Exxon Valdez Oil Spill
Restoration Project Annual Report

Pristane Monitoring in Mussels

Restoration Project 99195
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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Study History: This project was initiated in FY96. This is the fourth 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 monthly from March to September at 34 stations in Prince William Sound (PWS) to evaluate inter-annual and geographic variability, and to examine whether these results may be related to the marine survival of pink salmon in PWS. Results show that pristane accumulation by mussels averaged across stations throughout PWS was near the 5-year average, indicating that favorable conditions for early marine survival of pink salmon and other zooplanktivores continued through 1999. Stations where mussels accumulated the greatest concentrations of pristane clustered west of a line running from Montague Strait to Valdez Narrows, as in previous years. Attempts to monitor pristane in mussels during winter were largely unsuccessful, but analysis of results from prior years clearly indicates an unknown winter source of pristane throughout PWS, suggesting a significant winter energy source for zooplanktivorous fishes.

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

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

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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. Mussels were collected periodically from 34 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.

Results from 1999 indicate another year of high zooplankton forage abundance for pink salmon and other zooplanktivores in western PWS (figure 2). For PWS as a whole, the average amount of pristane accumulated by mussels was similar to previous years, with no consistent annual trends apparent. As in previous years, stations where pristane is accumulated to the highest concentrations by mussels are located on the western side of PWS, indicating abundant zooplankton forage for there during Spring. Most of the stations where pristane accumulation is low were consistently located on the eastern side of PWS, indicating poor zooplanktivore forage abundance there.

Results from the winter samplings of 1997 and 1998 confirm the existence of a substantial increase of pristane in mussels during winter across most of PWS (figure 3). Pristane concentrations were higher in 1997 than in 1998, exceeding 10,000 ng/g at 3 stations during mid-February in 1997. The sole mussel sample collected from Chenega Bay during early February 1999 also had a relatively high pristane concentration, as did 3 samples collected from the nearby Foxfarm stations in 1996. These results indicate that some unknown process mediates production of pristane from an unknown source for accumulation by mussels during mid-winter. This suggests that there may be other species of Calanoid copepods resident in PWS, or that life histories of known copepods in PWS are more complex, in order for a pristane source of this magnitude be available throughout most of PWS at this time of year.

The winter pristane results clearly indicate that an energetically important forage source is widely available during mid-winter in PWS, and this has significant trophodynamic implications for this ecosystem. These results imply significant primary production during winter, and winter availability of some Calanoid life stage perhaps derived from diapausing populations to zooplanktivores. Also, these results indicate that zooplanktivores other than juvenile pink salmon mediate transfer of pristane from copepods to mussels.

Unfortunately, the logistical challenges of studying the sources and zooplanktivores mediating pristane transfer from these sources to mussels during winter are formidable, and will probably require a much more substantial effort to pursue successfully. Our failed attempt to monitor this process more intensively by aircraft-based sampling in 1999 due to bad weather suggests that either boat- or resident-based sampling will be required, with a substantial commensurate increase in cost per collected sample.

Monitoring results of pristane in mussels collected from stations near hatcheries in PWS were compared with marine survivals of hatchery-reared pink salmon over the past 5 years, and this indicated a significant association between pristane increases in mussels at stations near hatcheries following mass releases of juvenile pink salmon, and the survival of released juveniles. This association can be rationalized by the foraging dynamics of pink salmon and the pristane accumulation dynamics of mussels. This association suggests that pristane monitoring may provide a basis for anticipating marine survivals of pink salmon cohorts in PWS.

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), 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 important prey of many predator species. 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 3 objectives given in the detailed project description:

1. Measure pristane concentrations in mussels collected during spring and summer from 30 stations in Prince William Sound to evaluate inter-annual variability.

2. Determine the existence and location of regions inside Prince William Sound where the energy conversion of objective 1 is consistently above average, and synthesize these data over time and geographic location each succeeding project year.

3. Measure pristane concentrations in mussels collected during fall and winter in Prince William Sound to further resolve the timing of the fall increase.

Methods

Mussel Collection

The seasonal variability of pristane concentrations in mussels (*Mytilus trossulus*) is based on collections from 34 stations in PWS (figure 1, table 1). Mussels were collected monthly from most stations beginning early March through early September for a total of 7 collection periods and 206 mussel samples (collection of some samples was prevented by weather). One sample was also collected from Chenega Bay in mid-February. Collected mussels are stored frozen and analyzed for whole-body pristane concentration.

Of the 34 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. 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 the frequency of laboratory artifacts above detection limits less than 1%. 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.

Data Analysis

Pristane Accumulation Index

Quantitative comparisons of the amount of pristane accumulated by mussels across stations and through time are based on a pristane accumulation index (PAI), calculated as:

$$PAI = (t_2 - t_1)\,[P]_1 + \sum_{i=2}^{I-1} \frac{(t_{i+1} - t_{i-1})\,[P]_i}{2} + (t_I - t_{I-1})\,[P]_I \approx \int_{t_1}^{t_I} [P]\,dt$$

where $[P]_i$ is the pristane concentration measured in mussels collected at time t_i, for mussels collected on I successive samplings throughout the collection season from the same station. This approximation method is used because it does not require equally spaced sampling intervals, or that sampling begin and end on exactly the same dates among different sites, and missed samplings are readily accomodated. These are considerable advantages of practicality for a long-term sampling program involving many stations that may not always be accessible due to poor weather. It is, however, necessary that [P] at t_1 and at t_I be near the annual minimum concentration, and that the number of samplings (I) be sufficiently numerous to adequately describe the shape of the accumulation profile in mussels.

Pristane Productivity Index

Interannual comparison of pristane accumulation by mussels averaged across stations is based on a pristane productivity index (Σ PAI), calculated as the PAIs summed across 25 selected stations. These 25 stations were selected because they are the most consistently sampled stations during the period 1995 through 1999. Two stations that had been included in previous calculations of this index were excluded this year, Hanning Bay and Johnstone Point. Sampling at these two stations will be discontinued in future because of persistent accessibility problems encountered during past sampling, and because results from previous years have indicated that pristane accumulation by mussels at these stations is usually low. Values of the PAI for Hanning Bay were consistently below the annual average PAI since inception of sampling, and at Johnstone Point the PAI exceeded tha annual average once only (in 1997).

Geographic Trends

Patterns in the geographic distribution of the PAI are evaluated by calculating the proportion of sampling years that the PAI at a station exceeds the average PAI for the respective year, and examining the geographic distribution of stations classified according to these proportions. That is, an average PAI may be calculated for each of the 5 sampling years 1009-1999, and stations with PAIs exceeding these averages are recorded. Stations that are consistently above-average in this sense are included in one classification, stations that are above-average in 4 of 5 years in another, etc., and the geographic distribution of stations in each class is examined. This procedure prevents differences in the average PAI among years from obscuring identification of stations where the PAI is relatively high (or low) most years.

Youth Area Watch (YAW) Collaboration

Youth Area Watch students (restoration project 99210) from Cordova, Chenega Bay, Tatitlek, Whittier, Valdez, Nanwalek, and Seldovia collected mussel samples in their hometowns for this project, thereby expanding the geographic coverage of the sampling effort. Poor weather prevented students from participating in mussel collecting flights in Prince William Sound with Auke Bay Lab staff this year, but 14 students and several teachers were able to visit the lab in February and March to learn more about the project and get some hands-on experience in pristane sample analysis.

Results

Pristane Concentrations in Mussels at Regular Monitoring Stations: Synopsis

The general geographic distribution of stations where high pristane concentrations were found in mussels is similar to that of previous years. West of a line running from Montague Strait to Valdez Narrows, pristane concentrations above 10,000 ppb (dry weight) were evident at several stations during May (figure 2). All of these stations either border or are down-current of the deep marine depression system of the northwestern sound. Except at Valdez, concentrations at stations in the eastern part of PWS remained low, usually below 1,000 ng/g.

Pristane concentrations increased substantially in mussels from stations in the southwest migration passages of the sound by early June, contrasting with more mixed fluctuations at northwestern stations (compare figures 4C and D). The highest concentrations observed during the history of this project were found at the Foxfarm stations on southwest Elrington Island, where one sample exceeded 170,000 ng pristane/g dry weight mussel tissue, and the nearest 4 stations were above 19,000 ng/g. But in the northwestern sound, pristane concentrations were lower at most stations compared with this time during previous years. A modest increase of pristane in mussels also occurred at Windy Bay by June (figure 4D).

