

Exxon Valdez Oil Spill
Restoration Project Annual Report

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

Restoration Project 01195
Annual Report

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

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EXXON VALDEZ OIL SPILL
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July 2002

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

Restoration Project 01195
Annual Report

Study History: This project was initiated in FY96. This is the sixth 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 biweekly from April through early July at 40 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 50% lower than in 2000, which in turn was less than half the average of the previous 5 years, indicating less favorable conditions for early marine survival of juvenile pink salmon and other zooplanktivores during 2000-2001 compared with the previous 5 years. 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. Returns of adult pink salmon to PWSAC hatcheries were relatively low for juvenile pink salmon released during spring 2000, consistent with the low pristane concentrations evident in mussels following the releases.

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

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

Citation: Short, J. and P. Harris. 2002. Pristane monitoring in mussels and predators of juvenile pink salmon & herring, *Exxon Valdez* Oil Spill Restoration Project Annual Report (Restoration Project 01195), Auke Bay Laboratory, Juneau, Alaska.

Table of Contents

List of Tables	4
List of Figures	4
Executive summary	4
Introduction	5
Objectives	6
Methods	6
Results	8
Discussion	9
Conclusions	11
Literature Cited	11
Table 1	13
Table 2	14

List of Tables

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

Table 2. Comparison of the pristane production index (PPI) and marine survival of pink salmon released from Prince William Sound Aquaculture Corporation (PWSAC) hatcheries, 1995-2000. Marine survival data from PWSAC.

List of Figures

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

Figure 2. Pristane concentrations in PWS mussels sampled during 2001 (A) April 6-10, (B) April 20-23, (C) May 6-11, (D) May 21-25, (E) June 5-7, (F) June 19-22, (G) July 5-7. Station locations are indicated by colored dots or open grey circles, where different colors indicate logarithmic ranges of pristane concentrations measured in mussels. Different colors indicate concentrations that are usually significantly different ($P < 0.05$). Open grey circles indicate stations that were not sampled during the indicated sampling interval.

Figure 3. Pristane accumulation index. Pristane concentrations at each of 25 index stations integrated over the sampling season. Results from these stations have been analyzed consistently from 1995 through 2001. Different colors indicate a doubling of ranges of results. The pristane productivity index (PPI) is the sum of results from the 25 stations.

Figure 4. Pristane productivity index, 1995 through 2001.

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, e.g. by 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 high concentrations of 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 40 stations (table 1, 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 2001 indicate a dramatic departure from the trend of increasing pristane production throughout PWS noted for previous years of this study. Time-integrated pristane concentrations summarized by the PPI were 3.13×10^6 ng-d for 2001, or 37% lower than in 2000 (which was 4.99×10^6 ng-d). In turn, the PPI for 2000 was less than half the 5-year average value of 10.4×10^6 for 1995 through 1999. These results suggest that marine foraging conditions for juvenile pink salmon appear to have continued to worsen since 1999. Aggregate marine survival was relatively poor (3.59%) for juvenile pink salmon released from Prince William Sound Aquaculture Corporation hatcheries during spring 2000, which was suggested by the low PPI calculated for that year. The even lower PPI calculated for 2001 suggests another year of relatively poor aggregate marine survivals for juvenile pink salmon released from PWSAC hatcheries in 2001.

As in previous years, the stations where pristane concentrations were greatest were located in the north and western parts of PWS. However, unlike results from 1995-1999, results for both 2000 and 2001 indicated that the peak accumulation of pristane by mussels occurred before juveniles were released from the PWSAC hatcheries, indicating that confounding by other zooplanktivores is significant, and that the *Neocalanus* prey base was substantially consumed prior to the releases of hatchery released pink salmon.

Introduction

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 elucidate some of the important natural factors that affect recruitment of juvenile salmon.

Objectives

This project has one objective given in the detailed project description:

1. Forecast marine survival of pink and chum salmon in PWS.

Methods

Mussel Collection

The seasonal variability of pristane concentrations in mussels (*Mytilus trossulus*) is based on collections from 40 stations in PWS (figure 1, table 1). Mussels were collected biweekly from most stations beginning early April through early July for a total of 7 collection periods and 272 mussel samples (collection of some samples was prevented by weather). Collected mussels were stored frozen and analyzed for whole-body pristane concentration.

Of the 40 stations monitored, 34 are sampled by ABL staff by small float-plane based out of Cordova. Four stations are located near Prince William Sound Aquaculture Corporation (PWSAC) hatcheries, and two were sampled by students in Valdez and Chenega Bay as part of Youth Area Watch, an Exxon Valdez Oil Spill Trustee sponsored program.

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.

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 $\pm 15\%$ 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 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 basis. Dry weights are 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 accommodated. 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 2001.

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 6 sampling years 1995-2000, 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 5 of 6 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.

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 3,000 ppb (dry weight) were evident at several stations during early May (figure 2C). Except for the Rocky Bay station, these stations either border or are down-current of the deep marine depression system of the northwestern sound. Concentrations in the eastern part of PWS remained low, usually below 1,000 ng/g.

As in 2000, pristane concentrations tend peaked earlier in spring 2001 compared with survey years prior to 2000. The highest concentrations usually occurred during the early-May

sampling period, with concentrations in early June comparable with those of mid-April (cf. figures 2B-2E). By mid-June, concentrations were similar to those of early April (cf. figures 2A and 2F).

In general, pristane concentrations in mussels were markedly lower compared with any of the prior survey years. The highest concentration occurred at Point Eleanor at 27,000 ng/g during the early May sampling, but unlike previous years, this was the only instance where the concentration exceeded 10,000 ng/g. Moderate concentrations (3000-10,000 ng/g) occurred at stations near Herring Point around the "black hole" area during early-May (figure 2C), and at three more northerly stations later in May (figure 2D), including Esther Island and Payday Point. Concentrations declined markedly at most other stations from early- to mid-May.

Interannual Trends

The markedly lower concentrations of pristane in mussels during 2001 is clearly reflected by the pristane productivity index (PPI). Time-integrated pristane concentrations summarized by the PPI were 3.13×10^6 ng-d, or 37% lower than the previous low of 4.99×10^6 ng-d reported for 2000 (figures 3 and 4). In comparison, the PPI varied little during the period 1995 through 1999, ranging from a low of 8.7×10^6 in 1995 to a high of 12.7×10^6 in 1998 (figure 4). The value in 1999 was 12.6×10^6 .

