Exxon Valdez Oil Spill
Restoration Project Annual Report

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

Restoration Project 96195
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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Pristane Monitoring in Mussels and Predators of Juvenile Pink Salmon & Herring

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Study History: This project was initiated in FY96. This is the first annual report for the project.

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. Analyses of pristane in mussels collected in 1995 and 1996 show that increases of pristane during spring occurs in 2 stages, and begins at stations near the marine trench system of northwest PWS. The first increase occurs in March and the second in early May. The onset of the second increase of early May was probably within 1 week in 1995 and 1996. The overall production of pristane-rich fecal material nearshore in PWS was similar in 1995 and 1996, suggesting similar feeding conditions for juvenile pink salmon and herring.

Pristane concentrations in mussels near Esther Hatchery tripled within 6 days following simultaneous release of 120 million pink salmon. The released juveniles preyed heavily on *Neocalanus* copepods and were observed defecating directly above monitored mussel beds, confirming them as major vector of pristane transmission.

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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Table of Contents

List of Tables

List of Figures

List of Appendices

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 fats 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 fat 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 from 27 stations once every 2 weeks in spring, and monthly during summer, and then analyzed for pristane to document seasonal concentration changes. Mussels were also collected from additional stations to evaluate the variability of pristane measurements in mussels over kilometer-scale distances, and to examine the response of concentrations in mussels following large-scale releases of juvenile pink salmon from hatcheries. In the laboratory, pristane is extracted from mussels with pentane and then isolated and measured by flame ionization gas-chromatography. Seasonal incorporation of pristane by mussels at a station is summarized by a pristane accumulation index (PAI), calculated as the numerical approximation of the integral of pristane concentration in mussels and time. The PAI is used to compare results among stations geographically. The sum of these PAI's across stations sampled the same year permits an indirect comparison of fecal production among years.

Pristane concentrations in mussels initially increased at stations peripheral to the marine trench system of northwest PWS by mid-March in both 1995 and 1996, but not in 1989. The cause of these late-winter increases is not known. A second increase occurred in late April, which also occurs first at stations peripheral to the marine trench system of northwest PWS, and progressively later at stations closer to the Gulf of Alaska. The onset of the second increase was within 1 week in 1995 and 1996, with 1995 the more delayed. Comparison of PAIs showed that most stations were not significantly different in 1995 and 1996, and the sum of these PAI's across stations were nearly identical for the 2 years. This indicates similar marine feeding conditions nearshore for juvenile pink salmon and herring during spring of these years.

Field experiments were conducted in 1996 to study the effects on pristane concentrations in mussels consequent to a large release of juvenile pink salmon from the Prince William Sound Aquaculture Corporation (PWSAC) hatchery at Esther Island in northwest PWS. About 120 million juvenile pink salmon were released from the hatchery the evening of 3 May. Mussels were collected from 3 stations within 5 km of the hatchery daily beginning about a week prior to the release and continuing about a week afterward. Pristane concentrations in the mussels increased sharply 2 to 6 days following the release, to concentrations that approached the highest observed in mussels sampled anywhere for this project. Two days after the release, the stomachs of released salmon typically contained more than a dozen *Neocalanus plumchrus*, which was the most prevalent ingested prey item both by mass and by numbers of individuals, and which were the most prevalent large zooplankton prey present in the immediate release area. Released salmon were also observed defecating directly above the monitored mussel collection stations. Together, these results confirm that juvenile pink salmon are a major vector for pristane transmission from copepods to mussels in PWS.

Other related field experiments showed that 2 alternative possible routes of pristane incorporation into mussels are probably minor. One possibility is that fecal pellets produced by the *Neocalanus* copepods themselves may contain pristane, and these pellets may be then ingested directly by the mussels (these copepods are themselves too large for mussels to ingest). However, pristane concentrations in fecal pellets collected from *Neocalanus* copepods were less than one tenth the concentrations of fecal matter produced by fish preying on these copepods. The copepod fecal pellets are produced at water column depths that are below the layer filtered by mussels, and the pellets sink relatively quickly. Compared with fecal matter produced by juvenile salmon, the copepod pellets are probably a minor source of the pristane found in mussels. Also, pristane concentrations measured in seawater that contained relatively high densities of *Neocalanus* copepods (>1 animal/L) were below method detection limits (about 25 ng pristane/L), suggesting that relatively little pristane is incorporated by mussels from seawater containing pristane dissolved out of the bodies of *Neocalanus* copepods.

This project enlisted the cooperation of the Prince William Sound Aquaculture Corporation (PWSAC) and the PWS Youth Area Watch (YAW) program (project 96210) to assist with collection of mussels from remote locations. Staff at PWSAC collected mussels from 4 hatchery locations in PWS, and YAW students collected mussels from several other

remote locations. These collections substantially lower project costs, because sample collection is the most expensive component of this project. It also enables explicit incorporation of PWSAC hatcheries into geographic and inter-annual comparisons of project results, so that nearshore feeding conditions assessed near the hatcheries may be directly related to the rest of PWS.

Students in the YAW program participated in a joint mussel collection cruise with this project. Staff from the Auke Bay Laboratory (ABL) demonstrated mussels collection methods and record keeping requirements to students on a YAW-sponsored cruise in PWS. Stations sampled during this and subsequent collection cruises showed that mussels had surprisingly high concentrations of pristane in February, 1996, and that mussels collected from a single location may be representative of surrounding area to distances of about a kilometer. These students also traveled to the ABL to learn more about the project and to participate in the sample analysis process. Staff at ABL presented a detailed explanation of the project, including the chemical analysis principles involved, followed by student participation in the mussel dissection, tissue processing, and chemical analysis procedures.

Another objective of this project in 1996 was to evaluate the dietary dependence of predators of juvenile pink salmon and larval herring on an alternative prey species, the copepod *Neocalanus plumchrus,* in PWS. Predators that may target *Neocalanus* copepods during years of high abundance may switch to juvenile pink salmon or herring during years of low copepod abundance, thereby intensifying predation pressure. If these predators prefer *Neocalanus* when abundant, then relatively high pristane concentrations should be present in them. To address this objective, samples of 5 fish and 1 squid species were to be collected by another project in May and in the fall, and analyzed for pristane. Unfortunately, the samples could not be collected, so this objective was not met.

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 Prince William Sound 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.

The proposed project will provide evidence that may be used to evaluate why populations of pink salmon and herring are not recovering. One of the major natural factors hypothesized as a constraint on the recovery potential for these species is prey-switching by predators on the

larval and juvenile stages. Under this hypothesis, predators are thought to concentrate on larval and juvenile pink salmon and herring predation in years of low copepod abundance, but switch their concentration to copepods in years of higher abundance. The proposed project addresses this hypothesis in two ways: first, by identifying unrecognized "pre-switching predators", and second, by indirectly monitoring survival through juvenile stages. Identification of prey-switching predators will permit subsequent evaluation of whether the identified species really do substantially determine recruitment success of pink salmon and herring.

Annual monitoring of pristane concentrations in mussels throughout Prince William Sound will permit an indirect evaluation of whether pink salmon and herring survival through the juvenile life stages primarily determines year class strength. In addition, the monitoring will identify important marine nursery areas for these species, the conservation of which may promote their recovery. Monitoring pristane in mussels will be necessary for at least 5 consecutive years to provide a minimal statistical basis for any observed relationship between variation of pristane concentrations in mussels and recruitment success of pink salmon and herring.

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 can be the dominant marine herbivores in Prince William Sound (PWS) during the spring phytoplankton bloom. They are consequently important prey during the reproductive period 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 conducted under this project at the Auke Bay Laboratory and in the field confirm that an important route of pristane accumulation in these filter feeders is through ingestion of fecal material derived from predators of *Calanus* and *Neocalanus*, e.g. 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.

These results suggest that tissue analysis of pristane may be used to investigate the PWS marine ecosystem in at least 3 ways. First, such analyses may identify predators that have a direct dietary dependence on *Calanus* and *Neocalanus*, and these predators may include heretofore unrecognized "prey-switching" species that switch predation to larval herring and

juvenile salmon in years of relatively low copepod abundance. Prey-switching has been hypothesized as major determinant of pink salmon and herring recruitment success in the SEA studies. Second, 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 commercially important predators such as herring and pink salmon. This may provide a relatively inexpensive indicator of survival through the early juvenile stages for these species. Finally, the monitoring program may 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 e.g. juvenile salmon and herring, which is necessary for determining the restoration of these resources.

Comparison of results from the 1996 field season with results for 1994 and 1995 consistently show a pristane "bloom" in mussels that begins in April at stations near the deep-water trench system of the northwestern sound, and radiates toward the Gulf of Alaska as the season progresses. These results support the idea that the trench system provides over-wintering habitat for *Calanus* and *Neocalanus*, and is therefore a kind of "essential marine habitat" for the copepods and predators that depend on them. Extensive field experiments were conducted to verify the hypothesized pathway of pristane into mussels. Juvenile pink salmon were confirmed as important vectors for the transmission of pristane to mussels through their feces. Two alternative pathways, direct uptake of pristane from seawater, and uptake via fecal pellets produced directly by *Calanus* and *Neocalanus*, were invalidated by the experiments. These tests were in part conducted in place of objective #1 below, which addresses the dietary dependence on *Calanus* and *Neocalanus* of fish and squid predators of pink salmon and larval herring. This objective was not met because insufficient samples of fish and squid were collected.

Objectives

This project has 3 objectives:

1. Measure concentrations of pristane in 500 tissue samples of 5 fish and 1 squid species to evaluate the dietary dependence of these juvenile pink salmon and larval herring predators on an alternative prey species, the copepod *Neocalanus plumchrus,* in PWS (FY96 only).

2. 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 (FY96 - FY00).

3. 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 (FY96 - FY00).

Methods

Mussel Collection

The seasonal variability of pristane concentrations in mussels (*Mytilus trossulus*) is based on collections from 36 stations in Prince William Sound (fig. 1, table 1). Mussels are collected biweekly, beginning about mid-March through June 1, then July 1 and August 1 for a total of 9 collection periods and 324 mussel samples. The collection frequency is initially higher 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 36 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. The remaining 6 stations are sampled by volunteer primary and secondary school students in collaboration with the PWS Youth Area Watch program (project 96210).

Mussels (20) 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.

Hatchery Release Study

An unanticipated, large-scale release of hatchery pink salmon into PWS presented an opportunity to study the response of mussels to pristane produced by the released juveniles. Over 120 million juvenile pink salmon were released from the PWSAC hatchery at Esther Island the evening of 3 May 1996. The release was timed to coincide with the establishment of a high population density of *Neocalanus spp.* in adjacent waters. Mussels were sampled from each of 3 stations within 5 km of the hatchery for 4 consecutive days prior to the release, and for 4 of the following 6 days following. Some of the released pink salmon were observed defecating over the sampled mussel beds, and were sampled for stomach content analysis to verify predation on *Neocalanus*. Also collected during this period were samples of seawater and of *Neocalanus spp.* fecal pellets to verify alternative pathways for pristane accumulation by mussels. Seawater samples were collected at least 200 m from shore.

Collection of Other Tissues

Insufficient samples were collected to pursue objective 1.

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 will be determined by adding a second internal standard prior to instrumental analysis. Method detection limits will be assessed annually for the mussel tissue matrix, and these detection limits will be assumed for the other matrixes analyzed. Based on previous performance, we anticipate accuracy of $\pm$ 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 $\pm$ 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 and percent lipid will also be determined in samples so that results may be analyzed on dry weight and lipid weight bases. Dry weights will be determined by heating samples at 60 C to constant final weight. Lipid proportions will be determined from weight loss due to dichloromethane extraction.

Data Analysis

Pristane concentrations in mussels are analyzed statistically using least-significant difference (LSD) criteria based on an extensive sampling of the error distribution for these measurements. An error distribution for log-transformed pristane concentrations in mussels is generated from 178 triplicate and 79 duplicate samples analyzed for the Exxon Valdez oil spill, which are contained in the EVTHD. These replicated samples were collected and analyzed by the same methods, and they all contained pristane concentrations above method detection limits. The variances of these replicates are homoscedastic after log transformation, so a distribution for differences of two random samples of the error distribution can be generated by Monte Carlo simulation. Based on this distribution of differences, the LSD at an $\alpha = 0.05$ type I error rate is about 1.015, which corresponds to a ratio of about 2.75 for un-transformed data. Thus, mussels from two different samples are judged significantly different if the ratio of the larger pristane concentration to the smaller is more than 2.75. The power of

this test to detect an actual increase of 3 is about 58%, again derived from Monte Carlo simulation of the error distribution. Since pristane concentrations in mussels typically increase by factors of greater than 10 during the season, the power of the sampling design is more than adequate.

