Exxon Valdez Oil Spill
Restoration Project Annual Report

Pristane Monitoring in Mussels and Predators of Juvenile Pink Salmon and Herring

Restoration Project 98195
Annual Report

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

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May 1998

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Study History: This project was initiated in FY96. This is the third annual report for the project. A similar demonstration project was conducted in FY94 and FY95 under Auke Bay Laboratory sponsorship which provided comparable data for those years.

Abstract: Pristane concentrations in mussels were monitored at 30 stations during spring and summer to compare nearshore feeding conditions for juvenile pink salmon and herring. The dominant-biomass zooplankter of PWS (*Neocalanus plumchrus*) contains about 1% pristane, a refractory hydrocarbon. Fecal material produced consequent to predation on *Neocalanus* contains un-absorbed pristane that may be accumulated by mussels, so pristane concentrations in mussels indicate the intensity of local predation. Comparison of pristane in mussels collected in 1994 through 1998 indicates increasingly favorable feeding conditions for these near-shore forage fish in PWS as a whole.

Key Words: *Exxon Valdez*, pristane, *Neocalanus spp.*, mussels, pink salmon, herring, Prince William Sound.

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

Citation: Short, J. and P. Harris. 1999. Pristane monitoring in mussels and predators of juvenile pink salmon and herring, *Exxon Valdez* Oil Spill Restoration Project Annual Report (Restoration Project 98195), Auke Bay Laboratory, National Marine Fisheries Service, National Oceanic and Atmospheric Administration, Juneau, Alaska.

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Executive Summary

The purpose of this project is to assess marine feeding conditions during juvenile life stages of pink salmon and herring in Prince William Sound (PWS). In spring, the principal prey of these juveniles is the copepod *Neocalanus plumchrus*, and annual copepod abundances may vary considerably. Predators of these juvenile fish (such as adult pollock) may also prey on the copepods, and may possibly select copepods during years of high abundance. Variability of these feeding conditions may therefore modulate recruitment of these commercially exploited fishes, by *e.g.* alleviating predation pressure during years when conditions are favorable. This project indirectly assesses energy conversion from *Neocalanus* copepods to nearshore, juvenile fish during spring, by monitoring a surrogate measure of fish fecal production in mussels.

Copepods in the genera *Calanus* and *Neocalanus* are apparently unique in their ability to biosynthesize a hydrocarbon called pristane. Pristane is derived from chlorophyll ingested by the copepods, and concentrations of pristane approach 1% in these animals. As a terminally-branched alkane, pristane dissolves into lipids and resists catabolic degradation, making it a

tracer molecule for the lipids produced by these copepods. When these copepods are ingested by fish, some of the pristane is excreted in fecal material as a result of incomplete lipid absorption in the intestine. The fecal material may disperse in the water column, and then be accumulated by mussels as they filter seawater for food. Thus, pristane accumulation by mussels may indirectly indicate the extent of predation on *Calanus* and *Neocalanus* by nearby juvenile fish, with high pristane concentrations in mussels during spring indicating simultaneously high abundances of copepods and fish.

Pristane concentrations in mussels have been shown to increase by orders of magnitude during spring in PWS. The sharpest increases occur in early May, about 2 weeks following the peak of the copepod bloom. This project evaluates whether systematic monitoring of these concentration changes in mussels at fixed stations throughout PWS may be related to early marine survival and subsequent recruitment of pink salmon and herring. Mussels were collected periodically from 30 stations (table1, figure 1) and analyzed for pristane to document seasonal concentration changes. In the laboratory, pristane is extracted from mussels with pentane and then isolated and measured by flame ionization gas-chromatography.

A series of field and laboratory studies were conducted in 1998 to elucidate the ecological pathway traversed by pristane from copepods to mussels in PWS. These experiments were sponsored by the Auke Bay Laboratory (ABL) as part of the agency contribution to this project. The field studies involved monitoring the effects of a mass-release of juvenile pink salmon from the Prince William Sound Aquaculture Corporation (PWSAC) Noerenberg hatchery on Esther Island on pristane concentrations in mussels and in ambient seawater. Zooplankton abundances were extremely high during May 1998 in Lake Bay where this hatchery is located (figure 2), and most of the juveniles remained within the Bay foraging for over a week following release. This contrasted with pink salmon behavior following a similar release in 1996 when zooplankton abundances within Lake Bay were much lower, and the released fish vacated the bay within 24 h in search of adequate forage. These differences were reflected by patterns of pristane accumulation by mussels between these two years. Pristane concentrations in mussels were greatest and appeared first at the station nearest the hatchery in both years, but in 1998, the increases at the two more distant stations was delayed about 2 days compared with 1996 (figure 3).

Comparison of daily seawater measurements before and after the pink salmon release confirmed that high population densities of *Neocalanus spp.* alone does not lead to detectable pristane concentrations, eliminating dissolution from zooplankton or zooplankton fecal pellets as alternative pathways of pristane incorporation by mussels.

Comparison of daily zooplankton abundances in early May near the Noerenberg hatchery showed that *N. plumchrus* was the overwhelmingly dominant zooplankter available in the surface layer, and that this availability responded strongly to local wind direction and strength. PWS received a succession of storms during April and May 1998, with little intervening calm weather. When sustained winds blew from the east, zooplankton densities were extremely high in Lake Bay and in Wells Passage just outside the Noerenberg hatchery, far exceeding previous records. When winds blew from the west, these densities declined sharply.

The laboratory studies demonstrated that fecal material produced by juvenile pink salmon feeding on the zooplankton available during May in PWS is a potent source of pristane for mussels. Pristane concentrations increased dramatically within a day in mussels exposed to whole feces. These studies showed that the bioconcentration factor for pristane is about $2x10^6$, confirming the ability of mussels to accumulate pristane from concentrations that are below detection limits of most methods of direct chemical analysis. Also, these studies showed that pristane accumulated by mussels from fecal material is retained substantially longer than when dissolved pristane is absorbed. These results indicate that mussels respond quickly to changes in pristane availability in seawater and retain the accumulation signal for weeks, so that stations where little seasonal increases of pristane in mussels are found reflect little zooplankton forage availability or forage activity by nearshore zooplanktivores, which characterizes the eastern half of PWS in 1998 as in previous years.

Other laboratory studies at ABL evaluated the dietary quality of the natural zooplankton forage assemblage to support pink salmon growth. Somewhat surprisingly, this diet was found to be relatively poor. Gross growth efficiency was less than 10%, so juvenile pink salmon must consume quite large daily rations of the natural forage to sustain adequate growth, and the efficiency of lipid utilization is very low. Hence, relatively high concentrations of lipid (and associated pristane) are excreted with the feces produced from this diet. Juvenile pink salmon are consequently especially effective at dispersing pristane into seawater, because they must consume large daily rations of *N. plumchrus*, and excrete most of the lipid in their feces.