Geographic Trends

Pristane concentrations were consistently above annual averages only at Point Eleanor during the period 1995 through 2001 (figure 1). The Foxfarm 1, Esther Island and Herring Point stations were above during 4 of the last 7 years. Five stations were variable, above the annual average during 3 or 4 of the last 7 years, including the AFK hatchery, Applegate Island, Fairmont Island, Perry Island and Point Pakenham.

In contrast, 7 stations were consistently below annual averages, and another 9 stations were below for 1 or 2 of the 6 years. These stations include all 7 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 coastline of Knight Island Passage (Main Bay, Chenega Island, and Fleming Island), 2 stations on the Naked Island complex (Naked Island and Storey Island, see figure 1), and the station on the east coast of Knight Island. These geographic trends are broadly similar with those of previous years (see previous annual reports).

Discussion

Inter-Annual Comparison

The results for 2001 indicate a second consecutive year of sharply lower pristane concentrations in mussels compared with survey years prior to 2000. The significance of inter-annual differences among PPI 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 PPI results from 1995 through 2001 indicates that the PPI for 2001 was significantly lower than for any previous year, including 2000 which was also sharply lower than prior years (figure 4).

The initial increase of pristane concentrations noted at stations in western PWS in early May (cf. figures 2B and 2C) were not likely caused by releases of hatchery fish. The first releases of hatchery-reared pink salmon occurred on May 7, 2001 at the AFK and WHN hatcheries on Evans and Esther Islands, and on May 11, 2001 at the Cannery Creek hatchery in Unakwik Inlet. Most of the early-May stations were sampled on May 6-7, 2002. Previous work indicates a delay of at least 3 days between a mass-release of pink salmon and an increase of pristane in mussels nearby (Short and Harris 1998). Hence, as in 2000, there appear to be other nearshore predators of *Neocalanus* zooplankton that release pristane-rich fecal material, and that have made a dominant contribution to the pristane signal in mussels. These may have included wild pink salmon, or other zooplanktivores such as sandlance, capelin, herring and pollock.

The temporal pattern of results in 2001 suggests that predation pressure on the *Neocalanus* zooplankton bloom may have been especially intense. Given that the increased pristane concentrations in mussels in early May were caused by zooplanktivores other than the hatchery-released pink salmon, the combined hatchery releases of 417 million juvenile pink salmon between May 7-31 would likely have intensified predation on *Neocalanus*. Despite these releases, pristane concentrations generally declined in the northwestern sound. This is consistent with the scenario proposed in the previous annual report, which involves the progressive exhaustion of the *Neocalanus* prey base for these zooplanktivores during early May, at least near the deep marine trench in the northwestern sound where *Neocalanus* are thought to overwinter.

Geographic Trends

Stations in the eastern sound had typically low PAI values as in previous years (figure 3). These results add another year of corroboration to 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 PWSAC hatcheries and streams in the western Sound.

Among the stations in the north and western parts of PWS where pristane concentrations may be above the annual average, there is considerable variability. Only one station is

consistently above the annual average for every year of this project (Point Eleanor). This suggests that foraging opportunities for juvenile salmon vary considerably among years.

Predicting Hatchery Survivals

Pristane results for mussels collected during spring, 2000 suggested that marine rearing conditions were relatively poor that year, suggesting poor marine survival for pink salmon juveniles. The aggregate marine survival of these pink salmon was in fact relatively low, and are weakly correlated with the PPI (table 2). However, these results do not include marine survivals for the Valdez Fisheries Development Association (VFDA) hatchery, which had record marine survivals for juvenile pink salmon released into Port Valdez in early May, 2000. The extent to which these fish forage on zooplankton in western PWS during May is not clear.

The very poor foraging conditions indicated by the PPI for 2001 suggests dismal marine survivals for pink salmon returning in 2002. The validity of this suggestion will be evident soon, when pink salmon return during August and September 2002.

We did not find a significant correlation between the maximum increase of pristane in mussels collected within 25 km of a hatchery, and the marine survival of pink salmon released by the hatchery at least 5 days prior to mussel collection, as in 2000.

Conclusions

1. Pristane accumulation by mussels has remained 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 continued to decline sharply compared with the period 1995-1999. The pristane production index (PPI) for 2001 was 37% lower than in 2000, which in turn was less than half the values of 1998 or 1999. These results suggest poor marine survivals for juvenile pink salmon released from hatcheries in spring, 2001.

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Short, J. and P. Harris. 1998. Pristane monitoring in mussels and predators of juvenile pink salmon & herring, *Exxon Valdez* Oil Spill Restoration Project Annual Report (Restoration Project 98195), Auke Bay Laboratory, Juneau, Alaska.

Table 1. Locations and abbreviations of mussel collection stations sampled for this project in 2000. The abbreviations are also used in figure 1.

Station Abbreviation	Station Name	Latitude			Longitude		
		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
CHIPP	Chippy Point	60	52	3	147	35	38
CONSH	Constantine Harbor	60	21	16	146	40	25
CULRP	Culross Passage	60	44	41	148	13	6
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
GRANB	Granite Bay	60	52	50	148	6	23
GREEI	Green Island	60	16	55	147	24	57
HERRP	Herring Point	60	28	28	147	47	27
HIDDB	Hidden Bay	60	42	57	148	6	12
ISLAB	Island Bay	60	48	4	147	59	27
KNIKL	Kniklik	60	50	51	147	37	46
LITTB	Little Bay	60	11	3	147	47	27
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
PAYDP	Payday Point	60	54	46	147	29	39
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
SHELB	Shelter Bay	60	7	47	147	55	8
SNUGC	Snug Corner Cove	60	44	8	146	37	32
STORI	Storey Island	60	43	41	147	27	2
WEAGL	West Eaglek Bay	60	49	46	147	44	25
WINDB	Windy Bay	60	34	22	148	57	29
WTWIB	West Twin Bay	60	43	32	147	59	35

Table 2. Comparison of the pristane production index (PPI) and marine survival of pink salmon released from Prince William Sound Aquaculture Corporation (PWSAC) hatcheries, 1995-2000. Marine survival data from PWSAC.

