Exxon Valdez Oil Spill
Restoration Project Final Report

Pristane in the Neritic Ecosystem of the Northern Gulf of Alaska

Restoration Project 02195
DRAFT Final Report

This final report has been prepared for peer review as
part of the *Exxon Valdez* oil Spill Trustee Council.
Peer review comments have not been addressed
in this final report

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Restoration Project 02195
DRAFT Final Report

Study History:
This project was initiated in 1996, and builds upon earlier sampling by NMFS Auke Bay Lab. Funding for synthesizing pristine monitoring as predicative tool was approved January 2000. This submission represents the first of four chapters of that final report.

Abstract: The extensive sampling of biota from the northern Gulf of Alaska (GOA) following the 1989 *Exxon Valdez* oil spill provides a unique opportunity to examine the distribution of pristane, a branched alkane hydrocarbon produced by copepods, in the neritic food web. Pristane is lipophilic and is resistant to degradation via β-oxidation, so it is moderately persistent in the marine food web, and serves as a natural chemical label for a substantial proportion of the lipid produced at the secondary level of trophic production in this ecosystem.

Chapter 1 of this report is directed towards synthesizing the results from hydrocarbon analyses of 3007 samples comprising 49 species to confirming prior speculation that pristane may serve as a natural chemical probe for food web analysis.
Subsequent chapters address the utility of pristane monitoring to assess feeding, condition and survival of the consumers of the copepods, including pink salmon.

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

Project Data:

Citation:

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June 10, 2004

Phil Mundy
Science Director
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Dear Dr. ~~Mundy~~, Phil

I'm pleased to submit for peer review chapter one of a draft final report for Project 02195, "Pristane in the Neritic Ecosystem of the Northern Gulf of Alaska"
Jeff Short is the Principal Investigator.

This project was initiated in January 2000 to synthesize six years of pristane monitoring largely funded by EVOS. Approval for the project was given prior to the council learning the extent of lingering oil in PWS, which as you know became a high priority for research. None-the-less the project has proceeded despite the PI's key involvement in those investigations. We believe this work will be a valuable contribution to the body of EVOS funded research. It represents a thorough investigation into a unique ecological measure of trophic linkages in the spill area and as such can serve as a tool to advance research under GEM.

This submission is the first chapter of what is anticipated to four-chapter document. The schedules for completing the remaining chapters are:
July 15 - Chapter 2
Sept 15 - Chapter 3
Nov 15 - Chapter 4

The intention is for each chapter to stand-alone and as such that they can be submitted that way to facilitate peer review.

We hope this submission and the schedule for final completion will elevate concerns, if any, as to the intention of NOAA and the Principal Investigator to meet their commitments and that this will satisfy contingencies placed on current funding requests.

Thank you for your consideration,

Pete Hagen

Pete Hagen, PhD
EVOS program manager.

cc Brenda Romas

CHAPTER 1

Abstract

The extensive sampling of biota from the northern Gulf of Alaska (GOA) following the 1989 *Exxon Valdez* oil spill provides a unique opportunity to examine the distribution of pristane, a branched alkane hydrocarbon produced by copepods, in the neritic food web. Pristane biosynthesis by *Calanus* copepods in the Atlantic Ocean has been previously demonstrated, and may constitute up to 1% of the dry mass of stage V copepodites and adults. I confirm here that similar concentrations are attained in Pacific Ocean species of *Calanus*, along with species of the closely related genus *Neocalanus*, but that concentrations in other GOA zooplankton genera are tenfold or more lower. Late-stage *Calanus* and *Neocalanus* copepodites account for half or more of the biomass of the spring zooplankton bloom in the neritic waters of the northern GOA and contain ~50% lipid on a dry weight basis. As a terminally-branched alkane, pristane is lipophilic and is resistant to degradation via β-oxidation, so it is moderately persistent in the marine food web, and serves as a natural chemical label for a substantial proportion of the lipid produced at the secondary level of trophic production in this ecosystem.

Results from hydrocarbon analyses of 3,007 samples comprising 49 species sampled for damage assessment studies of the *Exxon Valdez* oil spill, including birds, fishes, molluscs, crustaceans, plants, mammals and an echinoderm, demonstrate that the pristane introduced into the neritic food web of the northern GOA during spring by *Calanus* and *Neocalanus* copepodites gradually dissipates as it passes through successive consumer species and with time. The highest concentrations are found in zooplanktivorous species during spring, and are usually about tenfold below pristane concentrations in the copepodites. Pristane concentrations are highest in depot lipids, and are lowest in brains and blood, of species were multiple tissues were collected. Roughly tenfold or greater reductions occur with each trophic transfer, so pristane is not so refractory that it is biomagnified by the food chain. Pristane is probably introduced into intertidal and subtidal benthic food webs by feces produced by zooplanktivores preying on *Calanus* and *Neocalanus* copepodites, or perhaps by feces produced directly by these copepodites. Seasonal comparisons indicate that pristane concentrations in species dwelling in the intertidal, the subtidal benthos, the mid-water column, and in birds are nearly always higher during spring, consistent with production during the spring zooplankton bloom. Collectively these results confirm prior speculation that pristane may serve as a natural chemical probe for food web analysis.

Introduction

Chemical methods are often used to investigate marine food web dynamics. This approach typically involves the analysis of relatively persistent compounds that serve to label prey organisms, such as unusual aliphatic hydrocarbons and fatty acids (Blumer et al. 1964, Blumer et al. 1969, Paradis & Ackman 1977, Sargent and Whittle 1981, Stübing et al. 2003), or the analysis of stable isotopes of carbon and nitrogen as an indicator or

relative trophic position (Lajtha & Michener 1994). Pristane (2,6,10,14-tetramethylpentadecane) was the first aliphatic hydrocarbon proposed for this purpose (Blumer et al. 1964), in part because it is terminally branched and thus resistant to biochemical catabolism via β-oxidation (Pirnik 1977), conferring persistence (Blumer et al. 1969). Pristane is readily analyzed in marine tissues by gas chromatography, with detection limits on the order of 10 ng/g (Short et al. 1996). Despite these advantages, pristane analysis has not often been used for food web studies, in part because foundation studies to comprehensively identify the species that produce pristane biochemically, as well as studies to determine the distribution and persistence of pristane in tissues of their predators, are needed to provide context for the interpretation of new data.

The major biogenic source of pristane identified so far is copepods in the genus *Calanus*. These copepods introduce large quantities of pristane annually to mid- and high-latitude marine food webs at the secondary trophic level of production. Pristane is biosynthesized in these copepods from ingested chlorophyll (Avigan & Blumer 1968), and approaches 1% dry weight in adults or late copepodite stages of *C. finmarchicus*, *C. glacialis*, and *C. hyperboreus* (Blumer et al. 1964). These high concentrations have only been found in *Calanus* among zooplankton examined. Pristane concentrations in other Atlantic Ocean zooplankton, including other calanoid copepod genera, are lower by factors of at least 10 and usually more than 100 (Blumer et al. 1964), and only traces are found in phytoplankton (Blumer et al. 1971). *Calanus* copepods are mostly herbivorous, and are an important link in marine food webs between primary production and consumers at higher trophic levels, especially during spring phytoplankton blooms at sub-arctic latitudes where they may account for most of the spring zooplankton biomass near the seasurface (Parsons & Lalli 1988, Cooney et al 2001). Hydrocarbons are highly lipophilic, so pristane strongly associates with lipids of consumers that ultimately depend on *Calanus* copepods. The relatively high concentration of pristane produced annually by a large biomass of secondary producers, its lipophilicity, and its likely environmental persistence led to its proposal as a natural tracer molecule of trophic relationships (Blumer et al. 1964).

Pristane is nearly ubiquitous in marine organisms at widely varying concentrations that usually depend on how close the trophic connection is with marine zooplankton. Concentrations in lipids of zooplanktivores approach or exceed those found in *Calanus* spp., including basking and other planktivorous sharks (Kayama et al. 1969), herring (*Clupea harengus*) and sand lance (*Ammodytes americanus*; Ackman 1971), as do concentrations in stomach oils of procellariiform birds (Clarke & Prince 1976). Concentrations in lipid-rich tissues of pelagic fishes that do not feed directly on zooplankton are usually substantially lower, such as livers of cod (*Gadus morhua*), Greenland cod (*Gadus ogac*), Greenland halibut (*Reinhardtius hippoglossoides*), American plaice (*Hippoglossoides platessoides*), wolffish (*Anarhichas spp.*) and redfish (Sebastes marinus), in which pristane concentrations range from 1.86 – 99 μg/g wet weight (Johansen et al. 1977). However sperm whales (*Physeter macrocephalus*), which feed mainly on squid, contained as much as 240 μg/g in their blubber (Sano 1968), while

sockeye salmon (*Oncorhynchus nerka*), which are zooplanktivores, contained only 0.380 μg/g in their livers and 2.4 μg/g in their viscera (Sasaki et al. 1991), and pristane in oil from herring in the Baltic Sea contained less than 1% pristane found in oil from herring in the Atlantic Ocean (Linko & Kaitaranta 1976). Tissues of benthic invertebrates consistently contained pristane concentrations below about 2 μg/g (Johansen et al. 1977, Mackie et al. 1978, Mackie et al. 1974), as does the blubber of the Pacific walrus (*Odobenus rosmarus divergens*), which feed primarily on these invertebrates (Seagars & Garlich-Miller 2001). Pristane was not detected in any of four species of benthic epiphytes collected from a Carribean lagoon (Botello & Mandelli 1978). Concentrations in lipid-rich tissues of freshwater fishes or in carcasses of birds that depend on terrestrial food webs were also uniformly near or below the detection limits of the analyses used (Ackman 1971, Custer et al. 2001, Lopez et al. 2001).

Although the distribution of pristane in marine organisms as reported in the literature is broadly consistent with *Calanus* copepods as the primary source, other sources may exist, and comparisons among these studies must account for sampling from different parts of the world and decades apart. In contrast, samples of biota that were analyzed for hydrocarbons to assess the impacts of the 1989 *Exxon Valdez* oil spill in Prince William Sound (PWS), Alaska, provide an opportunity to evaluate the distribution of pristane in a regional subarctic marine food web during a period of a few consecutive years. Over 3,900 samples of biota were collected by government agencies from the affected part of the northern Gulf of Alaska (GOA) during the years immediately following the incident. The sampled biota included several species of birds, fish, mollusks, crustaceans, marine and terrestrial mammals and one echinoderm, and several different tissues were sampled from some of these species. The samples were analyzed by the same gas-chromatography (GC) method for a suite of aliphatic and aromatic hydrocarbons characteristic of crude oil, including pristane (Short et al. 1996), making this the largest self-consistent data set of its kind. Most of the analyzed samples were collected to evaluate the extent of pollution in environmental compartments where oil impacts were not obvious, hence the need for sensitive GC analyses. Hydrocarbons characteristic of the spilled oil were often not detected in these samples (Short & Heintz 1997), but pristane was, especially in mussels (*Mytilus trossulus*) collected before as well as after the spill (Short & Babcock 1996, Karinen et al. 1993).

Copepods of the genus *Neocalanus* dominate the near-surface zooplankton biomass during spring in the northern GOA affected by the spill (Cooney et al. 2001, Cooney 1986a, Cooney 1986b, Mackas et al. 1993, Miller et al. 1988), and this genus is closely related to *Calanus* (Bradford & Jillett 1974). If the concentrations of pristane in *Neocalanus* and *Calanus* copepods are comparable, then this ecosystem presents an instance of an extensively sampled food web receiving a large annual input of pristane from secondary production during spring.

I present here evidence that the high pristane concentrations found in Atlantic Ocean *Calanus* species also occur in Pacific Ocean species of the genera *Calanus* and

Neocalanus, but not in other zooplankton during spring in the northern GOA. I then present a summary of the seasonal distributions of pristane among the biota sampled for government-sponsored *Exxon Valdez* oil spill damage assessment studies, to evaluate whether distributions of pristane among species at different trophic levels are consistent with *Calanus* and *Neocalanus* copepods as primary pristane sources. These results provide an example of how pristane permeates a marine food web following introduction by secondary production during spring.

Study Area

Nearly all of the samples considered herein were collected from the vicinity of the path traversed by oil spilled from the T/V Exxon Valdez, including PWS in the northern GOA and extending along the Kenai and Alaska peninsulas to Chignik (Fig. 1). Most of these samples (83%) were collected from PWS, and the remainder from the spill-impacted region west of PWS (Fig. 1).

Prince William Sound is a complex fjord-type ecosystem with a seasurface area of about 8,800 km^2 (Schmidt 1977). Seawater depths exceed 700 m in the northwestern part of the sound. Direct deep-water exchange with the GOA is limited by a 180 m barrier sill on the continental shelf just outside PWS. The surface waters of PWS are flushed by the Alaska Coastal Current (ACC) that usually enters through Hinchinbrook Entrance and exits through Montague Strait (Niebauer et al. 1994, Vaughan et al. 2001) (Fig. 1). This flushing action is strongest in the fall and winter when wind-stress forcing by storms in the GOA is greatest (Niebauer et al. 1994, Vaughan et al. 2001), and freshwater runoff, which drives the ACC, is also greatest (Royer 1979). Surface-water from the GOA regularly introduces pelagic plankton communities into PWS, including *Neocalanus* copepods (Cooney 1986a, Cooney 1986b, Cooney 1993, Cooney et al. 2001, Vaughan et al. 2001).

Coastlines adjacent to the northern GOA west of PWS are heavily indented and contain numerous fjords smaller than PWS. The ACC continues along the Kenai Peninsula through Shelikof Strait between the Alaska Peninsula and the Kodiak Island complex.

The annual cycle of marine production follows a pattern typical of subarctic marine waters. Increased light and heat during spring together with calmer winds permit development of a stratified euphotic surface layer that leads to a strong spring phytoplankton bloom. This bloom peaks in April inside PWS and somewhat later in the coastal GOA, and is immediately followed by a strong zooplankton bloom (Eslinger et al. 2001, Cooney et al. 2001). The zooplankton bloom biomass consists mainly of Neocalanus cristatus, *N. plumchrus* (and perhaps *N. flemingeri* [Miller 1988], a species that has recently been distinguished from *N. plumchrus*), *Calanus marshallae*, *Metridia okhotensis* and *Pseudocalanus spp*. in PWS (Cooney 1986b, Cooney et al. 2001), and *N. cristatus*, *N. plumchrus*, *N. flemingeri*, and *E. bungii* in the open GOA (Mackas et al. 1993).

The coastal waters of the northwestern GOA are very productive, and have supported numerous important commercial fisheries, including five species of Pacific salmon (*Oncoryhnchus spp*.), Pacific halibut (*Hippoglossus stenolepis*), sablefish (*Anoplopoma fimbria*), Pacific herring (*Clupea harengus pallasi*) and walleye pollock (*Theragra chalcogramma*), as well as a diverse fauna of marine mammals and birds, both resident and migratory. Primary productivity is estimated at 100-225 g C m^{-2} yr^{-1} (Goering et al. 1973, Sambrotto & Lorenzen 1986), and most of the annual primary production occurs during the spring bloom (Goering et al. 1973). The biomass dominance of *Calanus* and *Neocalanus* copepods in the ensuing zooplankton bloom (~50% of biomass during May [Cooney et al. 2001]), together with the high lipid content of these copepods (~50% dry weight, [Sargent & Falk-Petersen 1988]) implies that a substantial proportion of the annual energy budget for this coastal marine ecosystem flows through these genera.

Methods

Zooplankton Samples

Three groups of copepods were collected for pristane analysis. The three collection groups are distinguished in Table 1 by their collection locations and seasons. The first group was collected from Lynn Canal and Chatham Strait in southeastern Alaska. These samples were preserved in buffered 5% formalin seawater immediately following collection during late June 1991, and analyzed December 1994 following species sorting of the preserved samples. The sorted species consisted mostly of stage V copepodites, and adult females. The second and third groups were collected during July 1998 and May 2000 respectively from PWS. These samples were sorted and identified immediately after collection and were stored frozen at -20 ˚C until analysis in December 1999 (second group) and May 2000 (third group). Discrete copepodite stages of *C. marshallae*, *N. cristatus*, and *N. plumchrus* were analyzed separately from the third group. Species and copepodite stages were determined following criteria given by Gardner and Szabo (1982). *Neocalanus plumchrus* was distinguished from *N. flemingeri* in the third group based on examination of the mandibular gnathobase and the ratio of cephalosome to prosome lengths following Miller (1988). These species were not distinguished in the first group, so identification of *N. plumchrus* in this group is presumptive.

Exxon Valdez Oil Spill Damage Assessment Samples

Comparison of hydrocarbon analysis results among the samples collected following the *Exxon Valdez* oil spill requires caution, because the samples were collected for a variety of assessment objectives by multiple government agencies and personnel. Sample collectors all followed the same collection, storage and documentation procedures, which involved use of dichloromethane-rinsed surfaces of collection and

dissection tools, and storage in pre-cleaned glass environmental sample jars at -20 ˚C as soon as possible after collection. Close geographical or temporal coordination of sample collection among different species was not possible because of biological constraints on species availability and conflicting sampling objectives. Sampling effort was therefore unevenly distributed among species, among tissues within species, among geographical locations and at different sampling times. The common and scientific names of the species considered herein, along with their foraging mode, are given in Table 2.

To facilitate comparisons among samples, the most broadly sampled tissues are emphasized in the presentation of the results. These include the livers and eggs of birds; whole bodies and eggs of fish; whole bodies of molluscs and plants; hepatopancreas and eggs of crustaceans; and blubber and livers of mammals. Relevant results for other less frequently sampled tissues or for stomach contents are also presented when these serve to corroborate or qualify the main body of results. I categorically excluded samples from consideration when they failed to meet any of the following criteria: the sampling location was outside the spill area (see Fig. 1) or was not available, the collection date was not available, the dry weight of the sample was not available, the sampled animal was not identified to species, the sampled matrix was feathers or an egg or mollusk shell, or the sample was likely contaminated by crude oil or refined petroleum products. I considered contamination likely if the ratio phytane to pristane was greater than 10%. Phytane (2,6,10,14-tetramethylhexadecane) is a branched hydrocarbon that is rarely found apart from petroleum sources (Blumer & Snyder 1965, Ackman & Zhou 2003), is about as persistent as pristane in the environment, and is present in *Exxon Valdez* cargo oil at nearly the same concentration as pristane (Wang et al. 2003). Application of these selection criteria reduced the number of samples considered to 3,007.

Most (57.7%) of the samples reported were collected within PWS during 1989 and 1990, and 22.7% were collected inside PWS during 1991 through 1995. Another 15.5% of samples were collected from outside PWS (but within the spill area, Fig. 1) during 1989 and 1990, and 4.5% were collected during 1991 through 1995. All but 2 of the samples collected after 1990 are molluscs, and of these 98.5% are bivalves.

All of the hydrocarbon data generated from government studies of the *Exxon Valdez* oil spill are archived in the *Exxon Valdez* Oil spill of 1989 State/Federal Trustee Council Hydrocarbon Database (EVTHD) at the Auke Bay Laboratory, and are available from the author.

Pristane Analysis

The chemical analysis of the oil spill samples for aliphatic hydrocarbons involved dichloromethane extraction of macerated tissues spiked initially with a suite of perdeuterated alkane internal standards, solvent concentration and exchange into hexane over steam, purification by silica gel/alumina column chromatography eluted with pentane, solvent concentration and alkane resolution by gas chromatography (GC) and

measurement by flame ionization (Short et al. 1996). Identification of pristane is based on GC elution time, with occasional confirmation by GC-mass spectrometry. The method for the zooplankton samples involved no alumina and less silica gel, because of the small tissue mass aliquots analyzed (< 0.1 g).

The accuracy of the pristane analyses were generally within ±15% based on comparison with authentic hydrocarbon standards prepared by the National Institute of Standards and Technology (NIST), and the coefficient of variation was generally less than ±20%. The method detection limit (MDL), defined as the estimated concentration associated with a 1% probability of type I detection error, was 0.0617 to 0.210 μg depending on the matrix and analytical laboratory. The corresponding MDL estimate for individual samples is the ratio of these values and the mass of the sample analyzed.

Dry Weight Determination

Zooplankton samples were air-dried at room temperature to constant weight for dry weight determination. The ratio of wet and dry weights of *Neocalanus plumchrus* CV copepodites collected from Prince William Sound was 6.1. Samples collected for the *Exxon Valdez* oil spill were heated overnight at 60 ˚C.

Data Analysis

Pristane concentrations are presented herein as μg pristane per gram dry tissue weight. Results are aggregated seasonally to evaluate seasonal variability and comparability. Pristane concentrations within each season, species, and tissue type are summarized by box plots indicating the median, 10%, 25%, 75% and 90% of the data for large sample sizes ($n > 5$), or the range ($n \leq 5$) in Figures 2 – 8.

The significance of differences between two samples is determined by the nonparametric Mann-Whitney U test (Mann & Whitney 1947).

Results

Zooplankton

Neocalanus and *Calanus* copepods contained the highest concentrations of pristane among the species surveyed (Table 1). Median pristane concentrations ranged from 2,440 to 8,020 μg/g in the CIV and CV copepodite stages of these species. The median was significantly lower in the CIV copepodite stage compared with the CV stage (P=0.024) of *C. marshallae*, but not of *N. plumchrus* (P=0.15). However, the pristane concentration of one sample of CIII *N. plumchrus* was only 731 μg/g, substantially lower than the lowest concentration in CIV copepodites (2,440 μg/g). Pristane concentrations in the formalin-preserved samples of *C. marshallae*, *N. cristatus*, and *N. plumchrus* from

southeastern Alaska were not significantly different than frozen samples collected from PWS (P>0.142).

Pristane concentrations were consistently less than 700 μg/g in the other zooplankton species survey, and were usually much less (Table 1). The median concentration in *M. okhotensis* was 660 μg/g, compared with concentrations less than 125 μg/g in the two other species of *Metridia*. *Euchaeta elongata* from southeastern Alaska contained 455 μg/g compared with 23.8 μg/g from PWS. The remaining species surveyed contained less than 125 μg/g, including *Pseudocalanus spp*. and the three euphausid species (*Thysanoessa spp*.)

Exxon Valdez Oil Spill Samples

Birds

The highest pristane concentrations in birds were found in the two zooplanktivorous species. One shearwater liver contained 1,960 μg/g (Fig. 2), and the stomach contents of the same animal contained 1,460 μg/g. The median concentration of 19 fork-tailed storm petrel eggs was 519 μg/g.

Substantial pristane concentrations were found in the five piscivorous bird species. Median pristane concentrations in livers of black kittiwakes, marbled murrelets, and pigeon guillemots were 137 μg/g, 46.1 μg/g and 1.15 μg/g (Fig. 2). The liver of one common loon sampled during spring 1989 contained to 229 μg/g. Median pristane concentrations in eggs of bald eagles and black kittiwakes were similar, with ranges of 1.17 μg/g to 62.2 μg/g for bald eagles and 1.51 μg/g to 61.7 μg/g for black kittiwakes (Fig. 2). Median pristane concentrations were higher in bald eagle eggs sampled during spring compared with summer of 1989 and of 1990, but the significance of these differences was marginal (P<0.10). Twenty-two blood samples from bald eagles were analyzed, but pristane was usually not detected.

Birds feeding primarily nearshore or intertidal invertebrates contained lower concentrations of pristane than the piscivorous birds. The invertebrate feeders include 3 shorebirds (black turnstone, rock sandpiper, and surfbird) and 5 sea ducks (Barrow's and common goldeneye, harlequin duck, surf scoter, and white-winged scoter). Among these 8 species, median pristane concentrations in livers were highest in black turnstones and surfbirds sampled during winter, at 5.11 μg/g and 12.9 μg/g respectively. The median for black turnstone livers was 1.05 μg/g in summer 1989 compared with 5.11 in winter 1990, but the difference is not significant (P=0.5). In contrast, pristane concentrations in livers of rock sandpipers and of all the sea ducks were lower, ranging from below MDL to 2.68 μg/g across all 39 liver samples of these species. Although no seasonal trends were evident in these shorebird and sea duck samples, these samples were usually collected during fall or early winter, rather than the spring and summer collections typical of most

other species in this survey, so comparisons among these species requires allowance for seasonal trends.

The only samples collected from an obligate avivore were peregrine falcon eggs. Median pristane concentrations in eggs collected during spring or summer 1990 were less than 1 μg/g, but ranged to 12.2 μg/g in the 12 samples analyzed (Fig. 2).

Fish

Pristane concentrations were highest in Pacific herring and juvenile pink salmon. Muscle and viscera of Pacific herring had median concentrations near 100 μg/g, compared with 11.6 μg/g to 21.6 μg/g in reproductive tissues and eggs (Fig. 3). Pristane concentrations in pink salmon increased substantially following migration to seawater. The median concentration in alevins just prior to emergence from gravels of incubation streams was 4.57 μg/g, compared with median concentrations in seawater-resident fry during spring and summer of 57.9 μg/g and 142 μg/g, which are significant increases (alevins vs spring juveniles, $P<0.001$; spring vs summer juveniles, $P<0.004$).

Pristane concentrations were generally lower in the other fishes. The median pristane concentration in whole juvenile dusky rockfish sampled during fall 1989 was 34.6 μg/g, compared with median concentrations of 7.10 μg/g to 13.3 μg/g in whole prickleback species sampled during spring or summer. During spring, one juvenile Pacific cod contained 8.09 μg/g, but three others contained less than 0.090 μg/g (Fig. 3).

Molluscs

The highest pristane concentrations among mollusks occurred in suspension-feeders sampled during spring. The suspension-feeders sampled include bay mussels, butter clams, littleneck clams, Pacific oysters, and weathervane scallops. Bay mussels contained the highest median concentrations among these suspension-feeders at 2.34 μg/g to 3.51 μg/g during spring, compared with 0.179 μg/g to 0.234 μg/g during summer, and this seasonal difference was highly significant ($P<0.001$; Fig. 4). Some bay mussels contained concentrations greater than 50 μg/g during spring, comparable with concentrations of some piscivorous fish. Springtime concentrations were significantly greater than summer for all the other suspension-feeders where data are available for comparison ($P<0.01$ in all cases).

Increased concentrations of pristane in bay mussels during spring compared with summer is a consistent pattern evident each year from 1989 through 1995 (Fig. 5). The differences between spring and summer concentrations are significant in each of these years ($P<0.001$) except 1991 ($P=0.065$).

Pristane concentrations attained seasonally maximum values in bay mussels, butter clams, and littleneck clams during May. In 1989, pristane concentrations increased from less than 0.5 μg/g in March and early April to over 63 μg/g in early May, then gradually subsided to concentrations generally less than 1 μg/g by August, and this

pattern repeated each subsequent year through 1995. The available data for butter clams and for littleneck clams are consistent with the trends evident for bay mussels, but the maximum pristane concentrations did not exceed 10 μg/g.

Pristane concentrations were lower in the deposit-feeding bivalves compared with the suspension-feeders. The deposit-feeders sampled include Kennerley's venus, macoma clams, razor clam, and sunset clams. Median pristane concentrations were near or below MDL in these bivalves, and the highest concentration found was 0.623 μg/g (Fig. 4). The deposit-feeders were not extensively sampled during spring, precluding seasonal comparisons.

Pristane concentrations in periwinkle snails, intertidal algal and detrital grazers, were comparable with the suspension-feeding bivalves. Median concentrations were 1.51 μg/g in spring 1989 and 0.504 μg/g in summer 1990 (Fig. 4).

Crustaceans, Echinoderm and Plants

Substantial but variable pristane concentrations occurred in the hepatopancreas of all three crab species sampled. Median concentrations were 39.1 μg/g in king crab hepatopancreas, and ranged from 3.26 μg/g to 27.1 μg/g in Tanner crab and from 0.261 μg/g to 4.53 μg/g in Dungeness crab (Fig. 6). Maximum pristane concentrations near 100 μg/g to more than 400 μg/g were evident in all three species.

Concentrations in crab eggs or ovaries were generally lower than in the hepatopancreas, with median concentrations of eggs ranging from below MDL to 15.1 μg/g across the three crab species and the sampling seasons. Maximum concentrations were also lower, the highest value being 33.2 μg/g (Fig. 6).

Spot shrimp eggs contained somewhat higher pristane concentrations than the crab eggs. Median concentrations of shrimp eggs were 7.90 μg/g, and the maximum value was 223 μg/g (Fig. 6).

The median pristane concentration in the suspension-feeding barnacle during spring was 1.74 μg/g, comparable with concentrations in suspension-feeding molluscs (Figs. 3 and 6). Barnacles were not sampled during other seasons, precluding comparison.

Concentrations of pristane in green sea urchin gonads were low compared with the crustacean tissues, with a median of 0.257 μg/g and a maximum of 2.26 μg/g (Fig. 6).

The median concentration of pristane in rockweed collected during spring from Montague Island was 5.68 μg/g, and ranged from 4.53 μg/g to 9.02 μg/g (Fig. 6). One eelgrass sample concurrently collected from the same location contained 5.04 μg/g.

Pristane concentrations were below detection limits in six samples of rockweed collected in late summer/early fall from sites along the Alaska Peninsula.

Mammals

Substantial concentrations of pristane were found in some tissues of the piscivorous harbor seal, harbor porpoise and sea lion. The large number of tissue types sampled from harbor seals provides an indication of the distribution of pristane within this animal. The highest median pristane concentrations occurred in the mammary, followed by blubber, milk, kidney, heart, ovary, liver, blood, and brain in descending order. Median concentrations in the mammary were 294 μg/g in the spring and 52.9 μg/g in the summer, and were between 2.82 μg/g and 50.9 μg/g in blubber, milk, kidney, and heart (Fig. 7). Maximum concentrations ranged to several hundred μg/g in the mammary and ranged to 50 – 150 μg/g in blubber, milk, kidney and liver, while minimum concentrations were near or below MDL in blubber, kidney and liver. The median concentration in liver was 0.590 μg/g but exceeded 10 μg/g in 2 samples. Comparatively low concentrations (< 9 μg/g) were consistently found in the blood, brain, heart, lung and ovary.