Pristane concentrations generally declined by late June, and continued to decline through late August to concentrations comparable with the those of early March (compare figures 4A and G) except at Point Pakenham in the northwestern sound. At Point Pakenham, pristane concentrations were unusually high during early March through early May (figures 4A through C), then declined for the rest of the season before increasing again by late August (figures 4D through G).

The pristane concentration in the sole mid-winter sample collected this year at Chenega Bay was over 4,500 ng/g, well above concentrations present at most stations during the subsequent March 8-22 sampling period.

Interannual Trends

Total pristane accumulation by mussels averaged across stations was similar to results from previous years. Time-integrated pristane concentrations summarized by the Σ PAI index were 10.3 x 10^6 for 1999, which is near the 5-year average value of 9.7 x 10^6 for 1995 through 1999. The Σ PAI index has varied little during this period, ranging from a low of 8.8 x 10^6 in 1995 to a high of 11.0 x 10^6 in 1998.

Geographic Trends

Pristane concentrations were consistently above annual averages at only two stations during the period 1995 through 1999. These two stations were at Point Eleanor on northern Eleanor Island , and at the Foxfarm stations on southern Elrington Island (figure 1). Two other stations were above annual averages during 4 of the 5 years, at the AFK hatchery on Evans Island and at Point Pakenham. Five stations were variable, above the annual average during 2 or 3 of the last 5 years, and all 5 these stations were clustered around Perry Island in the northwestern sound. These 5 variable stations include Applegate Island, Esther Island, Fairmont Island, Herring Point, and Perry Island (see figure 1).

In contrast, 13 stations were consistently below annual averages, and another 6 stations were below for 4 of the 5 years. These 19 stations include all 10 of the stations eastward of a line running from Montague Strait to Valdez Narrows, 3 stations in distal fjords (Cannery Creek, Decision Point, and Division Point), 3 stations along the western coasline of Knight Island Passage (Main Bay, Chenega Island, and Fleming Island), and 2 stations on the Naked Island complex (Naked Island and Storey Island; see figure 1).

Winter Sampling

Our attempts to sample stations during winter 1999 and later during fall 1999 were almost completely thwarted by weather. One sample was collected by volunteers at Chenega village. Attempts to collect samples from stations by aircraft were prevented by poor weather, despite repeated and persistent attempts, including one trip to Cordova for a week waiting unsuccessfully for weather improvements. Weather improvements were in general too brief to exploit successfully, in part because of the delay caused by the personnel transport time from Juneau to

Cordova. However, an early-February and an early-March sampling trip were completed in 1998, but the results of those samplings were not presented in the previous annual report because the data analysis was incomplete at the time the report was prepared. These results follow here, because they bear directly on the third objective of this project.

Pristane concentrations were relatively high at stations in western PWS during early February 1998. Concentrations were highest at stations westward of a line running from Montague Strait to Valdez Narrows, and were highest at Chenega Island (5,200 ng/g) followed by Fleming Island (4,300 ng/g; see figure 3A). These concentrations declined uniformly by early March (figure 3B). Eastward, pristane concentration remained generally low and unchanged from early February through early March.

Discussion

Inter-annual Trends

This project now has 5 consecutive years of comparable data, which permits evaluation of geographic and temporal trends with increased confidence. Data analysis was incomplete when the previous annual report for this project was submitted, but this deficiency has been corrected, so the following discussion of geographical and temporal trends includes newly reported data for both 1998 and 1999. Previous discussions of such trends relied on data collected from 1994 through 1997, and data collection in 1994 was considerably less comprehensive than in subsequent years. Consequently, the measure for inter-annual comparison of the degree of pristane accumulation by mussels across the sound as a whole (i.e., the Σ PAI index), was modified to be consistent across stations during 1995 through 1999 instead of 1994 through 1997. This revision allows the number of stations included in the Σ PAI calculation to increase from 20 to 26 making the index more robust and representative of the Sound.

The significance of inter-annual differences among Σ PAI results has been estimated by calculating a least-significant-difference (LSD) criterion based on Monte Carlo re-sampling procedure described in the 1997 annual report for this project (Short and Harris, 1997). These results showed that an LSD of 22% is significant at the 95% confidence level. Application of this criteria to the Σ PAI results from 1995 through 1999 indicates that the Σ PAI for 1998 was significantly higher than for 1995, but that all other comparisons among these years are not significant.

We had previously reported an increasing trend for this index from 1994 through 1997 (Short and Harris 1997), but the appearance of this trend was caused by the low Σ PAI value for 1994. This low value was likely an artifact of the low number of sampling times conducted in 1994, when most of the 20 stations included in the original un-revised Σ PAI were sampled only 2 or 3 times that year. This probably introduced a bias toward a lower value for the Σ PAI index compared to subsequent years, when the number of sampling periods included in the calculation was at least 4 and usually more than 5. The revised Σ PAI is more robust because more stations are included and more sampling periods compared with 1994, and the results for 1995 through 1999 provide less compelling evidence for an increasing trend of this index. Still, these results do support the conclusion that index values have remained high during this period, when zooplankton forage has been abundant during spring, and marine survival of pink salmon have remained high, at least in comparison with marine survivals typical during the mid-1970s.

Geographic Trends

One clear and persistent geographic trend has been the lower PAI values characteristic of stations eastward of a line running from Montague Strait to Valdez Narrows. This pattern is consistent with the hypothesis that the deep marine depression of the northwestern Sound provides overwintering habitat for diapausing *Neocalanus plumchrus/flemingerii*, and that copepodites produced by these diapausing adults in winter contribute substantially to the bulk of the zooplankton bloom during spring. This pattern is also consistent with the salmonid migration corridor from streams PWSAC hatcheries and in the western Sound. This corridor is generally believed to include Knight Island passage and the passages surrounding Bainbridge, Evans, Elrington and Latouche Islands in southwest PWS. Stations that are usually or consistently high in the PAI are located along this corridor, as are the 5 variable stations. The variability of these 5 stations is likely the result of stochastic influences on the distribution of *Neocalanus* zooplankton and of juvenile pink salmon during spring across spatial scales on the order of 10 km.

The consistently high PAIs observed at the Foxfarm stations are likely influenced by the large AFK hatchery releases of juvenile pink salmon 15 km to the northeast, and by advection of Neocalanus zooplankton along the salmonid migration corridor out of PWS. This advection (the "river" part of the "river/lake hypothesis" proposed by the SEA program), would tend to concentrate zooplankton and juvenile pink salmon in the southwestern ends of the passages surrounding Bainbridge, Evans, Elrington and Latouche Islands.

The persistent high PAI values associated with the station at Point Eleanor are less readily explained, especially because other stations in the vicinity include the 5 most variable stations. Point Eleanor is at least 35 km from the nearest hatchery and juvenile pink salmon would have to transit substantial stretches of open water to get there. It seems odd that hatchery pink salmon would consistently migrate to Point Eleanor, but not to any of the other stations in the vicinity. Alternatively, populations of other zooplanktivorous fishes such as sandlance in the vicinity of Point Eleanor may provide an explanation, but this remains speculative.

Two other anomalous stations appear in the 5-year record of PAIs, Windy Bay and Point Pakenham. Modest PAI values were found for the Windy Bay station during 1998, and a very high value occurred for 1994. These may reflect the presence of a productive wild pink salmon run in Windy Creek adjacent to the station, and a nearby marine depression in Orca Inlet that may provide over-winter habitat for diapausing *Neocalanus* copepods. Point Pakenham seems unusual because it is situated midway up a long narrow fjord, and had above-average PAI values for 4 of the last 5 years unlike other stations similarly situated (at Decision Point, Division Point and Cannery Creek). The high PAI values at Point Pakenham may simply be due to simultaneous advection of *Neocalanus* zooplankton and juvenile pink salmon from wild or hatchery origin, but the results from winter sampling at this station and elsewhere in PWS (including Windy Bay) indicate that *Neocalanus* life histories may be substantially more diverse and complex than is usually assumed, and that contributions of pristane to mussels mediated by zooplantivores other than juvenile pink salmon are important.