Preliminary results from the monitoring component of this project indicate that 1998 was another year of high zooplankton forage abundance for pink salmon and other zooplanktivores in western PWS (figures 4 and 5). Consequently, returns in the 1997 brood year of pink salmon as adults in fall, 1999 are forecast to be high, comparable with adult returns in 1998. Pristane concentrations in mussels remained generally low in the eastern part of PWS, indicating poor forage conditions for zooplanktivores there.

Introduction

Determination of the causes of the dramatic declines in populations of pink salmon and herring following the *Exxon Valdez* oil spill requires an assessment of the natural factors that affect recruitment of these species, because any toxic effects of the spill may otherwise be confounded with these natural factors. In addition, these natural factors impose constraints on the recovery potential of these species. Pink salmon and herring are identified as species that have not recovered. If the recent population declines of these two species are the result of changes in the basic ecology of PWS due to natural phenomena (e.g. El Niño), then recovery of these populations to pre-spill levels may not be possible, and the criteria for recovery must recognize these changes.

This project provides evidence that may be used to evaluate the recovery of pink salmon and herring. Annual monitoring of pristane concentrations in mussels throughout PWS provides an indication of pink salmon survival through the juvenile life stages, the period that primarily

determines year class strength.

Pristane is a hydrocarbon biosynthesized from chlorophyll by herbivorous copepods in the genera *Calanus* and *Neocalanus.* These copepods are the only proven modern marine source of pristane (Avigan & Blumer 1968) (it also occurs in petroleum), and they typically contain concentrations that approach 1% dry weight (i.e. 10,000,000 ppb). As a branched alkane, pristane is highly lipophilic and resistant to metabolic degradation, which suggests that it may be a useful "tracer" molecule that would quantitatively label fats in predators of these copepods (Blumer *et al.,* 1964). The low detection limit (about 100 ppb) of the inexpensive analytical method further suggests the utility of pristane as a natural indicator of energy flow from these copepods to higher trophic level predators.

Calanus and *Neocalanus* copepods are marine zooplankters about 3 - 8 mm in length, and are the dominant marine herbivores in Prince William Sound (PWS) during the spring phytoplankton bloom. They are consequently important prey of many predator species. Important direct predators of *Calanus* and *Neocalanus* copepods identified in PWS include storm petrels, herring, and juvenile pink salmon. In addition, pristane concentrations that range to 50,000 ppb (dry weight) are evident in filter feeding organisms such as mussels and some clams during spring. Experiments repeated at the Auke Bay Laboratory (ABL) and in the field in 1996 and in 1998 demonstrate that the route of pristane accumulation in these filter feeders is through ingestion of fecal material derived from predators of *Calanus* and *Neocalanus*, especially juvenile pink salmon. Pristane concentrations in PWS mussels may therefore reflect the timing and simultaneous abundance of *Calanus* and *Neocalanus* and their predators in seawater adjacent to sampled mussels.

A regular monitoring program for pristane in mussels during spring could provide a quantitative basis for comparing inter-annual energy flow through *Calanus* and *Neocalanus* to pink salmon. This may provide a relatively inexpensive indicator of survival through the early juvenile stages for these species. The monitoring program may also identify locations where this flow is consistently high, i.e. critical marine habitats. These approaches may clarify some of the important natural factors that affect recruitment of juvenile salmon, which is necessary for evaluating recovery of this species.

Objectives

This project has 2 objectives given in the detailed project description:

1. Measure pristane concentrations in mussels collected biweekly during spring from 30 stations in Prince William Sound to evaluate inter-annual variability of energy conversion from *Neocalanus* copepods to their nearshore predators.

2. Determine the existence and location of regions inside Prince William Sound where the energy conversion of objective 2 above is consistently above average, and synthesize these data over time and geographic location each succeeding project year.

This project was substantially augmented by agency-sponsored research in 1998. This research focussed on elucidating the transport pathway of pristane from copepods to mussels through a series of field and laboratory studies. Preliminary results of these studies are presented below as an aid to interpreting the monitoring data.

Methods

Mussel Collection

The seasonal variability of pristane concentrations in mussels (*Mytilus trossulus*) is based on collections from 30 stations in PWS (figure 1, table 1). Mussels are collected monthly beginning early February through late August, with additional biweekly samples collected in April and May for a total of 10 collection periods and 300 mussel samples. The higher collection frequency is to more accurately establish the onset of the initial rise of pristane concentrations in the mussels, which is correlated with the zooplankton bloom and may vary from year to year. Collected mussels are stored frozen and analyzed for whole-body pristane concentration.

Of the 30 stations monitored, 26 are sampled by ABL staff by small float-plane based out of Cordova. Another 4 stations are located near Prince William Sound Aquaculture Corporation (PWSAC) hatcheries, and are sampled by volunteer PWSAC staff. Additional stations are sampled by volunteer primary and secondary school students in collaboration with the PWS Youth Area Watch program (project 98210).

Ten mussels are collected from selected mussel beds and placed into a plastic bag together with collection documentation (i.e. date, time, location, collector). Selected mussels are usually in the length range 20 - 45 mm. Mussels are collected along a transect parallel with the shoreline; 1 mussel is collected every consecutive meter. Previous results archived in the *Exxon Valdez* Oil Spill of 1989: State/Federal Trustee Council Hydrocarbon Database 1989 - 1995 (EVTHD) indicates that pristane concentrations in mussels collected in this way are representative of entire mussel beds.

Pristane Analysis

The chemical analysis of pristane involves pentane extraction of macerated tissues, lipid removal with silica gel, and separation and measurement of pristane by gas chromatography equipped with a flame ionization detector. Pristane concentrations are determined by the internal standard method, with deuterated hexadecane added to the pentane initially as the internal standard. Pristane identification is based on retention time relative to the internal standard. Quality control samples include method blanks, spiked method blanks, and reference sample analyzed with each batch of 20 samples to verify method accuracy, precision, and absence of laboratory introduced artifacts and interferences. Recovery of the internal standard is determined by adding a second internal standard prior to instrumental analysis. Method detection limits are assessed annually for the mussel tissue matrix, and these detection limits are assumed for the

other matrixes analyzed. Based on previous performance, we anticipate accuracy of ± 15% of National Institute of Science and Technology (NIST)-certified values for the spiked blank and reference samples, precision of 95% of reference samples within ± 15% of sample means, and laboratory artifacts below detection limits more than 99% of the time. This level of analytical performance will insure that variability due to sample analysis is negligible compared with variability among replicate mussel samples.

