analysis is based on the assumption that the various sites are a random sample of all possible sites within each area

(Hurlbert 1984). The sites where large samples of CWT juvenile salmon were caught were generally places where fish were consistently abundant. This relationship between the sites and fish abundance was unavoidable, because only about one fish in a thousand was coded-wire tagged. As a result, a large number of fish needed to be scanned for CWTs. There were no other sites within each of the areas where fish were consistently abundant to our knowledge. Thus, the group of sites included in the analysis likely represents a nearly complete set of all sites where fish were consistently abundant within each area.

(2) growth differences caused by food consumption rate or prey composition

The observed growth differences do not appear to be caused by differences in food consumption rate between areas. Differences in food consumption rate between areas may be caused by differences in food abundance, fish density, water temperature, or the presence of currents that deliver food to the fish (Cooney et al. 1981). In the present study, stomach fullness and bioenergetics were used to evaluate if food consumption rate was likely different between areas. Stomach samples were collected at various times during daylight hours, but a systematic study of diel feeding periodicity was not conducted. Diel feeding periodicity has been documented in juvenile pink salmon (Parker and Vanstone 1966; Parker 1969; Simenstad et al. 1980; Godin 1981). In general, stomach fullness is low at night indicating a cessation of feeding and then increases during the day (Bailey et al. 1975; Simenstad et al. 1980; Godin 1981). The magnitude of the change in stomach fullness with time of day has varied considerably among studies. In the present study, time of day was not a significant factor related to stomach fullness. An evaluation of feeding bioenergetics indicated that differences in prey density between areas may not have affected food consumption rate during May 1989, because the fish were likely able to obtain maximum daily ration at the lowest prey density measured during this time period. Differences in prey density between areas may have affected food consumption rate in June 1989. However, stomach fullness was not significantly different between areas in either May or June, 1989 (Table 9). Sturdevant et al. (in press) also found no significant difference in stomach fullness of juvenile pink and chum salmon between oiled and non-oiled areas of PWS in 1989.

The observed growth differences do not appear to be caused by differences in prey composition between areas. The proportion of the diet comprised of large calanoid copepods may affect gastric evacuation rate and feeding rate. Gastric evacuation rate is generally greater when fish consume small versus large food particles (Fange and Grove 1979; Jobling 1980; Jobling 1981). However, the feeding rate of juvenile pink salmon was 10 and 30 mg hr^{-1} at maximum ration when the fish consumed small (*Pseudocalanus spp.*) and large (*Neocalanus plumchrus*) copepods, respectively (Parsons and LeBrasseur 1973). The handling time per calorie of food obtained is thus three times lower when large rather than small copepods are consumed (Parsons and LeBrasseur 1973). The greater net energetic gain associated with consumption of large copepods indicates that large copepods likely support a higher growth rate and are selected as prey (LeBrasseur 1969; Calow and Townsend 1981; Townsend and Winfield 1985). Results from laboratory studies indicate that juvenile pink salmon select large copepods (2-4 mm length) over small copepods and four other

zooplankton species (Parsons and LeBrasseur 1973). In the present study, large copepods comprised a greater proportion of the diet in the moderately-oiled area near the AFK Hatchery compared with the lightly-oiled area along the southern coast of Knight Island in both 1989 and 1990 (Table 9). There were no differences in the proportion of the diet comprised of large copepods between areas where fish from the WHN Hatchery were recovered (Table 9).

(3) growth differences caused by ocean temperature

Differences in ocean temperature cannot account entirely for growth differences between areas in 1989. Water temperature has a direct effect on the metabolic and gastric evacuation rates of fish (Brett and Groves 1979; Fange and Grove 1979). Under conditions of high food abundance, fish are able to acquire maximum daily ration, so temperature largely regulates growth (Shelbourn et al. 1973). It appears that juvenile pink salmon were able to acquire maximum daily ration in May, 1989 when zooplankton abundance was very high in PWS. Ocean temperatures were lower ($P=.075$) in the moderately-oiled area near the AFK Hatchery compared with the lightly-oiled southern coast of Knight Island in 1989. However, analysis of covariance indicated that the mean growth of juvenile pink salmon adjusted for the effect of temperature was still significantly lower ($P=.010$) in the moderately-oiled area near the AFK Hatchery compared with the lightly-oiled southern coast of Knight Island in 1989. Continued lower growth in the moderately-oiled area near AFK Hatchery in 1990 and 1991 was likely due to lower temperatures in this area compared with the southern coast of Knight Island. There were no temperature differences between areas where fish from the WHN Hatchery were recovered in 1989, 1990 or 1991.

(4) growth differences associated with hydrocarbon exposure

The significant differences in growth rate between moderately-oiled, and lightly- and non-oiled areas in 1989 are associated with significant differences in the frequency of P4501A staining. The frequency of P4501A staining in gill tissues closely coincided with observed levels of shoreline oil contamination in each sampling area (GIS Technical Group 1991). This association is evidence that the differences in P4501A staining between areas were caused by oil contamination from the EVOS. Carls et al. (in press b) also found an association between P4501A induction in juvenile pink salmon, shoreline oil contamination, and hydrocarbon tissue content. In the lightly-oiled area along the southern coast of Knight Island, the frequency of P4501A staining declined significantly between May and June (Table 6). This result is expected for fish that originated in the moderately-oiled area near the AFK Hatchery and migrated into the lightly-oiled area along the southern coast of Knight Island (Kloepper-Sams and Stegeman 1989). However, a decline in hydrocarbon concentrations between May and June may also explain the observed reduction in P4501A staining among fish from the southern coast of Knight Island. Induction of P4501A enzymes was not detected in juvenile pink salmon in 1990 (Carls et al. in press b).

(5) level of hydrocarbon exposure sufficient of cause reduced growth

All necessary evidence is not available at the present time to determine if exposure to hydrocarbons from the EVOS was at a level sufficient to cause the estimated reduction in growth. The question of level of exposure of juvenile pink salmon to EVOS oil in 1989 is linked to questions regarding the route of contamination. Several routes of contamination likely caused the level of exposure to EVOS oil that triggered induction of P4501A enzymes leading to reduced growth in juvenile pink salmon.

It does not appear that the observed reduction in growth was caused solely by exposure to the water-soluble fraction (WSF) of crude oil. Exposure of juvenile pink salmon to 0.40 mg L^{-1} of the WSF of Cook Inlet crude oil caused a 0.8% BW day^{-1} reduction in growth rate in the laboratory (Moles and Rice 1983). This concentration of WSF is substantially greater than levels measured in the water column in PWS within a few weeks after juvenile pink salmon were released from PWS hatcheries (Short and Rounds 1993; Neff 1990).

Ingestion of oil-contaminated prey or oil particles appears to be the most important route of contamination. The occurrence of oil particles in juvenile salmon stomachs (Sturdevant et al. in press), the degree of hydrocarbon contamination of visceral tissues, the ratio of total aromatics to total hydrocarbons, and the frequency, intensity, and occurrence of tissue types staining for P4501A activity led Carls et al. (in press b) to conclude that ingestion of oil particles or oil-contaminated prey was likely the primary route of contamination for both juvenile pink and chum salmon in PWS in 1989. Based on laboratory data, ingestion of prey contaminated with an estimated polyaromatic hydrocarbon (PAH) concentration of 0.29 mg per g of food would cause a 1% BW day^{-1} reduction in the growth of juvenile pink salmon (Mark Carls, National Marine Fisheries Service, personal communication). Hydrocarbon concentrations were not measured in zooplankton or epibenthic invertebrate samples collected in PWS in 1989. However, the estimated PAH concentration (393 mg g^{-1}) in carcasses of fish that exhibited a 1.4% BW day^{-1} growth reduction in the laboratory was roughly equal to that measured in juvenile pink salmon carcasses (326 mg g^{-1}) from oiled areas of PWS in 1989 (Mark Carls, National Marine Fisheries Service, personal communication). Ingestion of oil particles by zooplankton and epibenthic invertebrates has been documented in other areas. After the tanker Arrow spill, pelagic copepods ingested oil particles (Conover 1971) that were uniformly distributed to at least 80 m depth (Forrester 1971). Meiofauna and epibenthic invertebrates, such as harpacticoid copepods, are also known to ingest particulate oil (Landrum 1989).

In the present study, the frequency of P4501A staining was significantly different between areas in gill tissues and not in intestinal tissues. However, intensity of staining was not considered and this may influence how different tissues reflect hydrocarbon exposure. The route of contamination cannot be inferred from the frequency of occurrence of P4501A staining in gill pillar and gill epithelial cells, because P4501A enzymes are induced in these tissues by both intraperitoneal administration of PAH and exposure of fish to waterborne PAH (Miller et al. 1989; Stegeman et al. 1991).

A third possible route of contamination is linked to the behavior of juvenile pink salmon. Juvenile pink salmon commonly feed at the sea surface and leap from the water while feeding (Cooney et al. 1981; Heard 1991). The sea-surface microlayer may concentrate non-polar compounds such as petroleum hydrocarbons that are toxic to fish (Cross et al. 1987; Kocan et al. 1987). We frequently observed schools of juvenile pink salmon feeding and leaping through sheen in oiled areas of PWS in 1989. Further analysis of samples from an oil-ingestion experiment (Carls et al. in press a) is expected to provide additional quantitative data to more firmly establish that exposure to hydrocarbons from the EVOS was at a level sufficient to cause the estimated reduction in growth.

The observed reduction in growth in oil-contaminated areas was likely caused primarily by the increased metabolic cost associated with hydrocarbon metabolism and depuration. The reduction in growth caused by oil exposure in the laboratory (Rice et al. 1975; Moles and Rice 1983; Schwartz 1985; Carls et al. in press a) appears to be partly due to the increased metabolic demand associated with hydrocarbon depuration (Rice et al. 1977; Thomas and Rice 1979). Carls et al. (in press a) postulated that necrosis of the gastrointestinal tract may also reduce assimilation efficiency in juvenile pink salmon fed oil-contaminated prey. Veronique et al. (1992) documented a decline in food conversion efficiency but not feeding rate in Atlantic salmon (*Salmo salar*) parr exposed to a sublethal concentration of a Hibernia oil-water mixture. However, when high concentrations of hydrocarbons are introduced into the diet, feeding rate will decline (Schwartz 1985; Carls et al. in press a). In the present study, stomach fullness was not different between areas in 1989 (Table 9). The greater frequency of P4501A staining in moderately-oiled areas compared with lightly- and non-oiled areas (Table 7) is direct evidence that juvenile pink salmon in oiled areas expended greater amounts of energy to metabolize and depurate hydrocarbons leaving less energy available for somatic growth.

Effect of oil contamination on survival to the adult stage

Exposure of juvenile pink salmon to hydrocarbons from the EVOS appeared to reduce survival to the adult stage by 1.7 to 2.2% in 1989 (Table 14). Reduced growth in oiled nearshore habitats likely caused reduced survival, because slow-growing juvenile salmon are vulnerable to predators for a longer time (Parker 1971; Healey 1982; West and Larkin 1987). Mortenson et al. (1991) concluded that the growth of juvenile CWT pink salmon rearing in Auke Bay, Alaska was significantly related to survival to the adult stage in three out of four years. Mortenson et al. (1991) postulated that changes in predator abundance may have caused interannual changes in the growth-survival relationship. The observed interannual differences in the growth-survival relationship in the present study may have also been caused by interannual changes in predation rate. Predation rate may also differ among areas. The estimated reduction in survival attributed to oil contamination in the present study is based on the assumption that the growth-survival relationship was not different for fish rearing in different areas of PWS.

CONCLUSIONS

Goal A: Determine the effect of oil contamination from the EVOS on the migratory behavior of juvenile pink salmon in PWS.

A relatively large number of juvenile pink salmon from AFK Hatchery migrated out of the moderately-oiled area near the hatchery to the lightly-oiled southern coast of Knight Island in 1989. A similar northward migration of juveniles was not observed in 1990 and 1991. Although, this behavior was associated with oil contamination, it cannot be attributed to it due to a lack of understanding regarding natural environmental effects on migratory behavior. There was no apparent effect of oil contamination on the migratory behavior of juvenile CWT pink salmon released from the WHN hatchery in 1989.

Goal B: Determine the effect of oil contamination from the EVOS on juvenile pink salmon growth in nearshore marine nursery areas in PWS.

Exposure to hydrocarbons from the EVOS in oil-contaminated nearshore nursery habitats appeared to reduce the growth rate of juvenile CWT pink salmon by 0.76 to 0.94% BW day^{-1} in 1989. The growth of juvenile CWT pink salmon was significantly lower ($P < .05$) in oiled areas compared with non-oiled and lightly-oiled areas in 1989. The growth of juvenile CWT pink salmon was not significantly lower in oiled areas compared with non-oiled and lightly-oiled areas in 1990 and 1991. The observed differences in growth rate do not appear to be due to measurement or sampling error, or differences in food consumption rate, prey composition, or water temperature. The observed differences in growth rate were associated with a significantly greater ($P < .05$) frequency of P4501A enzyme induction in oiled areas compared with non-oiled and lightly-oiled areas in 1989. The greater frequency of P4501A enzyme induction in oiled areas is direct evidence that fish in oil-contaminated habitats expended energy to depurate hydrocarbons leaving less energy available for somatic growth. Insufficient data is available at the present time to determine if the level of hydrocarbon exposure of juvenile pink salmon was sufficient to cause the estimated reduction in growth rate attributed to oil contamination.

Goal C: Determine the effect of oil contamination from the EVOS on the fry-to-adult survival of pink salmon that reared in oiled nearshore nursery areas in PWS.