Winter Pristane Source

Results from the winter samplings of 1997 and 1998 confirm the existence of a substantial increase of pristane in mussels during winter across most of PWS. Pristane concentrations were higher in 1997 than in 1998, and exceeded 10,000 ng/g at 3 stations during mid-February in 1997 (Short and Harris, 1997). In 1997, the winter increases of pristane appeared throughout PWS, but were more associated with the marine depression of northwest PWS in 1998. The sole mussel sample collected from Chenega Bay during early February 1999 also had a relatively high pristane concentration, as did 3 samples collected from the nearby Foxfarm stations in 1996. As noted in the 1997 annual report, these results together indicate that some unknown process mediates production of pristane from an unknown source for accumulation by mussels during mid-winter (Short and Harris, 1997). This suggests that there may be other species of Calanoid copepods resident in PWS, or that life histories of known copepods in PWS are more complex, in order for a pristane source of this magnitude be available throughout most of PWS at this time of year.

The winter pristane results clearly indicate that an energetically important forage source is widely available during mid-winter in PWS, and this has significant trophodynamic implications for this ecosystem. These results imply significant primary production during winter, and winter availability of some Calanoid life stage perhaps derived from diapausing populations to zooplanktivores. Also, these results indicate that zooplanktivores other than juvenile pink salmon mediate transfer of pristane from copepods to mussels.

Unfortunately, the logistical challenges of studying the sources and zooplanktivores mediating pristane transfer from these sources to mussels during winter are formidable, and will probably require a much more substantial effort to pursue successfully. Our failed attempt to monitor this process more intensively by aircraft-based sampling in 1999 due to bad weather suggests that either boat- or resident-based sampling will be required, with a substantial commensurate increase in cost per collected sample.

Predicting Hatchery Survivals

A strong association appears to exist between pristane increases in mussels near PWSAC hatcheries immediately following mass releases of juvenile pink salmon, and the number of adults returning to the hatcheries 16 months later. This association may be formally stated as follows. Let $\{m_{i,j,25}\}$ denote the set of j mussel sampling stations near the i^{th} hatchery, where J(i) is the total number of stations within 25 km of the i^{th} hatchery, and the total number of hatcheries is I (=3). For each hatchery, assume mussels are collected and analyzed for pristane from all the stations $\{m_{i,j,25}\}$ just prior to a mass release of juvenile pink salmon, and again two to three weeks later. Pristane concentrations in mussels near hatcheries often increase substantially during this interval because of fecal production by the released salmon. From the analysis results, the change of the pristane concentration in mussels at each station may be calculated for this two to three week interval. Let $m_{i,j=j',25}$ indicate the station (j') within 25 km of the i^{th} hatchery where the *maximum* concentration increase of pristane is observed among the sampled mussels, and let $\Delta P(m_{i,j=j',25})$ denote the magnitude of this increase. Finally, let $N_{r,i}$ denote the number of adult survivors of the released cohort that return to the ith hatchery. The association of $N_{r,i}$ and $\Delta P(m_{i,j=j',25})$ is modeled simply as $N_{r,i} = a\,[\Delta P(m_{i,j=j',25})] + b + \epsilon_i$ (eq 1), that is the number of surviving pink salmon is related to the maximum increase of pristane in mussels collected anywhere within 25 km of a hatchery two to three weeks following release of juveniles, ϵ_i is the error for the i^{th} hatchery.

Regression of adult pink salmon returns with the associated $\Delta P(m_{i,j=j',25})$ at each of 3 PWSAC hatcheries for 5 brood years (1994-1998) produces a very highly significant association ($P<0.0003$, df = 13) wherein $\Delta P(m_{i,j=j',25})$ explains 62% of the variability of $N_{r,i}$ among hatcheries and across brood years. The strength of this association strongly suggests that survival through the early marine residence period largely determines recruitment to the returning adult cohort population.

The rationale for $\Delta P(m_{i,j=j',25})$ as a predictor variable derives from physical and biological constraints. After juvenile pink salmon commence marine residence, they must locate adequate prey densities to support rapid growth to avoid increasing vulnerability to predation or starvation. At 3 cm initial body length and swimming at 1 body length per second, these juveniles can travel a maximum of about 2.5 km per day, and hence are semi-planktonic. The pristane-producing *Neocalanus sp.* dominate the zooplankton of PWS in early May when juvenile pink salmon begin marine residence, and concentrations of these zooplankters together with juvenile pink salmon may appear adjacent to shorelines in response to wind-, tidal- or density-driven surface currents. These concentrations of juveniles and their *Neocalanus sp.* prey therefore likely have a strong random component, hence the need for a network of stations surrounding the hatcheries. A large increase of pristane in mussels near a hatchery following release of juvenile hatchery pink salmon indicates that zooplankton prey were successfully located by a substantial portion of the released salmon. The maximum observed pristane increase $\Delta P(m_{i,j=j',25})$ is therefore used as an indicator of the most favorable feeding conditions in the vicinity of a hatchery. This is where growth and survival are likely greatest, and hence where the greatest contributions to numbers of returning adults occur.

The two to three week sampling interval is suggested by the uptake and depuration kinetics of pristane associated with fecal material produced by pink salmon and accumulated by mussels. Both laboratory and field experiments have shown that increased pristane concentrations appear in mussels within a few hours to a few days following introduction of pristane-laden fecal material to mussels, whereas the depuration half-life is two to three weeks. Also, juvenile pink salmon probably need to begin rapid growth within the first week of marine residence or face severe predation.

The 25 km radius criterion for identifying the mussel collection stations associated with a particular hatchery $\{m_{i,j,25}\}$ corresponds with the distance juvenile pink salmon can swim during their first 10 days of marine residence. Surface currents may well transport juveniles faster than this, but these are unlikely unidirectional across 25 km distances given the heavily indented shoreline characteristic of PWS. Failure to locate abundant prey within 25 km of a hatchery would likely result in substantial weakening and increased vulnerability to predation.

The absence of density dependence implicit in eq 1 may be a consequence of the factors integrated by the $\Delta P(m_{i,j=j',25})$. Intuitively, no fecal material will be produced by juveniles that are starving, or that are killed by predators. However, it is unclear at present what functional relationship is to be expected between the amount of fecal material produced by a population of juvenile salmon that are growing and also being killed by predators, and the number of survivors. From this perspective, eq 1 must be regarded as an empirical approximation.

The regression based on eq 1 may be used to predict the numbers of returning adults to the PWSAC hatcheries. Application of this regression to the 1999 pristane results leads to predictions of comparatively low numbers of adults returning to the northern pink salmon hatcheries at Esther Island and at Cannery Creek (less than 4% marine survival), but very high survival of AKF pink salmon. The very high predicted AFK survival, >20%, results from the very high pristane increases observed at the 1999 Foxfarm stations. However, juvenile pink salmon from the AFK hatchery were released into a substantial diatom bloom of *Chaetoceros spp.* in spring 1999, which can eventually kill these fish caused by physical abrasion of gill tissues. Marine survivals of the AFK fish may therefore be lower than predicted because many of these fish may survive long enough to produce substantial quantities of pristane-laden feces, but ultimately die from exposure to *Chaetoceros spp.* (personal communication, Bud Perrine, PWSAC).

Conclusions

1. Pristane accumulation by mussels has been consistently highest in western PWS, consistent with the extent of over-winter habitat for Neocalanus copepods provided by the deep marine depression of northwestern PWS.

2. The intensity of pristane accumulation by mussels averaged across PWS as a whole has remained approximately constant during the previous 5 years, with most variability among stations between years confined to 5 stations adjacent to the deepest part of the deep marine

depression. These results indicate continued high abundances of zooplankton forage during spring throughout this period, and continued high marine survivals of pink salmon.

3. Winter sampling in 1996 through 1999 indicates significant pristane accumulation by mussels during February throughout this period. These results imply that an important winter forage source is available to zooplanktivors, and that other zooplanktivors are important intermediaries of this tranfer in addition to juvenile pink salmon.

4. The association of the pristane increase near hatcheries within 2-3 weeks following mass releases of juvenile pink salmon and marine survival of the released salmon suggests that pristane monitoring may have value as a method for prediciting hatchery survivals. Predicted marine survivals for PWSAC hatcheries are relatively low for the northern hatcheries (<4%), but high for the southern hatchery (AFK; > 20%).

Literature Cited

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Blumer, M., M. M. Mullin, and D. W. Thomas. 1964. Pristane in the marine environment. Helgoländer Wissenschaftliche Meeresuntersuchungen **10**:187-201.

Exxon Valdez oilspill of 1989: State/Federal trustee council hydrocarbon database, 1989-1995. Available from Bonita Nelson, Auke Bay Laboratory, 11305 Glacier Highway, Juneau, Alaska 99801-8626.