Percent moisture is determined in samples so that results may be analyzed on dry weight weight basis. Dry weights will be determined by heating samples at 60 C to constant final weight.

ABL-Sponsored Experiments

Field Component:

The field studies examined the effects of juvenile pink salmon released *en mass* from a hatchery on nearby plankton densities and on pristane concentrations in seawater and mussels. Seventy million salmon were released the evening of May 1, 1998 from the PWSAC Noerenberg Hatchery on Esther Island. The average wet weight of the released fish was 0.485g. Three monitoring stations were occupied daily beginning 5 days prior to the release through 7 days following except when prevented by weather, complemented by occasional samples collected from Lake Bay in front of the hatchery. These 3 stations are located along shorelines within 5 km of the hatchery (figure 2). Daily sampling at each station included duplicate vertical plankton tows, and samples of mussels and seawater. The plankton samples were collected from 30 m hauls of 0.5 mm mesh, 0.25 m^2 plankton net for population density and species composition determination, within 100 m of shorelines where mussel samples were collected. Mussels were collected for pristane analysis to determine the temporal response to the salmon release. Four-liter seawater samples were collected and filtered through a 1.5 μ glass fiber filter, and the filters and filtrate analyzed separately to determine particulate and dissolved pristane in the ambient seawater.

Laboratory Component:

Two experiments measuring pristane uptake and depuration by mussels were conducted at ABL during summer 1998. These experiments were designed to assess the dynamics and time-scales of pristane uptake and depuration, to help interpret results from pristane measurements in mussels collected from PWS. The first experiment examined accumulation of pristane dissolved in seawater by mussels, and subsequent depuration. Mussels were exposed to 0.5 ppb pristane for 14 d, followed by sampling over a 30 d depuration period. The second experiment examined accumulation of pristane associated with feces produced by juvenile pink salmon feeding on *Neocalanus* copepods. Four experimental conditions were employed, where mussels were exposed to seawater containing: (1) whole feces from *Neocalanus*-fed pink salmon juveniles, (2) homogenized feces from same, (3) whole feces from *Artemia*-fed pink salmon juveniles, and (4) nothing (control treatment). The *Artemia*-fed treatment served as a "feeding-level" control. The same exposure and depuration periods were used as for the dissolved pristane

experiment.

The pristane-laden feces used for the second uptake and depuration experiment were produced by feeding juvenile pink salmon zooplankton collected during the May 1998 field study at Esther Island. Zooplankton were collected in a 0.5 mm mesh, 0.25 m^2 plankton net towed horizontally, filtered on a 0.5 mm mesh screen, and frozen in ice-cube trays. The wet weight biomass of these cubes was consistently more than 95% late-stage *Neocalanus*, and mostly *N. plumchrus* stage IV-V. The frozen zooplankton cubes were allowed to dissolve in tanks containing juvenile pink salmon twice daily, and provided the sole source of food for these fish. Fish were fed for 1-h and then transferred to defecation tanks for 11-h, and the feces produced were collected, with two feeding cycles each day. The *Artemia*-fed fish were treated similarly, except they were fed *Artemia*. The amount of food consumed and the weight gained by each treatment group of fish was recorded to evaluate gross growth efficiencies supported by the two diets.

Results

Pristane Concentrations in Mussels at Regular Monitoring Stations

The large number of additional samples collected for the field and laboratory components of the ABL-sponsored experiments has resulted in a temporary delay in sample and data processing. These additional samples have more than doubled the analytical load. All of the samples are now analyzed, and data entry into the database is nearly complete. However, the data analysis has only recently begun. Hence, the results reported here are preliminary and incomplete.

The general geographic distribution of stations where high pristane concentrations were found in mussels is similar to that of previous years. Pristane concentrations above 10,000 ppb (dry weight) were evident at several stations in western PWS during May (figures 4 and 5). All of these stations were either bordering or down-current of the deep marine depression system of the northwestern sound. Except at Windy Bay, concentrations at stations in the eastern part of PWS remained low, usually below 1,000 ng/g.

Pristane concentrations increased at the western stations the middle of April, and were greatest at the end of May, generally declining thereafter through late August. This temporal pattern is also similar to that of previous years. The very high concentrations found during May in western PWS suggest that feeding conditions during the initial marine residence of juvenile salmonids in the sound continue to be very favorable.

ABL-Sponsored Experiments

Field Component:

Neocalanus plumchrus was extremely abundant in the vertical zooplankton tows. This species usually accounted for more than 99% of the zooplankton biomass collected, and densities of this species usually ranged from 0.25 to 2 individuals per liter at the 3 sampling stations occupied (figure 2). Densities near the low end of this range were found in plankton watch samples collected by the Noerenberg hatchery from Lake Bay, and are the highest densities ever recorded there, exceeding previous records by a factor of more than 3. The fluctuations in densities at the 3 sampling stations appeared to vary in response to prevailing 24-h wind direction. Winds blowing from the east for a day or more were associated with the highest zooplankton densities observed, and conversely for similar winds blowing from the west. These trends were observed concurrently in the duplicate samples at all 3 stations.

Zooplankton densities did not vary much in response to release of the juvenile pink salmon from the hatchery. Densities of *N. plumchrus* exceeded 0.25 individuals per liter in Lake Bay a week following the release, with no declining trend evident either in Lake Bay or at the 3 sampling stations just outside the Bay.

The juvenile pink salmon showed little evidence of migration from Lake Bay during the first week following the release. Schools of released juveniles were only occasionally observed at the sampling sites outside the Bay during the week following release. However, pink salmon fecal casts characteristic of feeding on *N. plumchrus* appeared in the seasurface layer in Lake Bay on the third day following the release. By the sixth day, these casts were so abundant they attracted several hundred seabirds, mostly gulls, kittiwakes and terns, that foraged on the casts by pecking at the water surface while swimming about. Predation on *N. plumchrus* by the released fish was confirmed by gut-content analysis of individuals captured within two days of the release, which contained an average of 20 *N. plumchrus* per stomach, and constituted >99% of the stomach content biomass.

Dissolved and particulate pristane concentrations in seawater were usually near or below the method detection limit of 25 ng/L (parts per trillion) at all the stations sampled. However, dissolved pristane increased to about 500 ng/L in Lake Bay a week after the release.

Pristane concentrations in mussels at the 3 sampling stations increased substantially following the hatchery salmon release. The increase at the station nearest the hatchery was concurrent with the release, but increases at the other two stations were delayed a week (figure 3). Concentrations at all three stations more than doubled after the release, and exceeded 10,000 ng/g at the two stations nearest the hatchery.