Exposure of juvenile pink salmon to hydrocarbons from the EVOS in oil-contaminated nearshore nursery habitats appeared to reduce survival to the adult stage by 1.7 to 2.2% in 1989. The growth of juvenile CWT pink salmon in 1989 was significantly related ($P < .05$) to survival to the adult stage. Thus, reduced juvenile growth in oiled areas in 1989 likely caused reduced survival to the adult stage. The adult pink salmon return to PWS in 1990 was thus lower than if the EVOS had not occurred.

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TABLES 1-14

Table 1: Mean growth rates (% body weight day^{-1}) of coded-wire tagged juvenile pink salmon from three release groups in Prince William Sound, 1989-1991. Early-fed, (fed 1-2 weeks and released at high zooplankton abundance), unfed (fed 2-5 days and released at high zooplankton abundance), and late-fed (fed 1-2 weeks and released during declining zooplankton abundance). AFK-Armin F. Koernig Hatchery, WHN-Wally H. Noerenberg Hatchery, and CCH-Cannery Creek Hatchery.

		Early Fed			Unfed			Late Fed			
Year	Hatchery	n	avg	SE	n	avg	SE	n	avg	SE	P[1]
1989	AFK	21(252)	4.12	.21	13(51)	2.98	.34	11(97)	3.15	.59	.421
	WHN	16(151)	4.14	.31	9(24)	4.44	.24	2 (5)	8.13	.10	.002
	CCH	3 (18)	5.38	.41	3(15)	4.30	.28	4(24)	4.69	.42	.486
1990	AFK	20(266)	4.42	.10	13(97)	3.77	.14	6 (6)	5.46	.26	< .001
	WHN	18 (81)	3.93	.23	6(17)	3.73	.32	7 (9)	5.86	.63	.013
	CCH	8 (20)	4.95	.16	4 (7)	4.91	.54	14(79)	5.17	.51	.047
1991	AFK	6(300)	3.04	.17	4(53)	3.11	.24	3(91)	5.36	.27	< .001
	WHN	9(239)	3.75	.15	6(24)	2.95	.25	6(22)	2.56	.44	.054
	CCH	5 (25)	3.22	.14	-	-	-	2 (4)	2.86	.13	.228

[1] Level of statistical significance for the comparison.

Table 2: Mean growth rates (% body weight day^{-1}) of coded-wire tagged juvenile pink salmon in early-fed groups released from the Armin F. Koernig Hatchery and recovered during May and June, 1989. Significance levels (P) from an analysis of variance are indicated for the oil, time, and oil-by-time interaction terms in the model. Sample size expressed as number of sites. Number of individual coded-wire tagged juvenile pink salmon included in the analysis indicated in parentheses. A: analysis including Squirrel Bay site; B: analysis not including Squirrel Bay site.

	Recovery Area	Combined average	May			June			Significance		
			n	avg	SE	n	avg	SE	Oil	Time	OilxTime
A	Light-oil	4.93	2 (66)	4.59	.21	2 (23)	5.27	.21	.189	.541	.033
A	Oil	3.70	5 (64)	3.87	.13	5 (49)	3.53	.13			
B	Light-oil	4.93	2 (66)	4.59	.23	2 (23)	5.27	.23	.034	.502	.069
B	Oil	4.17	4 (33)	4.31	.16	4 (48)	4.01	.16			

Table 3: Significance levels (P) associated with paired comparisons of juvenile pink salmon growth rates in oiled and non-oiled areas of Prince William Sound during May and June, 1989. A: analysis including Squirrel Bay site; B: analysis not including Squirrel Bay site.

	Recovery Area	Month	Light-oil		Oil	
			May	June	May	June
A	Light-oil	May	-	.071	.035	.008
A	Light-oil	June	-	-	.002	.001
A	Oil	May	-	-	-	.126
B	Light-oil	May	-	.105	.394	.112
B	Light-oil	June	-	-	.028	.011
B	Oil	May	-	-	-	.257

Table 4: Mean growth rates (% body weight day^{-1}) and length-weight parameter estimates for coded-wire tagged juvenile pink salmon in early-fed groups released from Armin F. Koernig (AFK) and Wally H. Noerenberg (WHN) hatcheries in Prince William Sound, 1989-1991.

Year	Hatchery	Recovery Area	Growth				Condition		
			n	avg	SE	P[1]	slope[2]	SE	P
1989	AFK	Light-oil	4 (89)	4.93	.20	.034	3.53	.05	.529
		Oil	8 (81)	4.17	.14		3.20	.29	
1990	AFK	Light-Oil	3 (14)	4.80	.28	.103	2.63	.01	.513
		Oil	13(225)	4.46	.08		2.84	.16	
1991	AFK	Light-oil	1 (43)	3.38	-	.085	-	-	-
		Oil	5(272)	2.97	.21		-	-	
1989	WHN	Non-oil	4(113)	5.16	.33	.019	2.96	.33	.585
		Oil	6 (29)	4.22	.13		2.75	.21	
1990	WHN	Non-oil	6(129)	3.93	.37	.767	3.38	.31	.313
		Oil	3 (12)	3.81	1.46		3.01	.09	
1991	WHN	Non-oil	4(116)	3.59	.19	.883	3.72	.17	.009
		Oil	3(118)	3.52	.06		2.52	.09	

[1] Level of statistical significance for the comparison.
[2] Slope parameter estimate of linear regression of ln (weight) on ln (length).

Table 5: Analysis of covariance of natural logarithm otolith radius length versus fish body weight and otolith increment count versus fish age, respectively. Slow and fast growing fish are defined as those exhibiting growth rates above and below the overall mean growth for all years combined.

Parameter	Slow Growth	Fast Growth	P[1]
Otolith Radius Length			
Intercept	-3.803	-3.368	.283
Slope	0.017	0.016	.407
sample size (n)	337	351	-
Otolith Increment Count			
Intercept	3.498	1.601	.459
Slope	1.189	0.971	.014
sample size (n)	337	351	-

[1] Level of statistical significance for the comparison.

Table 6: Percent frequency of occurrence of cytochrome P4501A enzyme induction in tissues of juvenile pink salmon sampled along the southern coast of Knight Island during May and June in 1989. Fisher exact test probabilities (P) of a type I error (2-tail) for pairwise comparisons between months.

Organ	Tissue	May	June	P[1]
Gill	pillar cells	67	0	<.001
	epithelium	4	0	1.000
	endothelium of gill arches	4	0	1.000
	gill buds	0	0	-
	pharyngeal epithelium	4	0	1.000
Liver	hepatocytes	6	9	1.000
	sinusoidal endothelium	0	0	-
	central veins	0	0	-
Kidney	duct epithelium	0	0	-
	sinusoidal endothelium	0	0	-
Intestine	cecal epithelium	45	0	.002

[1] level of statistical significance for the comparison.

Table 7: Percent frequency of occurrence of cytochrome P4501A enzyme induction in tissues of juvenile pink salmon sampled in non-oiled, lightly-oiled, and moderately-oiled nearshore habitats in Prince William Sound in 1989. Fisher exact test probabilities of a type I error (2-tail) for pairwise comparisons between areas.

		WHN Hatchery			AFK Hatchery		
Organ	Tissue	mod-oiled	non-oiled	P[1]	mod-oiled	light-oiled	P
Gill	pillar cells	75	0	<.001	100	67	.015
	epithelium	94	0	<.001	27	4	.068
	endothelium of gill arches	6	0	1.000	0	4	1.000
	gill buds	0	0	-	0	0	-
	pharyngeal epithelium	0	0	-	0	4	1.000
Liver	hepatocytes	8	12	1.000	9	6	1.000
	sinusoidal endothelium	0	0	-	9	0	.379
	central veins	0	0	-	9	0	.423
Kidney	duct epithelium	0	0	-	0	0	-
	sinusoidal endothelium	0	0	-	0	0	-
Intestine	cecal epithelium	14	9	1.000	21	45	.175

[1] level of statistical significance for the comparison.

Table 8: Median prey composition (% stomach contents weight) and stomach fullness (% body weight) of juvenile pink salmon during May and June in Prince William Sound, 1989-1990.

Prey Category	WHN Hatchery May	WHN Hatchery June	WHN Hatchery P[1]	AFK Hatchery May	AFK Hatchery June	AFK Hatchery P
1989						
large copepod	39.9	1.1	.001	52.4	23.6	<.001
small copepod	8.8	3.0	.208	5.3	10.0	.347
harpacticoid copepod	18.2	0.1	<.001	15.4	0.1	.026
stomach fullness	3.2	4.3	.984	2.6	2.1	.180
sample size (n)	12	18		10	25	
1990						
large copepod	5.4	0.9	.040	9.1	14.3	.679
small copepod	2.7	4.6	.673	17.2	5.2	.585
harpacticoid copepod	1.6	0.0	.002	17.8	0.7	<.001
stomach fullness	0.9	1.7	.929	2.4	2.8	.328
sample size (n)	6	10		7	40	

[1] level of statistical significance for the comparison.

Table 9: Median prey composition (% stomach contents weight) and stomach fullness (% body weight) of juvenile pink salmon in oiled and non-oiled areas of Prince William Sound, 1989-1991.

Tissue	WHN Hatchery mod-oiled	WHN Hatchery non-oiled	WHN Hatchery P[1]	AFK Hatchery mod-oiled	AFK Hatchery light-oiled	AFK Hatchery P
1989						
large copepod	6.8	2.5	.780	37.7	29.1	.089
small copepod	3.3	5.1	.180	12.3	2.2	.005
harpacticoid copepod	13.1	1.4	.397	1.1	16.3	.245
stomach fullness	4.0	3.5	.916	2.4	2.0	.337
sample size (n)	10	20		20	15	
1990						
large copepod	3.2	1.4	.525	22.9	0.3	.020
small copepod	2.7	5.1	.554	7.0	3.7	.261
harpacticoid copepod	0.6	0.0	.234	2.0	0.0	.017
stomach fullness	0.8	4.2	.061	2.4	3.1	.179
sample size (n)	9	7		34	13	
1991						
large copepod	0.0	3.5	.390	52.1	16.7	-
small copepod	1.6	7.6	.211	30.0	47.8	-
harpacticoid copepod	6.3	6.1	.590	5.9	32.0	-
stomach fullness	2.3	1.8	.497	3.5	2.0	-
sample size (n)	6	7		10	1	

[1] level of statistical significance for the comparison.

Table 10: Least-squares mean ocean temperatures (°C) in oiled and non-oiled areas where juvenile pink salmon released from the Wally H. Noerenberg (WHN) and Armin F. Koernig (AFK) hatcheries were recovered in 1989, 1990, and 1991.

Year	Hatchery	Recovery Area	Mean Temperature	Sample Size (n)	P[1]
1989	AFK	Light Oil	8.9	25	.075
		Oil	8.0	11	
1990	AFK	Light Oil	11.5	11	.0001
		Oil	9.1	28	
1991	AFK	Light Oil	8.6	3	.290
		Oil	7.9	18	
1989	WHN	Non-oil	9.9	18	.811
		Oil	9.8	17	
1990	WHN	Non-oil	12.0	14	.355
		Oil	12.5	5	
1991	WHN	Non-oil	11.2	11	.719
		Oil	11.0	20	

[1] level of statistical significance for the comparison.

Table 11: Analysis of covariance of mean growth of juvenile CWT pink salmon in relation to number of fry released, ocean temperature, and zooplankton settled volume. Mean growth rates (% body weight day^{-1}) by size of release group are adjusted for covariates.

Variable	Mean Growth	Parameter	SE	P[1]
Intercept	-	1.83	1.08	.096
number fry released:				
(< 50 million)	4.70	.82	.73	.265
(> 50 million & < 150 million)	4.15	.27	.59	.653
(>150 million)	3.88	-	-	-
ocean temperature	-	.30	.15	.050
zooplankton volume	-	-.06	.13	.619

[1] level of statistical significance for the variable.

Table 12: Temperature-specific growth (% body weight day^{-1}) at maximum daily ration, food consumption (% body weight day^{-1}), and gross conversion efficiency (%) for a 1.0 g pink salmon estimated from a bioenergetics model.

Temp(°C)	Growth	Food Consumption	Conversion Efficiency
4.0	1.8	7.8	23
6.0	2.3	9.8	23
8.0	2.7	11.7	23
10.0	3.2	13.7	23
12.0	3.6	15.6	23
14.0	4.1	17.6	23

Table 13: Bioenergetics model estimate of the time (hours) required for a 1 g pink salmon to obtain maximum daily ration when feeding on *Pseudocalanus spp.* and *Neocalanus plumchrus*.

	Prey Biomass (g wet wt m^{-3})					
Temp	.001	.010	.100	1.000	1.500	2.000
Pseudocalanus spp.						
4.0	22.1	10.5	9.4	9.2	9.2	9.2
6.0	27.6	13.1	11.7	11.5	11.5	11.5
8.0	33.1	15.7	14.0	13.8	13.8	13.8
10.0	38.6	18.3	16.3	16.1	16.1	16.1
12.0	44.1	20.9	18.6	18.4	18.4	18.4
14.0	49.6	23.5	20.9	20.7	20.7	20.7
Neocalanus plumchrus						
4.0	4.8	3.2	3.1	3.1	3.1	3.1
6.0	6.0	4.1	3.9	3.8	3.8	3.8
8.0	7.2	4.9	4.6	4.6	4.6	4.6
10.0	8.4	5.7	5.4	5.4	5.4	5.4
12.0	9.6	6.5	6.2	6.1	6.1	6.1
14.0	10.8	7.3	6.9	6.9	6.9	6.9

Table 14: Predicted survival to adult (%) for juvenile CWT pink salmon that reared in oiled and non-oiled areas of Prince William Sound in 1989.

