Exxon Valdez Oil Spill
Restoration Project Final Report

Pristane Monitoring in Mussels

Restoration Project 02195
Final Report

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Abstract: Pristane (2,6,10,14-tetramethylpentadecane) concentrations in mussels (*Mytilus trossulus*) increase abruptly during spring in Prince William Sound (PWS), Alaska. This increase is mainly due to ingestion by mussels of pristane-laden feces produced by near-shore zooplanktivores, especially juvenile pink salmon (*Oncorhynchus gorbuscha*). Examination of the trophic and temporal distribution of pristane found in 3,007 samples implicates *Neocalanus* copepods, which often dominate the zooplankton biomass in PWS during spring, as the source of pristane. Juvenile pink salmon, preying on *Neocalanus*, produce pristane-laden feces that are accumulated by mussels 52 times more efficiently than is dissolved pristane. Releases *en masse* of ~ 10^8 juvenile pink salmon from a hatchery at the peak of the *Neocalanus* bloom were immediately followed by increases in pristane concentrations of nearby mussels monitored during 1996 and 1998. Accumulation of dissolved pristane, or of fecal pellets produced by *Neocalanus* copepods, were substantially less important pathways of pristane transfer to mussels. The transfer pathway to mussels via feces produced by zooplanktivores preying on *Neocalanus* is the basis for a potential linkage between pristane accumulation by mussels and survival of juvenile pink salmon, because it reflects indirectly the magnitude of *Neocalanus* prey consumed. Annual survival values of hatchery pink salmon were weakly correlated ($P = 0.10$) with pristane concentrations monitored in mussels at 25 stations distributed throughout PWS from 1995 through 2001. Although *Neocalanus* copepods are considered important forage for juvenile pink salmon, feeding experiments reported herein confirm previous studies implicating growth inhibition by pristane, implying a lower forage value of *Neocalanus* than is usually assumed.

Key Words: *Exxon Valdez*, pristane, *Neocalanus spp.*, mussels, pink salmon.

Project Data: The data herein include pristane concentrations in tissues and excreta of marine biota from the Gulf of Alaska. These data are stored as Excel workbooks or in an Access database. The data custodian is Jeffrey Short, Auke Bay Laboratory, 11305 Glacier Highway, Juneau Alaska 99801-8626, Ph: 907.789.6065, email: Jeff.Short@noaa.gov.

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Executive Summary

Two species of large calanoid copepods, *Neocalanus plumchrus* and *Neocalanus flemingerii*, often dominate the zooplankton biomass in Prince William Sound (PWS), Alaska during spring, and hence occupy an important niche in the marine food web by connecting primary productivity to a wide variety of predators in upper trophic levels. Their dominance arises from the presence of deep (> 400 m) depressions inside PWS, which are necessary for their diapause and reproduction. *Neocalanus plumchrus* and *N. flemingerii* have a life span of one year and reproduce at depth in winter, after which the adults (i.e. stage VI copepodites) die. The naupliar offspring develop through five naupliar stages as they rise to shallower waters in late winter, metamorphosing to copepodites in time to begin grazing the incipient phytoplankton bloom in early spring. The copepodites develop rapidly from copepodite stage I to stage V by late spring, then seek deep water to begin diapause by the beginning of summer. This strategy allows them to efficiently graze the spring phytoplankton bloom, but it also exposes them to zooplanktivorous predators, often juvenile fish, whose survival depends on growing rapidly during spring to avoid their own predators. Hence, *Neocalanus* copepods, along with copepods in the closely-related genus *Calanus*, are usually considered as important forage for zooplantivores not only in PWS, but also in the pelagic North Pacific and North Atlantic oceans, where their deep-water reproductive habitat is ubiquitous.

Calanus and *Neocalanus* copepods biosynthesize unusually high concentrations of pristane from ingested chlorophyll, and may attain concentrations of ~1% of dry weight in adults or in late-stage copepodites. Pristane (2,6,10,14-tetramethylpentadecane: $(CH_3)_2CH\text{-}(CH_2)_3\text{-}CH(CH_3)\text{-}(CH_2)_3\text{-}CH(CH_3)\text{-}(CH_2)_3\text{-}CH(CH_3)_2$) is an alkane hydrocarbon that is terminally branched and thus relatively resistant to biochemical catabolism via β-oxidation. Hence, pristane is more persistent than un-branched alkanes when released into the environment.

Concentrations of pristane often increase abruptly in mussels (*Mytilus trossulus*) during spring in PWS. Increases of several hundred-fold are common, especially in mussels on shorelines of western PWS adjacent to the deep marine depressions there. These increases typically begin in April, peak during May, and then gradually decline to low values during the fall and winter months by late August.

The primary goal of this research is to elucidate the ecological pathway followed by pristane from *Neocalanus* copepods to mussels. The large sizes of late-stage *N. plumchrus* or of *N. flemingerii* copepodites usually preclude direct ingestion by mussels, so other routes must account for the springtime concentration increases in mussels. Three likely pathways are direct uptake of pristane dissolved into seawater from the copepods, ingestion of fecal pellets produced by them, or ingestion of feces produced by zooplanktivores that prey on *Neocalanus sp*. Objectives of this research include comparative evaluation of the importance of these three pathways in PWS, and assessment of whether interannual variation of pristane accumulation by mussels during spring can be related to interannual variation of marine survival of juvenile pink salmon (*Oncorhynchus*

gorbuscha).

Juvenile pink salmon are zooplanktivorous and remain within a few tens of meters of the shoreline during their first few weeks of marine residence in spring to avoid predation. Several hundreds of millions of juvenile pink salmon migrate to the marine waters of PWS, and hence are one of the most common zooplanktivores inhabiting the near shore during spring. Because of their numbers, migration timing, and habitat preference, juvenile pink salmon are likely to be an important intermediary in the transfer of pristane from *Neocalanus sp.* to mussels. Other zooplanktivorous fishes are either much less restricted to the very near shore (such as Pacific herring, *Clupea harengus*), or are usually less abundant (e.g., the *Stichaeidae* such as blennies, pricklebacks and cockscombs).

If mussels accumulate pristane mainly through ingestion of feces produced by near-shore zooplanktivores, the pristane increase in mussels may correspond with the abundance of zooplankton forage available to juvenile pink salmon, and hence the favorability of feeding conditions. If so, pristane accumulation by mussels would reflect concurrent abundances of the juvenile salmon and their prey, and hence might serve as an index of carbon transfer to the juveniles, which may in turn provide a particularly sensitive proxy index for early marine survival. Because marine mortality is thought to be greatest and most variable during the initial period of marine residence, such a proxy index might have predictive value that could be used to improve management of the fishery on pink salmon.

The seasonal pattern of increases of pristane in mussels is evident in the results of hydrocarbon analyses conducted for the 1989 *Exxon Valdez* oil spill (EVOS). This event stimulated the most comprehensive hydrocarbon analysis of a marine food web in history, and pristane was among the hydrocarbons analyzed because of its presence in crude oil. The hydrocarbon data engendered by the spill provide an opportunity to examine how a moderately persistent organic compound that is introduced at the level of secondary production permeates a marine food web. The first chapter of this report is devoted to summarizing these results with the aim of determining if the distribution and temporal variation of pristane in this food web are consistent with *Neocalanus* copepods as the pristane source.

The ecological pathway traversed by pristane from *Neocalanus* to mussels is the subject of a series of laboratory and field experiments that are presented in chapters 2 and 3, respectively. The laboratory experiments involved monitoring the dynamics of pristane uptake and depuration by mussels exposed to dissolved pristane, or to pristane-laden feces produced by juvenile pink salmon fed a natural zooplankton assemblage that was collected from PWS during spring, which consisted almost entirely of *Neocalanus sp*. The growth of these juvenile pink salmon was also monitored, and compared to growth of cohorts fed nearly equivalent rations of brine shrimp (*Artemia sp.*) as a control treatment. These experiments established the ability of mussels to bioconcentrate pristane, and the characteristic time scales of accumulation and of depuration. They also established the nutritive value relative to brine shrimp of the natural zooplankton assemblage present in PWS during the peak of the juvenile pink salmon outmigration period.

The field experiments reported in chapter 3 focus on the ecological response to release *en masse* of ~ 100,000,000 juvenile pink salmon from a hatchery in PWS near the peak of the springtime zooplankton bloom in 1996 and again in 1998. Pristane concentrations in mussels were monitored at stations near the release point for several days prior to the releases through several days afterward. Concentrations of pristane dissolved in seawater and of pristane associated with particulate matter in seawater were also monitored, as were zooplankton abundances. Fecal pellets produced mainly by *N. plumchrus* or *N. flemingerii* were collected and analyzed for pristane which, combined with zooplankton concentrations and their fecal production rates, allowed assessment of the importance of these fecal pellets as a vector of pristane transfer to mussels. When combined with the results of the laboratory experiments (which used zooplankton collected during the 1998 field experiments), the relative importance of the three alternative pathways of pristane accumulation by mussels can be clearly established.

The final chapter (chapter 4) is devoted to an assessment of whether the results of long-term monitoring of pristane concentrations in PWS mussels during spring and summer bear any relation to variation in the marine survival of hatchery-released pink salmon. Mussels were collected biweekly during spring and biweekly or monthly during summer from a network of 27 sampling stations distributed throughout PWS from 1995 through 2001. Pristane concentration increases were determined for each station sampled, and results were examined for spatial and temporal patterns. An index summarizing the overall springtime increase and subsequent decline was developed for comparison with the annual marine survival values of the hatchery pink salmon. Four hatcheries in PWS release ~ 500,000,000 juvenile pink salmon annually during spring, and the returns of adults to these hatcheries provides an especially firm basis for estimating marine survival.

Comparison of the distribution of pristane concentrations among the biota samples analyzed for the 1989 *Exxon Valdez* oil spill (EVOS) with concentrations in *Neocalanus* copepods confirms these copepods are the dominant source of pristane in the marine food web of PWS. Other potential biological sources, such as zooplankton in genera other than *Neocalanus* or *Calanus*, are negligible in comparison to the magnitude of pristane introduced annually by *Neocalanus sp*., and catastrophic inputs from anthropogenic sources such as the EVOS are rare but may be comparable during the years that such catastrophes occur.

The main ecological pathway followed by pristane from *Neocalanus* to mussels involves ingestion of pristane-laden feces produced by near-shore zooplanktivores that prey on *Neocalanus.* This pathway can account for large (i.e. hundred-fold or greater) increases of pristane concentrations in mussels. Juvenile pink salmon are often the most important of the zooplanktivores that mediate this transfer because of their preference for habitats that are very close to shorelines during the initial phase of their marine residence, when they may defecate directly on mussel beds during high tides. Mussels may also accumulate smaller increases of pristane from the dissolved state, or from fecal pellets produced by *Neocalanus* when abundances of these copepods are high.

Biosynthesis of pristane may afford *Neocalanus* and *Calanus* copepods a measure of chemical

protection from predation, because of the inhibitory effect of pristane on growth of juvenile fishes. Compared with alternative prey, juvenile salmonids that prey mostly on *Neocalanus* copepods experience markedly slower growth per unit ingested ration, which may prolong exposure of juveniles to size-selective predation by their predators. Any resulting increase in mortality would decrease predation pressure on *Neocalanus* copepods. Hence, the nutritive value of these copepods may be substantially lower than is usually assumed, not only in PWS, but throughout the North Pacific and North Atlantic oceans as well, where these two taxa are seasonally common potential forage for zooplanktivores.

The inhibitory effect of pristane on salmonid growth complicates interpretation of the relation between pristane increases in mussels during spring and the marine survival of juvenile pink salmon in PWS. Absent this effect, large increases of pristane in mussels on shorelines near salmon hatcheries or near reproductive habitat of wild pink salmon would imply favorable conditions for carbon transfer from *Neocalanus* to pink salmon and other zooplanktivores, because of the concurrently high abundances of *Neocalanus* and of zooplanktivores necessary for large increases of pristane in mussels to occur. However, growth-inhibition by pristane suggests that these abundant forage conditions may not translate into favorable survival conditions, if juveniles consuming *Neocalanus* are more vulnerable to predation when predators are abundant.

Despite the complications introduced by the inhibitory effect of pristane on salmonid growth, comparison of an integrative index of pristane accumulation by mussels throughout PWS with the combined survivals of hatchery pink salmon was suggestive if not statistically significant. A more focused monitoring program that tracks zooplankton composition and predator abundance, when combined with a region-wide index of zooplankton abundance might be more readily related to the marine survival of pink salmon, which has the potential to improve management of the fishery through prediction of recruitment at some level of significant accuracy.

Chapter 1

DISTRIBUTION OF PRISTANE IN THE NERITIC ECOSYSTEM OF THE NORTHERN GULF OF ALASKA

Abstract

Biosynthesis of pristane, a terminally-branched alkane hydrocarbon, by *Calanus* copepods in the Atlantic Ocean produces up to 1% of the dry mass of stage V copepodites and adults. We confirm here that similar concentrations are attained in Pacific Ocean species of *Calanus* and *Neocalanus*, but concentrations in other GOA zooplankton genera are at least an order of magnitude 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. Pristane is lipophilic and is somewhat recalcitrant, 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 49 species comprising 3,007 samples collected during damage assessment studies for the 1989 *Exxon Valdez* oil spill, including birds, fishes, molluscs, crustaceans, plants, mammals and an echinoderm, demonstrate that the pristane introduced into the food web by *Calanus* and *Neocalanus* copepodites during spring gradually dissipates as it passes through successive consumer species and with time. Pristane concentrations in zooplanktivorous species during spring are usually about an order of magnitude below concentrations in the copepodites. Roughly tenfold or greater reductions occur with each successive trophic transfer. Pristane concentrations are highest in adipose tissues, and are lowest in brains and blood, of species for which multiple tissues were collected. Pristane is probably introduced into intertidal and subtidal benthic food webs by feces produced by *Calanus* and *Neocalanus* copepodites, or by zooplanktivores preying on 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.

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 and Ackman 1977, Sargent and Whittle 1981, Parrish et al. 2000, Iverson et al. 1997, 2002, 2004, Stübing et al. 2003), or the analysis of stable isotopes of carbon and nitrogen as an indicator of relative trophic position (Lajtha and 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 recalcitrant to biodegradation (Pirnik 1977, Schaeffer et al. 1979), 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^{-1} (Short et al. 1996). Despite these advantages, pristane analysis has not often been used for food web studies, in part because foundation studies to 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 required to provide context for the interpretation of new data.

The major biogenic source of pristane identified 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. Pristane is biosynthesized in these copepods from ingested chlorophyll (Avigan and 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 the zooplankton genera examined. Pristane concentrations in other zooplankton collected from the Atlantic Ocean, including other calanoid copepod genera, were 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 subarctic latitudes where they may account for most of the spring zooplankton biomass near the sea surface (Parsons and Lalli 1988). 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 environmental persistence led to its proposal as a natural tracer molecule of predation relationships (Blumer et al. 1964).

Pristane is nearly ubiquitous in marine organisms at widely varying concentrations that usually depend on trophic distance from marine zooplankton, especially *Calanus spp*. 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 and 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 Atlantic 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 :g g^{-1} wet weight (Johansen et al. 1977). However sperm whales (*Physeter macrocephalus*), which feed mainly on squid, contained as much as 240 :g g^{-1} in their blubber (Sano 1968), while sockeye salmon (*Oncorhynchus nerka*), which are facultative zooplanktivores, contained only 0.380 :g g^{-1} in their livers and 2.4 :g g^{-1} in their viscera (Sasaki et al. 1991). Pristane in oil from herring in the Baltic Sea contained less than 1% of the pristane concentration found in oil from herring in the Atlantic Ocean (Linko and Kaitaranta 1976), consistent with the low abundance of *Calanus* copepods in the Baltic Sea (Hernroth and Ackefors 1979). Tissues of benthic invertebrates consistently contain pristane concentrations below about 2 :g g^{-1} (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 and Garlich-Miller 2001). Pristane was not detected in any of four species of benthic epiphytes collected from a Carribean lagoon (Botello and 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 (Ackman 1971, Custer et al. 2001, Lopez-Leitón 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 of 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 (EVOS) 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, molluscs, 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 data set of its kind that is internally consistent with respect to sampling and chemical analysis methods. 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 and Heintz 1997), but pristane was, especially in mussels (*Mytilus trossulus*) collected both before and after the spill (Short and Babcock 1996, Karinen et al. 1993).

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

We present here the results of pristane analyses of Pacific Ocean species of the genera *Calanus* and *Neocalanus*, and in other zooplankton sampled during spring in the northern GOA, to determine whether the high pristane concentrations found in Atlantic Ocean *Calanus* species also occur in Pacific Ocean species of this and other copepod genera. We then present a summary of the seasonal distributions of pristane among the biota sampled for government-sponsored EVOS damage assessment studies, to evaluate whether distributions of pristane among species at different trophic levels are consistent with *Calanus* and *Neocalanus* copepods as sources.

Study Area

Nearly all of the samples 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. 1.1). Most of these samples (83%) were collected from PWS, and the remainder from the spill-impacted region west of PWS (Fig. 1.1).

Prince William Sound is a complex fjord-type ecosystem with a sea surface area of about 8,800 km^2 (Schmidt 1977). 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. 1.1). 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).

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 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 *N. flemingerii* [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. flemingerii*, 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 and 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, [Båmstedt 1986, Duesterloh 2002]) 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 groups are

distinguished in Table 1.1 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 5% buffered 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 from PWS during July 1998 and May 2000 respectively. 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 Samples

Comparison of hydrocarbon analysis results among the samples collected for the EVOS requires caution, because these samples were collected for a variety of assessment objectives by multiple government agencies and personnel. Samplers 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 impossible 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 1.2.

All of the hydrocarbon data generated from government studies of the EVOS 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. Details of the sample collection methods are contained in the final reports of the principal investigators of the studies funded by the *Exxon Valdez* Trustee Council, and are available from the Council in Anchorage, Alaska. These studies are identified in the EVTHD for each sample contained therein.

To facilitate comparisons among samples, the most broadly sampled tissues are emphasized in the presentation of the results. These include 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. We 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. 1.1) or was not recorded in the database, the collection date was not recorded, the dry weight of the sample was

not recorded, the sampled animal was not identified to species, the sampled matrix was feathers or an egg or mollusc shell, or the sample was likely contaminated by crude oil or refined petroleum products. We 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 (Dean and Whithehead 1961, Blumer and Snyder 1965, Ackman and 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. 1.1) 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.

Pristane Analysis

The chemical analysis of aliphatic hydrocarbons in the oil spill samples 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, and solvent re-concentration followed by alkane resolution with gas chromatography (GC) with 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 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. 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 for dry weight determination.

Data Analysis

Pristane concentrations are presented as :g 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 1.2 – 1.8.

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

Results

Zooplankton

Neocalanus and *Calanus* copepods contained the highest concentrations of pristane among the species surveyed (Table 1.1). Median pristane concentrations ranged from 2,440 to 8,020 :g g^{-1} 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 :g g^{-1}, substantially lower than the lowest concentration in CIV copepodites (2,440 :g g^{-1}). 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 :g g^{-1} in the other zooplankton species analyzed, and were usually much less (Table 1.1). The median concentration in *M. okhotensis* was 660 :g g^{-1}, compared with concentrations less than 125 :g g^{-1} in the two other species of *Metridia*. *Euchaeta elongata* from southeastern Alaska contained 455 :g g^{-1} compared with 23.8 :g g^{-1} from PWS. The remaining species surveyed contained less than 125 :g g^{-1}, 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 :g g^{-1} (Fig. 1.2), and the stomach contents of the same animal contained 1,460 :g g^{-1}. The median concentration of 19 fork-tailed storm petrel eggs was 519 :g g^{-1}.

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 :g g^{-1}, 46.1 :g g^{-1} and 1.15 :g g^{-1} (Fig. 1.2). The liver of one common loon sampled during spring 1989 contained 229 :g g^{-1}. Median pristane concentrations in eggs of bald eagles and black kittiwakes were similar, with ranges of 1.17 :g g^{-1} to 62.2 :g g^{-1} for bald eagles and 1.51 :g g^{-1} to 61.7 :g g^{-1} for black kittiwakes (Fig. 1.2.). Median pristane concentrations were higher in bald eagle eggs sampled during spring compared with summer 1989 and 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 on intertidal invertebrates contained lower concentrations of pristane than did the piscivorous birds. The invertebrate feeders include three shorebirds (black turnstone, rock sandpiper, and surfbird) and five 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 :g g^{-1} and 12.9 :g g^{-1} respectively. The median for black turnstone livers was 1.05 :g g^{-1} in summer 1989 compared with 5.11 :g g^{-1} 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 :g g^{-1} across all 39 liver samples of these species. Although no seasonal trends were evident in these shorebird and sea duck samples, they 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 for the possibility of 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 :g g^{-1}, but ranged to 12.2 :g g^{-1} in the 12 samples analyzed (Fig. 1.2).

Fish

Pristane concentrations were highest in Pacific herring and juvenile pink salmon. Muscle and viscera of Pacific herring had median concentrations near 100 :g g^{-1}, compared with 11.6 :g g^{-1} to 21.6 :g g^{-1} in reproductive tissues and eggs (Fig. 1.3). 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 :g g^{-1}, compared with median concentrations in seawater-resident fry during spring and summer of 57.9 :g g^{-1} and 142 :g g^{-1}, 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 :g g^{-1}, compared with median concentrations of 7.10 :g g^{-1} to 13.3 :g g^{-1} in whole prickleback species sampled during spring or summer. During spring, one juvenile Pacific cod contained 8.09 :g g^{-1}, but three others contained less than 0.090 :g g^{-1} (Fig. 1.3). Pollock (*Theragra chalcogramma*), which are commercially important in PWS and the GOA, were unfortunately not sampled, because their pelagic habitat was considered to be at low risk of contamination from the oil spill.

Molluscs

The highest pristane concentrations among molluscs occurred in some of the suspension-feeders sampled during spring. The suspension-feeders sampled include bay mussels, butter clams, Kennerley's venus clams, littleneck clams, Pacific oysters, sunset clams, weathervane scallops and razor clams (Table 1.2). Bay mussels contained the highest median concentrations among

these suspension-feeders at 2.34 :g g^{-1} to 3.51 :g g^{-1} during spring, compared with 0.179 :g g^{-1} to 0.234 :g g^{-1} during summer, and this seasonal difference was highly significant ($P < 0.001$; Fig. 1.4). Some bay mussels contained concentrations greater than 50 :g g^{-1} during spring, comparable with concentrations of some piscivorous fish. Pristane concentrations during spring were significantly greater than summer for all the other suspension-feeders where data are available for comparison ($P < 0.01$) except for razor clams, which had greater concentrations in summer compared with spring (Fig. 1.4).

Increased concentrations of pristane in bay mussels during spring compared with summer is a consistent pattern evident each year from 1989 through 1995 (Fig. 1.5). 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 (Figs. 1.4 and 1.5). In 1989, pristane concentrations increased from less than 0.5 :g g^{-1} in March and early April to over 63 :g g^{-1} in early May, then gradually subsided to concentrations generally less than 1 :g g^{-1} by August, and this pattern repeated each subsequent year through 1995. The available data for butter clams and littleneck clams are consistent with the trends evident for bay mussels, but maximum pristane concentrations did not exceed 10 :g g^{-1}.

Pristane concentrations in periwinkle snails, intertidal algal and detrital grazers, were comparable with the suspension-feeding bivalves. Median concentrations were 1.51 :g g^{-1} in spring 1989 and 0.504 :g g^{-1} in summer 1990 (Fig. 1.4). Pristane concentrations were usually below MDL in the deposit-feeding *Macoma* clams (Fig. 1.4).

Crustaceans, Echinoderm and Plants

Substantial but variable pristane concentrations occurred in the hepatopancreas of all three crab species sampled. Median concentrations were 39.1 :g g^{-1} in king crab hepatopancreas, and ranged from 3.26 :g g^{-1} to 27.1 :g g^{-1} in Tanner crab and from 0.261 :g g^{-1} to 4.53 :g g^{-1} in Dungeness crab (Fig. 1.6). Maximum pristane concentrations near 100 :g g^{-1} to more than 400 :g g^{-1} 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 :g g^{-1} across the three crab species and the sampling seasons. Maximum concentrations were also lower, the highest value being 33.2 :g g^{-1} (Fig. 1.6).

Spot shrimp eggs contained somewhat higher pristane concentrations than the crab eggs. Median concentrations of shrimp eggs were 7.90 :g g^{-1}, and the maximum value was 223 :g g^{-1} (Fig. 1.6).

The median pristane concentration in the suspension-feeding barnacle during spring was 1.74 :g g^{-1}, comparable with concentrations in suspension-feeding molluscs (Figs. 1.3 and 1.6). Barnacles were not sampled during other seasons.

Concentrations of pristane in green sea urchin gonads were low compared with the crustacean tissues, with a median of 0.257 :g g^{-1} and a maximum of 2.26 :g g^{-1} (Fig. 1.6).

The median concentration of pristane in rockweed collected during spring from Montague Island was 5.68 :g g^{-1}, and ranged from 4.53 :g g^{-1} to 9.02 :g g^{-1} (Fig. 1.6). One eelgrass sample concurrently collected from the same location contained 5.04 :g g^{-1}. 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 :g g^{-1} in the spring and 52.9 :g g^{-1} in the summer, and were between 2.82 :g g^{-1} and 50.9 :g g^{-1} in blubber, milk, kidney, and heart (Fig. 1.7). Maximum concentrations ranged to several hundred :g g^{-1} in the mammary and ranged to 50 – 150 :g g^{-1} 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 :g g^{-1} but exceeded 10 :g g^{-1} in 2 samples. Comparatively low concentrations (< 9 :g g^{-1}) 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 :g g^{-1} and 175 :g g^{-1}, similar to the values that range from 0.333 :g g^{-1} to 138 :g g^{-1} in six samples of sea lion blubber (Fig. 1.8). Liver concentrations ranged from below MDL to 679 :g g^{-1} in harbor porpoise and sea lion, encompassing the range found in harbor seal livers (Fig. 1.8). Concentrations in sea lion brains were consistently low (< 1.29 :g g^{-1}), similar to harbor seal brains.

Pristane concentrations ranged from 37.3 :g g^{-1} to 628 :g g^{-1} in killer whale blubber, but were only 5.26 :g g^{-1} in the single sample of liver (Fig. 1.8). Blubber contained 8.97 :g g^{-1} and 8.02 :g g^{-1} in single samples from grey and minke whales, and liver concentrations ranged from 0.136 :g g^{-1} to 3.58 :g g^{-1} 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 :g g^{-1} in fat or liver (Fig. 1.8), and

was detected only once in 55 sea otter blood samples at 0.179 :g g^{-1}.

Pristane concentrations were 1.03 :g g^{-1} or less in 45 samples of feces from brown bears collected from the Alaska Peninsula during spring, and was 0.684 :g g^{-1} in a single liver sample collected (Fig. 1.8). Concentrations of pristane were below MDL in five samples of Sitka black-tailed deer livers (Fig. 1.8).