Release Year	Number Released	Number Returning	Marine Survival	Pristane Production Index (PPI)
1995	407,787,101	13,750,087	3.37%	8,671,150
1996	418,587,142	19,287,167	4.61%	9,201,431
1997	295,663,840	23,111,900	7.82%	8,672,668
1998	347,221,007	26,279,599	7.57%	12,740,401
1999	388,222,261	20,475,091	5.27%	12,550,526
2000	390,843,348	14,012,760	3.59%	4,985,376

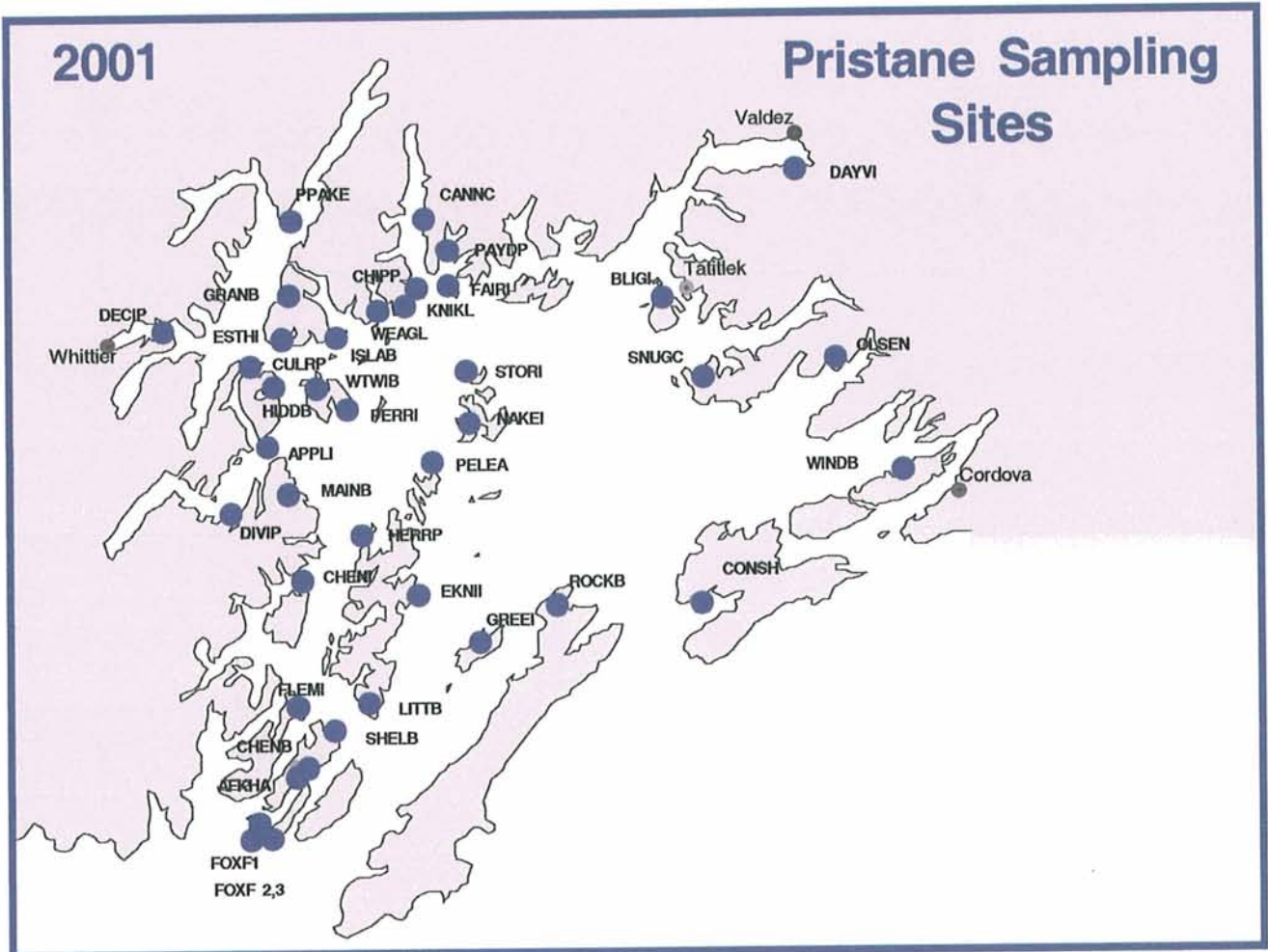


Figure 1.