Hatchery	Recovery Area	Mean Growth	Predicted Survival	Mean Difference
WHN	Non-oil	5.16	8.1	2.2
	Oil	4.22	5.9	
AFK	Light-oil	4.93	7.5	1.7
	Oil	4.17	5.8	

FIGURES 1-14

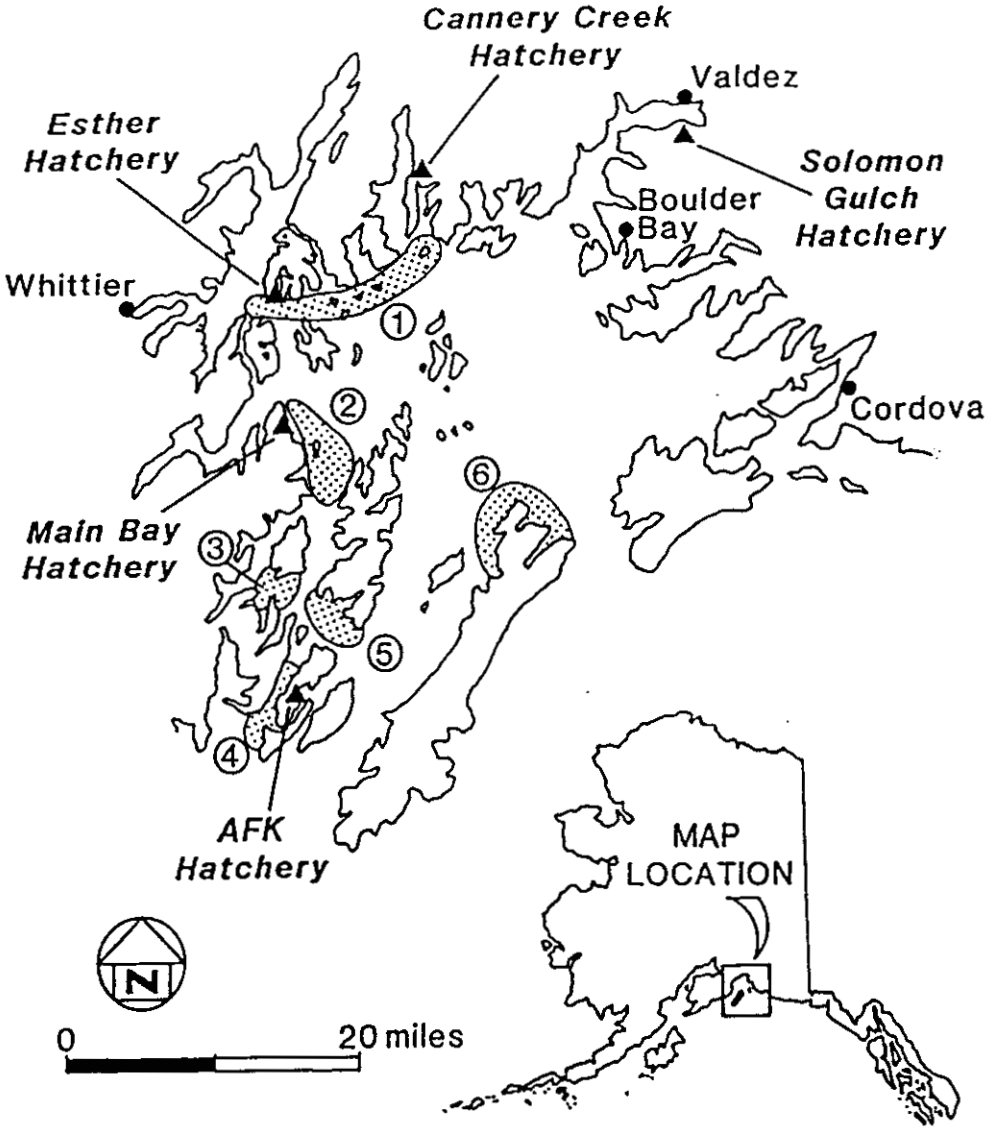


Figure 1: Six areas sampled for juvenile coded-wire tagged pink salmon in Prince William Sound, 1989.

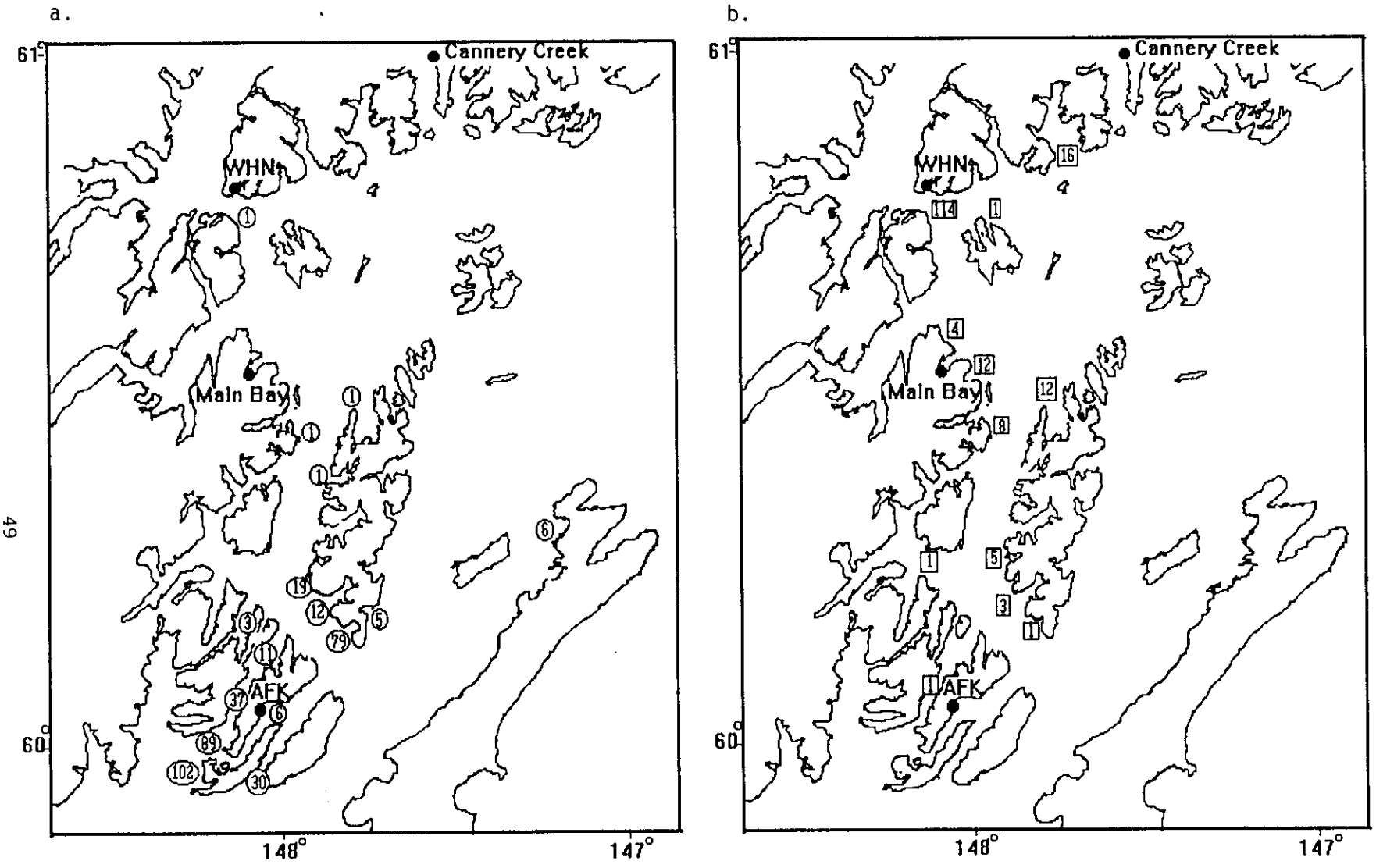


Figure 2: Geographic distribution of coded-wire tag recoveries in Prince William Sound in 1989: (a) fish released from the Armin F. Koernig Hatchery, (b) fish released from the Wally H. Noerenberg Hatchery. Enclosed values indicate the number of coded-wire tagged juvenile pink salmon recovered at each site.

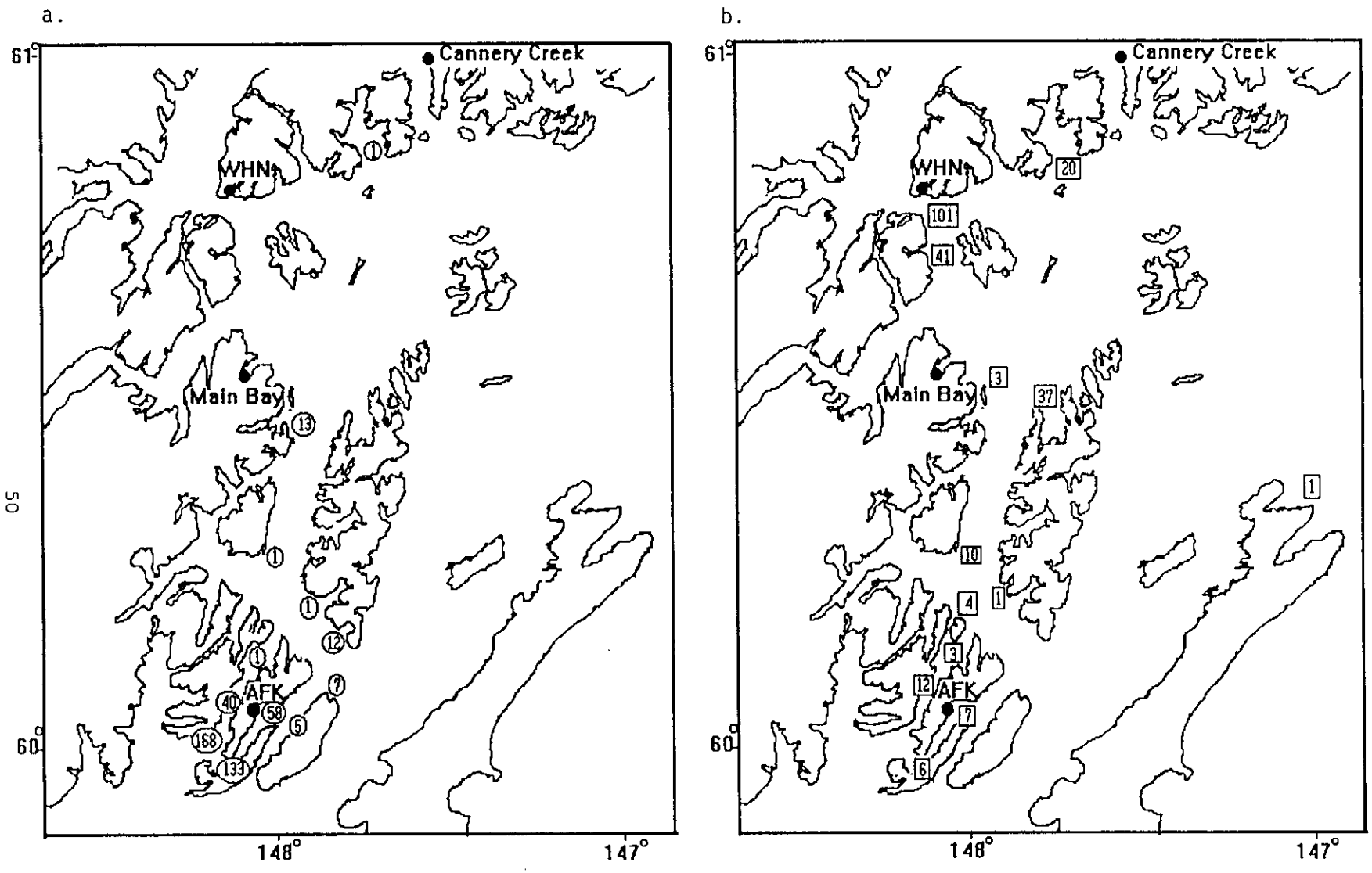


Figure 3: Geographic distribution of coded-wire tag recoveries in Prince William Sound in 1990: (a) fish released from the Armin F. Koernig Hatchery, (b) fish released from the Wally H. Noerenberg Hatchery. Enclosed values indicate the number of coded-wire tagged juvenile pink salmon recovered at each site.