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 (Blumer et al. 1964), indicating that Pacific Ocean species of both *Calanus* and *Neocalanus* possess the ability to biosynthesize pristane from ingested chlorophyll. Pristane concentrations in stage V copepodites of Atlantic Ocean species *C. finmarchichus*, *C. glacialis*, and *C. hyperboreus* ranged from 4,500 – 9,200 :g g^{-1}, 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 :g g^{-1}, and also do not vary seasonally from spring through summer nor geographically from southeast Alaska to PWS, a distance of ~ 500 km (Table 1.1). Although the sample sizes of copepods used to characterize the pristane concentrations in these species are small, the concentrations are probably typical of the respective populations in the regions sampled, at least for the Pacific Ocean species. The mean pristane concentration of mixed stages IV and V *C. marshallae, N. flemingerii,* and *N. plumchrus* collected from PWS during spring with a 505 :m-mesh plankton net was 7,490 ∀ 3,250 :g g^{-1}. This mean concentration was derived from twelve plankton net tows, each containing several thousands of these copepods (see Ch. 2). The general agreement of the mean pristane concentration of the zooplankton tows and the results for the constituent individual zooplankters indicates the concentrations measured in the individual zooplankters are not anomalous, but typical of the populations sampled. Also, 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 weak oxidizers such as formaldehyde.

Members of both *Neocalanus* and *Calanus* inhabiting high latitudes of the northern hemisphere accumulate substantial lipid reserves for sustenance during prolonged periods of diapause and for subsequent reproduction (Conover 1988). Their phylogenetic and life-history similarities suggest that *Neocalanus* copepods may 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 gut, but in either case the increasing pristane concentration with copepodite life stage in *N. plumchrus* and *C. marshallae* (Table 1.1) 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.

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 have been 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, for example, *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 GOA 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 a large proportion 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 are generally coherent with the production of pristane by *Calanus* and *Neocalanus* copepods. *Calanus* and *Neocalanus* copepods may account for ~ 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} sea surface (assuming a wet:dry weight ratio of 6.1 and a pristane concentration of 0.6%: 0.15 g m^{-3} x 50 m x $(6.1)^{-1}$ x 0.006 = 7.4 mg pristane m^{-2}). Averaged over the 8,800 km^2 surface area of PWS, this implies production on the order of at least 65 tons of pristane annually by these copepods. In comparison, this about the same as the amount of pristane introduced into PWS by the crude oil spilled from the T/V *Exxon Valdez* (assuming a spill volume of 43,000 m^3 (Wolfe et al. 1994), an oil density of 0.87 kg l^{-1}, and a pristane concentration of 1.89 g kg^{-1} oil (Wang et al. 2003) leads to 43,000,000 l x 0.87 kg l^{-1} x 1.89 g kg^{-1} = 71 tons). That other annual inputs of anthropogenic petroleum products to PWS are much smaller than the amount introduced by the EVOS is confirmed by the very low concentrations of petroleum hydrocarbons in mussels analyzed for monitoring seawater quality there (Payne et al. 2003). Hence, neglecting catastrophic oil spills, PWS is inoculated annually with a substantial dose of pristane generated almost entirely by *Calanus* and *Neocalanus* copepods, which then permeates the marine food web through trophic transfers involving predation, and by fecal production associated with these transfers or with the copepods themselves.

Distribution of Pristane in the Marine Food Web of the Northern Gulf of Alaska
The large number of species, tissues and samples analyzed for hydrocarbons following the

EVOS is by far the most comprehensive chemical survey of a marine ecosystem for the distribution of hydrocarbons among the constituent biota. Other oil spills before or since the EVOS (e.g. the Amoco Cadiz, CNEXO 1981), or pre-development surveys of marine biota potentially at risk from offshore oil and gas exploration (e.g. Johansen et al. 1977) have had far fewer resources available for the chemical analysis of affected biota, and large-scale monitoring programs for hydrocarbons in marine biota (e.g. the US benthic surveillance program, Lauenstein et al. 1993) are limited to one or a few species. The exceptional breadth of sampling for the EVOS has the potential of providing an especially detailed portrayal of the distribution of pristane in the food web, provided that proper allowance is made for certain problems regarding sample comparability and interpretation. Comparison of the results from the EVOS is problematic because of the sampling inconsistencies among the species, tissues and seasons. None of these samples were collected randomly, so the results are probably biased from multiple sources, and the magnitude and direction of these biases are unknown. Also, the sample sizes were very small (< 5) for many of the species and tissues examined. Nonetheless, even a non-random survey of the distribution of pristane can provide a useful indication of the approximate variability of the biota and the tissues sampled, of the most dominant trends regarding biomagnification or dissipation across trophic levels, and might also provide evidence suggestive of unexpected distribution pathways.

Equilibrium thermodynamics provides a basis for deriving expected distributions of lipophilic organic contaminants such as hydrocarbons among components of food webs (e.g. Clark et al. 1990). In this approach, contaminant concentrations in all the discrete chemical phases of the ecosystem, including the lipid and aqueous compartments of the biota, and the respired media (i.e. water and air) are assumed to be at a state of chemical equilibrium, implying no inherent tendency of these concentrations to change with time. Observed deviations of concentrations from these expected values are then taken as evidence of (1) non-equilibrium conditions, which may result from kinetic limitation of contaminant transport among phases, (2) active transport, where biologically mediated processes may enhance or suppress rates of contaminant transport, or (3) metabolic transformation of the contaminant, which usually involves biochemical detoxification pathways to promote the removal of the contaminant from an organism. All three of these must be considered in the distribution of pristane among biota of the northern GOA.

The basic thermodynamic parameter used to describe the distribution of a contaminant among immiscible phases is the partition coefficient. The partition coefficient is the ratio of fugacity capacities of a contaminant in two phases (fugacity capacity is the ratio of a volatile solute's concentration in a solvent and its vapor pressure; Kelly et al. 2004), and ideally is determined in the all of the phases of interest. Such determinations are often difficult in practice, and an approximate measure of the partition coefficient, the octanol-water distribution coefficient, K_{ow}, is often used as a proxy (Kelly et al. 2004). The octanol-water distribution coefficient for a contaminant is the ratio of the contaminant concentration in *n*-octanol and water (i.e. $K_{ow} = [X]_{octanol}/[X]_{water}$; [X] = contaminant concentration). This coefficient may then be used to predict the approximate distribution of a contaminant within an organism or among organisms within a contaminated environment, and is usually discussed in terms of its common logarithm (i.e. log

K_{ow}). In particular, the log K_{ow} provides a convenient and standardized measure of the tendency of a contaminant to bioaccumulate, and to biomagnify within a food web. Bioaccumulation refers to the increased contaminant concentration within an organism that results from the passive partitioning of the contaminant between the aqueous phase comprising the water compartment and the respired medium of an organism (where concentrations are assumed equal), and the lipid compartment of the organism. Hence, the log K_{ow} provides a measure of the expected contaminant concentration in the lipid compartment of the organism for a given aqueous concentration, which may be compared with observed concentrations when expressed on a lipid mass basis (i.e. lipid-normalized concentrations). For example, a contaminant with a log K_{ow} value of five would be expected to attain a lipid concentration 100,000-fold greater than the ambient aqueous concentration, and if the lipid content is 1% of the organism wet weight, then the whole-body concentration of the contaminant would be reduced to 1,000-fold greater than the ambient aqueous concentration.

Biomagnification may occur when an organism accumulates a contaminant through its diet, and the affinity of the contaminant for the lipid phase is sufficiently great that diffusion losses of the contaminant back to the ambient aqueous phase are outweighed by the rate of accumulation. Biomagnification also implies that the rate of metabolic transformation of the contaminant by the organism is negligible, and that the organism is able to somehow concentrate the contaminant above the lipid-normalized concentrations present in its prey, the details of which remain unclear (Kelly et al. 2004). It has been found empirically that non-metabolizable contaminants having log K_{ow} values exceeding five tend to biomagnify in food webs (Kelly et al. 2004).

The log K_{ow} value of pristane has not been reported, but an approximate value may be inferred from uptake experiments by marine mussels, which have little capacity to metabolize hydrocarbons (Lee et al. 1972, Mironov and Shchekaturina 1979). The whole-body bioaccumulation factor of pristane (i.e. the ratio of the somatic concentration and the dissolved aqueous concentration) in mussels (*Mytilus trossulus*) is about 2,000 (Murray et al. 1991, and Ch. 2, this report). Assuming a lipid concentration of 1% of wet weight (Kluytmans et al. 1975), the lipid-normalized partition coefficient would be on the order of 200,000, approximately equivalent to a log K_{ow} value of 5.3. This value is near the threshold for biomagnification, provided metabolic transformation is negligible. This implies that pristane incorporated by an organism would eventually become distributed among its constituent tissues at approximately equal concentrations per unit lipid. However, the ability to metabolize pristane has been demonstrated in fish (Cravedi et al. 1985, Le Bon et al. 1987, 1988a) and in the rat (Tulliez and Bories 1975, Le Bon et al. 1988b), so it is likely this capability is widespread among vertebrates, and might be present among some invertebrates also. Hence, pristane would not be expected to biomagnify appreciably in a marine food web, because of its low inherent tendency based on its partition coefficient value, and because of the ability of most organisms occupying the upper trophic levels (i.e. the vertebrates) to metabolize it.

The foregoing discussion provides a framework for interpreting the observed distribution of pristane among the biota analyzed for the EVOS. Clearly, the expected distribution of pristane

among tissues within an organism, or among different organisms should account especially for variation in the lipid content of the sampled tissues, the metabolic capability of the organism to transform pristane, and kinetic limitations on the transport of pristane among the sampled compartments. The breadth of sampling conducted after the EVOS provides an opportunity to examine whether the results are consistent with expectations based on equilibrium thermodynamics at both the organism and the ecosystem levels, and the extent to which processes such as transport rate limitation and metabolic degradation must be invoked to explain discrepancies from those expectations.

At the organism level, the distribution of pristane in harbor seal tissues provides an example of how the variability of pristane concentrations may be considerable among different tissues and among the individuals sampled. Among individuals, the more than 500-fold variation in pristane concentrations in blubber (Fig. 1.7), which is almost entirely lipid, is probably the result of differences in diet, and the time elapsed since ingestion of pristane-laden prey. Although harbor seals may prey on a wide spectrum of fish, cephalopods and crustaceans, especially pollock and octopus (*Octopus sp.*), with smaller but substantial reliance on capelin (*Mallotus villosus*), eulachon (*Thaleichthys pacificus*) and Pacific herring (Pitcher 1980), they tend to forage site-specifically, taking prey that are readily available within a home range of a few tens of km (Iverson et al. 1997). Seals that prey on species that are more closely connected trophically to *Neocalanus* or *Calanus* zooplankton would be expected to consume a higher ration of pristane, and these seals would tend to have higher concentrations of pristane in their lipid tissues, just as Atlantic Ocean herring contain much more pristane than Baltic Sea herring (Linko and Kaitaranta 1976) owing to the scarcity of *Calanus* copepods in the Baltic Sea (Hernroth and Ackefors 1979). Once incorporated into storage lipids or into lipid-rich cell membranes, pristane may remain unavailable to metabolic degradation if the rate of lipid or cell turnover is low. For example, somatic tissues of rats and of rainbow trout fed single doses retain traces of pristane for weeks, despite metabolic transformation and elimination of up to 99% of the absorbed pristane (Tulliez and Bories 1975, Cravedi and Tulliez 1982, Le Bon et al. 1988b), almost entirely by liver enzymes (Cravedi et al. 1989). Some of the variability of pristane concentrations among comparable tissues of different individuals may be attributed to differences in the efficiency of pristane-degrading activity among individuals, but coefficients of variation on the order of 25% in radioisotope studies of the pristane degradation rate in rainbow trout (Le Bon et al. 1987) and the rat (Le Bon et al. 1988b) indicate this is a relatively minor source of variability in comparison with dietary variability and differences in lipid content. Metabolic competence to degrade pristane therefore does not preclude retention at slowly-declining concentrations in lipid-rich tissues on a time scale of weeks.

Much of the additional variation of pristane concentrations across different tissues of the harbor seal may be the result of differences in lipid content. Whereas adipose tissue is almost entirely composed of lipid, the (dry weight) lipid content of the heart and liver of harbor seals is on the order of 10 – 30% (Ackman and Hooper 1974). Expression of pristane concentrations in the lipid of these tissues would likely reduce the variability within each tissue type, and make concentrations among different tissue types more similar, with the noteworthy exception of the

brain. The low concentrations of pristane in the blood of harbor seals (as well as of bald eagles) is likely the result of the ready availability of pristane in blood to pristane-degrading enzymes in the liver and the low lipid content of the dry matter of blood. The even lower concentrations of pristane in harbor seal brain (Fig. 1.7) is probably the result of exclusion by the blood-brain barrier, which limits diffusion of molecules larger than glucose from the blood to brain tissues (Newsholme and Leech 1983), effectively excluding fatty acids and pristane. This is noteworthy because brain tissue contains about 10% lipid on a wet weight basis (Folch et al. 1951), equivalent to about 40% lipid on a dry weight basis given that the ratio of dry and wet weights of harbor seal brain tissues sampled averaged 0.24. Despite the substantially greater proportion of lipid in brain compared with blood on a dry weight basis, the blood-brain barrier effectively excludes pristane from brain tissue.

Although the variability in the distribution of pristane among harbor seal tissues and individuals may be quite large (Fig. 1.7), detection of pristane in lipid-rich tissues of an organism may still be taken as evidence of a trophic relation to *Neocalanus* or *Calanus* copepods in marine ecosystems such as the northern GOA, where other sources of pristane are probably negligible. The converse may not necessarily be true, however, because of this large variability. Low concentrations in an individual may be the result of especially active metabolism for pristane degradation, or a diet that has weak trophic connection to *Neocalanus* or *Calanus* copepods. Variation in the lipid content among tissues sampled is also an important factor to consider (e.g. Fig. 1.7). Across species, lipid content may vary by a factor of 20 among whole-body samples of fish and shellfish in this region (Iverson et al. 2002). But giving these caveats due consideration, pristane concentrations would be expected to generally decrease in species that can metabolize it, and be relatively low in species or tissues that have low lipid content, but otherwise should remain about the same following a trophic transfer. The pristane distribution patterns depicted in Figures 1.2 – 1.8 broadly reflect these expected trends.

The collective results from the EVOS samples shows that 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 than 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. 1.2), herring and juvenile pink salmon among the fish (Fig. 1.3), and *Euchaeta elongata* and *Metridia spp*. among the zooplankton (Table 1.1). These species may all prey directly on *Calanus* and *Neocalanus* copepodite stages, and all had pristane concentrations of several hundred :g g^{-1}. Pristane concentrations are lower by an order of magnitude 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. 1.2), and the piscivorous marine mammals (Fig. 1.8). In contrast with these neritic species, pristane concentrations are usually much lower in species associated with the intertidal.

Part of the decline of pristane concentrations in the direct consumers of *Calanus* and *Neocalanus*

copepods is because of their lower lipid content. For example, lipid accounts for ~ 1% of the wet weight of juvenile pink salmon and 3 – 10% of Pacific herring in PWS (Iverson et al. 2002). Assuming 80% water content, these wet weight proportions would be equivalent to corresponding dry weight proportions of ~ 5 – 50% for these two species, compared with lipid proportions of the copepods of 50% or more (Båmstedt 1986, Duesterloh 2002). Hence reduction by factors of up to ~ 5 in wet weight pristane concentrations in these two consumer species may be attributed to their lower lipid content, and the remainder of the reduction is probably the result of metabolic degradation. Corrections for differences in lipid content among the other species and tissues sampled are comparable in magnitude because of similar ranges of lipid and water contents, suggesting that metabolic degradation by vertebrate consumers may be the most important factor accounting for the progressive declines of pristane concentrations in the higher-order consumer groups depicted in the pelagic/neritic compartment in Fig. 1.9.

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 :g g^{-1}. 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. 1.2). 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.

Although plausible, direct predation by mussels on *Calanus* or *Neocalanus* copepodites is not likely to be a major pathway of pristane accumulation by mussels. While 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 concentrations on the order of 500 :g g^{-1}. This is much higher than tissue concentrations found in mussels during spring (Fig. 1.5). Juvenile pink salmon prey heavily on *Neocalanus* copepodites during spring in PWS (Cooney et al. 1981, Sturdevant et al. 1996, Willette 1996, Willette et al. 2001), where they remain close to the shoreline during the first few weeks of their marine residence (Healey 1980, Cooney et al. 1981, Willette 2001). 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.

Feces produced by birds preying on nearshore zooplanktivores such as juvenile pink salmon which in turn prey directly on *Neocalanus* copepodites is another plausible but probably minor route for pristane incorporation by mussels. The highest pristane concentrations in juvenile pink salmon (Fig. 1.3) were more than an order of magnitude lower than concentrations in *Neocalanus* copepodites (Table 1.1), and concentrations in feces produced by birds preying on these juveniles would be lower by another one or two orders of magnitude because most of the pristane ingested by birds would be assimilated along with the ingested lipids. Hence, fecal concentrations of pristane produced by birds would likely be 10% or less compared with pristane concentrations in feces of zooplanktivorous fishes, but this route might still be important in some instances if aggregations of nearshore fish feeding on *Neocalanus* copepodites are attacked by aggregations of piscivorous birds.

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 (Figs. 1.2, 1.4, 1.6 and 1.8). The somewhat elevated (~ 1 – 10 :g g^{-1}) 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 :g g^{-1}, 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. 1.6). Hence, egg deposition is another route by which pristane may be introduced to the intertidal.

The very variable concentrations of pristane in benthic foragers (crabs and shrimp) may reflect occasional opportunities to prey on *Calanus* and *Neocalanus* copepods directly. Pristane concentrations in spot shrimp eggs and Dungeness crab hepatopancreas were substantial in the fall (Fig. 1.6), when *Calanus* and *Neocalanus* copepods descend to deep (> 300 m) waters during diapause (Fulton 1973, Damkaer 1977, Conover 1988, Miller and Clemons 1988, Miller 1993). The continental shelf and most of PWS is shallower than 300 m, so these copepods may encounter the benthic interface instead, where they may be vulnerable to predation by benthic foragers. 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 (Fig. 1.5) provides additional evidence that *Calanus* and *Neocalanus* copepods are the dominant source of pristane in the region sampled. 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 provides strong evidence 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 more than a thousand-fold (Murray et al. 1991, and Ch. 2, this report).

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^{-1} 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.

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. 1.2), with concentrations more comparable with piscivorous birds. The source and route of pristane to livers of these two bird species during winter is unclear.

The dissipation of pristane in biota following production during spring is consistent with expectations based on its relatively low log K_{ow} value and its biochemical transformation by organisms that are metabolicly competent to degrade it, with time scales for its dispersion and removal from the food web of weeks to months. Pristane dissolved into seawater may be photo-oxidized (Rontani and Giusti 1987), or may be degraded by the microbial community (Pirnik 1977, Schaeffer et al. 1979, 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).

The nearshore food web of the northern GOA may be roughly represented by springtime pristane

concentrations in the sampled biota (Fig. 1.9). This representation is consistent with documented trophic relationships (Table 1.2). 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. The decline at each trophic transfer may be attributed to (1) metabolic degradation in the livers of these predators, and (2) dilution by consumption of other prey that have less dependence on carbon derived from *Calanus* or *Neocalanus* copepods. 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.

Uses of Pristane as a Tracer Molecule

The trophic distance from *Calanus* or *Neocalanus* copepods as depicted in Fig. 1.9 is not the same as the absolute trophic level of a species in the food web. For example, both Pacific herring and sea otters occupy almost identical trophic levels (3.1 and 3.2 respectively, Okey and Pauly 1998) in the northern GOA, but these two species have very different trophic distances from *Neocalanus* copepods (Fig. 1.9). This is because these two species rely most heavily on different parts of the food web. Pacific herring are zooplanktivorous, and prey directly on *Neocalanus* copepods which are at trophic level 2, whereas sea otters rely most heavily on inter- and subtidal shellfish, especially suspension-feeding clams, which are also at trophic level 2 (Okey and Pauly 1998). Hence, the scheme depicted in Fig. 1.9 may serve to complement analysis of trophic level based on mass-balance considerations (Okey and Pauly 1998) or stable nitrogen isotopes (Lajtha and Michener 1994, Kline and Pauly 1998) by providing an indication of the food-web branch occupied by a species, but cannot be used as an alternative method for determining the absolute trophic level.

Despite the various factors that contribute to the variability of pristane in these biota, the coherence of the distribution of pristane in the sampled biota of the northern GOA as depicted in Fig. 1.9 suggests that detection of pristane may serve as a reasonably reliable indicator of trophic distance from *Calanus* or *Neocalanus* copepods in this region, provided confounding anthropogenic sources of pristane can be confidently discounted. Obviously, further work will be necessary to establish the magnitude of these linkages and their ecological importance more clearly, including at minimum better assessments of variability of pristane concentrations among individuals on a lipid-normalized basis, better estimates of the persistence of pristane in adipose tissues of species that can metabolize it in their livers, and elucidation of the details of the fecal transport pathways depicted in Fig. 1.9.

Inclusion of pristane might lead to substantial improvements in current methods based on lipid analysis for elucidating dietary dependencies of marine predators, for separating breeding stocks of marine fishes, and as a proxy measure of marine carbon imported with anadromous fishes to terrestrial food webs. Provided the variability of metabolic activity for pristane degradation is comparable to variability in the transformation of ingested fatty acids, pristane may be a useful addition to the lipids analyzed for inferring the dietary dependencies of harbor seals (Iverson et al. 1997, 2002) and other marine vertebrates (Iverson et al. 2004), at least in food webs where

Calanus or *Neocalanus* copepods are important.

To the extent that the variability of pristane in the blubber of harbor seals reported here (Fig. 1.7) is due to differences in diet among the individuals sampled, inclusion of pristane may enhance the power of such efforts to distinguish actual dietary differences. Elucidation of these dietary dependencies may in turn help to identify habitat dependencies that are important for the viability of the populations studied. The large difference in pristane concentrations of herring oils from the Baltic and North Seas (Linko and Kaitaranta 1976) indicates that pristane analysis may be helpful in distinguishing stocks of herring and perhaps other fishes, which may be of considerable use in the management of commercially exploited species. Available evidence indicates 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, and a similar approach might find useful application to other terrestrial mammals associated with anadromous streams.

Pristane might also serve as a useful proxy of carbon subsidy from fecal material to marine benthic communities in regions where *Neocalanus* or *Calanus* copepods are important in the pelagic food web. In addition, 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 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 *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 resists degradation but it is not as refractory as halogenated organic pollutants, and does not magnify through the food chain. Fish and rats are capable of transforming pristane to more excretable metabolites,

which suggests that other vertebrates are also. Despite this capability, pristane accumulates in lipid storage compartments of vertebrates and may persist at least for weeks. Pristane concentrations tend to decline with the number of trophic transfers among subsequent consumer species, because of its low inherent tendency for food-chain biomagnification, metabolic degradation by vertebrate predators, and dilution by alternate prey that are more weakly dependent on carbon derived from *Calanus* or *Neocalanus* copepods. Defecation 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.

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 GOA 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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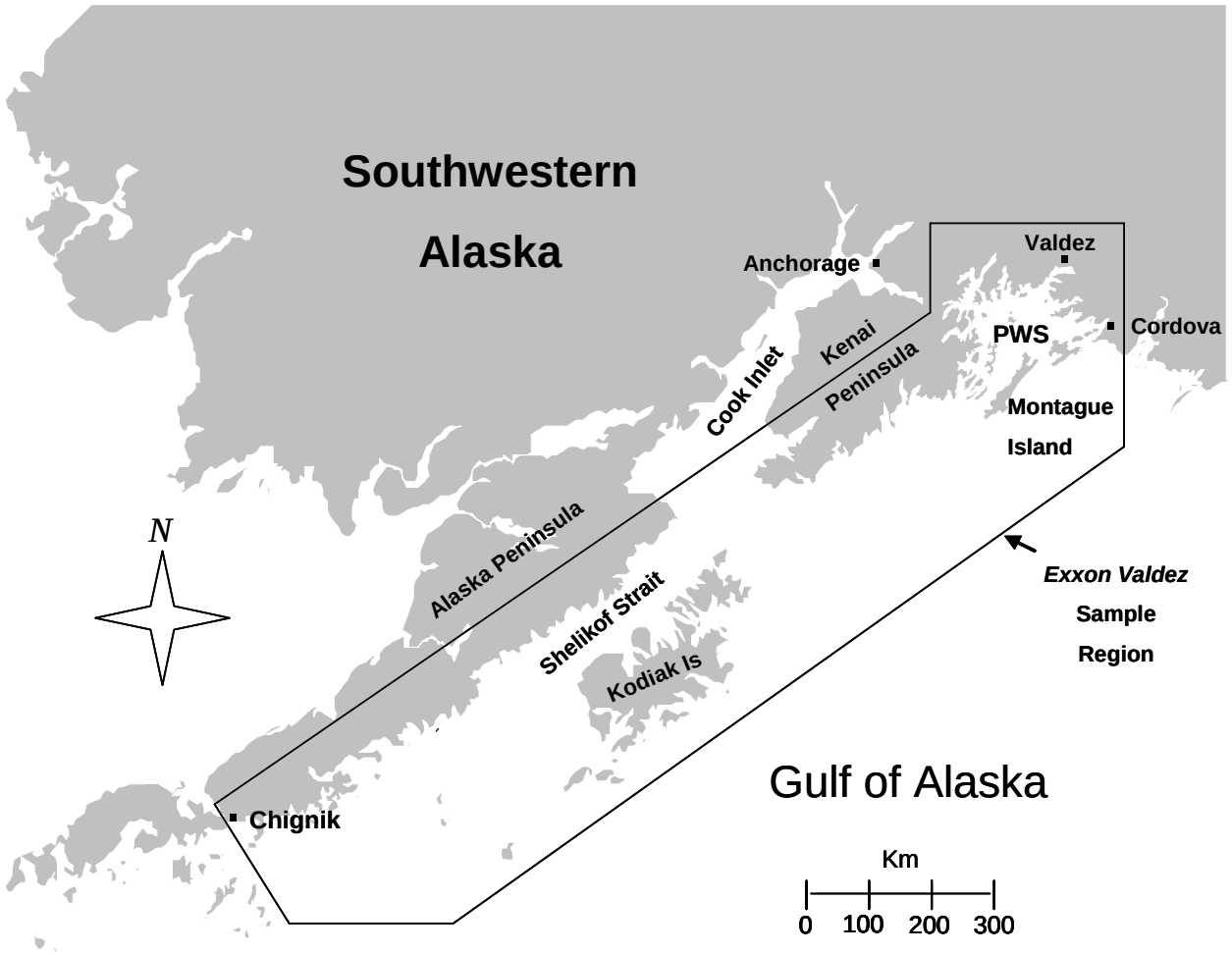

Figure 1.1. Region of the northwestern Gulf of Alaska where biota samples were collected.