Propagation of errors for derived indexes indicates that 66% increases of the pristane accumulation index (PAI) are significant at the $\alpha = 0.05$ type I error rate. The PAI represents the productivity of near-shore *Neocalanus* consumers in one sampling season. The PAI is calculated as the product of pristane concentration and sampling interval, and is an approximation of the integral of concentration and time at each station. The power of these criteria to detect an actual doubling of the PAI is about 80%, estimated by Monte Carlo simulation. The power to detect differences among years for the sum of the PAI's across stations is even greater, due to the larger number of measurements involved: increases of 22% are significant, and the power to detect such increases when they occur is about 50%.

Results

Pristane Concentrations in Mussels

Substantial concentrations of pristane were present in mussels at many of the western stations during the first sampling period of 96 March 18-25 (fig. 2A). Concentrations ranged to 3,790 ng/g dry weight at Esther Island and to 3,100 at Point Pakenham. Concentrations in the range 1,000 - 3,000 ng/g were present at about half the remaining stations adjacent to the deep marine trench system of the northwest sound. Pristane concentrations were consistently less than 1,000 ng/g in the eastern sound. This pattern of pristane concentrations remained little changed through the end of April (fig. 2B & 2C).

Pristane concentrations increased sharply in early May (fig. 2D & 2E). Concentrations exceeding 10,000 ng/g occurred at 8 stations sampled May 1 - 20. Most of these stations were also associated with the marine trench system of the northwest sound, although concentration increases occurred at stations in the rest of the sound as well during this period. The highest concentration observed was 69,600 ng/g at Point Eleanor, and concentrations exceeding 20,000 ng/g occurred at Esther Island and Foxfarm.

Pristane concentrations begin to decline at most stations by the end of May (fig. 2F), and this decline continues through late-July/early-August (fig. 2G & 2H). The highest pristane concentrations observed during the last sampling period were 4,120 and 4,540 ng/g at Main Bay and Point Pakenham, and concentrations at all the remaining stations were less that 1,900 ng/g.

Pristane Accumulation Index (PAI)

The highest PAI were associated with stations adjacent to the marine trench system of the northwest sound (fig. 3). The highest PAI was 2,510,000 ng-d/g at Point Eleanor followed by

1,100,000 ng-d/g at Esther Island and 841,000 ng-d/g at Foxfarm. the PAI was consistently less than 300,000 ng-d/g at southeastern stations. The total PAI summed across stations was 10,900,000 ng-d/g.

Hatchery Release Study

Pristane concentrations in mussels near Esther Hatchery increased sharply following the juvenile salmon release there. Concentrations about tripled within 2 to 6 days after the release (fig. 4). Released juveniles were captured by dip-net the day following release, and analysis of stomach contents indicated near-exclusive predation on *Neocalanus spp.* These juveniles were captured at the sampled mussel beds, and were observed defecating directly above the beds.

Pristane concentrations were also measured in fecal pellets produced by *Neocalanus spp.* Copepods collected by 0.5 mm mesh plankton net were kept is seawater for 1 - 2 hrs in a stainless steel bowl lined with 0.064 mm mesh plankton netting. *Neocalanus spp.* were the numerically dominant copepods collected, and were removed from the bowl with the liner. The fecal pellets remaining in the bowl were concentrated by gravity into successively smaller volumes after discarding supernatants, then dried and stored at -20 C until analysis. The pristane concentration of the pellets was 80,200 ± 35,700 ng/g dry weight (95% CI, n = 8). The sinking rates of these pellets ranged from 2.1 to 3.0 m/hr, based on the time required to fall through a 22.5 cm column of 31 ‰ seawater at 5 C.

Pristane concentrations in seawater were consistently below method detection limits (about 25 ng/L), both before and after pink salmon were released from the hatchery.

Youth Area Watch (YAW)

Staff from this project participated on a YAW cruise from January 30 to February 2, 1996 to explain the project and to demonstrate mussel collection methods to students from 5 PWS communities. Mussels were collected from the Foxfarm station on southern Elrington Island (Foxfarm 1), and from two additional stations established about 1 km in opposite directions (Foxfarm 2 & 3) from Foxfarm 1. The additional stations were established to examine variability of pristane concentrations in mussels across kilometer spatial scales, and sampling at these stations continued during the regular collection periods of the rest of the season. Pristane concentrations in mussels were surprisingly high in early February, ranging from 2,430 to 4,640 ng/g. Concentrations were significantly different for only 1 of the 9 samplings (May 16 - 20), when concentrations were 5,080 ng/g at the Foxfarm 1 station, compared with 35,900 ng/g at Foxfarm 3.

Four YAW students visited the ABL during April 10 - 12 to learn about the project and participate in the sample analysis process. Staff at ABL presented a detailed explanation of the

project, including the chemical analysis principles involved, followed by student participation in the mussel dissection, tissue processing, and chemical analysis procedures.

Supplemental educational materials, produced under this project on beach topography, were used by YAW students that participated on the May 1996 cruise of the Kenai Explorer out of Seward.

Discussion

1. Association with marine trench system

The cause of the relatively high pristane concentrations detected in mussels of northwest PWS in March 1996 is obscure. Comparable concentrations were also detected late March of 1995 (Appendix I, fig. A-1A), but not in 1989 (EVTHD). The association of mussels that had higher concentrations of pristane in March of 1995 and 1996 with stations either adjacent to the marine trench system of northwest PWS or else down-current of it suggests that the proximate pristane source may be *Neocalanus spp.* copepodites produced in the deeper parts of the marine trench system. However, the process that permits incorporation of pristane into mussels is not clear. Direct ingestion of copepodites by mussels is a possibility, although the pristane content of copepodites is not known. The low pristane concentrations of mussels in March 1989, a year of exceptionally high *Neocalanus spp.* abundance (Salmon, personal communication), suggests that incorporation of copepodites by mussels is not a substantial route of pristane accumulation. Similarly, herring were very abundant in PWS in 1989 (Brown, personal communication), so fecal material produced by herring predation on *Neocalanus spp.* in March 1989 was probably not a substantial accumulation route either. Alternatively, pollock have invaded PWS since 1989 and may filter-feed on *Neocalanus spp.*, so perhaps the March accumulations of pristane in 1995 & 1996 in mussels are due to fecal production by pollock feeding on *Neocalanus spp.*, but this is speculative at present.

2. Timing comparison of 1995 & 1996

The sharp increase of pristane concentrations in mussels during early May, 1996 is consistent with 1995 results to within about a week. Comparison of results for 1995 and 1996 suggests that the May increase may have been delayed somewhat in 1995, but timing differences of 1 week are at the limit of resolution of the sampling frequency.

3. PAI comparison of 1995 & 1996

Comparison of 1995 and 1996 PAI values indicates that differences at most stations were

not significant. Significant increases in 1996 occurred at Esther Island and at Point Eleanor, and significant decreases at Fairmont Island, Olsen Bay, and Perry island, based on the LSD criteria for significance. Differences at remaining stations were not significant. The total PAI was similar in 1995 and 1996 at 10,300,000 ng-d/g and 10,900,000 ng-d/g (compare fig. 3 with Appendix I, fig. A-2) The stations that contributed most to the total PAI were northwest of a line running along Bligh Island to Montague Strait both years. The geographic distribution pattern and similarity of total PAI for both years indicates similar conditions conducive for near-shore fecal production derived from predation on *Neocalanus spp.* in 1995 and in 1996. To the extent that these conditions modulate juvenile salmonid survival during the early marine phase, these results suggest that juvenile salmonid marine survivals during these years should be similar. In addition, the consistent patterns of geographic distribution both years suggests continued higher productivity of northwest PWS.

4. Variability across km distances

The similarity of pristane concentrations found in mussels from the 3 Foxfarm stations indicates that sampling from mussel beds may be representative of km-sized areas, at least when abundances of copepods and their predators are high. The Foxfarm stations are located on the migration path of juvenile salmon released from the AFK hatchery on Evans Island. Released salmon essentially flood the area, and probably prey heavily on *Neocalanus spp* that are flushed from PWS through the southwest passages. The general concordance of results for the Foxfarm stations therefore suggests that significant differences among stations elsewhere in PWS may not be simply due to differences of small-scale factors such as local water circulation.

5. Mechanism & Response Times

The hatchery release study provided considerable evidence confirming fish feces produced consequent to predation on *Neocalanus spp.* by juvenile pink salmon as the route of pristane accumulation by mussels. The observations that juveniles released from the hatchery preyed heavily on *Neocalanus spp.*, but spent considerable time near-shore and defecated directly above mussel beds, which consequently led to substantial increases in pristane concentrations in mussels confirms pink salmon as an important vector mediating transfer of pristane from copepods to mussels in PWS. The fact that subsequent pristane concentrations in mussels at Esther Island (23,900 ng/g) are within a factor of 3 of the highest concentrations observed in PWS (69,600 ng/g at Point Eleanor) suggests that pink salmon juveniles have the ability to account for all of the concentration increases observed at most stations. These results restrict the alternative species that may cause significant contributions to pristane concentration increases in mussels in May to near-shore residents that prey on *Neocalanus spp.* (such as sandlance). In contrast, the contribution of *Neocalanus spp.* predators that feed and defecate farther off-shore is probably not substantial, due to the brief residence time of the fecal material produced in the upper part of the water column accessible by mussels.

The contribution of pristane in fecal pellets produced by *Neocalanus spp.* to mussels is probably negligible, because the residence times of these pellets is also brief, and the pristane concentration is considerably lower than that of pink salmon feces during periods of heavy predation on *Neocalanus spp.* The density of these copepods usually increases substantially over the first few hundred meters from shoreline. In order for mussels to incorporate fecal pellets produced by these copepod, the residence time of the pellets in the mixed layer must be sufficiently long to permit horizontal transport to mussel beds. The measured sinking rates suggest that this residence time is less 1 day, since most of the pellets are produced at depths below 5 - 10 m. These pellets are therefore probably a minor source of pristane in mussels. The failure to detect pristane in seawater indicates that pristane dissolved into seawater from the bodies of living *Neocalanus spp.* was not a major source either. It is therefore tentatively concluded that the May increase of pristane concentrations in mussels is due to feces produced by juvenile pink salmon, or by other predators of *Neocalanus spp.* that occupy the same feeding niche.

Conclusions

1. Increases of pristane in mussels during spring begins a stations adjacent to the marine trench system of northwest PWS and then radiates outward toward the Gulf of Alaska.

2. Pristane increases appeared to occur in 2 stages in 1995 and 1996, with the first stage in March and the second in early May. The March increase did not appear in 1989. The onset of the second increase of early May was probably within 1 week in 1995 and 1996.

3. Juvenile pink salmon are a major vector of pristane transmission from *Neocalanus spp.* to mussels by preying on *Neocalanus spp.* and then defecating near mussel beds. Any other major vectors probably occupy the same feeding niche as juvenile pink salmon during spring in PWS.

4. The production of pristane-rich fecal material nearshore in PWS was similar in 1995 and 1996, suggesting similar feeding conditions for the early marine stages of pink salmon during these 2 years.

5. Samples of mussels collected from single beds may be representative of conditions at kilometer spatial scales, at least when abundances of juvenile pink salmon and of *Neocalanus spp.* are simultaneously high.

6. The relation of pristane concentrations in mussels to pink salmon catches and escapements the following year will be an on-going major goal of this study in the future.

Literature Cited

Avigan, J., and M. Blumer. 1968. On the origin of pristane in marine organisms. Journal of Lipid Research **9**:350-352.

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.

Other References

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Salmon, D. K. 1994. Personal communication - fax of Prince William Sound coastal convergence and zooplankton abundance at the PWSAC AFK hatchery.