Laboratory Component:

Pristane concentrations in mussels increased substantially within a day of exposure to dissolved pristane. When exposed to 500 ng/L dissolved pristane, concentrations increased from about 100 ng/g initially to about 3,000 ng/g after 2 d of continuous exposure. At the end of the 14 d exposure period, concentrations exceeded 10,000 ng/g, indicating a bioconcentration factor of about 2×10^6 (assuming a wet:dry weight ratio of 10). Concentrations declined by a factor of

10 two weeks after transfer to clean seawater, and declined by another factor of 2 the following two weeks.

Mussels exposed to feces produced by juvenile pink salmon feeding on the zooplankton assemblage collected near the Noerenberg hatchery accumulated extremely high concentrations of pristane. The mussels that were exposed to whole and to homogenized feces contained about 175,000 ng/g and 375,000 ng/g, respectively, at the end of the 14 d exposure period. These accumulations depurated more slowly than when accumulated from dissolved pristane. At the end of the four week depuration period, pristane concentrations in mussels declined by factors of about 8 and 5 for the mussels exposed to whole and homogenized feces.

Estimates of gross growth efficiencies of the juvenile pink salmon used to produce feces in these experiments indicated that *Artemia* is more nutritious than the natural zooplankton assemblage collected from PWS. When calculated on a dry weight basis, as the ratio of weight gained to food consumed, these fish gained more than twice as much weight from unit consumption of *Artemia* compared with the natural zooplankton assemblage.

Discussion

The behavior of juvenile pink salmon released *en mass* from the Noerenberg hatchery in 1998 contrasted sharply with a similar release in 1996. In 1996, a comparable biomass (130 million juvenile pink salmon at a mean weight of 0.28 g) was released *en mass* one evening in early May. Population densities of *N. plumchrus* were lower by a factor of about 10 in Lake Bay compared with 1998. The released fish depleted the resident *N. plumchrus* within Lake Bay the night following the release, and the following day could be observed migrating as a continuous band along the eastward and westward shores out of the Bay. Lake Bay was substantially vacated by the released juveniles within a week following release, and had dispersed to several tens of km from the hatchery. In contrast, fish released in 1998 mostly remained within the Bay to feed on the abundant *N. plumchrus* present that year. These observations are consistent with the expectation that juvenile pink salmon will actively migrate in search of adequate forage following the onset of free marine residence, and will cease migration when adequate forage is encountered.

Juvenile pink salmon appear to be very dependent on high densities of *N. plumchrus* in PWS to support growth. In the laboratory experiments, juveniles that consumed in excess of 6% body weight per day of the identical plankton assemblage available to them in PWS barely grew at all, suggesting that feeding rates as high as 20% - 30% of body weight per day may be necessary to support adequate growth. This would require nearly continuous feeding in the field, because a 0.4 g pink salmon consuming 0.08 g of *N. plumchrus* weighing about 3 mg each would need to capture about 1 per hour on average. Note that during the weeks following free marine residence in PWS, there is little else but *N. plumchrus* available as forage in the planktonic spectrum.

Although rich in lipids, *Neocalanus spp.* may not be rich in other factors such as protein necessary for growth, leading to inefficient lipid utilization. On a dry weight basis, *Neocalanus*

spp. contain about 10 times as much lipid as *Artemia* per unit mass, but less than half the protein. Lipid droplets are visible fecal casts of the released pink salmon under low magnification, and their consumption by seabirds corroborates them as a source of available calories. Consequently, much of the pristane present in the *Neocalanus spp.* consumed by these juvenile pink salmon is excreted.

The field and laboratory results confirm pink salmon as a major vector for pristane incorporation by mussels. Juveniles must consume large bodily proportions of *N. plumchrus* because that is the only forage available, and these are so inefficiently utilized that much of the pristane in these copepods is excreted as feces. The laboratory uptake and depuration experiments confirm that feces produced in this way are potent sources of pristane for mussels, and uptake of pristane by mussels in the field following the hatchery releases are consistent with the dynamics of pristane accumulation observed in the laboratory experiments.

The timing of the pristane increases in mussels at the Esther Island stations reflects the migration behavior of the released pink salmon. After the pink salmon juveniles were released in 1996 and 1998, increased pristane concentrations in mussels were greatest and appeared first at the Brass Beach station nearest the hatchery (figure 3). In 1998, the increases at the two more distant stations was delayed about 2 days compared with 1996. In 1996, the released juveniles immediately vacated the inner harbor and commenced foraging on the more abundant *N. plumchrus* populations available just outside Lake Bay in Wells Passage. The laboratory uptake experiments confirm that mussels can accumulate substantial burdens of pristane when exposed to increased ambient concentrations within about a day. These field observations therefore suggest that high pristane concentrations in mussels reflect process that are quite local, involving intense nearshore predation on high abundances of *Neocalanus spp.*

The extreme nearshore habitat preference of juvenile pink salmon, combined with continuous predation on *N. plumchrus* (if only for lack of alternative prey), suggests that they are the dominant vector for introduction of pristane into mussels. During early marine residence in April and May, juvenile pink salmon have a greater preference for the nearshore habitat (i.e. within 50 m of the shoreline) than any other zooplanktivorous forage fish in the marine ecosystem of PWS. The mere presence of *N. plumchrus* in the water column is not sufficient to account for pristane concentrations observed at the monitoring stations, because mussel concentrations did not respond to the extremely high densities of *N. plumchrus* at the temporary monitoring stations on Esther Island prior to the hatchery release. Other forage fish that prey on *N. plumchrus* in spring may also be vectors, but significant contributions from them to the mussel signal require formation of dense schools nearshore. Pristane-laden fecal material produced by fish more that 50 m from shore probably contributes little to mussels, because of fecal casts lost through sinking or dispersion. The concurrent high nearshore densities of *N. plumchrus* and juvenile pink salmon following the hatchery releases led to pristane concentrations in mussels in the range 10,000 to 20,000 ng/g in 1996 and in 1998, which are near the highest values observed in mussels at the regular monitoring stations elsewhere in PWS. If these high values elsewhere do not result from juvenile pink salmon, then the alternate fish species involved must prey on *N. plumchrus* at comparable rates, and form predominantly nearshore schools of comparable density.

The response of *N. plumchrus* abundance to microscale winds, together with migration propensity of juvenile pink salmon to locate prey, may explain the rather high interannual variability of pristane accumulation by mussels at the regular monitoring stations. High concentrations of pristane in mussels is found consistently from one year to the next at relatively few of the stations routinely monitored. It seems clear that the source of the springtime populations of *N. plumchrus* in PWS is the deep marine depression of the western sound, which provides overwintering habitat for diapausing adults. If formation of nearshore plankton patches of sufficient density to attract juvenile pink salmon depends on microscale winds, then very high interannual geographic variability would result. Hence, the existing monitoring network of 30 stations is probably necessary to cope with such high geographic variability interannually, especially for construction of a robust summary index such as the Pristane Accumulation Index (PAI) used in previous years to compare the sound as a whole interannually.