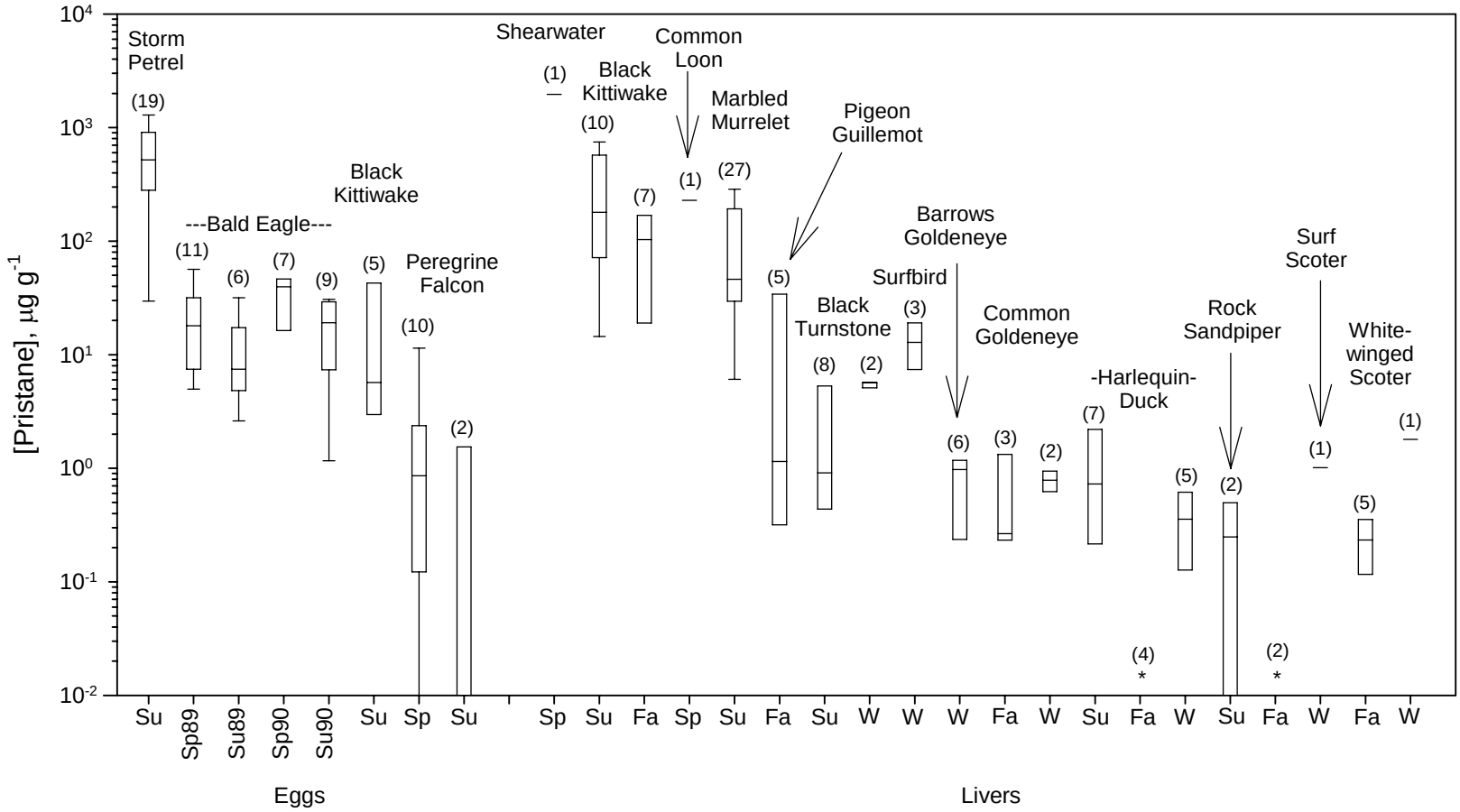

Figure 1.2. Pristane concentrations in eggs and in livers of birds. Number of samples analyzed in parentheses. The horizontal lines indicate the 10th, 25th, median, 75th and 90th percentiles of the distributions. Asterisks indicate concentrations below method detection limits. Sp = spring, Su = summer, Fa = fall, W = winter sampling; "89" = 1989, "90" = 1990.

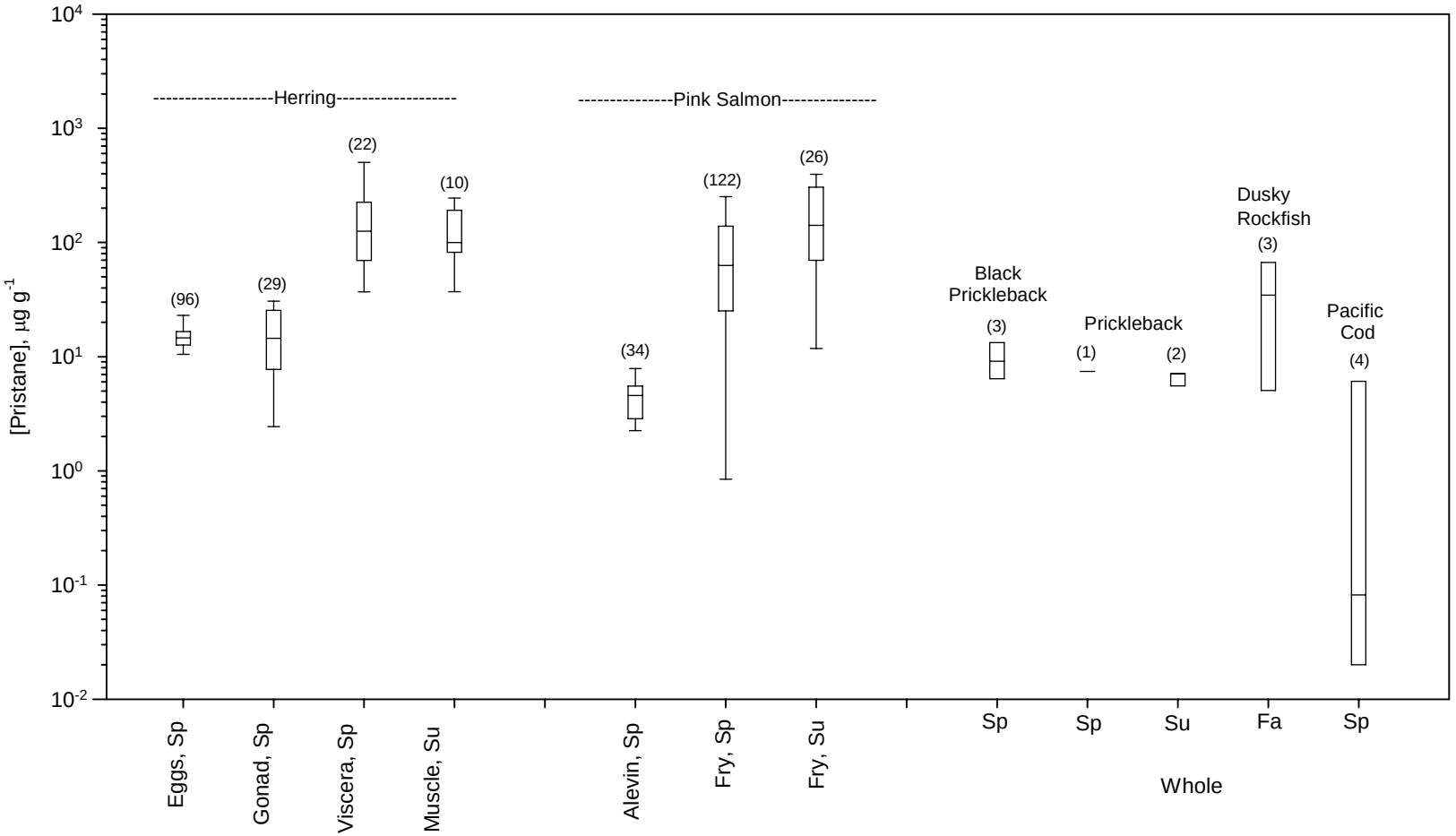

Figure 1.3. Pristane concentrations in tissues of fish. Symbols and abbreviations as in Figure 1.2.

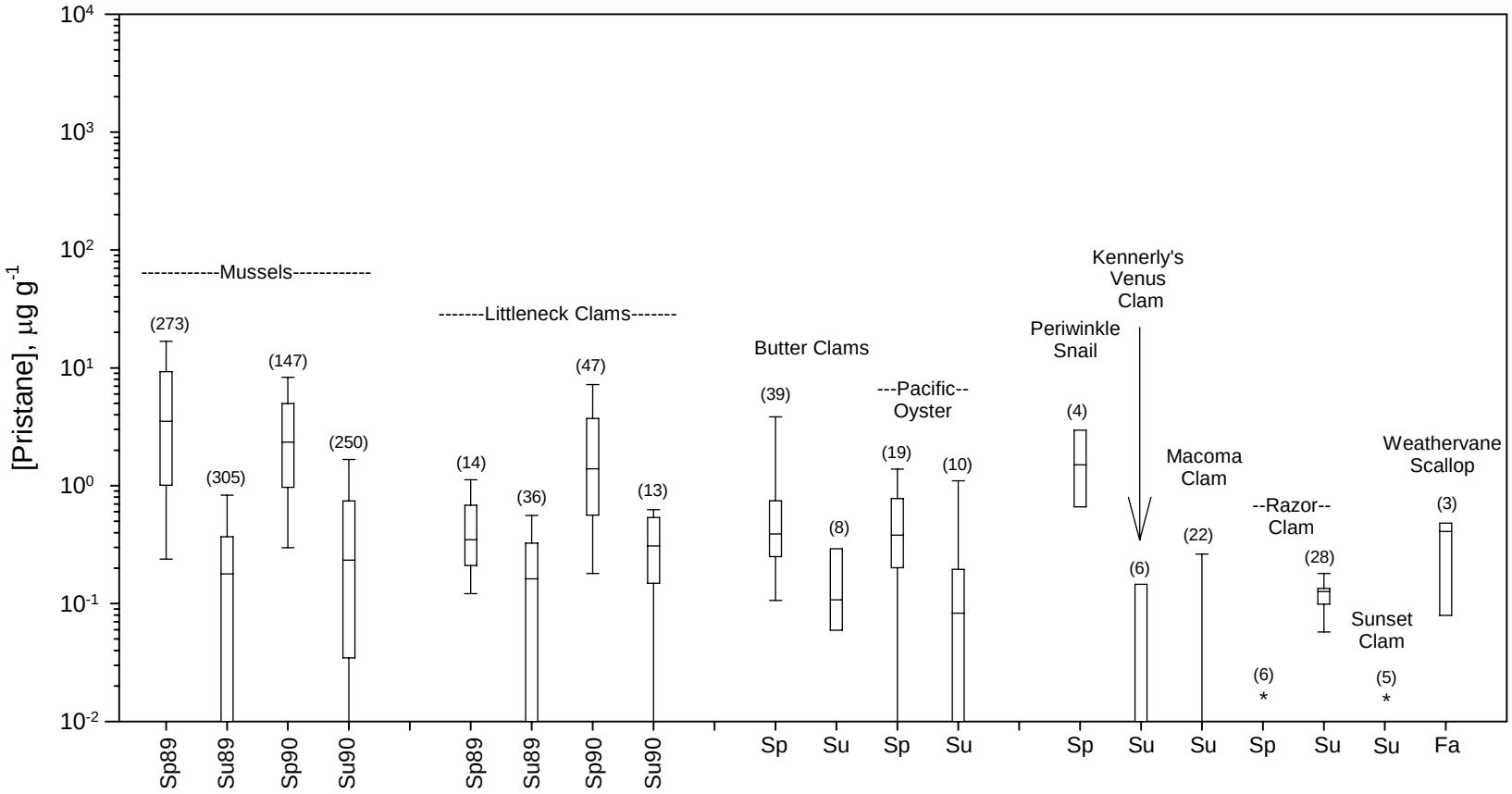

Figure 1.4. Pristane concentrations in molluscs. Symbols and abbreviations as in Figure 1.2.

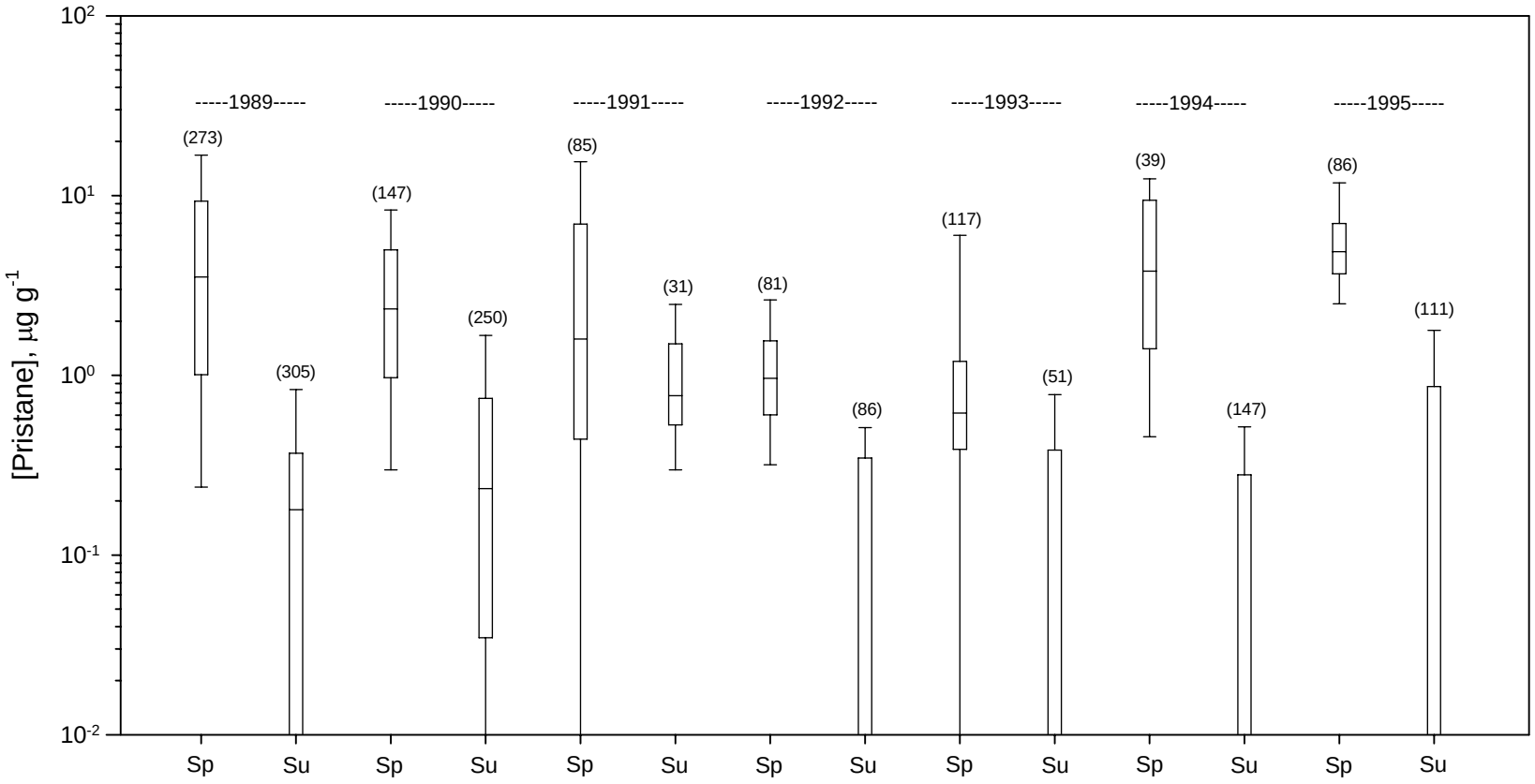

Figure 1.5. Pristane concentrations in bay mussels (*Mytilus trossulus*). Symbols and abbreviations as in Figure 1.2.

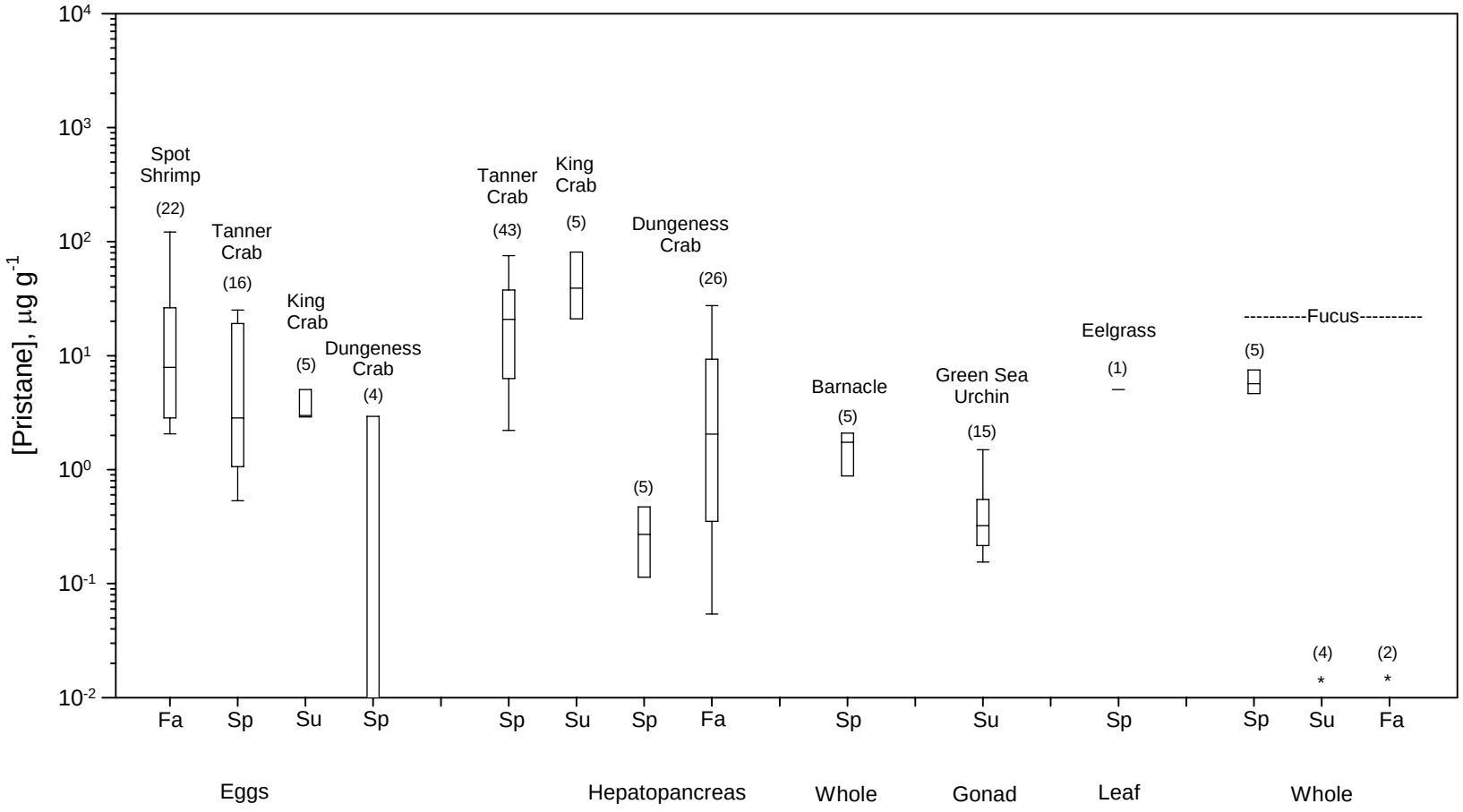

Figure 1.6. Pristane concentrations in crustaceans, sea urchin and plants. Symbols and abbreviations as in Figure 1.2.

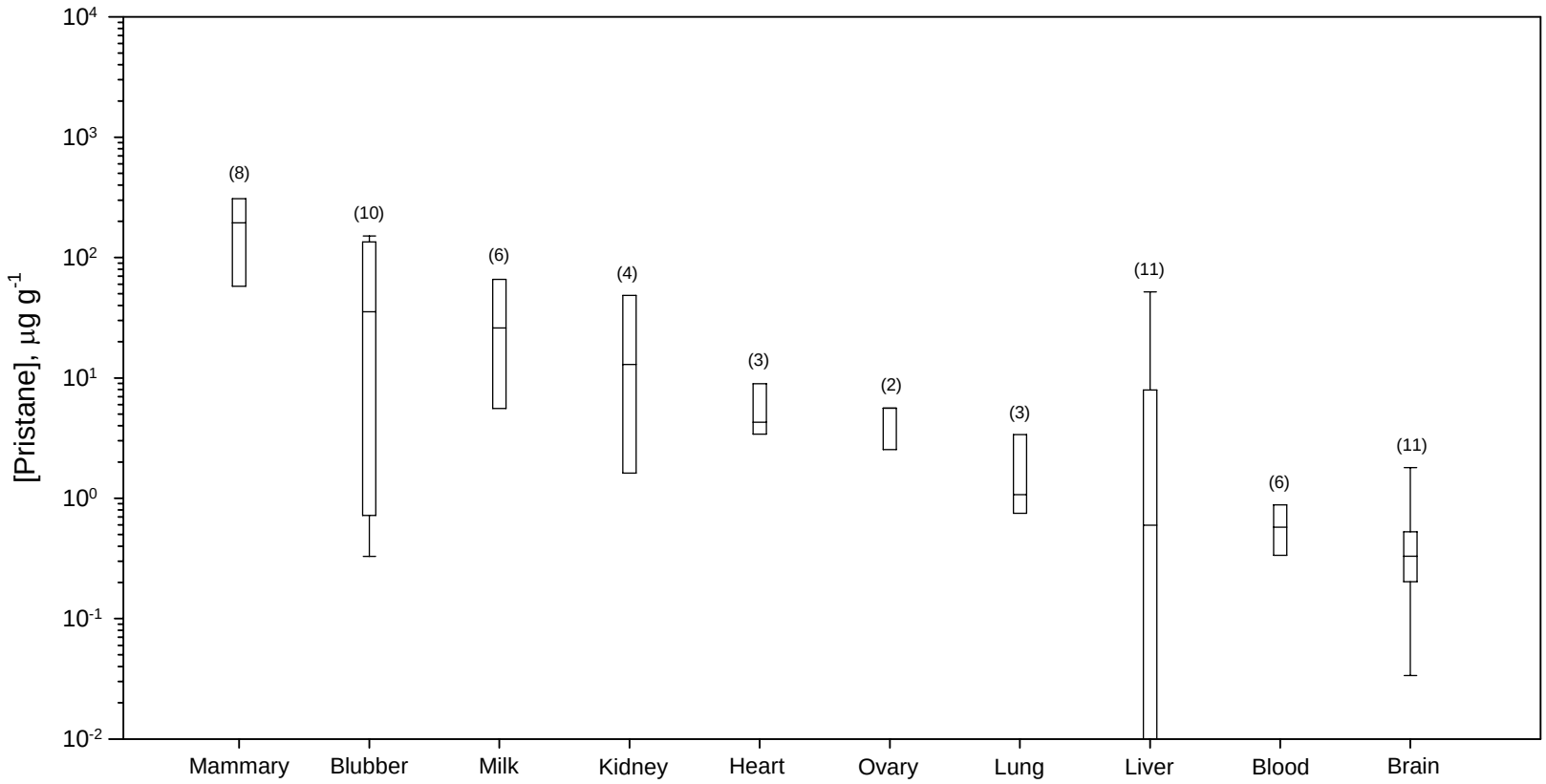

Figure 1.7. Pristane concentrations in harbor seal (*Phoca vitulina*) tissues. Symbols and abbreviations as in Figure 1.2.

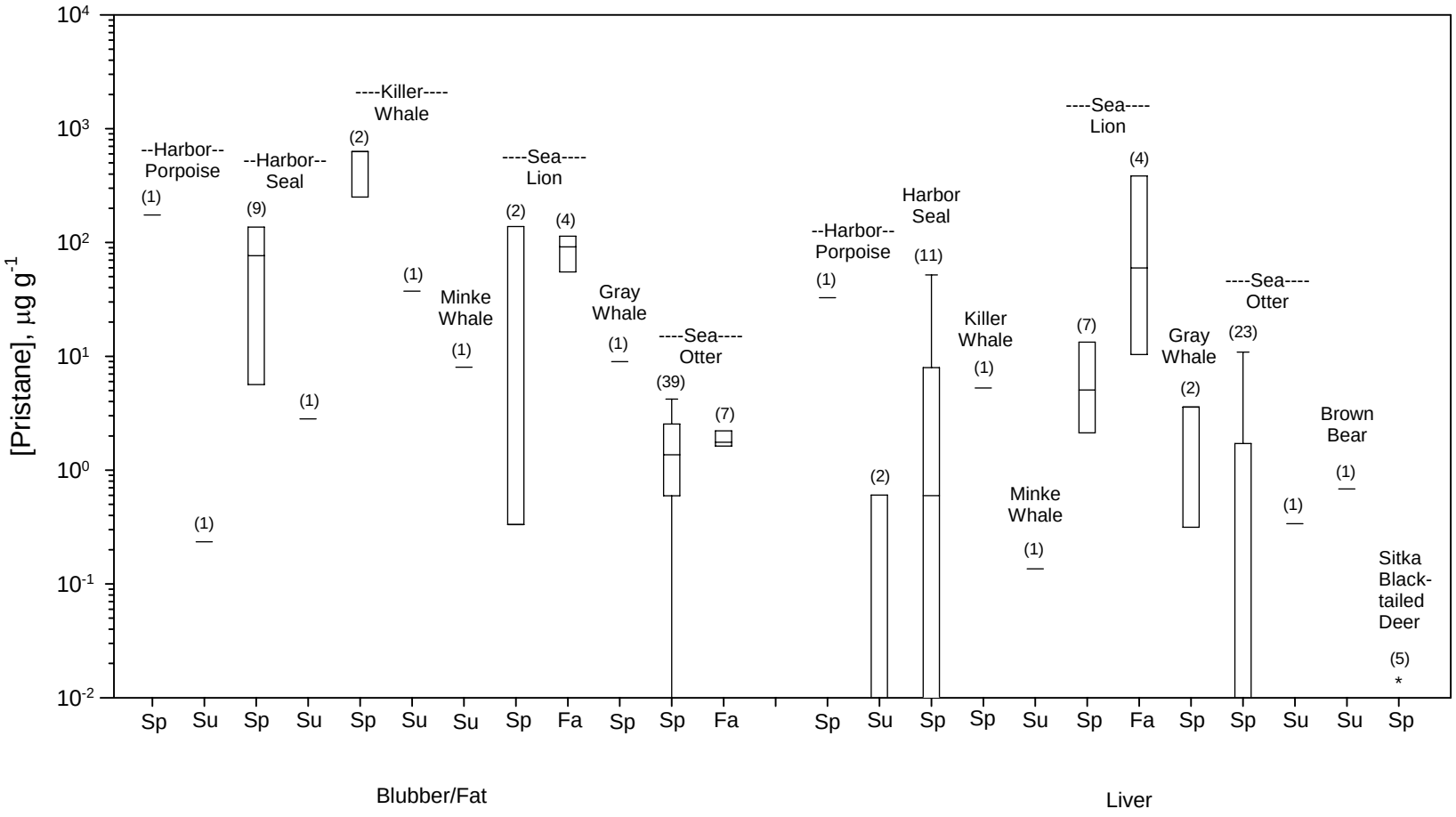

Figure 1.8. Pristane concentrations in mammal blubber and liver. Symbols and abbreviations as in Figure 1.2.