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

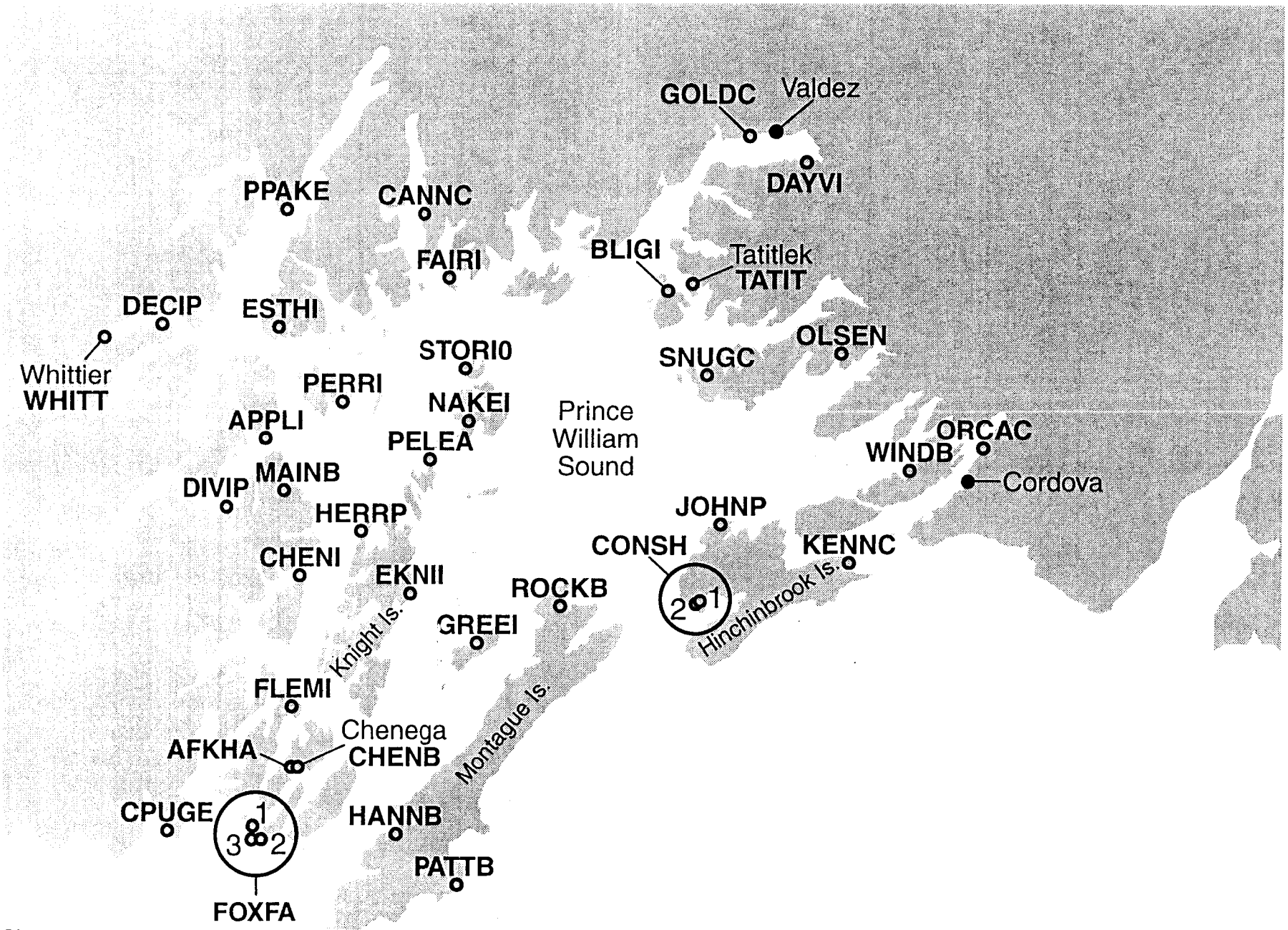
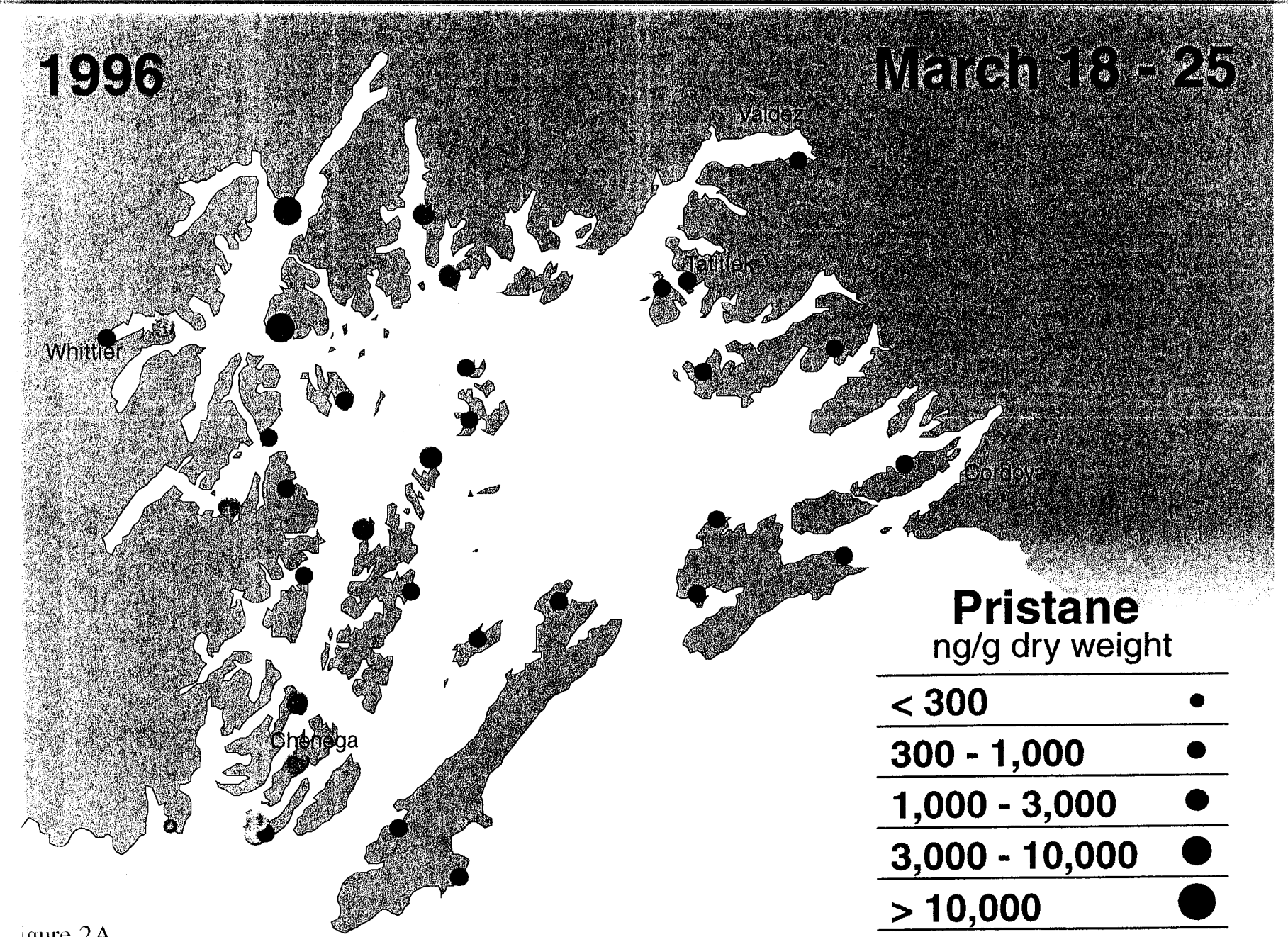
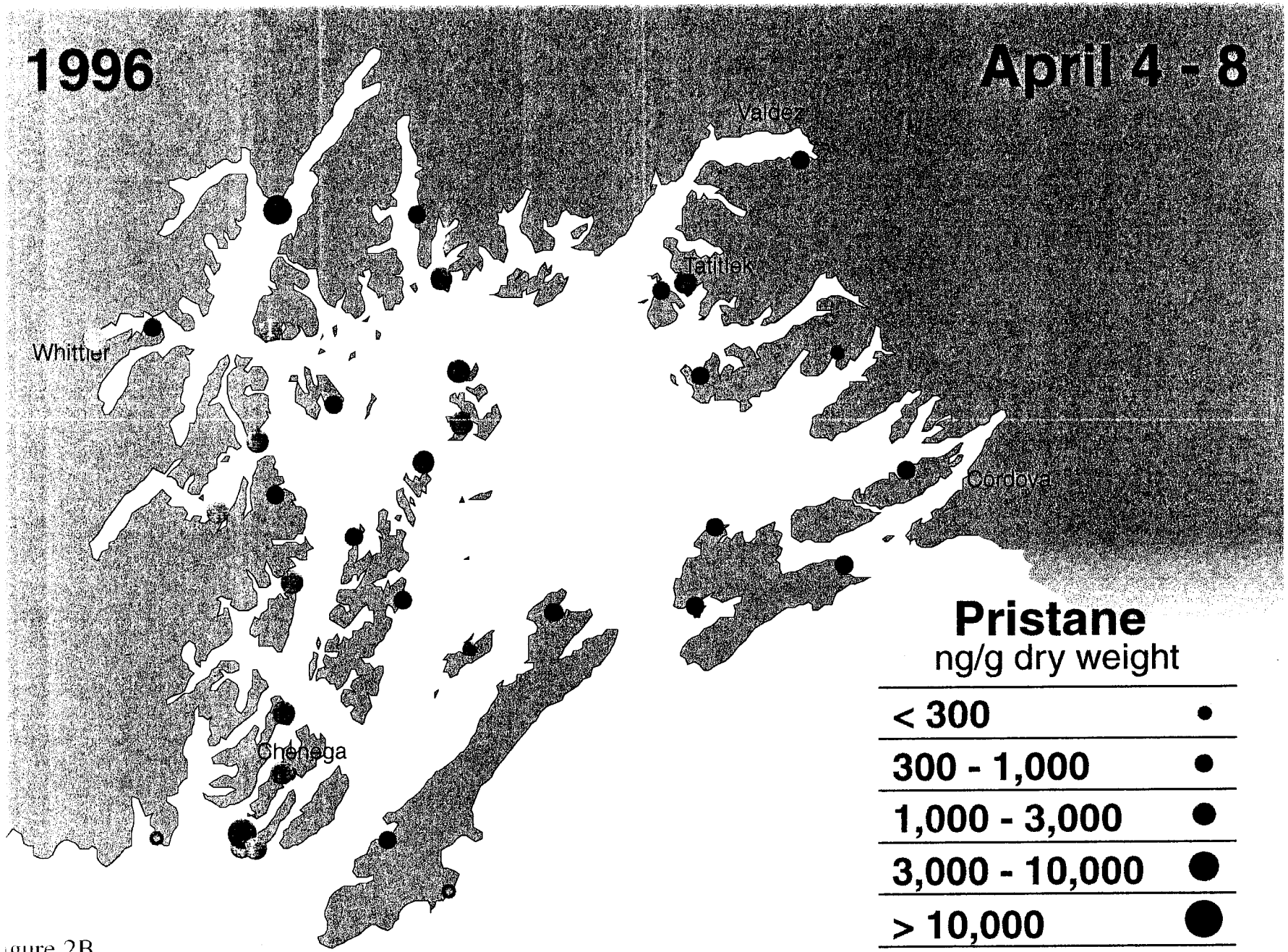

GOLDC
Valdez
DAYVI
PPAKE
CANNC
FAIRI
BLIGI
Tatitlek
TATIT
DECIP
ESTHI
OLSEN
SNUGC
STORI0
Whittier
WHITT
PERRI
NAKEI
APPLI
PELEA
Prince
William
Sound
ORCAC
WINDB
Cordova
MAINB
DIVIP
HERRP
JOHNP
CONSH
KENNC
CHENI
EKNII
ROCKB
Knight Is.
GREEI
Hinchinbrook Is.
FLEMI
Montague Is.
Chenega
AFKHA
CHENB
CPUGE
HANNB
PATTB
FOXFA