The distribution of pristane in the harbor porpoise and sea lion were consistent with the pattern evident in harbor seals. The two blubber samples from harbor porpoise had pristane concentrations of 0.234 μg/g and 175 μg/g, similar to the values that range from 0.333 μg/g to 138 μg/g in six samples of sea lion blubber (Fig. 8). Liver concentrations ranged from below MDL to 679 μg/g in harbor porpoise and sea lion, encompassing the range found in harbor seal livers (Fig. 8). Concentrations in sea lion brains were consistently low (<1.29 μg/g), similar to harbor seal brains.
Pristane concentrations ranged from 37.3 μg/g to 628 μg/g in killer whale blubber, but were only 5.26 μg/g in the single sample of liver (Fig. 8). Blubber contained 8.97 μg/g and 8.02 μg/g in single samples from grey and minke whales, and liver concentrations ranged from 0.136 μg/g to 3.58 μg/g in these two whales.

Pristane concentrations were consistently low in sea otter blood, fat, and livers compared with the piscivorous marine mammals. Pristane was less than 14 μg/g in fat or liver (Fig. 8), and was detected only once in 55 sea otter blood samples at 0.179 μg/g.

Pristane concentrations were 1.03 μg/g or less in 45 samples of feces from brown bears collected from the Alaska Peninsula during spring, and was 0.684 in a single liver sample collected (Fig. 8). Concentrations of pristane were below MDL in five samples of Sitka black-tailed deer livers (Fig. 8).

Discussion

Analogous Role of Pristane in Pacific and Atlantic Ocean Members of Calanus and Neocalanus

The high concentrations of pristane reported here for Pacific Ocean species of *Calanus* and *Neocalanus* are comparable with concentrations reported previously for Atlantic Ocean *Calanus* species, indicating that the ability to biosynthesize pristane from ingested chlorophyll attributed to the Atlantic *Calanus* species may be extended to Pacific species of both *Calanus* and *Neocalanus*. Pristane concentrations in stage V copepodites of Atlantic Ocean species *C. finmarchichus*, *C. glacialis*, and *C. hyperboreus* ranged from 4,500 – 9,200 μg/g, and did not appear to vary significantly from April through August, or geographically within a geographic range of > 300 km (Blumer et al. 1964). Pristane concentrations in stage V copepodites of the Pacific Ocean species *C. marshallae*, *N. cristatus*, and *N. plumchrus* reported here range from 1,960 – 8,850 μg/g, and also do not vary seasonally from spring through summer nor geographically from southeast Alaska to PWS, a distance of ~500 km (Table 1). Note that the insignificant differences between pristane concentrations in formalin-preserved compared with frozen samples of *C. marshallae* or *N. cristatus* indicates that prolonged contact with formaldehyde has little effect on pristane concentration, which is not surprising given the chemical resistance of alkanes to oxidation by weak oxidizers such as formaldehyde.

Members of both *Neocalanus* and *Calanus* genera inhabiting high latitudes of the northern hemisphere accumulate substantial lipid reserves for sustenance during prolonged periods of diapause and for subsequent reproduction (Conover, 1988). Blumer et al. (1964) proposed that *Calanus* copepods biosynthesize pristane to increase buoyancy, which could permit fuller utilization of lipid reserves during diapause, and the same rationale applies to *Neocalanus*. These phylogenetic and life-history similarities suggest that *Neocalanus* copepods probably share with *Calanus* copepods the ability to biosynthesize pristane from chlorophyll. Avigan and Blumer (1968) were unable to resolve whether this biosynthetic competence is associated with the copepod's anabolic metabolism or with the microbial community inhabiting their intestine, but in either case the increasing pristane concentration with copepodite life stage in *N. plumchrus* and *C. marshallae* (Table 1) suggests pristane production parallels oil droplet formation. Analysis of tropical and Antarctic members of these genera may provide additional insight regarding the role of pristane in the life histories of these copepods.

Pristane in Other Zooplankton Species

Zooplankton genera other than *Calanus* and *Neocalanus* may acquire pristane through similar pathways of anabolic synthesis, or through predation. *Pseudocalanus spp*. are herbivorous (Mauchline 1998), so the lower pristane concentrations found in these zooplankters may be acquired through a less active biosynthetic pathway compared with *Calanus* or *Neocalanus* species. The other zooplankton analyzed here or by Blumer et al. (1964) are either omnivorous or carnivorous, and pristane in these species may be acquired through ingestion of the younger copepodite stages of *Calanus* or *Neocalanus*, or of later copepodite stages or adults of e.g. *Pseudocalanus spp*.

Dominance of Calanus and Neocalanus as Sources of Pristane in the Northern Gulf of Alaska

Evidence supporting the identification of *Calanus* and *Neocalanus* copepods as the major source of pristane in the northern Gulf of Alaska includes: (1) the fact that, of all the biota and tissue types sampled, pristane concentrations are highest in these copepods, (2) *Calanus* and *Neocalanus* copepods account for most of the zooplankton biomass in the spring zooplankton bloom, (3) pristane concentrations in other species decline with increasing trophic transfers from these copepods, and (4) patterns of seasonal variability in the biota sampled are generally coherent with the production of pristane by *Calanus* and *Neocalanus* copepods. *Calanus* and *Neocalanus* copepods may account for nearly 50% of the zooplankton biomass during May (Cooney et al. 2001), and pristane concentrations exceed those of other potential zooplankton producers of pristane by a factor of ~ 50, implying ~98% of the pristane produced during spring is produced by *Calanus* and *Neocalanus* copepods. The combined wet weight biomass of *Calanus* and *Neocalanus* copepods in the uppermost 50 m of seawater is ~0.15 g/m^3 in May (Cooney et al. 2001), equivalent to ~7 mg pristane/m^2 seasurface (assuming a wet:dry weight ratio of 6.1 and a pristane concentration of 0.6%). Averaged over the 8,800 km^2 surface area of PWS, this implies production on the order of 65,000 tons of pristane annually by these copepods. In comparison, this is more than 800-fold the amount of pristane introduced into PWS by the crude oil spilled from the T/V *Exxon Valdez* (assuming spill volume data from Wolfe et al. 1994, and oil density and composition data from Wang et al. 2003). Prince William Sound is thus annually inoculated with a substantial dose of pristane generated by *Calanus* and *Neocalanus* copepods, which then permeates the marine food web through trophic transfers involving predation, and perhaps also by fecal production associated with these transfers or with the copepods themselves.

Comparison of pristane burdens among the species sampled is hampered somewhat by the lack of uniformity of tissues sampled. Pristane concentrations span a nearly 100-fold range in the harbor seal tissues sampled, and are highest in the lipid storage tissues, and lowest in blood and brain (Fig. 7). The relatively low pristane concentration in the blood, not only of harbor seals but also of bald eagles and sea otters, suggests that blood may not be an appropriate tissue for monitoring pristane (or indeed other contaminant) burdens. The low concentrations in the brain suggests that pristane does not cross the blood-brain barrier effectively. Conversely, the relatively high concentrations in the lipid-rich tissues indicates that lipid normalization (i.e. expression of pristane concentrations as mass pristane per unit mass lipid) might give a more accurate indication of trophic relationships among the predator species complex (Laws 1993). But provided appropriate allowance is made for the likely differences in lipid content of the species and tissues sampled, the progress of the pristane introduced into the marine food web of PWS may still be roughly traced.

The trend of generally declining pristane concentrations with trophic transfers in the marine-dependent food web extends from the neritic components to the intertidal and

the benthos. Pristane concentrations in the neritic predators of *Calanus* and *Neocalanus* copepods are generally higher that any of the other species sampled, but are lower than the concentrations in the copepods themselves. These neritic predators include the fork-tailed storm petrel and shearwaters among the birds (Fig. 2), herring and juvenile pink salmon among the fish (Fig. 3), and *Euchaeta elongata*, and *Metridia spp*. among the zooplankton. These species may all prey directly on *Calanus* and *Neocalanus* copepodite stages, and all had pristane concentrations of several hundred μg/g. Pristane concentrations are lower by factors of 10 or more in species that prey on these neritic copepod consumers, including the bald eagle, black kittiwake, common loon, marbled murrelet and pigeon guillemot among the birds (Fig. 2), and the piscivorous marine mammals (Fig. 8). In contrast with these neritic species, pristane concentrations are usually much lower in species associated with the intertidal.

The route by which pristane enters the intertidal is not entirely clear. None of the species associated with the intertidal have pristane concentrations as high as the neritic predators of *Calanus* or *Neocalanus* copepods, but they often contain concentrations approaching 10 μg/g. Pricklebacks and black pricklebacks in the intertidal may opportunistically prey on *Calanus* or *Neocalanus* when available, and juvenile dusky rockfish definitely do (Yang 1993) (Fig. 2). The fact that pristane concentrations are generally higher in suspension-feeding compared with deposit-feeding molluscs in the intertidal suggests that the acquired pristane may be associated with dispersed organic particulate material in the water column. This material could be *Calanus* or *Neocalanus* copepodites, or dispersed fecal material produced by the neritic predators of *Calanus* or *Neocalanus* copepods, or feces of the copepods themselves.

While plausible, direct predation by mussels on *Calanus* or *Neocalanus* copepodites is not likely to be a major pathway of pristane accumulation by mussels. Mussels may effectively prey on some smaller (~0.25 mm length) mesozooplankton (Wong et al. 2003) and may occasionally capture mobile zooplankton as large as 3 – 6 mm length (Davenport et al. 2000), escape responses of even the naupliar stages of copepods are usually adequate to avoid capture by mussels (Green et al. 2003).

Incorporation of fecal material produced by efficient predators of *Calanus* or *Neocalanus* copepodites is probably a more important pathway of pristane accumulation by mussels. Although feeding experiments using *Calanus finmarchichus* have shown that lipid assimilation by herring and rainbow trout (*Salmo gairdneri*) is very efficient, with less than 5% of total ingested lipid excreted in feces (Sargent et al. 1979), the high concentration of pristane in these copepods means that the feces produced would still contain pristane concentration on the order of 500 μg/g. Such fecal concentrations of pristane are much higher than tissue concentrations found in mussels during spring (Fig. 5). Juvenile pink salmon prey heavily on *Neocalanus* copepodites during spring in PWS (Sturdevant et al. 1996, Willette 1996), where they remain close to the shoreline during the first few weeks of their marine residence (Cooney et al. 1981). Dispersion of feces produced by nearshore piscine predators of *Neocalanus* might provide a pristane-laden

form of organic material that could be readily ingested by mussels. However, this route of incorporation is hypothetical. So is the possibility that mussels may incorporate pristane-laden feces produced directly by *Neocalanus* copepodites, because the concentration of pristane in feces from these copepodites has not been measured.

Regardless of the pristane transport pathway, intertidal deposit-feeders and herbivores have consistently low concentrations of pristane, as do most of their predators, including sea otters, the sea ducks, and rock sandpipers. The somewhat elevated (~ 1 – 10 μg/g) pristane concentrations associated with the eelgrass, rockweed and periwinkle snails sampled during spring are probably the result of herring eggs deposited on these plants. The samples of these three species that contained the higher concentrations were all from Montague Island and were collected during a herring spawning event which was particularly extensive in 1989. Herring eggs contain pristane at concentrations above 10 μg/g, so these may have been the vector for pristane transmission to these plants and the periwinkle snail. Pristane is below MDL in rockweed collected later in the year (Fig. 6). Hence, egg deposition is another route by which pristane may be introduced to the intertidal.

The very variable concentrations of pristane in the benthic foragers (crabs and shrimp) may reflect occasional opportunities to prey on *Calanus* and *Neocalanus* copepods directly. Pristane concentrations in spot shrimp eggs and in Dungeness crab hepatopancreas were substantial in the fall (Fig. 6), when *Calanus* and *Neocalanus* copepods descend to deep (> 300 m) waters during diapause (Conover 1988). The continental shelf and most of PWS is shallower than 300 m, so these copepods may encounter the benthic interface instead, and may then be vulnerable to predation by benthic foragers that learn to target them as prey. Whatever the cause, pristane concentrations were also found to be highly variable in pink shrimp (*Pandalus borealis*) compared with the other species sampled during the most extensive survey of pristane in marine biota before this one (Johansen et al. 1977). The pristane concentrations in king and in Tanner crabs, as well as in some of the spot shrimp and dungeness crab samples, may arise mostly through predation or scavaging on first- and second-level consumers of *Calanus* and *Neocalanus* copepods. In contrast, pristane concentrations are uniformly low in the green sea urchin, which grazes epibenthic algae, and in the terrestrial mammals, consistent with an absence of autochthonous sources of pristane in the nearshore benthic or the terrestrial communities.

The timing of the annual spring increase of pristane in suspension-feeding molluscs provides additional evidence that *Calanus* and *Neocalanus* copepods are the dominant source of pristane in the sampled region. Pristane concentrations consistently increase sharply just following the spring zooplankton bloom every year, and begin to decline in June just as *Calanus* and *Neocalanus* copepods begin their ontogenetic migration to deeper water. Mussels depurate half their burden of accumulated hydrocarbons in ~1 – 4 weeks, with the longer periods following longer exposures (Pruell et al. 1986, Mason 1988a, 1988b), so the decline of pristane concentrations in mussels

throughout the summer reflects the decline of pristane production by *Calanus* and *Neocalanus* copepods, lagged by a few weeks. The absence of increases in pristane concentrations in suspension-feeding molluscs later in the summer argues strongly against the presence of another important source of pristane available to this food web, at least during summer, given the ability of these organisms to concentrate hydrocarbons from seawater into their tissues by factors of nearly 10^6 (Murray et al. 1991).

The annual increases of pristane concentrations in mussels during spring were associated with a concurrent pulse of pristane-laden particulate material to the benthos in PWS. Pristane concentrations in sediments collected by sediment traps deployed at 10 – 20 m depths were much higher during spring, when concentrations ranged as high as 1.1 mg pristane/g dry sediment, and these concentrations were also concurrent with increases found in benthic sediments adjacent to the traps (Sale et al. 1995). Traps deployed during other seasons, including winter, had much lower pristane concentrations in the collected sediments, which further corroborates the spring production of pristane by *Calanus* and *Neocalanus* copepods as the major source of pristane.

Although nearly all the evidence regarding the distribution and seasonal variability of pristane implicates *Calanus* and *Neocalanus* copepods as the dominant natural source, two exceptions stand out. Pristane concentrations in livers of black turnstones and in surfbirds were higher than expected in winter compared with other birds that forage in the intertidal (Fig. 2), with concentrations more comparable with piscivorous birds. The source and route of pristane to livers of these two birds during winter is unclear.

Pristane in the Marine Food Web

The dissipation of pristane in biota following production during spring implies that pristane is not bioconcentrated in the food chain, but is biochemically transformed and dispersed on time scales of weeks to months. Pristane dissolved into seawater may be photo-oxidized (Rontani & Giusti 1987), or may be degraded by the microbial community (Pirnik 1977, Alvarez 2003). Pristane in fecal material exported to the benthos may also be vulnerable to microbial degradation, although pristane incorporated into carbonate oozes of the deep seafloor may persist for several millenia (Ohkouchi et al. 1997). Fish are capable of metabolizing accumulated pristane (Cravedi et al. 1985, 1989), and it is likely that other vertebrates share this capability. However, once incorporated into depot lipids, pristane may persist in these organisms for months and perhaps years periods despite catabolic competence (Cravedi & Tulliez 1982), especially if the rate of lipid turnover is slow.

The nearshore food web of the northern GOA may be roughly represented by springtime pristane concentrations in the sampled biota (Fig. 9). This representation is consistent with known trophic relationships, and with results from other measurements of pristane in marine biota. Pristane concentrations decline about tenfold with each trophic

level beginning with the *Calanus* and *Neocalanus* copepod producers through their first, second and third level predators. Transport of pristane to the benthos and to the intertidal is probably mediated by fecal production from *Calanus* and *Neocalanus* copepods or their predators, and perhaps augmented by ontogenetic migration of these copepods to the benthos. Further work will be necessary to establish the magnitude of these linkages and their ecological importance.

Uses of Pristane as a Tracer Molecule

Pristane has several potential uses as a natural biological tracer in food-web studies. Available evidence suggests that natural terrestrial sources of pristane may be negligible in comparison with pristane imported with anadromous salmonids to riparian systems (Ackman 1971), where pristane may serve as an independent proxy for carbon from marine lipids. For example, pristane analysis of depot lipid or of feces from brown bears might distinguish bears that prey mainly on fish from those preying on mammals. Similarly, pristane analyses may help to clarify carbon subsidies to benthic communities, or to evaluate suspected trophic linkages in food webs through comparison of concentrations in predators with their potential prey fields. In addition, the large difference in pristane concentrations of herring oils from the Baltic and North Seas (Linko and Kaitaranta 1976) suggests that pristane analysis may be helpful in distinguishing stocks of herring and perhaps other fishes. Pristane could also be useful in physiological studies, including studies on assimilation efficiency of ingested lipids, and studies on lipid turnover rates. Finally, pristane analyses may serve to elucidate suspected contaminant pathways in ecosystems. The pattern of pristane dispersion in the ecosystem comprising the biota surveyed here suggests that these contaminant pathways may often be subtle and unexpected.

Conclusions

As in Atlantic Ocean *Calanus* species, biosynthesis of pristane occurs in Pacific Ocean species of copepods in the genera *Calanus* and *Neocalanus*, producing concentrations that increase with ontogenetic copepodite development to near 1% dry mass in stage V copepodites. Pristane biosynthesis may occur in other herbivorous calanoid copepods such as *Pseudocalanus spp*., but concentrations in these species are lower by factors of ~100, and these species are considerably smaller than late-stage copepodites of *Calanus* or *Neocalanus* (e.g. 2 mm total length vs 4 – 8 mm). The large size and high concentrations of pristane in stage V copepodites of the *Calanus* and *Neocalanus* species that dominate the biomass of the annual spring zooplankton bloom implies these copepodites are by far the major source of pristane for the neritic ecosystem of the northern Gulf of Alaska.

The pristane introduced with the spring pulse of secondary production serves as a natural chemical label for the associated lipid produced by stage V *Calanus* and *Neocalanus* copepodites, and these pristane-labeled lipids permeate the food web at least

through fall, initially through direct predation on these copepods by zooplanktivorous birds and fishes. Pristane concentrations tend to decline with the number of trophic transfers among subsequent consumer species. Excretion of pristane-labeled lipids that are not assimilated by consumers, especially the zooplanktivores, may provide a pathway for pristane to the intertidal and the subtidal benthos, but fecal material produced by the *Calanus* and *Neocalanus* copepodites might also be important, and requires further study for resolution.

As a terminally-branched hydrocarbon pristane resists degradation through β-oxidation but it is not as refractory as halogenated organic pollutants, and does not magnify through the food chain. Fish are capable of transforming pristane to more excretable metabolites, which suggests that other vertebrates are also. Despite this capability, pristane accumulates in vertebrate depot lipids and may persist at least for weeks, and possibly much longer. The distribution of pristane incorporated by birds and mammals suggests that measurement of pristane per unit lipid may provide additional insight into the way pristane is transferred trophically, and that blood sample analysis for pristane is a very insensitive indicator of lipid concentrations of pristane. These attributes make pristane a candidate as a tracer compound for food web analysis, for physiological studies, and as an adjunct for studying the permeation of food webs by lipophilic organic pollutants. The results of this survey of pristane in the neritic food web of the Gulf of Alaska confirm an earlier suggestion by Blumer et al. (1964) that pristane may be a useful label for probing marine food webs.

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Tables

Table 1. Concentrations of pristane in zooplankton from Prince William Sound (PWS) and Southeastern Alaska (SEAK). Sp = Spring, Su = Summer.

Species	Region	Season	Median [Pristane] (µg/g)	Range (µg/g)	Samples Analyzed n	Individuals/ Sample n
Chiridius spp.	PWS	Su	15.6		1	25
Calanus marshallae – CV & adult female	SEAK	Su	6240	5630-6390	3	1
Calanus marshallae - CV	PWS	Sp	6520	5450-7300	6	1
Calanus marshallae - CIV	PWS	Sp	1030	606-1620	3	1
Eucalanus bungii – CV & adult female	SEAK	Su	107	61.6-205	3	1
Euchaeta elongata – CV & adult female	SEAK	Su	455	388-600	3	1
	PWS	Su	23.8	23.6-24.0	2	2
Metridia pacifica - female	PWS	Su	30.8		1	28
Metridia okhotensis – CV & adult female	SEAK	Su	660	509-672	3	1
Metridia lucens – CV & adult female	SEAK	Su	86.6	74.8-121	3	1
Neocalanus cristatus – CV	SEAK	Su	4460	3960-6070	3	1
Neocalanus cristatus - CV	PWS	Sp	2440	1960-5190	5	1
Neocalanus plumchrus – CV	SEAK	Su	7890	6980-11300	3	1
Neocalanus plumchrus - CV	PWS	Sp	8020	4010-8850	5	1
Neocalanus plumchrus - CIV	PWS	Sp	4430	2660-6700	5	1
Neocalanus plumchrus - CIII	PWS	Sp	731		1	4
Pseudocalanus spp	PWS	Sp	117		1	16
Thysanoessa inermis	PWS	Su	39.0	34.8-43.2	2	1
Thysanoessa raschii	PWS	Su	9.09		1	1
Thysanoessa spinifera	PWS	Su	3.42	1.84-5.00	2	1

Table 2. Common and scientific names of species compared in this study that were sampled during the Natural Resources Damage Assessment effort for the 1989 *Exxon Valdez* oil spill in Prince William Sound, Alaska, and their foraging mode. AE = algal epiphytes, AV = avivore, BI = benthic invertebrates, DF = deposit feeder, II = intertidal invertebrates, PP = primary producer, PV = piscivore, SF = suspension feeder, TH = terrestrial herbivore, TM = predator of terrestrial mammals, TO = terrestrial omnivore, ZV = zooplanktivore.

Species Name	Foraging Mode	Reference
I. Birds		
Bald Eagle, *Haliaeetus leucocephalus*	PV, AV, TM	Ehrlich et al. 1988
Barrow's Goldeneye, *Bucephala islandica*	BI, II	DeGange & Sanger 1986
Black Kittiwake, *Rissa tridactyla*	PV, BI	DeGange & Sanger 1986
Black Turnstone, *Arenaria melanocephala*	II	Ehrlich et al. 1988
Common Goldeneye, *Bucephala clangula*	BI	Ehrlich et al. 1988
Common Loon, *Gavia immer*	PV, BI	Ehrlich et al. 1988
Fork-tailed Storm Petrel, *Oceanodroma furcata*	PV, ZV	DeGange & Sanger 1986
Harlequin Duck, *Histrionicus histrionicus*	BI, II	DeGange & Sanger 1986
Marbled Murrelet, *Brachyramphus marmoratus*	PV, BI	DeGange & Sanger 1986
Peregrine Falcon, *Falco peregrinus*	AV	Ehrlich et al. 1988
Pigeon Guillemot, *Cepphus columba*	PV, BI	DeGange & Sanger 1986
Rock Sandpiper, *Calidris ptilocnemis*	II	Ehrlich et al. 1988
Shearwater, *Puffinus spp.*	PV, ZV	DeGange & Sanger 1986
Surfbird, *Aphriza virgata*	II	Ehrlich et al. 1988
Surf Scoter, *Melanitta perspicillata*	BI	Ehrlich et al. 1988
White-winged Scoter, *Melanitta fusca*	BI	DeGange & Sanger 1986
II. Fish		
Black Prickleback, *Xiphister atropurpureus*	ZV	Hart 1973
Dusky Rockfish (juvenile), *Sebastes ciliatus*	BI, ZV	Yang 1993
Pacific Herring, *Clupea harengus*	ZV	Hart 1973
Pacific Cod (juvenile), *Gadus macrocephalus*	BI, ZV, PV	Yang 1993
Pink Salmon (juvenile), *Oncorhynchus gorbuscha*	ZV	Rogers et al. 1979

Prickleback, *Anoplarchus purpurescens*	ZV, BI, AE	Hart 1973

III. Molluscs

Bay Mussel, *Mytilus trossulus*	SF	O'Clair & O'Clair 1998
Butter Clam, *Saxidomus giganteus*	SF	O'Clair & O'Clair 1998
Kennerley's Venus, *Humilaria kennerleyi*	DF	
Littleneck Clam, *Protothaca staminea*	SF	O'Clair & O'Clair 1998
Macoma Clam, *Macoma balthica*	DF	O'Clair & O'Clair 1998
Pacific Oyster, *Crassostrea gigas*	SF	
Periwinkle Snail, *Littorina sp.*	AE	O'Clair & O'Clair 1998
Razor Clam, *Siliqua patula*	DF	
Sunset Clam, *Gari californica*	DF	
Weathervane Scallop, *Patinopecten caurinus*	SF	

IV. Crustaceans

Barnacle, *Balanus cariosus*	SF	O'Clair & O'Clair 1998
Dungeness Crab, *Cancer magister*	BI, PV	O'Clair & O'Clair 1998
King Crab, *Paralithodes camtschaticus*	BI, PV	Cunningham 1969
Spot Shrimp, *Pandalus platyceros*	BI	Butler 1980
Tanner Crab, *Chionoecetes bairdi*	BI	Brethes et al. 1982

V. Echinoderm

Sea Urchin, *Strongylocentrotus droebachiensis*	AE	O'Clair & O'Clair 1998

Common Name
VI. Mammals

Brown Bear, *Ursus arctos*	TO	
Gray Whale, *Eschrichtius robustus*	BI, PV	Calkins 1988
Harbor Porpoise, *Phocoena phocoena*	PV	Calkins 1988
Harbor Seal, *Phoca vitulina*	PV	Calkins 1988
Killer Whale, *Orcinus orca*	PV, MM	Calkins 1988
Minke Whale, *Balaenoptera acutorostrata*	ZV, PV	Calkins 1988
Sitka Black-Tailed Deer, *Odocoileus hermionus*	TH	O'Clair & O'Clair 1998
Sea Lion, *Eumetopias jubatus*	PV	Calkins 1988
Sea Otter, *Enhydra lutris*	BI	Calkins 1988

VII. Plants

Eelgrass, *Zostera marina*	PP
Rockweed, *Fucus spp.*	PP

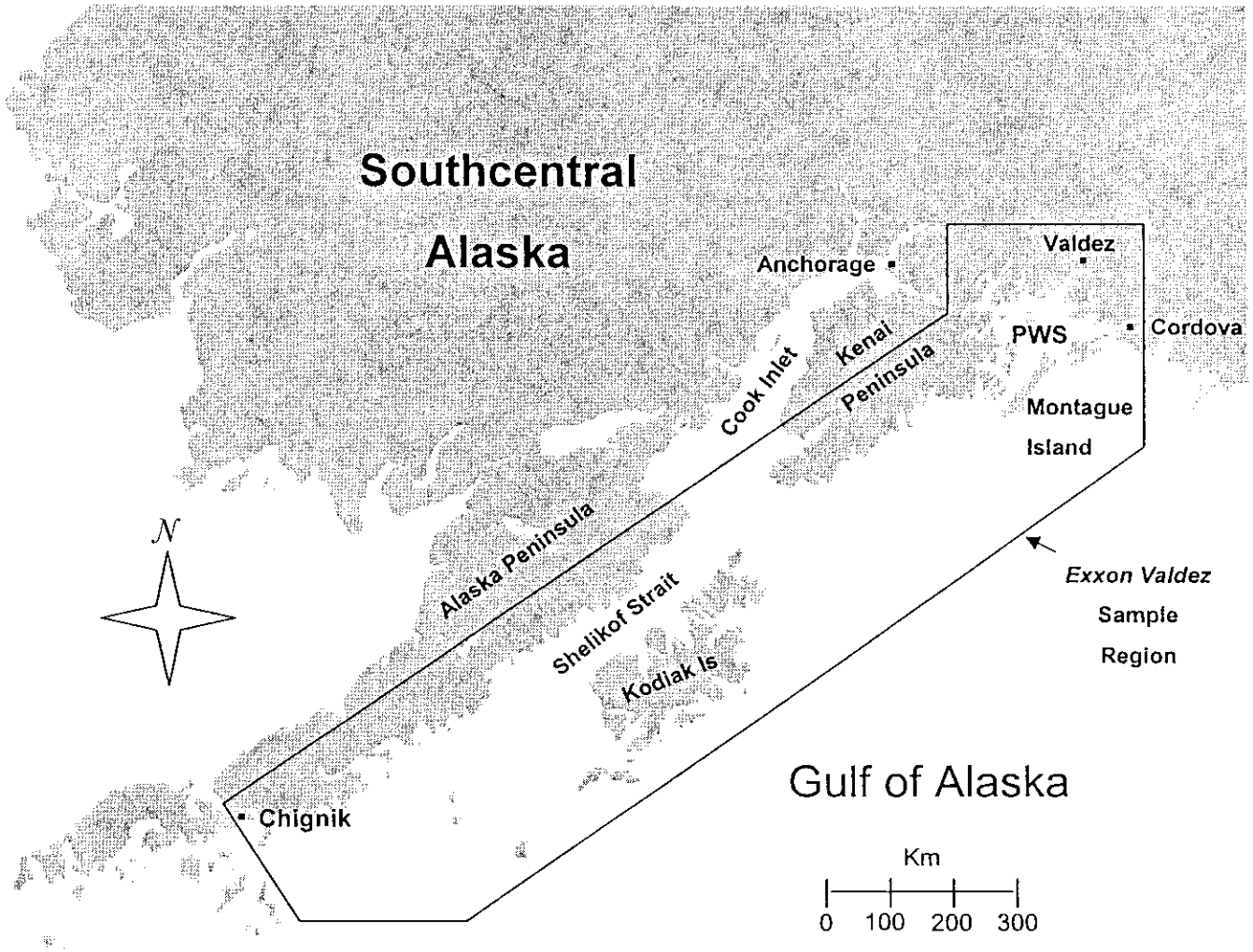

Figure 1. Map of the northwestern Gulf of Alaska where samples of biota were collected to assess impacts of the 1989 *Exxon Valdez* oil spill.