Short, J. and P. Harris. 1997. Pristane monitoring in mussels and predators of juvenile pink salmon & herring, *Exxon Valdez* Oil Spill Restoration Project Annual Report (Restoration Project 97195), Auke Bay Laboratory, Juneau, Alaska.

Table l. Locations and abbreviations of mussel collection stations sampled for this project in 1999. The abbreviations are also used in figure 1.

		Latitude			**Longitude**		
Station Abbreviation	**Station Name**	**Deg N**	**Min**	**Sec**	**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
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
FOXF1	Fox Farm 1	59	58	15	148	8	22
FOXF2	Fox Farm 2	59	58	7	148	6	36
FOXF3	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
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

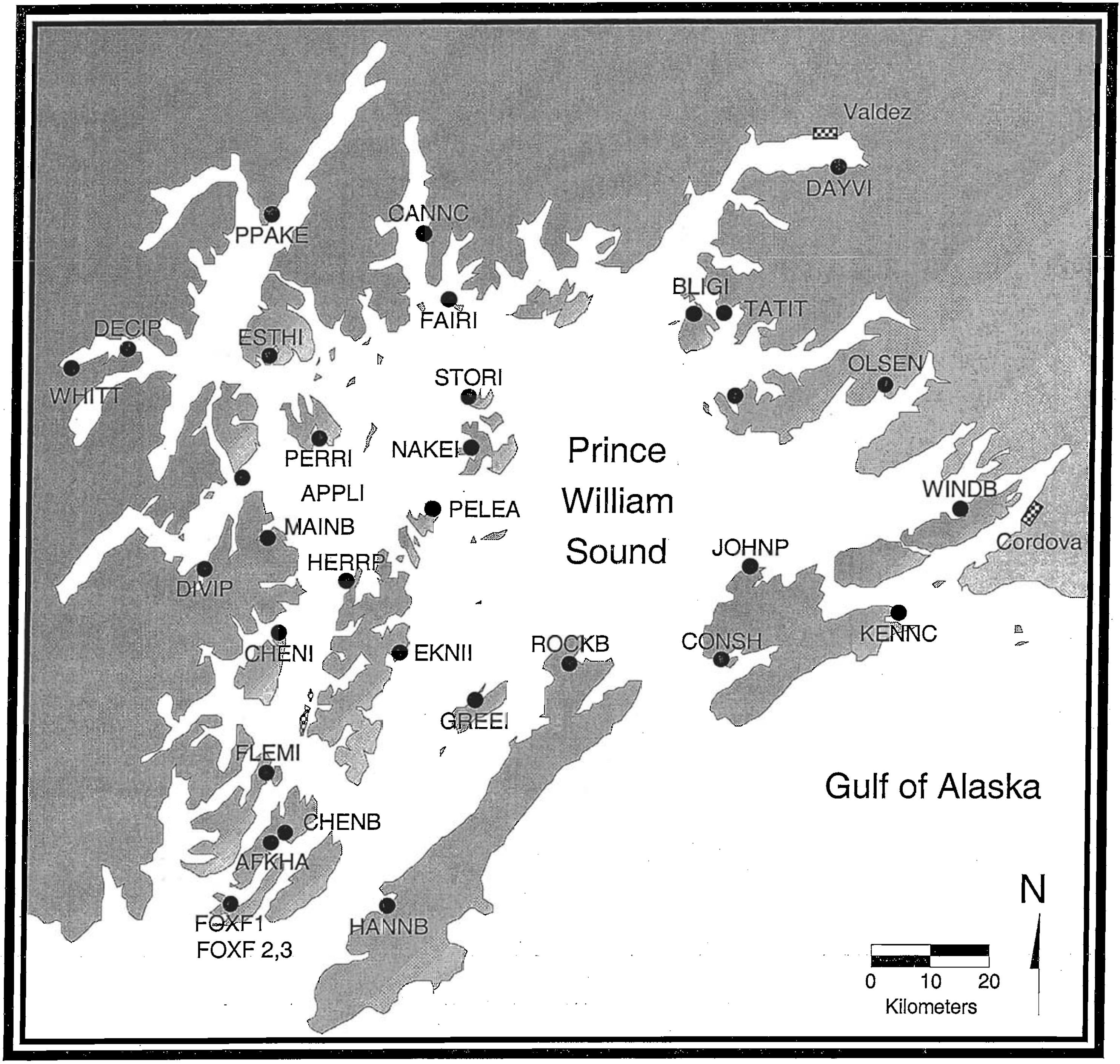
Valdez
DAYVI
PPAKE
CANNC
FAIRI
BLIGI
TATIT
DECIP
ESTHI
WHITT
OLSEN
STORI
NAKEI
PERRI
APPLI
Prince
William
Sound
WINDB
PELEA
MAINB
Cordova
JOHNP
HERRP
DIVIP
CHENI
EKNII
ROCKB
CONSH
KENNC
GREEI
FLEMI
Gulf of Alaska
CHENB
AFKHA
FOXF1
FOXF 2,3
HANNB
N
0
10
20
Kilometers

Figure 1.

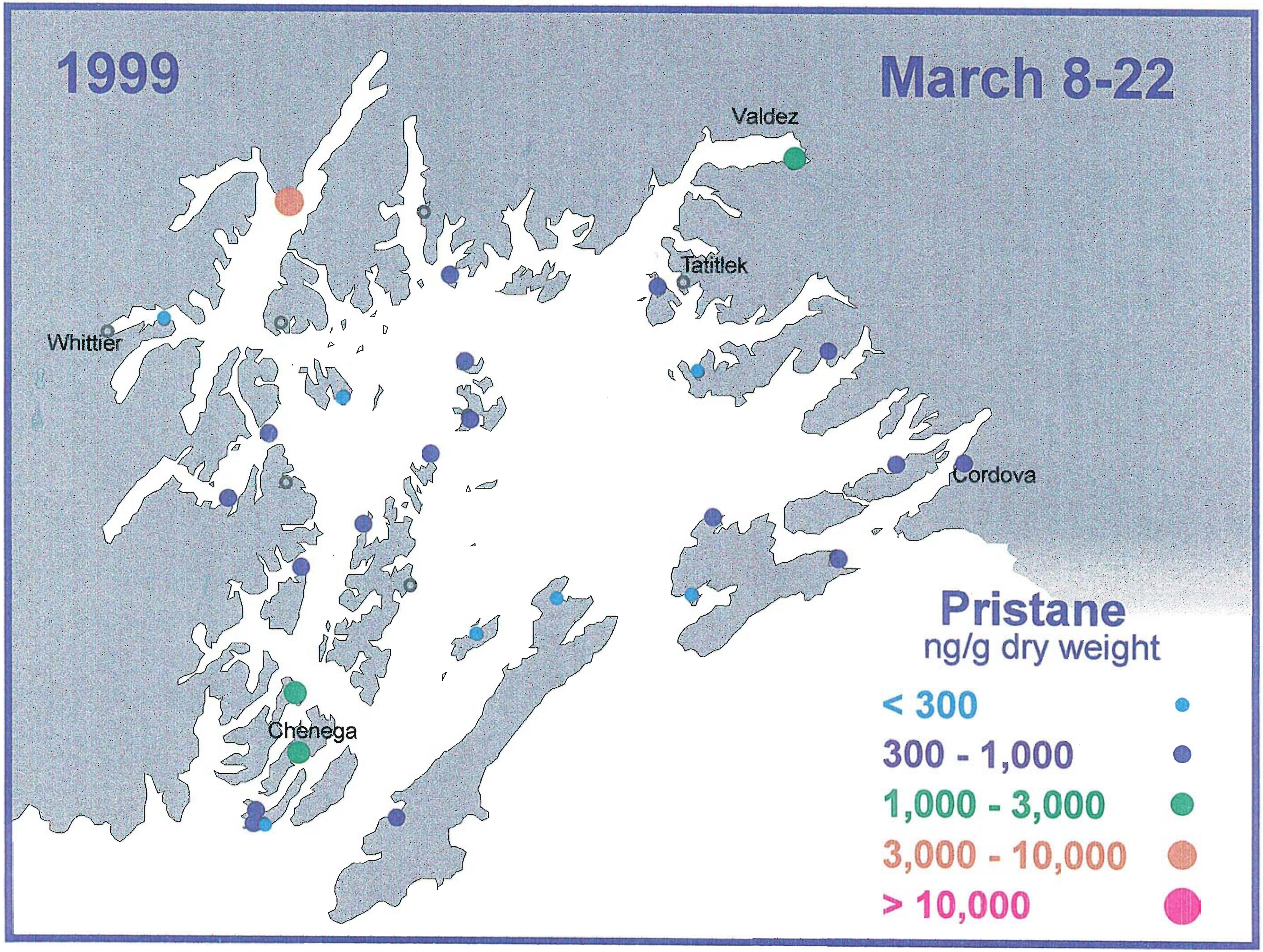
1999
March 8-22
Valdez
Tatitlek
Whittier
Cordova
Chenega
Pristane
ng/g dry weight
< 300
300 - 1,000
1,000 - 3,000
3,000 - 10,000
> 10,000

Figure 2A.