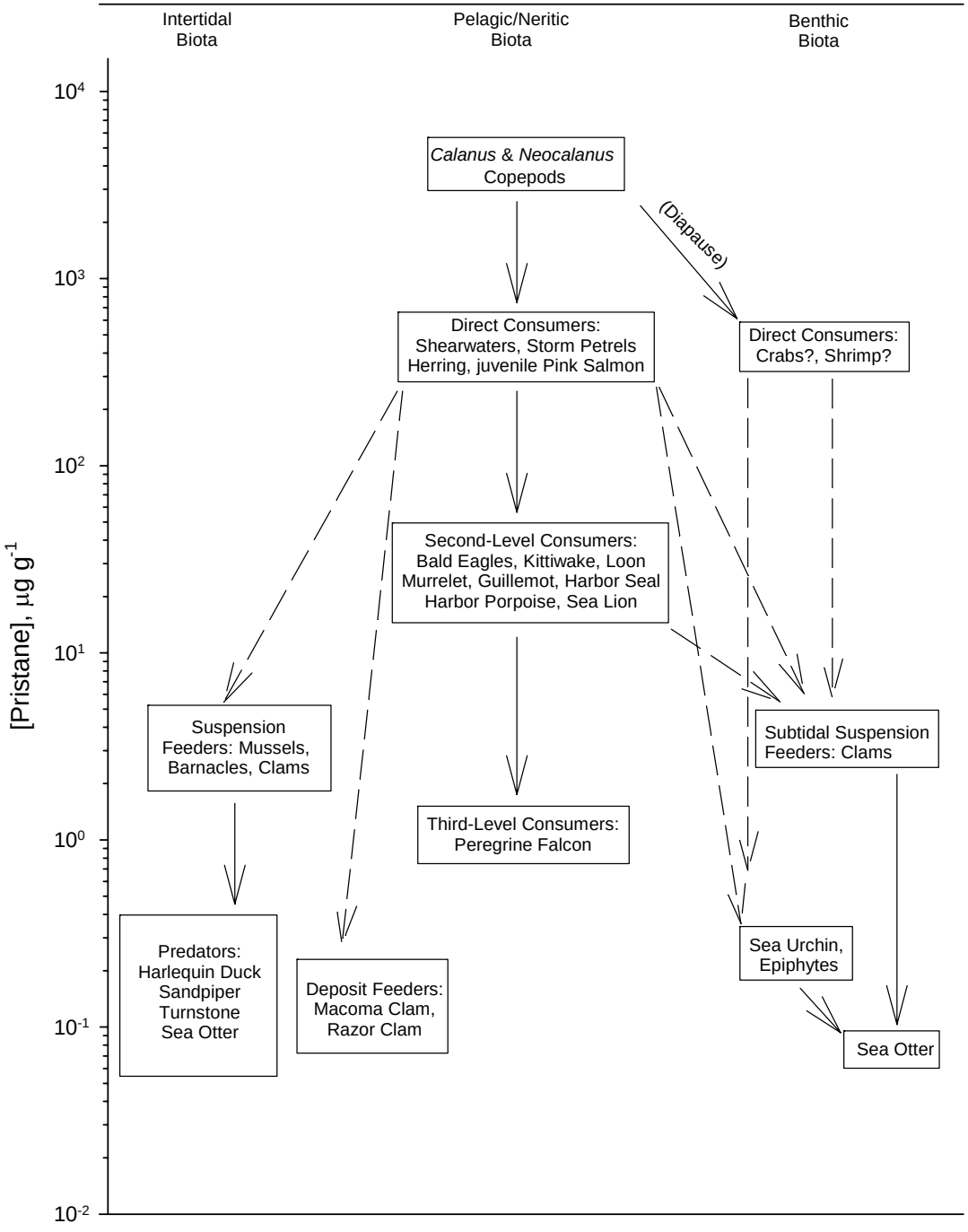

Figure 1.9. Generalized trophic relationships based on pristane concentrations. Solid arrows indicate ingestion of pristane from predation, dashed arrows indicate hypothesized ingestion of pristane associated with fecal material.

Tables

Table 1.1. Concentrations of pristane in zooplankton. PWS = Prince William Sound, SEAK = Southeastern Alaska; Sp = Spring, Su = Summer.

Species	Region	Season	Median [Pristane] (:g g^{-1})	Range (:g g^{-1})	Samples Analyzed n	Individuals/ Sample n
Chiridius spp.	PWS	Su	15.6		1	25
Calanus marshallae – CV and 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 and adult female	SEAK	Su	107	61.6-205	3	1
Euchaeta elongata – CV and 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 and adult female	SEAK	Su	660	509-672	3	1
Metridia lucens – CV and 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 1.2. Common and scientific names and foraging modes of species. Species compared in

this study were sampled during the Natural Resources Damage Assessment effort for the 1989 *Exxon Valdez* oil spill in Prince William Sound, Alaska. Foraging modes: 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 and Sanger 1986
Black-Legged Kittiwake, *Rissa tridactyla*	PV, BI	DeGange and 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 and Sanger 1986
Harlequin Duck, *Histrionicus histrionicus*	BI, II	DeGange and Sanger 1986
Marbled Murrelet, *Brachyramphus marmoratus*	PV, BI	DeGange and Sanger 1986
Peregrine Falcon, *Falco peregrinus*	AV	Ehrlich et al. 1988
Pigeon Guillemot, *Cepphus columba*	PV, BI	DeGange and Sanger 1986
Rock Sandpiper, *Calidris ptilocnemis*	II	Ehrlich et al. 1988
Shearwater, *Puffinus spp.*	PV, ZV	DeGange and 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 and 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 and O'Clair 1998
Butter Clam, *Saxidomus giganteus*	SF	O'Clair and O'Clair 1998
Kennerley's Venus, *Humilaria kennerleyi*	SF	Abbott 1954
Littleneck Clam, *Protothaca staminea*	SF	O'Clair and O'Clair 1998
Macoma Clam, *Macoma balthica*	DF	O'Clair and O'Clair 1998
Pacific Oyster, *Crassostrea gigas*	SF	Abbott 1954
Periwinkle Snail, *Littorina sp.*	AE	O'Clair and O'Clair 1998
Razor Clam, *Siliqua patula*	SF	Abbott 1954
Sunset Clam, *Gari californica*	SF	Abbott 1954
Weathervane Scallop, *Patinopecten caurinus*	SF	Abbott 1954
IV. Crustaceans		
Barnacle, *Balanus cariosus*	SF	O'Clair and O'Clair 1998
Dungeness Crab, *Cancer magister*	BI, PV	O'Clair and 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 and O'Clair 1998
VI. Mammals		
Brown Bear, *Ursus arctos*	TO	McCarthy 1989
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 and 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	

Chapter 2

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*), and to compare growth of these pink salmon with growth of their cohorts fed similar rations of brine shrimp (*Artemia sp.*). The PWS zooplankton used as food contained 7,450 ∀ 3,250 :g g^{-1} pristane (95% CI; dry mass basis), and feces derived from them contained 383 ∀ 72.8 :g g^{-1} pristane. The absorption efficiencies of tissue mass and of pristane by pink salmon fed PWS zooplankton were 74.6 ∀ 8.49% and 98.7 ∀ 0.108%. In comparison, *Artemia sp*. used as a control diet contained 1.24 ∀ 0.597 :g g^{-1} pristane, and pristane was usually not detectable in feces derived from them. The efficiency of tissue mass absorption of *Artemia* was 88.4 ∀ 0.731%. Pink salmon fed *Artemia* grew about four times faster than those fed PWS zooplankton, and gross growth efficiencies of the *Artemia*-fed fish were about three times greater. The difference in growth between the two diets is attributed mainly to growth inhibition by pristane in the PWS zooplankton-fed fish. Mussels accumulated pristane about 52 times faster from dispersed feces than from dissolved pristane. The bioaccumulation factor for dissolved pristane was 2,000, compared with 175,000 for pristane accumulated from feces. Mussels exposed to partially dispersed feces accumulated pristane less rapidly than did mussels exposed to completely dispersed feces. Mussels exposed to dispersed feces derived from *Artemia*-fed fish accumulated readily detectable pristane concentrations from exposure concentrations less than ~ 0.020 :g l^{-1} (parts per trillion). These results indicate that predation by nearshore zooplanktivores on *Calanus* and *Neocalanus* copepodites in PWS, exemplified by juvenile pink salmon, is an important route by which pristane is transferred from these copepodites to mussels.

Introduction

Chemical analysis of lipids may provide useful insights into aquatic food-web dynamics. Biosynthesis of unusual and environmentally persistent compounds may serve as labels for tracing nutrient transport among environmental compartments (Corner et al. 1986). Comparative analyses of food and resulting feces are necessary to determine digestibility and absorption efficiency (e.g., Sargent et al. 1979). One of the first lipids proposed for such purposes is pristane (2,6,10,14-tetramethylpentadecane; 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, and Ch. 1, this report). Pristane is relatively persistent in the environment because it is terminally branched, and hence relatively resistant to ∃-oxidation.

Every spring, bay mussels (*Mytilus trossulus*) in Prince William Sound (PWS), Alaska rapidly accumulate pristane, with concentrations subsiding to background levels by late summer (see Ch. 1, this report). *Neocalanus plumchrus* and *N. flemingerii* have a life span of one year and reproduce at depth in winter, after which the adults (i.e. stage VI copepodites) die. The naupliar offspring develop through five naupliar stages as they rise to shallower waters in late winter, metamorphosing to copepodites in time to begin grazing the incipient phytoplankton bloom in early spring. The copepodites develop rapidly from copepodite stage I to stage V by late spring, then seek deep water to begin diapause by the beginning of summer. In the surface waters of PWS during spring these copepodites may dominate the zooplankton biomass (Cooney 1986a, Cooney 1986b, Kirsch et al. 2000, 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 accumulation of pristane by mussels (Ch. 1, this report). Concentrations of pristane in mussels may increase up to several thousand fold over 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. 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 ~ 0.5 mm, 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 is rare.

Other potentially significant 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. Pristane may diffuse from the lipid compartment of copepodites into the ambient seawater. 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 bioaccumulation factor may be determined from comparison of the uptake and depuration rates of pristane in mussels exposed to a constant ambient concentration of pristane. 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, and Ch. 1, this report). The pristane content of *Neocalanus* copepodite feces has not been reported, but this concentration, when combined with production and sinking rates of copepodite feces, would permit an assessment of the importance of this route of pristane incorporation by mussels. Because of the difficulty of collecting sufficient copepod feces for direct experimental determination of pristane bioaccumulation by mussels, the importance of this route will be evaluated on the basis of a field

observations reported in chapter 3 of this report.

Another less direct but possibly important route of pristane incorporation by mussels may be through ingestion of dispersed feces produced by predators of *Neocalanus* copepodites. Feces of fish fed *Calanus helgolandicus* stage V copepodites contained significant amounts of pristane, although absolute concentrations were not reported (Prahl et al. 1985). Juvenile pink salmon (*Oncorhynchus gorbuscha*) are an abundant zooplanktivore in PWS during spring, although their biomass is less than 1% that of some other zooplanktivores such as juvenile Pacific herring (*Clupea pallasi*) or pollock (*Theragra chalcogramma*) (Okey and Pauly 1998). Wild stocks and hatcheries combined produce over a half-billion juvenile pink salmon annually (Johnson et al. 2002) 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 to avoid predation (Healey 1980, Cooney et al. 1981, Willette 2001), and this behavior may place them immediately above mussel beds during high tides. Juvenile pink salmon prey heavily on copepods, especially *Neocalanus* and *Calanus* in PWS (Cooney et al. 1981, Sturdevant et al. 1996, Willette 1996, Willette et al. 2001). 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 concentrations of pristane on the order of 500 $\mu g\ g^{-1}$. This is much higher than tissue concentrations found in mussels during spring, which are usually less than 20 $\mu g\ g^{-1}$ (Ch. 1, this report). Their feeding habits, nearshore residence, and numbers suggest 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 would facilitate interpretation of the annual spring increase of pristane concentrations in mussels, which might prove useful for indirectly monitoring *Calanus* and *Neocalanus* zooplankton abundances, and perhaps the early marine survival of zooplanktivorous fishes such as pink salmon and herring. Marine survival of pink salmon is thought to be determined during the initial period of marine residence (Parker 1962, Parker 1968, Ricker 1976, Hartt 1980, Peterman 1987, Karpenko 1998, Willette et al. 2001), 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 may promote rapid growth, reducing the period of maximum vulnerability to predation and thus increasing population survival (Parker 1971, Healey 1982a, West and Larkin 1987, Willette et al. 1999, Willette 2001, 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 compare two of the pathways by which pristane can be accumulated by mussels that are amenable to laboratory manipulation. These two pathways are ingestion of pristane-laden feces produced by juvenile pink salmon fed *Neocalanus* copepods,

and absorption of pristane dissolved in seawater. Comparison with a third pathway, involving ingestion by mussels of fecal pellets produced by *Neocalanus* copepods, will be based on a companion field study conducted in PWS during spring (Ch. 3, this report). In the present study, We determined the concentration of pristane in feces produced by juvenile pink salmon fed zooplankton collected during the annual spring bloom from PWS, and then measured the uptake and depuration dynamics of pristane in mussels exposed to these feces or to pristane dissolved in seawater. These measurements supply constraints on the relative importance of dissolved vs fecal-associated pristane as sources of pristane for mussels in PWS. The 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.

The secondary objective is to evaluate the growth efficiency of juvenile pink salmon reared on *Neocalanus* copepodites. Gross growth efficiencies as high as 45% have been assumed for evaluations of the impact of juvenile pink salmon on their zooplankton forage base in PWS (Cooney 1993, Cooney and Brodeur 1998, Boldt and Haldorson 2002), but these may be too high in view of the inhibitory effect of pristane on fish growth reported previously (Luquet et al. 1983, 1984). The feeding experiments reported here provided 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, and the amount of fecal material produced per unit growth, and may also provide insight into the growth dynamics of the juveniles relying on a natural diet.

Methods

Laboratory experiments were conducted by feeding juvenile pink salmon a diet of frozen zooplankton collected from PWS during spring, and exposing mussels to the fecal material produced. The mass of zooplankton consumed and of 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 frozen brine shrimp (*Artemia sp*.) as a control comparison. Mussels were also exposed to pristane dissolved in seawater, to compare the uptake and depuration rates of ingested pristane with those of pristane absorbed passively from seawater. These were all compared with mussels exposed to the same ambient seawater but without addition of any pristane. Pristane accumulation was measured at approximately equal interval ratios in mussels throughout their two week exposure, and during a four week depuration period following exposure.

Experimental Animal Collection

Zooplankters were collected in PWS during 23 – 30 April 1998 over a series of 12 samplings. At each sampling a 505 :m-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 polypropylene ice cube trays with a metal spatula and stored frozen at -20 EC. About 1 g of the drained zooplankton from each sampling was preserved in 5% formalin-seawater for determination of species composition.

Juvenile pink salmon were collected from the inner bay at Little Port Walter on Baranof Island 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 weight of 1.81 ∀ 0.0859 g wet wt. (95% CI, n = 9; sample sizes were usually at least the minimum necessary to achieve meaningful statistical power here and following). Small fish were selected because of the limited amount of zooplankton collected from PWS available for the feeding experiments. These fish were fed a commercial diet formulated for salmon hatcheries (Biodiet), were offered food twice daily and were allowed to feed to satiation. On 16 July, fish selected opportunistically for the feeding experiments were fed twice daily on the zooplankton collected earlier from PWS, or on a frozen commercial *Artemia sp*. fish-food product (Sally's Frozen Brine Shrimp, San Francisco Bay Brand, containing minima of 5.02% crude protein, 0.24% crude fat, 0.29% crude fiber, and 92.5% moisture, and packaged as a 5 mm thick sheet of frozen copepods wrapped in polyethylene packaging material).

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, 600 individuals ranging from 2 – 4 cm shell length were selected opportunistically from the 900 mussels collected, and were distributed sequentially among each of 12 glass pans, resulting in 50 mussels in each pan. 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 EC, and the seawater in the trays was replaced once daily until the fecal exposures began on 20 July. The remaining ~ 300 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. The 31 l seawater used for these experiments was pumped from a depth of 15 m in Auke Bay, with particles larger than ~ 100 :m removed by filtration through sand, allowing passage of most phytoplankton and small zooplankton.

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 sp*. Feces produced by groups 2 and 3 were macerated 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 temperatures ranging from 7.3 – 8.5

EC. Treatment trays were placed within and among the seawater bath containers in a sequence determined using the random number generator on a Hewlett-Packard model 32S II handheld calculator.

Fish in each replicate were offered 7 g (wet wt) of frozen zooplankton from PWS or frozen *Artemia sp*., depending on treatment, at 8 am and again at 8 pm daily, and allowed to feed for 1 h, beginning 20 July through 4 August 1998. The daylength is approximately 17 h at this latitude and time of year. Just prior to feeding, each group of 20 fish was transferred together by dip net to a separate tray for feeding. After feeding, each group was again transferred by dip net to another tray, where they remained until the next feeding period. The fecal material defecated during the previous 11 h was collected from the first tray by filtering the seawater through 202 :m-mesh plankton netting after fish had been removed. Fecal material from the feeding period was collected by pipette and stored in a polypropylene container at -20 EC for pristane analysis and for determination of the ratio of wet and dry weight of the feces. The weight of this material was included with the weight produced during the previous defecation period for measurement of total fecal weight 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 zooplankters were collected by filtration through a 202 :m-mesh net and weighed.

Two reserve pools of 25 and 12 juvenile pink salmon were offered rations of zooplankton from PWS or *Artemia sp*., respectively, that were identical (on a wet weight basis) with the rations offered fish in the experimental replicates. The reserve pool fish were used to replace dead or moribund fish in the treatment groups. When feeding the fish in the reserve pools, zooplankton were first thawed in seawater and then collected with a 202 :m-mesh net to determine the ratio of frozen weight to zooplankton weight recovered. This ratio was used to adjust the tissue weight of frozen zooplankton for water losses caused by freezing and thawing, because this water mass was not available for consumption by fish. The mass of food consumed by fish in the treatment groups was estimated as the difference in the tissue weight of zooplankton offered (adjusted for water losses on thawing), and the tissue weight of zooplankton recovered by dip net at the end of each feeding period.

Pink Salmon Growth and Feeding

For estimating growth rates, juvenile pink salmon were weighed near the middle and again at the end of the experiment 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 weight. Consumption of food altered fish weight significantly, so weighings were conducted at least 6 h after the morning feeding period but before the evening feeding period. We estimated the instantaneous growth rate as $k = t^{-1} \ln(W_f \ W_i^{-1})$, where t is the number of days between weighings, and W_f and W_i are the final and initial masses. Two fish died during the interval between weighings, which were replaced from the reserve pools, and the weight of fish in the treatment was corrected for the difference in weights between the dead and replacement fish. Fish from two of the HF-A replicates were inadvertently mixed on the fifth day of the experiment, so weighings for growth rate determination of these two treatment replicates were based on observations from this day and end of the feeding period, whereas the initial weighings for the other treatment groups

occurred on the fourth day of the experiment (Table 2.1).

Food consumption rations were determined for each feeding period and treatment group replicate as the ratio of food consumed and the estimated weight of fish. Food consumed was calculated as the difference between the mass of food offered at the beginning of each 12-h feeding period, and the mass that remained unconsumed at the end. The weight of fish at the beginning of each feeding period was calculated on the assumption of exponential growth between measurements of fish weights, with $W_{t'} = W_i \exp(kt')$, where $W_{t'}$ is the weight of fish in a treatment group replicate at time t', and k is the instantaneous growth rate calculated as described above.

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 EC. 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. Each experimental treatment comprised three groups as replicates, with each replicate containing 50 mussels. 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 38 l of seawater containing 50 mussels. 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 :g l^{-1} solution of pristane in seawater. This concentration is near the upper limit of concentrations that might be found in natural seawater (Blumer et al. 1964), and was chosen to mimic an environmentally relevant exposure condition. 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 :g 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 throughout the 14-day 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. Four-liter 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.

Dry Weight Determination

The ratio of dry and wet weights of tissue and of fecal samples was determined by drying a weighed sample aliquot at 60 EC 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). The wide variability of this ratio for PWS zooplankton is likely the result of differences in zooplankton composition (especially the proportion of small gelatinous zooplankters), differences in the extent of seawater drainage during collection and differences in rupturing caused by freezing the zooplankton. Because of this variability, conversions involving zooplankton from PWS were batch-specific. Ratios for other sample types were less variable as follows: *Artemia sp.,* 0.0903 ∀ 0.00360 (n = 6); feces from PWS zooplankton, 0.105 ∀ 0.00571 (n = 29); feces from *Artemia sp.*, 0.0718 ∀ 0.0114 (n = 24); mussels, 0.112 ∀ 0.00149 (n = 180). These mean values were used for these respective samples in calculations.

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

Pristane Analysis

The chemical analysis of tissue samples for pristane involved pentane 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, 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 weight 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 0.041 :g l^{-1}.

Data Analysis

Except for the kinetic constants characterizing pristane uptake and depuration by mussels, Student's t-test was used to calculate confidence intervals and for tests of significance between pairs of treatments.

We assumed 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:

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

During depuration, P_{ex} is zero, and eq 1 simplifies to $P = P_{int} e^{-k2(t-14)}$ (eq 3), where P_{int} is the concentration at the end of the 14 day exposure period. We fitted the pristane concentration measurements in mussels simultaneously to eqs 2 and 3 for the uptake and depuration phases of exposure, using the pristane concentration estimated at the end of the exposure period as the value for P_{int} in eq 3 for depuration, and using least-squares error minimization to find simultaneous best-fit estimates for k_1, k_2 and P_{int}.

We 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 estimated value of P at each sampling time in place of each actual data point (subject to the constraint that the result be non-negative), 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.

We also used a bootstrap method to estimate the significance of differences between pairs of depuration constants k_1 or k_2. We calculated the difference between each of the 1,000 iterated estimates of the two *k*'s compared, in the order of the smaller subtracted from the larger of the two *k*'s estimated initially, and counted the proportion that were negative or zero. This proportion is taken as the probability of Type I error (i.e., the significance level).

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 weight 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 1,590 – 16,000 :g g^{-1} (mean 7,490 ∀ 3,250 :g 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 weight recovered after thawing in the reserve trays and the initial frozen weight was surprisingly low and variable, ranging from 0.245 – 0.392 (mean 0.306 ∀ 0.0376, n = 9). Freezing likely disrupted cell membranes allowing loss of cellular contents, which may have included variable amounts of the lipid droplets of *Calanus* and *Neocalanus* copepods. Similar losses of mass on thawing occurred with frozen *Artemia sp.*, where ratios ranged from 0.339 – 0.640 (mean 0.490 ∀ 0.0507, n = 15). The mean pristane concentration of frozen *Artemia sp*. was 1.24 ∀ 0.597 :g g^{-1} (n = 3).

Concentrations of pristane in feces derived from PWS zooplankton ranged from 137 – 660 :g g^{-1} (mean 383 ∀ 72.8 :g g^{-1}, n = 21). Concentrations in feces derived from *Artemia sp.* ranged from < MDL to 30.8 :g g^{-1}, the latter being less than three times the MDL for that sample. These samples had relatively high values of the MDL because of the small sample aliquot sizes (< 20 mg) available for analysis. The mean MDL for these samples was 21.9 :g g^{-1}, and ranged from 9.02 – 81.2 :g g^{-1}.

Juvenile Pink Salmon Feeding and Growth

Juvenile pink salmon grew significantly faster when fed *Artemia sp.* compared with zooplankton from PWS. The mean instantaneous growth rate for the *Artemia*-fed fish was k = 0.0209 ∀ 0.00368 d^{-1} (n = 3), more than four times faster than fish fed zooplankton from PWS (k = 0.00502 ∀ 0.00251 d^{-1}, n = 6; $P < 0.001$) (Table 2.1). 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 ∀ 0.0418 (n = 3) and 0.0657 ∀ 0.0340 (n = 6), respectively. These differences were not the result of differing rations. Fish fed *Artemia sp.* consumed a mean of 10.9 ± 0.561% (n = 3) of their wet weight in (wet) *Artemia sp.* per day, equivalent to 0.988 ∀ 0.0559% of dry *Artemia sp.* per day (Table 2.1). In comparison, fish fed PWS-zooplankton consumed a mean of 7.66 ± 0.554% (n = 6) of their wet weight in (wet) PWS-zooplankton per day, equivalent to 0.931 ∀ 0.0943% of dry PWS-zooplankton per day (Table 2.1). Hence, on a dry weight basis of food consumed, the ingestion rates of *Artemia sp.* and of PWS-zooplankton were nearly identical.

Fish absorbed a greater proportion of *Artemia* than PWS zooplankton. Fish absorbed 88.4 ∀ 0.731% (n = 3) of the *Artemia sp*. ingested compared with 74.6 ∀ 8.49% (n = 6) for the PWS zooplankton-fed fish, a significant ($P < 0.001$) difference. Hence, the *Artemia*-fed fish grew faster in part because they consumed about 5% more food and absorbed it more completely (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.

Pristane was very efficiently absorbed by the PWS zooplankton-fed fish, with 98.7 ∀ 0.108% (n = 6) of ingested pristane absorbed. The absorption of pristane was significantly ($P < 0.001$) greater than the absorption of PWS zooplankton biomass by these fish. The absorption efficiency of pristane in *Artemia sp*. could not be accurately estimated because fecal concentrations were too frequently below MDL.

Uptake and Depuration of Pristane in Mussels

The assumed uptake and depuration functions (eqs 2 and 3) provide a reasonably good approximation of the measured concentrations in mussels exposed to dissolved pristane or to pristane contained in feces (Figs. 2.1 – 2.4). 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 (Fig. 2.1). The measured exposure concentration was 0.558 ∀ 0.817 :g l^{-1} (n = 4; the large confidence interval was caused by one sample containing 1.73 :g l^{-1}, which may have been caused by inadequate mixing before sampling). For calculation of the kinetic constants and BAF, We assume the nominal value of 0.5 :g l^{-1} based on the mass of pristane added. The estimates of the uptake and depuration constants are k_1 = 339 d^{-1} (95% CI: 263 – 417 d^{-1}) and k_2 = 0.169 d^{-1} (95% CI: 0.122 – 0.202 d^{-1}), giving a BAF estimate of 2,000. The depuration constant implies a half-life of ln 2 k_2^{-1} = 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 :g g^{-1} (wet weight basis; see Fig. 2.1 for dry weight basis), implying seawater concentrations near 0.030 :g l^{-1} during the exposure and depuration periods.