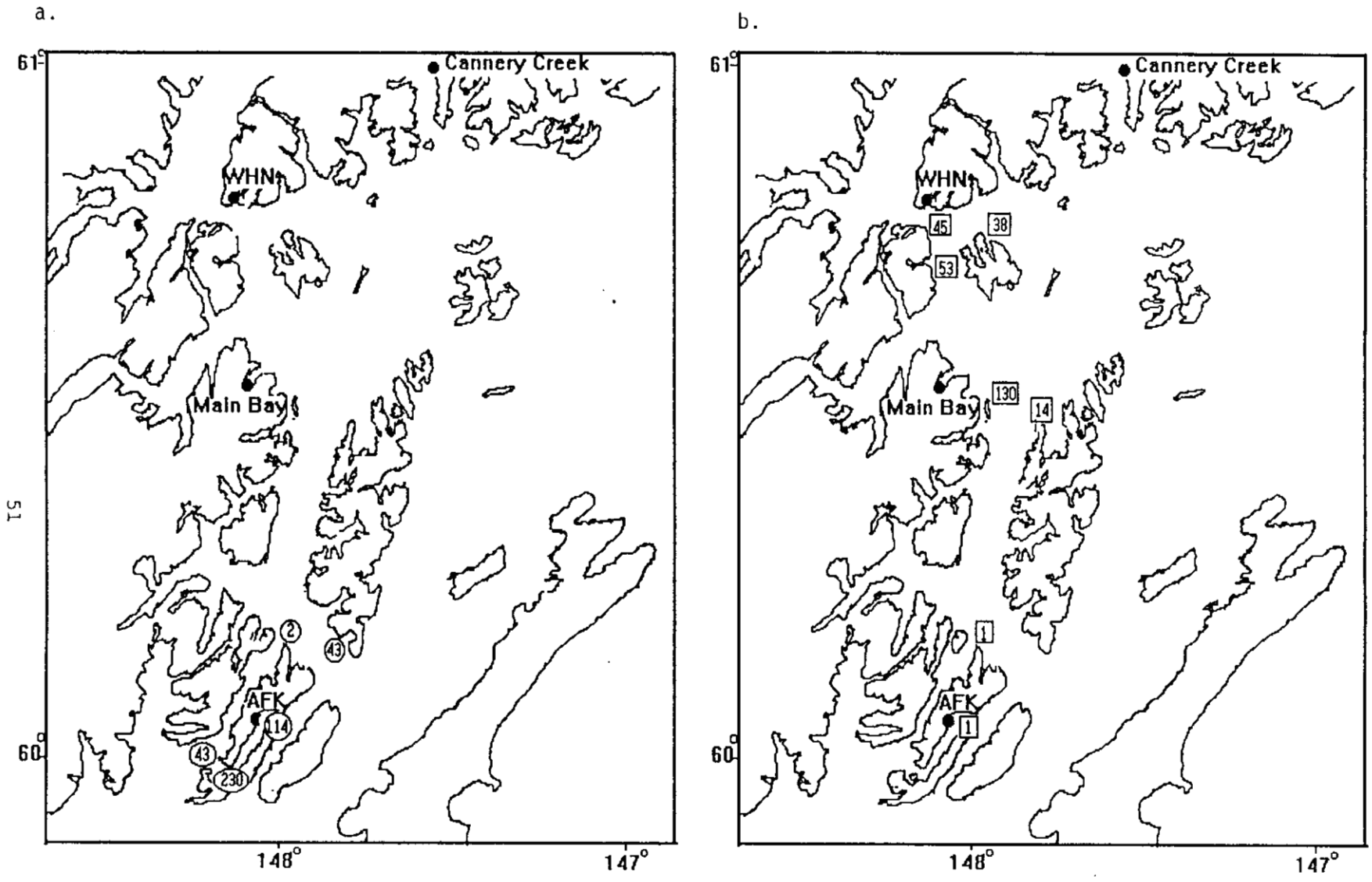


Figure 4: Geographic distribution of coded-wire tag recoveries in Prince William Sound in 1991: (a) fish released from the Armin F. Koernig Hatchery, (b) fish released from the Wally H. Noerenberg Hatchery. Enclosed values indicate the number of coded-wire tagged juvenile pink salmon recovered at each site.

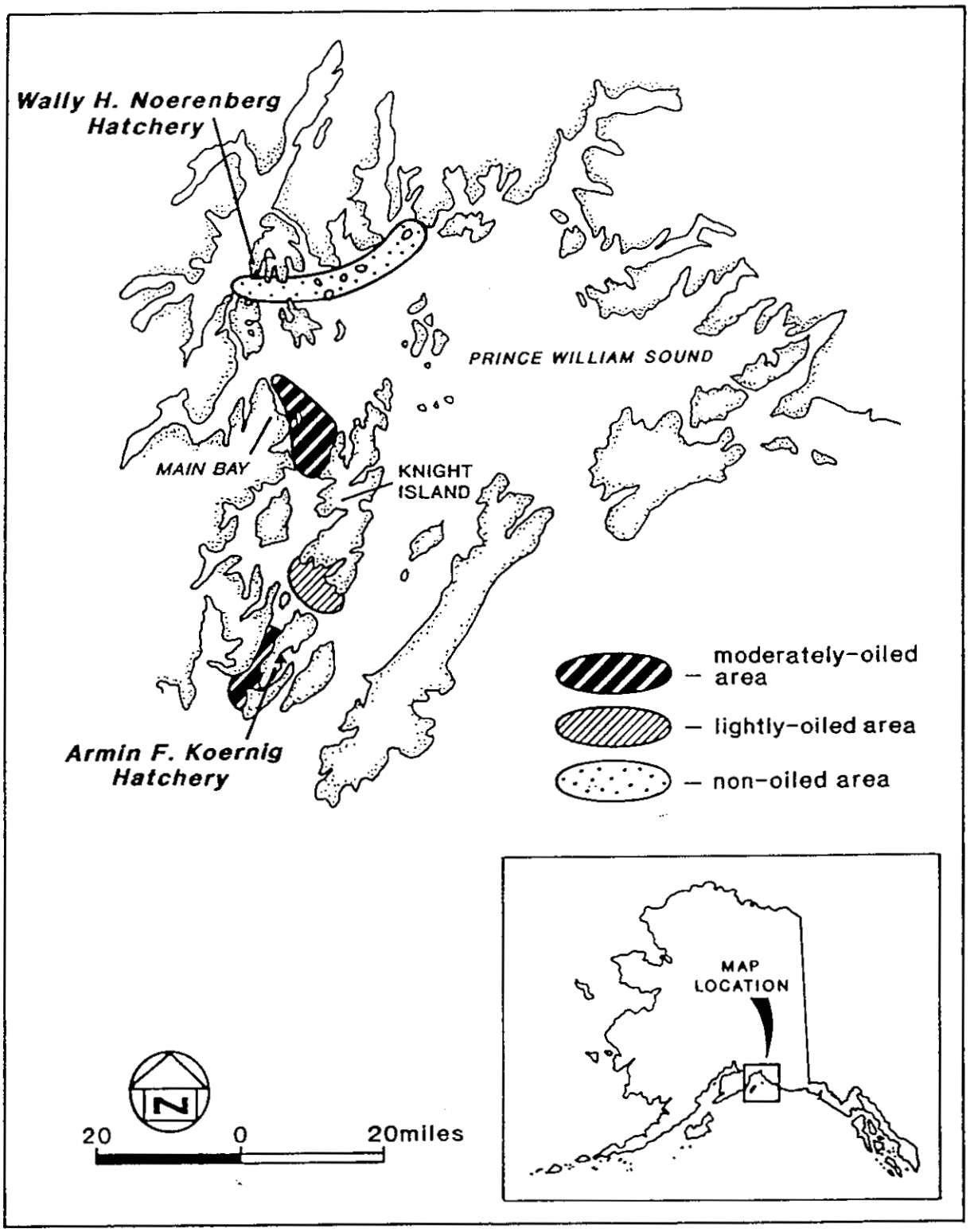


Figure 5: Paired oiled and non-oiled areas selected for comparison of growth rates of juvenile coded-wire tagged pink salmon released from the Wally H. Noerenberg and Armin F. Koernig hatcheries in 1989, 1990, and 1991.

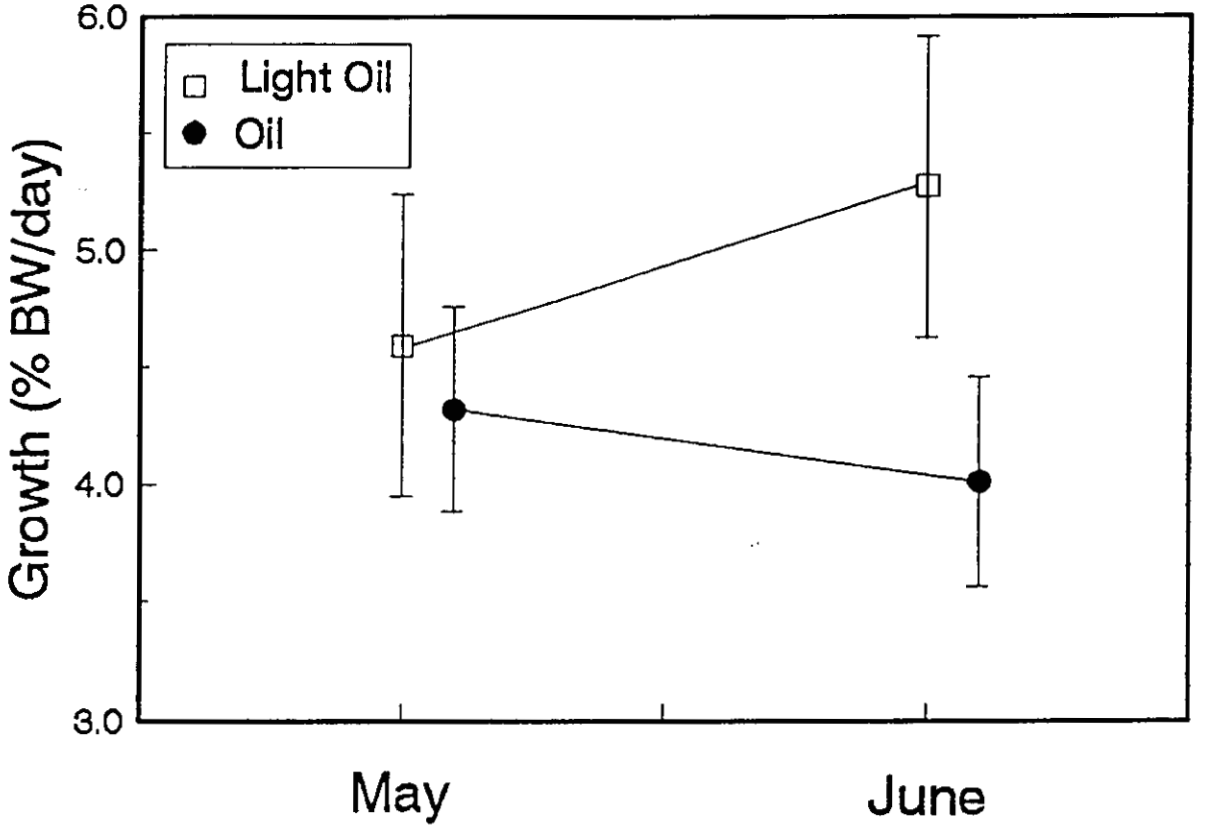
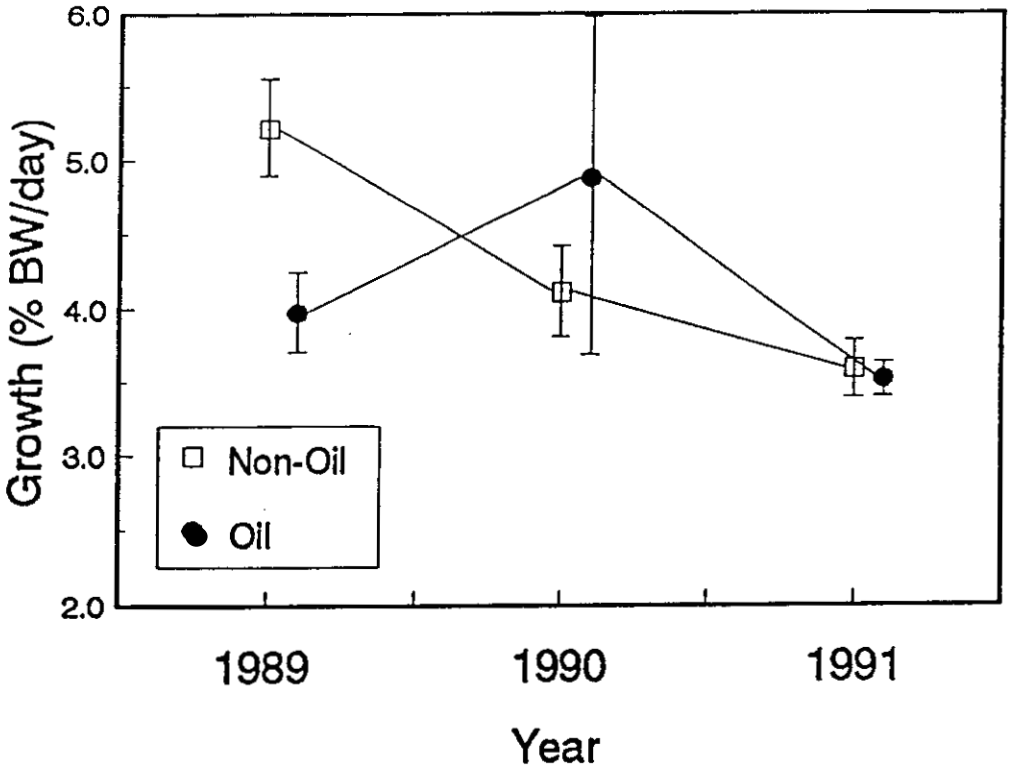


Figure 6: Mean growth with 95% confidence intervals for juvenile coded-wire tagged pink salmon in the early-fed group released from the Armin F. Koernig Hatchery and recovered in lightly oiled and non-oiled areas of Prince William Sound during May and June of 1989.

a.



b.