Figure 1


1996
March 18 - 25
Valdez
Tatitlek
Whittier
Cordova
Chenega
Pristane
ng/g dry weight
< 300
300 - 1,000
1,000 - 3,000
3,000 - 10,000
> 10,000

igure 2A


1996
April 4 - 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

igure 2B

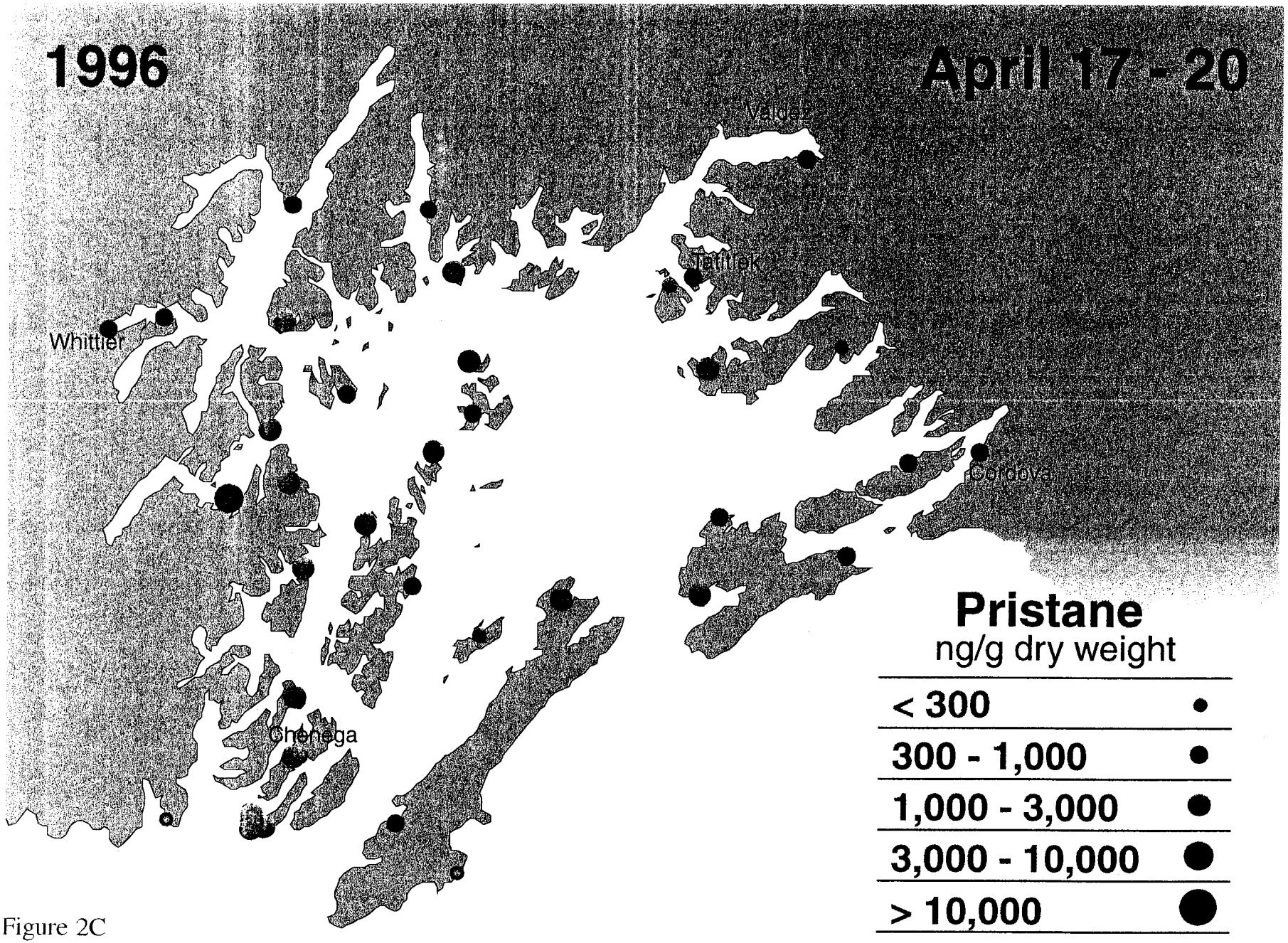

1996
April 17 - 20
Valdez
Tatitlek
Whittier
Cordova
Chenega
Pristane
ng/g dry weight
< 300
300 - 1,000
1,000 - 3,000
3,000 - 10,000
> 10,000

Figure 2C

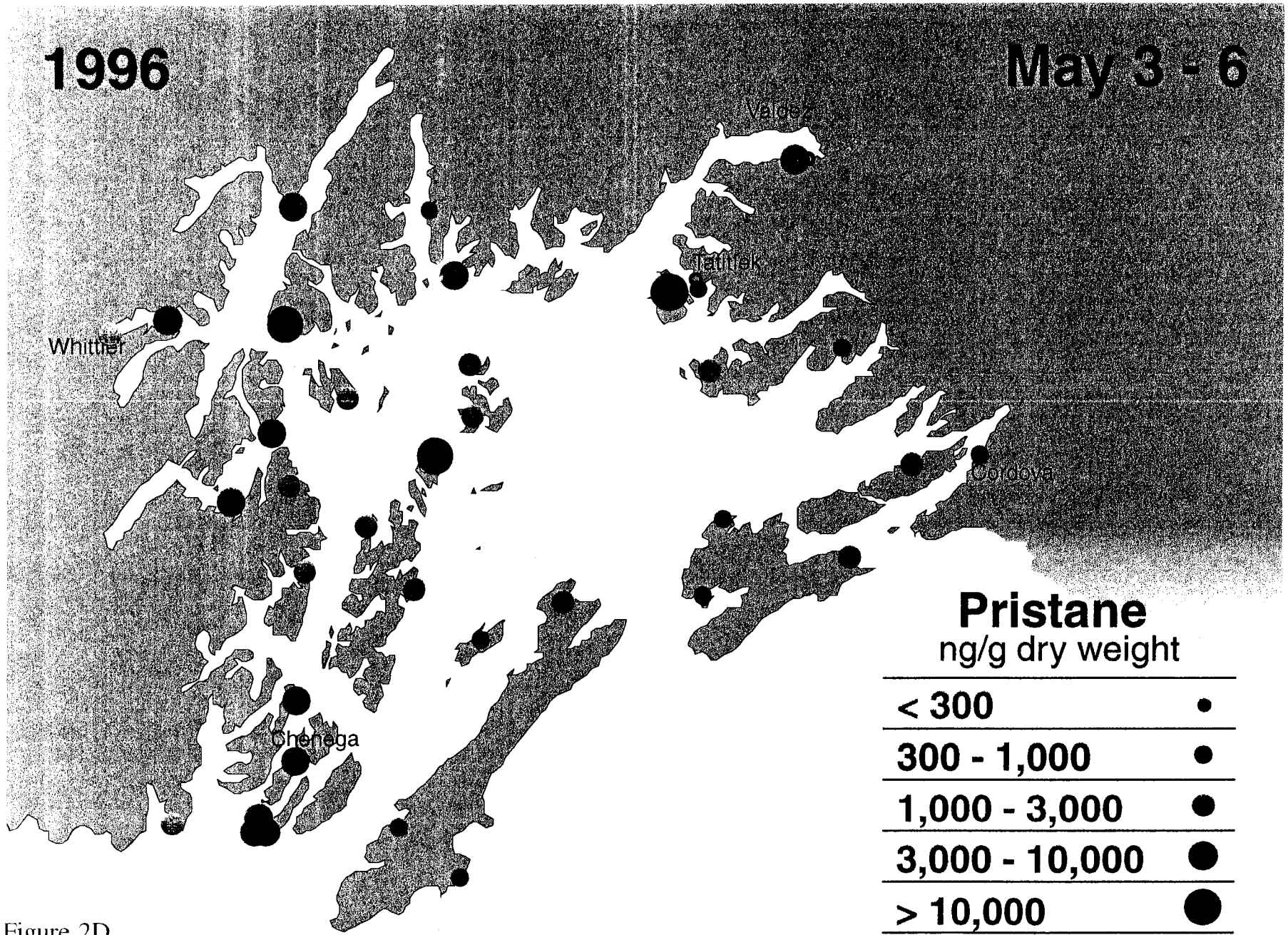
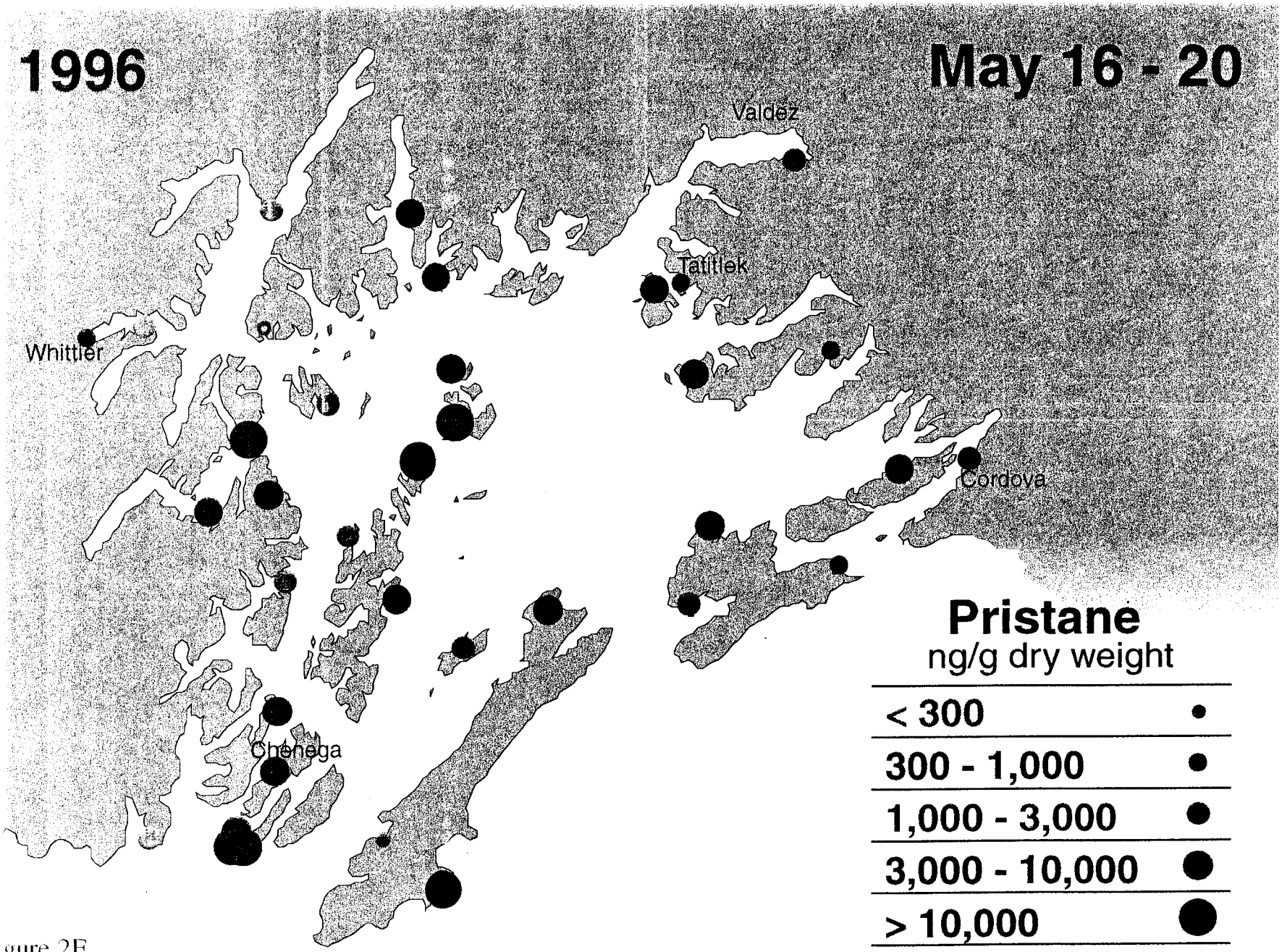
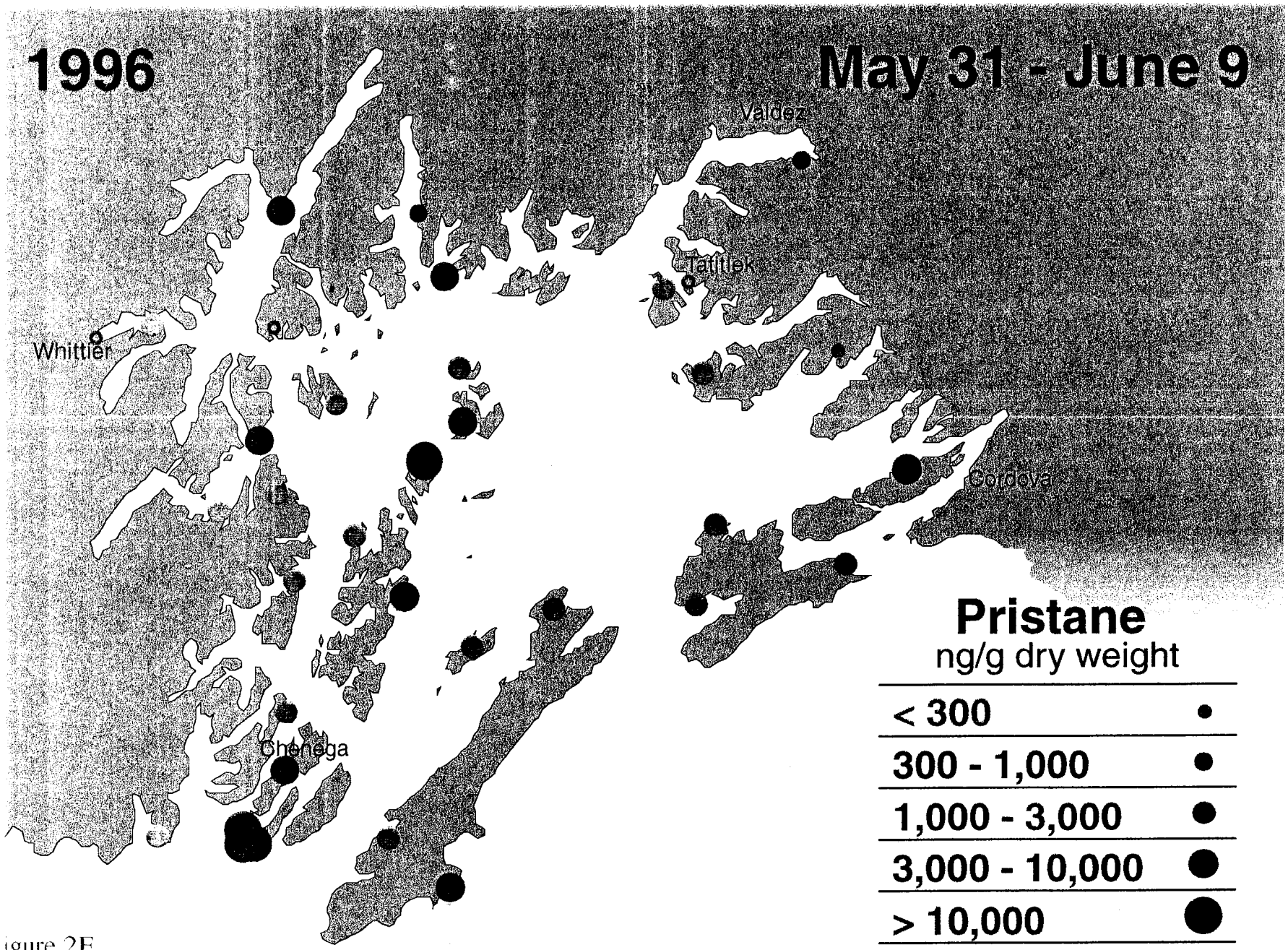