Mussels exposed to homogenized feces produced by pink salmon that were 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 (Figs. 2.1 and 2.2). The mean concentration of pristane added to the seawater as homogenized feces in the HF-P treatment was 0.317 ∀ 0.0163 :g l^{-1} (n = 84), slightly less than the 0.5 :g 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: 12,600 – 22,900 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 ($P = 0.012$) 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: 0.0571 – 0.135 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 that were fed zooplankton from PWS (WF-P treatment) accumulated pristane more slowly than mussels in the HF-P treatment (Figs. 2.2 and 2.3). The mean concentration of pristane added to the seawater was nearly the same as 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: 4,830 – 7,660 d^{-1}). The depuration constant k_2 for the WF-P treatment was 0.0992 d^{-1} (95% CI: 0.0668 – 0.127 d^{-1}), implying a pristane half-life of 7.0 d, which was not significantly different (P = 0.533) than the depuration constant for the HF-P treatment, but was significantly slower (P = 0.007) than the depuration rate of mussels that accumulated dissolved pristane. The BAF implied by the ratio of the kinetic constants is 64,200.

Mussels exposed to feces produced by the *Artemia*-fed pink salmon (HF-A treatment) accumulated readily detectable concentrations of pristane (Fig. 2.4), despite exposure concentrations near the limits of detectability (implying exposure concentrations < ~ 0.020 :g 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 > 0.23) than those of the other exposures to fecal material (k_2 = 0.0766 d^{-1}, 95% CI: 0.0372 – 0.126 d^{-1}), implying a pristane half-life of 9.1 d.

Discussion

The relative importance of pristane accumulation from the dissolved state compared with ingestion of food particles containing pristane can be roughly determined by the octanol-water partition coefficient (K_{ow}) of pristane (Kelly et al. 2004). The octanol-water distribution coefficient for a contaminant is the ratio of the contaminant concentration in *n*-octanol and water (i.e. $K_{ow} = [X]_{octanol}/[X]_{water}$; [X] = contaminant concentration). This coefficient is a measure of the tendency of a low-polarity chemical such as pristane to accumulate into a low-polarity solvent such as *n*-octanol or lipid in biological tissues. Empirically, organic compounds that have values of K_{ow} exceeding about 100,000 may biomagnify in biota, provided the accumulating organism has a negligible capability to metabolizing the compound (Kelly et al. 2004). Biomagnification may occur when an organism accumulates a contaminant through its diet, and the affinity of the contaminant for the lipid phase is sufficiently great that diffusion losses of the contaminant back to the ambient aqueous phase are outweighed by the rate of accumulation through ingestion.

The K_{ow} value of pristane has not been reported, but an approximate value may be inferred from the BAF result of ~ 2,000 reported here. Marine mussels have little capacity to metabolize hydrocarbons (Lee et al. 1972, Mironov and Shchekaturina 1979). Assuming a lipid concentration of 1% of wet weight (Kluytmans et al. 1975), and that all of the accumulated pristane was contained within the lipid compartment of the mussels, the lipid-normalized partition coefficient would be on the order of 200,000. This BAF value implies that pristane is near the threshold for food-chain biomagnification. The pristane accumulation and depuration results from the mussels exposed to pristane incorporated within fecal material support this conclusion. The 175,000-fold increase (at equilbrium) of pristane accumulated by mussels exposed to 0.317 :g l^{-1} of pristane in dispersed feces of the HF-P treatment implies an

equilibrium concentration of 55.5 :g g^{-1} in the mussels on a wet weight basis. The wet weight concentration of pristane in the feces is (383 :g g^{-1}) x (0.105) = 40.2 :g g^{-1}. The ratio of these values is near one, the value indicative of the biomagnification threshold. Expression of pristane concentrations per unit lipid would have little effect on this comparison. Assuming wet mussel tissue contains 1% lipid, a wet weight concentration of pristane of 55.5 :g g^{-1} in mussels implies a pristane concentration of (0.0000555 g g^{-1}) x $(0.01)^{-1}$ x 100% = 0.56% of lipid. The concentration of pristane stage V copepodites of *Neocalanus plumchrus* is ~ 0.8% (Ch. 1, this report), and these copepodites usually contain 50% or more of their dry weight as lipid (Båmstedt 1986, Duesterloh 2002). Assuming the relation of pristane and lipid is unaltered by passage through the intestinal tract, the concentration of pristane per unit lipid in the fecal material produced by the juvenile pink salmon would be on the order of 0.4%, comparable with the equilibrium concentration (0.55%) estimated for pristane in the lipids of mussels ingesting these feces. These results indicate mussels have little tendency to biomagnify pristane through ingestion of fecal material produced by juvenile pink salmon preying on *Neocalanus plumchrus*, which is consistent with the empirical findings based on the estimated K_{ow} of pristane (Kelley et al. 2004).

The results from the mussel uptake experiments indicate that pristane associated with feces produced by juvenile pink salmon is a substantially more available form of this hydrocarbon for mussels than is 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. Figs. 2.1 and 2.2) 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 :m (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. A similar result has been reported for the suspension-feeding copepod *Calanus helgolandicus*, which required exposure to much higher concentrations of dissolved naphthalene than of naphthalene bound to food particles to achieve equivalent internal concentrations (Corner et al. 1976).

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 nearly twenty times faster than did mussels exposed to dissolved pristane. The slower accumulation rate of pristane by mussels exposed to whole feces compared with the accumulation rate 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 Figs. 2.1 and 2.3).

Mussels exposed to feces derived from *Artemia sp.* accumulated pristane to higher concentrations than mussels exposed to dissolved pristane (cf. Figs. 2.1 and 2.4), despite the much lower seawater concentration of pristane associated with the *Artemia sp.*-derived feces. In fact, the likely exposure concentration of this treatment of ~ 0.020 :g 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. By assuming a BAF of 2,000 for the accumulation of dissolved pristane into mussels, an exposure concentration of 0.020 :g l^{-1} and a ratio of dry and wet weights of 0.1, the equilibrium concentration of pristane in dry mussel tissue is 0.40 :g g^{-1}, very near the tissue MDL for a 0.5 g dry weight tissue aliquot of 0.33 :g g^{-1}. Comparison with observed mussel tissue concentrations exceeding 10 :g g^{-1} (Fig. 2.4) 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 seawater solution 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). The same rationale was used to explain the slower depuration of naphthalene accumulated through the diet compared with accumulation from the dissolved state for *Calanus helgolandicus* (Corner et al. 1976).

Ingestion of pristane-laden feces may account for the increased concentrations of pristane often found in mussels of PWS during spring. Feces derived from fish fed zooplankton from PWS remain a relatively concentrated source of pristane. The mean concentration of pristane in feces produced by the PWS zooplankton-fed fish was 383 :g g^{-1}, which would make these feces the second most concentrated form of pristane in PWS during spring (see Ch. 1, this report). Although direct measurements have not been reported, the solubility of pristane in seawater is almost certainly less than 1 :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 :g g^{-1} wet weight, or about 20 :g g^{-1} dry weight, when mussels are exposed to seawater saturated with pristane. In PWS, concentrations in mussels during spring may exceed 50 :g g^{-1} dry mass (Ch. 1, this report), when ambient seawater concentrations are ~ 0.1 :g l^{-1} or

less (Short and Harris 1996), limiting mussel tissue concentrations to < 2 :g g^{-1} dry weight 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. Ingestion of fecal material produced by *Calanus* or *Neocalanus* copepods might also be a significant uptake pathway for mussels, if the concentration of pristane and the particle density of fecal pellets are sufficiently great. The importance of this pathway will be evaluated in a companion field study (Ch. 3, this report).

Our estimate of absorption efficiency for tissue mass of PWS zooplankton is substantially lower than that reported for rainbow trout (*Salmo gairdnerii*) fed frozen zooplankton consisting mainly of *Calanus hyperboreus*, where the estimated absorption efficiency was 94% for tissue mass (Sargent et al 1979). This may be a consequence of the correction we 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 sp*. was probably defecated by the juvenile pink salmon. The concentration of pristane in feces derived from *Artemia sp*. was probably ~ 10 :g g^{-1}, well above the concentration estimated in the *Artemia sp*. food (1.24 ∀ 0.597 :g g^{-1}). The absorption efficiency of ingested *Artemia sp.* tissue 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 sp*., which is near ~ 2.7% (dry weight 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 sp*. is too low to stimulate sufficient bile salts for efficient absorption of lipids and associated pristane.

The daily rations ingested by the pink salmon in the experiments reported here are comparable with rations of wild juveniles. Wild pink salmon typically consume 5 – 30% of their body weight per day in wet weight of prey (Simenstad et al. 1980, Godin 1981, Healey 1982b). The experimental pink salmon consumed 7.7% of their body weight of PWS zooplankton, and 11% of *Artemia sp*. daily (Table 2.1). The experimental pink salmon were therefore not stressed on account of food availability.

The growth rate of the juvenile pink salmon fed *Artemia sp*. is somewhat lower than that typical of wild juveniles, and may have been a consequence of the selection of small fish and handling stress. Wild juvenile pink salmon typically have daily growth rates of about 3% of body weight per day during the initial phase of their marine residence (Cooney et al. 1981, Willette 2001). Selection of the smallest fish available for these experiments in mid-July led to an average individual weight of 1.8 g, and these fish would have weighed ~ 0.3 g during when entering seawater some 90 days earlier. This amount of growth implies an instantaneous growth rate of ~ 2% d^{-1}, consistent with the continued growth of the fish that were fed *Artemia sp*. Selection of the smallest fish means their measured growth performance is probably an underestimate of the

growth rate of the population sampled, hence inferences regarding the sampled population growth under the experimental conditions used here must be done with caution. Also, the considerable daily handling stress of all treatment groups may have reduced growth somewhat, but this affected all the treatment groups equally, and hence cannot account for the low growth rate of the pink salmon that were fed zooplankton from PWS compared with those that were fed *Artemia sp*. Despite these caveats, the differences in the growth of fish that were fed zooplankton from PWS compared with fish that were fed *Artemia sp*. were so great that differences of approximately similar magnitude would probably be found in larger, faster-growing fish as well.

The low growth rate of juvenile pink salmon that were fed zooplankton from PWS is most likely due to the pristane content, because pristane inhibits growth of fish. Juvenile rainbow trout (*Salmo gairdnerii*, 13 g initial wet weight) ingesting a daily ration of ~ 1.5 g dry artificial food per g wet body weight that contained 1% pristane for 45 weeks had a mean instantaneous growth rate $k = 0.00748$ d^{-1}, compared with a control diet where $k = 0.0141$ d^{-1}, and the fish fed pristane required 67% more food to achieve equivalent weight gain (Luquet et al. 1983). Similar results were found during a follow-up experiment with larger rainbow trout (121 g), where fish ingesting identical rations (~ 2 g dry food per g wet body weight) containing either 1% pristane or no pristane had $k = 0.00349$ d^{-1} compared with $k = 0.00817$ 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 not very different than 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 feeding experiments with pristane added to artificial fish food conducted by Luquet et al. (1983, 1984) suggest that pristane reduced growth by interfering with lipid metabolism. These experiments demonstrated that pristane (as well as other saturated hydrocarbons) depressed appetite of fish, that pristane depressed growth when ingested rations fed with and without pristane were identical, and that the appetite depression did not appear until about 2 weeks after feeding on pristane-laden food began. This last observation argues against a palatability effect of pristane causing appetite depression. Instead, Luquet et al. (1984) suggest that appetite depression results from slower growth, not *vice versa*. These authors also noted lower liver mass, lower liver lipid content and liver hepatosomatic index (i.e., the ratio of liver and body masses) of fish ingesting pristane-contaminated food compared with those ingesting equivalent rations of uncontaminated food. These results imply that pristane inhibits fish growth through an unknown metabolic effect.

The growth inhibition of pristane on the juvenile pink salmon that were fed zooplankton from PWS may have been exacerbated by two other factors. First, *Calanus* and *Neocalanus* copepodites may not be as balanced a diet for juvenile pink salmon as *Artemia sp*. 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 to be efficiently captured by the 505 :m-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, although similar losses also affected the *Artemia* treatment, which supported the better fish growth nonetheless. Hence, it is unlikely that these losses would be sufficient to account for the large differences in growth observed between these two diets.

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 and Lalli 1988). The value placed by researchers on these copepods as prey items to their consumers has heretofore been based mainly 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 act as a chemical defense against predation, by prolonging the period of greatest vulnerability of their predators to size-dependent predation. Size-dependent mortality from predation has been implicated as an important factor for the survival of juvenile pink salmon (Parker 1971, Healey 1982a, West and Larkin 1987, Willette et al. 1999, Willette 2001, Willette et al. 2001).

Pristane was initially suggested to play a role in buoyancy regulation, allowing copepods to expend less energy to maintain position in the water column during diapause, and thereby conserve lipid stores (Blumer et al. 1964), but this hypothesis is problematic. The hypothesized advantage of pristane in buoyancy regulation was based in part on lipid utilization rates of active but starving copepods, which use lipid stores more rapidly than quiescent copepods in diapause (Hirche 1983, Campbell et al. 2004), leading to an exaggeration of the benefits of pristane as an agent to decrease buoyancy. *Calanus* and *Neocalanus* copepods are actually close to neutral buoyancy and consume only a small proportion of their storage lipids during diapause (Jónasdóttir 1999, Campbell and Dower 2003, Campbell et al. 2004), with the greater proportion of the lipids being used for gamete production (Jónasdóttir 1999). Also, if pristane concentrations on the order of 1% dry mass interacts somehow with lipids to reduce lipid utilization during dormant periods, it is not clear why nearly the same concentration would be produced by, for example*, Calanus marshallae*, which may have multiple generations annually and therefore relatively brief periods of diapause, and *Neocalanus spp*., which have only one generation annually and a correspondingly longer period of diapause (Conover 1988).

In contrast to the buoyancy regulation hypothesis, the inhibitory effect of pristane on growth of copepod predators would have a clear and direct benefit on the population fitness of copepods. By exposing their predators to increased risk of mortality, copepods reduce predation on themselves in two respects: there are fewer predators, and the remaining predators are smaller than they would otherwise be and hence less able to capture prey, because the search volume of predators varies directly with their size. This situation is analogous to the adverse effects certain phytoplankton may have on the reproductive capacity of their copepod predators (Ban et al. 1997, Ianora et al 1999, Paffenh fer 2002). In both cases, the reproductive potential of the predator population is reduced when chemically defended prey are consumed, by reduced fecundity of predators in the case of diatoms, and by outright mortality of fish in the case of *Calanus* and *Neocalanus* copepods. And in both cases, the damage inflicted on the predators involves death of the prey.

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. *Calanus* and *Neocalanus* copepods may account for half or more of the ingested biomass of juvenile pink salmon in PWS during spring (Cooney et al. 1981, Sturdevant et al. 1996, Willette 1996, Willette et al. 2001). Gross growth efficiency assumptions of 25% - 45% are possibly 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 North Pacific Oceans for trophic levels above *Calanus* and *Neocalanus* copepods, as a consequence of the lower gross growth efficiency associated with predators of 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

Feces produced by nearshore zooplanktivorous fishes, exemplified by juvenile pink salmon, feeding on *Calanus* and *Neocalanus* copepodites is an important 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 often account for the greatest prey biomass available to these fishes during spring, (4) pristane ingested with *Calanus* and *Neocalanus* copepodites is incompletely absorped by juvenile pink salmon and their feces remain a relatively concentrated source of pristane, and (5) mussels are more efficient, by factors ranging to ~ 50, at accumulating pristane from fish feces than from pristane dissolved in seawater.

The hatcheries operated by Prince William Sound Aquaculture Corporation 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 often release on the order of 10^8 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 following chapter.

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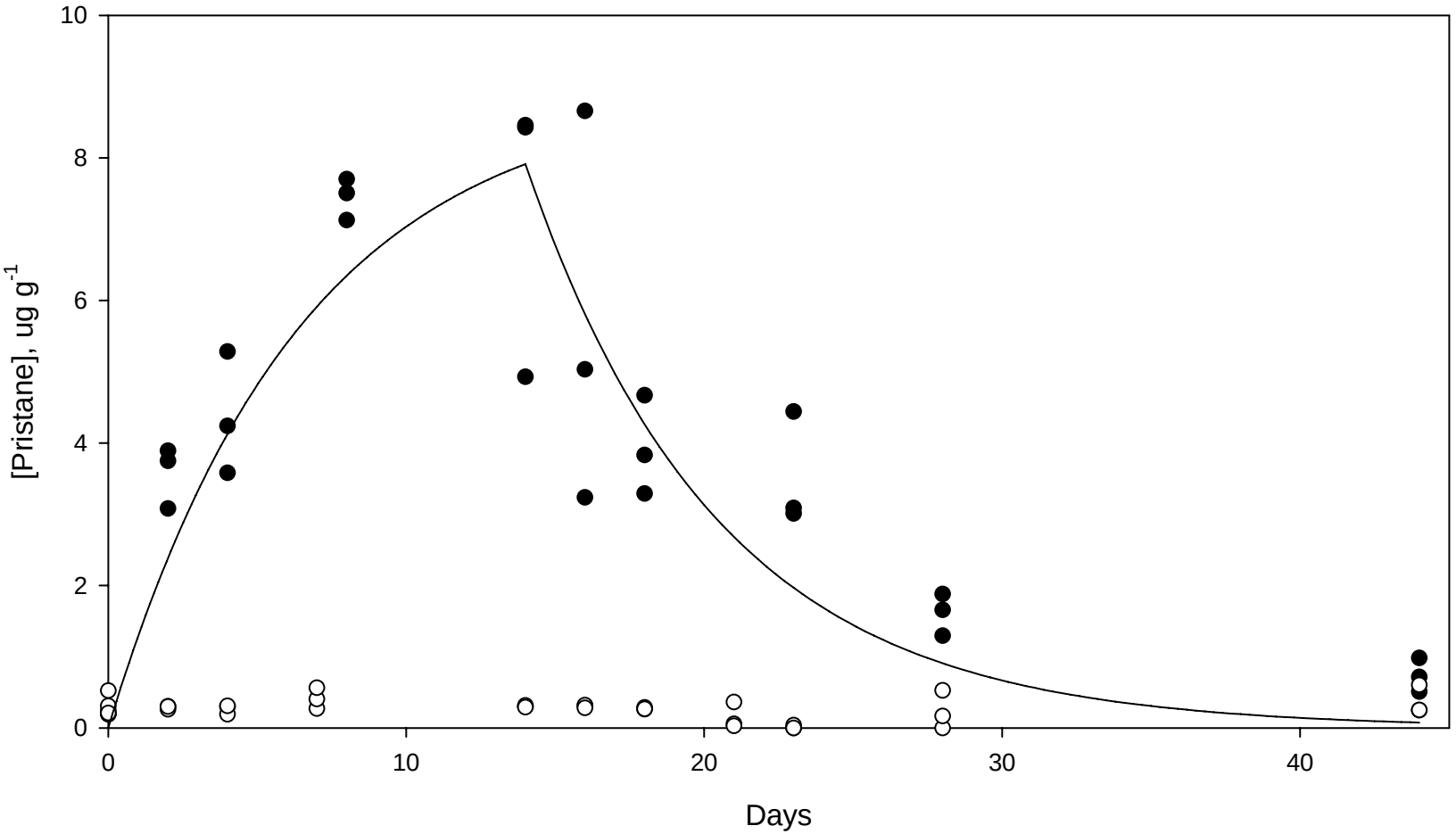

Figure 2.1. Accumulation and depuration of dissolved pristane by mussels. Mussels exposed to 0.5 mg l^{-1} dissolved pristane for 14 d, followed by a 30 d depuration period (filled circles), or to ambient laboratory seawater (open circles). Solid line indicates non-linear least-squares fit of accumulation and depuration functions assuming first-order kinetics for both processes (see Methods).

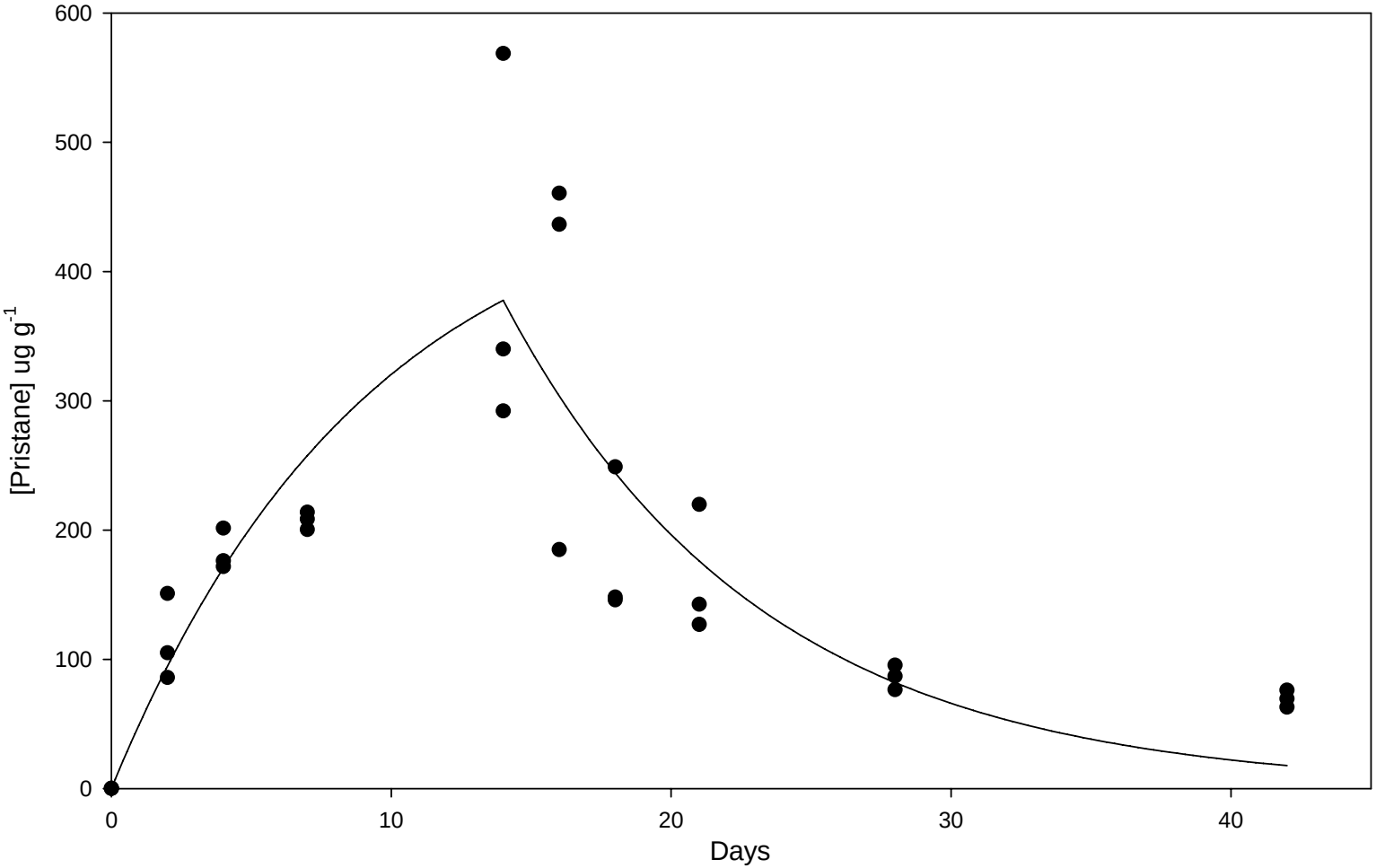

Figure 2.2. Accumulation and depuration of pristane from homogenized feces derived from PWS zooplankton by mussels. Mussels exposed to pristane for 14 d, followed by a 28 d depuration period. Solid line indicates non-linear least-squares fit of accumulation and depuration functions assuming first-order kinetics for both processes (see Methods).

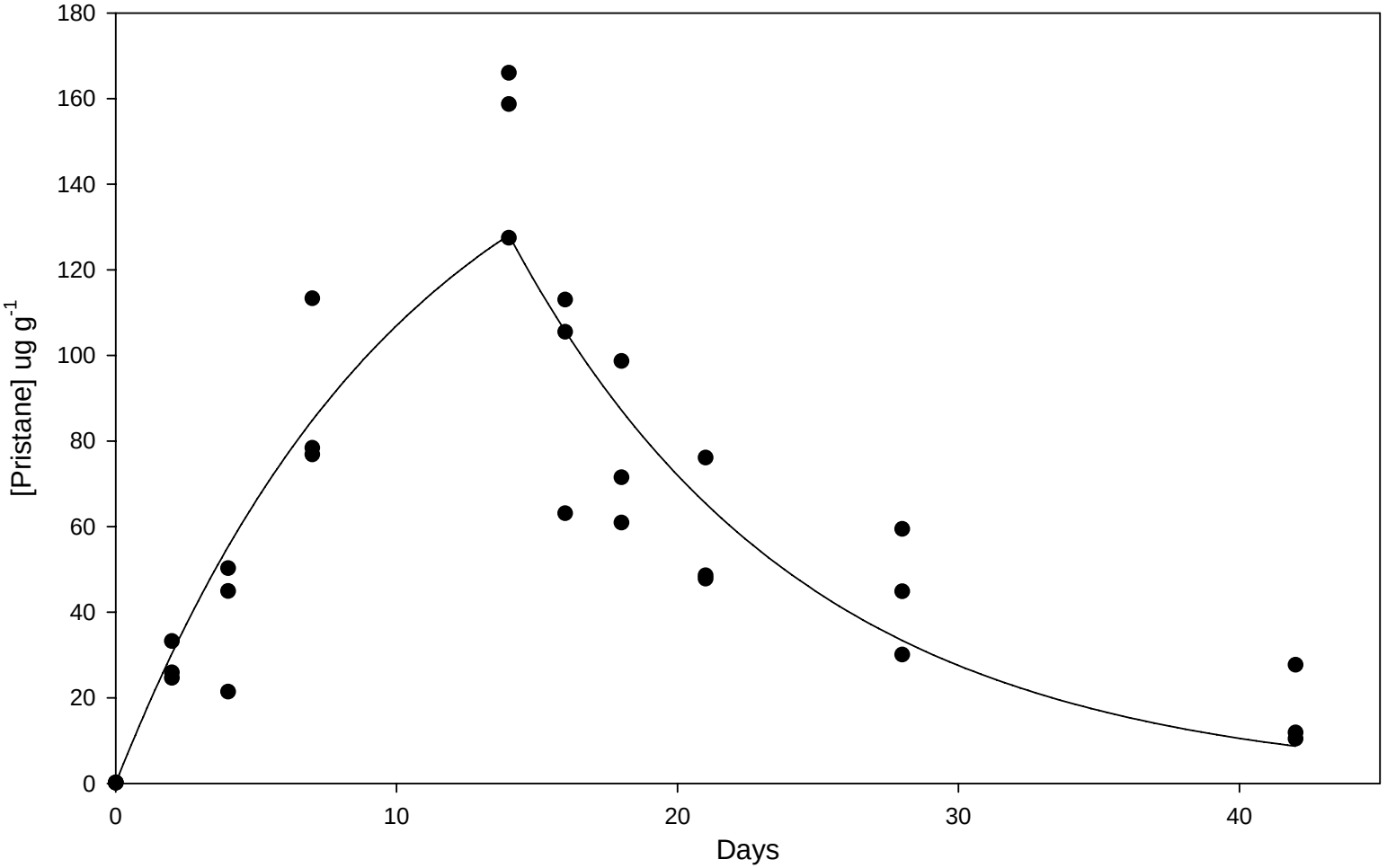

Figure 2.3. Accumulation and depuration of pristane from whole feces derived from PWS zooplankton by mussels. Mussels exposed to pristane for 14 d, followed by a 28 d depuration period. Solid line indicates non-linear least-squares fit of accumulation and depuration functions assuming first-order kinetics for both processes (see Methods).