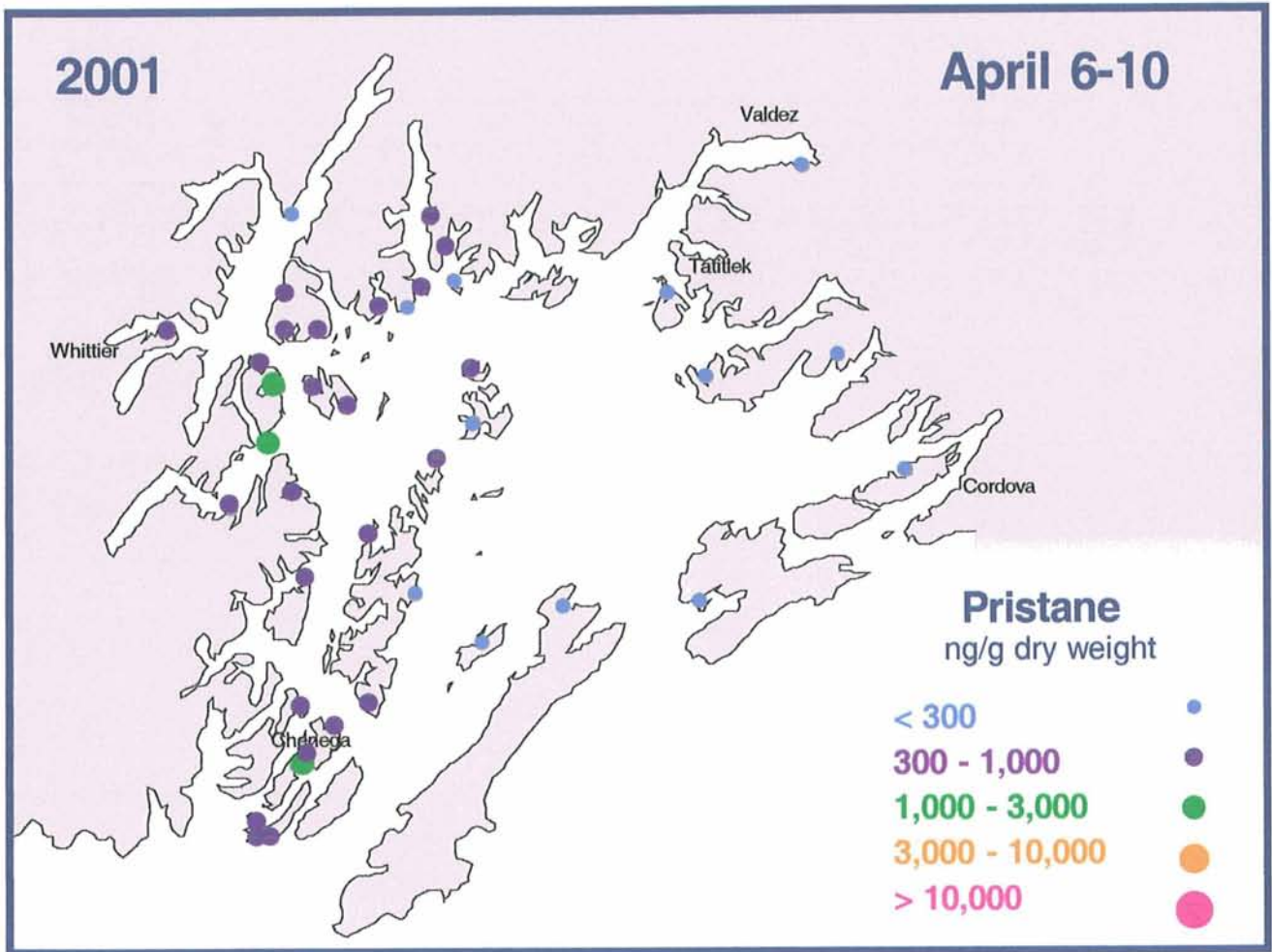


Figure 2A.

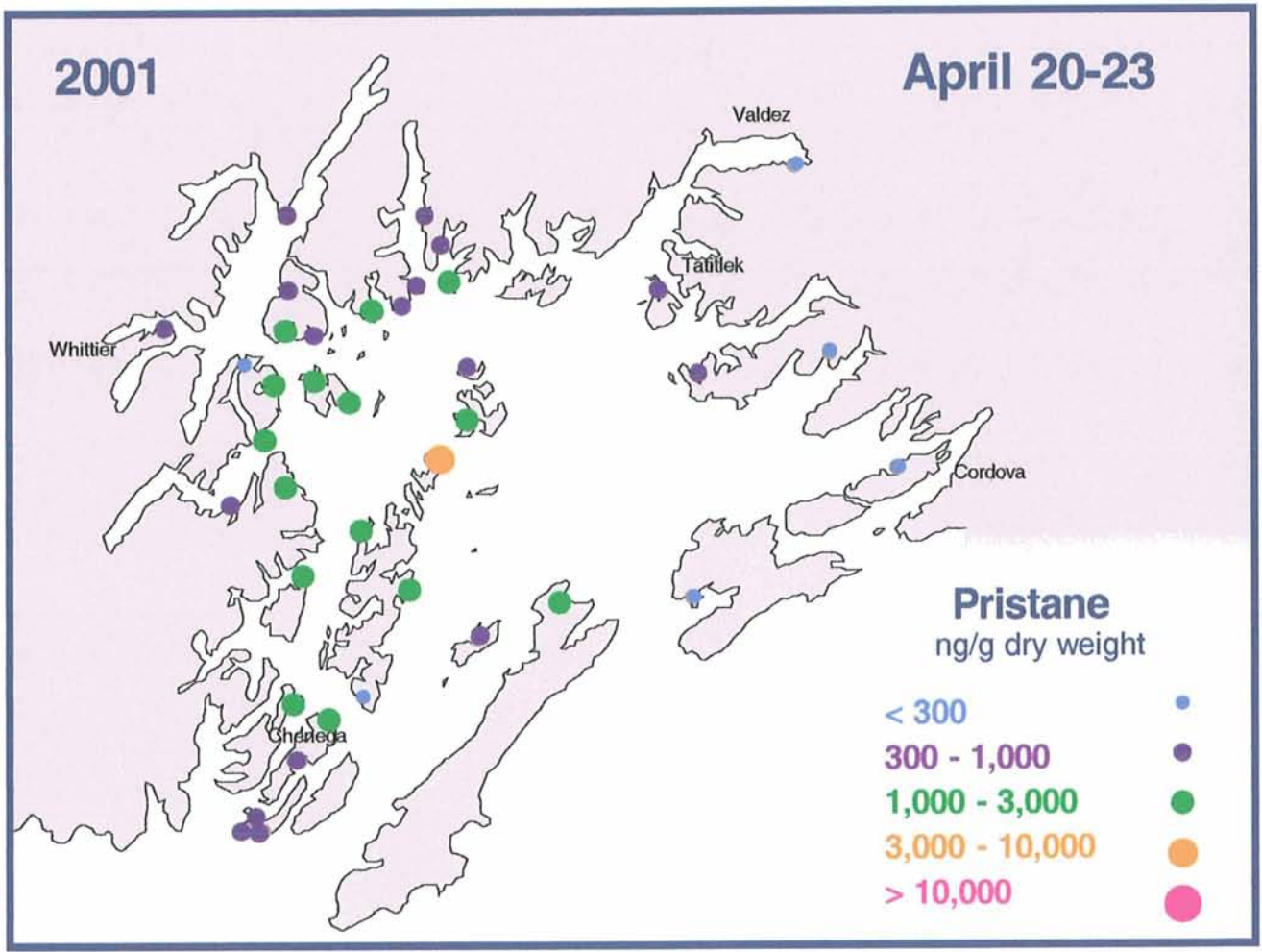


Figure 2B.