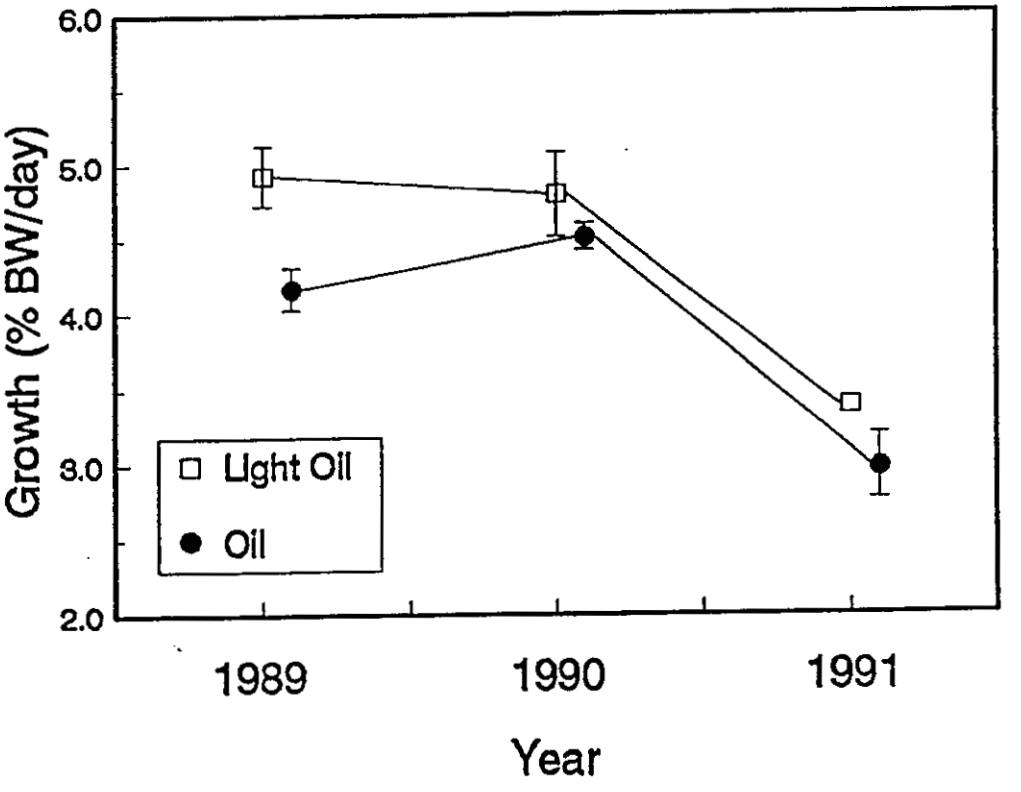


Figure 7: Mean growth with standard errors for juvenile coded-wire tagged pink salmon in the early-fed groups released from the (a) Wally H. Noerenberg and (b) Armin F. Koernig hatcheries and recovered in oiled and non-oiled areas of Prince William Sound, 1989-1991.

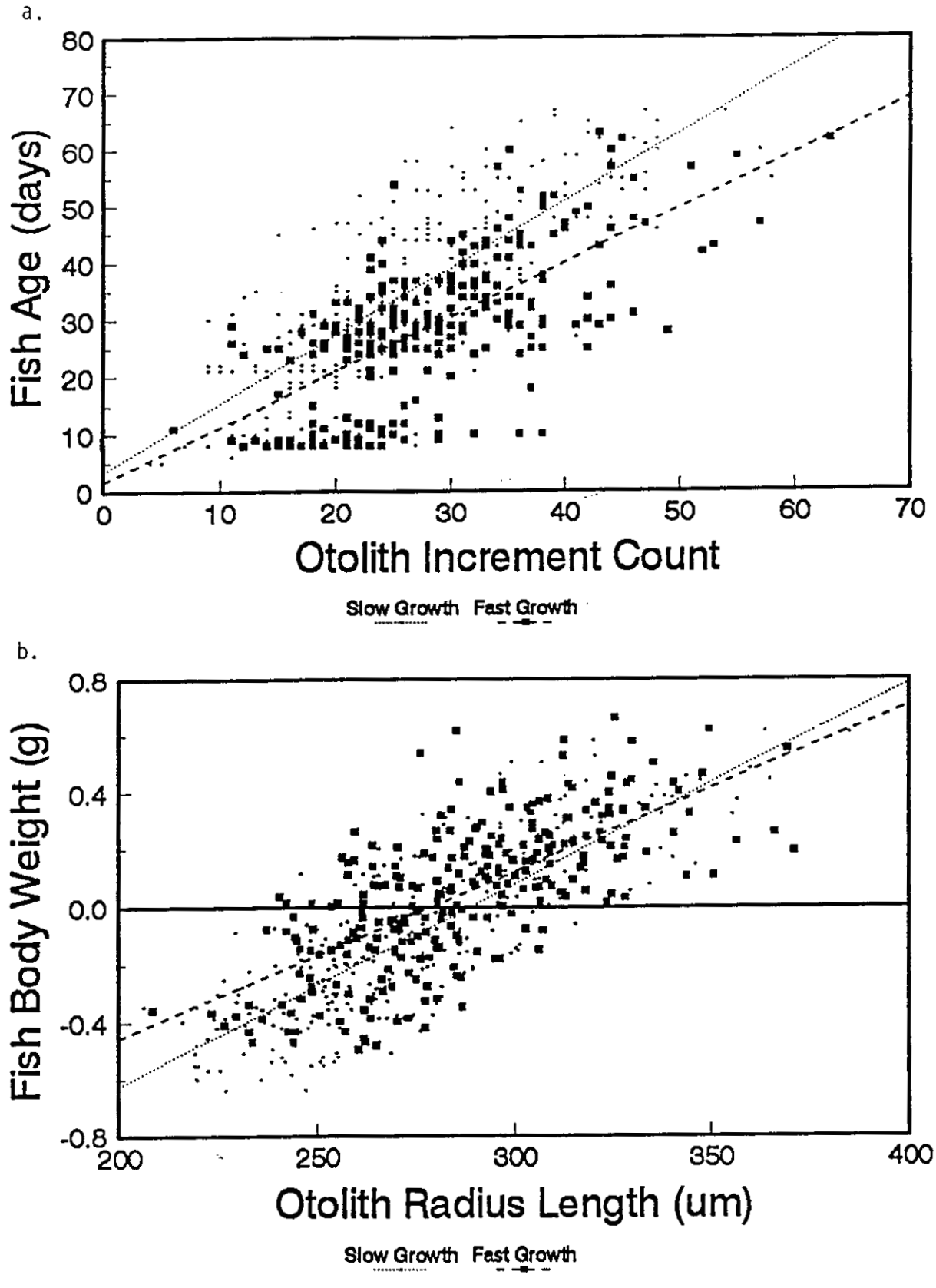
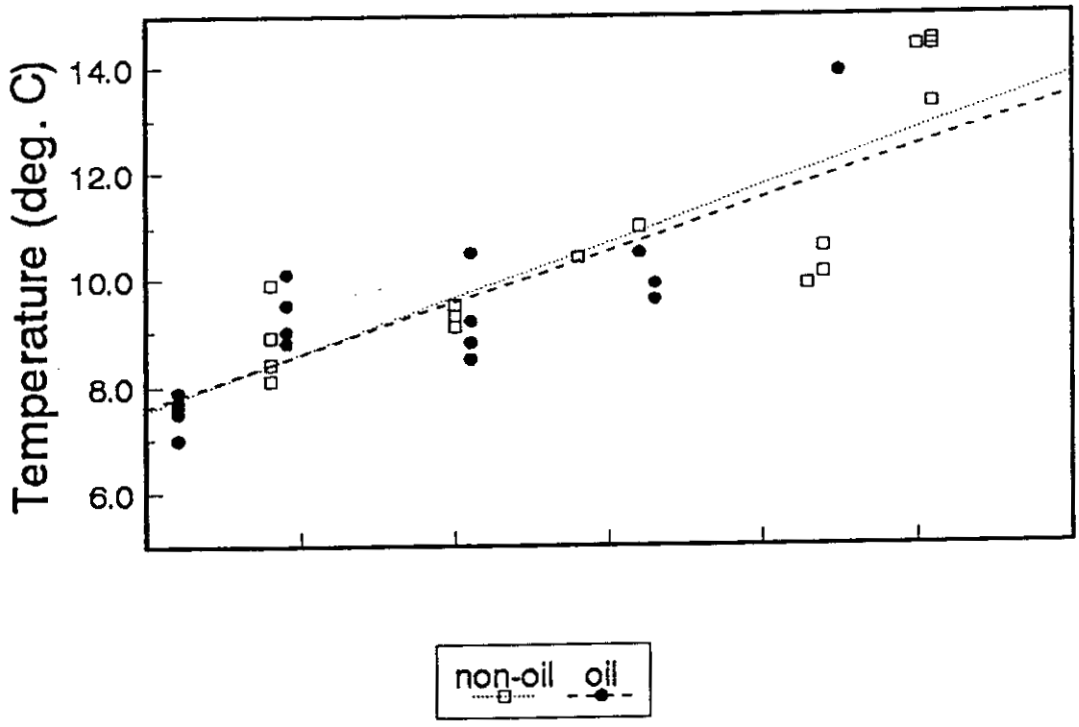


Figure 8: Relationships between (a) otolith increment count and fish age (release to recovery), and (b) otolith radius length and fish body weight for slow and fast growing juvenile coded-wire tagged pink salmon, 1990-1991.

a.



b.

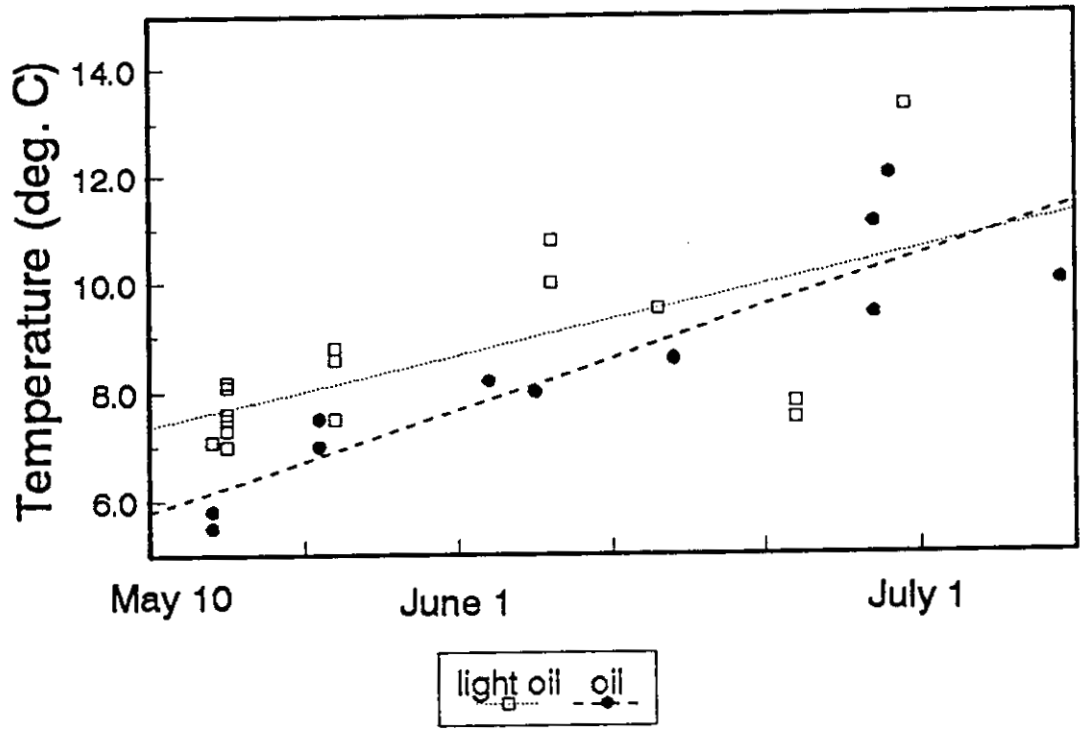


Figure 9: Relationships between ocean temperature and date in oiled and non-oiled areas where juvenile pink salmon released from the (a) Wally H. Noerenberg and (b) Armin F. Koernig hatcheries were recovered, 1989.

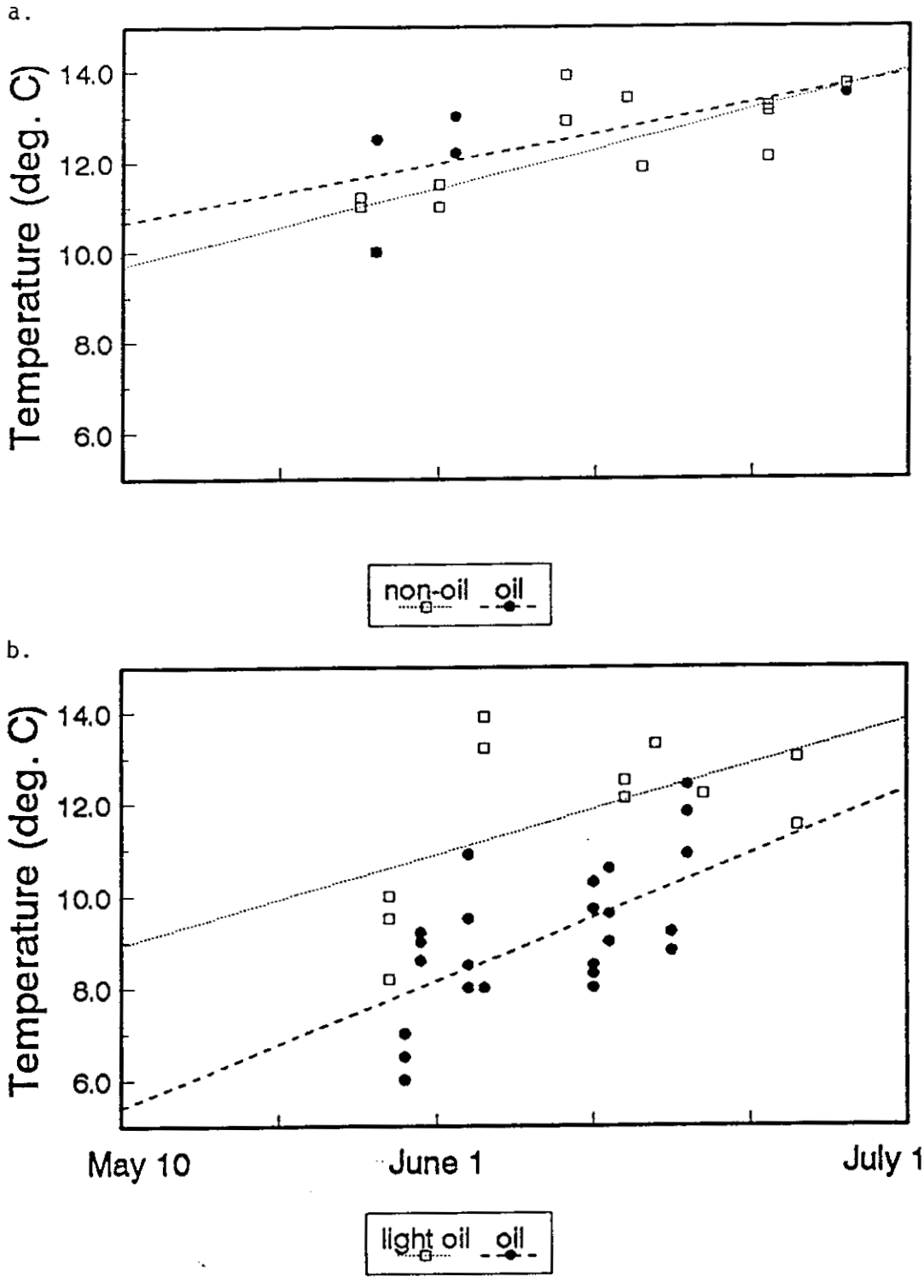


Figure 10: Relationships between ocean temperature and date in oiled and non-oiled areas where juvenile pink salmon released from the (a) Armin F. Koernig and (b) Wally H. Noerenberg hatcheries were recovered, 1990.