1996
May 3 - 6
Valdez
Tatitlek
Whittier
Cordova
Chenega
Pristane
ng/g dry weight
< 300
300 - 1,000
1,000 - 3,000
3,000 - 10,000
> 10,000

Figure 2D


1996
May 16 - 20
Valdez
Tatitlek
Whittier
Cordova
Chenega
Pristane
ng/g dry weight
< 300
300 - 1,000
1,000 - 3,000
3,000 - 10,000
> 10,000

gure 2E


1996
May 31 - June 9
Valdez
Tatitlek
Whittier
Cordova
Chenega
Pristane
ng/g dry weight
< 300
300 - 1,000
1,000 - 3,000
3,000 - 10,000
> 10,000

igure 2F

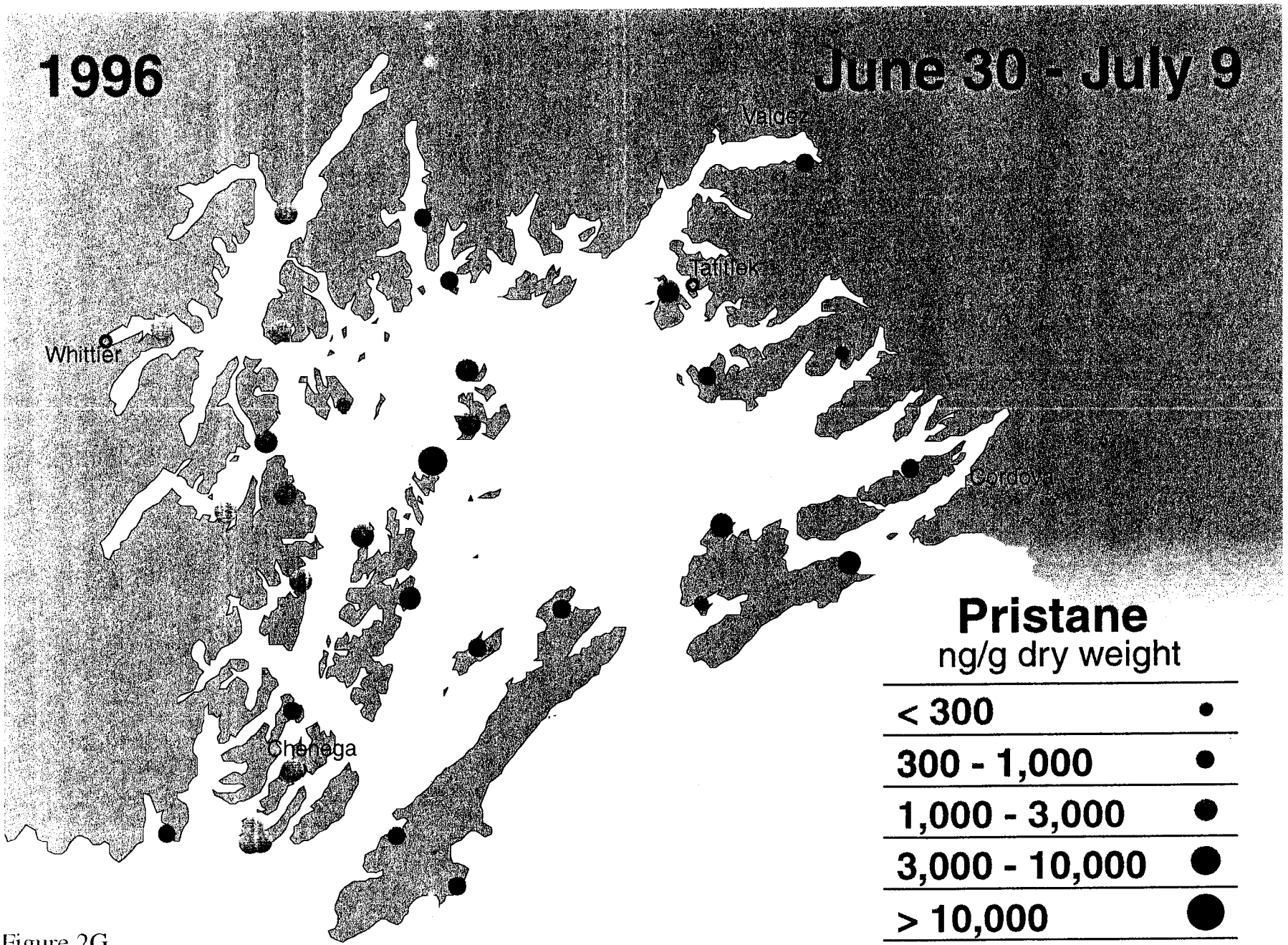

1996
June 30 - July 9
Valdez
Tatitlek
Whittier
Cordova
Chenega
Pristane
ng/g dry weight
< 300
300 - 1,000
1,000 - 3,000
3,000 - 10,000
> 10,000

Figure 2G

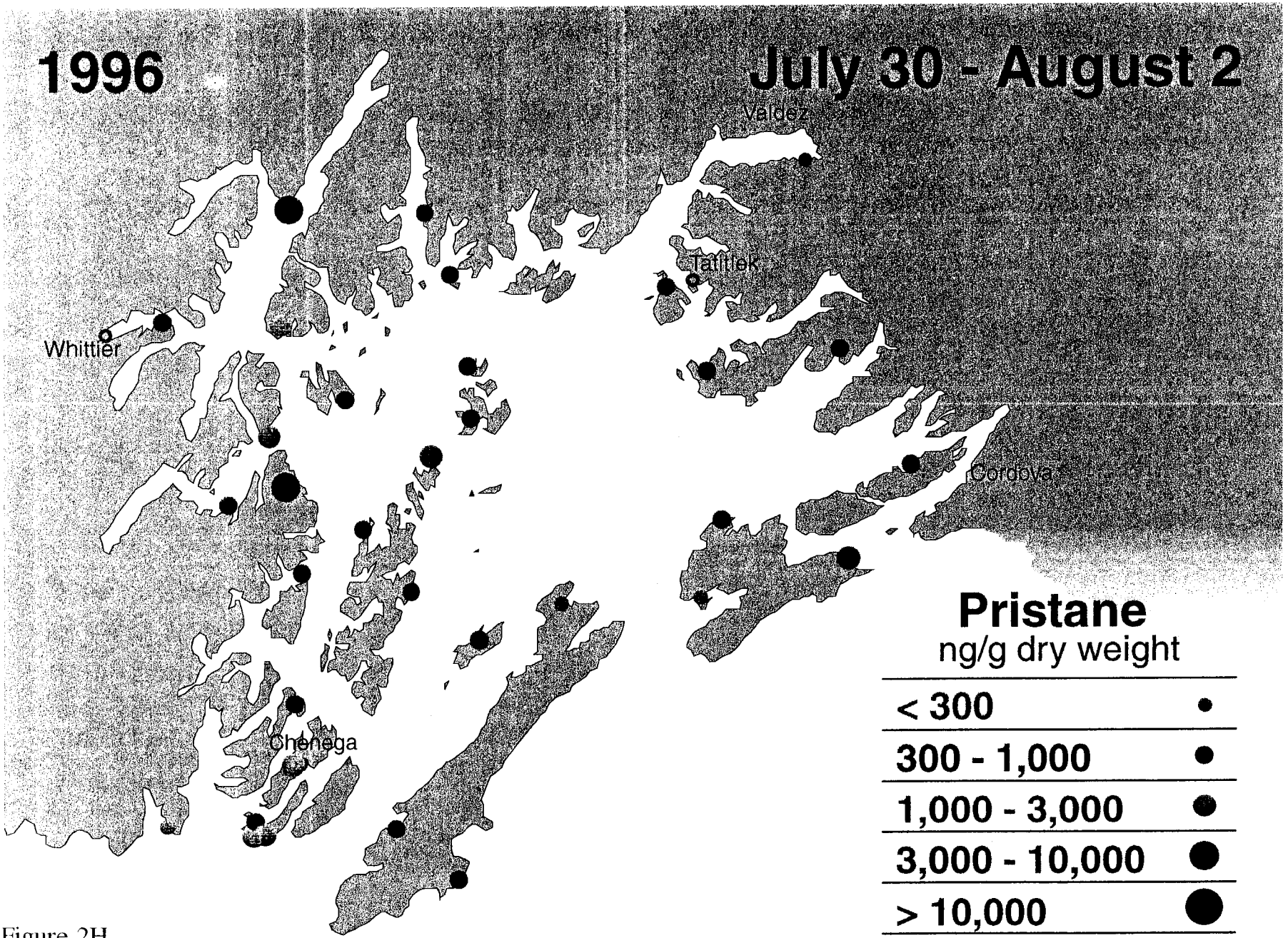
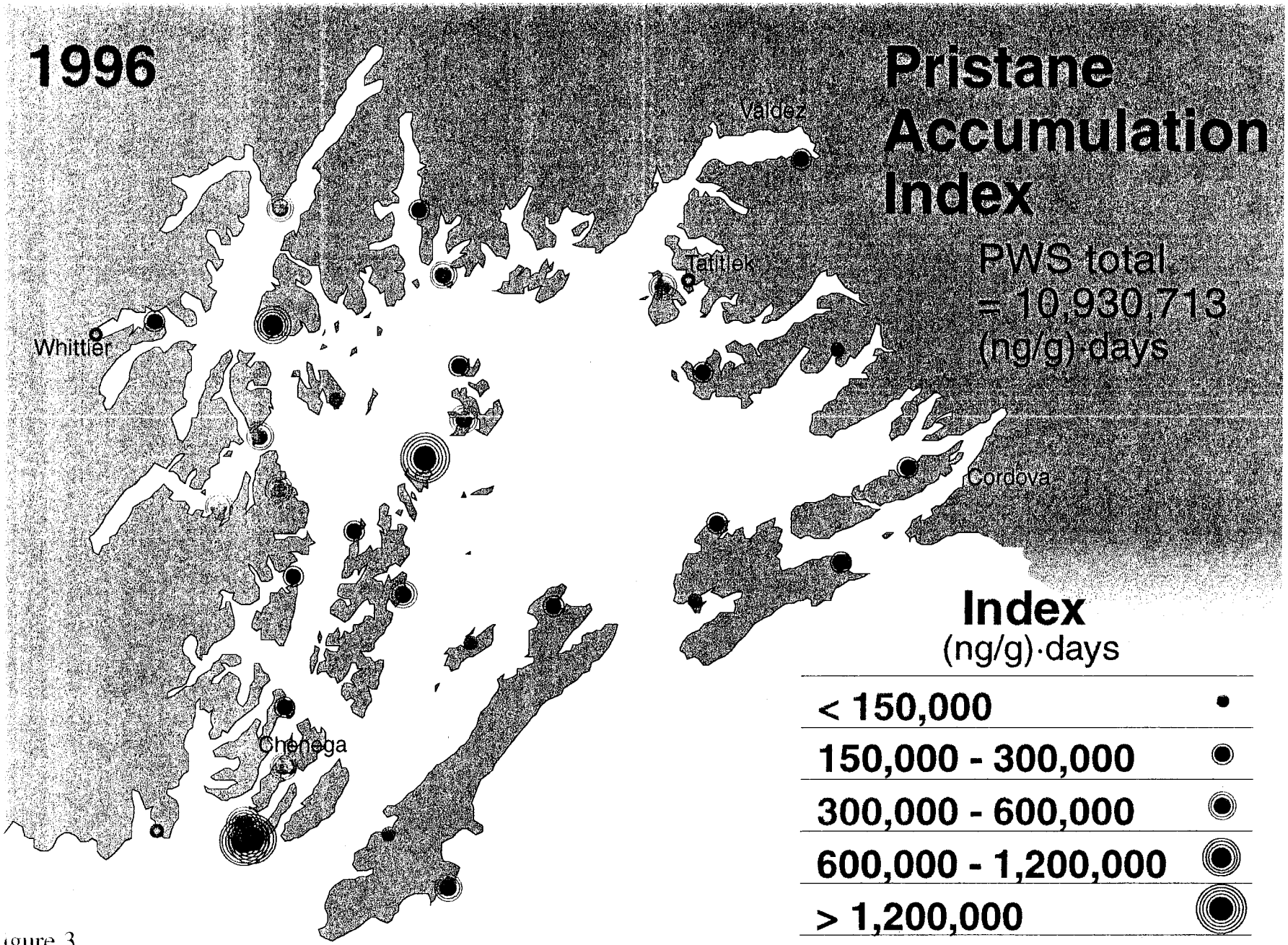
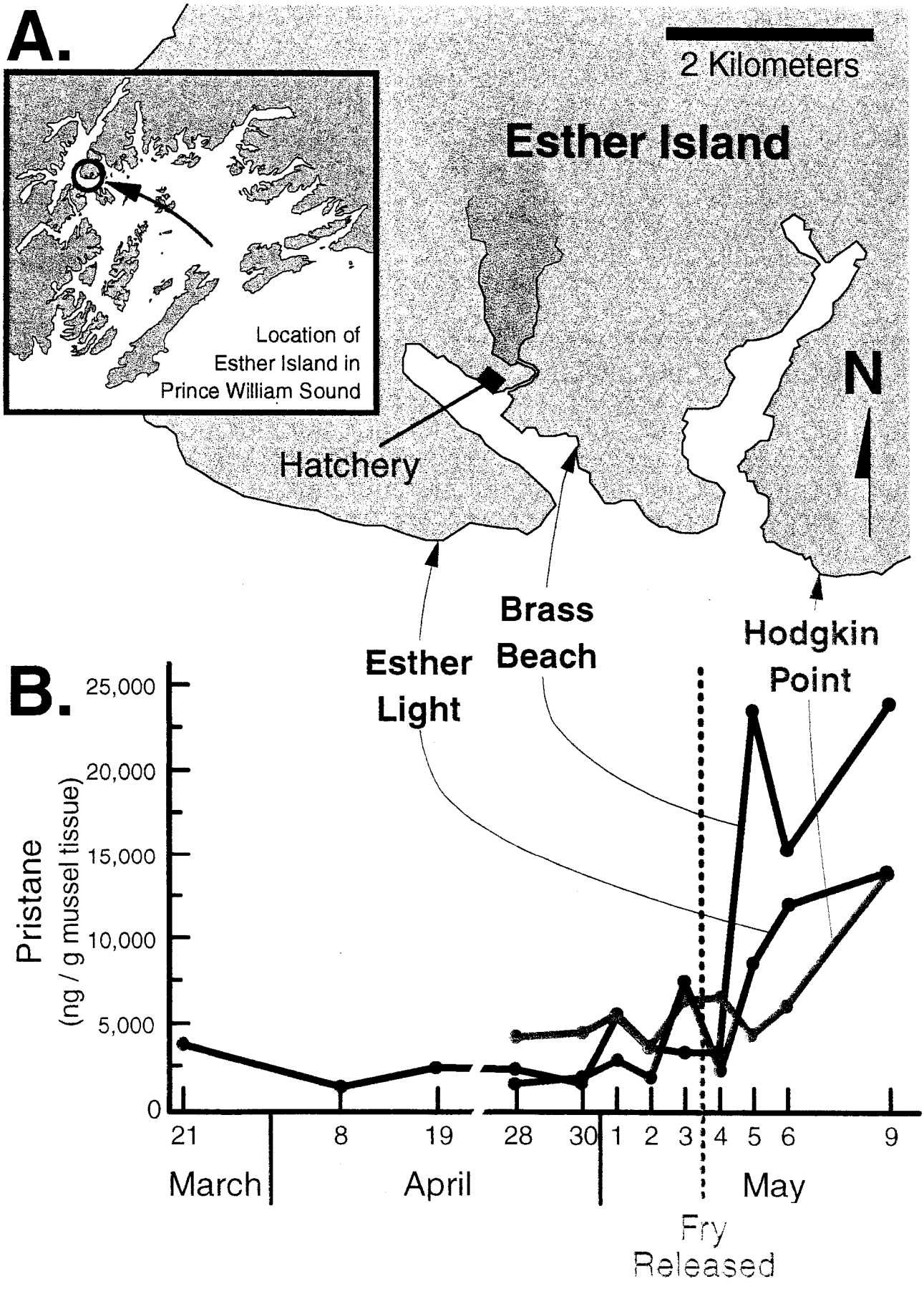