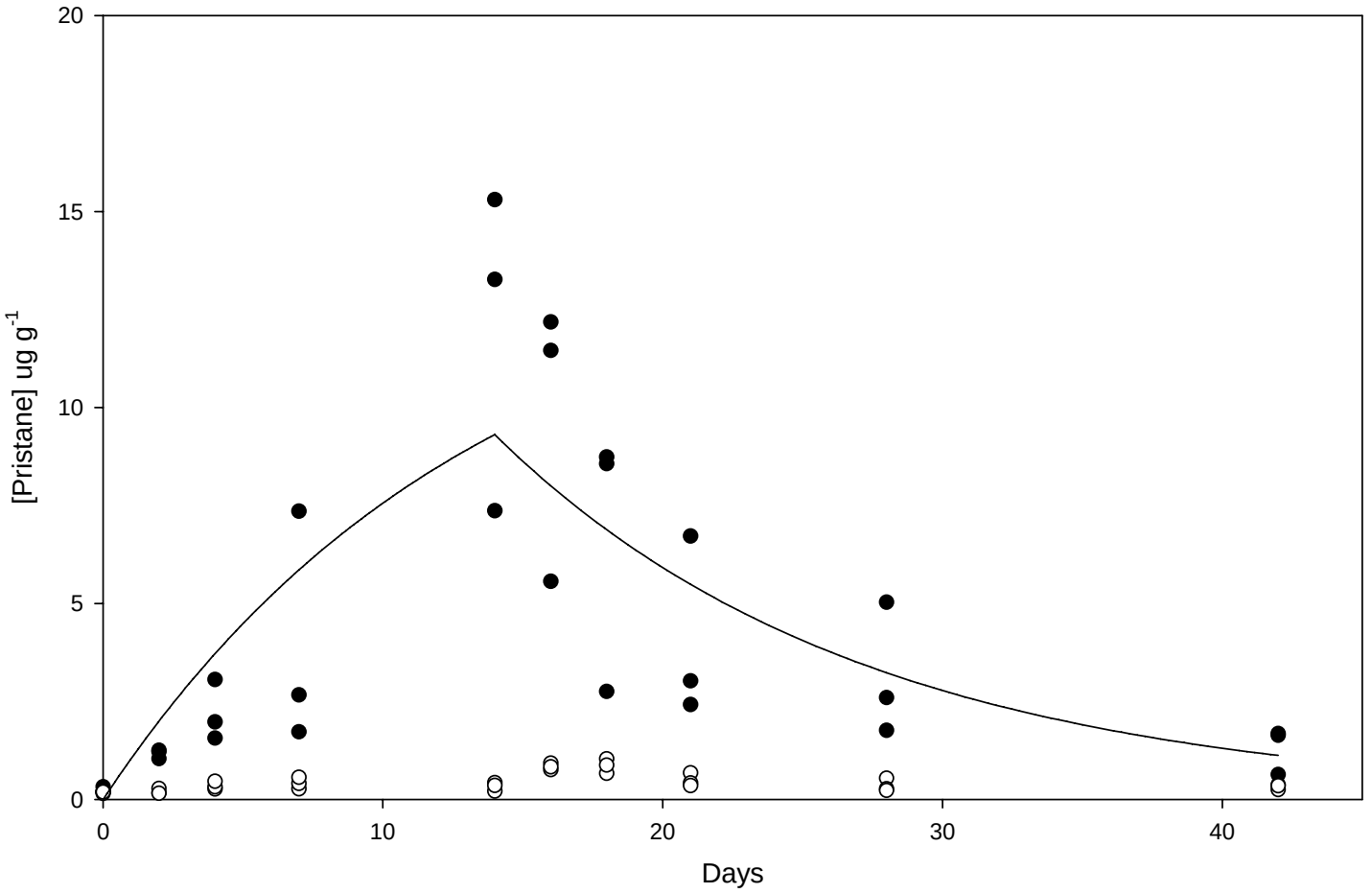

Figure 2.4. Accumulation and depuration of pristane from homogenized feces derived from *Artemia sp*. by mussels. Mussels exposed to pristane for14 d, followed by a 28 d depuration period (filled circles), or exposed to ambient laboratory seawater (open circles). Solid line indicates non-linear least-squares fit of accumulation and depuration functions assuming first-order kinetics for both processes (see Methods).

Tables

Table 2.1. Growth of juvenile pink salmon. Weights of 20 juvenile pink salmon in each of the treatment group replicates (see Methods) are given for the beginning and end of the growth determination interval. See Methods section for calculation of growth rate constants. Food consumption is based on the number of successive measurements of food consumed per unit estimated total wet body weight indicated in parentheses.

Zooplankton Type:	PWS-Zooplankton							*Artemia sp.*		
Treatment Group:		HF-P			WF-P				HF-A	
Replicate:	1	2	3	1	2	3		1	2	3
Total weights (g), initial:	37.2	40.3	38.6	38.3	38.1	33.5		40.9	40.5	42.5
final:	39.1	41.6	39.2	41.4	39.7	35.4		48.1	47.1	52.0
Days between weighings:	9	9	9	9	9	9		7.76	7.76	9
Growth Rate (k x 10^2):	0.554	0.353	0.171	0.865	0.457	0.613		2.09	1.95	2.24
Mean Food Consumption: (Wet Food/Wet Body Wt., x 100%)	8.21 ±0.823 (n = 18)	7.23 ±0.736 (n = 18)	7.54 ±0.881 (n = 18)	7.25 ±0.692 (n = 18)	7.30 ±0.775 (n = 18)	8.43 ±0.788 (n = 18)		11.0 ±1.09 (n = 15)	10.7 ±1.36 (n = 15)	11.1 ±1.03 (n = 18)
(Dry Food/Wet Body Wt., x 100%)	1.03 ±0.213	0.868 ±0.192	0.891 ±0.223	0.850 ±0.207	0.889 ±0.207	1.06 ±0.223		0.989 ±0.0988	0.965 ±0.123	1.01 ±0.0932

Chapter 3

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 investigated the role of 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 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, along with zooplankton composition and abundances.

Pristane concentrations increased up to ~ 10 :g g^{-1} (dry weight) in mussels after releases of juvenile pink salmon. Pristane concentrations in seawater were usually below detection limits (0.041 :g l^{-1}), limiting the contribution to pristane burdens of mussels to ~ 2 :g g^{-1} from dissolved pristane. The pristane concentration of fecal pellets produced by *Neocalanus* was 80.2 :g g^{-1}, which limited the contribution to mussels from this source to no more than ~ 3 :g g^{-1}. Analysis of stomach contents and visual observations of behavior confirmed the fecal pathway mediated by fish as the primary cause of the concentration increase of pristane in mussels following release of the fish.

Zooplankton sampling indicated that *Neocalanus* abundance declined by more than half the week following the release, and is attributed to consumption by released fish. Decline 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* and *Acartia* copepods doubled as the *Neocalanus* population declined.

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 for many species (e.g. starfish) analysis of stomach contents is impractical. 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 lower-trophic level 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 (2,6,10,14-tetramethylentadecane; 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, and Ch. 1, this report). Pristane is relatively persistent in the environment because it is terminally branched, and hence is more resistant to ∃-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 and 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 clear 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 :g g^{-1} (dry weight basis), returning to low concentrations by late summer (Ch. 1, this report). The source of pristane is mainly stage IV and V *Neocalanus plumchrus*, *Neocalanus flemingerii* and *Calanus marshallae* copepodites (Ch. 1, this report). Bay mussels may accumulate pristane through direct ingestion of *Neocalanus* and *Calanus* copepodites, through absorption of pristane dissolved into seawater from *Neocalanus* and *Calanus* copepodites, from ingestion 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 at equilibrium of the wet weight concentration of pristane in mussels and the concentration of pristane dissolved in ambient seawater). Concentrations of dissolved pristane as high as ~ 0.2 :g l^{-1} have been measured in PWS during spring (Short and Harris 1996), and the BAF of pristane in mussels is ~ 2,000 (Ch. 2, this report), which implies maximum concentrations in mussels accumulated from dissolved pristane would be ~ 0.4 :g g^{-1} wet tissue weight, or ~ 4 :g g^{-1} dry tissue weight. 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, and Ch. 1, this report), 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 a very

important route of pristane from these copepods to mussels (Ch. 2, this report).

Prince William Sound is a nearly ideal setting for evaluating the relative importance of the alternative transfer pathways of pristane from *Neocalanus* and *Calanus* copepods to mussels. The zooplankton biomass is dominated by these genera during spring, (Cooney et al. 2001, 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 (Johnson et al. 2002). 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 fish. Juvenile pink salmon are zooplanktivorous, and *Neocalanus* and *Calanus* copepods are important 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 feces produced by the released fish on pristane transfer from copepods to mussels.

Objectives of 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. Marine survivals of pink salmon populations are thought to be determined during the initial period of marine residence (Parker 1962, Parker 1968, Ricker 1976, Hartt 1980, Peterman 1987, Karpenko 1998, Willette et al. 2001), 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 data were collected during spring, 1996 and again during spring, 1998 near the Wally H. Noerenberg salmon hatchery (WHN), operated by the Prince William Sound Aquaculture Corporation (PWSAC). This hatchery is located on the north shore of Lake Bay, a small embayment on the southern coast of Esther Island in northwestern PWS (Figure 3.1). Juvenile pink salmon are reared in marine net pens a few weeks prior to their release into PWS. The mean mass of a released juvenile was 0.30 g in 1996 and 0.485 g in 1998. About 1.3 x 10^8 and ~ 7.0 x 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 EC during both years of the study.

During both years we 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 within 3 km of the release point (Figure 3.1). We also captured juvenile pink salmon just prior to release and a few days afterward to examine their stomach contents and, in 1996, to determine their whole-body concentrations of pristane. In 1996, we collected bulk zooplankton for species composition and for collecting zooplankton fecal pellets for pristane analysis and for sinking rate determination. In 1998, we 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 3.1). We also collected zooplankton fecal pellets for determination of the wet and dry weight ratio. Following are details of these collections and the analyses performed on them.

Sampling Stations and Mussel Collection

The three monitoring stations are denoted as Lake Bay (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 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 3.1). The shore at the LB station is a small, protected indentation of the coastline, mostly bedrock with mussels present in scattered clumps at a beach slope of about 15E. The EL station had a dense horizontal band of mussels attached to a steep (> 50E) bedrock face where an access ladder to a navigational light is 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 EC 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 3.1). One of the duplicates was filtered through a 1.5 :m 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 EC until analysis for pristane.

Zooplankton Sampling and Species Composition Determination
In 1996, zooplankters were collected near the PWSAC plankton sampling station 2 in the mouth of Lake Bay (Figure 3.1). At each sampling a 1.75 m long, 505 :m-mesh plankton net with a 0.5-m diameter circular 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 zooplankters 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, zooplankters were collected along with seawater samples near each of the three mussel collection stations, where seawater depths were ~ 50 m (LB) or ~ 100 m (EL and HP) and the seafloor slope was > 30E. Two collections were made at each station, one with a 202 :m-mesh plankton net and another with a 505 :m-mesh net, both having the same shape and size of the net used in 1996. Both nets 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 were 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. Zooplankters 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 zooplankters were stored in 5% formalin in seawater for determination of species composition. The 202 :m-mesh samples were used for determination of species abundance, biomass, and variability among stations, and the 505 :m-mesh samples were treated as duplicates for the large zooplankton captured to evaluate sampling variability within stations.

Zooplankters were identified to genus and usually 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 weights 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 3.1). These collections involved three vertical tows from 20 m depth to the surface of a 0.5 m diameter, 243 :m-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 202 :m-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 dry overnight at ~ 50 EC, and then were transferred to a small vial and stored at -20 EC 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 31l, temperature 6 EC).

Juvenile Pink Salmon Collection and Stomach Content Analysis
Released and un-released juvenile pink salmon were collected to compare stomach contents and pristane concentrations. Schools of released fish were located by visual inspection from a skiff, and individuals were captured by dip-net two days after release in 1996, and 1 – 3 days after in 1998. Stomach contents of captured fish that were preserved in 5% formalin in seawater were examined by dissecting out then incising the stomach, and rinsing the exposed contents onto a zooplankton counting plate. Zooplankton in pink salmon stomachs were identified to genus, and unusual items (e.g. dipterans) were also noted. Pristane analysis was performed on whole fish stored frozen at -20 EC.

Dry Weight Determination
The ratio of dry and wet weights of tissue was determined by drying weighed sample aliquots at 65 EC for 24 h. This ratio was 0.113 $\forall$ 0.00907 (n = 27) for mussels collected in 1996, and 0.0962 $\forall$ 0.00399 (n = 42) in 1998. The ratio was 0.541 $\forall$ 0.167 (n = 4) for zooplankton fecal pellets. Pristane concentrations are expressed on a dry weight basis.

Pristane Analysis
Pristane analysis of whole organisms 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, 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 0.041 :g l^{-1}.

Data Analysis

The significance of differences between daylight and night-time abundances of *Neocalanus plumchrus/flemingerii* and of *Pseudocalanus sp.* were evaluated by the paired t-test. Samples collected at 2200 h on 30 April and near midnight on 7 May 1998 were pooled for this test. For *Neocalanus plumchrus/flemingerii*, morning and night-time samples collected from each station with the 505 :m-mesh nets were paired, as were samples collected with the 202 :m-mesh nets, and the t-test was applied to the sum of these samples to increase the power of the test. For *Pseudocalanus sp.*, the t-test was applied only to samples collected with the 202 :m-mesh net.

The significance of changes in the pristane concentrations of mussels and in the abundances of zooplankton during the monitoring period was evaluated with a one-way repeated measures analysis of variance (RM-ANOVA). The measurements were repeated at each of the sampling stations on each of the days sampled, and the sampling days are considered as the treatments in this analysis. Pristane concentration data in mussels, and zooplankton abundance data were log-transformed prior to the RM-ANOVA, which then satisfied assumptions of normality (determined by the Kolmogorov-Smirnoff test) and equality of variance (determined by the Levene median test).

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, and were observed defecating above 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. *Neocalanus* and *Calanus* were somewhat more abundant at the PWSAC station in the mouth of Lake Bay. Weather conditions were generally calm during the weeks 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 (*Larus sp*.) and kittiwakes (*Rissa tridactyla*) on the seventh day after the release, when they could be seen floating on the sea surface 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 generally increased in mussels beginning at least 2 days after the juvenile pink salmon were released in both 1996 and 1998 (Figures 3.2 and 3.3), and the RM-ANOVA indicated that overall these changes were highly significant ($P \leq 0.005$). 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.

Pristane in Seawater

Nearly all of the pristane concentration measurements were below detection limits (0.041 :g l^{-1}). Four samples contained detectable pristane concentrations associated with particulate material in 1996, the highest being 0.083 :g l^{-1}. Three samples (of 27) contained detectable dissolved pristane in 1998, the highest being 0.107 :g l^{-1}, and three contained particulate-pristane (maximum 0.066 :g l^{-1}). These concentrations are too low to resolve contributions from dissolved pristane and pristane associated with particulate matter, or to evaluate temporal trends.

Pristane in Fecal Pellets

The mean pristane concentration in fecal pellets produced by the zooplankton collected in 1996 was 80.2 $\forall$ 35.7 :g g^{-1} (95% CI, n = 8). These pellets were ~ 500 :m in length and ~ 80 :m 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 202 and the 505 :m-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.

Among pairs, the relative frequency of higher abundances in the smaller mesh net was 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, *N. 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 505 :m-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 was produced by either *N. 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 3.1). 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 3.4). Abundances declined simultaneously to between a half to less than a third at the three monitoring stations, and this decline was highly significant (RM-ANOVA, P = 0.007). Before the release, the median abundance of *N. 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 3.5). In concert with the decline of *N. plumchrus/flemingerii*, abundances and biomass of *Acartia sp.* and of *Pseudocalanus sp.* increased by factors of three and two, respectively, (Figures 3.4, 3.6 and 3.7), and these increases were highly significant (RM-ANOVA, $P \leq 0.002$).

The species composition of zooplankton collected in the afternoon and night samples were not notably different than the morning samples (Figures 3.4 – 3.7). Abundances of *Neocalanus plumchrus/flemingerii* or of *Pseudocalanus sp.* were not significantly different in the morning compared with the night-time samples (paired t-test; P = 0.132, df = 11 for N. plumchrus/flemingerii, and P = 0.547, df = 5 for *Pseudocalanus sp.*), and these two generic groups accounted for at least 83% of the zooplankton biomass sampled on 30 April or on 7 May 1998, regardless of the hour of sampling. This indicates little diurnal variation 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 3.2). 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 (Table 3.2). Whereas *Neocalanus* usually accounted for most of the biomass, some individuals contained none of these but numerous *Pseudocalanus sp*. instead (Appendix 12). 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 :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 :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 numerical responses of zooplankton in the immediate vicinity to the hatchery releases of juvenile pink salmon. The following discussion 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 small 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 increased considerably in mussels following the hatchery releases of pink salmon (Figures 3.2 and 3.3) but concentrations of pristane in seawater did not. The highest concentration of pristane measured in seawater during this study was 0.107 :g l^{-1}, which implies a wet weight tissue concentration of pristane in mussels of 0.215 :g g^{-1} at equilibrium. Assuming a ratio of dry and wet tissue weights of 0.1, this is equivalent to 2.15 :g g^{-1} dry weight. This upper limit is comparable with concentrations observed in mussels prior to release of the hatchery pink salmon (Figures 3.2 and 3.3), 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 portion of the pristane burden when abundances of

copepod predators are low, 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 comparable to accumulation of dissolved pristane. The contribution from pristane in copepod fecal pellets 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 $\forall$ 35.7 :g 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 (Ch. 2, this report).

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 $C_p = p\ B\ z\ P_p^{-1}$, 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 P_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, 1956, Corner et al. 1972, Gaudy 1974). Seawater rarely contained more than one *N. plumchrus/flemingerii* l^{-1} in the zooplankton samples of the uppermost 30 m from 1998 (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 *N. 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 *N. 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 weights of the fecal pellets produced by *N. 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 (80.2 :g pristane g^{-1} dry weight) x (0.541) = 0.00127 :g l^{-1}. At equilibrium the corresponding concentration of pristane in mussels would be 175,000 x 0.00127 :g l^{-1} x 0.001 l g^{-1} wet tissue = 0.222 :g g^{-1} wet tissue, or about 2.22 :g g^{-1} dry tissue. Hence, pristane associated with fecal pellets produced by *N. 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 3.1). 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 3.2). 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 salmon caught 25 h after release from the hatchery in 1998 (Table 3.2). 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. This is probably an underestimate, because it does not account for the gastric turnover rate, which may be as fast as two to three times a day (Healey 1982). Assuming an average wet tissue weight of 1 mg copepod^{-1} for a mixture consisting of equal proportions of stage 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 505 :m-mesh tows was 75%, the ratio of dry and wet fecal weight was 0.117, and the concentration of pristane in the feces produced was 383 :g g^{-1} (dry mass basis; Ch. 2, this report). This implies daily introduction of ~ 11 g 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 would travel a maximum of 3.3 km in each of two directions the first day, covering 6.6 km of shoreline. Assuming also these fish remain within 50 m of the shoreline and an average seawater depth of 25 m leads to an approximate volume of seawater receiving the feces produced by the pink salmon of 8.3 x 10^6 m^3, and a corresponding concentration of pristane associated with feces of ~ 0.0013 :g l^{-1}. The BAF of pristane associated with these feces when completely dispersed was estimated at 175,000 (Ch. 2, this report), so a pristane concentration of 0.0013 :g l^{-1} associated with dispersed feces could result in a daily increase in the mussel tissue concentration of ~ 0.24 :g g^{-1} on a wet tissue weight basis, or ~ 2.4 :g g^{-1} on a dry tissue mass basis (assuming a ratio of dry to wet mussel tissue weight of 0.1). This is broadly consistent with the increase of the pristane concentration observed in mussels at the LB station in 1998.

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 *N. 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 (0.383 :g mg^{-1}) = 0.0056 :g pristane l^{-1}, which is comparable with the estimate of 0.0027 :g 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 ~ 9.4 :g 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 25 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 in Prince William Sound

Each of the three ecological pathways considered here contribute to 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. These two pathways would account for gradual increases during April to mussel concentrations of 4 – 5 :g g^{-1} at most. Abundances of these copepods are lower most years compared to 1998, so peak concentrations of pristane in mussels from these two pathways 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, which may cause pristane concentrations in mussels to increase on the order of ~ 2.4 :g g^{-1} per day. If increases of this magnitude were sustained over several days, this pathway may account for pristane concentration increases of tens of :g g^{-1} in mussels.

Factors contributing to the springtime increases of pristane in PWS mussels include simultaneously high abundances of *Neocalanus* or *Calanus* copepods and their predators, and 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* biomasses were 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. 2001). 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 3.6). In 1996, hatchery records indicate that substantial abundances of zooplankton were present at the stations monitored by PWSAC staff (Figure 3.1), and visual inspection of these samples showed they contained mostly *Neocalanus* and *Pseudocalanus*.

The results of the night-time sampling indicate that the zooplankton prey field biomass remained dominated by *Neocalanus* and *Pseudocalanus sp.* throughout the day and night, so the juvenile pink salmon released from the hatchery did not have access to a substantially different zooplankton prey field at night. The absence of a substantial shift in the zooplankton composition may have been a result of sampling within 50 m of the shoreline in seawater depths of ~ 100 m or less. These shallow depths may have been too distant horizontally to be reached in substantial numbers by zooplankton predators residing at deeper depths during daylight hours.

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 3.2). 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 3.3). 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 3.4 and 3.6) was probably caused by the predation of the salmon on the copepods. *Neocalanus* copepods have completed about a quarter of their year-long life cycle by May, so natural mortality due to senescence is likely to be negligible. 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* per day following the release as estimated above would reduce the copepod population abundance by 450 individuals m^{-3} over four days (Figure 3.4) in 8.9 x 10^6 m^3 of seawater, which volume is equivalent to a shoreline distance of 3.6 km seasurface at a depth of 25 m and a distance from shore of 50 m. This distance is consistent with the distances of the zooplankton sampling stations from the point where the juvenile salmon were released. 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. 2001).

Reduction of the *Neocalanus plumchrus/flemingerii* population apparently allowed a corresponding rapid increase in the populations of *Acartia sp*. and of *Pseudocalanus sp*. These three copepod genera are all mainly herbivores (Mauchline 1998) that are actively growing during spring, and hence compete for phytoplankton production. 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 202 :m-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. Note that 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 in the pelagic food web: 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 their high pristane content (4 – 8 mg g^{-1} dry body mass for stage IV – V copepodites; see Ch. 1, this report) seriously impairs growth of juvenile salmonids, including pink salmon (Luquet et al. 1983, 1984, and Ch. 2, this report). 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 505 :m-mesh nets of this study. This implies that juvenile salmon would have to consume about 45% of their body weight daily

to support growth of 3% wet body weight d^{-1} reported for juvenile pink salmon in PWS (Cooney et al. 1981, Willette 1996, 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, a foraging strategy to maximize growth would probably require predation 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*, or to search for alternative prey in other habitats, such as harpacticoid copepods in the benthos (Cooney et al. 1981, Sturdevant et al. 1996).

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 April to mid-May, when abundances of *Neocalanus* were greatest (Figures 9 and 10 in Cooney et al. 1981, Figure 6 in Willette et al. 2001). These latter two observations are also consistent with growth inhibition from the pristane content of *Neocalanus* copepods. Although estimates of the carrying-capacity of PWS for juvenile pink salmon indicate depletion of zooplankton abundances is unlikely except perhaps on small, localized spatial scales, such as the immediate vicinity of hatcheries just after a mass-release of juveniles (Cooney 1983, Cooney and Brodeur 1998, Boldt and Haldorson 2002), these estimates did not account for growth inhibition by pristane. The growth efficiencies assumed or implied by these estimates exceed 25%, whereas if growth is inhibited through ingestion of pristane, depletion of zooplankton abundances may be considerably greater than these estimates have indicated.

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 the 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 the abundances of zooplantivorous fishes in PWS 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 :g g^{-1} dry tissue weight. Substantially 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 lost through consumption of *Neocalanus* by the released fish.