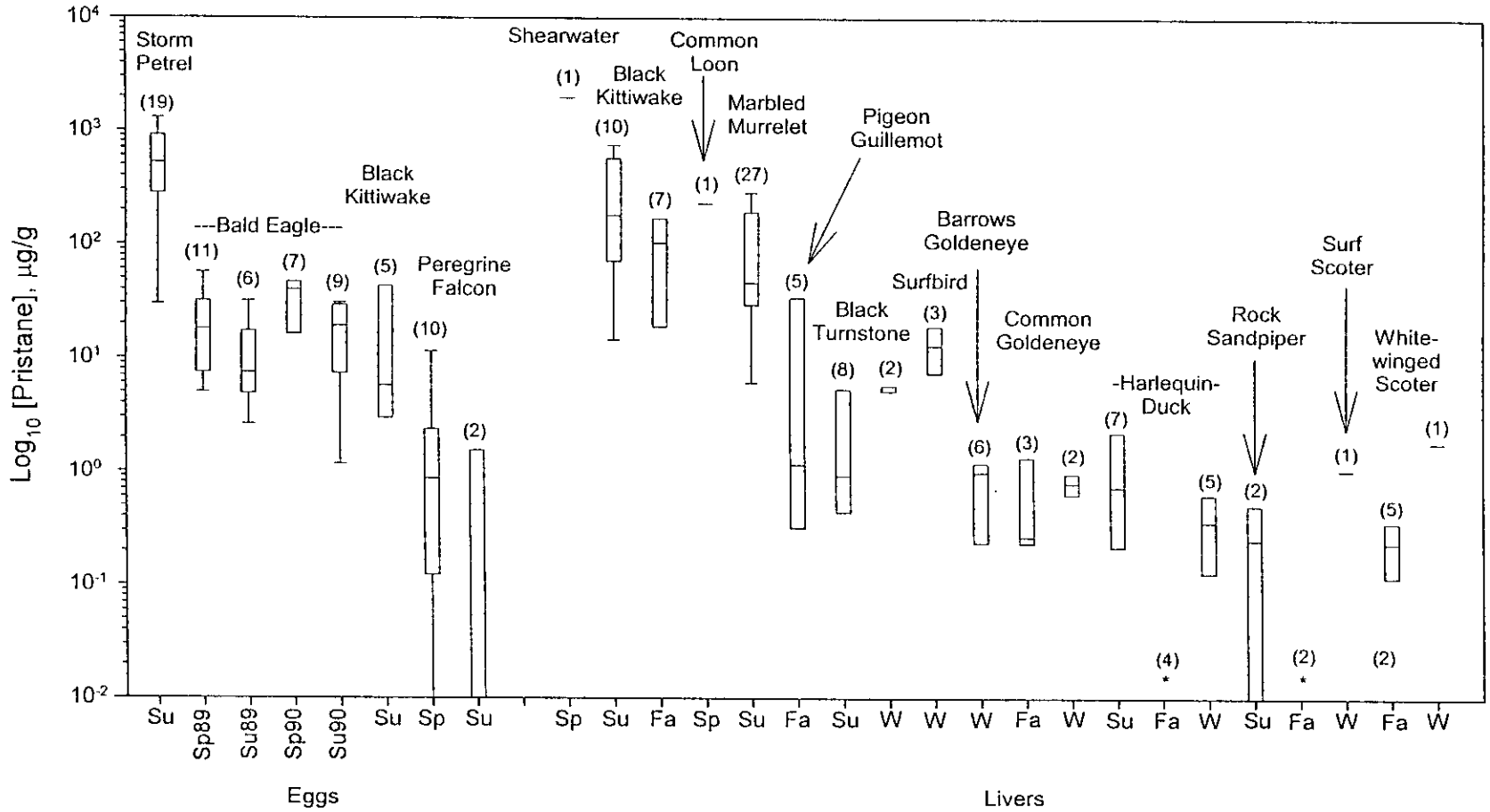

Figure 2. Distributions of pristane concentrations in eggs and in livers of birds sampled from Prince William Sound, Alaska, following the *Exxon Valdez* oil spill. Numbers in parentheses indicate the number of samples analyzed. The horizontal lines indicate the 10th, 25th, median, 75th and 90th percentiles of the distributions. Asterisks indicate concentrations below method detection limits. Sp = spring, Su = summer, Fa = fall, W = winter sampling; "89" = 1989, "90" = 1990.

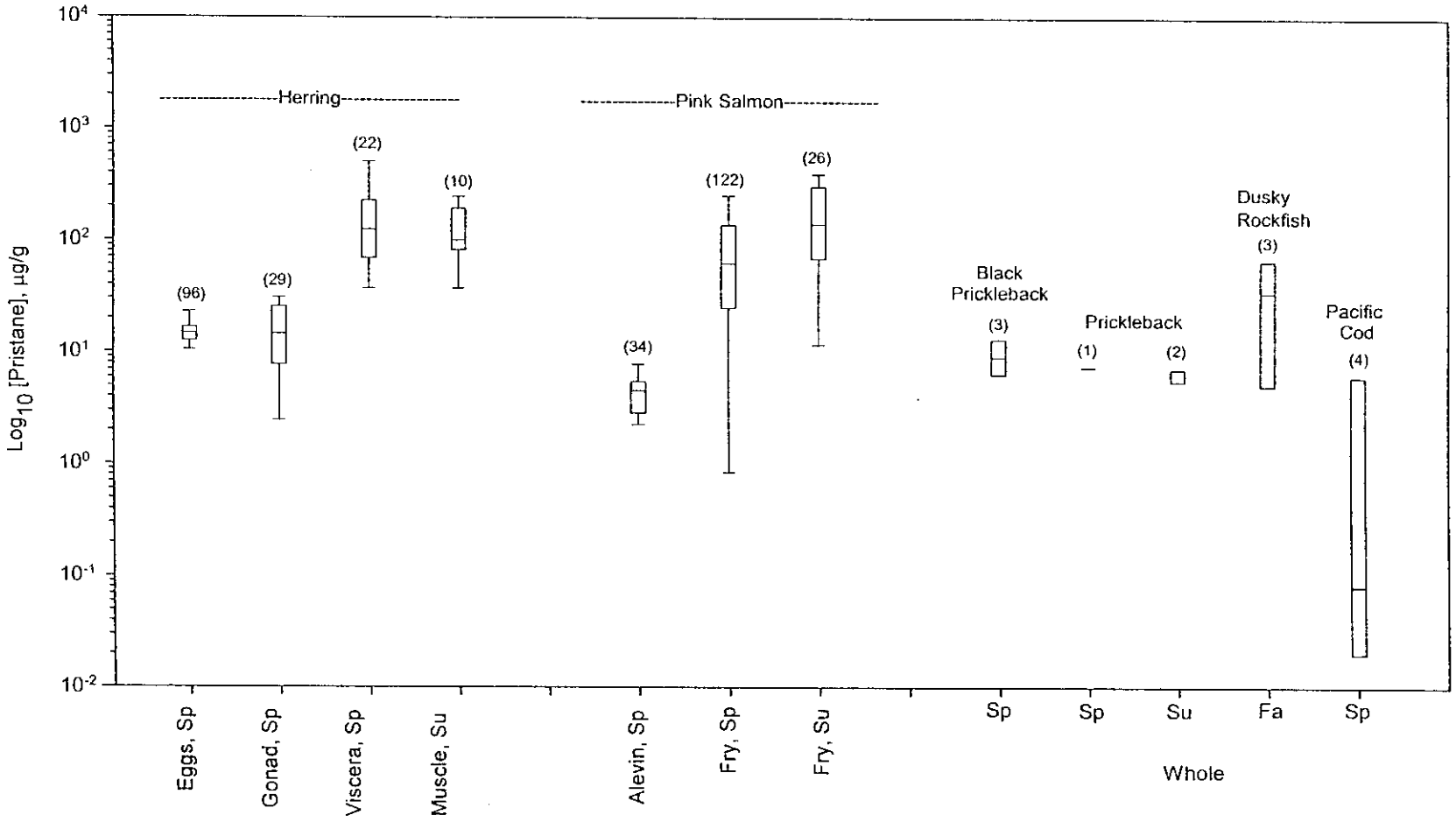

Figure 3. Distributions of pristane concentrations in tissues of fish sampled from Prince William Sound, Alaska, following the *Exxon Valdez* oil spill. Other symbols and abbreviations as in Figure 2.

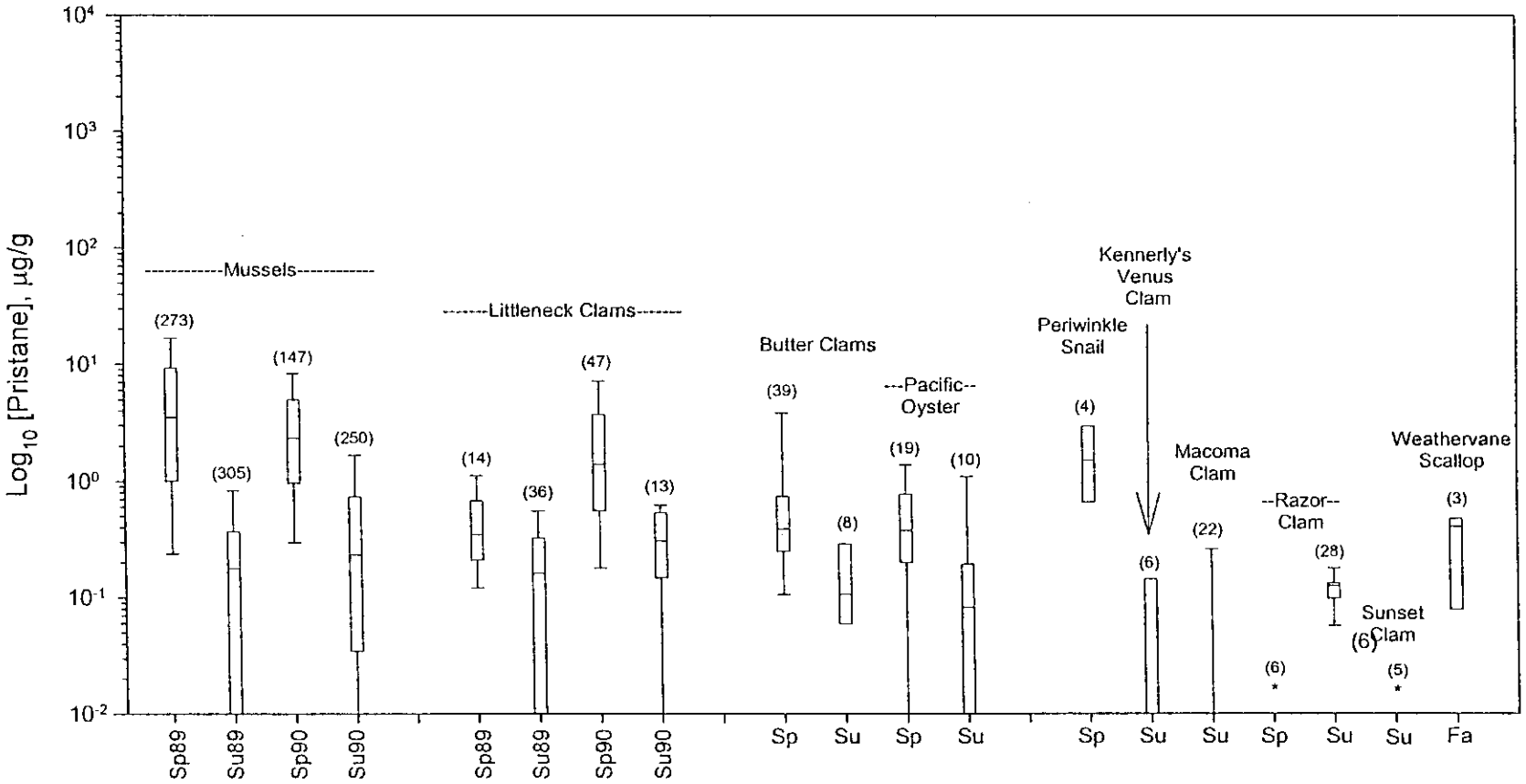

Figure 4. Distributions of pristane concentrations in molluscs sampled from Prince William Sound, Alaska, following the *Exxon Valdez* oil spill. Other symbols and abbreviations as in Figure 2.

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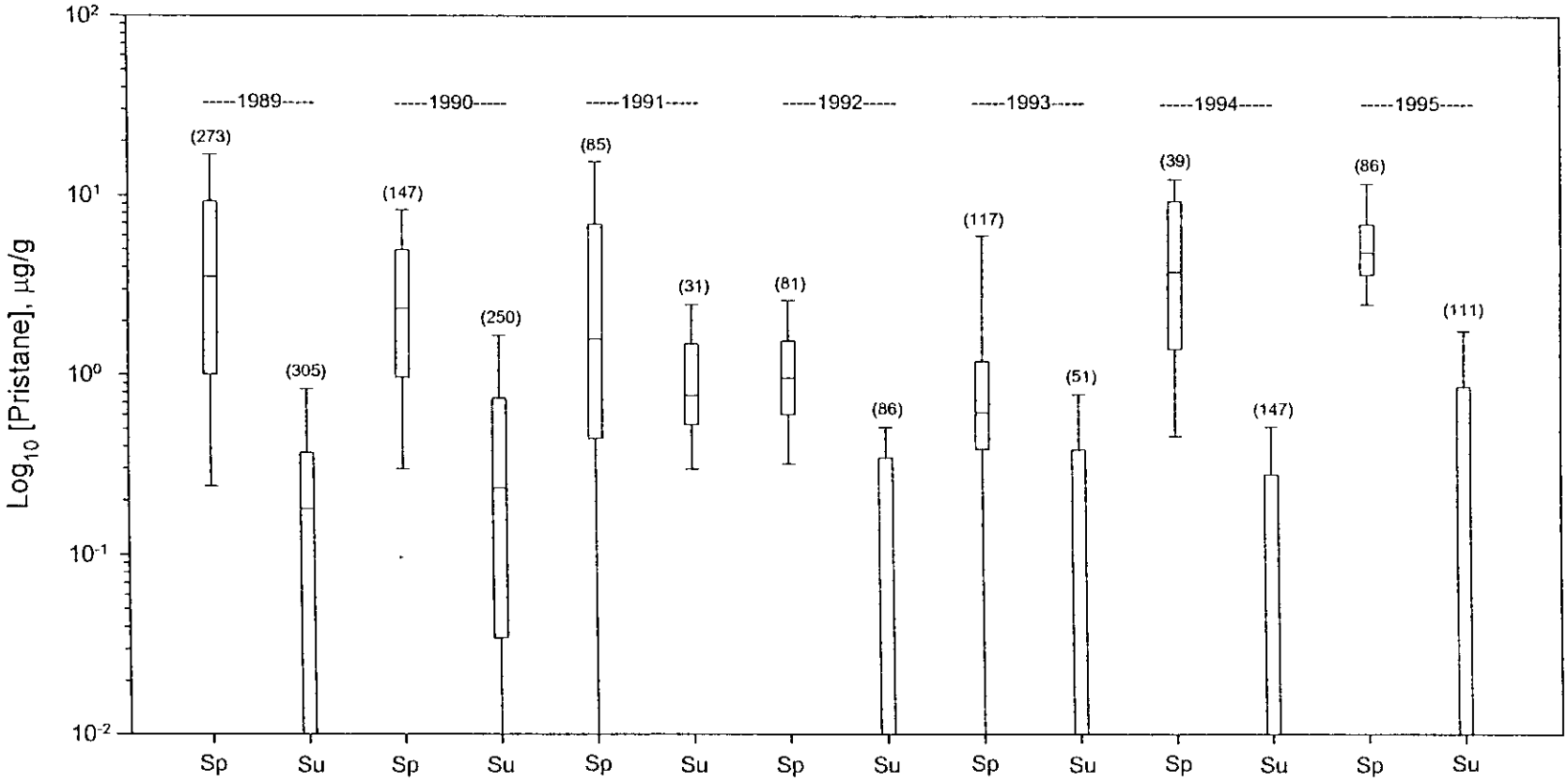

Figure 5. Distributions of pristane concentrations in bay mussels (*Mytilus trossulus*) sampled from Prince William Sound, Alaska, from 1989 through 1995. Other symbols and abbreviations as in Figure 2.

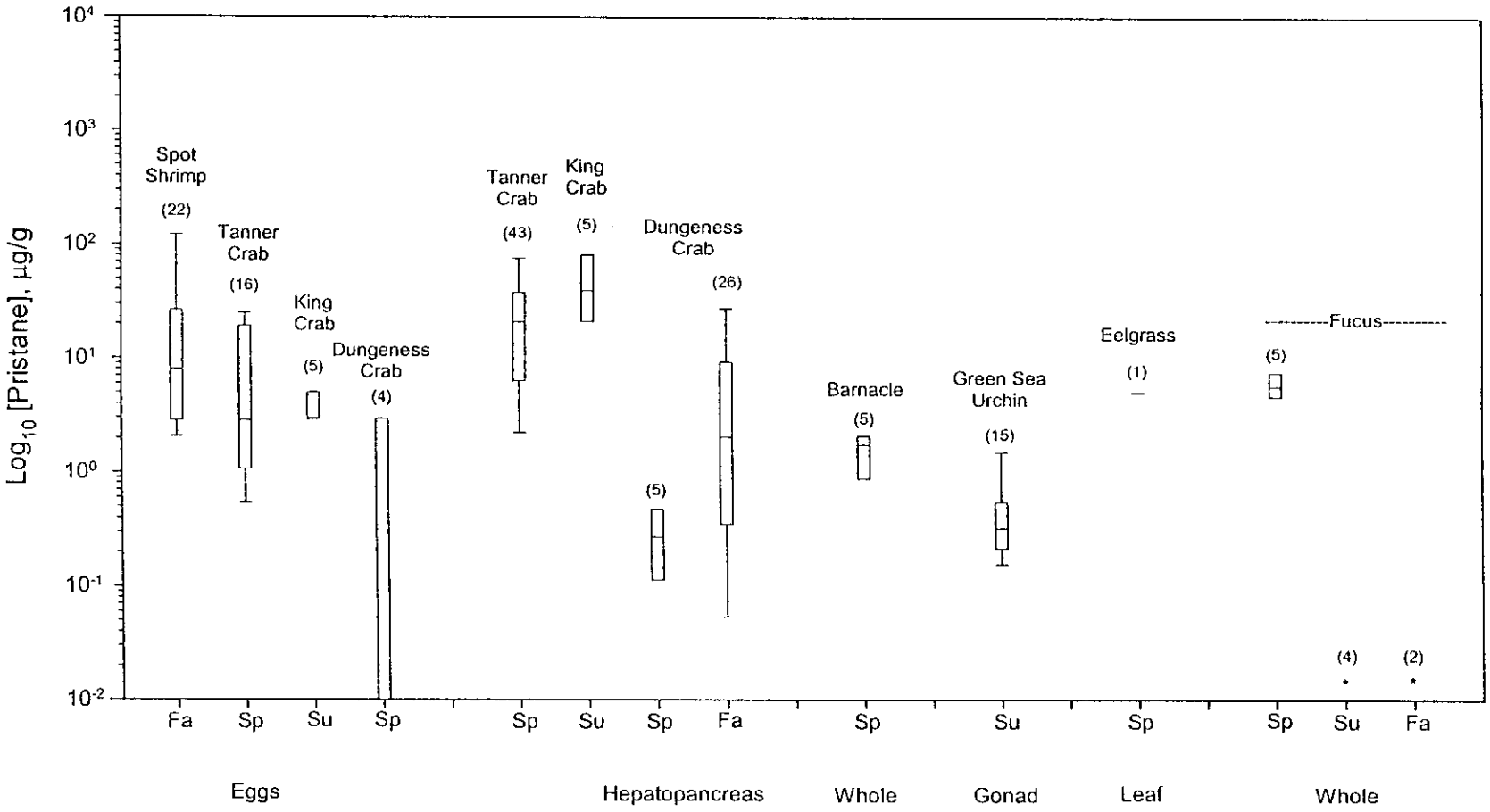

Figure 6. Distributions of pristane concentrations in crustaceans, sea urchin and plants sampled from Prince William Sound, Alaska, following the *Exxon Valdez* oil spill. Other symbols and abbreviations as in Figure 2.

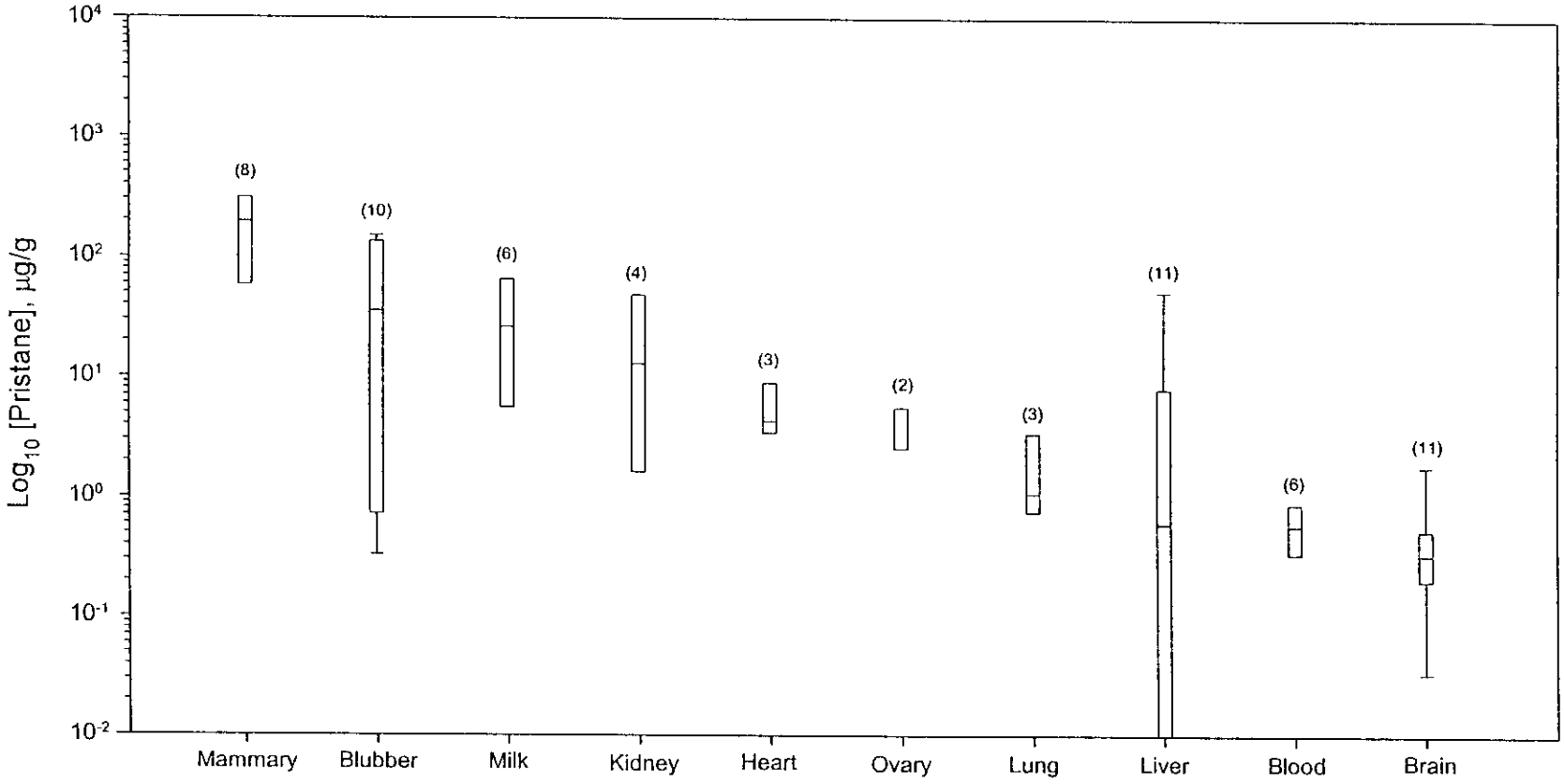

Figure 7. Distributions of pristane concentrations in harbor seal (*Phoca vitulina*) tissues sampled from Prince William Sound, Alaska, following the *Exxon Valdez* oil spill. Other symbols and abbreviations as in Figure 2.

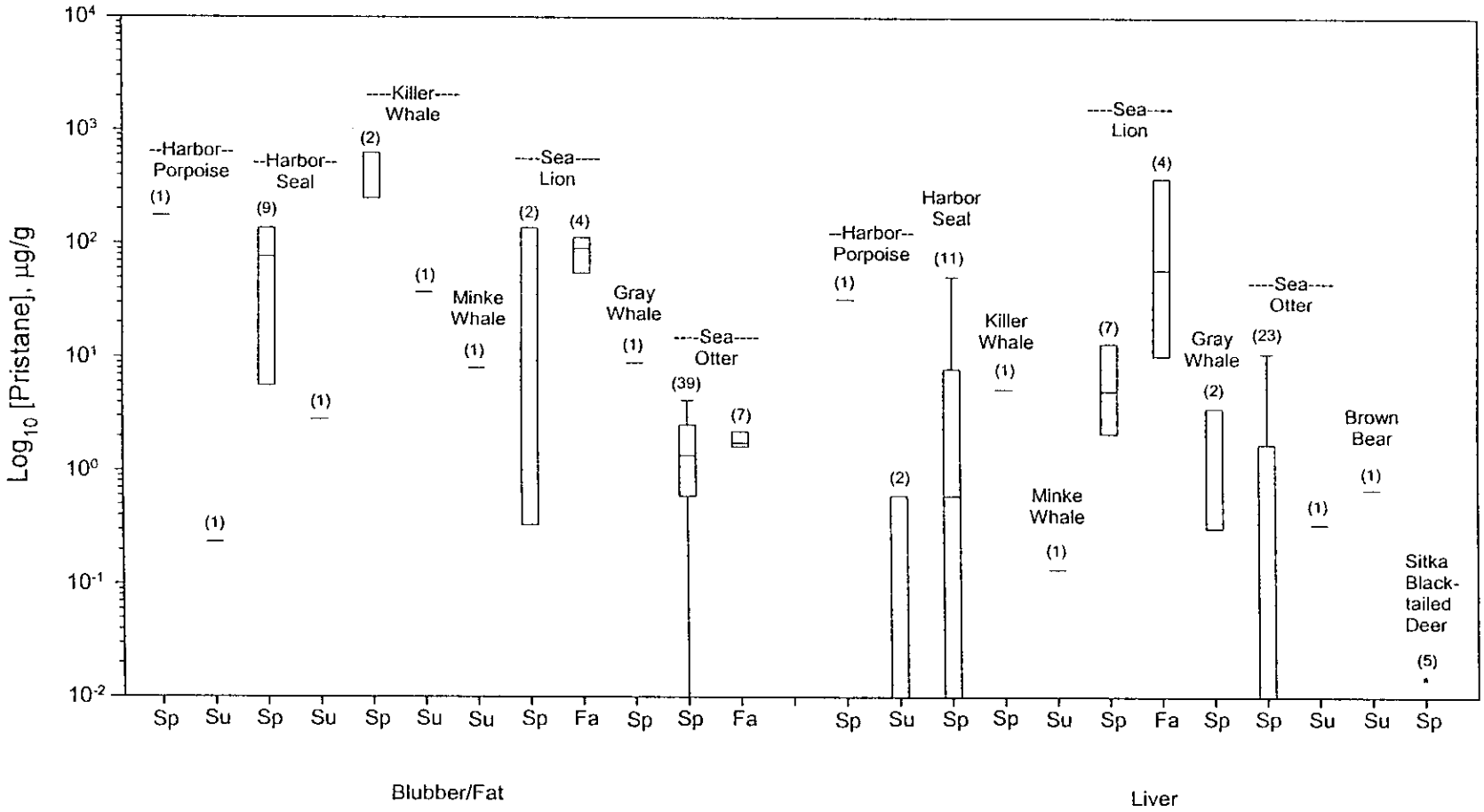

Figure 8. Distributions of pristane concentrations in mammal blubber and liver sampled from Prince William Sound, Alaska, following the *Exxon Valdez* oil spill. Other symbols and abbreviations as in Figure 2.