Continued sampling of this network of stations in future years, at least during the zooplankton bloom in May, will likely provide a much more robust basis for evaluating large-scale changes in zooplankton production within PWS. In particular, concurrent absence of the springtime increase of pristane in mussels throughout PWS would be compelling evidence of an incipient year-class failure for salmonids, given the geographic coverage of the monitoring station network and the retention of pristane by mussels for weeks following exposure. Other approaches, such as directly monitoring zooplankton abundances with vertical plankton-net tows at the same number of stations, would provide only "snapshot" data and would likely be much more expensive to acquire.

Although the data analysis is not yet complete to calculate the PAI for 1998, the high concentrations of pristane observed at numerous stations by the end of May suggest a continued high value for the PAI. This index integrates results across all the stations in PWS for the whole year for interannual comparison, and has increased continuously since 1994. These increases have been associated with high salmonid survival among hatchery and wild salmon in PWS during these years. The PAI has been used to predict high survivals within this project since 1996, and the preliminary projection for adult pink salmon returns in 1999 remains high.

The zooplankton forage base produced within PWS has been consistently substantial since the inception of this project, which has hindered evaluation of the utility of the PAI as a predictive tool. Until a really bad year arrives, with very low production of large *Neocalanus* copepods, substantiation of the utility of this project in the field will remain elusive, at least at the scale of the sound as a whole. Predictive utility at a finer spatial scale, such as differentiating marine survival of salmon released by PWSAC hatcheries, is considerably more tenuous because of the interaction of migratory propensities of the juveniles and unpredictable locations of plankton patches induced by currents and winds.

We cooperated with Youth Area Watch (project 98210) to involve 24 students students from 6 communities in the spill area in the pristane monitoring project. Students collected mussels in their howmetowns on the same schedule we sampled. We attended a teacher training session in Anchorage in February to explain the the science behind the project and sampling protocol to new teachers. Eleven Youth Area Watch students and six teachers visited Auke Bay

Lab in April to observe and participate in sample preparation and analysis and 6 students assisted on sampling trips in the sound later in the spring.

Conclusions

1. Combination of laboratory and field evidence suggests that juvenile pink salmon are the dominant vector mediating incorporation of pristane into mussels.

2. Juvenile pink salmon may be considerably more dependent on high abundances of *Neocalanus spp.* zooplankton than previously recognized, because of the low nutritional quality of this diet.

3. Abundances of *Neocalanus spp.* zooplankton and pristane concentrations in mussels were extremely high during May, 1998 in western PWS, forecasting high early marine survival rates for the 1997 brood year of pink salmon in western PWS as a whole. Returns of this brood year as adults in 1999 are forecast to be high, comparable with returns of adults in 1998. Pink salmon production in eastern PWS will remain poor.

4. The ability of this project to detect differences among marine survivals of pink salmon released from different hatcheries in PWS is more doubtful, owing to a large stochastic component in the geographic location of adequate zooplankton patches for forage.

5. The likelihood of this monitoring project to detect large, interannual sound-wide changes in zooplankton forage availability to juvenile pink salmon during the initial marine residence phase remains high, but this cannot be confirmed until a year of poor production arrives. Zooplankton production in western PWS has remained high by historical standards since the inception of this project.

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Table 1. Locations and abbreviations of mussel collection stations sampled for this project. The abbreviations are also used in figure 1.

Station Abbreviation	Station Name	Latitude Deg N	Min	Sec	Longitude Deg W	Min	Sec
AFKHA	AFK Hatchery	60	3	8	148	3	30
APPLI	Applegate Island	60	37	30	148	8	10
BLIGI	Bligh Island	60	52	2	146	44	59
CANNC	Cannery Creek Hatchery	60	59	39	147	32	19
CHENB	Chenega Bay	60	3	47	148	1	10
CHENI	Chenega Island	60	23	11	148	0	4
CONSH	Constantine Harbor	60	21	16	146	40	25
CPUGE	Cape Puget	59	57	35	148	28	48
DAYVI	Dayville	61	5	13	146	16	40
DECIP	Decision Point	60	48	21	148	28	35
DIVIP	Division Point	60	28	55	148	17	13
EKNII	East Knight Island	60	20	49	147	38	32
ESTHI	Esther Island (WN Hatchery)	60	47	7	148	3	30
FAIRI	Fairmont Island	60	52	51	147	26	17
FLEMI	Fleming Island	60	10	29	148	2	3
FOXFA1	Fox Farm 1	59	58	15	148	8	22
FOXFA2	Fox Farm 2	59	58	7	148	6	36
FOXFA3	Fox Farm 3	59	58	10	148	10	22
GREEI	Green Island	60	16	55	147	24	57
HANNB	Hanning Bay	59	57	12	147	42	56
HERRP	Herring Point	60	28	28	147	47	27
JOHNP	Johnstone Point	60	29	1	146	34	15
KENNC	Kenny Cove	60	25	24	146	7	23
MAINB	Main Bay	60	32	0	148	3	30
NAKEI	Naked Island	60	39	3	147	26	24
OLSEN	Olsen Bay	60	44	30	146	11	58
PATTB	Patton Bay	59	52	40	147	26	15
PELEA	Point Eleanor	60	34	33	147	33	49
PERRI	Perry Island	60	40	40	147	54	50
PPAKE	Point Pakenham	60	0	23	148	5	7
ROCKB	Rocky Bay	60	20	14	147	7	32
SNUGC	Snug Corner Cove	60	44	8	146	37	32
STORI	Storey Island	60	43	41	147	27	2
TATIT	Tatitlek	60	51	48	146	41	6
WHITT	Whittier	60	46	42	148	40	0
WINDB	Windy Bay	60	34	22	148	57	29