1996
July 30 - August 2
Valdez
Tatitlek
Whittier
Cordova
Chenega
Pristane
ng/g dry weight
< 300
300 - 1,000
1,000 - 3,000
3,000 - 10,000
> 10,000

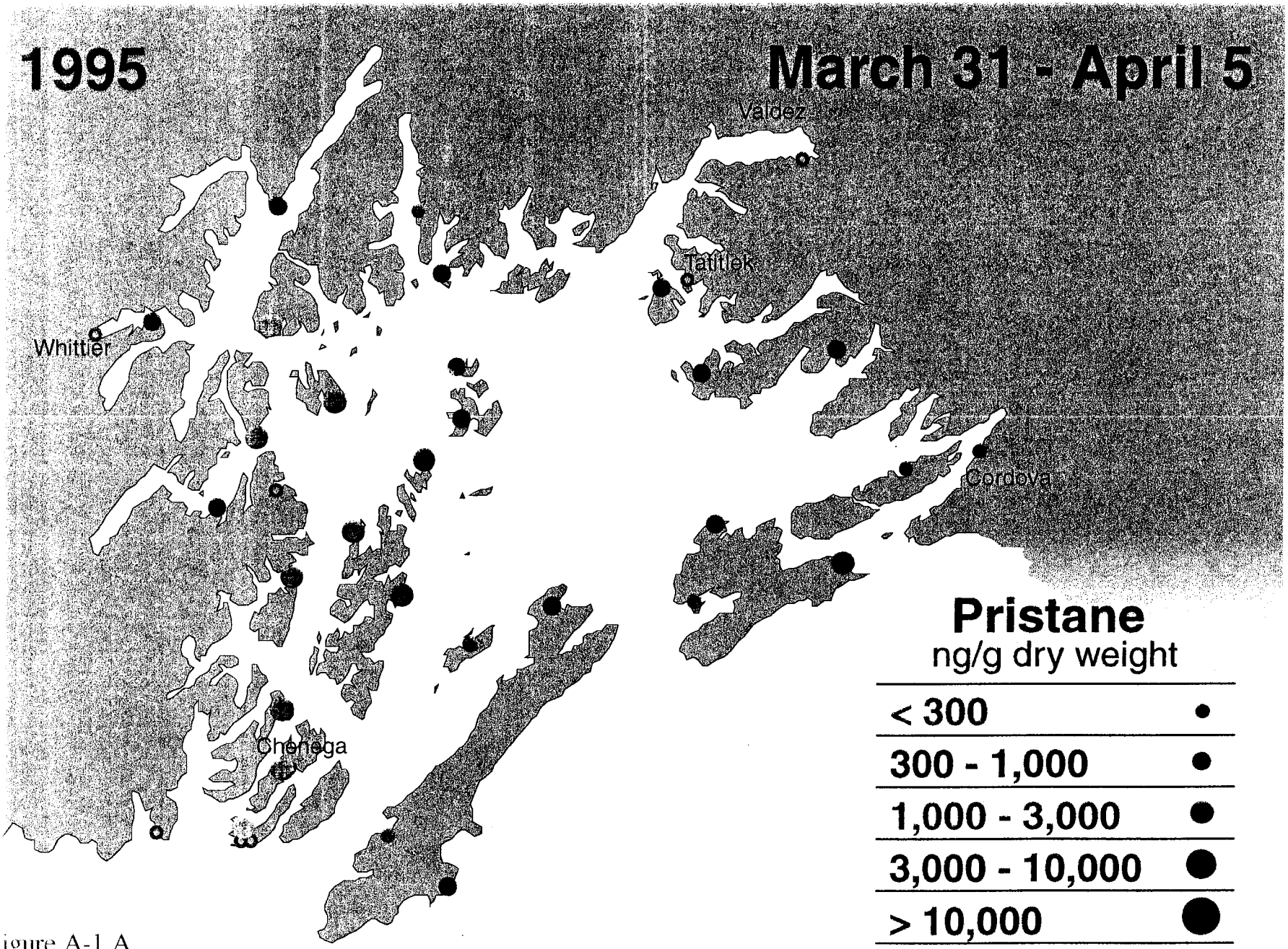
Figure 2H


1996
Pristane Accumulation Index
PWS total = 10,930,713 (ng/g)·days
Valdez
Tatitlek
Whittier
Cordova
Chenega
Index
(ng/g)·days
< 150,000
150,000 - 300,000
300,000 - 600,000
600,000 - 1,200,000
> 1,200,000

igure 3


A.
2 Kilometers
Esther Island
Location of
Esther Island in
Prince William Sound
Hatchery
N
Esther
Light
Brass
Beach
Hodgkin
Point
B.
Pristane
(ng / g mussel tissue)
25,000
20,000
15,000
10,000
5,000
0
21
8
19
28
30
1
2
3
4
5
6
9
March
April
May
Fry
Released

Appendix I


1995
March 31 - April 5
Valdez
Tatitlek
Whittier
Cordova
Chenega
Pristane
ng/g dry weight
< 300
300 - 1,000
1,000 - 3,000
3,000 - 10,000
> 10,000

igure A-1 A

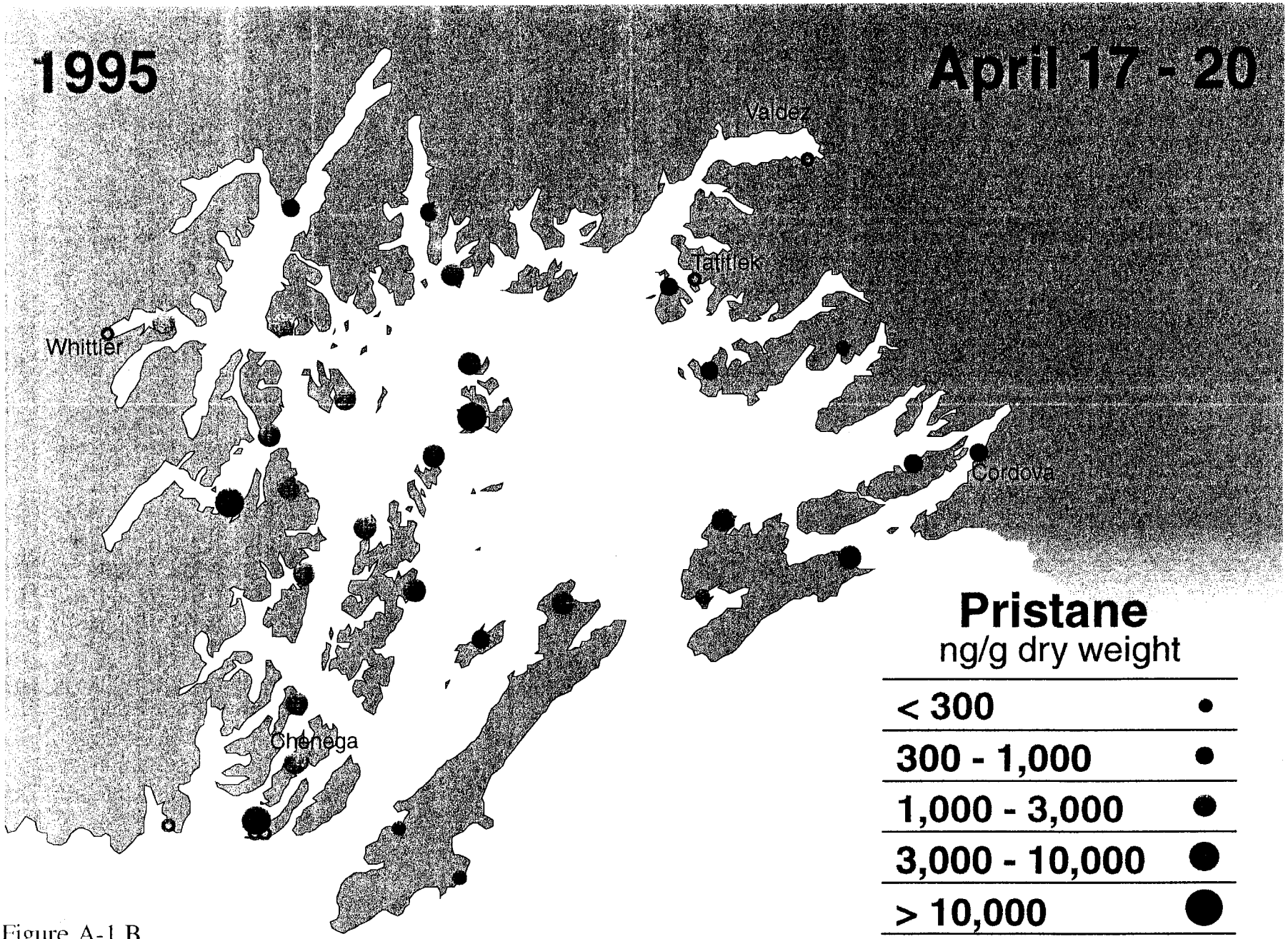

1995
April 17 - 20
Valdez
Tatitlek
Whittier
Cordova
Chenega
Pristane
ng/g dry weight
< 300
300 - 1,000
1,000 - 3,000
3,000 - 10,000
> 10,000