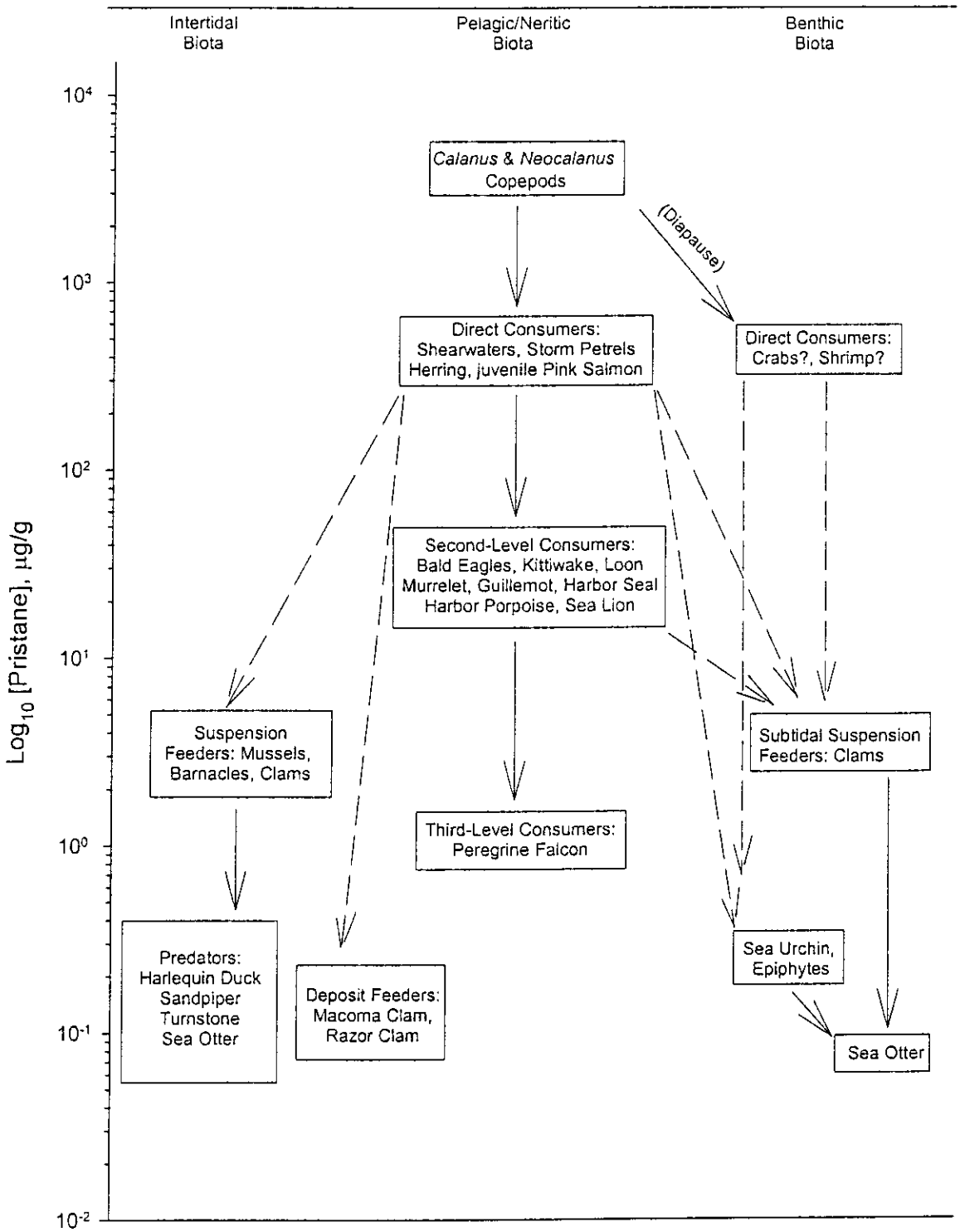

Figure 9. Generalized trophic relationships based on pristane concentrations measured in biota of Prince William Sound, Alaska. Solid arrows indicate ingestion of pristane from predation; dashed arrows indicate hypothesized ingestion of pristane associated with fecal material.

Exxon Valdez Oil Spill
Restoration Project Final Report

Pristane in the Neritic Ecosystem of the Northern Gulf of Alaska
CHAPTER 2:
Accumulation of Pristane by Mussels (*Mytilus trossulus*) Mediated by Juvenile Pink Salmon (*Oncorhynchus gorbuscha*) Predation on *Neocalanus* Copepods I: Laboratory Study

Restoration Project 02195
DRAFT Final Report
< CHAPTER 2 >

This final report has been prepared for peer review as
part of the *Exxon Valdez* oil Spill Trustee Council.
Peer review comments have not been addressed
in this final report

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June 2004

Restoration Project 02195
DRAFT Final Report
<CHAPTER 2 >

Study History:
This project was initiated in 1996, and builds upon earlier sampling by NMFS Auke Bay Lab. Funding for synthesizing pristine monitoring as predicative tool was approved January 2000. This submission represents the first of four chapters of that final report.

Abstract: The extensive sampling of biota from the northern Gulf of Alaska (GOA) following the 1989 *Exxon Valdez* oil spill provides a unique opportunity to examine the distribution of pristane, a branched alkane hydrocarbon produced by copepods, in the neritic food web. Pristane is lipophilic and is resistant to degradation via β-oxidation, so it is moderately persistent in the marine food web, and serves as a natural chemical label for a substantial proportion of the lipid produced at the secondary level of trophic production in this ecosystem.

Chapter 1 of this report is directed towards synthesizing the results from hydrocarbon analyses of 3007 samples comprising 49 species to confirming prior speculation that pristane may serve as a natural chemical probe for food web analysis. Chapter one was submitted earlier for review.

Chapter 2 is a laboratory study that investigates the accumulation of pristane by mussels (Mytilus trossulus) mediated by juvenile pink salmon (Oncorhynchus gorbuscha) predation on Neocalanus copepods. Chapter 2 is attached to this draft.

Subsequent chapters address the utility of pristane monitoring to assess feeding, condition and survival of the consumers of the copepods, including pink salmon.

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

Project Data:

Citation:

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Figure 2. Accumulation and depuration of pristane by mussels exposed to homogenized feces derived from juvenile pink salmon fed zooplankton from Prince William Sound, Alaska for 14 d, followed by a 28 d depuration period. Solid line indicates non-linear least-squares fit of accumulation and depuration functions assuming first-order kinetics for both processes (see Methods).

Figure 3. Accumulation and depuration of pristane by mussels exposed to whole feces derived from juvenile pink salmon fed zooplankton from Prince William Sound, Alaska for 14 d, followed by a 28 d depuration period. Solid line indicates non-linear least-squares fit of accumulation and depuration functions assuming first-order kinetics for both processes (see Methods).

Figure 4. Accumulation and depuration of pristane by mussels exposed to homogenized feces derived from juvenile pink salmon fed *Artemia spp*.14 d, followed by a 28 d depuration period (filled circles), or exposed to ambient laboratory seawater (open circles). Solid line indicates non-linear least-squares fit of accumulation and depuration functions assuming first-order kinetics for both processes (see Methods).

Accumulation of Pristane by Mussels (*Mytilus trossulus*) Mediated by Juvenile Pink Salmon (*Oncorhynchus gorbuscha*) Predation on *Neocalanus* Copepods I: Laboratory Study

Abstract

Juvenile pink salmon (*Oncorhynchus gorbuscha*) were fed zooplankton from Prince William Sound (PWS), Alaska, to evaluate the role played by their feces in transferring pristane from *Calanus* and *Neocalanus* copepods to bay mussels (Mytilus *trossulus*). Pristane is a branched alkane biosynthesized from ingested chlorophyll by these copepods at concentrations that may approach 1% dry mass in stage CV copepodites. The PWS zooplankton used as food contained 749 ± 325 μg/g pristane (dry mass basis), and feces derived from them contained 383 ± 72.8 μg/g pristane. The assimilation efficiency of mass and of pristane by pink salmon fed PWS zooplankton was 0.746 ± 0.0849 and 0.855 ± 0.0121. In comparison, *Artemia spp*. used as a control diet contained 1.24 ± 0.597 μg/g pristane, and feces derived from them were usually near detection limits, ~ 10 μg/g pristane. Assimilation efficiency of *Artemia* consumed by pink salmon was 0.884 ± 0.00170. Pink salmon fed *Artemia* grew about four times faster than those fed PWS zooplankton at nearly identical rations, and gross growth efficiencies of the *Artemia*-fed fish were about three times greater. Most of the difference in growth between the two diets is attributed to growth inhibition by pristane in the PWS Zooplankton-fed fish.

Mussels exposed to dispersed feces derived from PWS zooplankton-fed pink salmon accumulated pristane about 50 times faster than mussels exposed to dissolved pristane. The bioaccumulation factor for pristane accumulated from the dissolved state was 2,000, compared with 175,000 for pristane accumulated from feces. Mussels exposed to partially dispersed feces accumulated pristane less rapidly than completely dispersed feces, and mussels exposed to completely dispersed feces

derived from *Artemia*-fed fish accumulated readily detectable pristane concentrations from exposure concentrations in the low parts per trillion. The combined results indicate that predation by nearshore zooplanktivores on *Calanus* and *Neocalanus* copepodites in PWS, exemplified by juvenile pink salmon, is possibly the dominant route by which pristane is transferred from these copepodites to mussels.

Introduction

Every spring, bay mussels (*Mytilus trossulus*) in Prince William Sound (PWS), Alaska rapidly accumulate pristane, a branched alkane hydrocarbon biosynthesized by marine copepods in the genera *Calanus* and *Neocalanus* (Short in prep [a]). Late-stage copepodites of these genera biosynthesize pristane from ingested chlorophyll (Avigan and Blumer 1964), attaining concentrations that approach 1% dry body mass (Blumer et al. 1964, Short in prep [a]). Pristane is relatively persistent in the environment because it is terminally branched, and hence resistant to β-oxidation. *Neocalanus plumchrus*, *N. flemingerii* and *Calanus marshallae* copepodites develop to stage CV in the surface waters of PWS in early spring, when they may dominate the zooplankton biomass (Cooney 1986, Cooney et al. 2001). The pristane content of these copepodites increases with each development stage, and the appearance of CIV and CV copepodites in PWS surface waters is directly followed by rapid accumulation of pristane by mussels (Short in prep [a]). Concentrations of pristane in mussels may increase by factors ranging to several thousand during two to three weeks beginning mid-April, but the ecological pathway followed by pristane from *Calanus* and *Neocalanus* copepodites is not clear.

Although mussels may occasionally ingest mesozooplankton as large as late-stage *Calanus* and *Neocalanus* copepodites (Davenport et al. 2000), their ingestion rate is probably too low to account for the rapid increases of pristane concentrations during spring in PWS mussels. Escape responses of naupliar stages of calanoid

copepods in the flow field of blue mussels (*M. edulis*) are often effective (Green et al. 2003), and the escape responses of late-stage *Calanus* copepodites are considerably more effective (Landry 1978, Ohman 1988). Also, the internal diameter of the intake siphon of mussels is about 0.5 mm (Bayne 1976), similar to the diameter of stage IV or V *Neocalanus* copepodites, so successful capture of these copepodites by mussels implies relatively precise (and hence unlikely) geometric alignment of the major axis of the copepodite with that of the intake siphon of the mussel. These two factors insure that successful capture of *Neocalanus* copepodites by mussels, while possible, is rare.

Other pathways of pristane transfer from *Neocalanus* copepodites to mussels include accumulation of pristane dissolved into seawater from the copepodites, or ingestion of feces produced by the copepodites. The uptake of pristane dissolved in seawater may be assessed by measuring ambient concentrations during the spring zooplankton bloom, along with the bioaccumulation factor of pristane in mussels. The concentration of pristane in feces produced by *Neocalanus* copepodites has not been reported, but this concentration when combined with production and sinking rates of copepodite feces, and with mussel clearance rate would permit an assessment of the importance of this route of pristane incorporation by mussels.

Another less direct but potentially important route of pristane incorporation by mussels may be through ingestion of dispersed feces produced by predators of *Neocalanus* copepodites. In PWS, one of the most important predators is juvenile pink salmon (*Oncorhynchus nerka*). Wild stocks and hatcheries combined produce on the order of one billion juvenile pink salmon annually (Hilborn and Eggers 2000, Willette 1996) that migrate to marine waters during April and May (Kirkwood 1972, Olsen 1991), coincident with the spring zooplankton bloom (Cooney et al. 1995). Juvenile pink salmon generally remain close to shore during their initial marine residence (Healey 1980, Cooney et al. 1981), and prey heavily on copepods,

especially *Neocalanus* and *Calanus* in PWS (Sturdevant et al. 1996, Willette 1996). Although feeding experiments using *Calanus finmarchichus* have shown that lipid assimilation by rainbow trout (*Salmo gairdneri*) is very efficient, with less than 5% of total ingested lipid excreted in feces (Sargent et al. 1979), the high concentration of pristane in these copepods implies the feces produced would still contain a pristane concentration on the order of 500 μg/g. This is much higher than tissue concentrations found in mussels during spring, which are usually less than 20 μg/g (Short in prep [a]). Their feeding habits, nearshore residence, and numbers imply that juvenile pink salmon may provide an important ecological pathway for transferring pristane produced by *Neocalanus* copepodites to suspension-feeders such as mussels via fecal material produced through predation.

Knowledge of the ecological pathway followed by pristane from *Neocalanus* copepods to mussels may permit interpretation of the annual spring increase of pristane concentrations in mussels, which might prove useful for indirectly monitoring *Calanus* and *Neocalanus* zooplankton abundances, or perhaps the early marine survival of pink salmon. Year-class strength of pink salmon is thought to be determined during the initial period of marine residence (Parker 1968, Ricker 1976, Hartt 1980, Bax 1983), and if mussels accumulate pristane primarily from feces produced by nearshore zooplankton predators, then monitoring pristane increases in mussels during spring may provide an index of forage conditions for these predators. Abundant forage promotes rapid growth, which may reduce the period of maximum vulnerability to predation and thus increase population survival (Parker 1971, Healey 1982, West & Larkin 1987, Willette et al. 2001). Abundant forage has also been proposed to enhance survival of juvenile pink salmon by providing alterative prey to their predators (Willette et al. 2001), reducing predation pressure.

The primary objective of this study is to determine the concentration of pristane in feces produced by juvenile pink salmon fed zooplankton collected during

the annual spring bloom from PWS, and then to measure the uptake and depuration dynamics of pristane in mussels exposed to these feces or to pristane dissolved in seawater. These measurements will supply constraints on the relative importance of dissolved vs fecal-associated pristane as sources of pristane for mussels in PWS. A companion field study will incorporate these results into an overall comparison of the importance of dissolved pristane, pristane associated with feces produced by *Neocalanus* copepodites, and pristane associated with feces produced by predators of these copepodites as routes of pristane accumulation into mussels.

A secondary objective is to evaluate the growth efficiency of juvenile pink salmon reared mainly on *Neocalanus* copepodites. Gross growth efficiencies as high as 45% have been assumed in an evaluation of the impact of juvenile pink salmon on their zooplankton forage base in PWS (Cooney et al. 1998), but these may be optimistic in view of the inhibitory effect of pristane on fish growth reported previously (Luquet et al. 1983, 1984). The feeding experiments reported here provide an opportunity for direct measurement. A more precise estimate of growth efficiency permits a more accurate assessment of the potential impact of juvenile pink salmon on their *Neocalanus* prey, the amount of fecal material that must be produced per unit growth, and may provide insight into the growth dynamics of the juveniles relying on a natural diet.

Methods

The following laboratory experiments involved feeding juvenile pink salmon a diet of zooplankton collected from PWS during spring, and exposing mussels to the fecal material produced. The mass of zooplankton consumed and feces produced by the pink salmon, and their pristane concentrations were monitored throughout the feeding period, along with pink salmon growth. Mussels were exposed to whole

feces or to homogenized feces dispersed in seawater, or to homogenized feces produced by pink salmon fed brine shrimp (*Artemia* spp.) as a control comparison. Mussels were also exposed to pristane dissolved in seawater. These were all compared with mussels exposed to the same ambient seawater but without addition of pristane in any form. Pristane accumulation was measured in mussels throughout their exposure, and during a four week depuration period following exposure.

Experimental Animal Collection

Zooplankton were collected within 2 km of a hatchery operated by the Prince William Sound Aquaculture Corporation (PWSAC) on the southern shoreline of Esther Island in PWS from 23 – 30 April 1998 during a series of 12 samplings. At each sampling a 0.505 mm-mesh plankton net with a 0.5 m diameter opening and 1 L jar at the cod-end (to reduce compaction) was towed for 10 min at a depth of ~ 5 m during daylight. Captured zooplankton were rinsed from the net into a polypropylene tray, and then poured into a 0.5 mm-mesh circular metal sieve partially immersed in seawater and left covered with tinfoil to exclude light for 3 – 6 h to allow evacuation of zooplankton intestinal tracts. At the end of the zooplankton defecation period the sieve was removed from the seawater, allowed to drain for a few min., and the zooplankton were transferred to ~ 20 cm^3 compartments of ice cube trays with a metal spatula and stored frozen at -20 °C. About 1 g of the drained zooplankton from each sampling was preserved in 5% formalin-seawater for determining the species composition.

Juvenile pink salmon were collected from the inner bay at Little Port Walter in southeast Alaska, and were transported to the Auke Bay Laboratory by air in early July 1998. Several hundred of the smallest individuals were selected for the feeding experiments, and these had a mean initial mass of 1.81 ± 0.086 g wet wt. (95% CI, n = 9). These fish were fed a commercial diet formulated for salmon hatcheries, were

offered food twice daily and were allowed to feed to satiation. On 16 July, fish selected for the feeding experiments were fed twice daily on the zooplankton collected earlier from PWS, or on a commercial *Artemia spp*. fish-food product.

About 900 mussels (*Mytilus trossulus*) were collected from Tee Harbor, ~12 km north of the Auke Bay Laboratory in southeast Alaska, on 13 July 1998. For the fecal exposure experiments, 50 individuals ranging from 2 – 4 cm shell length were selected and distributed sequentially among each of 12 glass pans, and each pan was then placed inside a 38 L polypropylene tray filled with seawater and fitted with an air stone. The trays were placed in tanks of flowing seawater at 7.4 °C, and the seawater in the trays was replaced once daily until the fecal exposures began on 20 July. The remaining mussels were held in flowing seawater until 24 August, when 56 individuals within the same size range were placed into each of 6 glass trays for the dissolved pristane exposure experiment.

Zooplankton Consumption and Fecal Production by Pink Salmon

Juvenile pink salmon were sorted into three groups according to their zooplankton diet and treatment of their resulting feces. Groups 1 and 2 were fed the zooplankton collected from PWS, and group 3 was fed *Artemia* spp. Feces produced by groups 2 and 3 were macerated with a small ground-glass homogenizer prior to dispersion in the seawater aliquots used for the mussel exposures, while those of group 1 were left whole. These groups are denoted as "whole feces – pristane" (WF-P), "homogenized feces – pristane" (HF-P) and "homogenized feces – *Artemia*" (HF-A). Each treatment comprised triplicate feeding and fecal collection containers with separate groups of 20 juvenile pink salmon in each replicate. Each group of fish was kept in a 38 L polypropylene tray fitted with an air stone, and the tray was kept in a flowing seawater bath at seawater temperatures that varied from 7.3 – 8.5 °C.

Treatment trays were placed within and among the seawater bath containers in a sequence determined by a random number generator.

Fish in each replicate were offered 7 g (wet wt) of frozen zooplankton at 8 am and again at 8 pm daily, and allowed to feed for 1 h, beginning 20 July through 2 August 1998. Prior to feeding, fish were transferred by dip net to a separate tray for feeding. After feeding, fish were transferred by dip net to another tray, where they remained until the next feeding period. The fecal material excreted during the previous 11 h was collected from the first tray by filtering the seawater through 0.202 mm-mesh plankton netting after fish had been removed. Fecal material excreted during the feeding period was manually collected by pipette and saved for pristane analysis and for determination of the ratio of wet and dry mass of the feces. The mass of this material was included with the mass produced during the previous defecation period for measurement of total fecal mass produced, but was not included in material used for the mussel exposures because of the possibility of contamination by the zooplankton food. After removal of feces, unconsumed zooplankton were collected by filtration through a 0.202 mm-mesh net and the wet mass weighed.

I maintained two reserve pools of 25 and 12 juvenile pink salmon fed identical rations of zooplankton from PWS or *Artemia spp*., respectively, which I used to replace dead or moribund fish in the treatment groups. When feeding the fish in the reserve pools, I first thawed their zooplankton in seawater and collected the zooplankton with a 0.202 mm-mesh net to determine the ratio of frozen mass to zooplankton mass recovered. I used this ratio to adjust the masses of frozen zooplankton offered to the treatment group fish for water losses caused by freezing and thawing, because this water mass is not available for consumption by fish. I estimated the mass of food consumed by fish in the treatment groups as the difference in the mass of zooplankton offered (adjusted for water losses on thawing), and the mass of zooplankton recovered by dip net at the end of each feeding period.

Pink Salmon Growth

Juvenile pink salmon were weighed at the beginning, near the middle, and at the end of the feeding period by transferring all 20 of the fish in a treatment replicate to a bucket containing seawater on a scale with a dip net, and noting the increase in mass. Consumption of food altered fish mass significantly, so weighings used to estimate growth rates were done just prior to the next feeding when fish mass was lowest for the day. I estimated the instantaneous growth rate as $k = (1/t) \ln(W_f/W_i)$, where t is the number of days between weighings, and W_f and W_i are the final and initial masses. Eight fish died during the feeding period, of which four were from a single WF-P replicate during the first 4 days. Dead fish were replaced from the reserve pools, and the mass of fish in the treatment was corrected for the difference in masses between the dead and replacement fish. Also, fish from two of the HF-A replicates were inadvertently mixed on the fifth day of the feeding period, so weighings for growth rate determination were based on observations from the middle and end of the feeding period.

Mussel Exposures to Pristane

The 38 L polypropylene trays containing mussels exposed to pink salmon feces were also located at random in larger containers of flowing seawater that served to maintain temperature in the range 7.3 – 8.5 °C. Mussels were marked initially with red nailpolish, and were replaced with un-marked mussels when removed for pristane analysis to maintain an approximately constant ratio of tissue mass to exposure water volume. Feces collected for the HF-P and HF-A treatment groups were macerated in ~1 mL seawater with a Potter-Elvehjem tissue grinder, then mixed with the 38 L of seawater containing the 50 mussels of the treatment replicate. Feces collected for the WF-P treatment were left to soak for 24 h in a separate 38 L

polypropylene tray to allow time for the fecal material to disperse and for pristane to dissolve, and were then used for the WF-P exposures. A fourth treatment group consisted of 50 mussels per replicate exposed to seawater but no feces as a control. Seawater of all the treatment groups was replaced once every 12 h with freshly and independently prepared seawater containing feces appropriate for each group replicate. Five mussels were removed from each treatment replicate at 0, 2, 4, 7, and 14 d during the exposure period for pristane analysis, and again at 2, 4, 7, 14, and 28 d following the exposure period when the mussels were kept in flowing seawater with no fecal material added to monitor depuration of the accumulated pristane.

Another experiment involved exposure of mussels to a nominal 0.5 μg L^{-1} solution of pristane in seawater. Three replicates were exposed to this pristane solution, and another three replicates were exposed to ambient seawater as a control treatment. Each treatment replicate consisted of 50 mussels in a glass tray, and the three trays of the 0.5 μg pristane L^{-1} seawater exposure were placed in a fiberglass tank containing 475 L seawater to which 0.222 mg pristane dissolved into 1.0 mL acetone was added. The control treatment replicates were placed in a similar tank, and both tanks were equipped with circulation pumps and air stones. Seawater in the two tanks was replaced twice daily during the exposure period. Five marked mussels were removed (with replacement from the reserve pool of un-marked mussels) from each treatment replicate at 0, 2, 4, 8, and 14 d during the exposure period, and again at 2, 4, 8, 14, and 32 d following the exposure period when the mussels were kept in flowing seawater with no pristane added to monitor depuration of the accumulated pristane. Four L aliquots of seawater were sampled for pristane analysis at the beginning of three and again at the end of two 12 h exposure episodes to verify exposure concentration.

Two mussels died during the first (fecal-exposure) experiment and none during the second (dissolved pristane) exposure. Mussels attached themselves to the

glass pan with byssal threads and their shells remained slightly opened throughout the exposure and depuration periods of both experiments, indicating active seawater pumping.

Wet:Dry Mass Determination

The ratio of dry and wet masses of tissue and fecal samples was determined by drying a weighed sample aliquot at 65 °C for 24 h. This ratio varied considerably among different batches of zooplankton collected from PWS, ranging from 0.0625 to 0.267 (mean 0.134 ± 0.0352, n = 12). Because of this variability, conversions involving zooplankton from PWS were batch-specific. Ratios for other sample types were less variable as follows: *Artemia spp.,* 0.0903 ± 0.0036 (n = 6); feces from PWS zooplankton, 0.105 ± 0.0057 (n = 29); feces from *Artemia spp.*, 0.0718 ± 0.0113 (n = 24); mussels, 0.112 ± 0.0015 (n = 180). These mean values were used for these respective samples in calculations.

Pristane concentrations are expressed on a dry mass basis, except in calculations involving the kinetic constants the characterize pristane uptake and depuration in mussels, and growth and assimilation efficiencies of juvenile pink salmon, which are on a wet mass basis.

Pristane Analysis

The chemical analysis of tissue samples for pristane involved dichloromethane extraction of macerated tissues spiked initially with perdeuterated *n*-hexadecane as an internal standard, solvent concentration and exchange into hexane over steam, purification by silica gel/alumina column chromatography eluted with pentane, solvent re-concentration, resolution of alkanes by gas chromatography (GC) and measurement by flame ionization (Short et al. 1996). Identification of pristane is

based on GC elution time. The method for the zooplankton samples involved no alumina and less silica gel, because of the small tissue mass aliquots analyzed (< 0.05 g dry mass vs ~ 0.5 g for mussels).

The seawater samples were spiked with an acetone solution containing the same perdeuterated internal standard used for the tissue analyses, then extracted twice into 100 mL aliquots of dichloromethane. The dichloromethane extracts were combined and exchanged into 1 mL hexane over steam, and then analysed by the GC analysis used for the tissue samples.

The accuracy of the pristane analyses were generally within ±15% based on comparison with an authentic hydrocarbon standard prepared by the National Institute of Standards and Technology (NIST), and the coefficient of variation was generally less than ±20%. The method detection limit (MDL), defined as the estimated concentration associated with a 1% probability of type I detection error, is 0.115 μg for tissue samples. The corresponding MDL estimate for individual samples is the ratio of this value and the mass of the sample analyzed. No comparable MDL estimate is available for pristane in seawater, so the ratio of the tissue MDL and the seawater aliquot volume (4 L) is assumed, resulting in a MDL of 28 ng L^{-1}.

Data Analysis

Except for the kinetic constants characterizing pristane uptake and depuration by mussels, the confidence level of intervals associated with summary sample statistics is 95%, calculated as $\pm\ tsn^{-1/2}$, where t is Student's t, s is the standard deviation, and n is the number of observations.

I assume that mussels accumulate and depurate pristane according to the following first-order kinetic process:

$$\frac{dP}{dt} = k_1 P_{ex} - k_2 P \qquad \text{(eq 1)}$$

where P_{ex} in the external concentration of pristane in the exposure seawater (assumed constant), P is the concentration in mussel tissue, and k_1 and k_2 are rate constants for uptake and depuration, respectively. The solution to this equation is:

$$P = \frac{P_{ex} k_2}{k_1}\left(1 - e^{-k_2 t}\right) \qquad \text{(eq 2)}$$

During depuration, P_{ex} is zero, and eq 1 simplifies to $P = P_{int}\, e^{-k_2 t}$ (eq 3). I fit the pristane concentration measurements in mussels piecewise to eqs 2 and 3 for the uptake and depuration phases of exposure, using the mean concentration at the end of the 14 d uptake period as P_{int} in eq 3 for depuration, and using least-squares error minimization to find simultaneous best-fit estimates for k_1 and k_2.

I used a non-linear bootstrap method to estimate 95% confidence intervals for k_1 and k_2. This involved randomly associating (with replacement) the observed data errors to the best fit estimates of eqs 2 and 3 in place of each actual data point, and re-estimating k_1 and k_2, the process repeated 1,000 times. The 95% confidence interval is estimated as the bounds of upper and lower 2.5% of values in the tails of this distribution. This method preserves the error distribution without making assumptions about it (Efron and Tibshirani 1993), and permits simultaneous estimation of k_1 and k_2 while making full use of the available data.

I also used a bootstrap method to estimate the significance of differences between pairs of depuration constants k_1 or k_2. I calculated the difference between each of the 1,000 iterated estimates of k and the median value for each experiment to generate a list of 1,000 errors. To assess the significance of differences between two

experiments, I algebraically added two errors selected at random (with replacement) from the combined lists of errors 1,000 times, and found the proportion of resulting sums that exceeded the difference between the original estimates of k for the two experiments. This proportion is taken as the probability of Type I error (*i.e.* the significance level). Otherwise, the significance of differences among pairs of calculated statistics is determined by Student's t test.

The bioaccumulation factor (BAF) for pristane in mussels is the ratio of the tissue and exposure concentrations at equilibrium, with the tissue concentration expressed on a wet mass basis (Barron 1994), and may be calculated directly from eq 1 when equated with zero as the ratio of the kinetic constants k_1 and k_2.

Results

Pristane in Zooplankton and Feces

Pristane concentrations in the thawed batches of zooplankton collected from PWS were variable, ranging from 159 – 1,600 μg g^{-1} (mean 749 ± 325 μg g^{-1}, n = 12). This was not the result of variable species composition among the batches, because all contained more than 95% Stages IV and V *Neocalanus plumchrus*, *N. flemingeri* or *Calanus marshallae* (mass basis). This variability may instead have been caused by differences in losses during thawing. The ratio of wet PWS zooplankton mass recovered after thawing in the reserve trays and the initial frozen mass was surprisingly low and variable, ranging from 0.245 – 0.392 (mean 0.307 ± 0.0376, n = 9). Freezing likely disrupted cell membranes allowing cytosol loss, which may have entrained variable amounts of the lipid droplets of *Calanus* and *Neocalanus* copepods. Similar losses of mass on thawing occurred with frozen *Artemia spp.*, where ratios ranged from 0.339 – 0.558 (mean 0.490 ± 0.0507, n = 15).

The mean pristane concentration of frozen *Artemia spp*. was 1.24 ± 0.597 μg g^{-1} (n = 3).

Concentrations of pristane in feces derived from PWS zooplankton ranged from 137 – 660 μg g^{-1} (mean 383 ± 72.8 μg g^{-1}, n = 21). Concentrations in feces derived from *Artemia spp.* ranged from < MDL to 30.8 μg g^{-1}, the latter being less than four times the MDL for that sample. Twelve of the 21 samples analyzed were above 50% of the MDL value, and the mean of these samples was 15.3 ± 5.04 μg g^{-1}.