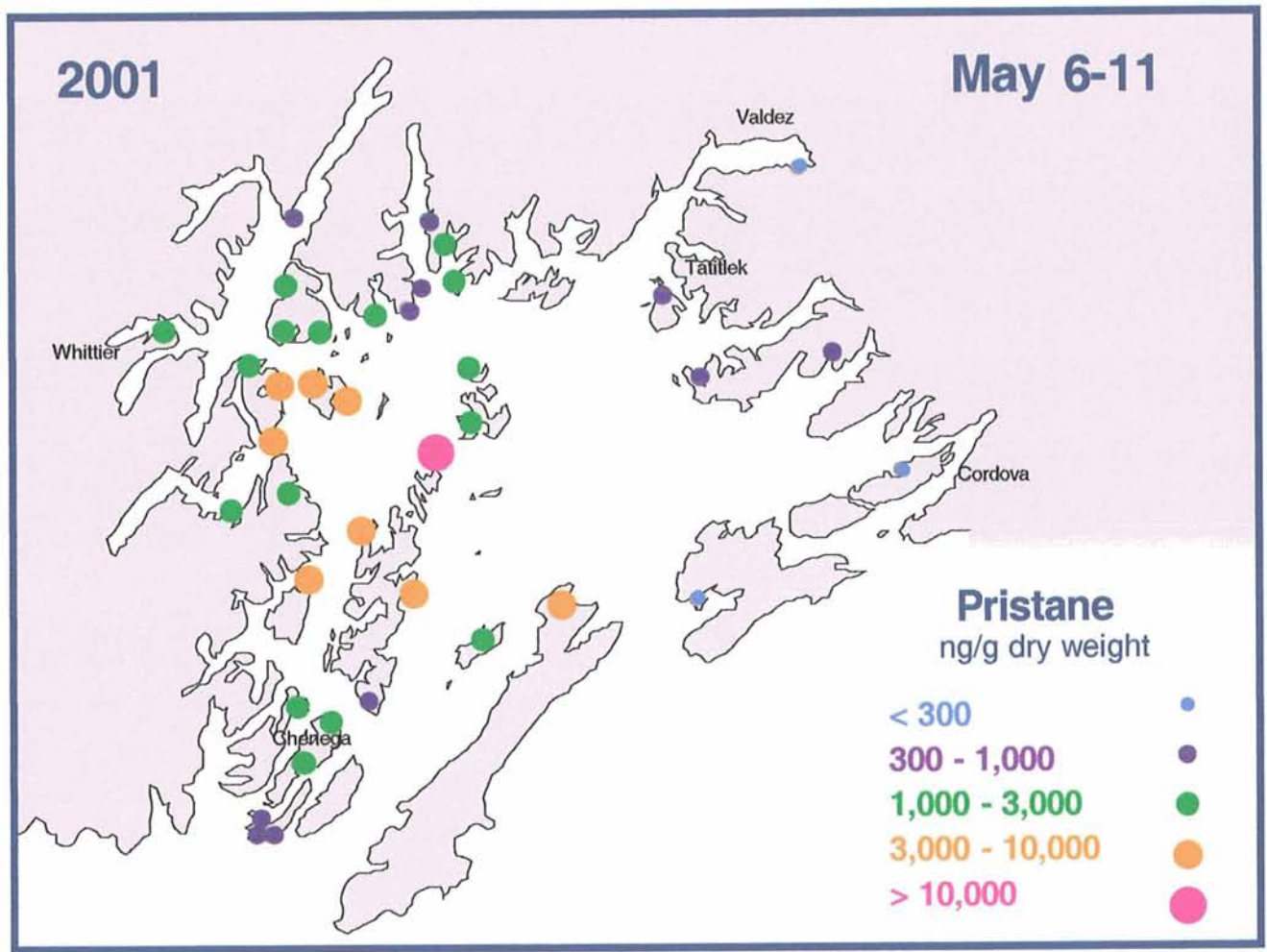


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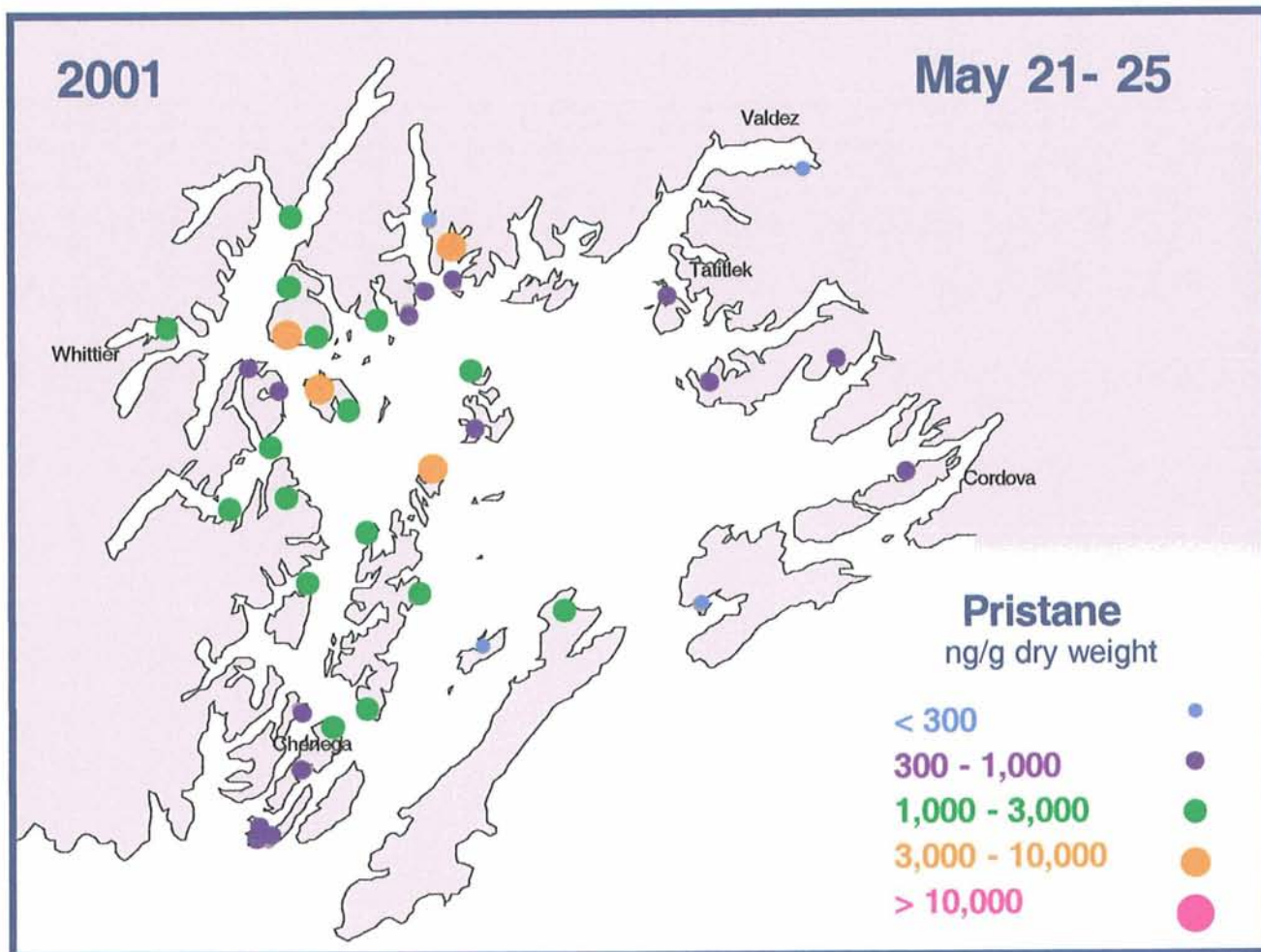