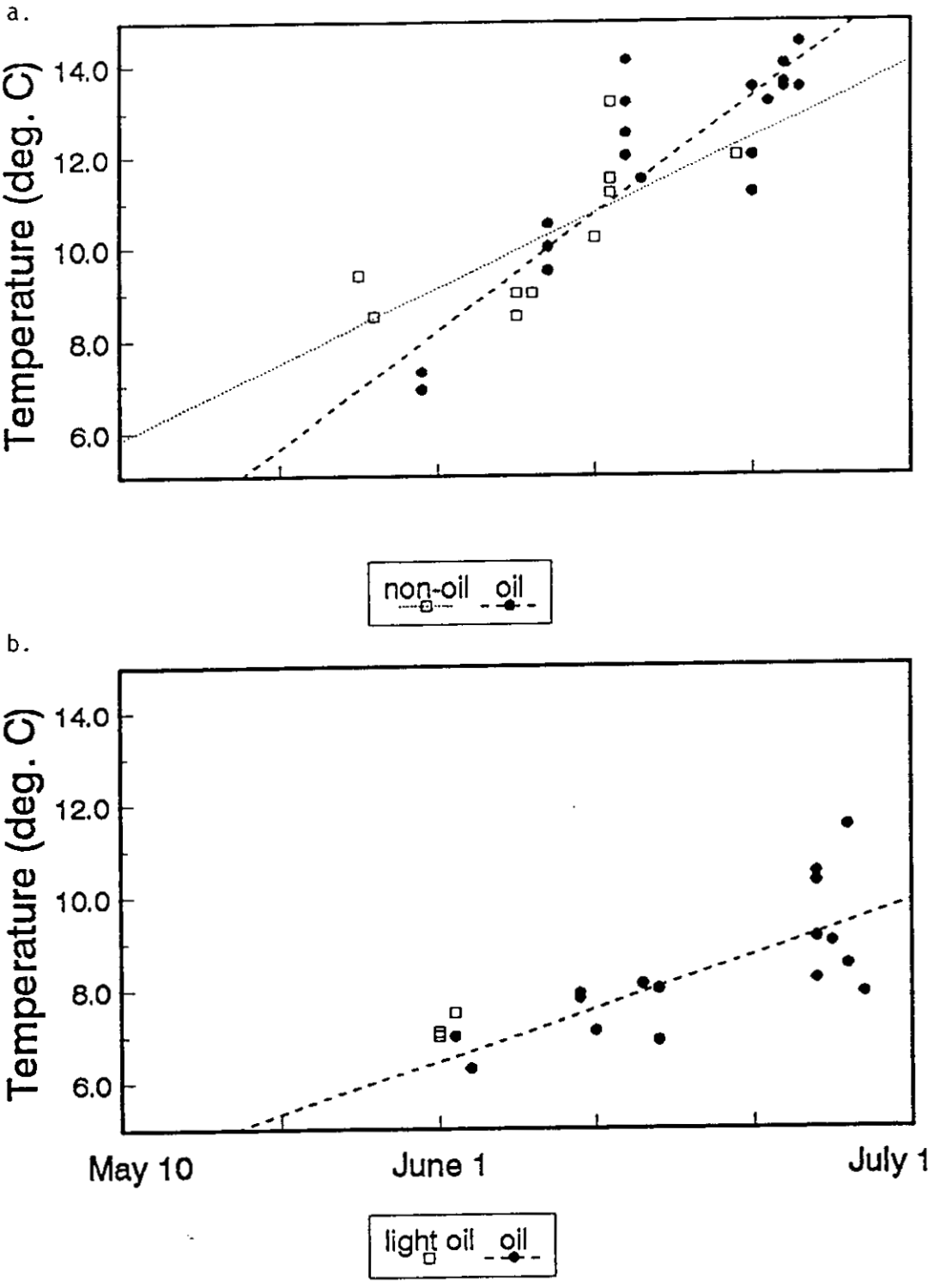


Figure 11: Relationships between ocean temperature and date in oiled and non-oiled areas where juvenile pink salmon released from the (a) Wally H. Noerenberg and (b) Armin F. Koernig hatcheries were recovered, 1991.

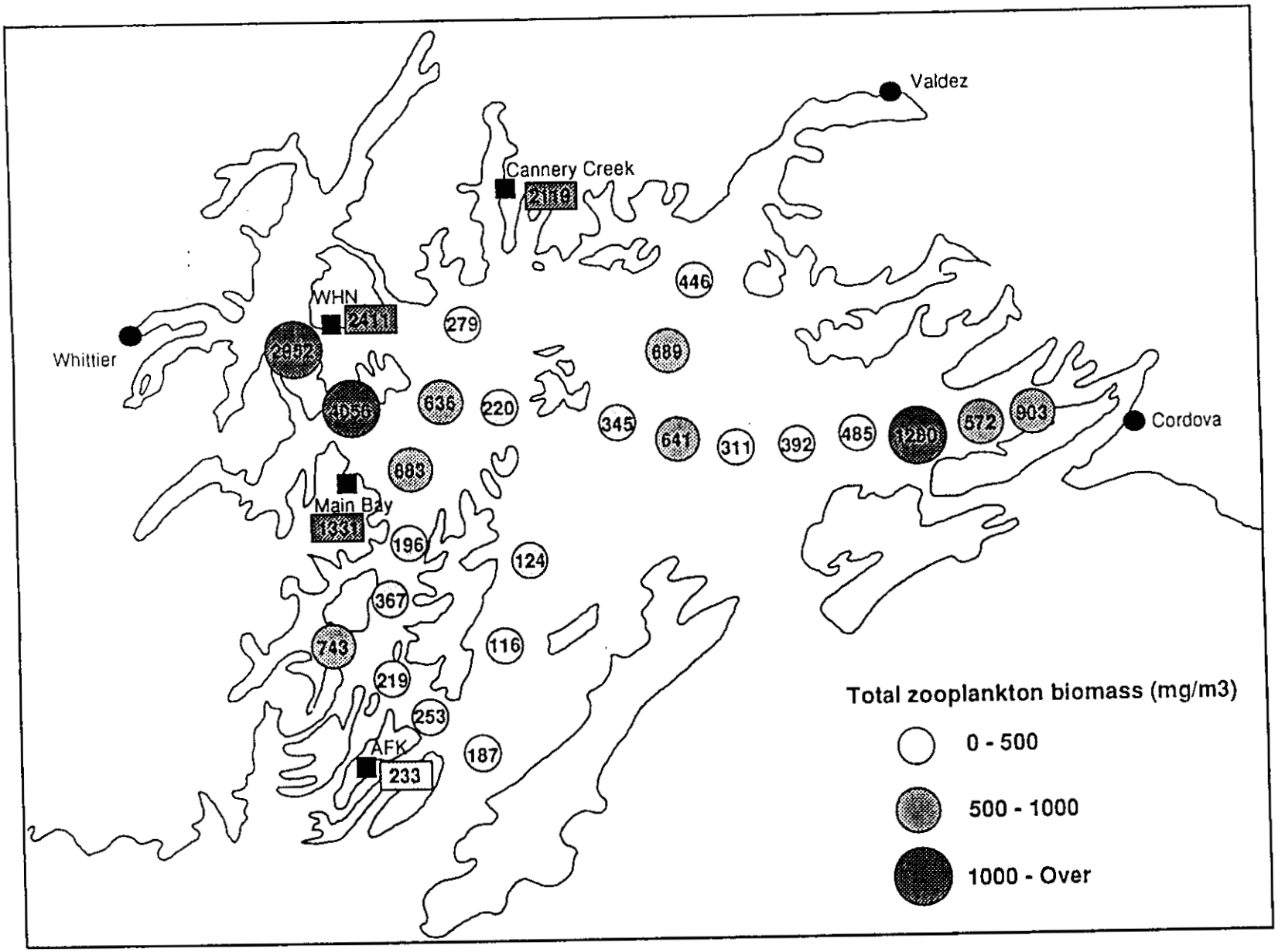


Figure 12: Distribution of zooplankton in the upper 20 m of Prince William Sound during May, 1991: (a) total zooplankton biomass, (b) abundance of large calanoid copepods, (c) abundance of small calanoid copepods, and (d) abundance of 'other' zooplankton.

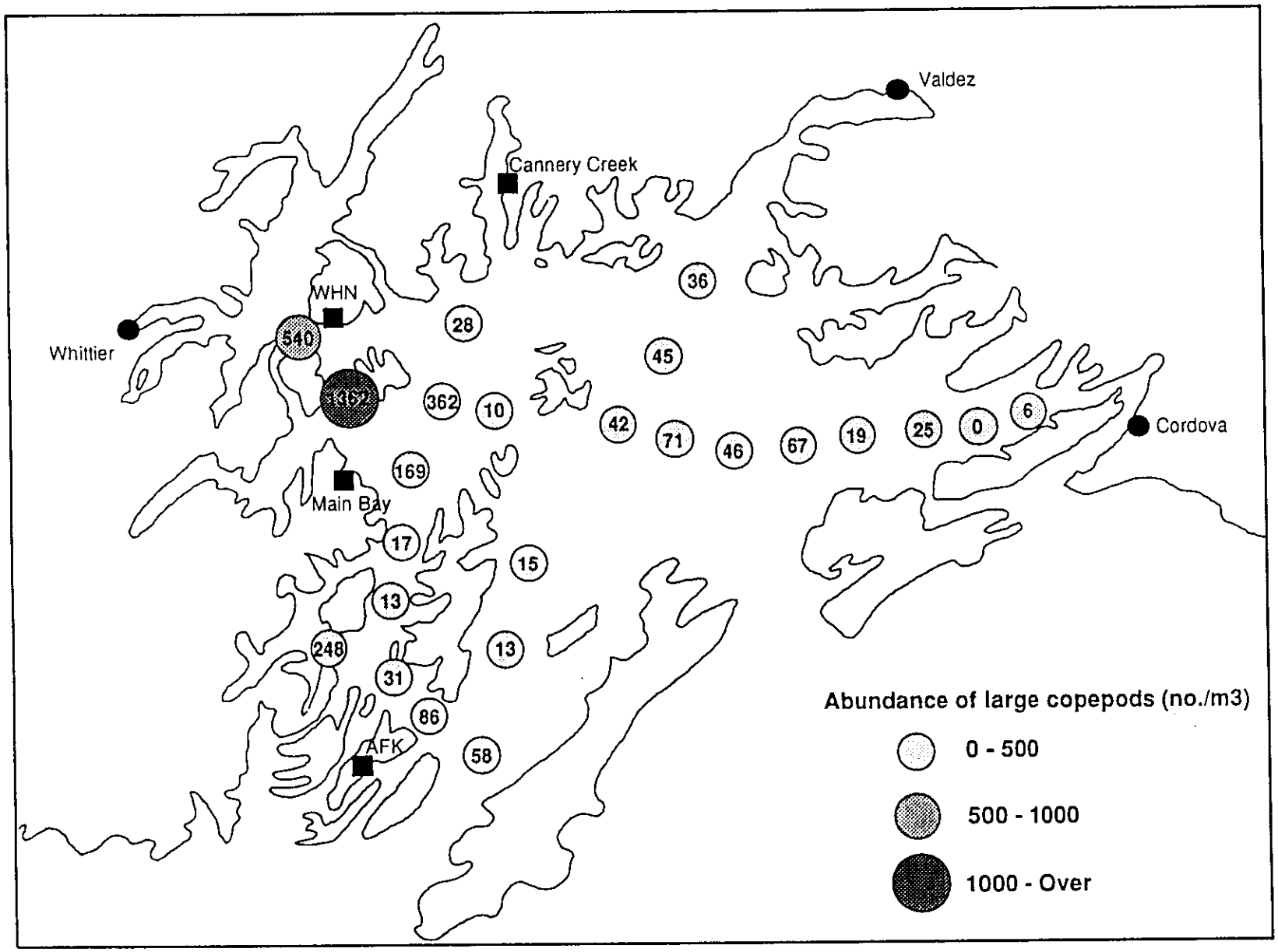

Valdez
Cannery Creek
WHN
Whittier
540
1572
362
10
28
36
45
42
71
46
67
19
25
0
6
Cordova
169
Main Bay
17
15
13
248
13
31
86
AFK
58
Abundance of large copepods (no./m3)
0 - 500
500 - 1000
1000 - Over

Figure 12(b)