Juvenile Pink Salmon Feeding and Growth

Juvenile pink salmon grew significantly faster when fed *Artemia spp.* compared with zooplankton from PWS. The mean instantaneous growth rate for the *Artemia*-fed fish was $k = 0.0209 \pm 0.0037$ d^{-1} (n = 3), more than four times faster than fish fed zooplankton from PWS ($k = 0.00502 \pm 0.00251$ d^{-1}, n = 6; $P < 0.001$). This difference was not the result of differing rations. The *Artemia*-fed fish consumed 0.995 ± 0.0063% (n = 3) of their wet body mass per day in dry *Artemia spp.*, compared with 0.904 ± 0.064% (n = 6) for the PWS zooplankton-fed fish. Gross growth efficiencies (*i.e.* the ratio of body growth and wet mass of zooplankton consumed, or K_1) were correspondingly greater for the Artemia- compared with the PWS zooplankton-fed fish, with $K_1 = 0.185 \pm 0.0418$ (n = 3) and 0.0657 ± 0.0340 (n = 6), respectively.

The Artemia-fed fish had significantly ($P < 0.001$) higher assimilation efficiencies (*i.e.* the ratio of zooplankton mass absorbed and consumed, A, neglecting urinary losses) than the PWS zooplankton-fed fish, with $A = 0.884 \pm 0.00170$ (n = 3) and 0.746 ± 0.0849 (n = 6), respectively. Hence, the *Artemia*-fed fish grew faster in part because they consumed slightly (~10%) more food and assimilated it more efficiently (88.4% vs 74.6%) compared with fish fed zooplankton from PWS, but

these differences are not sufficiently great to account for the fourfold increase in growth rate.

The assimilation efficiency of pristane in PWS zooplankton (*i.e.* the ratio of pristane mass absorbed and consumed, A_p) was 0.855 ± 0.0121 (n = 6), significantly ($P < 0.001$) higher than the assimilation efficiency of PWS zooplankton biomass. The assimilation efficiency of pristane in *Artemia spp*. could not be accurately estimated because fecal concentrations were too frequently below MDL, but the fact that these fecal concentrations were near MDLs of ~ 10 μg g^{-1} implies that A_p for pristane in *Artemia spp.* is substantially lower than in PWS zooplankton.

Uptake and Depuration of Pristane in Mussels

The assumed uptake and depuration functions (eqs 2 and 3) provide a fair approximation of the measured concentrations in mussels exposed to dissolved pristane or to pristane contained in feces (Figures 1 – 4). The variability of the measured pristane concentrations increases substantially with concentration in all exposure treatments, and is greatest at the end of the exposure period and near the beginning of the depuration period.

Mussels rapidly accumulated dissolved pristane (Figure 1). The measured exposure concentration was 0.558 ± 0.817 μg L^{-1} (the large confidence interval was caused by one sample containing 1.73 μg/L), and I assume the nominal value of 0.5 μg L^{-1} based on the mass of pristane added for calculation of the kinetic constants and BAF. The estimates of the uptake and depuration constants are k_1 = 339 d^{-1} (95% CI: 272 – 442 d^{-1}) and k_2 = 0.169 d^{-1} (95% CI: 0.123 – 0.220 d^{-1}), giving a BAF estimate of 2,000. The depuration constant implies a half-life of ln 2/k_2 = 4.1 d for accumulated pristane. The concentration of pristane in mussels exposed to seawater with no pristane added was consistently less than 0.060 μg g^{-1} (wet mass basis; see

Figure 1 for dry mass basis), implying seawater concentrations near 30 ng L^{-1} during the exposure and depuration periods.

Mussels exposed to homogenized feces produced by pink salmon fed zooplankton from PWS (HF-P treatment) accumulated pristane to much higher concentrations than when exposed to the dissolved pristane, despite nearly equivalent exposure concentrations (Figures 1 and 2). The mean concentration of pristane added to the seawater as homogenized feces in the HF-P treatment was 0.317 ± 0.0163 μg L^{-1} (n = 84), slightly less than the 0.5 μg L^{-1} exposure to dissolved pristane. The rate constant for pristane uptake in mussels of the HF-P treatment was 17,600 d^{-1} (95% CI: 13,600 – 23,400 d^{-1}), which is 52 times faster than dissolved pristane was accumulated. Depuration of pristane accumulated by mussels in the HF-P treatment was significantly slower ($P = 0.032$) than depuration of pristane accumulated from the dissolved form. The depuration constant k_2 for the HF-P treatment was 0.101 d^{-1} (95% CI: 0.0644 – 0.141 d^{-1}), implying a pristane half life of 6.9 d. The BAF implied by the ratio of the kinetic constants is 175,000, far higher than the BAF for dissolved pristane.

Mussels exposed to whole feces produced by pink salmon fed zooplankton from PWS (WF-P treatment) accumulated pristane more slowly than mussels in the HF-P treatment (Figures 2 and 3). The mean concentration of pristane added to the seawater was identical with that of the HF-P treatment, but the rate constant for pristane uptake in the WF-P treatment was about a third as rapid (k_1 = 6,360 d^{-1}, 95% CI: 5,150 – 7950 d^{-1}). The depuration constant k_2 for the WF-P treatment was 0.0992 d^{-1} (95% CI: 0.0644 – 0.141 d^{-1}), implying a pristane half-life of 7.0 d, which was not significantly different ($P = 0.482$) than the depuration constant for the HF-P treatment, but was significantly slower ($P = 0.019$) than the depuration rate of mussels that accumulated dissolved pristane. The BAF implied by the ratio of the kinetic constants is 64,100.

Mussels exposed to feces produced by the *Artemia*-fed pink salmon (HF-A treatment) accumulated readily detectable concentrations of pristane (Figure 4), despite exposure concentrations near the limits of detectability (implying exposure concentrations < ~ 10 ng L^{-1}). The uptake rate constant could not be accurately estimated because the exposure concentration was too often below the MDL, but the depuration rate constant was not significantly lower ($P > 0.19$) than those of the other exposures to fecal material ($k_2 = 0.0766\ d^{-1}$, 95% CI: 0416 – 0.126 d^{-1}), implying a pristane half-life of 9.1 d.

Discussion

The results from the mussel uptake experiments clearly demonstrate that pristane associated with feces produced by juvenile pink salmon is a much more available form of this hydrocarbon for mussels compared with dissolved pristane. The 52-fold increase in the rate of pristane accumulation by mussels exposed to dispersed, pristane-laden feces (HF-P treatment) compared with exposure to dissolved pristane (cf. Figures 1 and 2) reflects the greater efficiency of particle-capture by these suspension-feeders compared with passive absorption of dissolved pristane. This greater efficiency is a consequence of the lower entropy of pristane associated with feces compared with the dissolved state. In a unit volume of seawater, pristane in fecal material is concentrated in a relatively few particles compared with the molecular scale of dissolved pristane. Suspension-feeders such as mussels are adapted to collect these particles efficiently by filtration, capturing particles as small as 1 μm (Vahl 1972). The accumulation rate of pristane from the dissolved state is limited by the diffusion rate across the seawater boundary layer adjacent to mussel tissues, an inherently slow process compared with particle filtration. Mussels thus

incorporate a much higher proportion of pristane from a unit volume of inspired seawater when concentrated in fecal particles compared to dissolved form.

The high efficiency of particle-capture by mussels is also evident in the other two experimental treatments. Mussels exposed to the whole feces derived from PWS zooplankton, which were allowed to partially disintegrate in seawater for 24 h prior to introduction to the mussels, still accumulated pristane much faster than they accumulated dissolved pristane. The slower accumulation rate compared with accumulation of homogenized feces is because only a fraction of the whole feces disintegrated to particle sizes available to the mussels. Dissolution of pristane prior to incorporation by mussels was clearly a negligible process, because even if all the pristane in the feces dissolved, comparison with the results for the dissolved pristane uptake experiment show that this would have accounted for less than ~ 5% of the pristane burden accumulated by the mussels exposed to the whole feces (compare Figures 1 and 3). Mussels exposed to feces derived from *Artemia spp.* accumulated pristane to higher concentrations than mussels exposed to dissolved pristane (cf. Figures 1 and 4), despite the much lower seawater concentration of pristane associated with the *Artemia spp*.-derived feces. In fact, the likely exposure concentration of this treatment of ~ 10 ng L^{-1} would be difficult to detect by direct analysis of 4 L aliquots of seawater, as would the resulting tissue concentrations of mussels exposed to this concentration of dissolved pristane. Assuming a BAF of 2,000 for the accumulation of dissolved pristane into mussels, an exposure concentration of 10 ng L^{-1} and a ratio of dry and wet masses of 0.1 gives an equilibrium concentration of pristane in dry mussel tissue of 200 ng g^{-1}, very near the tissue MDL for a 0.5 g dry mass tissue aliquot. Comparison with observed mussel tissue concentrations exceeding 10,000 ng g^{-1} (Figure 4) that resulted from exposure to pristane associated with *Artemia*-derived feces illustrates the ability of mussels to bioconcentrate fecal-associated pristane from very low ambient exposures.

The slower depuration rate of pristane from mussels exposed to pristane-laden feces compared with those exposed to dissolved pristane is probably because of the additional time required for ingested pristane to migrate to the externally-exposed tissues of mussels. Pristane absorbed from the dissolved form accumulates initially on the externally-exposed tissue surfaces (especially the gills, which account for most of the externally-exposed surface area), where the reverse process of depuration occurs readily. Pristane ingested with fecal material is transported directly to the innermost tissues, and additional time is required for it to migrate to the gills and other external tissue surfaces where depuration occurs. This additional time is reflected in the longer half-lives of ingested pristane (7 – 9 d) compared with pristane absorbed from solution (~ 4 d). These half-lives are comparable with those reported previously for mussels briefly exposed to dissolved hydrocarbons (Mason 1988).

Ingestion of pristane-laden fecal material may account for the very high concentrations of pristane occasionally found in mussels of PWS during spring. Although direct measurements have not been reported, the solubility of pristane in seawater is almost certainly less than 1 $\mu g\ L^{-1}$, based on comparison with solubilities of *n*-alkanes that have comparable molecular mass (Sutton and Calder 1974). The BAF derived from the mussels exposed to dissolved pristane reported herein is 2,000, implying a maximum pristane concentration of 2 $\mu g\ g^{-1}$ wet mass, or about 20 $\mu g\ g^{-1}$ dry mass, when mussels are exposed to seawater saturated with pristane. In PWS, concentrations in mussels during spring may exceed 50 $\mu g\ g^{-1}$ dry mass (Short in prep), when ambient seawater concentrations are ~0.1 $\mu g\ L^{-1}$ (Short and Harris 1996), limiting mussel tissue concentrations to < 2 $\mu g\ g^{-1}$ dry mass when accumulated from dissolved pristane only. Uptake of dissolved pristane clearly cannot account for the higher concentrations observed in these mussels during spring, but ingestion of fecal material produced by nearshore zooplanktivores such as juvenile pink salmon could.

Feces derived from zooplankton from PWS remain a concentrated source of pristane. The juvenile pink salmon assimilated 74.6% of the dry mass ingested, of this dry mass lipids were preferentially assimilated, based on the assimilation efficiency of pristane (85.5%). The difference between assimilation efficiencies of mass and of pristane implies that the pristane concentration in feces is reduced from that in zooplankton by a factor of $(1 - A_p)/(1 - A) = 0.57$, to concentrations of ~ 380 $\mu g\ g^{-1}$, which would make these feces the second most concentrated form of pristane in PWS during spring (cf. Short in prep [a]). My estimates of assimilation efficiencies for mass and for pristane are substantially lower than those for mass and lipid reported for rainbow trout (*Salmo gairdnerii*) fed frozen zooplankton consisting mainly of *Calanus hyperboreus*, where estimated assimilation efficiencies were 94% for mass and 96% for lipid (Sargent et al 1979). This may be a consequence of the correction I used for water loss on thawing. Without this correction, the assimilation efficiencies reported here would be nearly identical with those reported by Sargent et al. (1979), but it is not clear whether a similar correction was used by Sargent et al. (1979).

In contrast with the PWS zooplankton, nearly all of the pristane contained in *Artemia spp*. was excreted by the juvenile pink salmon. The concentration of pristane in feces derived from *Artemia spp*. was probably ~ 10 μg/g, well above the concentration estimated in the *Artemia spp*. food (1.24 ± 0.597 μg/g). The assimilation efficiency of ingested Artemia spp. mass was 88.4%, suggesting that little of the ingested pristane was assimilated. This might be the result of the much lower lipid content of the *Artemia spp*., which is near ~2.7% (dry mass basis) according to the supplier. The lipid content of late stage *Calanus* and *Neocalanus* copepodites is much higher, usually 50% or more (Båmstedt 1986, Duesterloh 2002), so it may be that the lipid content of *Artemia spp*. is too low to stimulate sufficient bile salts for efficient assimilation of lipids and associated pristane.

The low growth rate of juvenile pink salmon fed zooplankton from PWS is almost certainly due mainly to the pristane content, because pristane itself inhibits growth of fish. Juvenile rainbow trout (*Salmo gairdnerii*, 13.5 g initial wet mass) fed a diet containing 1% pristane during a 45 week period had a mean instantaneous growth rate $k = 0.0073\ d^{-1}$ compared with a control diet where $k = 0.014\ d^{-1}$, despite nearly equivalent daily rations of ingested food, and the fish fed pristane required 67% more food to achieve the same increase in mass (Luquet et al. 1983). Similar results were found during a follow-up experiment with larger rainbow trout (121 g), where fish fed a diet containing 1% pristane had $k = 0.0035\ d^{-1}$ compared with $k = 0.011\ d^{-1}$, and the pristane fed fish required nearly three times the food ingested by control fish to achieve equivalent growth (Luquet et al. 1984). These instantaneous growth rates are comparable with those reported here, as are the food conversion efficiencies (the ratio of K_1 for the PWS zooplankton- and *Artemia*-fed fish is 2.8, implying the PWS zooplankton-fed fish must ingest 2.8 times more food than the *Artemia*-fed fish to achieve equivalent growth). The results presented here for juvenile pink salmon and by Luquet et al. (1983, 1984) indicate that pristane inhibits fish growth substantially, and is the primary reason why pink salmon grew so poorly on a diet of zooplankton from PWS.

The growth inhibition of pristane on the juvenile pink salmon fed zooplankton from PWS may have been exacerbated by two other factors. First, *Calanus* and *Neocalanus* copepodites may not present as balanced diet for juvenile pink salmon as *Artemia spp*. Although rich in lipid, *Calanus* and *Neocalanus* copepodites may be deficient in other nutrients essential for rapid growth. Juvenile pink salmon in PWS would have access to a broader spectrum of prey than those captured and frozen for my feeding experiments, such as zooplankton too small or too large to be efficiently captured by the 0.505 µm-mesh plankton net, or prey found in other habitats accessible to pink salmon such as harpacticoid copepods in benthic sediments or insects at the seasurface. These other prey may supply the nutrients necessary for

rapid growth that may be lacking in *Calanus* and *Neocalanus* copepodites. Second, nutrients essential for rapid growth may have been lost from the *Calanus* and *Neocalanus* copepodites captured from PWS during thawing. The ~ 70% reduction in mass on thawing suggests considerable losses of water-soluble nutrients, and perhaps some of these were essential for juvenile pink salmon growth.

Calanus and *Neocalanus* copepods are an important link in marine food webs between primary production and consumers at higher trophic levels, especially during spring phytoplankton blooms at sub-arctic latitudes where they may account for most of the spring zooplankton biomass near the seasurface (Parsons & Lalli 1988). The high value of these copepods as prey items to their consumers has heretofore been based solely on their high lipid, and hence caloric, content. The countervailing effect of growth inhibition caused by pristane suggests that the energetic value based only on caloric content assigned to these copepods in models of marine food webs requires revision. In fact, it may be that the relatively high concentrations of pristane biosynthesized by these copepods may act as a chemical defense against predation, by prolonging the period of greatest vulnerability of their predators to size-dependent predation. It has also been suggested that pristane allows more complete use of lipid reserves during diapause by increasing buoyancy, thereby reducing the swimming energy needed to maintain position in the seawater column (Blumer et al. 1964). Protection from predation through growth inhibition of consumers may be another advantage of pristane production by *Calanus* and *Neocalanus* copepods.

The inhibitory effect of pristane on juvenile pink salmon growth has serious implications for growth models and estimates of prey impacts of juvenile pink salmon in PWS. Gross growth efficiency assumptions of 25% - 45% are probably high by factors of 3 – 6, implying that juvenile pink salmon may need to consume a correspondingly greater proportion of prey to achieve estimated growth rates in the field. Growth inhibition by pristane may also prolong the period of maximum

vulnerability to size-dependent predation, and models of population trajectories in PWS (*e.g.* Willette et al. 2001) may be improved by recognition of this inhibitory effect. At a larger scale, the growth inhibition caused by pristane may lead to lower assessments of the carrying capacity of the north Atlantic and Pacific Oceans for trophic levels above *Calanus* and *Neocalanus* copepods, as a consequence of the lower gross growth efficiency associated with predators these abundant and widely distributed prey. Finally, the low gross growth efficiency for PWS zooplankton indicates that pink salmon produce considerably more abundant feces than would be estimated on the assumption of a value of ~25%, making fecal production by nearshore zooplanktivorous fishes an even more important pathway followed by pristane to mussels than would be otherwise assumed.

Conclusions

I conclude that fecal production by nearshore zooplanktivorous fishes, exemplified by juvenile pink salmon, feeding of *Calanus* and *Neocalanus* copepodites is probably the dominant pathway followed by pristane from the copepodite sources to mussels during spring in PWS. Five factors contribute to the importance of this pathway: (1) juvenile pink salmon remain close to shorelines during early marine residence, in close proximity to mussel beds in PWS, (2) pristane inhibits fish growth, so zooplanktivorous fishes must consume inordinately large rations of *Calanus* and *Neocalanus* copepodites to sustain growth, (3) *Calanus* and *Neocalanus* copepodites account for the greatest prey biomass available to these fishes during spring, (4) pristane is incompletely assimilated by juvenile pink salmon and their feces remain a rich source of pristane, and (5) mussels are more efficient, by factors ranging to ~50, at accumulating pristane from feces than from pristane dissolved in seawater.

The hatcheries operated by PWSAC provide a unique opportunity to evaluate the importance of juvenile pink salmon as a conduit for pristane from *Calanus* and *Neocalanus* copepodites to mussels in the field. These hatcheries release on the order of 75 x 10^6 juvenile pink salmon *en masse* at the height of the zooplankton bloom during spring, flooding the adjacent shorelines with these fish when *Calanus* and *Neocalanus* copepodites are most abundant. Monitoring pristane in seawater, mussels, and in fecal pellets produced by *Calanus* and *Neocalanus* copepodites would permit assessment of the importance of fecal material produced by juvenile pink salmon as a vehicle for pristane transfer to mussels, and is the focus of the sequel to this paper (Short in prep [b]).

Acknowledgement

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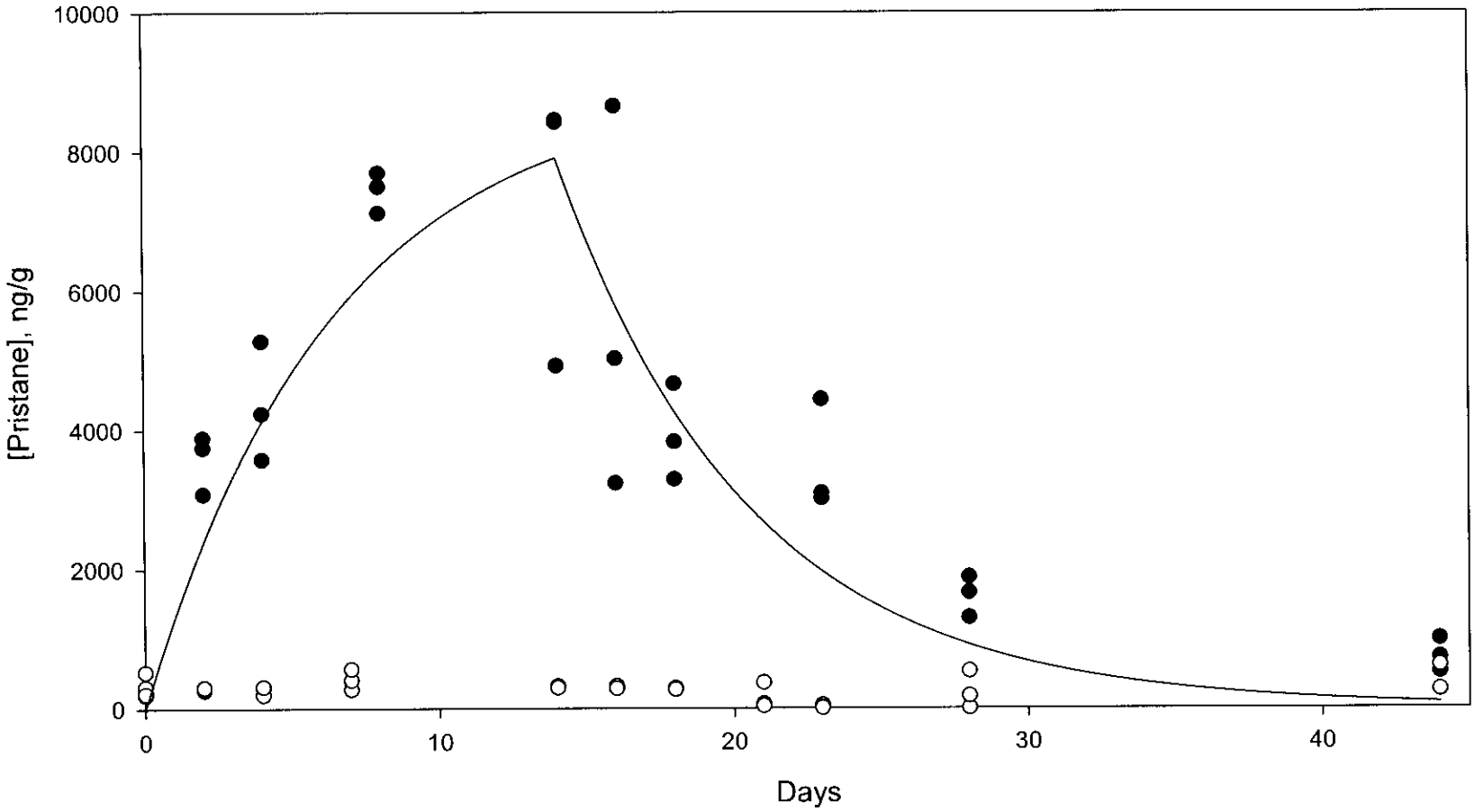

Figure 1. Accumulation and depuration of pristane by mussels exposed to 0.5 μg/L dissolved pristane for 14 d, followed by a 30 d depuration period (filled circles), or to ambient laboratory seawater (open circles). Solid line indicates non-linear least-squares fit of accumulation and depuration functions assuming first-order kinetics for both processes (see Methods).

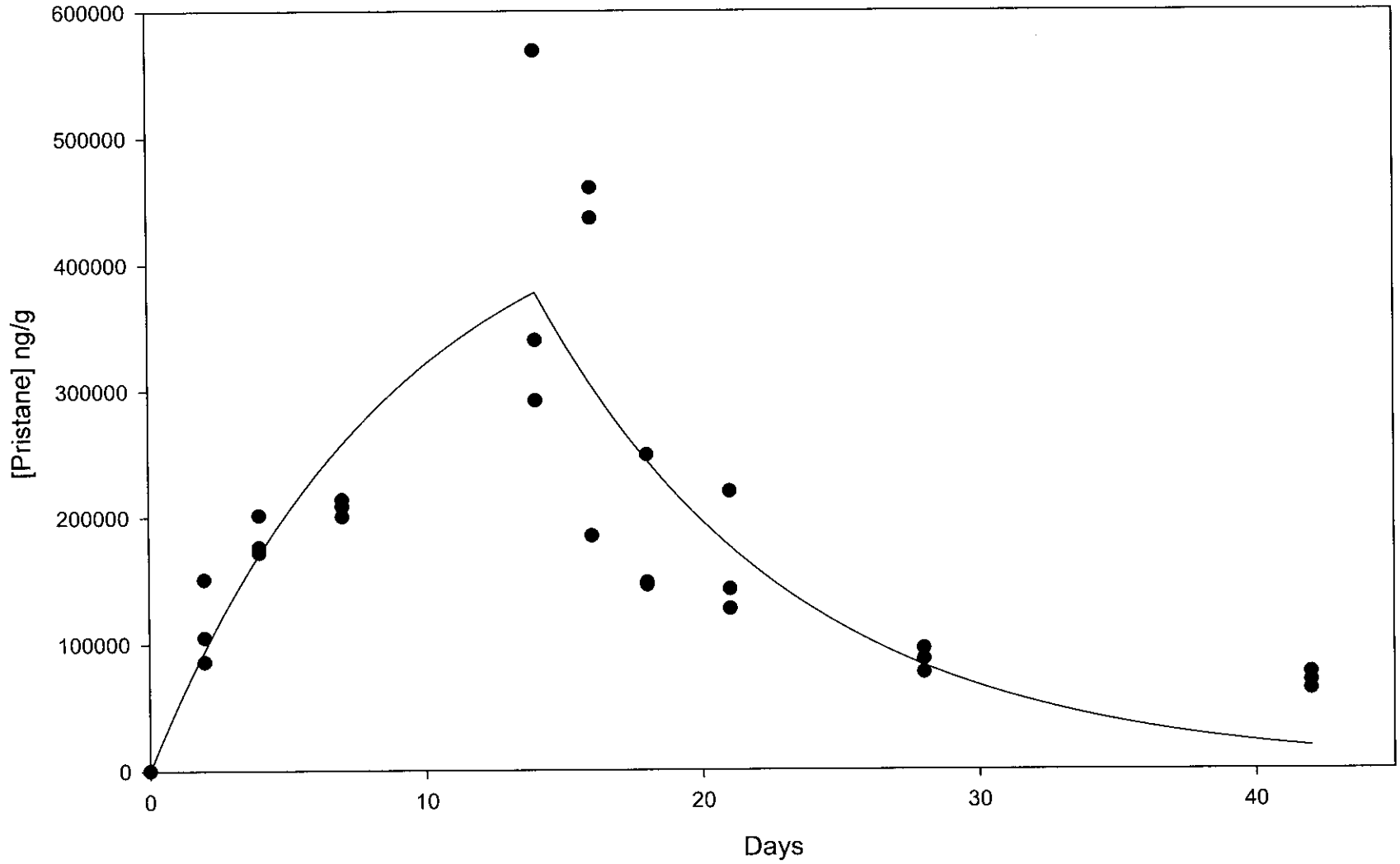

Figure 2. Accumulation and depuration of pristane by mussels exposed to homogenized feces derived from juvenile pink salmon fed zooplankton from Prince William Sound, Alaska for 14 d, followed by a 28 d depuration period. Solid line indicates non-linear least-squares fit of accumulation and depuration functions assuming first-order kinetics for both processes (see Methods).

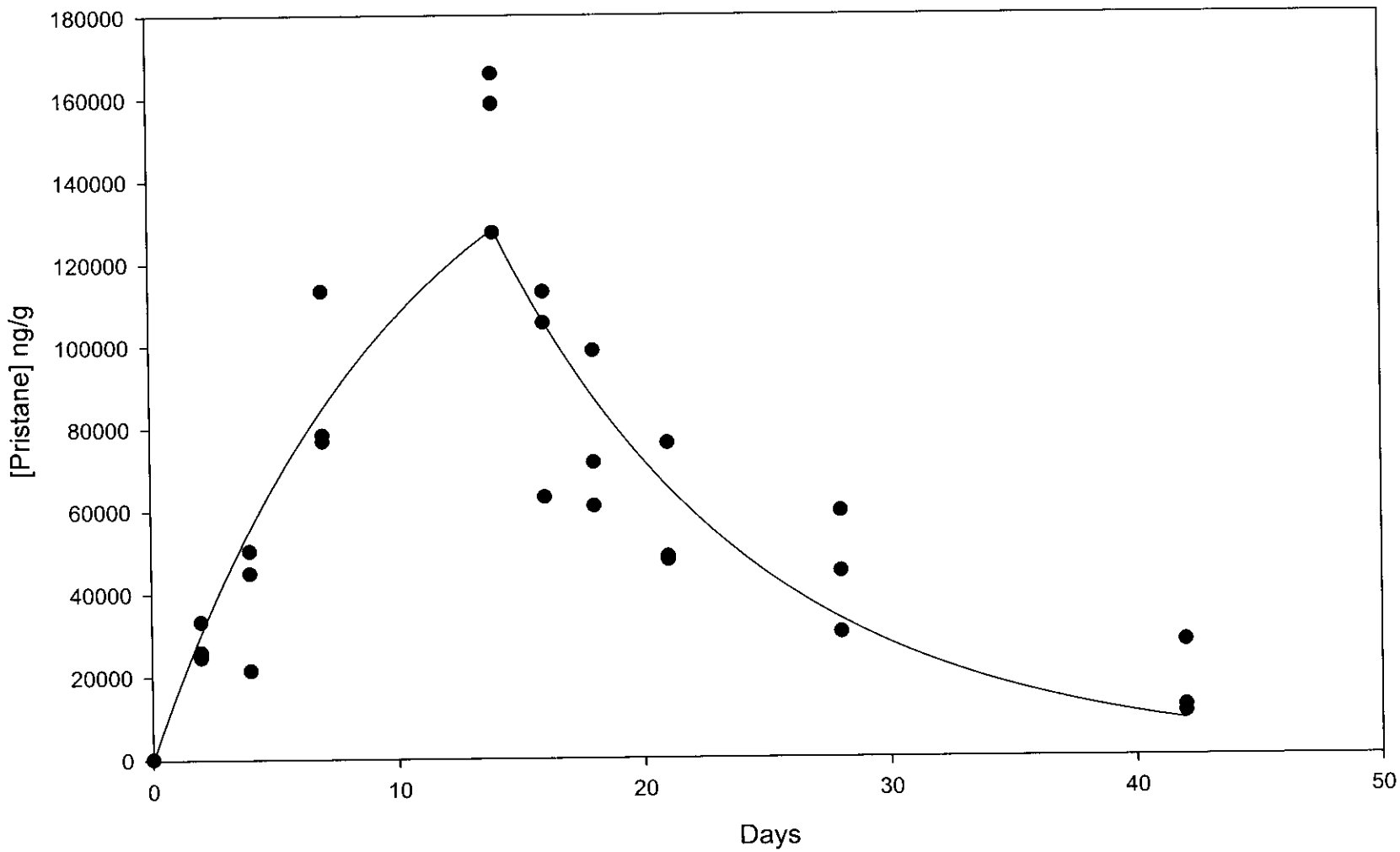

Figure 3. Accumulation and depuration of pristane by mussels exposed to whole feces derived from juvenile pink salmon fed zooplankton from Prince William Sound, Alaska for 14 d, followed by a 28 d depuration period. Solid line indicates non-linear least-squares fit of accumulation and depuration functions assuming first-order kinetics for both processes (see Methods).