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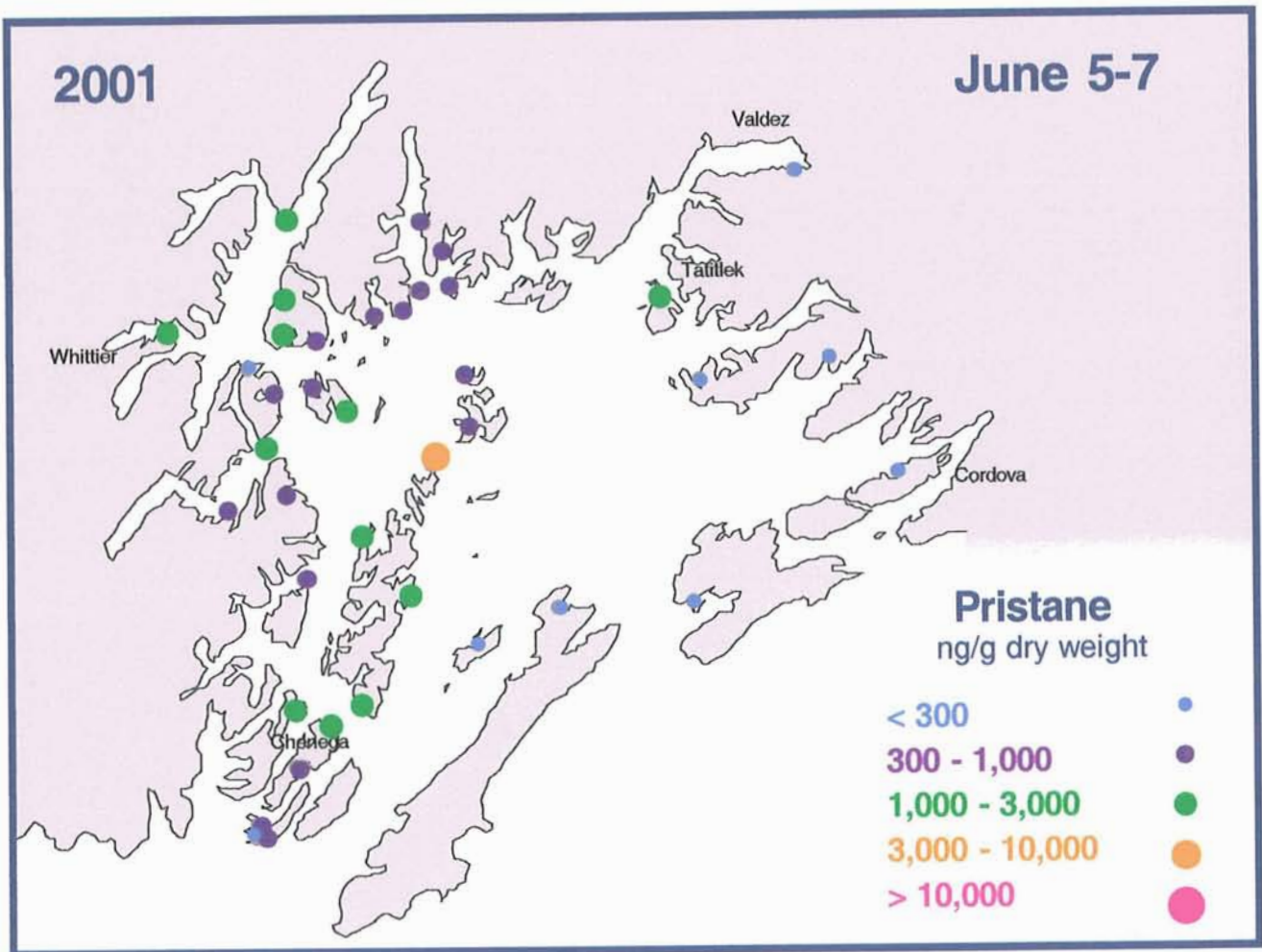


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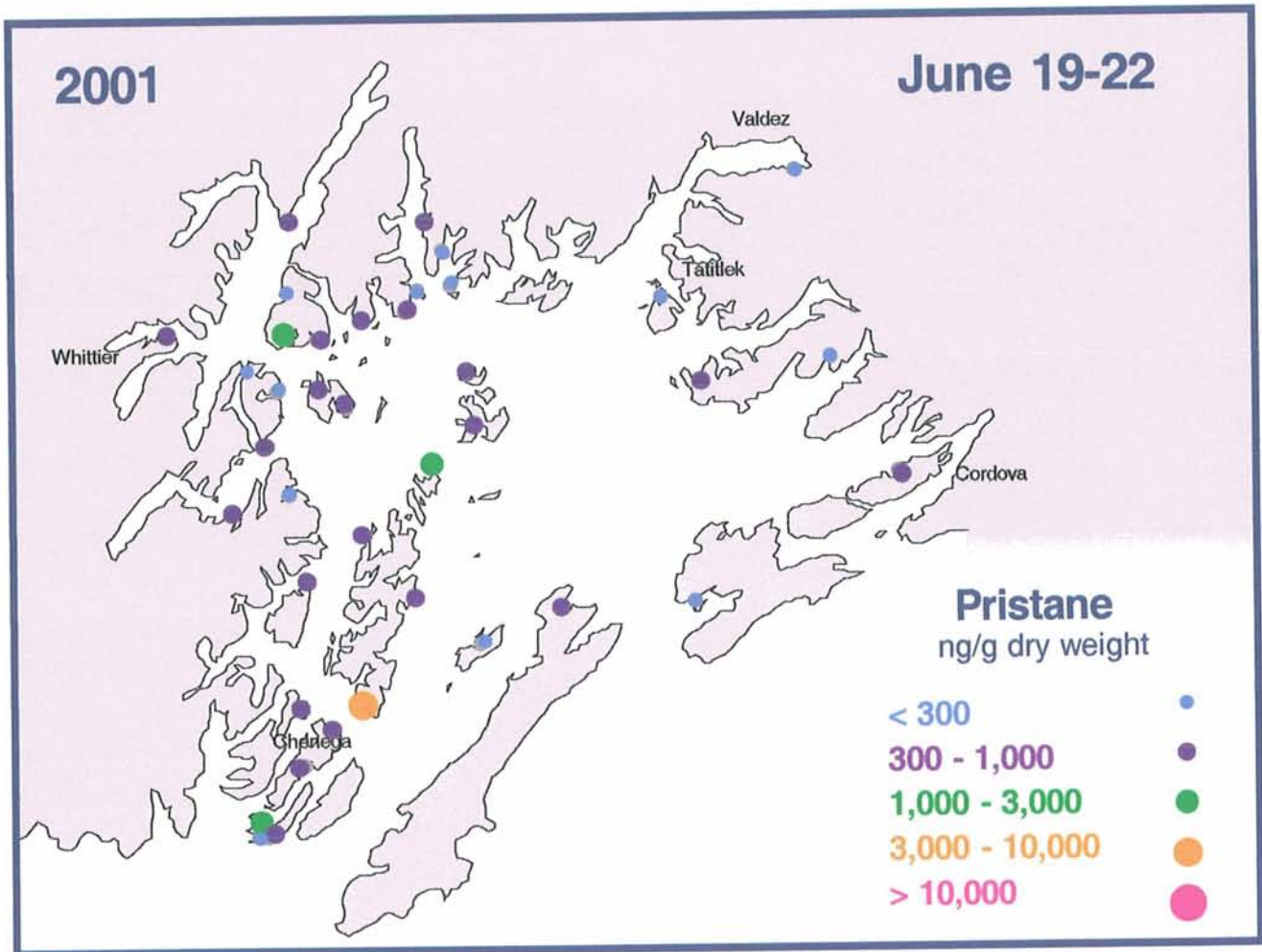