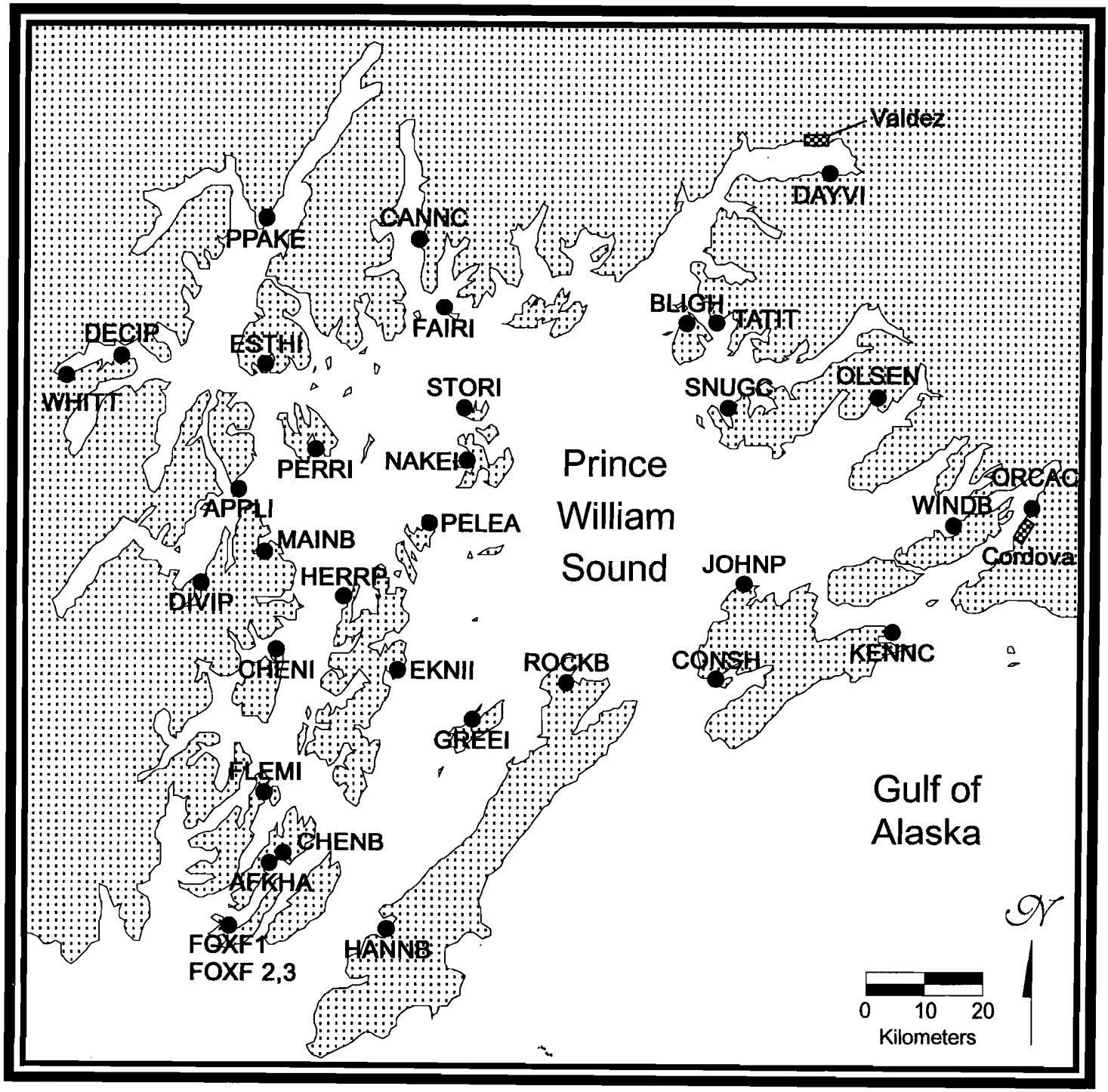

Figure 1. Mussel collection stations in PWS. Abbreviations are defined in table 1, where latitudes and longitudes are presented.

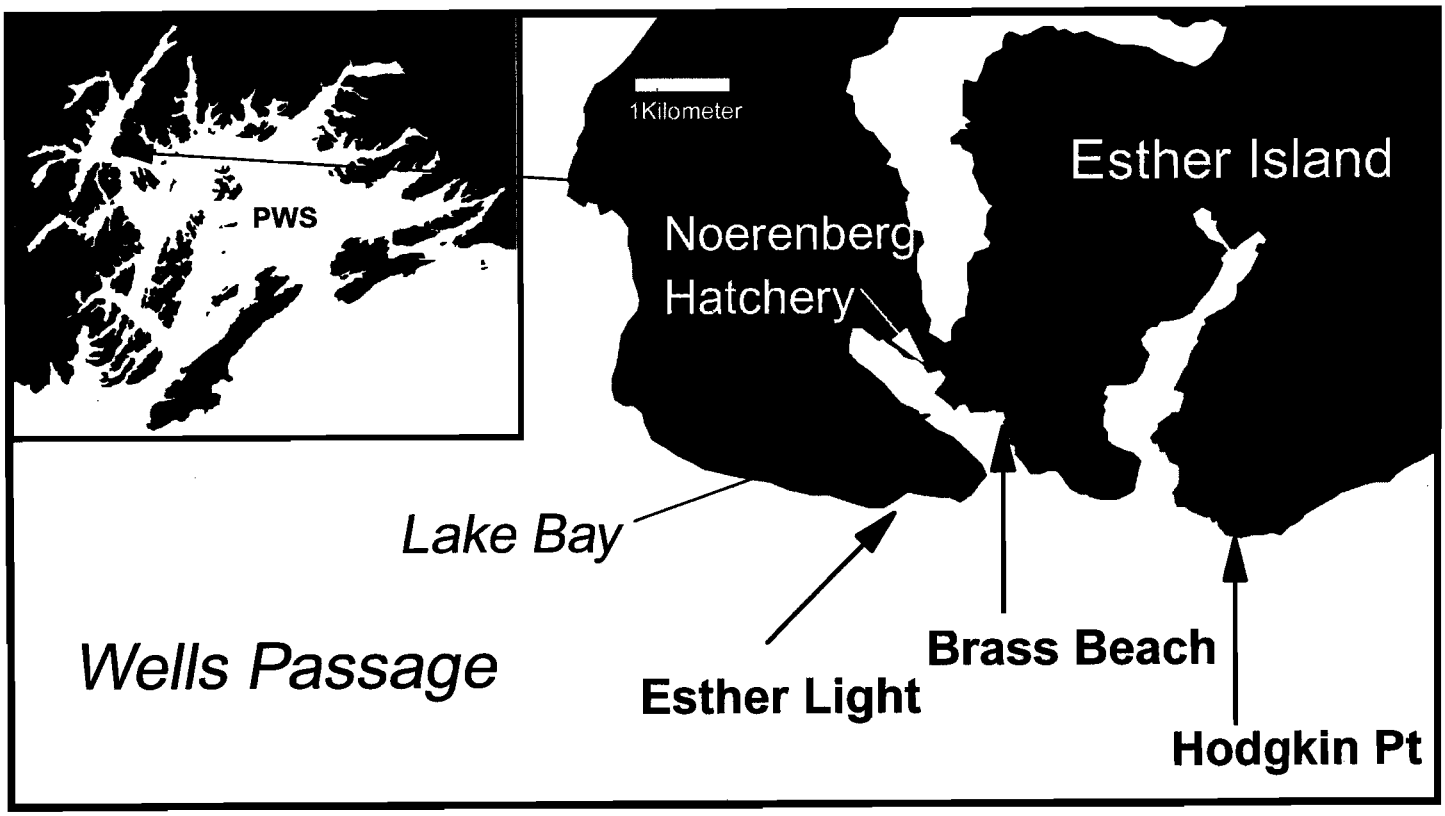

Figure 2. Temporary monitoring stations occupied to evaluate effects of the mass-release of juvenile pink salmon from the PWSAC Noerenberg hatchery at Lake Bay on Esther island.