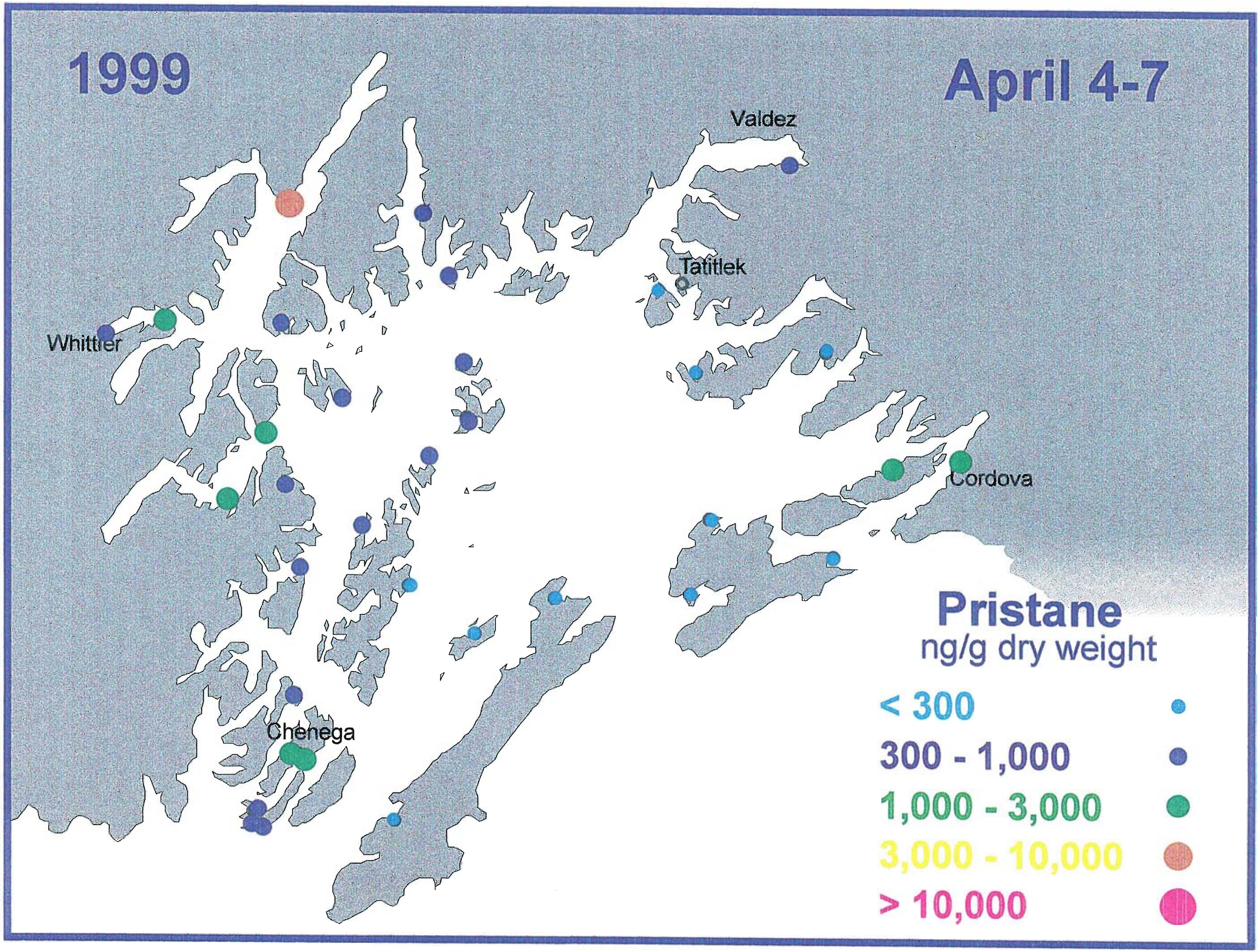
1999
April 4-7
Valdez
Tatitlek
Whittier
Cordova
Chenega
Pristane
ng/g dry weight
< 300
300 - 1,000
1,000 - 3,000
3,000 - 10,000
> 10,000

Figure 2B.

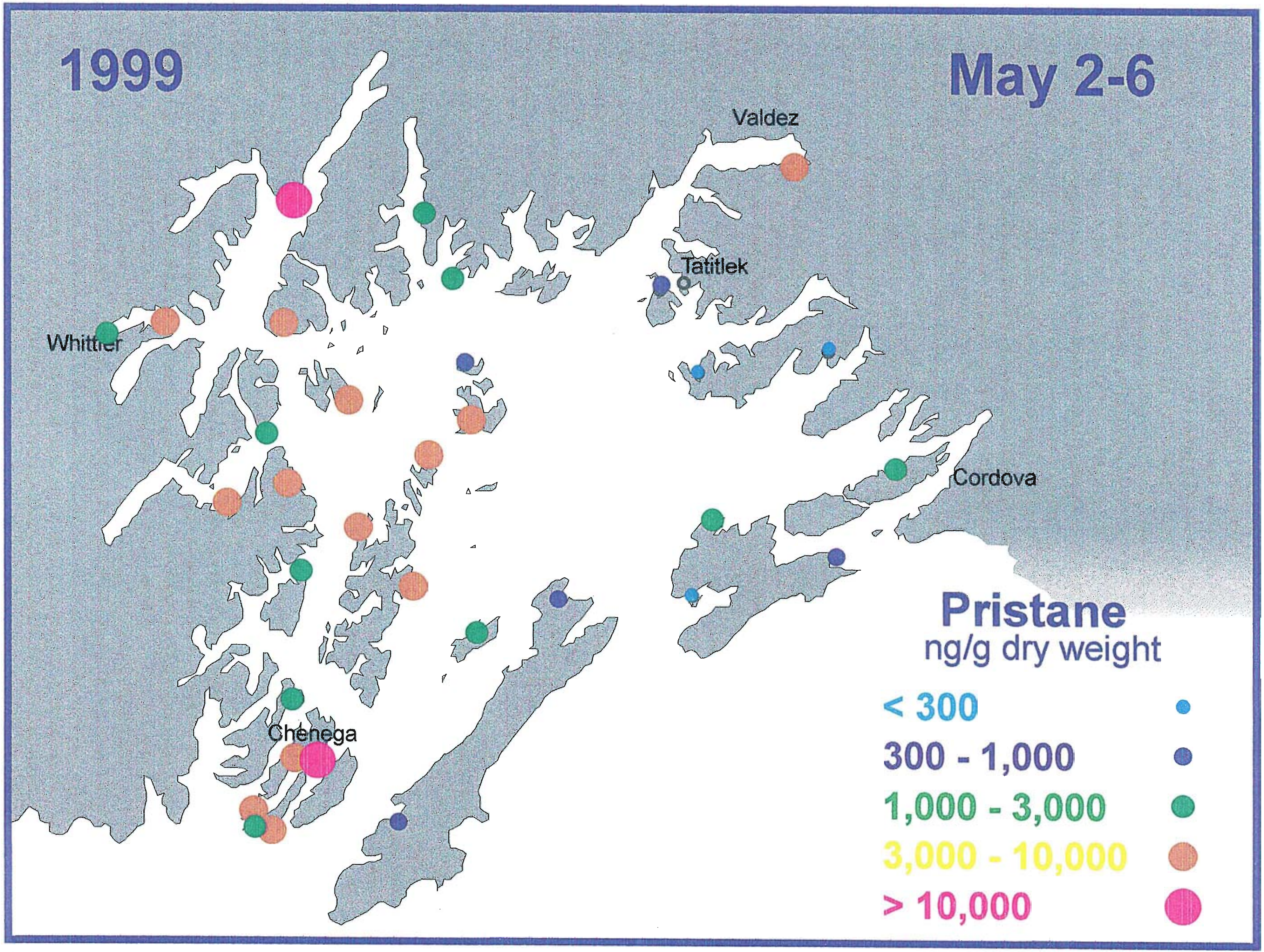
1999
May 2-6
Valdez
Tatitlek
Whittier
Cordova
Chenega
Pristane
ng/g dry weight
< 300
300 - 1,000
1,000 - 3,000
3,000 - 10,000
> 10,000

Figure 2C.