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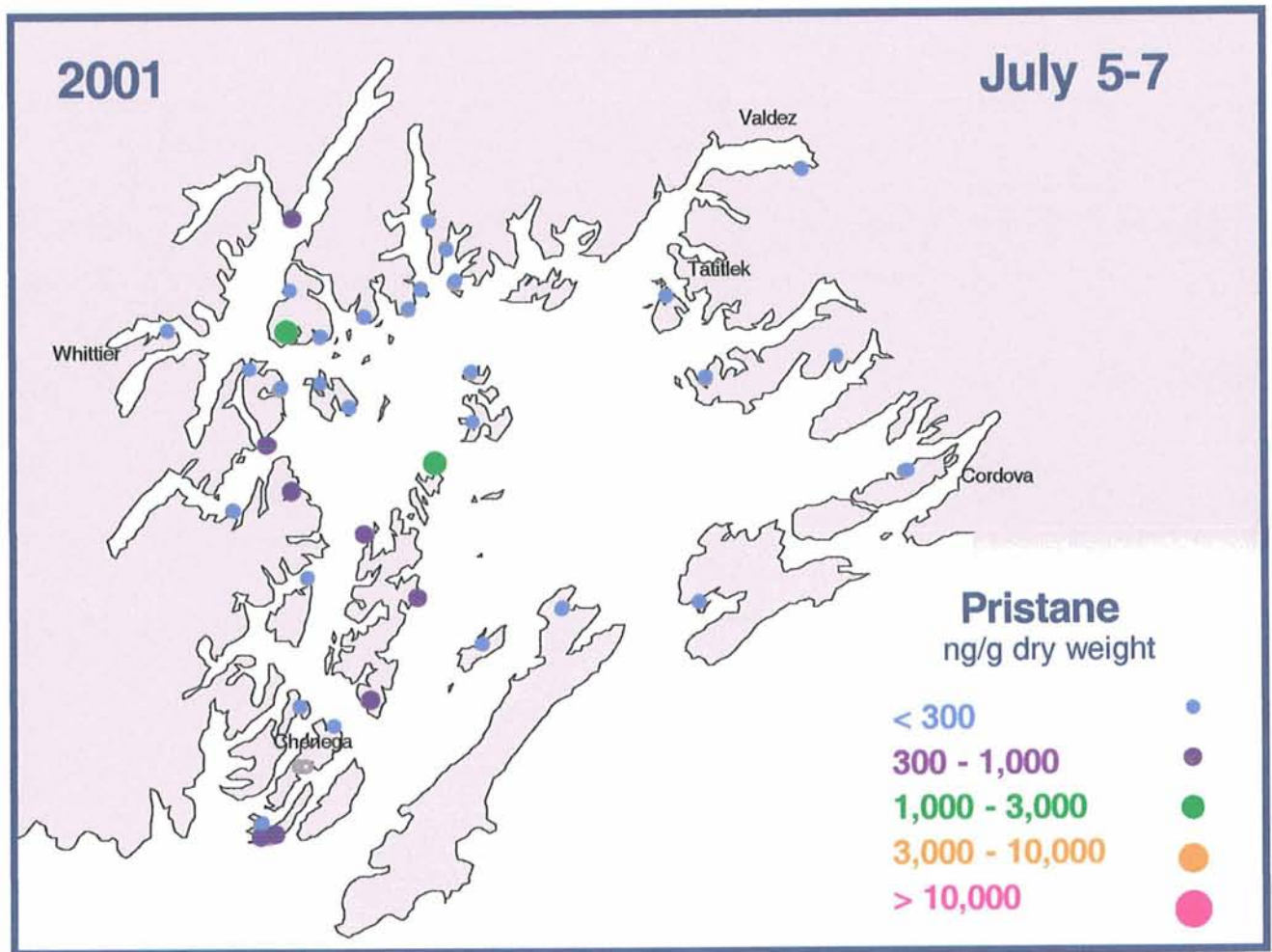


Figure 2G.