Figure A-1 B

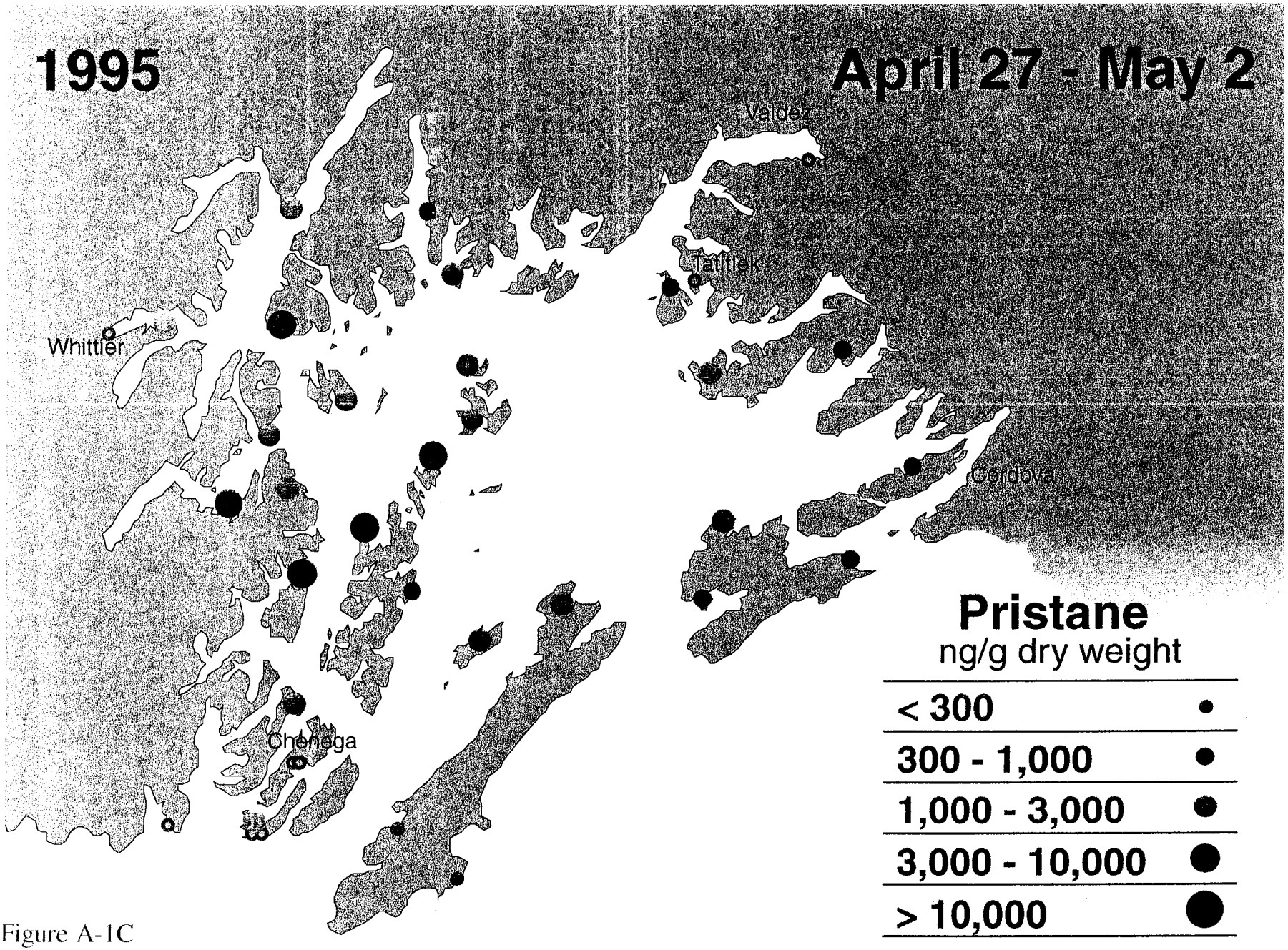
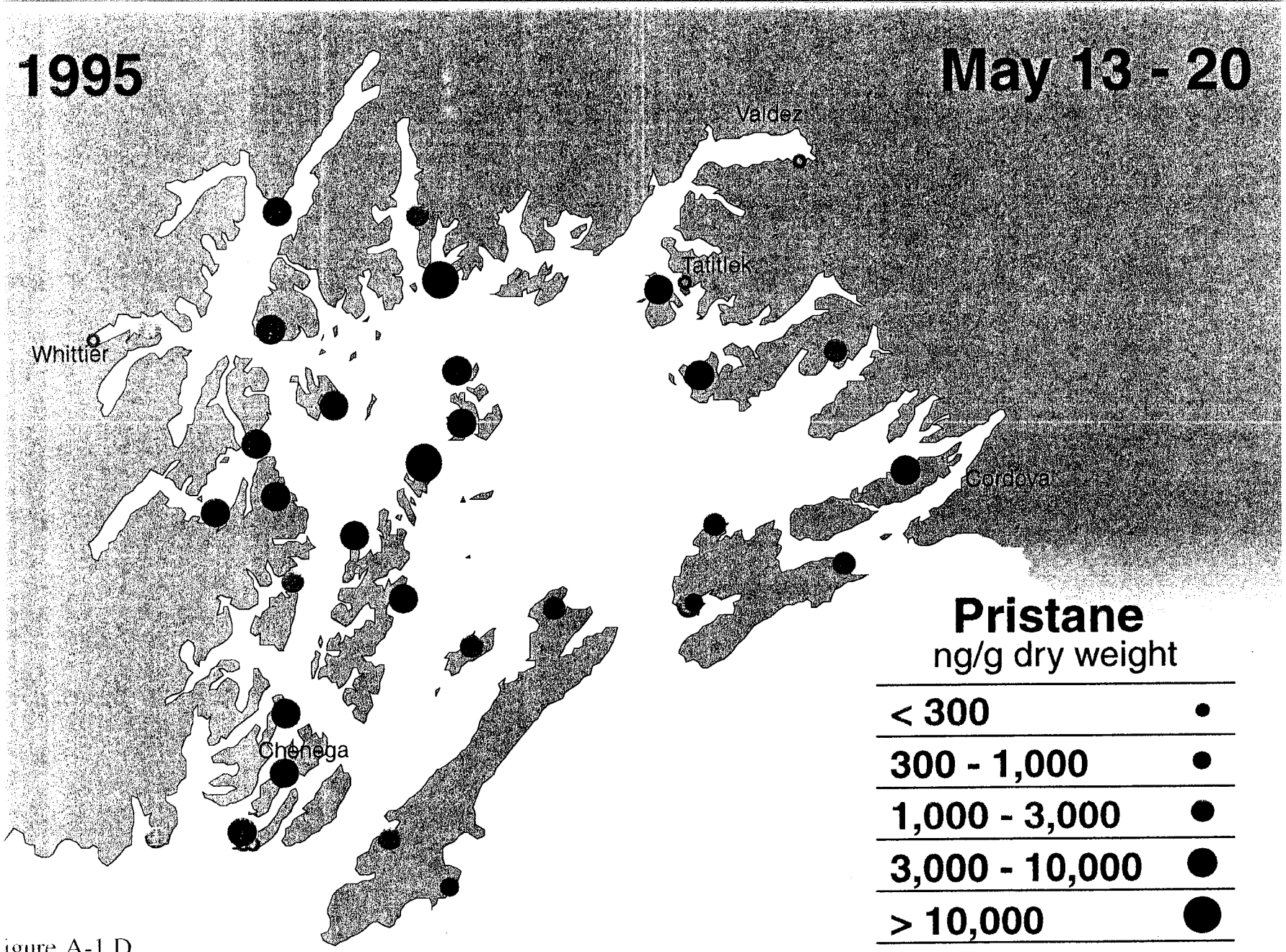

1995
April 27 - May 2
Valdez
Tatitlek
Whittier
Cordova
Chenega
Pristane
ng/g dry weight
< 300
300 - 1,000
1,000 - 3,000
3,000 - 10,000
> 10,000

Figure A-1C


1995
May 13 - 20
Valdez
Tatitlek
Whittier
Cordova
Chenega
Pristane
ng/g dry weight
< 300
300 - 1,000
1,000 - 3,000
3,000 - 10,000
> 10,000