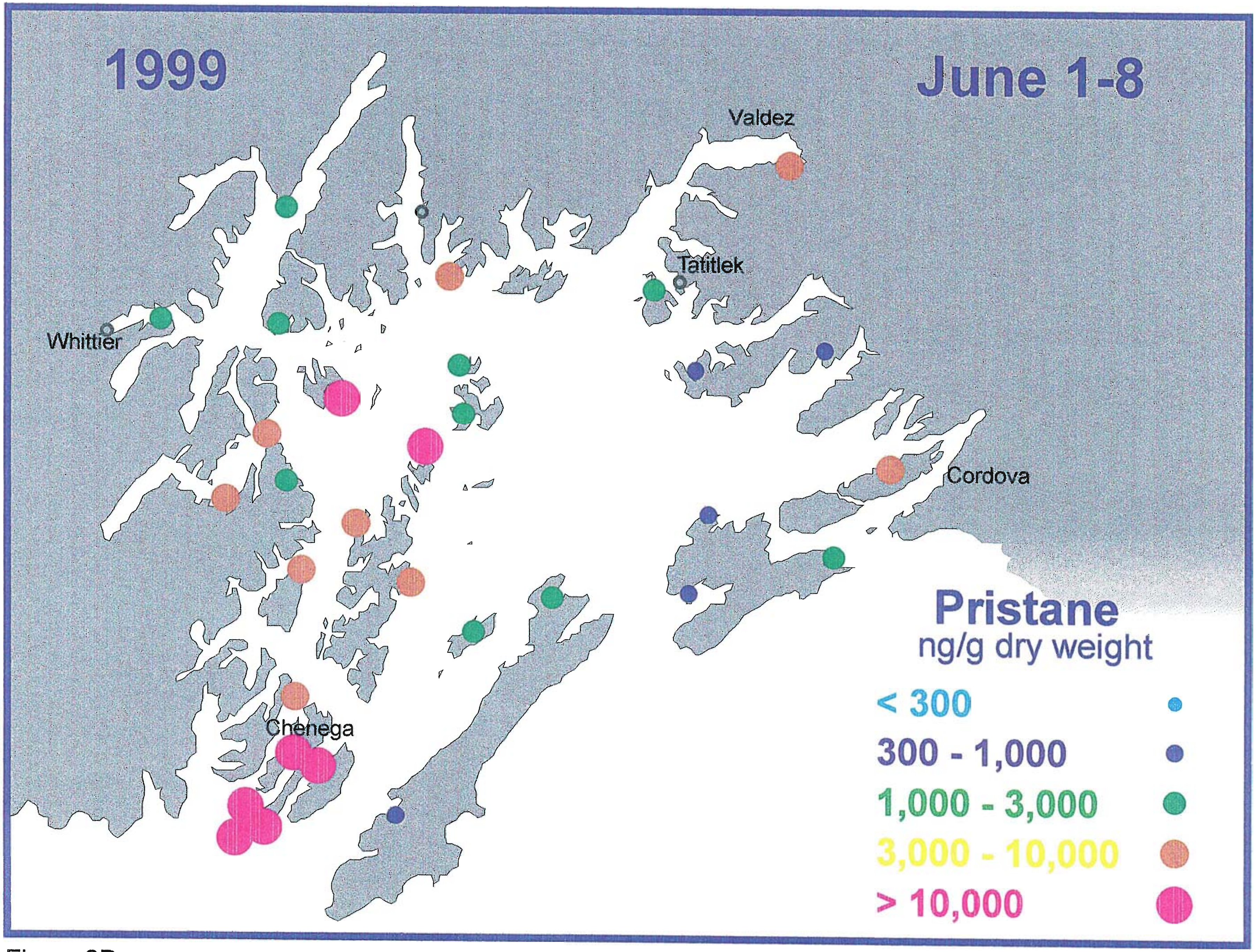
1999
June 1-8
Valdez
Tatitlek
Whittier
Cordova
Chenega
Pristane
ng/g dry weight
< 300
300 - 1,000
1,000 - 3,000
3,000 - 10,000
> 10,000

Figure 2D.

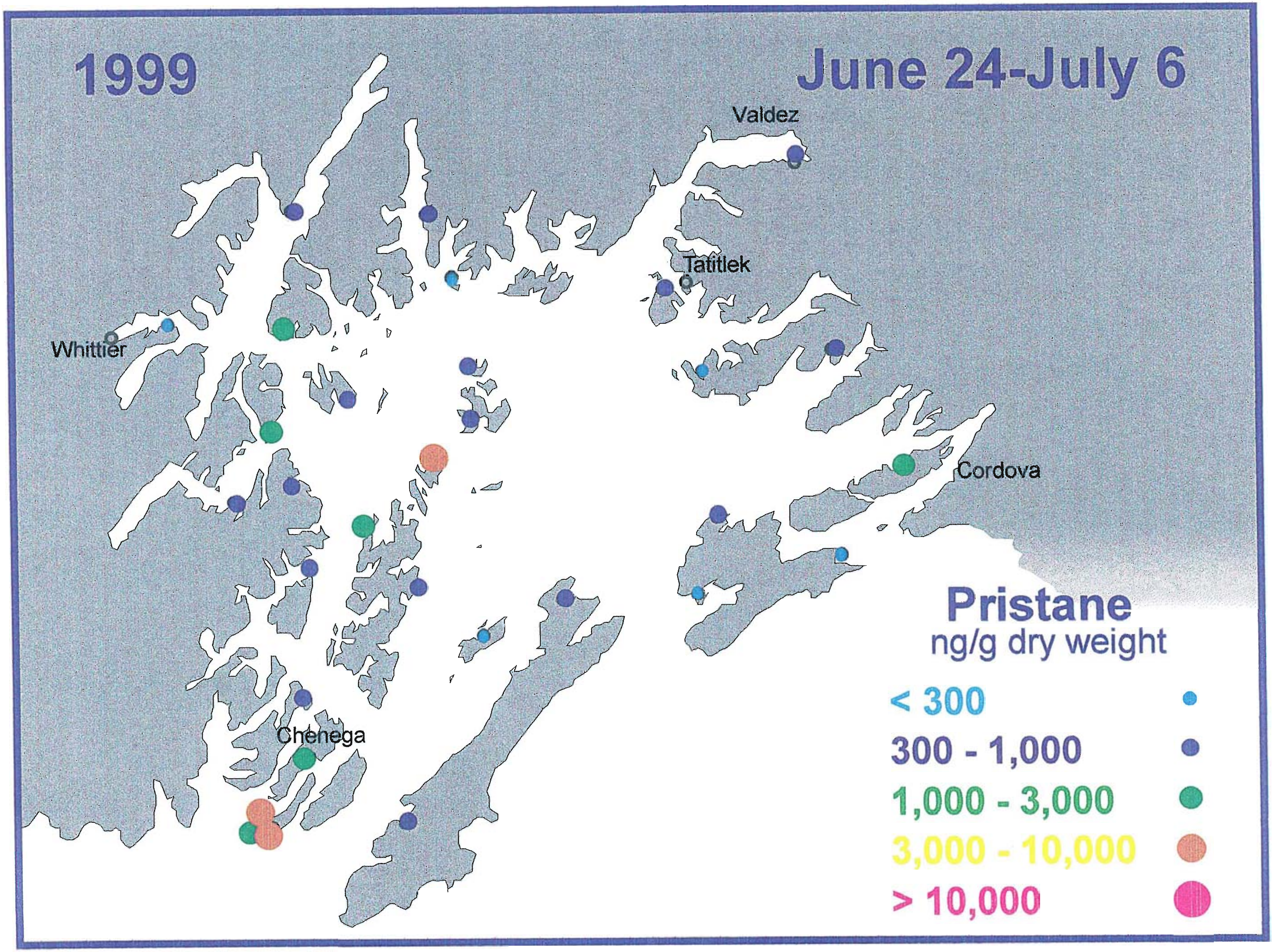
1999
June 24-July 6
Valdez
Tatitlek
Whittier
Cordova
Chenega
Pristane
ng/g dry weight
< 300 ●
300 - 1,000 ●
1,000 - 3,000 ●
3,000 - 10,000 ●
> 10,000 ●

Figure 2E.