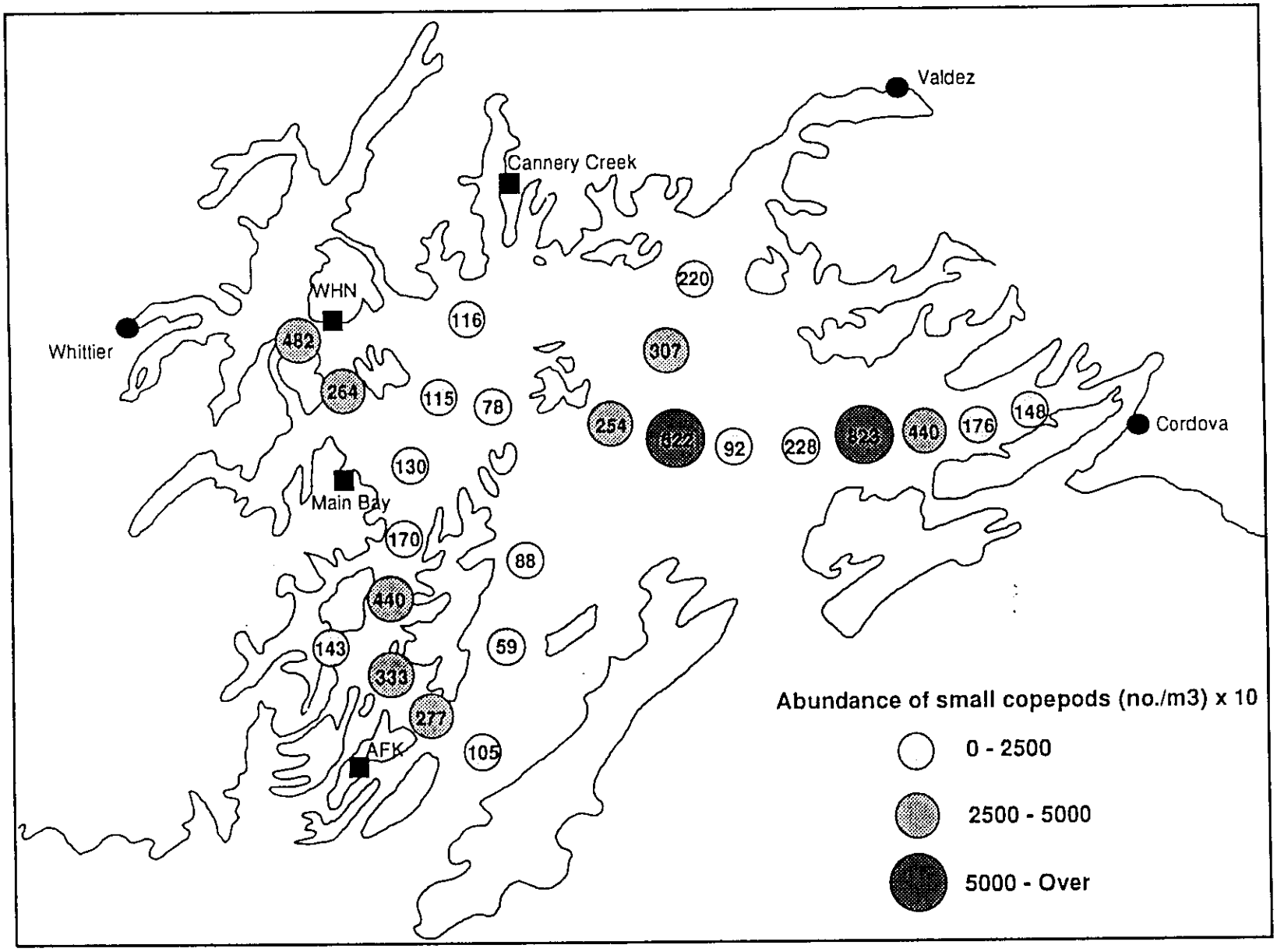

Valdez
Cannery Creek
WHN
Whittier
Cordova
Main Bay
AFK
482
264
116
115
78
220
307
254
92
228
440
176
148
130
170
88
440
143
59
333
277
105
Abundance of small copepods (no./m3) x 10
0 - 2500
2500 - 5000
5000 - Over

Figure 12(c)

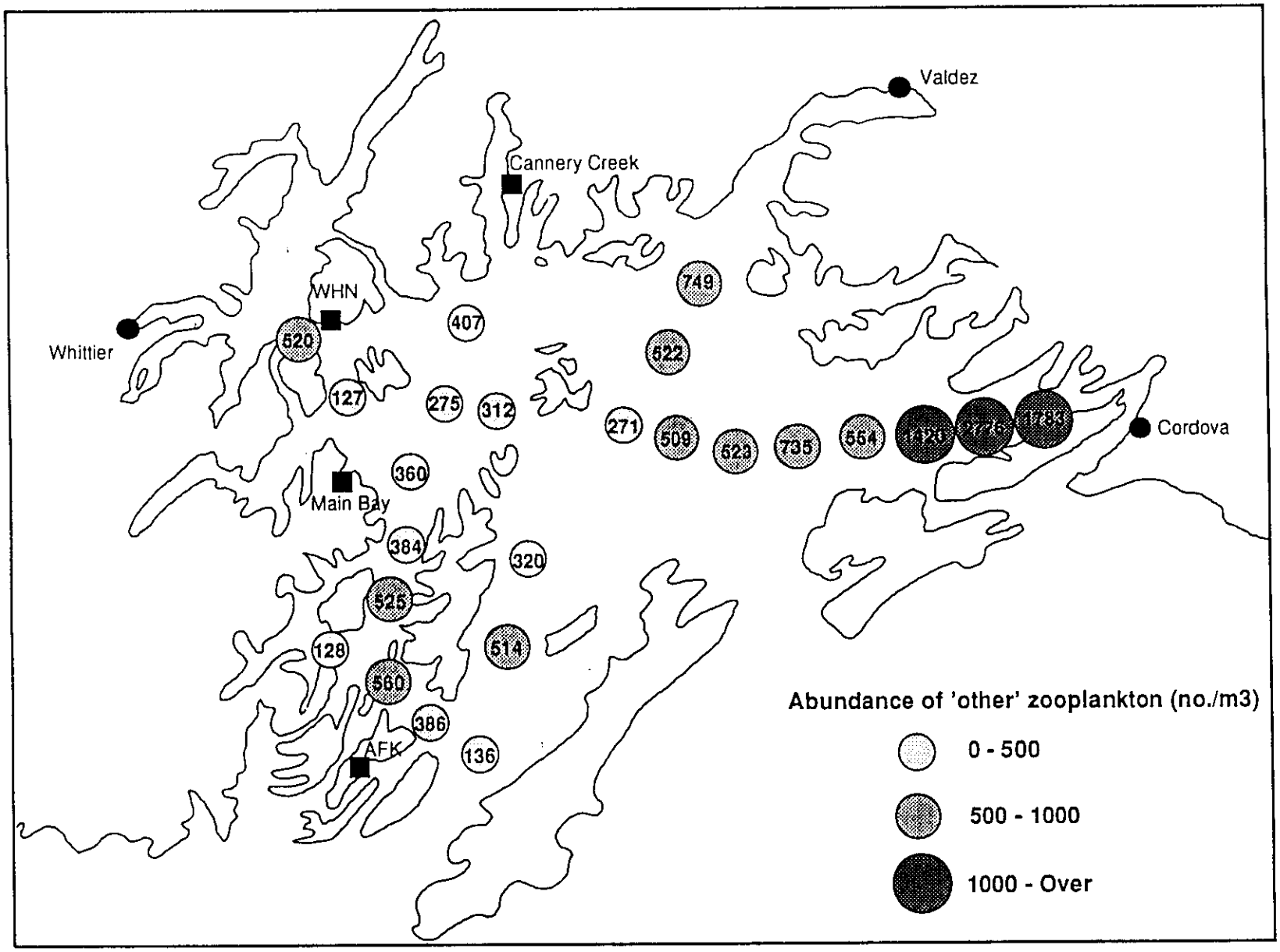

Valdez
Cannery Creek
WHN
Whittier
520
407
749
522
127
275
312
271
509
623
735
554
Cordova
360
Main Bay
384
320
525
514
128
560
386
AFK
136
Abundance of 'other' zooplankton (no./m3)
0 - 500
500 - 1000
1000 - Over

Figure 12(d)

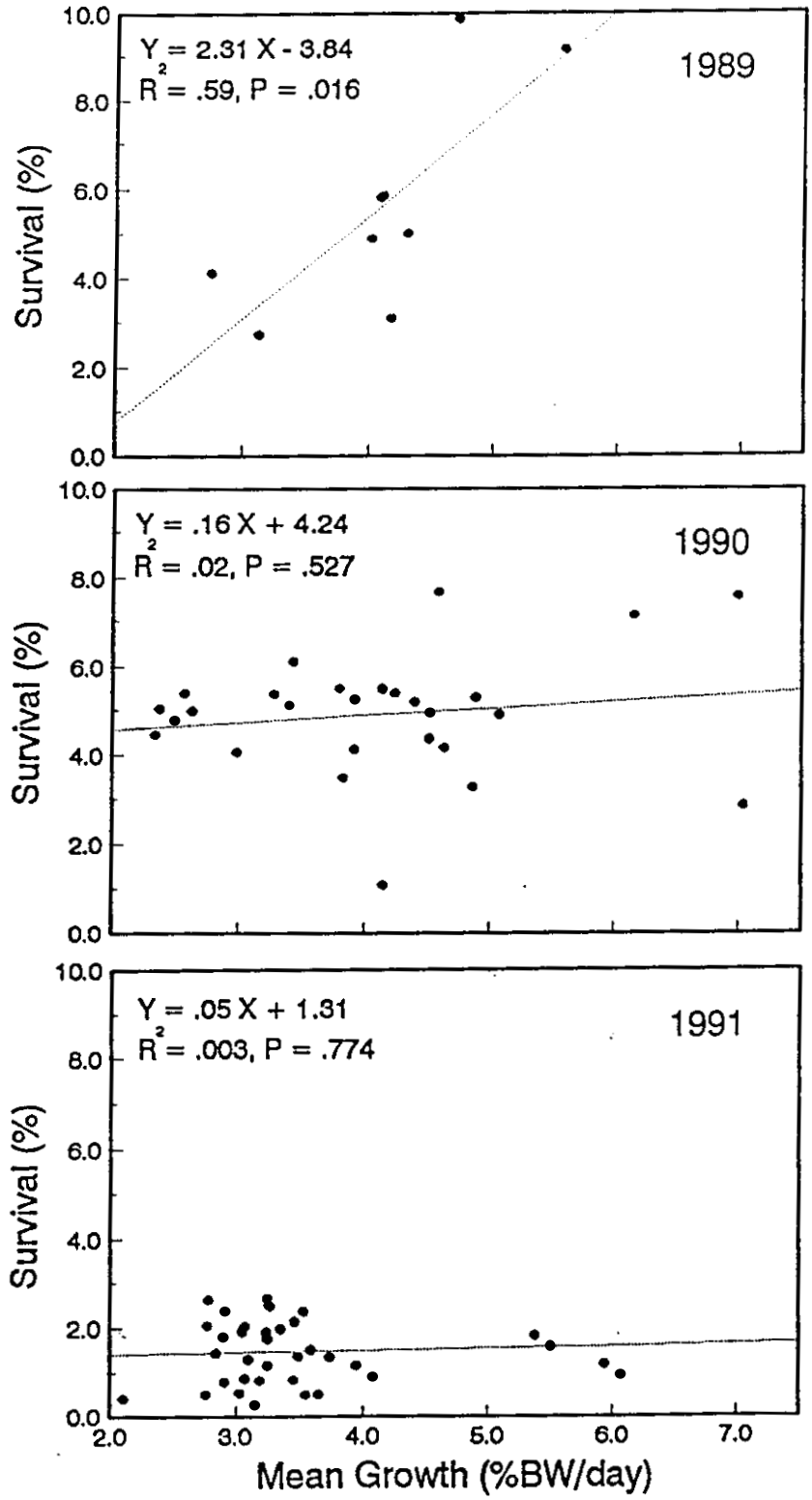


Figure 13: Relationship between fry-to-adult survival and mean growth of coded-wire tagged juvenile pink salmon released from hatcheries in Prince William Sound in (a) 1989, (b) 1990, (c) 1991.

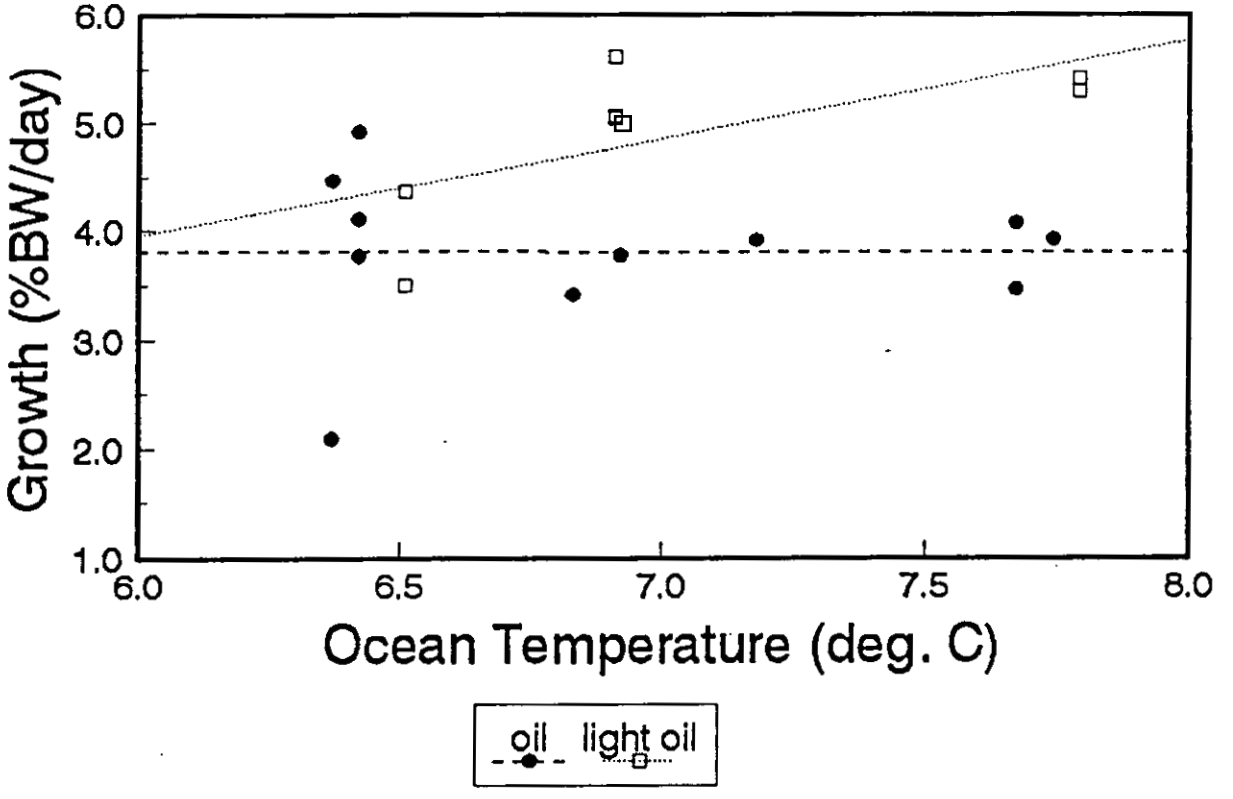


Figure 14: Relationship between mean growth of coded-wire tagged juvenile pink salmon in the early-fed group released from the Armin F. Koernig Hatchery and ocean temperatures in lightly-oiled and oiled areas of Prince William Sound, 1989.