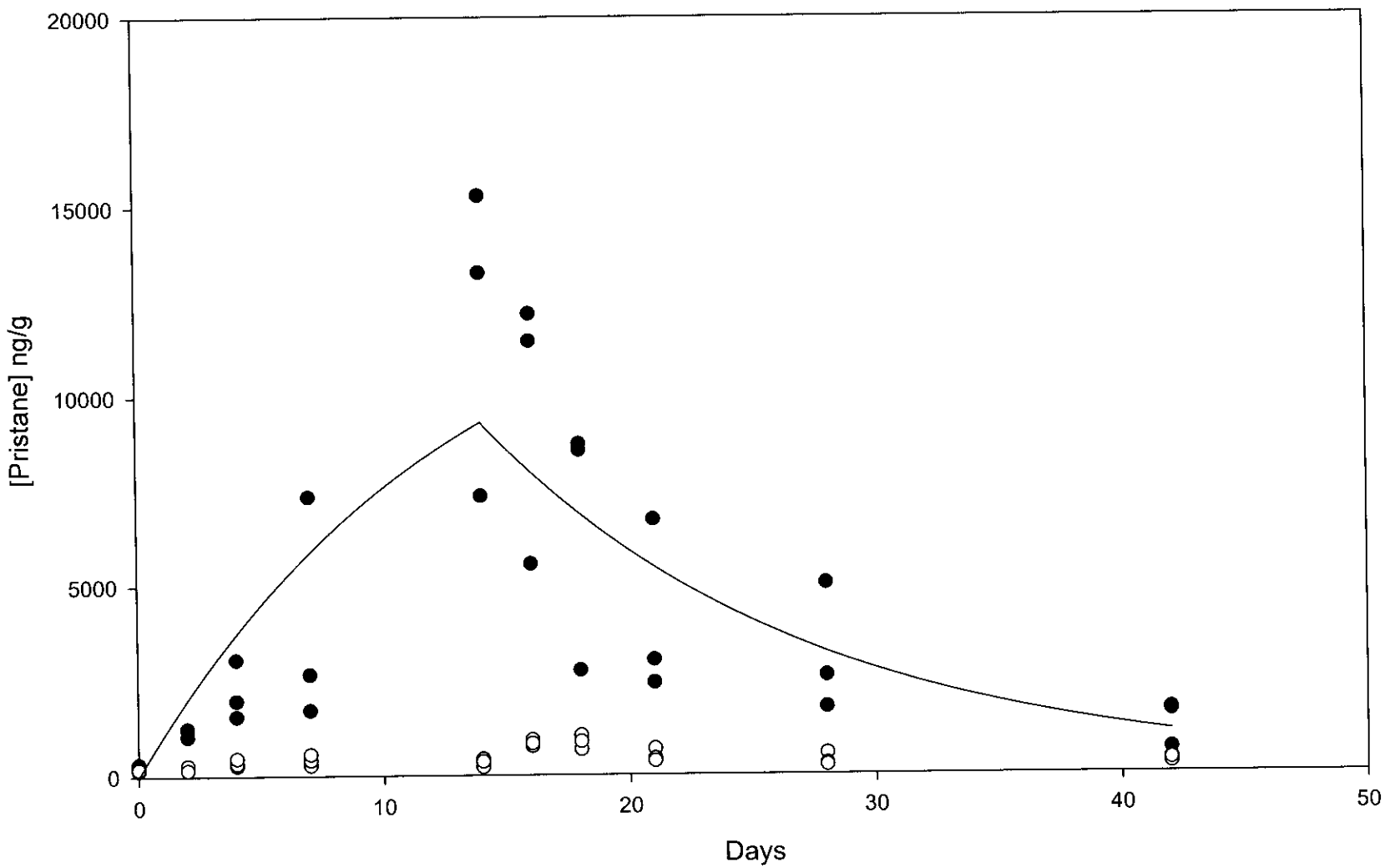

Figure 4. Accumulation and depuration of pristane by mussels exposed to homogenized feces derived from juvenile pink salmon fed *Artemia spp.*14 d, followed by a 28 d depuration period (filled circles), or exposed to ambient laboratory seawater (open circles). Solid line indicates non-linear least-squares fit of accumulation and depuration functions assuming first-order kinetics for both processes (see Methods).

Exxon Valdez Oil Spill
Restoration Project Final Report

Pristane in the Neritic Ecosystem of the Northern Gulf of Alaska

CHAPTER 3:

Accumulation of Pristane by Mussels (*Mytilus trossulus*) Mediated by Juvenile Pink Salmon (*Oncorhynchus gorbuscha*) Predation on *Neocalanus* Copepods II: Field Study

Restoration Project 02195
DRAFT Final Report
< CHAPTER 3 >

This final report has been prepared for peer review as
part of the *Exxon Valdez* oil Spill Trustee Council.
Peer review comments have not been addressed
in this final report

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Restoration Project 02195
DRAFT Final Report
<CHAPTER 3 >

Study History:
This project was initiated in 1996, and builds upon earlier sampling by NMFS Auke Bay Lab. Funding for synthesizing pristine monitoring as predicative tool was approved January 2000. This submission represents the third of four chapters of that final report.

Abstract: The extensive sampling of biota from the northern Gulf of Alaska (GOA) following the 1989 *Exxon Valdez* oil spill provides a unique opportunity to examine the distribution of pristane, a branched alkane hydrocarbon produced by copepods, in the neritic food web. Pristane is lipophilic and is resistant to degradation via β-oxidation, so it is moderately persistent in the marine food web, and serves as a natural chemical label for a substantial proportion of the lipid produced at the secondary level of trophic production in this ecosystem.

Chapter 1 of this report is directed towards synthesizing the results from hydrocarbon analyses of 3007 samples comprising 49 species to confirming prior speculation that pristane may serve as a natural chemical probe for food web analysis. Chapter one was submitted earlier for review.

Chapter 2 is a laboratory study that investigates the accumulation of pristane by mussels (Mytilus trossulus) mediated by juvenile pink salmon (Oncorhynchus gorbuscha) predation on Neocalanus copepods. This chapter was submitted earlier for review

Chapter 3 contains the results of a field study aimed at investigating the role played by juvenile pink salmon (*Oncorhynchus gorbuscha*) in the transfer of pristane from copepods to bay mussels (*Mytilus trossulus*) during spring in Prince William Sound (PWS), Alaska. This chapter is attached is contained in this submission.

Subsequent chapters address the utility of pristane monitoring to assess feeding, condition and survival of the consumers of the copepods, including pink salmon.

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

Project Data:

Citation:

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Accumulation of Pristane by Mussels (*Mytilus trossulus*) Mediated by Juvenile Pink Salmon (*Oncorhynchus gorbuscha*) Predation on *Neocalanus* Copepods II: Field Study

Abstract

This field study was aimed at investigating the role played by juvenile pink salmon (*Oncorhynchus gorbuscha*) in the transfer of pristane from copepods to bay mussels (*Mytilus trossulus*) during spring in Prince William Sound (PWS), Alaska. Pristane is a branched, saturated aliphatic hydrocarbon produced mainly by copepods in the genera *Neocalanus* and *Calanus*, and these copepods dominate the springtime zooplankton biomass in PWS. Mussels may accumulate dissolved pristane, pristane associated with copepod fecal pellets and with feces produced by fish preying on copepods. Pristane concentrations were monitored in mussels and in the dissolved and particulate phases of seawater at three stations one week before through one week after releases of ~10^8 juvenile pink salmon from a hatchery in PWS in 1996 and again in 1998. Zooplankton composition and abundances were also monitored a these stations.

Pristane concentrations increased ~10,000 ng g^{-1} (dry mass) in mussels at the station nearest the point where the juvenile salmon were released both years. Similar increases eventually occurred at the more distant stations in 1996, but at only the intermediate station in 1998. Pristane concentrations in seawater were usually below detection limits (41 ng L^{-1}) both years, limiting the contribution to pristane burdens of mussels to ~2,000 ng g^{-1} from dissolved pristane. The pristane concentration of fecal pellets produced by *Neocalanus* was 80.2 μg g^{-1}, and calculations based on copepod

abundance indicate mussels would accumulate no more than ~3,000 ng g^{-1} from this source. Stomach content analysis of the released fish and visual observations confirmed the fecal pathway mediated by fish predation on copepods as the primary cause of the increase of pristane in the mussels following release of the fish.

Zooplankton sampling indicated the *Neocalanus* abundance declined by more than half at all three monitoring stations the week following the release, and is attributed to consumption by the released fish. Removal of *Neocalanus* apparently released smaller copepods including *Pseudocalanus*, the other major component of zooplankton biomass, and *Acartia* from competition for food, and the population of late-stage *Pseudocalanus sp.* and *Acartia* copepods doubled as the *Neocalanus* population declined. These population responses of the smaller copepods might reinforce the inhibitory effects of pristane on salmon growth as a means of shielding *Neocalanus* from predation.

Introduction

Investigations of marine food webs must often rely on indirect methods to infer trophic relationships. The feeding habits of marine fauna are frequently difficult to determine by direct observation, and many species are not amenable to analysis of their stomach contents. In such cases chemical analysis of marker compounds may be necessary to gain insight into dietary dependencies. Analysis of lipids has gained increasing attention over the last two decades (Iverson et al. 2004, Howell et al. 2003,

Walton et al. 2000, Graeve et al. 1997, Grahl-Nielsen and Mjaavatten 1991, Fraser et al. 1989), because some species biosynthsize unusual (and often essential) fatty acids that may serve as chemical tracers, and because of the introduction of capillary gas chromatography for the analysis of these compounds, which has tremendously increased the ease of isolating and identifying closely-related lipids.

One of the first lipids proposed as a chemical marker compound for food-web investigations is pristane (Blumer et al. 1964), a branched alkane hydrocarbon biosynthesized by marine copepods in the genera *Calanus* and *Neocalanus*. Late-stage copepodites of these genera biosynthesize pristane from ingested chlorophyll (Avigan and Blumer 1968), attaining concentrations that may approach 1% dry body mass (Blumer et al. 1964, Short in prep [a]). Pristane is relatively persistent in the environment because it is terminally branched, and hence resistant to β-oxidation. *Calanus* and *Neocalanus* copepods are an important link in marine food webs between primary production and consumers at higher trophic levels, especially during spring phytoplankton blooms at sub-arctic latitudes where they may account for most of the spring zooplankton biomass near the seasurface (Parsons & Lalli 1988). Despite these advantages, pristane analysis has not often been used for food web studies, in part because little is known about the ecological pathways followed by it.

One ecological pathway followed by pristane that is not self-evident is from *Neocalanus* and *Calanus* copepods to suspension-feeding organisms such as clams and

mussels. In Prince William Sound (PWS), Alaska, pristane concentrations in bay mussels (*Mytilus trossulus*) often increase dramatically during spring to concentrations as high as ~50 μg g^{-1} (dry mass basis), returning to low concentrations by late summer (Short in prep [a]). The source of pristane is mainly stage IV and V *Neocalanus plumchrus*, *Neocalanus flemingerii* and *Calanus marshallae* copepodites (Short in prep [a,b]). Bay mussels may accumulate pristane through absorption of pristane dissolved into seawater from *Neocalanus* and *Calanus* copepodites, from ingestion of copepodites or of fecal pellets produced by these copepodites, or from ingestion of fecal pellets produced by predators of these copepodites.

Direct ingestion of late stage *Neocalanus* or *Calanus* copepodites by mussels is possible but unlikely, because these copepodites are nearly as large as the diameter of the intake siphon of bay mussels, and are usually able to escape the incurrent stream of mussels (Green et al. 2003). Accumulation of dissolved pristane by mussels is limited by the ambient concentration of pristane in seawater in PWS, and by the bioaccumulation factor (BAF, *i.e.* the ratio of the wet mass concentration of pristane in mussels and the concentration of pristane dissolved in ambient seawater). Concentrations of dissolved pristane as high as ~0.2 μg L^{-1} have been measured in PWS during spring (Short and Harris 1996), and the BAF of pristane in mussels is ~2,000 (Short in prep [b]), which implies maximum concentrations in mussels accumulated from dissolved pristane of ~0.4 μg g^{-1} wet tissue mass, or ~4,000 ng g^{-1} dry tissue mass. This suggests uptake by mussels of dissolved pristane may be significant. Pristane was undetected in feces of stage CV

copepodites of *Calanus helgolandicus*, but the concentration of pristane in these copepods is lower by factors of several hundred compared with other species of *Calanus* or *Neocalanus* (Prahl et al. 1984, Blumer et al. 1964, Short in prep [a]), so the importance of this pathway as a route of pristane accumulation by PWS mussels is unclear. However, the laboratory study accompanying the field study presented here indicated that fecal matter produced by fish preying on *Neocalanus* and *Calanus* copepods may be very important, and possibly the dominant route of pristane from these copepods to mussels (Short in prep [b]).

Prince William Sound is a nearly ideal setting for evaluating the relative importance of alternative transfer pathways of pristane from *Neocalanus* and *Calanus* copepods to mussels. The zooplankton biomass is dominated by these genera during spring, (Short in prep [b], Cooney et al. 2001a, Cooney 1986a, Cooney 1986b), facilitating evaluation of pristane in zooplankton feces and of pristane dissolved from these copepods into seawater. Five large salmon hatcheries are located within PWS, each releasing up to ~10^8 juvenile salmon (mainly pink salmon, *Oncorhynchus gorbuscha*, from four of the hatcheries) during spring (Hilborn and Eggers 2000, Willette 1996, Willette 2001, Willette et al. 2001). Three of the pink salmon hatcheries are located in areas remote from population centers, and pink salmon are usually released *en masse* at the peak of the zooplankton bloom, which effectively floods the vicinity with these fish. Juvenile pink salmon are zooplanktivorous, and *Neocalanus* and *Calanus* copepods are their primary prey in PWS (Willette et al. 2001, Sturdevant et al. 1996, Willette 1996,

Cooney et al. 1981). The concurrent release of large numbers of juvenile pink salmon from a discrete source in a remote area, when *Neocalanus* and *Calanus* copepods dominate their zooplankton prey, provides an especially favorable opportunity to evaluate the influence of the released fish on pristane transfer from copepods to mussels via feces produced by predation of the fish on the copepods.

My objectives in this study are to evaluate the relative importance of pristane dissolved in seawater, pristane in fecal pellets produced by *Neocalanus* and *Calanus* copepods, and pristane in fecal material produced by juvenile pink salmon as proximal sources of pristane accumulated by bay mussels in PWS. Knowledge of the ecological pathway followed by pristane from copepods to mussels may permit a more detailed interpretation of the annual spring increase of pristane concentrations in mussels. This might prove useful for indirectly monitoring *Calanus* and *Neocalanus* zooplankton abundances, and possibly the intensity of consumption of these copepods by their near-shore predators. Year-class strength of pink salmon is thought to be determined during the initial period of marine residence (Parker 1968, Ricker 1976, Hartt 1980), and if mussels accumulate pristane primarily from feces produced by near-shore zooplankton predators, then monitoring pristane increases in mussels during spring might provide an index of forage conditions for these predators and the relative success of their feeding, both geographically and inter-annually.

Methods

Field observations for this study occurred during spring, 1996 and again during spring, 1998 near the Wally H. Noerenberg hatchery (WHN), operated by the Prince William Sound Aquaculture Corporation (PWSAC). This salmon hatchery is located on the shore of Lake Bay, a small embayment on the southern coast of Esther Island in northwestern PWS (Figure 1). Juvenile pink salmon are reared in marine net pens a few weeks prior to their release into PWS. The mean mass of the released juveniles was 0.30 g in 1996 and 0.485 g in 1998. About 1.3×10^8 and ~7.0×10^7 juvenile pink salmon were released the evenings of 3 May 1996 and of 1 May 1998, respectively, on falling tides to aid dispersal of the released fish. Sea surface temperatures ranged 4.5 – 6.9 °C during both years of the study.

During both years I monitored daily concentrations of pristane dissolved in seawater and of pristane accumulated by bay mussels, beginning about one week prior through about one week following the release date, at each of three monitoring stations established near the release point (Figure 1). I also captured juvenile pink salmon just prior to release and a few days afterward, and determined their stomach contents and, in 1996, whole-body concentrations of pristane. In 1996, I collected bulk zooplankton for species composition and for collecting zooplankton fecal pellets for pristane analysis and for sinking rate determination. In 1998, I collected zooplankton from each of the three monitoring stations to determine species composition, relative abundance, variability

among stations and collection times, and to compare with standardized zooplankton collections made by the WHN staff at two stations in the area (Figure 1). I also collected zooplankton fecal pellets for determination of the wet and dry mass ratio. Following are details of these collections and the analyses performed on them.

Sampling Stations and Mussel Collection

The three stations established to monitor the effects of the released pink salmon on pristane concentrations of mussels and of seawater are denoted as Esther Hatchery (LB), Esther Light (EL) and Hodgkin's Point (HP). The LB station is located near the mouth of Lake Bay, 2.12 km to the east of the pink salmon release area, and the other two stations are located about 1.11 km to the west (EL) and 2.53 km to the east (HP) from the LB station, with another small embayment about the size of Lake Bay between the LB and HP stations (Figure 1). The beach at the LB station is a small, protected indentation of the coastline, mostly bedrock with mussels present in scattered clumps on mostly bedrock at a beach slope of about 15°. The EL station had a dense horizontal band of mussels attached to a steep (> 50°) bedrock face where an access ladder to a navigational light was located. The HP station is a small, irregular reef extending seaward from Esther Island that descended abruptly to deep (200 m) water on the seaward face. Mussels were collected from the scattered bands near the top of the reef. At each station and collection event, at least 20 mussels with shell lengths ranging from 2 – 4 cm were collected *ad libitum* as near as possible to the center of the vertical tidal range of the

mussel bed, with at least 1 m separating each mussel collected. Collected mussels were stored in polyethylene bags at -20 °C until analysis for pristane. Mussel samples were collected daily, weather permitting.

Seawater Sampling

In 1996, seawater samples were collected in duplicate at the PWSAC plankton sampling station 2 in the mouth of Lake Bay (Figure 1). One of the duplicates was filtered through a 1.5 µm glass fiber filter to distinguish dissolved pristane from pristane associated with particles, and these are compared with analysis of the other un-filtered duplicate. Single samples were collected within 100 m of each of the three mussel sample stations in 1998, and each was filtered as in 1996. Samples were collected daily, weather permitting.

Seawater samples were collected by submerging a 4-L glass jar to a depth of ~ 10 cm and removing the lid. Unfiltered samples and sample filtrates were spiked with perdeuterated *n*-hexadecane and sequentially extracted twice with 100-mL aliquots of dichloromethane within 2 h of collection. The glass fiber filter containing the filtered material was wrapped in aluminum foil and stored at -20 °C until analysis for pristane.

Zooplankton Sampling and Species Composition Determination

In 1996, zooplankton were collected near the PWSAC plankton sampling station 2 in the mouth of Lake Bay (Figure 1). At each sampling a 1.75 m long, 0.505 mm-mesh plankton net with a 0.5 m diameter opening and 1 L jar at the cod-end (to reduce compaction) was towed obliquely for 10 min at a depth of ~ 5 m during daylight. The captured zooplankton were concentrated by filtration with a 0.5 mm-mesh sieve, and about 1 g was transferred to a 5% solution of formalin in seawater for determination of species composition. Fecal pellets defecated by the remaining zooplankton were collected for pristane analysis (see below).

In 1998, zooplankton were collected along with seawater samples near each of the three mussel collection stations. Two collections were made at each station, one with a 1.75 m long by 0.202 mm-mesh plankton net and another with a 1.75 m by 0.505 mm-mesh net. Both nets had a 0.5 m diameter opening and were hauled vertically from 30 m depth to the surface at ~ 1 m s^{-1}, sweeping a calculated seawater volume of 5.89 m^3. The aspect ratio of these nets exceeded 5.4, and hence are more than 90% efficient (Tranter and Smith 1968), so the calculated seawater volume swept during the vertical tows is presumed accurate for calculation of zooplankton abundances. Zooplankton were collected at ~ 0800 h – 0900 h each day the weather allowed sample collection. Additional samples were collected ~1500 h on 29 April, ~2200 h on 30 April, and near

midnight on 7 May to evaluate whether the composition or abundances varied diurnally. A total of 36 vertical tows were collected with each mesh size. Captured zooplankton were stored in 5% formalin in seawater for determination of species composition. The 0.202 mm-mesh samples were used for determination of species abundance, biomass, and variability among stations, and the 0.505 mm-mesh samples were treated as duplicates for the large zooplankton captured to evaluate sampling variability within stations.

Zooplankton were usually identified to genus and often to species. *Neocalanus plumchrus* was not distinguished from *N. flemingerii*, which are hereafter denoted as *N. plumchrus/flemingerii.* Copepodite stages of *N. plumchrus/flemingerii* , *N. cristatus* and *Calanus marshallae* were also identified. Samples were split before sorting with a plankton splitter. Split samples contained at least 100 individuals. Species and copepodite stages were determined following criteria given by Gardner and Szabo (1982). Zooplankton biomass was calculated from abundances assuming wet tissue masses of 12 mg ind^{-1} for combined stages of *N. cristatus*, 1 for combined stage IV and V of *N. plumchrus/flemingerii* or *C. marshallae*, 0.1745 for *Limacina*, 0.142 for *Pseudocalanus*, 0.121 for *Oithona*, 0.0519 for *Acartia,* 0.0333 for *Oikopleura*, and 0.02 for bryozoan larvae (from M. Sturdevant, NMFS, Auke Bay Laboratory, personal communication). A value of 0.1 mg ind^{-1} was arbitrarily assumed for the other species encountered, which probably overestimates their contribution to biomass but they were encountered so rarely that this bias is likely negligible.

Samples of zooplankton were collected twice weekly by PWSAC staff as part of their plankton watch program at two locations, one within Lake Bay and the other at the mouth of the bay (Figure 1). These collections involved three vertical tows from 20 m depth to the surface of a 0.5 m diameter, 0.243 mm-mesh plankton net at each station. The settled volume of phytoplankton and zooplankton in the combined tows was determined by allowing plankton to settle in a graduated conical flask for 24 h. Results of this program are used in part to determine juvenile salmon release dates at PWSAC hatcheries.

Collection of Zooplankton Fecal Pellets

Zooplankton captured in 1996 were rinsed from the net into a polypropylene tray, and then poured into a 0.5 mm-mesh circular metal sieve partially immersed in seawater and left covered with tinfoil to exclude light for 3 – 6 h to allow evacuation of zooplankton intestinal tracts. The sieve rested in a stainless steel bowl with 0.202 mm-mesh plankton netting attached to the bottom of the sieve to exclude small zooplankton that may have passed through the metal sieve from the bottom of the bowl. The sieve and plankton mesh were carefully removed from the bowl after the zooplankton defecation period, leaving a layer of green zooplankton fecal pellets visible on the bottom of the bowl. Most of the seawater was removed from the bowl by siphon, and any remaining material other than fecal pellets was removed by pipette or with zooplankton forceps. The accumulated fecal pellets were transferred with seawater to a conical glass

vial, and most of the seawater was removed by pipette after the pellets had settled. The pellets were rinsed three times with distilled water to remove salt, with most of the water removed by pipette each time after the pellets had settled. After the last rinse the fecal pellets were transferred to an aluminum dish and allowed to air dry overnight, and then were transferred to a small vial and stored at -20 °C for pristane analysis.

Determination of Zooplankton Fecal Pellet Sinking Rate

The sinking rate of one sample of zooplankton fecal pellets was determined by measuring the range of times required for individual pellets to fall through a 22.5 cm column of ambient seawater (salinity 31‰ , temperature 6 °C).

Juvenile Pink Salmon Collection and Stomach Content Analysis

Released and un-released juvenile pink salmon were collected to compare stomach contents and pristane concentrations. Released fish were captured by dip-net two days after release in 1996, and 1 – 3 days after in 1998. Stomach contents were examined in fish preserved in 5% formalin in seawater, and pristane analysis was performed on fish stored frozen at -20 °C. Zooplankton in pink salmon stomachs were usually identified to genus. Unusual items (*e.g.* dipterans) were also noted.

Dry:Wet Mass Determination

The ratio of dry and wet mass of tissue was determined by drying weighed sample aliquots at 65 °C for 24 h. This ratio was 0.113 ± 0.00907 (n = 27) for mussels collected in 1996, and 0.0962 ± 0.00399 (n = 42) in 1998, a significant (P = 0.002, Mann-Whitney U test) difference. The ratio was 0.541 ± 0.167 (n = 4) for zooplankton fecal pellets. Pristane concentrations are expressed on a dry mass basis.

Chemical Analysis of Pristane in Tissues and Seawater

Pristane analysis of tissue samples and of particulate material filtered from seawater involved pentane or dichloromethane extraction of macerated tissues or of filters spiked initially with perdeuterated *n*-hexadecane as an internal standard, solvent concentration and exchange into hexane over steam, purification by silica gel/alumina column chromatography eluted with pentane, solvent re-concentration, resolution of alkanes by gas chromatography (GC) and measurement by flame ionization (Short et al. 1996). Identification of pristane is based on GC elution time.

The dichloromethane extracts of seawater samples were combined and exchanged into 1 mL hexane over steam, and then analysed by the GC analysis used for the tissue samples.

The accuracy of the pristane analyses were generally within ±15% based on comparison with an authentic hydrocarbon standard prepared by the National Institute of Standards and Technology (NIST), and the coefficient of variation was generally less than ±20%. The method detection limit (MDL), defined as the estimated concentration associated with a 1% probability of type I detection error, is 0.162 μg for tissue samples. The corresponding MDL estimate for individual samples is the ratio of this value and the mass of the sample analyzed. No comparable MDL estimate is available for pristane in seawater, so the ratio of the tissue MDL and the seawater aliquot volume (4 L) is assumed, resulting in a MDL of 41 ng L^{-1}.

Data Analysis

The significance of differences between two samples is determined by the nonparametric Mann-Whitney U test (Mann & Whitney 1947). For each mussel monitoring station within each year, the test was applied to two sets of samples, one consisting of samples collected from the first mussel collection until the day after release of juvenile pink salmon from the hatchery, and the other consisting of all samples collected thereafter. Samples collected the day after the releases are included in the first sample set because laboratory experiments indicate mussels require at least 24 h to respond to changes in the pristane content of ambient seawater (Short in prep [b]). For zooplankton samples, the first sample set consisted of samples collected prior to and including the day of the salmon release, in comparison with the second set consisting of

all samples collected thereafter. When temporal differences at individual stations were not judged significant ($P < 0.05$), samples from the three stations were pooled and the test re-applied to determine whether the absence of significance was not a consequence of insufficient statistical power.

Results

General Observations

Most of the juvenile pink salmon in the 1996 release dispersed rapidly from the inner bay. The following day it appeared the majority of released fish were migrating along the western shore of Esther Island, although groups including thousands of juveniles could be found in the vicinity of the mussel monitoring stations for several days following the release, and were observed defecating above the mussel beds at high tides. Cursory examination of the PWSAC plankton watch samples collected after the release revealed few large copepods such as *Neocalanus* or *Calanus* at the station within Lake Bay, and the pink-colored fecal casts characteristic of juvenile pink salmon preying on these copepods were rarely evident within the bay. Large copepods, including *Neocalanus* and *Calanus* were somewhat more abundant at the PWSAC station in the mouth of Lake Bay. Weather conditions were generally calm the week before and after the pink salmon release.

In contrast with 1996, a substantial proportion of the juvenile pink salmon released in 1998 remained within the inner part of Lake Bay for several days. Pink colored fecal casts were abundant within the inner bay by the third day following the release, floating on the seasurface or suspended in the upper few m of the water column, and were readily evident in the PWSAC plankton watch samples. These casts apparently attracted over a thousand gulls and kittiwakes on the seventh day after the release, when they could be seen floating on the seasurface and pecking the surface for the casts. Juvenile pink salmon were visually evident near the EL and LB stations the day following the release and again two days later, but could not be found in the vicinity of the HP station on either of these days. Weather conditions before and after the release were considerably less calm compared with those of 1996, with gales on 28 April, 3 May and 8 May 1998.

Pristane in Mussels

Pristane concentrations increased significantly ($P \leq 0.025$) at the LB station beginning at least 2 days after the juvenile pink salmon were released in both 1996 and 1998 (Figures 2 and 3). The median mussel concentrations increased nearly seven-fold in 1996 and nearly four-fold in 1998, and remained elevated beginning 2 – 3 days after the release. At the EL station, a significant ($P = 0.024$) increase of pristane in mussels occurred only in 1996, when a fourfold increase occurred following the release. In 1998, pristane concentrations appeared to increase in mussels at the EL station a week

following the release. At the HP station, no significant change in pristane concentration occurred in either 1996 or 1998, although the last sampling in 1996 at this station suggests a delayed increase, and similarly at the EL station in 1998.