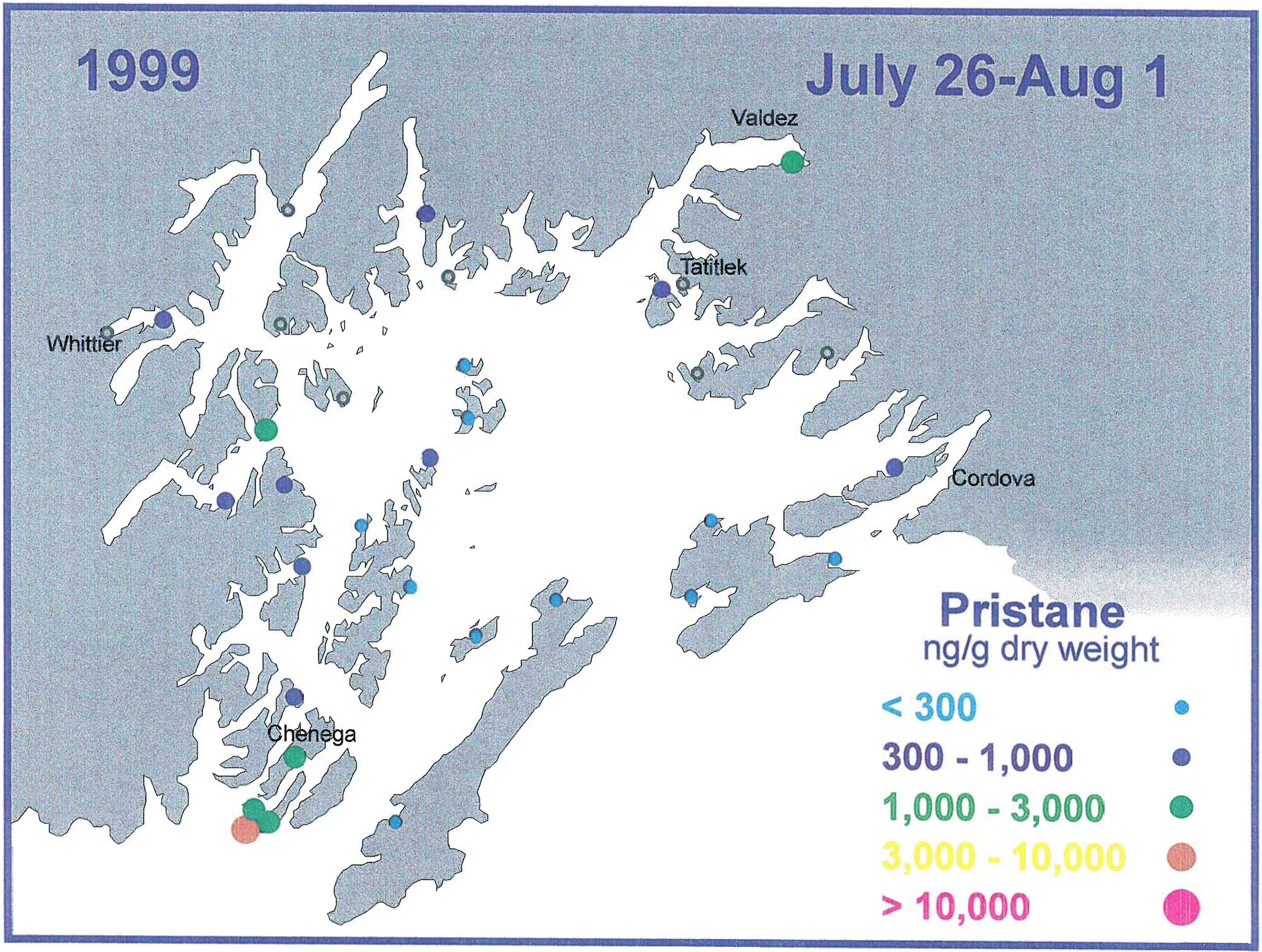
1999
July 26-Aug 1
Valdez
Tatitlek
Whittier
Cordova
Chenega
Pristane
ng/g dry weight
< 300
300 - 1,000
1,000 - 3,000
3,000 - 10,000
> 10,000

Figure 2F.

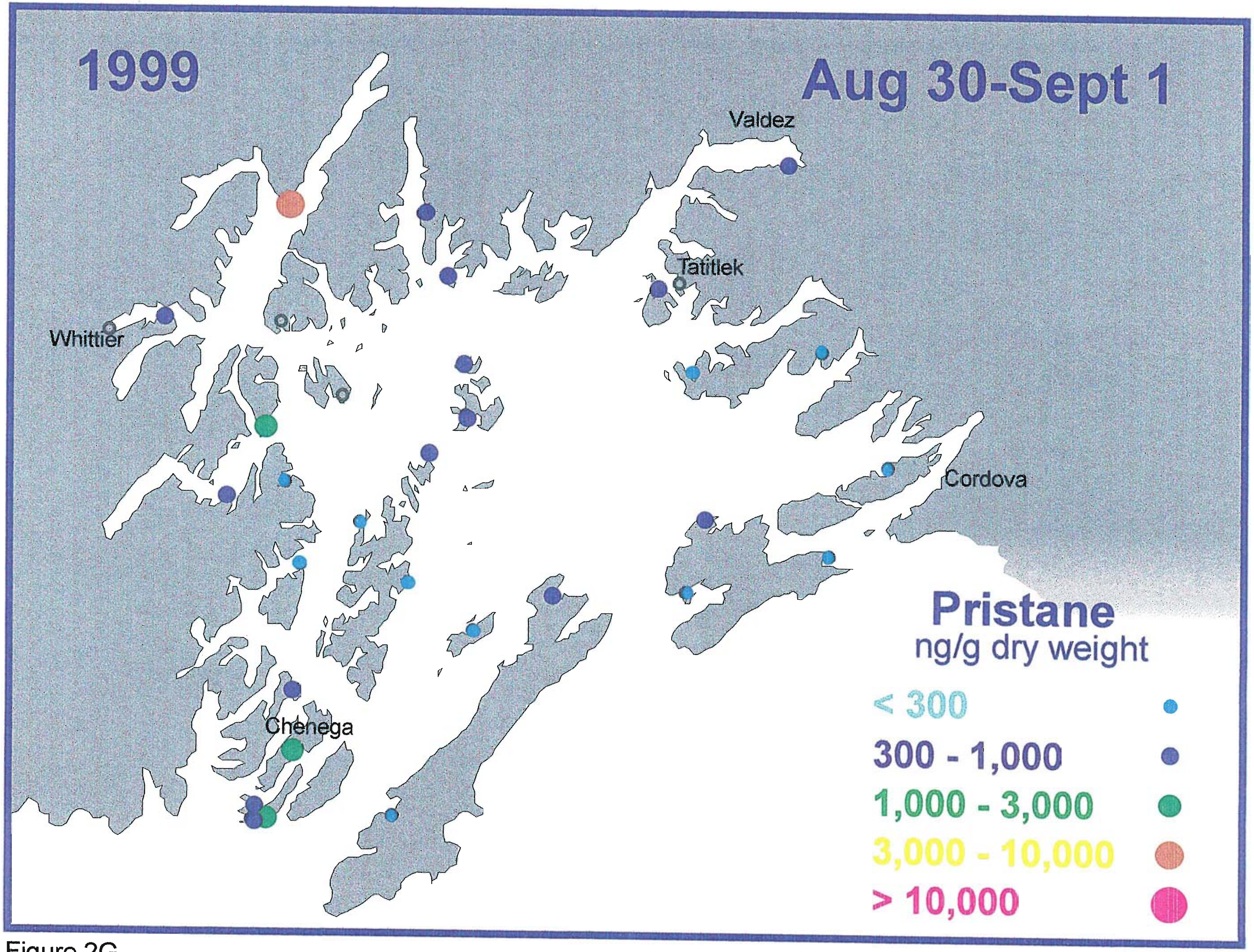
1999
Aug 30-Sept 1
Valdez
Tatitlek
Whittier
Cordova
Chenega
Pristane
ng/g dry weight
< 300
300 - 1,000
1,000 - 3,000
3,000 - 10,000
> 10,000

Figure 2G.