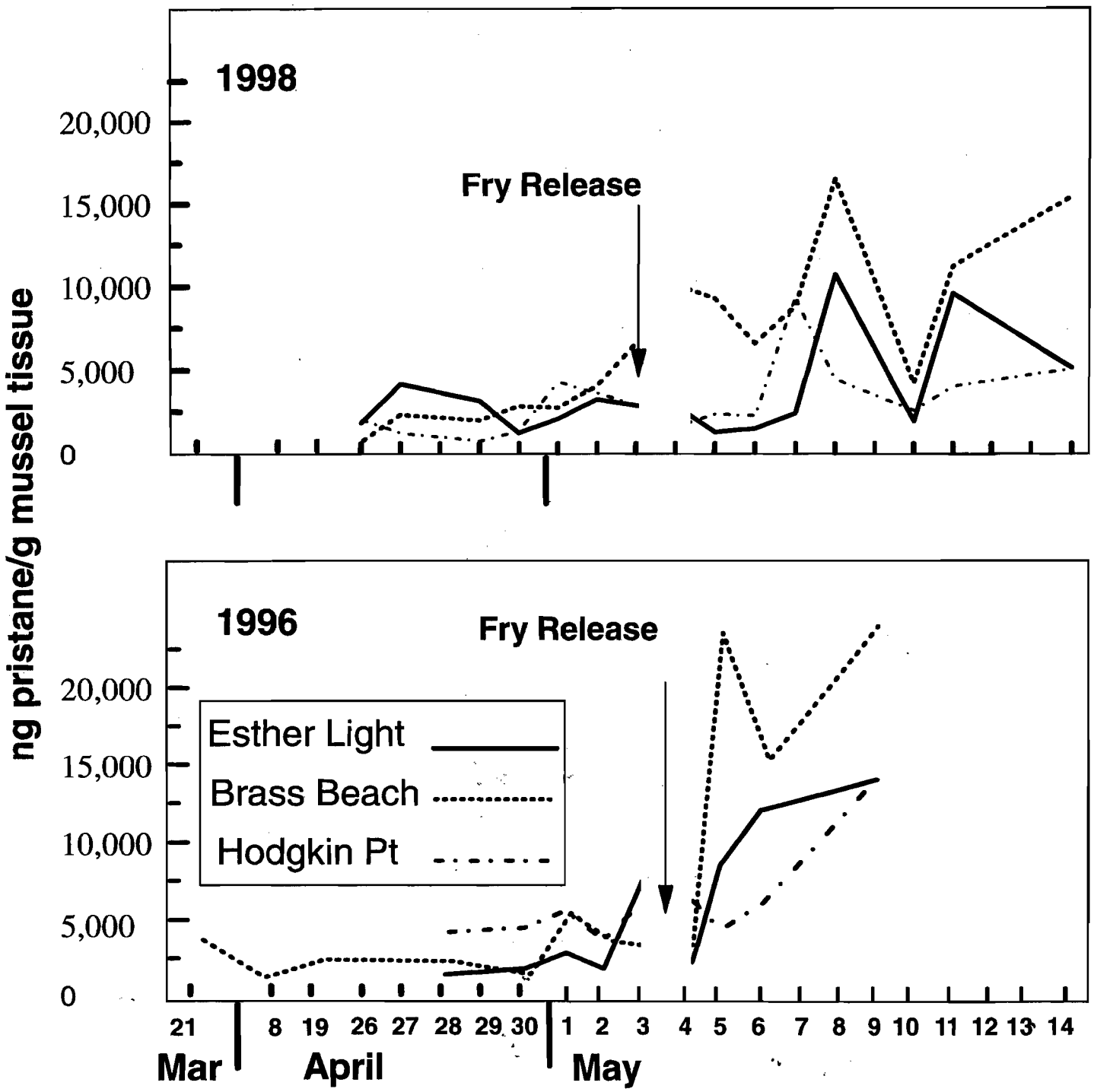

Figure 3. Pristane concentrations measured in mussels at three stations near the Noerenberg hatchery at Lake Bay on Esther island before and after mass-releases of juvenile pink salmon in 1996 and in 1998. See figure 2 for hatchery and sampling station locations.