Pristane in Seawater

Nearly all of the pristane concentration measurements were below detection limits (41 ng L^{-1}). Four samples contained detectable pristane concentrations associated with particulate material in 1996, the highest being 83 ng L^{-1}. Three samples (of 27) contained detectable dissolved pristane in 1998, the highest being 107 ng L^{-1}, and three contained particulate-pristane (maximum 66 ng L^{-1}). These concentrations are too low to resolve contributions from dissolved pristane and pristane associated with particulate matter, or to evaluate temporal trends.

Fecal Pellets

The mean pristane concentration in fecal pellets produced by the zooplankton collected in 1996 was 80.2 ± 35.7 μg g^{-1} (95% CI, n = 8). These pellets were usually ~500 μm in length and ~80 μm in diameter. The sinking rate of the pellets ranged from 2.1 – 3.0 m h^{-1}.

Zooplankton Abundance and Species Composition

Comparison of *Neocalanus plumchrus/flemingerii* abundances in the 0.202 and the 0.505 mm-mesh collection pairs at each station in 1998 usually agreed within a factor of two (*i.e.* the ratio of the higher and lower abundance at each station and sampling). Of the 36 collection pairs, 21 were within a factor of 1.5, 31 were within a factor of two and all were within a factor of three. Within each pair, the frequency of higher abundances in the smaller mesh net was 0.58, suggesting that these copepods did not avoid the smaller mesh net.

Neocalanus plumchrus/flemingerii and *Pseudocalanus sp*. were by far the most abundant zooplankton collected. In 1996, *Neocalanus plumchrus/flemingerii* stages IV and V copepodites usually accounted for 85% of the individuals and 98% of the biomass. *Pseudocalanus sp*. usually accounted for less than 5% of the individuals, but these small (<2 mm TL) copepods are not efficiently captured by the 0.505 mm-mesh net used. *Calanus marshallae* stages IV and V copepodites accounted for 2 – 15% of the individuals and of the biomass. Hence, nearly all of the zooplankton fecal pellet mass collected from these copepods were produced by either *Neocalanus plumchrus/flemingerii* or *Calanus marshallae*.

In 1998, the median abundances of *Neocalanus plumchrus/flemingerii* and *Pseudocalanus sp*. were 418 and 1,500 individuals m^{-3} (Table 1). These two genera

nearly always accounted for more than half the numbers of zooplankton, and consistently accounted for more than 80% of the biomass. Total biomass of zooplankton ranged from 0.276 – 2.81 g m^{-3} (median 0.783 g m^{-3}, n = 36). Other important contributors to the zooplankton in 1998 included *Acartia sp.*, bryozoan larvae, *Calanus marshallae*, *Oithona sp.*, and *Oikopleura sp.* Small (< 2 mm TL) hyperiid amphipods and pteropods (*Limacina helicina*), and chaetognaths (*Sagitta elegans*, < 10 mm TL) were also often present.

Abundances of *Neocalanus plumchrus/flemingerii* declined substantially at all three stations following release of the juvenile pink salmon in 1998 (Figure 4). Abundances declined simultaneously to between a half to less than a third at the three monitoring stations, and this decline was very highly significant ($P < 0.001$) at each station. Before the release, the median abundance of *Neocalanus plumchrus/flemingerii* was 710 individuals m^{-3}, declining to 261 m^{-3} afterwards. These declines in abundance are reflected by corresponding declines in biomass (Figure 5). In concert with the decline of *Neocalanus plumchrus/flemingerii*, abundances and biomass of *Acartia sp.* and of *Pseudocalanus sp.* increased by factors of three and two, respectively, (Figures 4, 6 and 7). There was insufficient statistical power to detect significant increases at every station independently, but the results for all stations combined were very highly significant ($P < 0.001$).

The species composition of zooplankton collected in the afternoon and night samples were not notably different than the morning samples (Figures 4 – 7). The zooplankton abundances were also similar. This indicates an absence of diurnal migration of the zooplankton community during the two weeks of this study.

Zooplankton and Pristane in Juvenile Pink Salmon

Neocalanus and *Pseudocalanus* accounted for nearly all of the prey found in the stomachs of juvenile pink salmon captured within three days of release from the hatchery (Table 2). These two genera comprised over 96% of individuals and 99% of the biomass. Other prey infrequently found include, in decreasing order, harpacticoid copepods, small insects, barnacle larvae, *Acartia* sp., juvenile pteropods, hyperiid amphipods, immature euphausiids, and one each of a decapod larvae, *Cumacea sp*., *Colembola sp*., and *Oithona sp*.

Individual pink salmon displayed considerable variability in their stomach contents. While *Neocalanus* usually accounted for most of the biomass, some individuals contained none of these but numerous *Pseudocalanus sp*. instead (Table 2). Differences in prey selection may have resulted from differences in sizes of the juvenile pink salmon captured, because the individuals that targeted *Pseudocalanus sp*. were invariably smaller than the median size of the unreleased fish. Also, in 1998, smaller pink salmon were captured at the EL station both 25 h and 65 h following release, but the small numbers of

captured animals and the opportunistic sampling method precludes inference about the pink salmon population at the EL station in 1998.

The juvenile pink salmon collected from all three sampling stations in 1996 contained considerable concentrations of pristane. Pristane concentrations ranged from 255 – 424 $\mu g\ g^{-1}$, and most of this was because of the *Neocalanus* in the stomachs of these fish. Unreleased fish at the hatchery contained 2.38 $\mu g\ g^{-1}$ pristane in 1996, and did not contain any zooplankton in 1996 or in 1998.

Discussion

Dispersion of fecal material produced by nearshore predators of *Neocalanus plumchrus/flemingerii* is clearly the dominant pathway followed by pristane from these copepods to mussels in PWS during spring. The importance of this pathway is strongly supported by the responses of pristane in the ecological compartments monitored, and by the ecological response to the hatchery releases of juvenile pink salmon, one of the most important nearshore predators of these copepods in PWS. The following discussion of the results of this study begins with consideration of two plausible alternative pathways, accumulation by mussels of pristane dissolved in seawater or of pristane associated with fecal pellets produced by copepods, which are shown to be minor in comparison with the pathway involving fecal material produced by predators of these copepods discussed subsequently.

Accumulation of Dissolved Pristane by Mussels

Accumulation of dissolved pristane by mussels is a significant but minor pathway because of the low ambient seawater concentrations, the bioaccumulation factor of mussels for pristane, and the fact that pristane concentrations abruptly increased in mussels following the hatchery releases of pink salmon (Figures 2 and 3) but concentrations of pristane in seawater did not. The highest concentrations of pristane measured in seawater during this study was 107 ng L^{-1}, which implies a wet mass tissue concentration of pristane in mussels of 215 ng/g at equilibrium. Assuming a ratio of dry and wet tissue mass of 0.1, this is equivalent to 2,150 ng g^{-1}. This upper limit is comparable with concentrations observed in mussels prior to release of the hatchery pink salmon (Figures 2 and 3), but is lower than concentrations in mussels at the LB station after the release by a factor of ~10. Hence, accumulation of dissolved pristane by mussels may account for a considerable portion of the pristane burden when predators of copepods are absent, but cannot account for the higher concentrations found after the hatchery releases.

Accumulation of Pristane in Copepod Fecal Pellets by Mussels

Accumulation by mussels of pristane associated with fecal pellets produced by *Neocalanus* and *Calanus* is probably more important than accumulation of dissolved pristane. The contribution from pristane in copepod fecal pellets to mussels may be

estimated from the concentration of pristane in the pellets, the concentration of pellets in seawater, and the BAF of mussels for particulate-bound pristane. The concentration of pristane in fecal pellets produced by *Neocalanus plumchrus/flemingerii* from PWS is reported here as 80.2 ± 35.7 μg g^{-1}. The BAF of pristane associated with organic particulate material (dispersed pink salmon feces) in mussels was measured in the accompanying laboratory study at 175,000 (Short in prep [b]).

The equilibrium concentration (denoted here as C_p) of fecal pellets in seawater produced by *Neocalanus plumchrus/flemingerii* may be estimated by equating the generation rate of the pellets with their loss rate from sinking. The result derived by Bienfang (1980) for a well-mixed column of seawater of depth z containing an homogenous distribution of identical copepods is $C_p = p\ B\ z\ \psi_p^{-1}$, where p is the pellet production rate per organism per unit time, B is the standing stock of organisms per unit volume producing pellets, and ψ_p is the sinking rate of the pellets produced. Well-fed *Calanus* copepods rarely produce more than about 4 pellets h^{-1} (Raymont and Gross 1942, Marshall and Orr 1955a, 1955b and 1956, Corner et al. 1972, Gaudy 1974). In PWS in 1998, seawater rarely contained more than one *Neocalanus plumchrus/flemingerii* L^{-1} (Table 1), giving a pellet generation rate ($p\ B$) of 4 pellets L^{-1} h^{-1}. Assuming the minimum observed sinking rate of ~2 m h^{-1}, the concentration of pellets at 5 m depth would therefore be 10 pellets L^{-1}, and this depth corresponds with maximum thickness of seawater that would be above mussels at high tide in PWS. The volume of a pellet produced by *Neocalanus plumchrus/flemingerii* in this study is ~ 2.5 x

10^{-6} mL (calculated from the volume of a cylinder having the length and diameter of the fecal pellets). The volume and sinking rate estimates reported here for fecal pellets from *Neocalanus plumchrus/flemingerii* are comparable with values reported by Bienfang (1980) for *Calanus spp*., who also reported a density of 1.17 g mL^{-1} for these copepods feeding on diatoms. The ratio of wet and dry mass of the fecal pellets produced by *Neocalanus plumchrus/flemingerii* was 0.541. Using these values an upper limit to the concentration of pristane associated with copepod fecal pellets in seawater in PWS may be estimated as (10 pellets L^{-1}) x (2.5 x 10^{-6} mL $pellet^{-1}$) x (1.17 g mL^{-1}) x (80,200 ng pristane g^{-1} dry mass) x (0.541) = 1.27 ng L^{-1}. At equilibrium the corresponding concentration of pristane in mussels would be 175,000 x 1.27 ng L^{-1} x 0.001 L g^{-1} wet tissue = ~225 ng g^{-1} wet tissue, or about 2,250 ng g^{-1} dry tissue. Hence, pristane associated with fecal pellets produced by *Neocalanus plumchrus/flemingerii* could measurably contribute to the pristane burden found in PWS mussels, but alone cannot account for the large increases in the concentrations observed during spring.

Accumulation of Pristane in Pink Salmon Feces by Mussels

The critical role played by the hatchery-released juvenile pink salmon in the transfer of pristane from *Neocalanus* copepods to mussels near the hatchery is supported by several lines of evidence. Because of their high abundances relative to other potential prey, *Neocalanus* and *Pseudocalanus* copepods are the most readily encountered prey for zooplanktivorous fishes in PWS (Table 1). Consumption of *Neocalanus* and

Pseudocalanus copepods was confirmed by the stomach content analysis in both 1996 and 1998, with *Neocalanus* the main prey taken on a biomass basis (Table 2). Observation of these pink salmon defecating directly above the monitored mussel beds confirms the validity of this pathway, and the quantitative importance of this pathway may also be assessed.

Estimates are available for the feeding rate of juvenile pink salmon on *Neocalanus* copepods, the efficiency of absorption of *Neocalanus* ingested by pink salmon, and the pristane content of the feces produced, which permit a rough assessment of the rate at which pristane is introduced into ambient seawater. A minimum feeding rate is directly available from the stomach content analysis of pristane caught 25 h after release from the hatchery in 1998 (Table 2). The median consumption rate of pink salmon caught at the LB station was 0.83 *Neocalanus* h^{-1} and was 0.375 h^{-1} at the EL station. Using the average of these multiplied by the number of fish released implies consumption of about 10^9 *Neocalanus* per day. Assuming an average wet tissue mass of 1 mg $copepod^{-1}$ for a mixture consisting of equal proportions of stave IV and stage V *Neocalanus plumchrus/flemingerii*, this is equivalent to daily consumption of ~1000 kg of these copepods. The absorption efficiency of pink salmon fed a copepod assemblage identical to that collected here in the 0.505 mm-mesh tows was 75%, the ratio of dry and wet fecal mass was 0.117, and the concentration of pristane in the feces produced was 383 $\mu g\ g^{-1}$ (dry mass basis; Short in prep. [b]). This implies daily introduction of ~11g pristane associated with feces into the seawater near the hatchery by the released pink

salmon. Assuming the cruising speed of the released pink salmon is 1 body length s^{-1}, these fish could travel a maximum of 3.3 km the first day. Assuming also these fish remain within 50 m of the shoreline and an average seawater depth of 50 m leads to an approximate volume of seawater receiving the feces produced by the pink salmon of 4.1 x 10^6 m^3, and a corresponding concentration of pristane associated with feces of ~1.4 ng L^{-1}. The BAF of pristane associated with these feces when completely dispersed was estimated at 175,000 (Short in prep [b]), so a pristane concentration of 1.4 ng L^{-1} associated with dispersed feces could result in a daily increase in the mussel tissue concentration of ~245 ng g^{-1} on a wet tissue mass basis, or ~2,450 ng g^{-1} on a dry tissue mass basis (assuming a ratio of dry to wet mussel tissue mass of 0.1). This is broadly consistent with the increase of the pristane concentration observed in mussels at the LB station in 1998, and similar calculations lead to the same conclusion for 1996.

The intensity of predation by hatchery-released pink salmon on *Neocalanus plumchrus/flemingerii* may also be inferred from the population changes of these copepods following the 1998 release. The decline in the median value of *Neocalanus plumchrus/flemingerii* abundance from 710 to 261 individuals m^{-3} over a 4-day period following release indicates a loss of ~0.5 individuals L^{-1}, or ~0.5 mg wet tissue mass L^{-1}. If all this were consumed by juvenile pink salmon, it would lead to (0.5 mg L^{-1}) x (0.25) x (0.117) x (383 ng mg^{-1}) = 5.6 ng pristane L^{-1}, which is comparable with the estimate of 1.4 ng L^{-1} d^{-1} based on the feeding rate of the pink salmon, and if completely dispersed

would cause an increase of the pristane concentration in mussels of ~9,400 ng g^{-1} at equilibrium.

The foregoing estimates of fecal-associated pristane available for uptake by mussels may be overestimated because feces produced by pink salmon may not fully disperse immediately, so some (possibly large) fraction of the feces produced may be present in fecal masses too large for mussels to ingest. However, volume of seawater occupied by pink salmon feces may also be overestimated, first because pink salmon cannot spend all their time cruising, but must stop to feed if feces are to be produced at all, and second because unlike other marine fish (Prahl et al. 1985), the feces produced by pink salmon preying on *Neocalanus* are nearly neutrally buoyant, as indicated by the floating fecal material inside Lake Bay in 1998, and so may be effectively retained within seawater depths less than 50 m within a day of production. Also, onshore winds may concentrate feces on or near shorelines, increasing their concentration near mussel beds, possibly considerably. Pristane-laden feces of pink salmon preying on *Neocalanus* is thus the most likely pathway to mussels, and is broadly consistent with the magnitude of pristane concentration increases observed in mussels immediately following releases of the hatchery pink salmon.

Accumulation of Pristane by Mussels

Four factors determine the magnitude of pristane increases in mussels during spring in PWS. Increasing populations of late-stage copepodites of *Neocalanus* and of

Calanus may introduce pristane into the surface seawater by dissolution and by production of pristane-laden fecal pellets, which may then be accumulated by mussels. This pathway would account for gradual increases during April to mussel concentrations of 2,000 – 3,000 ng g^{-1} at most. Abundances of these copepods are lower most years compared to 1998, so peak concentrations of pristane in mussels from this pathway would be correspondingly lower. The more dramatic increase in pristane concentrations during late April – early May is caused by feces produced from predation by zooplanktivorous fishes on these copepods. This pathway may account for pristane concentration increases of tens of thousands of ng g^{-1} in mussels. Required for such increases are simultaneously high abundances of *Neocalanus* or *Calanus* copepods, of their predators, and of favorable winds and currents that would concentrate and trap feces produced by the predators along shorelines containing mussel beds. The responses of pristane concentrations in mussels at the three stations monitored during this study reflect these requirements.

Neocalanus and *Pseudocalanus* biomass was unusually high at all three stations monitored near the hatchery in 1998. The average biomass of *Neocalanus* and of *Pseudocalanus* from 1994 through 1997 in PWS was 0.16 g m^{-3} and 0.08 g m^{-3}, respectively, in the upper 50 m of the water column in early May (Cooney et al. 2001a). In 1998, the biomass of *Neocalanus* in the upper 30 m sampled here was typically greater by a factor of three or more prior to release of the hatchery pink salmon, and the biomass of *Pseudocalanus* was also considerably higher, especially after the release (Figure 6).

In 1996, hatchery records indicate that substantial abundances of zooplankton were present at the stations monitored by PWSAC staff (Figure 1), and visual inspection of these samples showed they contained mostly *Neocalanus* and *Pseudocalanus*.

The hatchery releases of juvenile pink salmon in 1996 and in 1998 insured high abundances of these fish near the monitoring stations. Differences in the responses of pristane concentrations in mussels among the stations may in part reflect differences in the time required for the released fish to arrive at them. Large increases in mussel burdens of pristane first occurred at the station (LB) nearest the hatchery in both years, and in 1996 was followed by increases at the next nearest station (EL) and finally the most distant station (HP; Figure 2). This pattern is consistent with observations of fish leaving Lake Bay and migrating along the coast the day after the release, with an additional 1 – 2 days of travel needed for fish to reach the EL and HP stations, respectively. In 1998, when a large portion of the released fish remained within Lake Bay feeding on the abundant zooplankton there, fish took longer to disperse to the outlying monitoring stations, which may partially account for the delayed or absent increases of pristane in mussels at the EL and HP stations, respectively.

Surface currents may also have influenced the pattern of pristane increases observed in mussels following the hatchery releases of pink salmon. In 1996, the weather was mild, so feces deposited on or near mussel beds were less apt to be advected. In 1998 the weather was much less calm, and the EL and HP stations are more exposed

to wind-driven currents, which may also account in part for the delayed or absent increases of pristane in mussels at those stations (Figure 3). The LB station is in a more protected location, which may have contributed to the more pronounced pristane increase in mussels there.

Effects of Released Pink Salmon on Zooplankton Biomass

The abrupt decline of the *Neocalanus* population immediately following release of the hatchery pink salmon (Figures 4 and 6) was probably caused by the predation of the salmon on the copepods. It is of course possible that the coincident changes in the species composition of the zooplankton with the release of the juvenile pink salmon from the hatchery was simply the result of advection of a different water mass containing different proportions of these species, but three observations suggest predation by the released salmon on *Neocalanus* is a more likely cause. First, the reduction of the *Neocalanus* population abundance near the hatchery measured immediately following the pink salmon release agrees closely with the estimated predation impact of the pink salmon on the copepods, based on the stomach contents of the released fish the day following the release. Consumption of ~10^9 *Neocalanus* the day following the release as estimated above would reduce the copepod population abundance by 500 individuals m^{-3} in 2 x 10^6 m^{-3} of seawater, equivalent to a seasurface area of 40 km^2 at a depth of 50 m, and these dimensions are consistent with the range the juveniles could reasonably be expected to travel in a day. Second, the abrupt decline in the abundance of *Neocalanus*

was observed qualitatively in 1996, and it seems unlikely that such an abrupt change in zooplankton composition so precisely coincident with the hatchery releases would occur by advection twice. Third, previous sampling of PWS suggests that a zooplankton community dominated by *Pseudocalanus* biomass would be unusual in early May, the period of maximum *Neocalanus* biomass (Cooney et al. 2001a).

Reduction of the *Neocalanus plumchrus/flemingerii* population apparently allowed a corresponding rapid increase in the populations of *Acartia sp*. and of *Pseudocalanus sp*. When food is abundant copepodites of both *Acartia sp*. and *Pseudocalanus sp*. develop isochronally (Klein Breteler et al. 1994, Miller et al. 1977, Landry 1975), and can pass through a copepodite stage in a week or less, even at relatively cold temperatures (Klein Breteler and Schogt 1994). Early copepodite stages of these two genera are too small to be captured in the 0.202 mm-mesh net, but must be present at abundances comparable with the later stages that were captured, given the relatively rapid time scale of their development. Because isochronal development implies exponential growth (Miller et al. 1977), these species are able to respond rapidly to release from competition for food, with early copepodite stages rapidly recruiting to later stages. The abrupt reduction of *Neocalanus* abundance through predation by the released pink salmon would have left a considerable proportion of phytoplankton production available to surviving *Acartia sp*. and *Pseudocalanus sp*., allowing their corresponding numerical response. Interestingly, the total zooplankton biomass changed

little despite the abrupt loss of *Neocalanus* biomass, owing to the rapid response mainly by *Pseudocalanus sp*.

Implications for Monitoring Pristane in Mussels

Juvenile pink salmon and other zooplanktivorous fishes in PWS face an interesting foraging problem in early spring. Only two kinds of prey are readily available: large copepods dominated by *Neocalanus*, and small copepods dominated by *Pseudocalanus*. Preying on large copepods is inherently more efficient, especially in light of their very high lipid content (~50% dry weight; Båmstedt 1986, Duesterloh 2002), but they are chemically defended by their high pristane content (4 – 8 mg g^{-1} dry body mass for stage IV – V copepodites, Short in prep [a]), so that consuming them seriously impairs growth (Short in prep [b], Luquet et al. 1983, 1984). Because of their substantially smaller size, *Pseudocalanus* require more search effort to capture but they contain much less pristane (~0.1 mg g^{-1} dry tissue mass). In the laboratory study that accompanies this field study, juvenile pink salmon had gross growth efficiencies of only 6.6% when fed the zooplankton assemblage caught in the 0.505 mm-mesh nets of this study. This implies that juvenile salmon would have to consume about 45% of their body mass daily to support growth of 3% wet body mass d^{-1} reported for juvenile pink salmon in PWS (Willette et al. 2001) and this exceeds the estimated ration for the first day following release of the juveniles caught at the LB station by a factor of ten. Although pink salmon have been found to consume as much as 37% of their body mass daily in a

laboratory study (Mortensen 1983), such a high forage rate is probably impossible for most juveniles to sustain in the wild, even in 1998 when zooplankton abundances were relatively high. But if juveniles preying entirely on *Neocalanus* attain much lower ingestion rates, they will grow much more slowly than 3% d^{-1}, which would in turn expose them to greater risk of mortality from their own predators. Hence, an optimal foraging strategy to maximize growth would probably be to prey on *Pseudocalanus*, which a few juvenile pink salmon apparently did, judging from the large numbers of these copepods in their stomachs and the absence of any *Neocalanus* (Table 2).

Prey selection by zooplantivorous fishes in PWS might be influenced indirectly by the pristane content of *Neocalanus*. Feeding studies indicate that salmon do not avoid food contaminated by pristane, and ingestion of pristane does not depress appetite for the first two weeks on a pristane-laden diet (Luquet et al. 1984). It therefore seems likely that initial prey preferences of the juvenile pink salmon released by the hatchery may be arbitrarily determined, with some portion of the fish developing preference for and skill at capturing *Neocalanus*, some for *Pseudocalanus*, and some for either, consuming zooplankton in the order encountered. If so, the results of this study suggest that the pristane defense of *Neocalanus* may serve as a powerful factor diverting predation pressure away from these copepods. Fish that prey primarily on *Neocalanus* will enhance the growth and survival of their cohorts that target *Pseudocalanus* in three ways. First, by not preying on *Pseudocalanus*, more *Pseudoalanus* are available as prey to those fish that do. Second, to the extent fish preying on *Neocalanus* reduce the population of these

copepods, they augment the *Pseudocalanus* population by releasing these latter from competition with *Neocalanus* for phytoplankton food, making *Pseudocalanus* more abundant prey. These two factors enhance the ability of fish preying on *Pseudocalanus* to find food and hence grow faster. Finally, the fish preying primarily on *Neocalanus* will grow slower than their cohorts preying on *Pseudocalanus* because of growth inhibition by pristane, which will tend to shelter their cohorts from piscivores, because the fish feeding on *Neocalanus* would eventually become smaller and hence be more readily captured as prey. Even tiny differences in growth may translate into substantial difference in predation risk from predatory fish that are less than about tenfold larger than their prey (Miller et al. 1988). The net result is that fish that prey primarily on *Neocalanus* become the cohort members most vulnerable to predation, and their mortality reduces predation pressure on *Neocalanus*, while fish preying primarily on *Pseudocalanus* may become habituated to these prey, lessening predation pressure on *Neocalanus* still more.

Field evidence from other studies of hatchery-released pink salmon supports the hypothesis that releases *en masse* may deplete zooplankton abundances in the immediate vicinity, leading to slower growth (Willette et al. 1999) and lower energy density of the released pink salmon (Paul and Willette 1997). Growth of juvenile pink salmon was especially slow during early-mid May, when abundances of *Neocalanus* were greatest (see Figure 6 in Willette et al. 2001). These observations are also consistent with growth inhibition by the pristane content of *Neocalanus* copepods.

Understanding the trophic interactions among predators of *Neocalanus* and their prey is crucial for interpreting the annual increase of pristane concentrations in mussels during spring in PWS. The results of this study indicate these increases are mostly due to feces produced by zooplanktivorous fishes such as pink salmon inhabiting the near shore during spring, implying that mussel beds where increases are especially large are near relatively high concurrent abundances of *Neocalanus* copepods and their predators. Hence, a systematic survey of mussel beds for pristane increases during spring might indicate favorable feeding habitats for these predators. Comparison of the magnitude of these increases interannually might serve as a proxy indicator of interannual differences in intersection of concurrent abundances of *Neocalanus* copepods and their predators. These spatial and temporal variations of pristane increases in mussels during spring might also bear some relation to the early marine survival of juvenile pink salmon, but the details of any such relationship are unclear at present. High pristane concentrations in mussels may indicate abundant *Neocalanus* prey and nearshore zooplanktivorous fishes during spring, but these conditions may not translate directly to increased survival of fish. Because of the growth-inhibiting property of pristane, areas where pristane is abundant in mussels may also indicate particularly favorable opportunities for the predators of the zooplanktivorous fishes there. Indeed, growth inhibition by pristane in *Neocalanus* makes this food web especially sensitive to abundances of higher trophic level predators.

Conclusions

Each of three distinct ecological pathways may be followed by pristane from its source in *Neocalanus* and *Calanus* copepods to suspension-feeding mussels in PWS. When these copepods are abundant during spring, pristane dissolved into seawater from them, or pristane associated with fecal pellets produced by them may be accumulated by mussels to concentrations of a few thousand ng g^{-1} dry tissue mass. Much higher concentrations may be attained when zooplanktivorous fishes such as juvenile pink salmon are present, through ingestion by mussels of pristane-laden feces produced by these fishes preying on the copepods.

The large hatchery releases of juvenile pink salmon caused a detectable shift in the zooplankton community near the hatchery. Predation of the released fish on *Neocalanus* released the smaller *Pseudocalanus* and *Acartia* from competition for food, and these smaller species largely replaced the zooplankton biomass through consumption of *Neocalanus* by the released fish. This shift in the zooplankton community may indirectly help relieve predation pressure on *Neocalanus* by encouraging predation on *Pseudocalanus*, and by exposing fish that prey on *Neocalanus* to increased mortality risk, because fish preying on *Neocalanus* grow slower on account of growth inhibition by pristane.

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Table 1. Zooplankton composition near W. H. Noerenberg hatchery, spring, 1998. Summary of 36 vertical zooplankton tows from 30 m depth at three sampling station (EL, LB and HP; see Methods), from 26 April 1998 through 8 May 1998. Abbreviations of zooplankton are: AC = *Acartia sp.*, BL = bryozoan larvae, CM = *Calanus marshallae*, NFP = *Neocalanus plumchrus/flemingerii*, OIT = *Oithona sp.*, OKP = *Oikopleura sp.*, PSC = *Pseudocalanus sp.* Zooplankton abundance is individuals m^{-3}, and biomass is g m^{-3}.

	AC	BL	CM	NPF	OIT	OKP	PSC	Other	Total
Abundance									
Median	144	114	10.9	418	21.7	130	1500	27.2	2,620
Range	0.68 – 608	0 – 348	0 – 304	54.3 – 2031	0 – 261	0 - 630	217 – 11,000	4.58 – 94.9	815 – 12,600
Biomass									
Median	0.00747	0.00228	0.0109	0.418	0.00263	0.00434	0.213	0.00489	0.783
Range	3.52×10^{-5} – 0.0316	0 – 0.00695	0 – 0.304	0.0543 – 2.09	0 – 0.0316	0 – 0.0210	0.0309 – 1.57	7.63×10^{-4} – 0.0259	0.276 – 2.81

Table 2. Stomach contents of juvenile pink salmon near W. H. Noerenberg hatchery. Median (range) of pink salmon fork length (FL), and number of individuals and biomass (mg) of *Neocalanus sp*. and of *Pseudocalanus sp*. in stomachs of seven juvenile pink salmon following release from the hatchery in 1996 and 1998. The number of hours after the juveniles were released from hatchery net pens is indicated. "Unreleased" indicates results for cohorts retained within net pens.