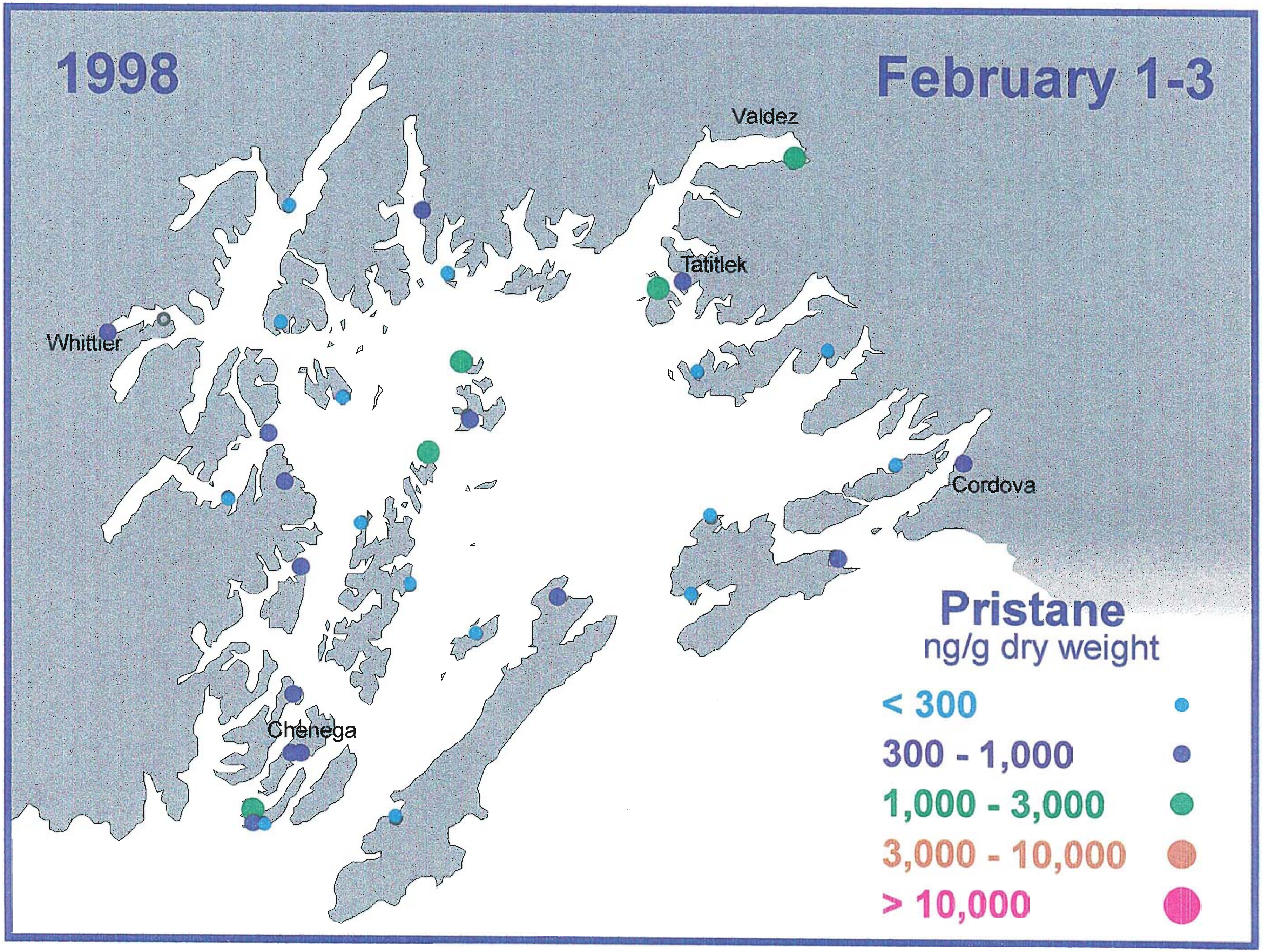
1998
February 1-3
Valdez
Tatitlek
Whittier
Cordova
Chenega
Pristane
ng/g dry weight
< 300 ●
300 - 1,000 ●
1,000 - 3,000 ●
3,000 - 10,000 ●
> 10,000 ●

Figure 3A.

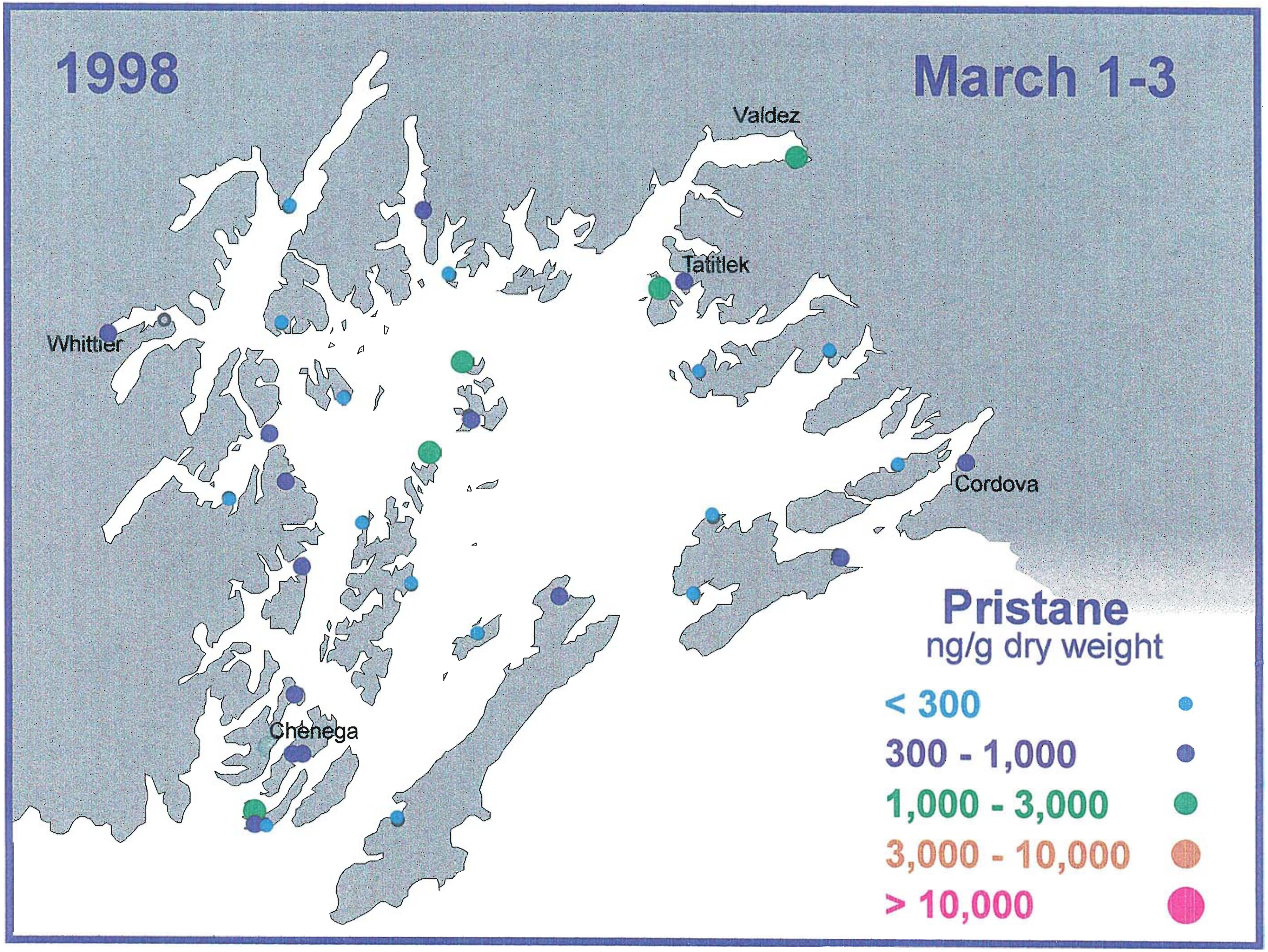
1998
March 1-3
Valdez
Tatitlek
Whittier
Cordova
Chenega
Pristane
ng/g dry weight
< 300
300 - 1,000
1,000 - 3,000
3,000 - 10,000
> 10,000

Figure 3B.