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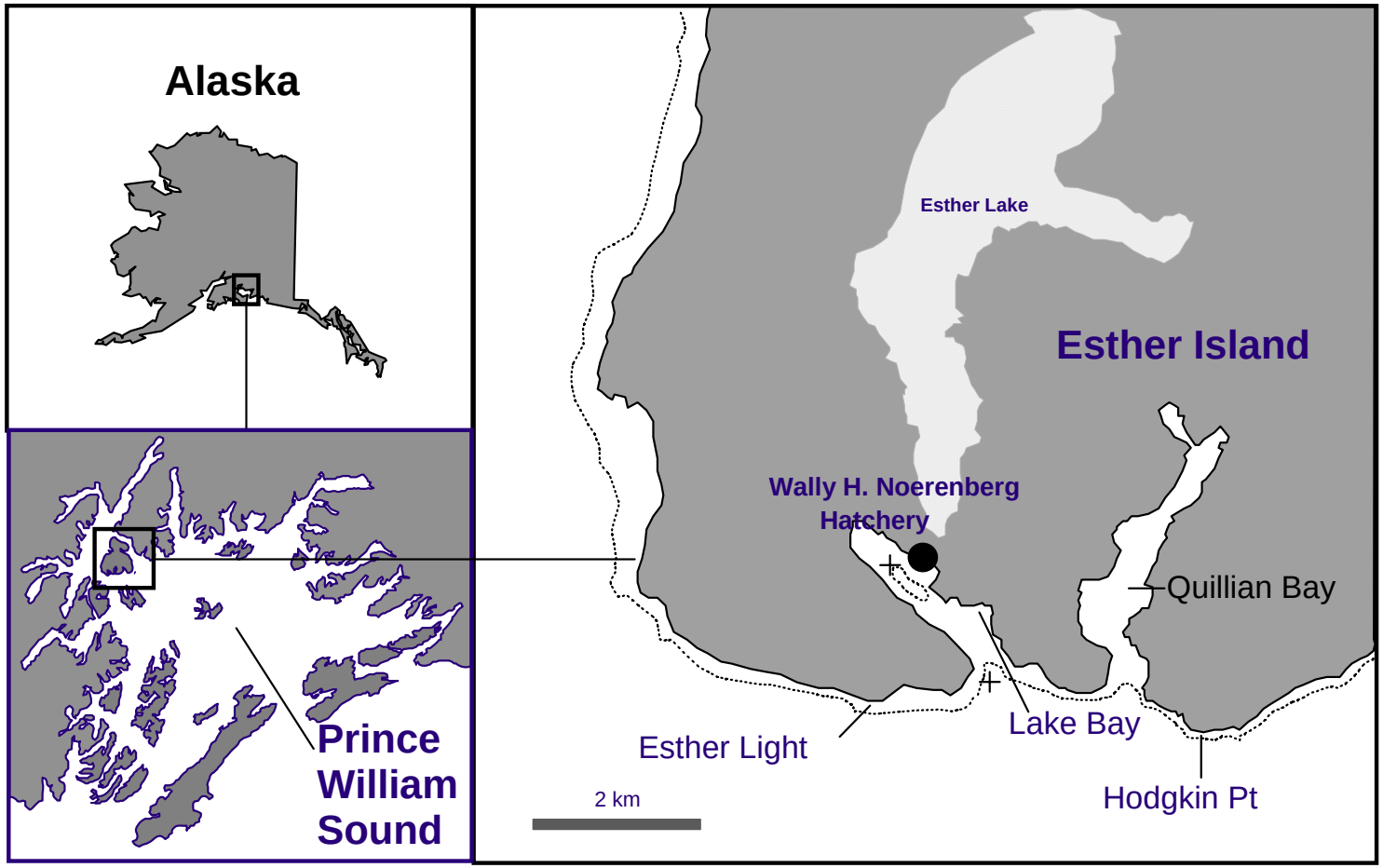


Figure 3.1. Map of study area. Lines indicate the mussel monitoring stations, and crosses the zooplankton sampling stations monitored by the Prince William Sound Aquaculture Corporation in relation to the W. H. Noerenberg hatchery. The dashed line along the coast indicates the approximate 50 m depth contour.

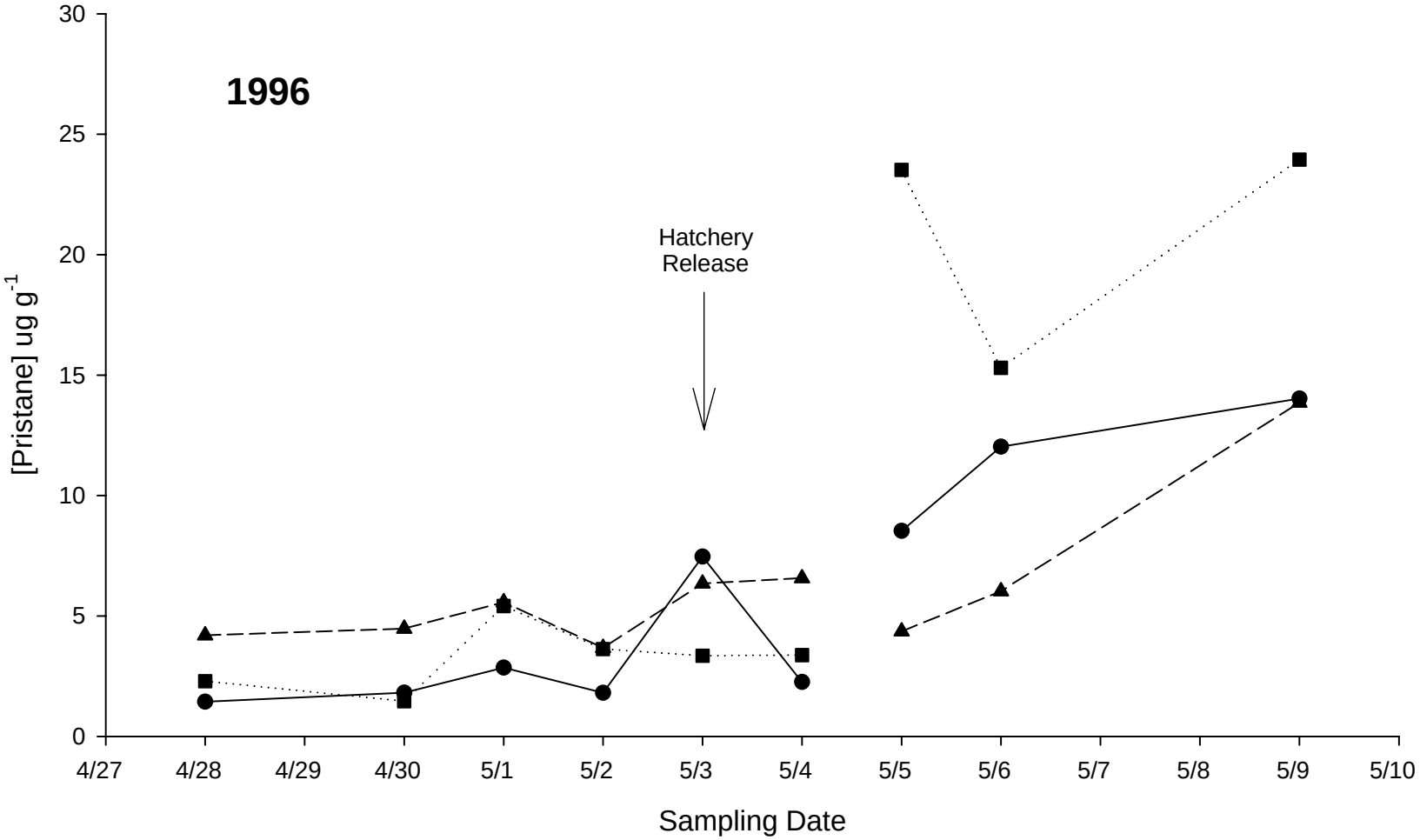
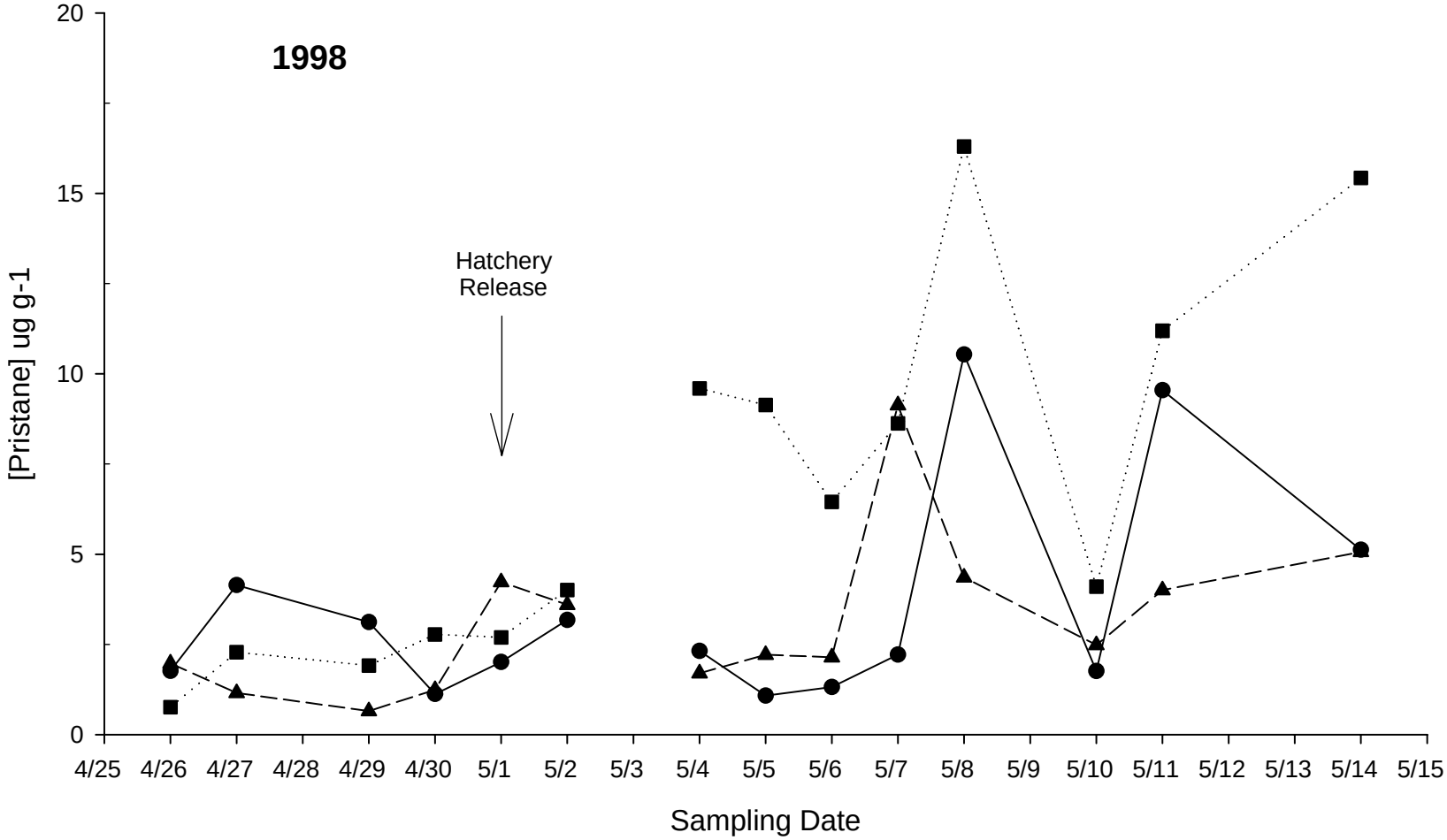


Figure 3.2. Pristane in mussels near W. H. Noerenberg hatchery, 1996. Stations: circle = Esther Light, square = Lake Bay, triangle = Hodgkins Point. Release date of 1.3 x 10^8 juvenile pink salmon from the hatchery is indicated by the vertical arrow.

Figure 3.3. Pristane in mussels near W. 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.

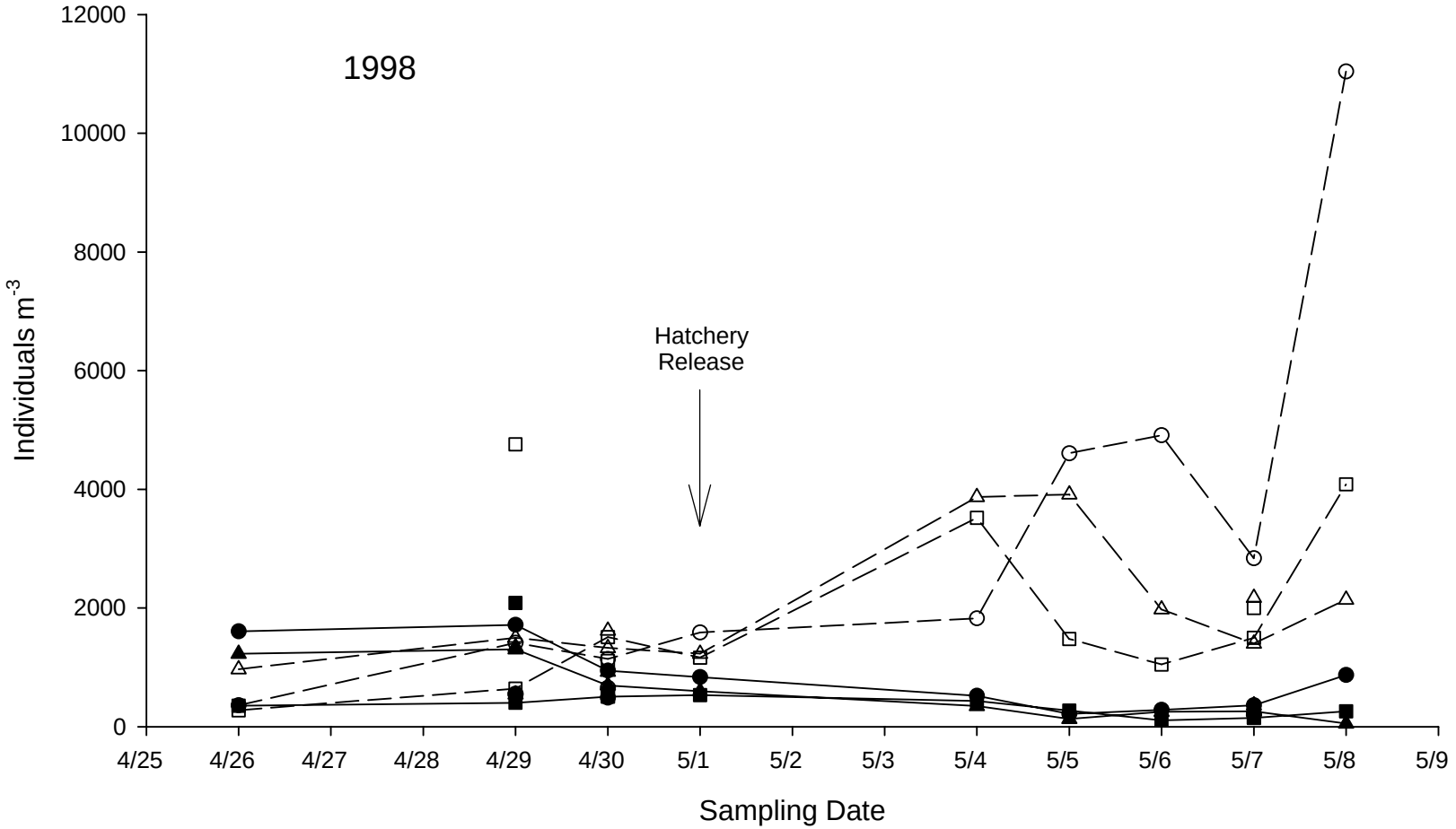


Figure 3.4. *Neocalanus* and *Pseudocalanus* copepod abundance. Stations: circle = Esther Light,square = Lake Bay, triangle = Hodgkins Point. *Neocalanus* = solid symbols and lines, *Pseudocalanus* = open symbols and dashed lines. Afternoon (4/29), late evening (4/30) and midnight (5/7) samples unconnected by lines, otherwise morning samples.

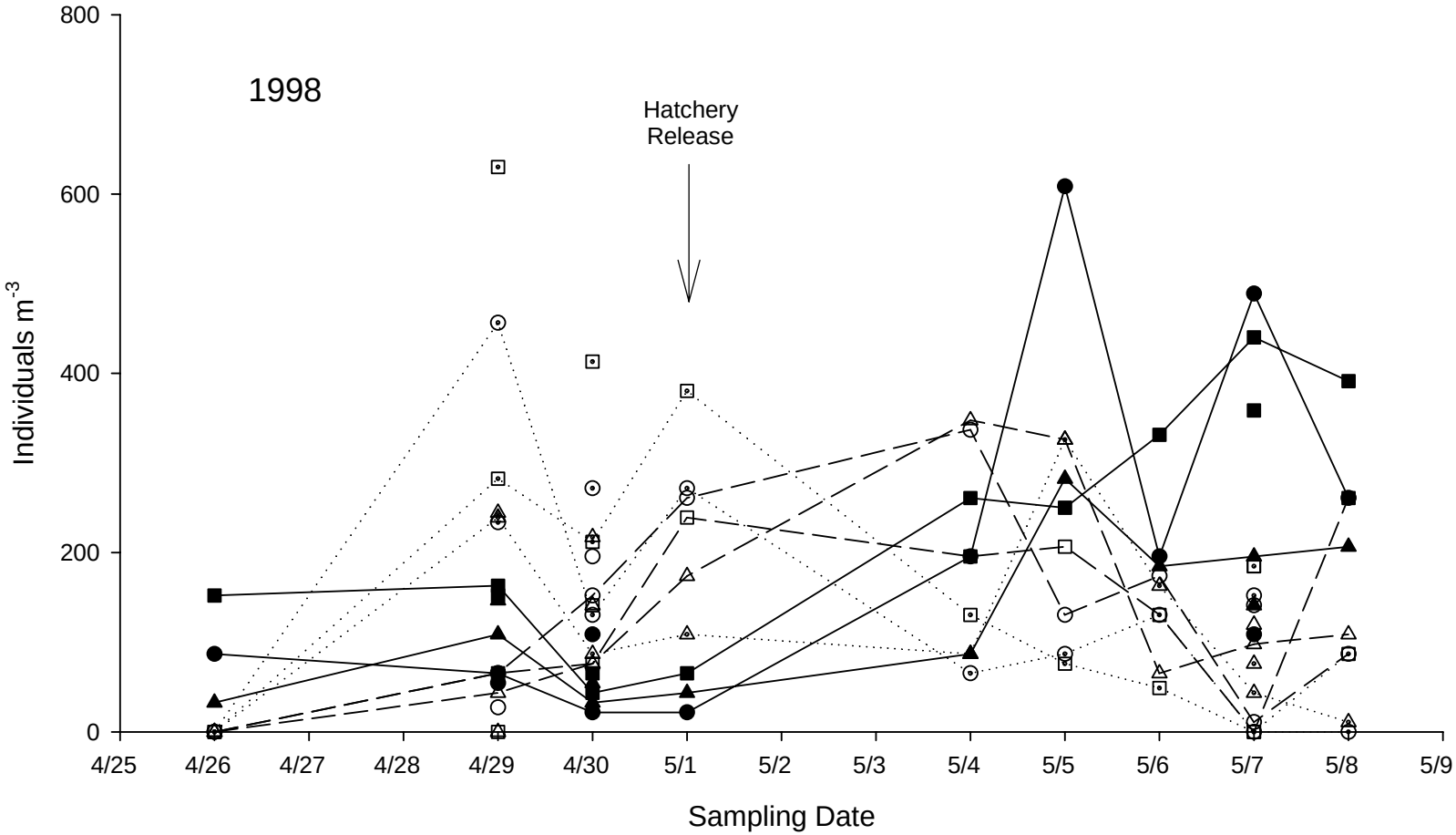


Figure 3.5. *Acartia*, and bryozoan larvae and *Oikopleura* abundance. Stations: circle = Esther Light, square = Lake Bay, triangle = Hodgkins Point. *Acartia* = solid symbols and lines, bryozoan larvae = open symbols and dashed lines, *Oikopleura* = dotted open symbols with dotted lines. Afternoon (4/29), late evening (4/30) and midnight (5/7) samples unconnected by lines, otherwise morning samples.

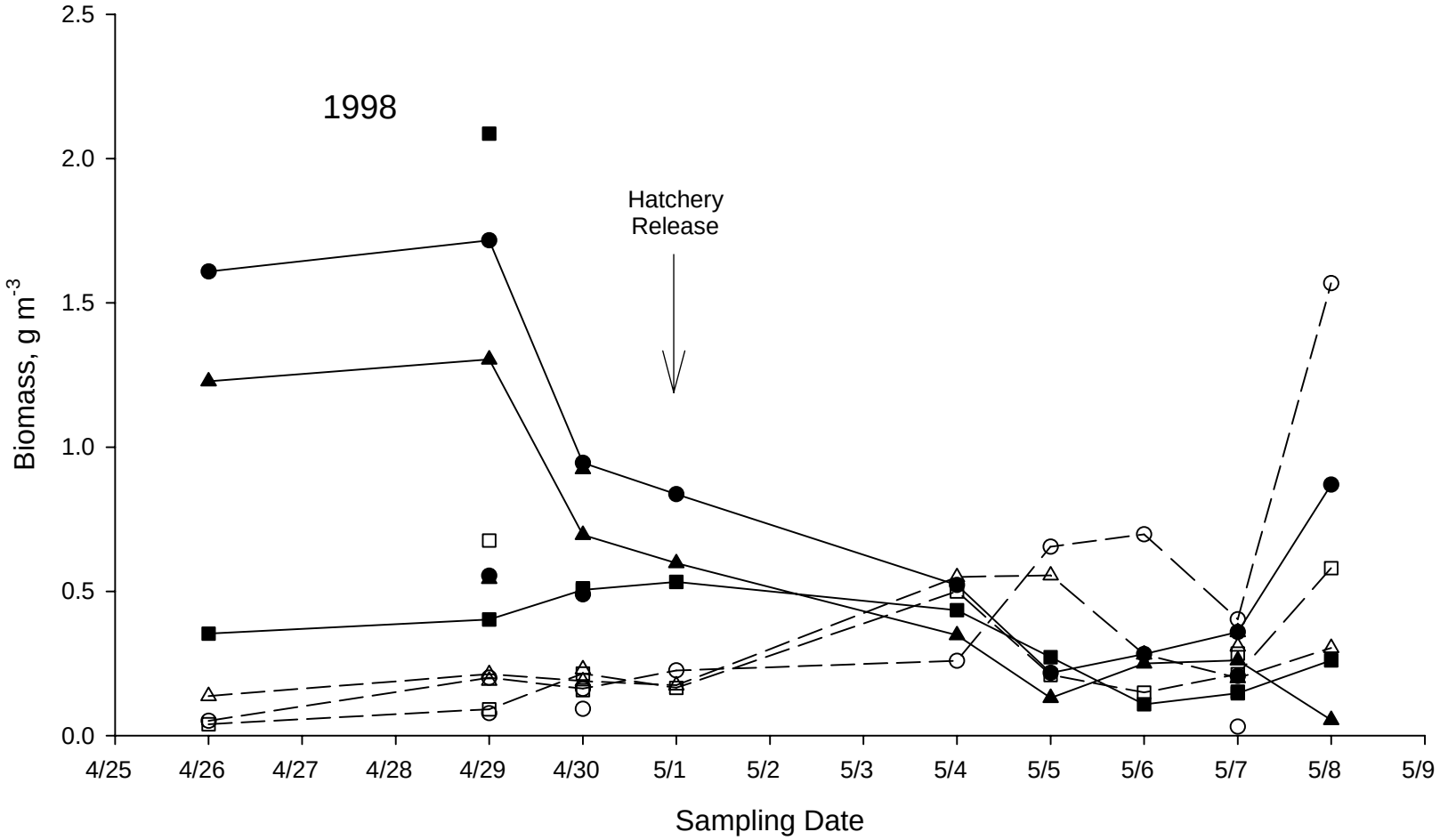


Figure 3.6. *Neocalanus* and *Pseudocalanus* biomass. Stations: circle = Esther Light, square = Lake Bay, triangle = Hodgkins Point. *Neocalanus* = solid symbols and lines, *Pseudocalanus* = open symbols and dashed lines. Afternoon (4/29), late evening (4/30) and midnight (5/7) samples unconnected by lines, otherwise morning samples.

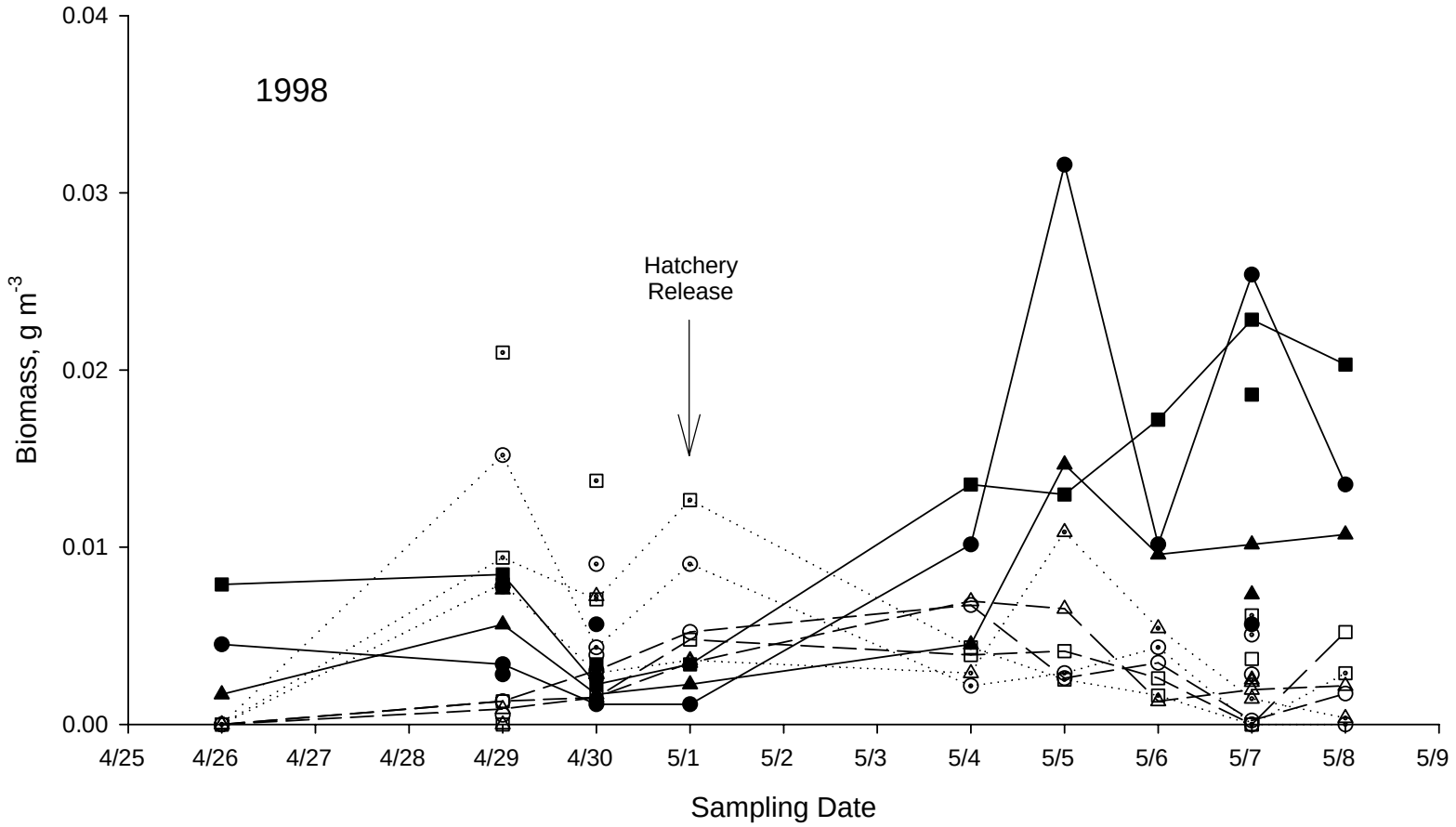


Figure 3.7. *Acartia*, bryozoan larvae and *Oikopleura* biomass. Stations: circle = Esther Light, square = Lake Bay, triangle = Hodgkins Point. *Acartia* = solid symbols and lines, bryozoan larvae = open symbols and dashed lines, *Oikopleura* = dotted open symbols with dotted lines. Afternoon (4/29), late evening (4/30) and midnight (5/7) samples unconnected by lines, otherwise morning samples.