	Hours		Pink				
	After		Salmon	*Neocalanus sp.*		*Pseudocalanus sp.*	
Year	Release	Station	FL (mm)	Individuals	Biomass	Individuals	Biomass
1996	0	Unreleased	38	0	0	0	0
			(33–40)				
	42	EL	35	6	6.00	34	4.83
			(32–36)	(2–10)	(2.00–10.0)	(0–82)	(0–11.6)
	42	LB	35	7	7.00	81	11.5
			(32–38)	(0–13)	(0–13.0)	(38–203)	(5.40–28.8)
	42	HP	35	9	9.00	29	4.12
			(33–38)	(0–12)	(0–12.0)	(19–129)	(2.70–18.3)
1998	0	Unreleased	38	0	0	0	0
			(35–40)				
	25	EL	32	9	9.00	51	7.24
			(30–36)	(0–28)	(0–28.0)	(2–59)	(0.284–8.38)
	25	LB	37	20	20.0	12	1.70
			(35–39)	(18–33)	(18.0–33.0)	(0–38)	(0–5.40)
	65	EL	32	5	5.00	13	1.85
			(30–33)	(3–11)	(3.00–11.0)	(5–17)	(0.710–2.41)
	65	LB	35	8	8.00	22	3.12
			(30–35)	(0–17)	(0–17.0)	(2–56)	(0.284–7.95)
	65	HP	38	5	5.00	12	1.70
			(37–39)	(3–7)	(3–7)	(3–49)	(0.426–6.96)

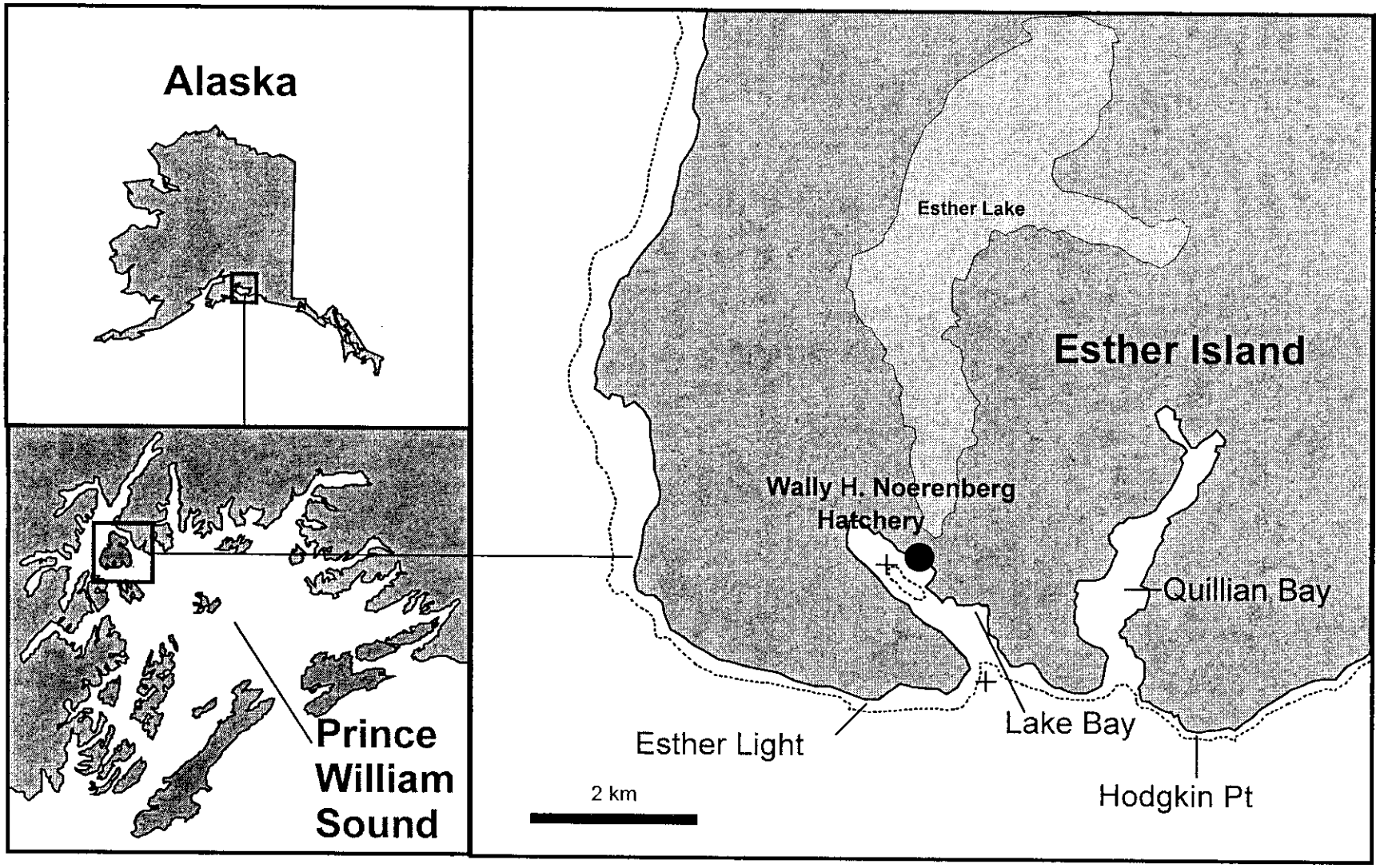

Figure 1. Map of study area. Lines indicate the mussel monitoring stations, and crosses the zooplankton sampling stations monitored by the Prince William Sound Aquaculture Corporation in relation to the Wally H. Noerenberg hatchery. The dashed line along the coast indicates the approximate 50 m depth contour.

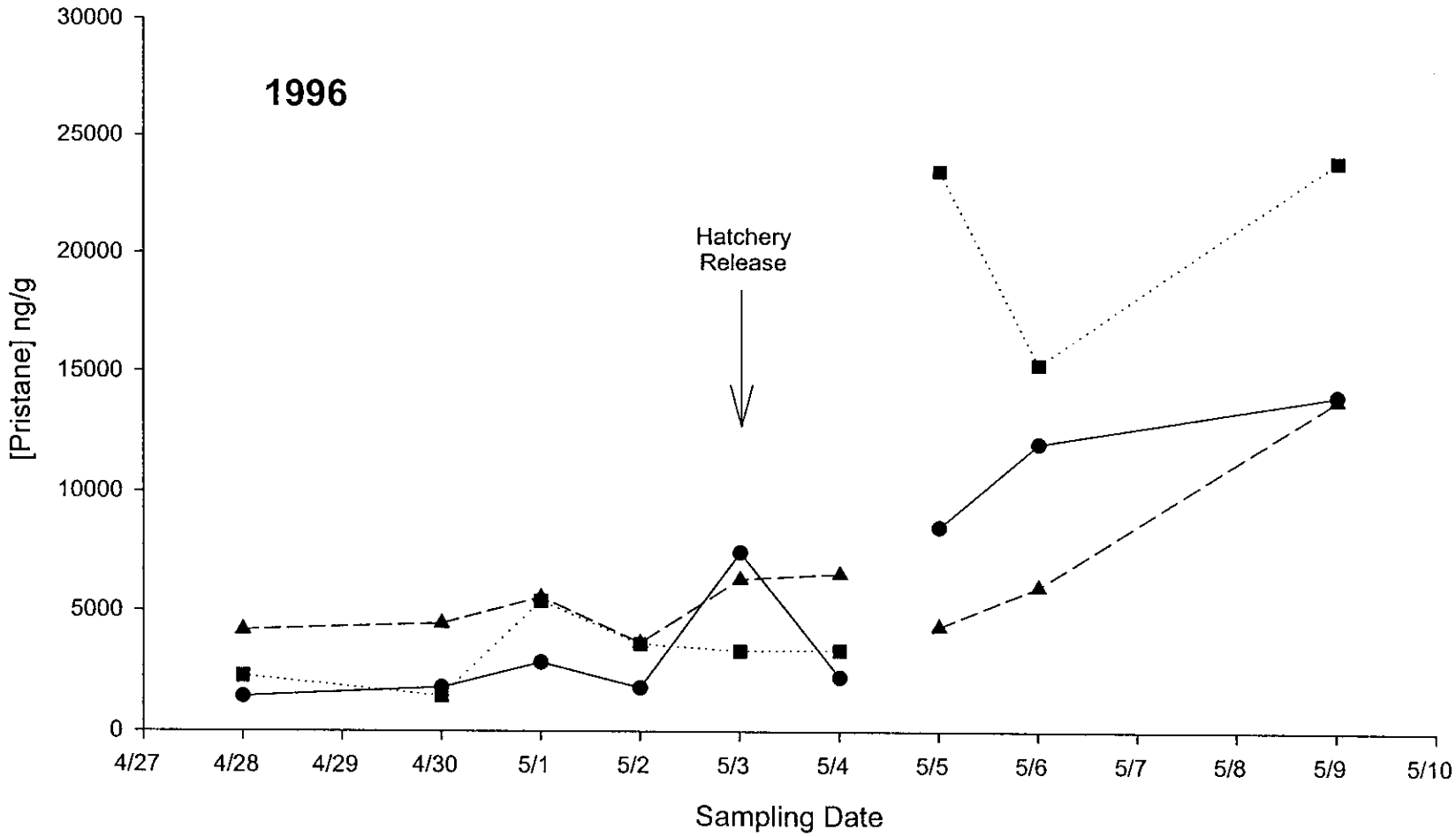

Figure 2. Pristane in mussels near Wally H. Noerenberg hatchery,1996. Stations: circle = Esther Light, square = Lake Bay, triangle = Hodgkins Point. Release date of 1.7 x 10^8 juvenile pink salmon from the hatchery is indicated by the vertical arrow.

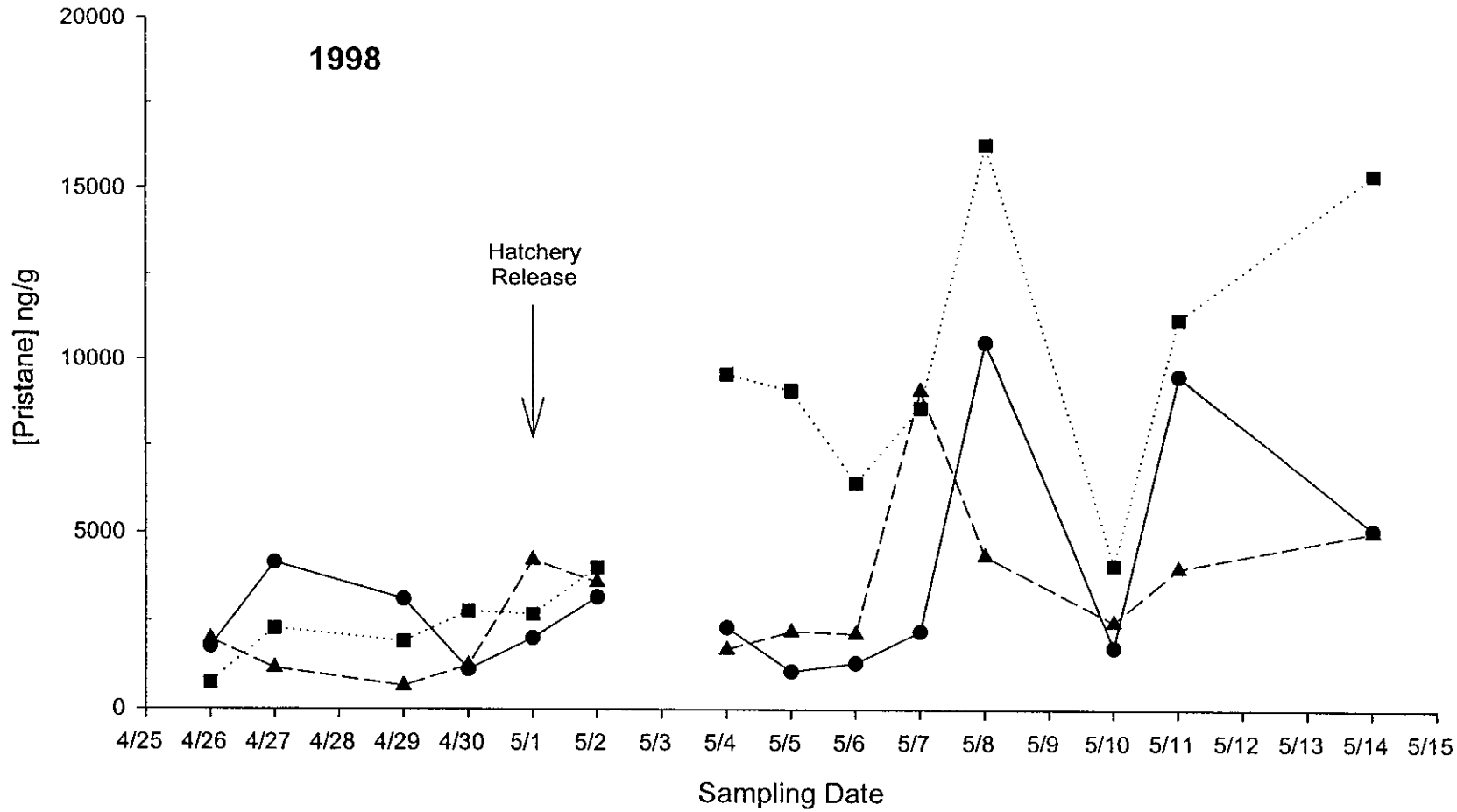

Figure 3. Pristane in mussels near Wally H. Noerenberg hatchery,1998. Stations: circle = Esther Light, square = Lake Bay, triangle = Hodgkins Point. Release date of 7.0 x 10^7 juvenile pink salmon from the hatchery is indicated by the vertical arrow.

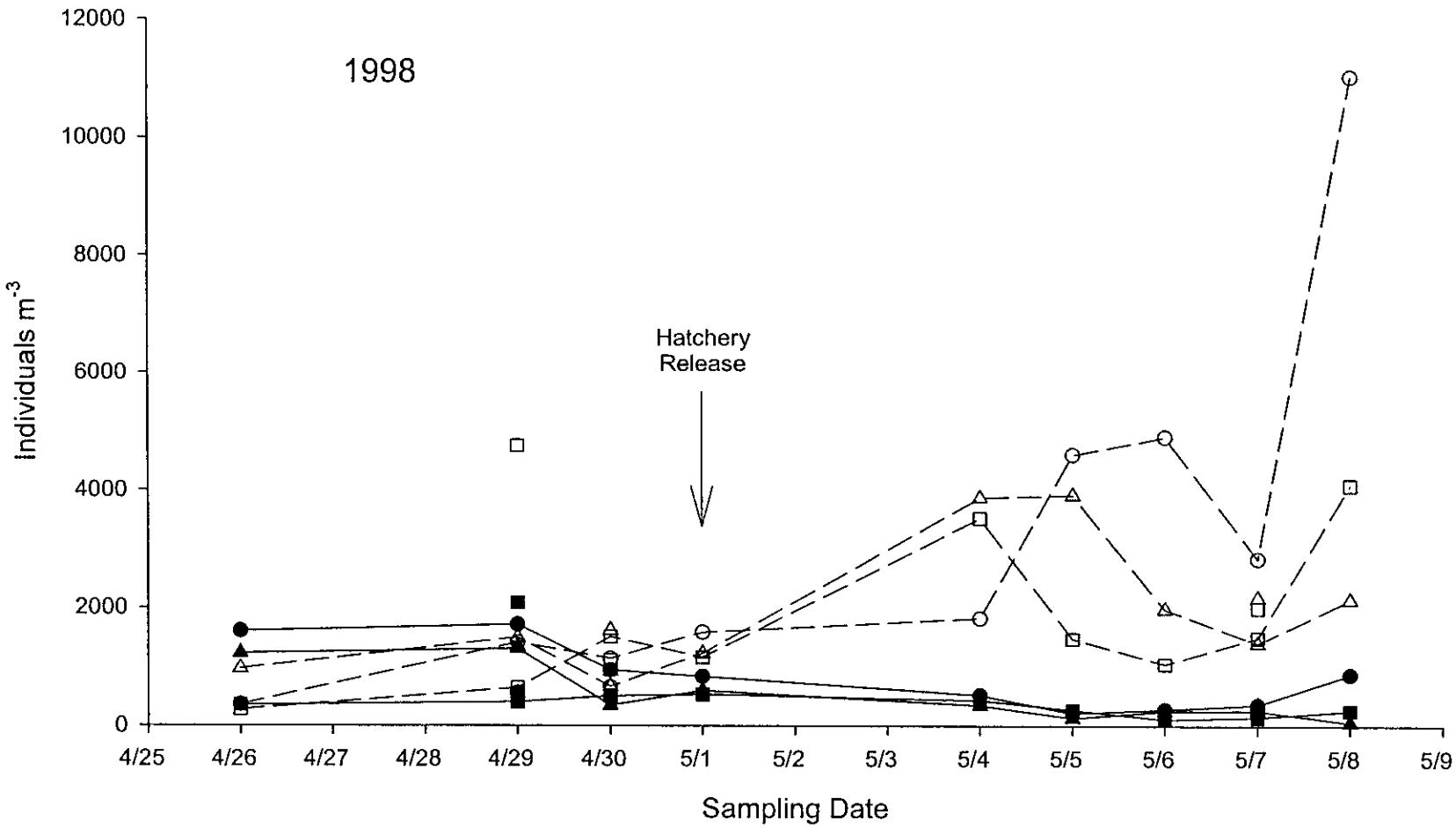

Figure 4. *Neocalanus* and *Pseudocalanus* copepod abundance. Stations: circle = Esther Light,square = Lake Bay, triangle = Hodgkins Point. *Neocalanus* = solid symbols and lines, *Pseudocalanus* = open symbols and dashed lines. Afternoon (4/29), late evening (4/30) and midnight (5/7) samples unconnected by lines, otherwise morning samples.

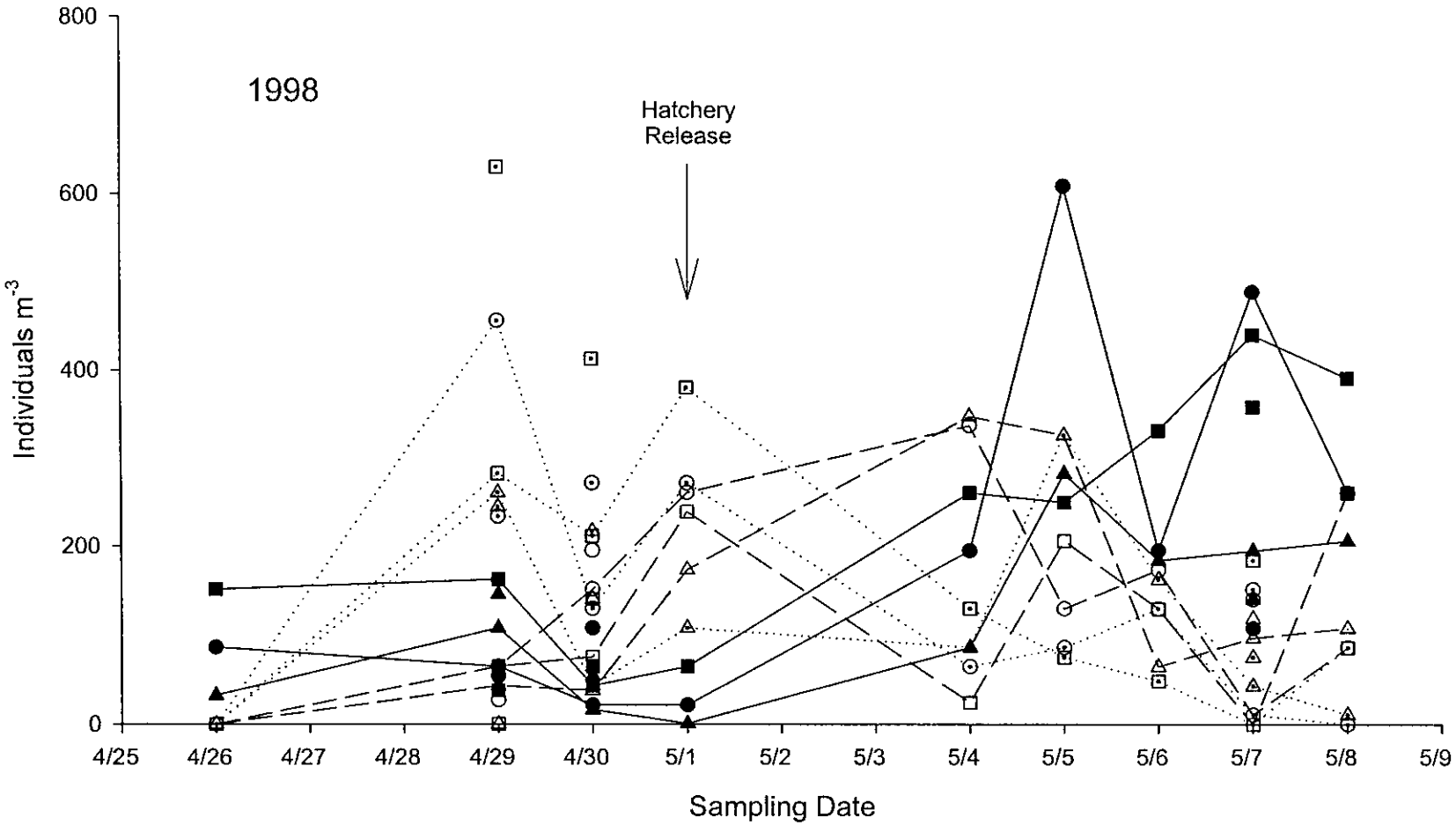

Figure 5. *Acartia*, and bryozoan larvae and *Oikopleura* abundance. Stations: circle = Esther Light,square = Lake Bay, triangle = Hodgkins Point. *Acartia* = solid symbols and lines, bryozoan larvae = open symbols and dashed lines, *Oikopleura* = dotted open symbols with dotted lines. Afternoon (4/29), late evening (4/30) and midnight (5/7) samples unconnected by lines, otherwise morning samples.

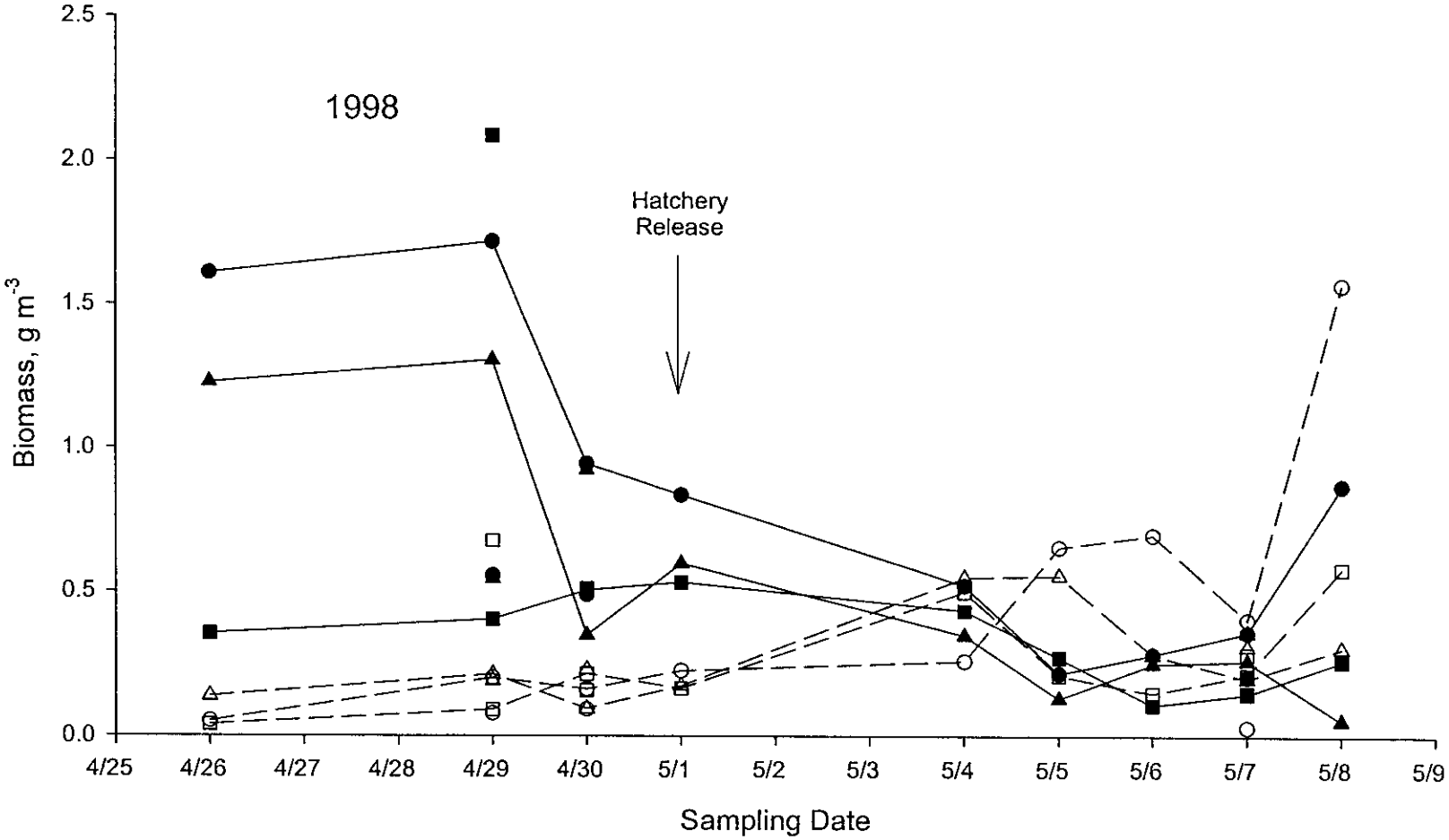

Figure 6. *Neocalanus* and *Pseudocalanus* biomass. Stations: circle = Esther Light, square = Lake Bay, triangle = Hodgkins Point. *Neocalanus* = solid symbols and lines, *Pseudocalanus* = open symbols and dashed lines. Afternoon (4/29), late evening (4/30) and midnight (5/7) samples unconnected by lines, otherwise morning samples.

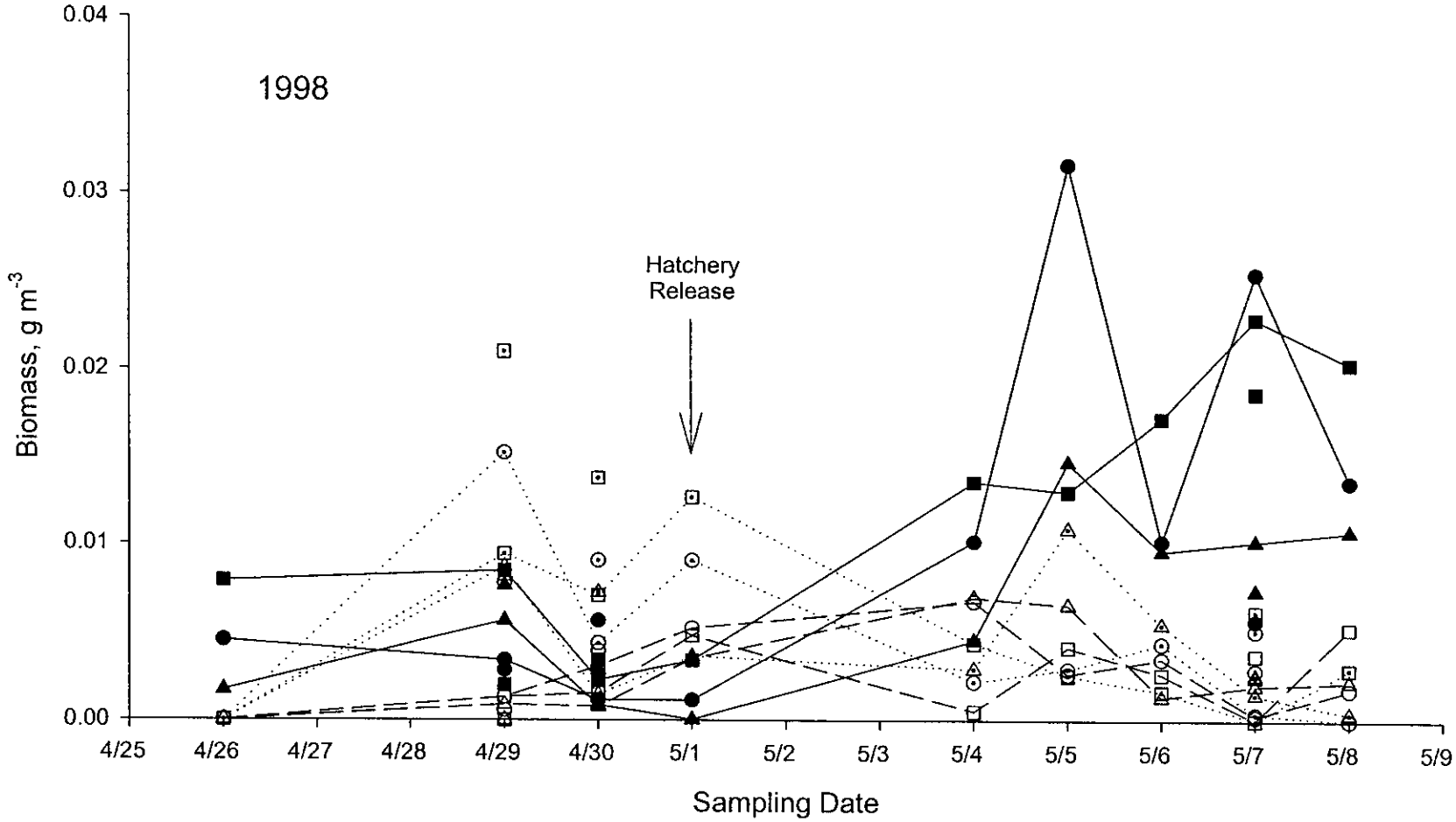

Figure 7. *Acartia*, and bryozoan larvae and *Oikopleura* biomass. Stations: circle = Esther Light,square = Lake Bay, triangle = Hodgkins Point. *Acartia* = solid symbols and lines, bryozoan larvae = open symbols and dashed lines, *Oikopleura* = dotted open symbols with dotted lines. Afternoon (4/29), late evening (4/30) and midnight (5/7) samples unconnected by lines, otherwise morning samples.