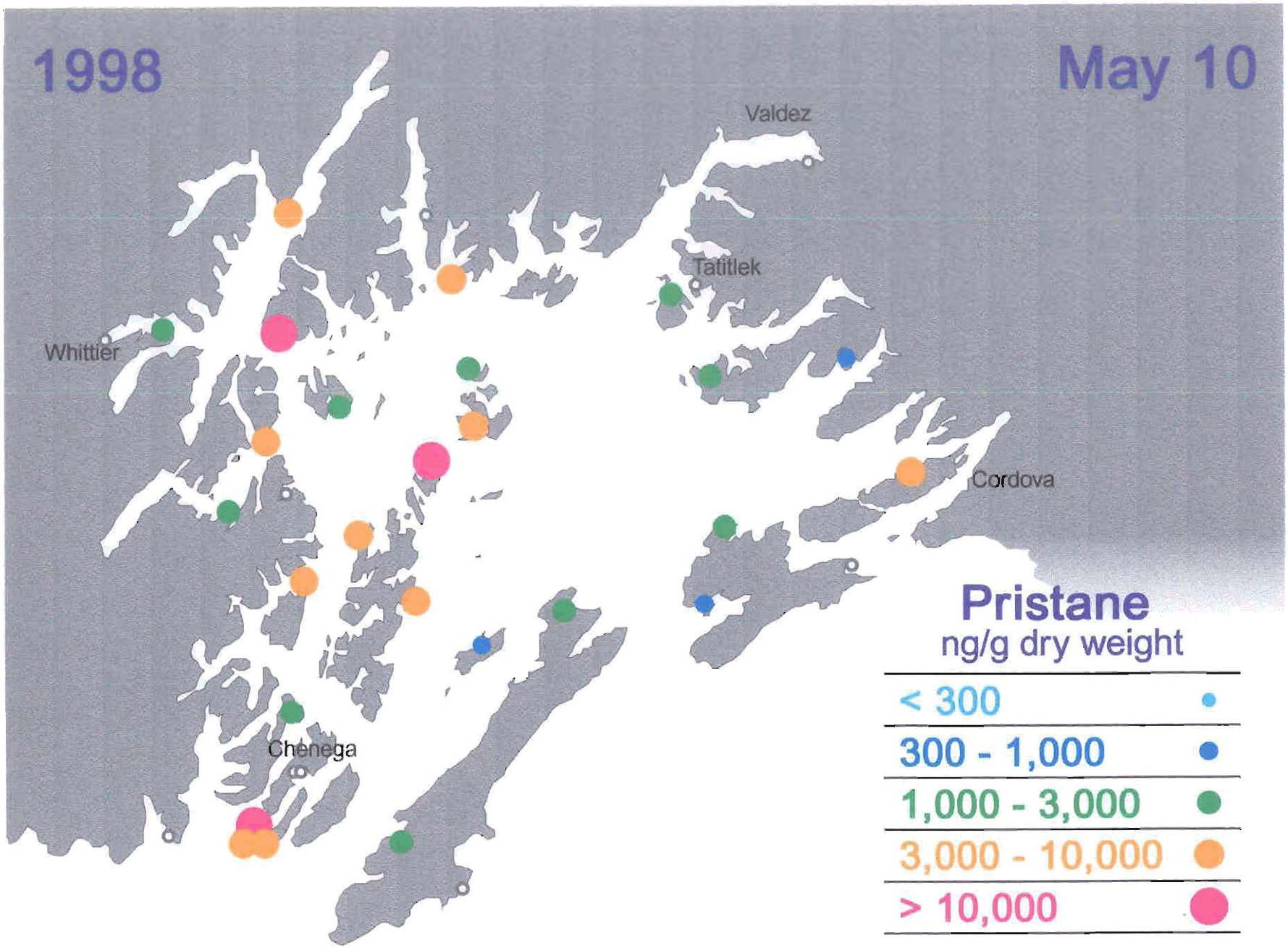
1998
May 10
Valdez
Tatitlek
Whittier
Cordova
Chenega
Pristane
ng/g dry weight
< 300
300 - 1,000
1,000 - 3,000
3,000 - 10,000
> 10,000

Figure 4.

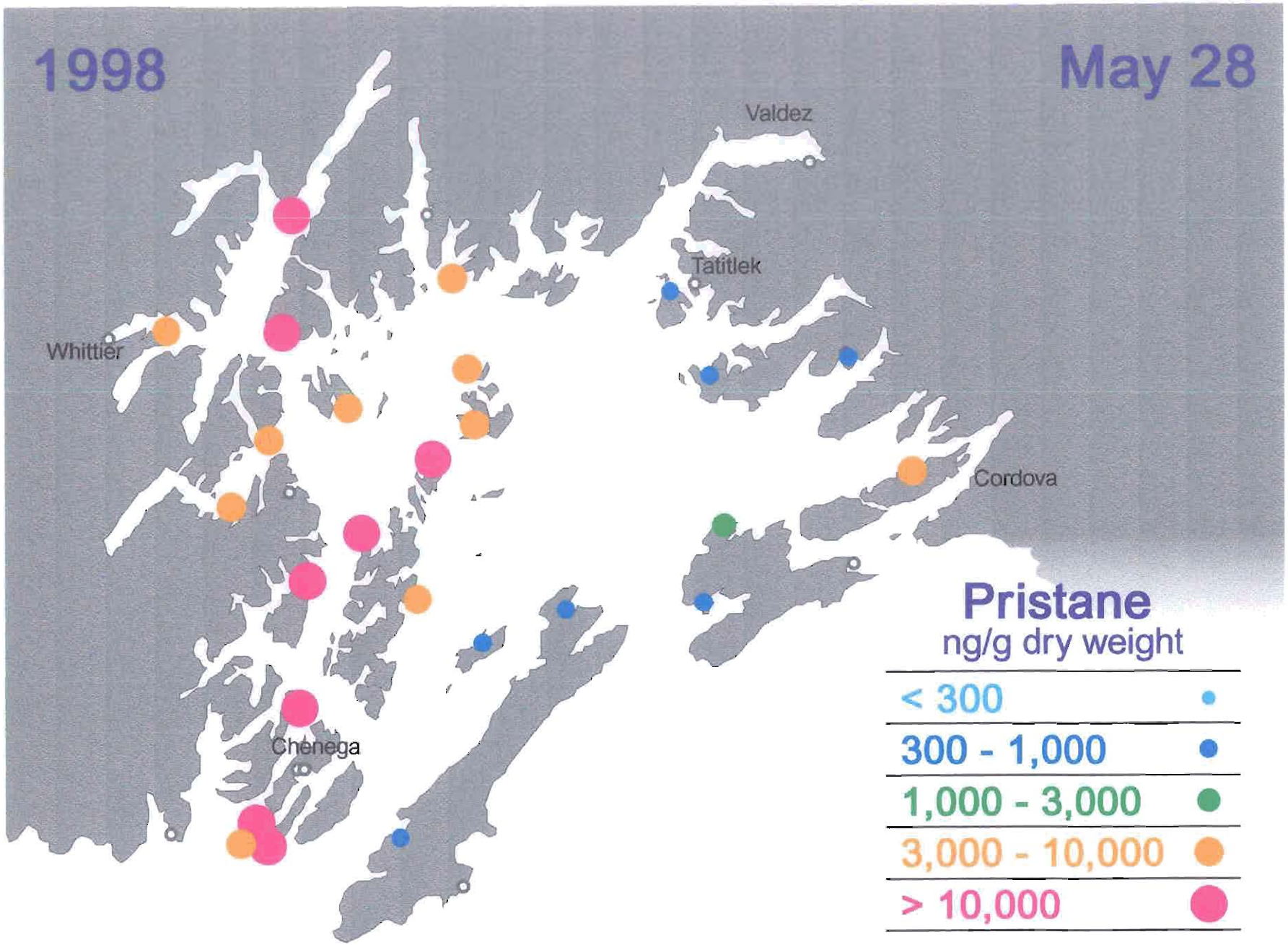
1998
May 28
Valdez
Tatitlek
Whittier
Cordova
Chenega
Pristane
ng/g dry weight
< 300
300 - 1,000
1,000 - 3,000
3,000 - 10,000
> 10,000

Figure 5.