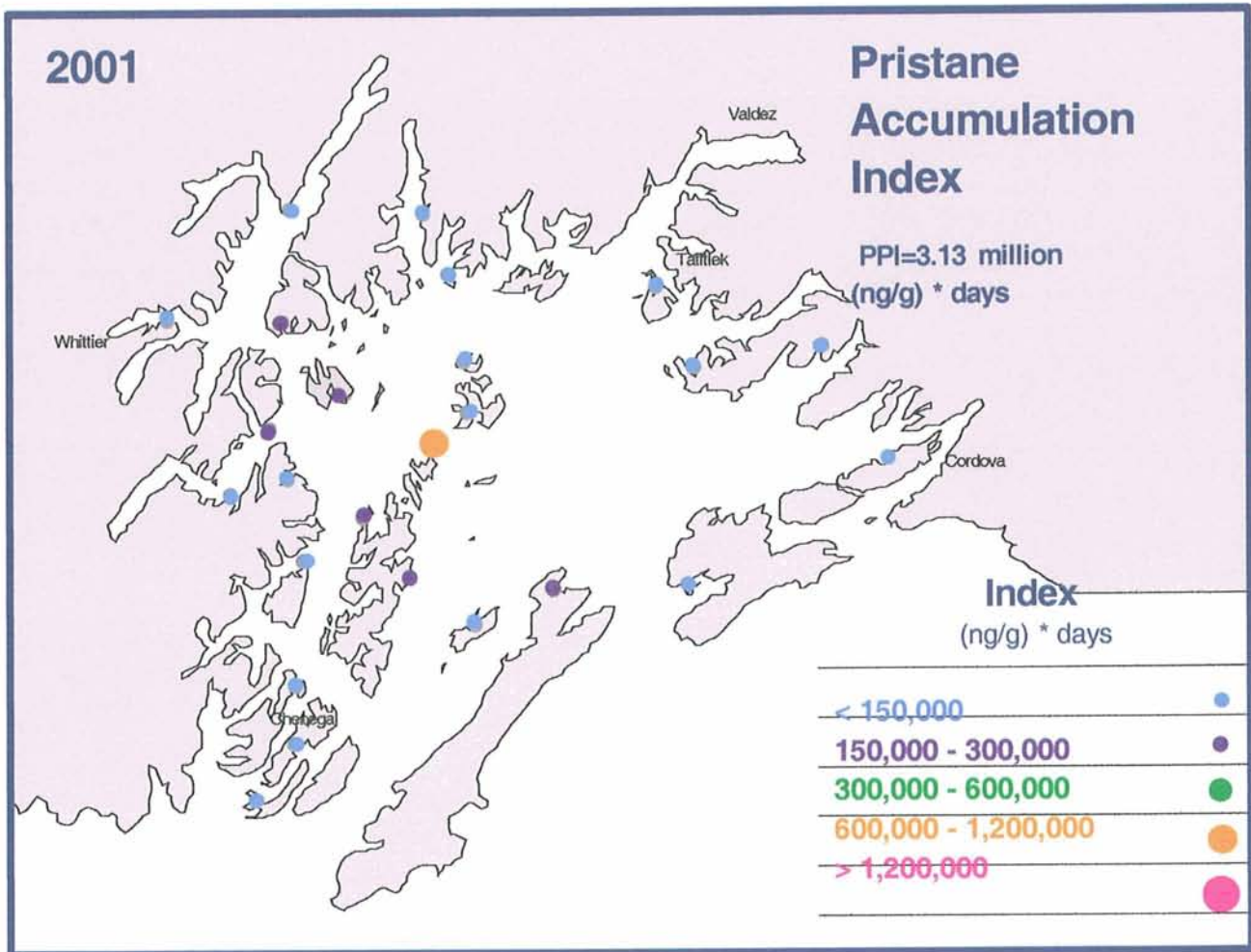


Figure 3.

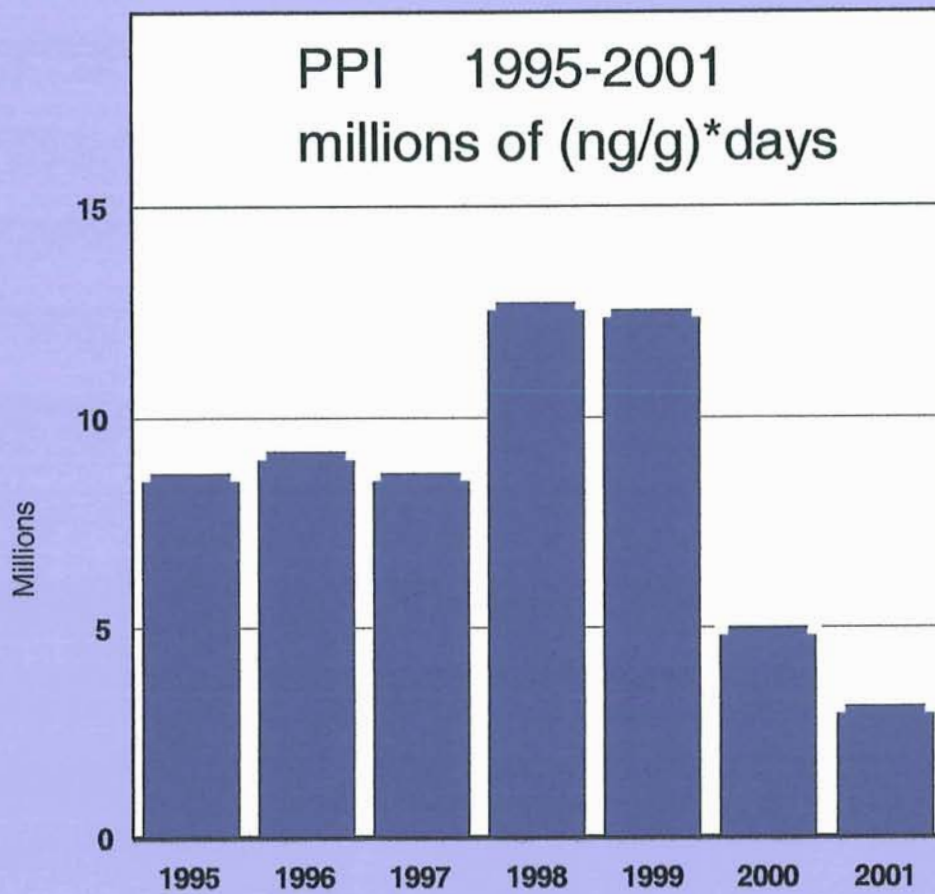


Figure 4.

29 July 2002

MEMORANDUM FOR: Robert Spies, PhD
FROM: Peter Hagen, PhD
SUBJECT: Annual Reports

Enclosed are two copies the annual reports for projects 00195 and 01195 by Jeff Short and Pat Harris titled: "Pristane Monitoring in Mussels and Predators of Juvenile Pink Salmon & Herring."

Also enclosed is a draft final report for project 99347 by Ron Heintz and Marie Larson for your review titled: "Fatty acid profile and lipid class analysis for estimating diet composition and quality at different trophic levels."

Enclosures

cc. Sandra Shubert, Restoration office (one copy of each)