igure A-1 D

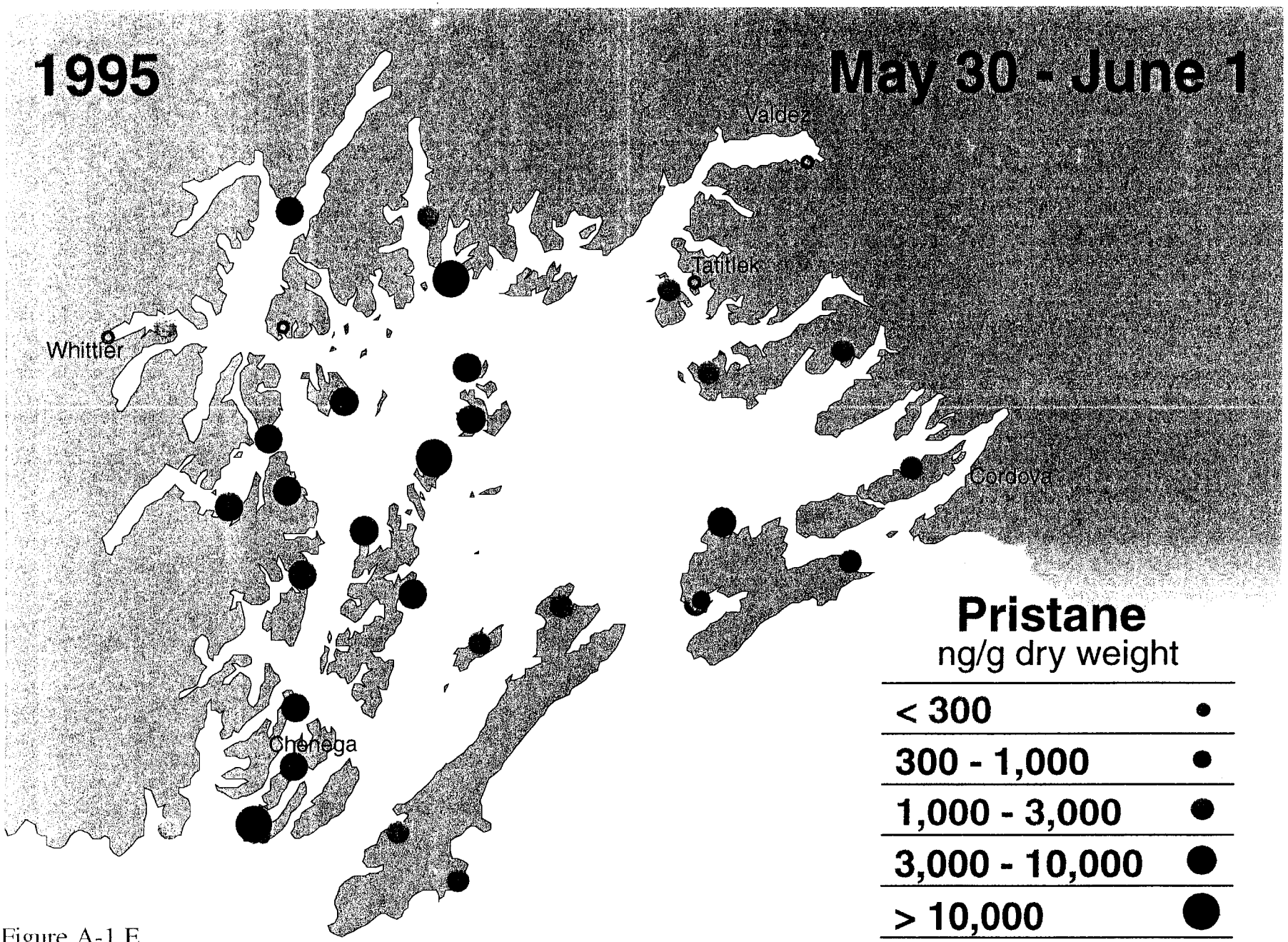
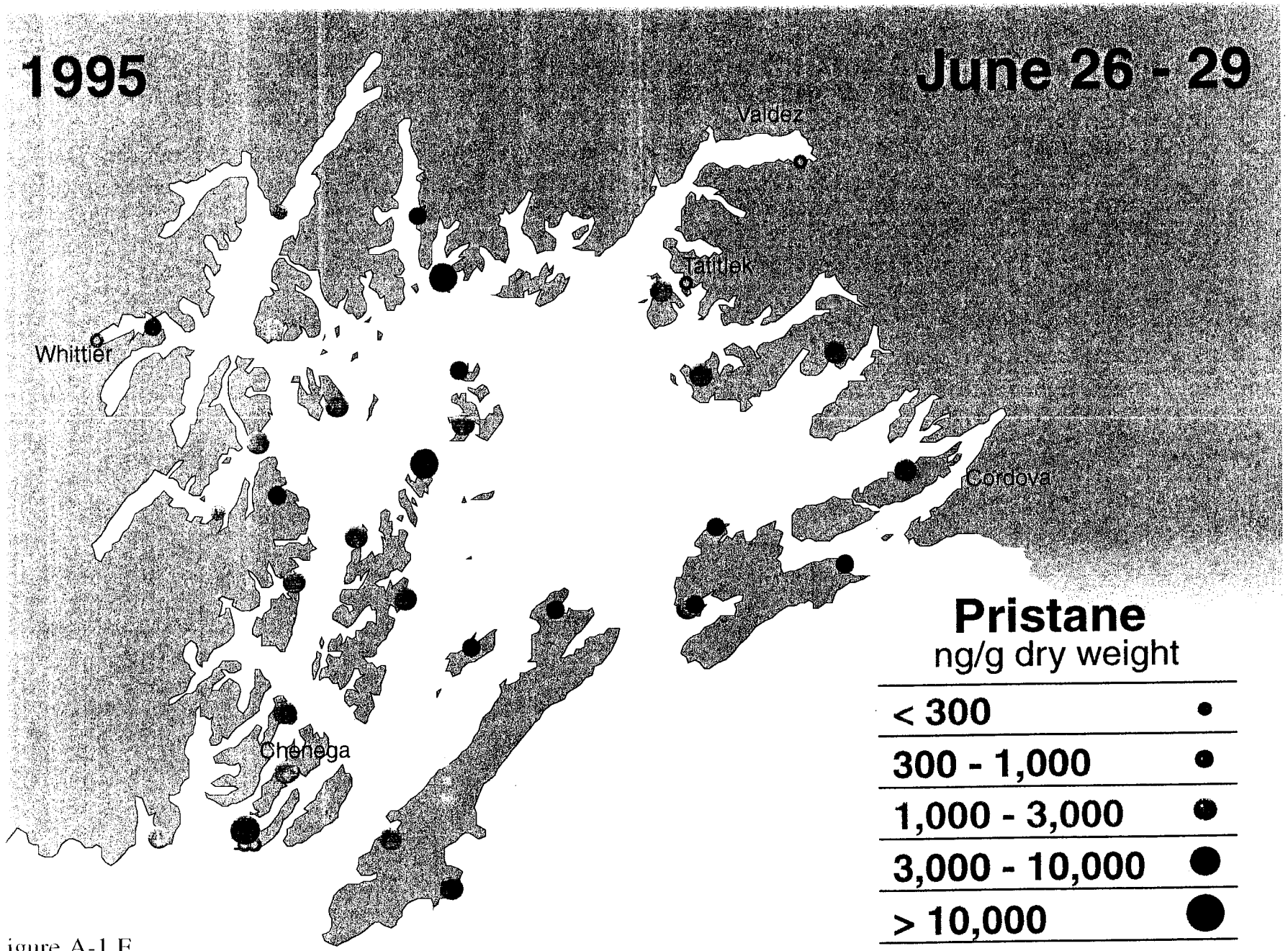
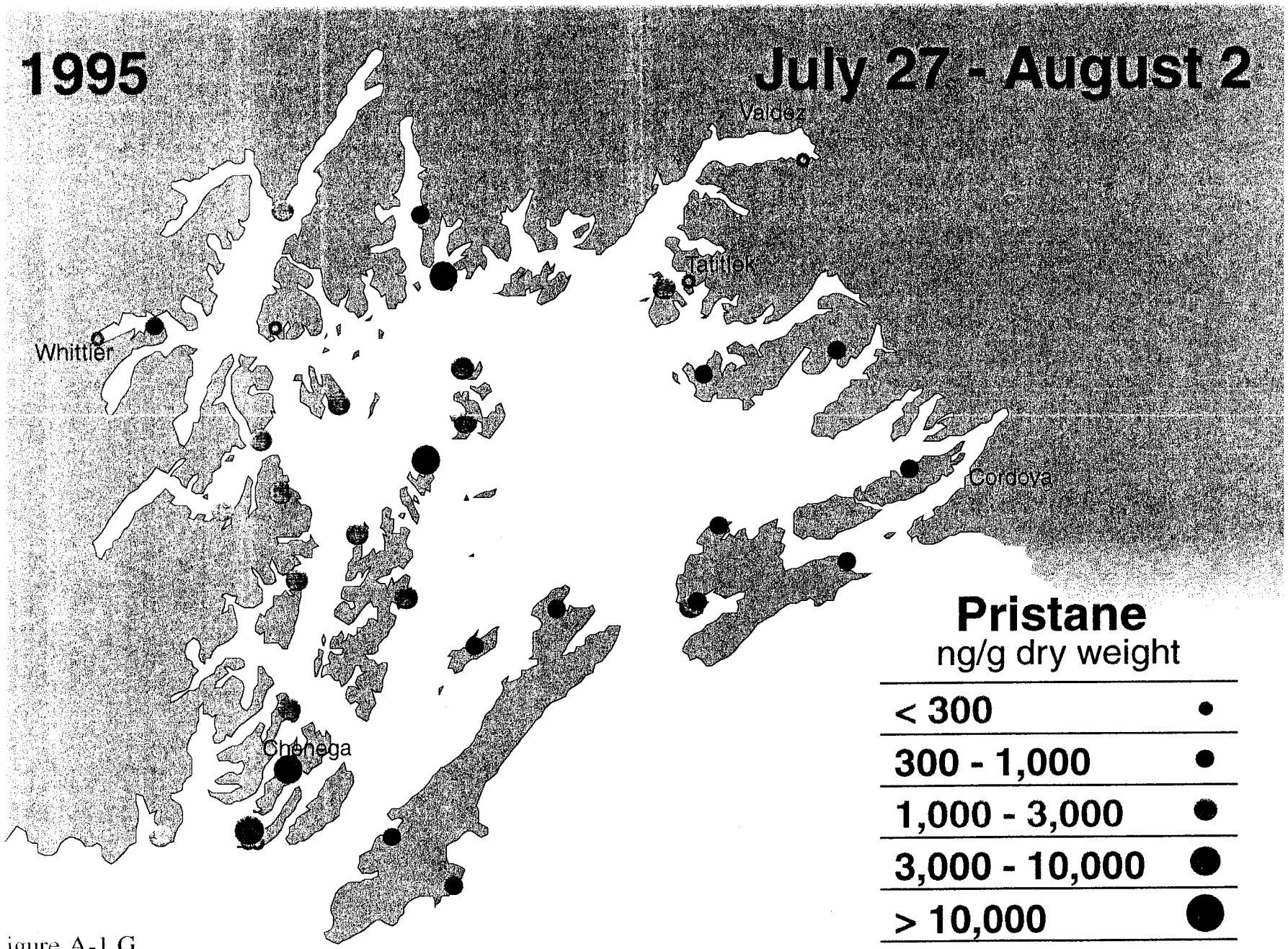

1995
May 30 - June 1
Valdez
Tatitlek
Whittier
Cordova
Chenega
Pristane
ng/g dry weight
< 300
300 - 1,000
1,000 - 3,000
3,000 - 10,000
> 10,000

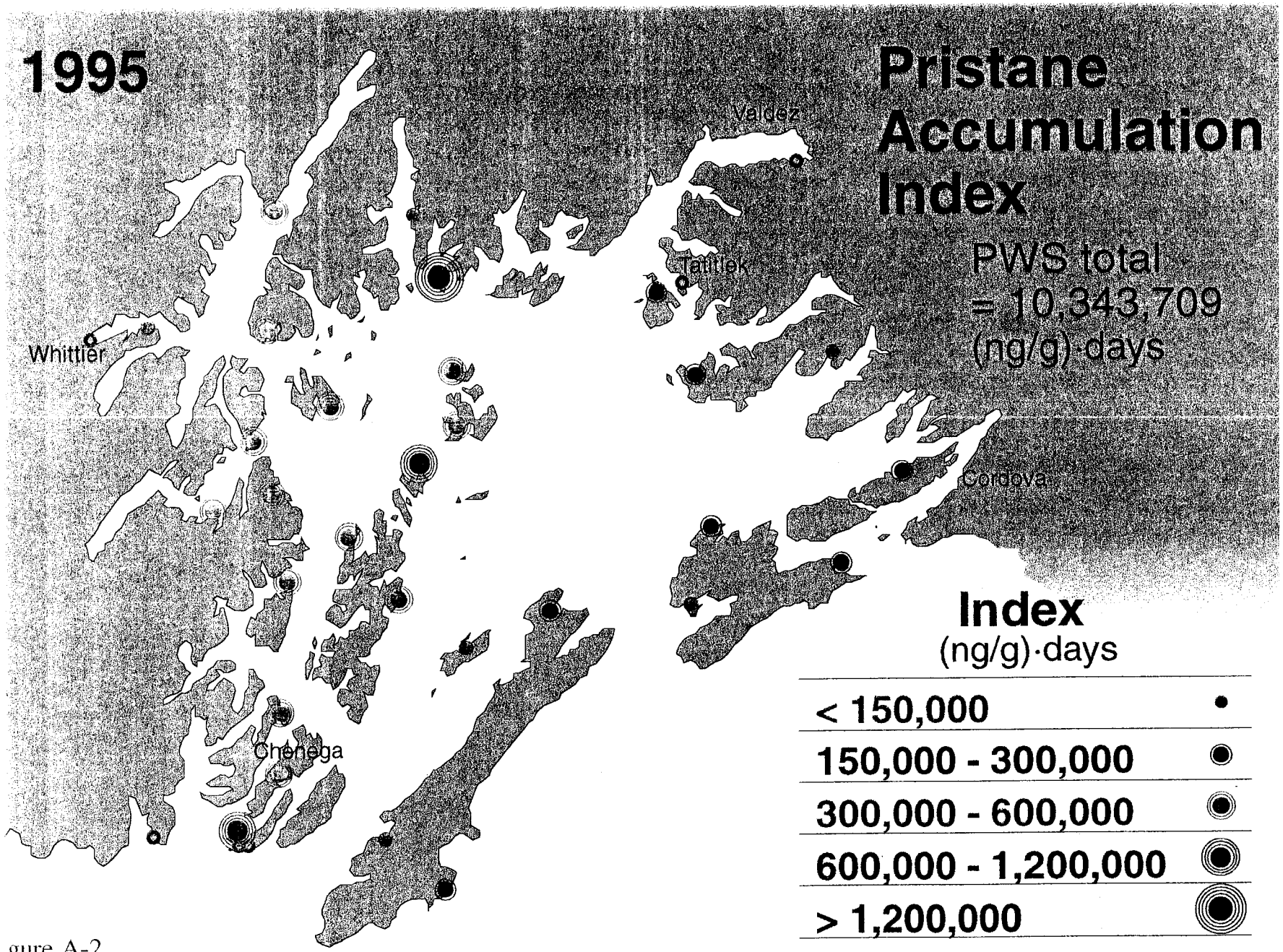
Figure A-1 E


1995
June 26 - 29
Valdez
Tatitlek
Whittier
Cordova
Chenega
Pristane
ng/g dry weight
< 300
300 - 1,000
1,000 - 3,000
3,000 - 10,000
> 10,000

igure A-1 F


1995
July 27 - August 2
Valdez
Tatitlek
Whittier
Cordova
Chenega
Pristane
ng/g dry weight
< 300
300 - 1,000
1,000 - 3,000
3,000 - 10,000
> 10,000

igure A-1 G


1995
Pristane Accumulation Index
PWS total = 10,343,709 (ng/g)·days
Valdez
Tatitlek
Whittier
Cordova
Chenega
Index
(ng/g)·days
< 150,000
150,000 - 300,000
300,000 - 600,000
600,000 - 1,200,000
> 1,200,000

gure A-2