APPENDIX I:

Summary of sample sizes obtained for P4501A analyses of juvenile pink salmon captured in Prince William Sound, 1989.

Table I-1: Summary of sampling locations, fish lengths, and sample sizes obtained for cytochrome P4501A analysis in 1989.

Date	Location	Target[1] Length (mm)	Actual[2] Length (mm)	Sample Size
WHN Hatchery: non-oiled				
May 18	Eaglek Island	48.5	41.8	7
June 8	Eaglek Island	57.3	58.8	7
June 8	Unakwik Inlet	50.0	51.3	6
June 24	Eaglek Island	73.5	69.5	6
June 24	Unakwik Inlet	60.0	60.8	6
July 1	Kiniklik	52.0	52.5	12
Total				44
WHN Hatchery: oiled				
May 19	Main Bay	41.7	40.3	7
May 19	Pt. Nowell	45.1	41.8	6
May 19	Falls Bay	43.1	40.1	6
May 31	Foul Bay	53.5	52.6	5
Total				24
AFK Hatchery: lightly oiled				
May 22	Little Bay	45.0	45.2	12
June 6	Little Bay	60.6	61.8	6
June 6	Mummy Bay	61.5	65.8	6
June 22	Little Bay	69.0	65.8	6
June 22	Squire Island	55.0	54.2	6
June 22	Squire Island	52.5	54.2	10
Total				46
AFK Hatchery: oiled				
May 21	Fox Farm Harbor	41.6	39.7	6
June 2	Fox Farm Harbor	40.0	38.5	6
June 5	Fox Farm Harbor	50.0	43.8	12
Total				24

[1] Mean length of coded-wire tagged fish captured at the site.
[2] Mean length of untagged fish selected for MFO analysis.

Table I-2: Summary of sample sizes obtained for cytochrome P4501A analyses by tissue type and sampling area. Asterisks indicate tissues in which at least ten samples were obtained from each sampling area.

Organ	Tissue	WHN Hatchery mod-oiled	WHN Hatchery non-oiled	AFK Hatchery mod-oiled	AFK Hatchery light-oiled	
Gill	pillar cells	16	27	15	40	*
	epithelium	16	27	15	39	*
	endothelium of gill arches	16	27	14	38	*
	gill buds	16	27	15	38	*
	Pharyngeal epithelium	15	25	15	38	*
Heart	atrial endothelium	3	15	7	19	
	ventricular endothelium	2	14	6	14	
	bulbus endothelium	1	7	0	2	
	aorta endothelium	1	1	0	1	
Liver	hepatocytes	13	23	11	29	*
	bile ducts	1	3	5	7	
	sinusoidal endothelium	12	22	11	29	*
	central veins	11	22	11	23	*
	portal veins	0	15	10	16	
	hepatic arteries	0	5	5	8	
Kidney	duct epithelium	11	23	14	27	*
	sinusoidal endothelium	15	24	12	27	*
	tubular epithelium	8	22	12	26	
	glomerular endothelium	7	22	12	26	
	vascular endothelium	7	22	12	27	
	pronephros epithelium	0	0	0	3	
Intestine	gastric epithelium	8	20	9	22	
	cecal epithelium	14	27	14	37	*
	ant. intestinal epithelium	8	11	3	11	
	post. intestinal epithelium	13	7	8	7	
	colonic epithelium	0	3	1	1	
	peripit endothelium	7	21	2	10	
Pancreas	bile duct	1	0	0	0	
	acinar cells	1	0	2	0	
	ductule cells	16	11	0	6	

APPENDIX II:

Summary of sample sizes obtained for coded-wire tagged juvenile pink salmon in three treatment groups released from three hatcheries in Prince William Sound, 1989-1991.

Table II-1: Sample sizes obtained for coded-wire tagged juvenile pink salmon in the 'early fed' group released from the Armin F. Koernig Hatchery, 1989-1991. See Figure 1 for a description of areas 1 through 6.

Year	Month	Area					
		1	2	3	4	5	6
1989	May				64	89	6
	June				50	24	
	July				15		
1990	May	1	7		67	12	
	June				157	2	
	July				25		
1991	May				17	43	
	June				255		

Table II-2: Sample sizes obtained for coded-wire tagged juvenile pink salmon in the 'early fed' group released from the Wally H. Noerenberg Hatchery, 1989-1991. See Figure 1 for a description of areas 1 through 6.

Year	Month	Area					
		1	2	3	4	5	6
1989	May	97	28	1	1	4	
	June	14	1		3	1	
1990	May	32	3		1		
	June	12	2		9	7	
	July				12		1
1991	May	41	15				
	June	75	103		2		

Table II-3: Sample sizes obtained for coded-wire tagged juvenile pink salmon in the 'early fed' group released from the Cannery Creek Hatchery, 1989-1991. See Figure 1 for a description of areas 1 through 6.

Year	Month	Area					
		1	2	3	4	5	6
1989	June	18					
1990	May	2					
	June	6	4		2	3	
	July				3		
1991	May	3					
	June	2	19				

Table II-4: Sample sizes obtained for coded-wire tagged juvenile pink salmon in the 'unfed' group released from the Armin F. Koernig Hatchery, 1989-1991. See Figure 1 for a description of areas 1 through 6.

Year	Month	Area					
		1	2	3	4	5	6
1989	May		1		8	1	
	June				32		
	July				9		
1990	May		2		16		
	June				66		
	July				13		
1991	May				8		
	June				45		

Table II-5: Sample sizes obtained for coded-wire tagged juvenile pink salmon in the 'unfed' group released from the Wally H. Noerenberg Hatchery, 1989-1991. See Figure 1 for a description of areas 1 through 6.

Year	Month	Area 1	2	3	4	5	6
1989	May	11	4				
	June	2			2	2	
1990	May	6					
	June	8	3				
1991	May	2	2				
	June	7	13				

Table II-6: Sample sizes obtained for coded-wire tagged juvenile pink salmon in the 'unfed' group released from the Cannery Creek Hatchery, 1989-1991. See Figure 1 for a description of areas 1 through 6.

Year	Month	Area 1	2	3	4	5	6
1989	June	13					
	July	2					
1990	May	1					
	June	2	3				
	July				1		

Table II-7: Sample sizes obtained for coded-wire tagged juvenile pink salmon in the 'late fed' group released from the Armin F. Koernig Hatchery, 1989-1991. See Figure 1 for a description of areas 1 through 6.

Year	Month	Area 1	2	3	4	5	6
1989	June		1		63	1	
	July				39		
1990	June				59		
	July				6		
1991	June				92		

Table II-8: Sample sizes obtained for coded-wire tagged juvenile pink salmon in the 'late fed' group released from the Wally H. Noerenberg Hatchery, 1989-1991. See Figure 1 for a description of areas 1 through 6.

Year	Month	Area					
		1	2	3	4	5	6
1989	June	2	3			2	
1990	June	3	1		2	3	
	July				2		
1991	May		2				
	June	11	9				

Table II-9: Sample sizes obtained for coded-wire tagged juvenile pink salmon in the 'late fed' group released from the Cannery Creek Hatchery, 1989-1991. See Figure 1 for a description of areas 1 through 6.

Year	Month	Area					
		1	2	3	4	5	6
1989	June	17					
	July	7					
1990	May						2
	June	17	28		3	28	
	July				5		
1991	June		4				

APPENDIX III:

Summary of coded-wire tagged juvenile salmon in the early-fed group released from the Armin F. Koernig and Wally H. Noerenberg hatcheries, 1989-1991.

Table III-1: Summary of coded-wire tagged juvenile salmon in the early-fed group released from the Armin F. Koernig (AFK) Hatchery in 1989 and recovered in the moderately-oiled area near the AFK Hatchery (area 4) and along the lightly-oiled southern coast of Knight Island (area 5).

Month	Area	Site	Sample Size (n)	Mean Growth (%BW day^{-1})
May	4	Aluklik Bay	4	4.11
	4	Fox Farm	15	3.77
	4	Panhat Point	10	4.47
	4	Prince of Wales Pass	4	4.92
	4	Squirrel Bay	31	2.09
	5	Little Bay	54	4.80
	5	Lucky Bay	19	5.08[1]
	5	Mummy Bay	12	4.37
June	4	Aluklik Bay	2	4.06
	4	Bettles Island	1	4.10[1]
	4	Fox Farm	41	3.72
	4	Panhat Point	2	4.32
	4	Prince of Wales Pass	3	3.95
	4	Squirrel Bay	1	1.58
	5	Little Bay	18	5.13
	5	Mummy Bay	5	5.40

[1]Sites not included in the split-plot analysis of variance because CWT juvenile salmon were not recovered in both May and June.

Table III-2: Summary of coded-wire tagged juvenile salmon in the early-fed group released from the Wally H. Noerenberg (WHN) Hatchery in 1989 and recovered in the non-oiled area near the WHN hatchery (area 1) and in the moderately-oiled area near Main Bay (area 2).

Month	Area	Site	Sample Size (n)	Mean Growth (%BW day^{-1})
May	1	Culross Island	86	4.72
	1	Eaglek Island	10	6.14
	2	Falls Bay	8	4.15
	2	Foul Bay	3	4.77
	2	Herring Bay	8	4.30
	2	Main Bay	2	3.85
	2	Point Nowell	7	4.03
June	1	Culross Island	12	5.02
	1	Eaglek Island	5	4.77
	2	Herring Bay	1	4.20

Table III-3: Summary of coded-wire tagged juvenile salmon in the early-fed group released from the Armin F. Koernig (AFK) Hatchery in 1990 and recovered in the moderately-oiled area near the AFK Hatchery (area 4) and along the lightly-oiled southern coast of Knight Island (area 5).

Month	Area	Site	Sample Size (n)	Mean Growth (%BW day^{-1})
May	4	Aluklik Bay	6	4.52
	4	Bettles Island	13	4.95
	4	Fox Farm	27	4.48
	4	Panhat Point	1	3.90
	4	Sleepy Bay	3	4.90
	4	Squirrel Bay	18	4.45
	5	Little Bay	12	5.32
June	4	Aluklik Bay	38	4.37
	4	Bettles Island	21	4.62
	4	Fox Farm	49	4.21
	4	Prince of Wales Pass	18	4.56
	4	Sleepy Bay	2	4.69
	4	Squirrel Bay	26	4.30
	4	Wilson Bay	3	4.08
	5	East Chenega Island	1	4.36
	5	Mummy Bay	1	4.72

Table III-4: Summary of coded-wire tagged juvenile salmon in the early-fed group released from the Wally H. Noerenberg (WHN) Hatchery in 1990 and recovered in the non-oiled area near the WHN hatchery (area 1) and in the moderately-oiled area near Main Bay (area 2).

Month	Area	Site	Sample Size (n)	Mean Growth (%BW day^{-1})
May	1	Culross Island	82	2.41
	1	Eaglek Island	7	5.22
	1	Hidden Bay	25	4.04
	2	Crafton Island	3	6.07
	2	Herring Bay	5	1.08
June	1	Culross Island	5	4.16
	1	Eaglek Island	4	3.64
	1	Hidden Bay	6	4.13
	2	Herring Bay	4	4.29

Table III-5: Summary of coded-wire tagged juvenile salmon in the early-fed group released from the Armin F. Koernig (AFK) Hatchery in 1991 and recovered in the moderately-oiled area near the AFK Hatchery (area 4) and along the lightly-oiled southern coast of Knight Island (area 5).

Month	Area	Site	Sample Size (n)	Mean Growth (%BW day^{-1})
May	4	Fox Farm	17	2.23
	5	Little Bay	43	3.38
June	4	Bettles Island	99	3.47
	4	Fox Farm	101	2.89
	4	Shelter Bay	2	3.28
	4	Squirrel Bay	53	2.98

Table III-6: Summary of coded-wire tagged juvenile salmon in the early-fed group released from the Wally H. Noerenberg (WHN) Hatchery in 1991 and recovered in the non-oiled area near the WHN hatchery (area 1) and in the moderately-oiled area near Main Bay (area 2).

Month	Area	Site	Sample Size (n)	Mean Growth (%BW day^{-1})
May	1	Culross Island	41	3.42
	2	Crafton Island	15	3.61
June	1	Esther Bay	1	4.09
	1	Hidden Bay	41	3.19
	1	Perry Island	33	3.67
	2	Crafton Island	94	3.56
	2	Herring Bay	9	3.41