Table 3.1. 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	$3.52x10^{-5}$ – 0.0316	0 – 0.00695	0 – 0.304	0.0543 – 2.09	0 – 0.0316	0 – 0.0210	0.0309 – 1.57	$7.63x10^{-4}$ – 0.0259	0.276 – 2.81

Table 3.2. 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)

Chapter 4

RELATIONSHIP BETWEEN PRISTANE ACCUMULATION BY MUSSELS (*MYTILUS TROSSULUS*) AND MARINE SURVIVAL OF PINK SALMON (*ONCORHYNCHUS GORBUSCHA*) IN PRINCE WILLIAM SOUND, ALASKA

Abstract

Pristane concentrations were monitored in bay mussels (*Mytilus trossulus*) from early spring through mid-summer each year of 1995 through 2001 at 27 stations in Prince William Sound (PWS), Alaska, to evaluate whether increases of pristane are related to the marine survival of pink salmon reared by PWS hatcheries. Mussels accumulate pristane through ingestion of feces produced by near-shore zooplanktivores such as juvenile pink salmon that prey on *Neocalanus* or *Calanus* copepods during spring, and large increases reflect high consumption of these copepods near shore.

Pristane increases were consistently greatest at stations on the western portion of PWS adjacent to the deepest seawater depths where *Neocalanus* copepods reproduce during winter. Pristane increases varied inconsistently among the stations monitored in western PWS from year to year, probably because of random components of factors affecting the distribution of copepod and zooplanktivore abundances rather than site-specific factors such as beach habitat type or proximity to a hatchery. Pristane accumulation by mussels at one of the monitored stations reflected accumulations by other mussels in the vicinity within about 2 km. Pristane accumulation by mussels throughout PWS remained at intermediate levels from 1995 through 1997, increased by ~ 50% during 1998 – 1999, then fell by factors of ~ 3 – 4 during 2000 – 2001, reflecting substantial interannual variability in the nearshore production of pristane at the stations monitored. Indexes of pristane accumulation by mussels were weakly correlated ($r = 0.667$, $P = 0.10$) with survival of hatchery pink salmon considered in aggregate, but were usually not correlated with survivals from individual hatcheries. Marine survival of pink salmon was not correlated among the four pink salmon hatcheries in PWS. A monitoring program that includes pristane concentrations in mussels during spring, augmented by data on zooplankton community composition and on predator abundances, holds promise for improving forecasts of pink salmon recruitment in PWS.

Introduction

In Prince William Sound (PWS), Alaska, foraging by nearshore zooplanktivorous fishes can be recorded by increases of pristane concentrations in mussels (*Mytilus trossulus*), which might provide an indirect and relatively inexpensive index of foraging conditions for these fishes (see Ch. 3, this report). Copepods in the genera *Neocalanus* and *Calanus* often dominate the springtime zooplankton biomass in PWS (Cooney 1986a, Cooney 1986b, Kirsch et al. 2000, Cooney et al. 2001), and stage V copepodites or adults contain substantial concentrations (~1% of dry mass) of pristane (2,6,10,14-tetramethyl-

hexadecane; Blumer 1964, and Ch. 1, this report). Pristane is biosynthesized by these copepods from chlorophyll (Avigan and Blumer 1968), and it is relatively persistent when released into the environment. Zooplanktivorous fishes preying on these copepod species produce feces containing traces of pristane, which can be efficiently accumulated by mussels after dispersion of the fecal matter in seawater (Ch. 2, this report).

Pristane concentrations in mussels often increase sharply in early spring by factors ranging to several thousand, mainly from accumulation of fecal material produced by near-shore zooplanktivorous fishes, and then decline during late spring and summer (Ch. 1, this report). Because a large increase in the concentration of pristane accumulated by mussels requires the simultaneous presence of *Neocalanus* and *Calanus* copepods and of their zooplanktivorous predators, the magnitude of the increase of pristane concentration in mussels may reflect the magnitude of carbon transfer from these copepods to their predators in immediately adjacent waters. Hence, monitoring the magnitude of pristane accumulation by mussels during spring may have some utility as an index of forage conditions for nearshore zooplantivorous fishes.

Favorable conditions for foraging on *Neocalanus* do not necessarily imply rapid growth of juvenile salmonids, however, because pristane inhibits their growth. Laboratory experiments show that ingestion of ~1% pristane in the (dry) diet, whether artificial fish food or in *Neocalanus* copepods, reduces food conversion efficiency by a factor of ~ 3 (Luquet et al. 1983, 1984, and Ch. 2, this report). Hence, fish that target *Neocalanus* or *Calanus* copepods must consume nearly three times the food compared with fish that do not to achieve equivalent growth, and they would produce three times the fecal material if equivalent growth were attained. During spring, the biomass of *Neocalanus* is roughly the same as the biomass of all other pelagic zooplankton combined (mostly *Pseudocalanus spp*.; Cooney et al. 2001, and Ch. 3, this report), but the *Neocalanus* are larger by a factor of ~ 5 or more. Preying on *Neocalanus* is thus efficient because of the relatively large ration presented by individuals of these copepods, but this advantage is partially offset by growth inhibition from the ingested pristane. As a result, juvenile fish that prey on zooplankton other than *Neocalanus* or *Calanus* may grow faster than fish that prey on these copepods, so the implication of large increases of pristane in mussels for the survival of juvenile zooplanktivorous fishes foraging nearby is not clear.

Prince William Sound is an especially favorable setting for investigating how the springtime increase of pristane in mussels may be related to the survival of juvenile pink salmon (*Oncorhynchus gorbuscha*). Juvenile pink salmon are often the dominant nearshore zooplanktivore during spring, especially following their release from hatcheries (Cooney et al. 1981). Pink salmon have a fixed two-year life span, are anadromous, and have high fidelity for their natal stream, characteristics which make them very attractive for study in the field. In Alaska, pink salmon typically spawn during summer, spend the fall and winter within freshwater-irrigated gravels of streams or intertidal beaches, and emerge during early spring to migrate to the sea (Heard 1991).

After ~ 16 months at sea they return to their natal habitat to spawn and then die. Because most surviving cohorts concurrently return to and concentrate at their natal stream, and are not interspersed among conspecifics of other year classes, it is relatively simple to monitor their marine survival. In PWS, pink salmon are reared at four large hatcheries, which may each release up to ~ 10^8 juveniles *en masse* (Johnson et al. 2002) bearing a thermally-induced otolith mark unique to the hatchery of origin (Willette 1996). This hatchery marking permits estimation of the contribution from the fishery to the marine mortality of the hatchery fish, permitting an especially accurate assessment of marine mortality prior to the fishery. The year-class strength of hatchery pink salmon is thought to be established during the initial phase of their marine residence, when mortality rates are greatest (Parker 1962, Parker 1968, Ricker 1976, Hartt 1980, Peterman 1987, Karpenko 1998, Willette et al. 2001). This combination of circumstances make hatchery reared pink salmon in PWS especially suitable for studying the relation between their marine survival and their foraging success during their initial marine residence, as reflected by the accumulation by mussels of fecal-borne pristane produced by the juvenile salmon.

The primary objective of the seven-year program monitoring pristane concentrations in mussels during spring in PWS is to provide a basis for evaluating whether increases of pristane could be related to the marine survival of pink salmon. Secondary objectives include characterization of the geographic pattern of these increases and their interannual variability, to determine whether portions of PWS had consistently higher increases than others.

Methods

Study Area

Prince William Sound is a small semi-enclosed sea adjacent to the northern Gulf of Alaska with a heavily indented shoreline, and containing numerous islands in the west (Figure 4.1). The sound was recently deglaciated (< 10,000 yr bp), and much of the shoreline consists of steep, rocky headlands and boulder-strewn beaches. Fine-particle beaches are not common, and are often found in "pocket beaches" of a few tens to hundreds of m length bracketed by rocky outcrops of similar lengths or longer. The maximum range of tidal excursion in PWS ~ 4.6 m, with a semi-diurnal tide typical of the Pacific coast of northwesthern North America.

A series of marine depressions at seawater depths below 400 m provide overwintering reproductive habitat for *Neocalanus* in PWS (Figure 4.1). Reproduction occurs during January and February in waters deeper than ~ 300 m during winter, followed by death of the adults, and the offspring develop while rising to the surface and grazing on the spring phytoplankton bloom (Fulton 1973, Damkaer 1977, Conover 1988, Miller and Clemons 1988, Miller 1993). This life-history strategy allows *Neocalanus* copepodites to efficiently incorporate the springtime maximum in primary production (Eslinger et al. 2001).

Mussels are common in the lower half of the intertidal of PWS, and are usually present on beaches or rock outcrops that are sufficiently protected from high-energy waves that would otherwise scour the substrate of epifauna. Winter storms may generate sustained wind speeds of 35 m s^{-1} or more, generating waves of several m height along exposed shorelines. The frequency of these storms declines with the approach of spring, although violent storms may occur during any season in PWS.

Three of the pink salmon hatcheries in PWS are operated by the Prince William Sound Aquaculture Corporation (PWSAC) and include the Armin F. Koernig (AFK) hatchery, the Wally H. Noerenburg (WHN) hatchery, and the Cannery Creek (CCH) hatchery. Another pink salmon hatchery is located in the Port of Valdez and is operated by the Valdez Fisheries Development Association (VFDA). The locations of these four hatcheries are shown in Figure 4.1. Outmigrating juvenile pink salmon tend to travel west along the northern margin of PWS and then south along the corridors formed by the islands in the western part of the sound. Returning adults follow the same path in reverse (Templin et al. 1996).

Mussel Collection

Mussels were collected from a network of 25 stations in PWS during each of the seven years of the monitoring period (Figure 4.1). Station names, locations and abbreviations are listed in Table 4.1. Each year, samples were collected during 7 – 9 synoptic collections beginning in March and ending in July or August. The rationale used to establish the locations of these stations required balancing broad geographic coverage with accessibility. Some of the stations were established near hatcheries operated by the Prince William Sound Aquaculture Corporation (PWSAC), and were sampled by hatchery staff. The remaining stations were visited by small aircraft equipped with floats to enable landing on the sea surface near remote beaches, and then moving the airplane to the beach to permit sample collection. The primary constraint on beach selection was the judgment of professional pilots regarding the likelihood that a plane could get to a beach under poor weather conditions. Despite these precautions, some stations occasionally could not be sampled because of weather.

Mussels were collected at two additional stations (Foxfarm 2 and Foxfarm 3) located ~ 2 km east and west of one of the 25 monitoring stations (Foxfarm 1) for all but the first year monitored, to assess the variability of results over km distances. At Point Pakenham in 2000, mussels were collected at each of 5 tidal elevations spanning the lower half of the intertidal to assess the effect of elevation on pristane content in mussels.

At each beach where mussels were collected, ten mussels were collected from selected mussel beds and placed into a polyethylene bag together with collection documentation. Selected mussels ranged 20 - 45 mm total length. Mussels were collected *ad libitum* within sampled mussel beds, with ~ 1 m separating each mussel collected. Mussels were

frozen at – 20 EC within 8 h of collection until analysis for pristane.

Dry Weight Determination
The ratio of dry and wet weight of tissue was determined by drying weighed sample aliquots at 65 EC for 24 h. This ratio was 0.110 ∀ 0.00160 (n = 1,975) for mussels collected during the seven years of the monitoring program.

Marine Survivals of Hatchery Pink Salmon
Marine survivals of hatchery pink salmon are estimated as the ratio of returning adults and the number of juveniles released. Estimates of the numbers of released juveniles are based on measurements of the numbers of eggs collected and estimates of mortality during rearing at the hatchery, and these mortality rates are usually small. Estimates of the numbers of adults returning to a hatchery are derived from two sources. First, all adult pink salmon caught within ~ 5 km of a hatchery during the period of the returning migration are assumed to have originated from the hatchery. Second, released juveniles received a hatchery identification mark, which was either a nearly microscopic wire bearing a binary code that was injected into the fish's snout, or was imprinted as variations in the daily circuli of otoliths by brief changes in water temperature. Wire tags or otoliths of adult pink salmon captured during the mixed-stock fishery within PWS are sampled and decoded by the Alaska Department of Fish and Game, and the proportion of the catch bearing a hatchery's mark is estimated based on the ratio of number of marked individuals among the sampled adults and the enumerated size of the landed catch.

Pristane Analysis
The chemical analysis of mussels for pristane involved pentane 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 accuracy of the pristane analyses was generally within ∀15% based on comparison with an authentic hydrocarbon standard prepared by the National Institute of Standards and Technology, 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 :g. The corresponding MDL estimate for individual samples is the ratio of this value and the mass of the sample analyzed. The mean MDL was 0.278 ∀ 0.00153 :g g^{-1} on a dry tissue mass basis (n = 1,975), and ranged from 0.0577 – 5.23 :g g^{-1}.

Data Analysis
The integrated accumulation of pristane by mussels at each site throughout the spring and summer is summarized by a pristane accumulation index (PAI), which is a step-wise

approximation of the integral of pristane concentration in mussels over time:

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

where $[P]_i$ is the pristane concentration measured in mussels collected at time t_i (i = 1,...,I), for mussels collected on I successive samplings throughout the collection season from the same site. This approximation method is used because it does not require equally spaced sampling intervals, or that sampling begin and end on exactly the same dates among different sites, and missed samplings are readily accommodated. These are considerable advantages of practicality for a long-term sampling program involving many stations that may not always be accessible due to poor weather. It is, however, necessary that $[P]$ at t_1 and at t_I be near the annual minimum concentration, and that the number of samplings (I) be sufficiently numerous to adequately describe the shape of the accumulation profile in mussels. These requirements were fulfilled for the results reported herein.

Interannual differences of overall pristane accumulation by mussels across all of PWS are compared using two measures. The first is simply the sum of the PAI's across the 25 stations sampled during each of the years monitored (ΣPAI). The second is the magnitude of the first principal component (PC1) of the matrix of correlations among PAI's of the 25 sampling stations (i.e. principal component analysis, or PCA). This PC1 is the linear combination of PAI's from the 25 stations that account for the greatest proportion of interannual PAI variability. The weighting factors for each station that define PC1 reflect the extent to which station PAI's are intercorrelated, hence PC1 has the effect of suppressing contributions from stations that are anomalous. The weighting factors that define PC1 are listed in Table 4.1.

Differences among pairs of pristane concentrations in mussels are evaluated statistically using least-significant difference (LSD) criteria based on an extensive sampling of the error distribution for these measurements. An error distribution for log-transformed pristane concentrations in mussels was generated from 178 triplicate and 79 duplicate samples analyzed for the *Exxon Valdez* oil spill, which are contained 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. These replicated samples were collected and analyzed by similar methods, and they all contained pristane concentrations above method detection limits. The variances of these replicates were homoscedastic after log transformation. A distribution for differences of two random samples of the error distribution can be generated by Monte Carlo simulation. Based on this distribution of differences, the LSD at $\alpha = 0.05$ type I error rate is about 1.015, which corresponds to a ratio of about 2.75 for un-transformed data. Thus, mussels from two different samples are judged significantly different if the ratio of the larger pristane concentration to the smaller is more than 2.75. The power of this test to detect an actual increase of 3 is about

58%, again derived from Monte Carlo simulation of the error distribution.

Propagation of errors for derived indices indicates that 66% changes in the PAI (eq 1) are significant at the $\alpha = 0.05$ type I error rate. The power of these criteria to detect an actual doubling of the PAI is about 80%, estimated by Monte Carlo simulation. The power to detect ΣPAI differences among years is greater, due to the larger number of measurements involved: differences of 22% are significant, and the power to detect such increases when they occur is about 50%.

The marine survival of juvenile pink salmon released by hatcheries in PWS is examined by correlation and by PCA to assess whether these survivals are intercorrelated among the four hatcheries across years. To evaluate the significance of the dominant eigenvector (PC1) of the correlation matrix of annual survivals among hatcheries, we randomly re-assigned the observed survivals (with replacement) and calculated the PC1 eigenvalue 1,000 times, and then we took the proportion of eigenvalues that were greater than the eigenvalue calculated for the original data as the estimate of the significance level (type I error rate). We used this same method to evaluate the significance of the PC1 associated with PCA of pristane concentrations in mussels from the three Foxfarm stations.

We used the Pearson correlation coefficient to evaluate whether a significant relationship exists between the results for pristane accumulation by mussels in PWS and the marine survival of juvenile pink salmon. We evaluated this relationship at two spatial scales. For PWS as a whole, we calculated the correlation coefficient of either the ΣPAI or the PC1 value (derived from the PCA of the pristane concentrations in mussels) and the survival of pink salmon released by all four hatcheries combined. We performed a similar comparison with survivals from individual hatcheries, to examine whether survivals from each hatchery were correlated with either of these two indices of pristane accumulation by mussels throughout PWS. At a smaller spatial scale, we calculated correlations using a reduced ΣPAI index (ΣPAI^r), which only included data on pristane in mussels from stations within ~ 50 km of a hatchery, and survivals from the hatchery. Results from each of the hatcheries were also combined to determine whether the aggregated indexes were correlated with respective hatchery survivals. Because spurious correlations may result from autocorrelation within the time series of the indices of pristane accumulation by PWS mussels or the survivals of pink salmon, we evaluated the significance of autocorrelation by linearly regressing each time series with itself lagged by one year, and calculating the probability of type I error for the slope of the regression.

Results

Spatial and Temporal Variability of Pristane in Mussels

Pristane concentrations in mussels increased during spring, often sharply, especially at stations on the western side of PWS. Results for 1995 are depicted in Figures 4.2 and

4.3, and the same seasonal pattern was repeated each succeeding year (Figures 4.4 – 4.5). Pristane increases were consistently greatest and most persistent at stations to the west of a line from Valdez Narrows through Montague Strait (Figure 4.1).

Pristane concentrations were consistently above annual station averages only at Point Eleanor during the period 1995 through 2001 (Figures 4.4 – 4.5). The Foxfarm 1, Esther Island and Herring Point stations were above their annual averages during 4 of the 7 years. Five stations were variable, above the annual average during 3 or 4 of the last 7 years, including the AFK hatchery, Applegate Island, Fairmont Island, Perry Island and Point Pakenham.

In contrast, 7 stations were consistently below annual averages, and another 9 stations were below for all but 1 or 2 of the 7 years. These stations include all 7 of the stations eastward of a line running from Montague Strait to Valdez Narrows, 3 stations in distal fjords (Cannery Creek, Decision Point, and Division Point), 3 stations along the western coastline of Knight Island Passage (Main Bay, Chenega Island, and Fleming Island), 2 stations on the Naked Island complex (Naked Island and Storey Island), and the station on the east coast of Knight Island (see Figure 4.1).

The ΣPAI index varied considerably during the seven-year study period (Table 4.2). The ΣPAI index had intermediate values ranging from 8,670 – 9,200 :g g^{-1} d during 1995-1997 that did not differ significantly using the LSD criterion (see Methods). The index was significantly higher in 1998 and 1999 at ~ 12,600 :g g^{-1} d, and was significantly lower in 2000 and 2001 at 3,000 – 5,000 :g g^{-1} d.

Pristane concentrations in mussels at the three Foxfarm stations were moderately correlated. Correlation coefficients among station pairs ranged from 0.57 to 0.89, and were very highly significant ($P < 0.001$, $df = 45$). The dominant eigenvalue of the PCA of these results accounted for 83% of the variation in the pristane concentrations among mussels, and was very highly significant ($P < 0.001$) based on comparison with dominant eigenvalues generated by PCA of randomly assigned concentrations among stations and years (see Methods).

Pristane concentrations in mussels varied little with vertical tide height within the mussel bed sampled a Point Pakenham in 2000. These concentrations ranged from 1,450 :g g^{-1} – 2,790 :g g^{-1} except for the sample from the lower edge of the mussel bed, where the concentration was 748 :g g^{-1}.

Coherence of Marine Survival of Hatchery Pink Salmon

The marine survivals of pink salmon released by the hatcheries in PWS were not significantly inter-correlated. The strongest correlation coefficient was $r = 0.68$ ($P = 0.10$, $df = 5$) between the AFK and WHN hatcheries. Other correlations were considerably weaker ($r < 0.47$) and sometimes negative. The eigenvalue of the dominant

eigenvector of the correlation matrix accounted for 49% of the variation in the survivals among hatcheries, and was not significant (P = 0.48). The marine survivals varied considerably among hatcheries, with no one hatchery consistently accounting for a disproportionate share of the aggregate survival of hatchery pink salmon in PWS (Figure 4.6).

Relation of Interannual Variability of Pristane in Mussels and Marine Survival of Pink Salmon

Taken across PWS as a whole, the marine survivals of hatchery pink salmon were only weakly related to springtime increases of pristane in mussels. Correlation of the marine survivals from the hatcheries combined against the ΣPAI index was not significant (r = 0.667, P = 0.10, df = 5; Figure 4.7), but nonsignificance may be due to the relatively low statistical power present. The correlation of survivals and the PC1 from the PCA of pristane concentrations in mussels produced similar results (r = 0.584, P = 0.17, Figure 4.8). The PC1 accounted for 35% of the variation in the concentrations of pristane in the mussels, and the weighting factors that define this PC1 indicate that contributions from the individual stations to the eigenvalue are broadly similar except at the CCH hatchery and the Foxfarm station (Table 4.1).

Marine survivals of pink salmon released from the CCH were strongly correlated with the ΣPAI index (r = 0.894, P = 0.0066) and with the PC1 from the PCA of pristane concentrations in mussels (r = 0.923, P = 0.0031), in contrast with correlations associated with the other hatcheries ($|r| < 0.529$, $P > 0.22$), where correlation coefficients were sometimes negative (Figures 4.7 and 4.8).

Correlations derived from analysis at smaller spatial scales were variable. Coefficients of regression of survivals with the (ΣPAI^r) index for each hatchery considered individually ranged from r = -0.361 to r = 0.830 (Figure 4.9), only the largest of which was significant (for CCH: P = 0.021). When the survival-(ΣPAI^r) data from the hatcheries were combined, the regression coefficient was r = 0.127, which was not significant (P = 0.51, df = 26; Figure 4.9).

Neither the marine survivals of hatchery pink salmon nor the ΣPAI index were significantly autocorrelated. The regression coefficients for the slopes of the autocorrelation regressions were r = 0.341 (P = 0.51, df = 4) and r = 0.400 (P = 0.432) for survival and for the ΣPAI index, respectively. Although these time series are too brief to permit a statistically powerful assessment of autocorrelation, these results suggest that contributions from autocorrelation to the correlations noted above between survivals and indices of pristane accumulation by mussels are likely negligible.

Discussion

The two most important pathways followed by pristane from its source in *Neocalanus* and *Calanus* copepods to mussels are feces produced by their predators, followed by

feces produced by the copepods themselves (Ch. 3, this report). Increases of pristane concentrations derived from copepod feces are probably limited to less than ~ 3 :g g^{-1} (Ch. 3, this report), but this may account for an appreciable portion of the pristane accumulated by mussels in late March (Figure 4.2). Feces produced through predation on these copepods contain higher concentrations of pristane and may be more readily transported to mussel beds (Ch. 3, this report), and the greater part of the pristane in mussels is probably accumulated through this pathway. The predators involved are likely zooplanktivores that reside close to shore such as sand-lance (*Ammodytes hexapterus*), gunnels (*e.g. Pholis laeta*), cockscombs (*Anoplarchus spp*.) and other Stichaeidae, and perhaps juvenile herring (*Clupea harengus*) (Mecklenburg et al. 2002). Outmigration of wild juvenile pink salmon usually does not begin until mid-April (Cooney et al. 1995), and hence is not likely a substantial contributor of pristane-laden fecal material for accumulation by mussels in late March.

The association of the largest increases of pristane concentrations in mussels during spring with proximity to the deepest seawater depths (Figures 4.2 – 4.3) suggests that the population of *Neocalanus spp*. that overwinters and reproduces within PWS may be the source of most of the pristane accumulated by the mussels. Thermohaline stratification conditions conducive to the spring phytoplankton bloom are first established during early to mid-March in the more protected embayments of PWS, especially in the northwestern portion of the sound, becoming more widespread throughout PWS during the ensuing 1 – 2 weeks (Eslinger et al. 2001, Wang et al. 2001, Gay and Vaughan 2001). Developing *Neocalanus* copepodites originating from the deepest parts of PWS are ideally situated to graze on the developing phytoplankton bloom (Figure 4.1). While evidence from stable carbon and nitrogen isotopes indicates that considerable proportions of adult *Neocalanus* overwintering in the marine depressions of PWS originate from the Gulf of Alaska, presumably entering PWS during summer or fall by advection (Kline 1999), it seems likely that much of the carbon incorporated in their offspring is derived from local phytoplankton production. Production of pristane-laden feces in this region would be greatly facilitated by releases of juvenile pink salmon from hatcheries, because the migration path followed by these fish generally coincides with the overwintering habitat of *Neocalanus spp*. (Figure 4.1). However, concurrent outmigration of wild juvenile pink salmon would also increase the abundance of near-shore zooplanktivores in eastern PWS, yet pristane increases in mussels there were substantially lower than western PWS, corroborating the importance of the local overwintering habitat for production of *Neocalanus* copepodites in spring.

The main reason pristane accumulation was so inconsistent across years at most of the monitoring stations is probably the result of random variation in the co-occurrence of *Neocalanus* copepods and their predators (especially juvenile pink salmon), rather than site-specific factors such as beach slope and aspect, beach particle size distribution, proximity to hatcheries or major pink salmon-producing streams, tidal elevation at sampling etc. The opportunistic selection of station locations did not permit much

consideration of site-specific factors beyond mussel availability, hence the beaches at these stations span a variety of morphological characteristics. Despite these differences, most mussels on beaches on the western side of PWS accumulated substantial concentrations of pristane during some, but rarely every year. This suggests that oceanographic factors that concentrate zooplankton and the foraging behavior that leads juvenile pink salmon to search for these concentrations often override the importance of site-specific factors associated with the selected monitoring-station location. Variable wind- and tidally-driven currents change the locations of these zooplankton concentrations on time scales of days to weeks, hence considerable interannual variability in the locations of these concentrations during spring is to be expected. Because of this, the fact that the monitoring stations were chosen opportunistically rather than randomly is less problematic than might otherwise be the case: the complex interaction of local winds and tidal currents with the heavily indented shorelines typical of PWS leads to a sampling environment that is characterized by a considerable degree of sampling isotropism. This conclusion is further corroborated by the degree of intercorrelation of pristane concentrations in mussels over distances of several km at the Foxfarm stations, and by the general absence of differences among mussels sampled across the vertical excursion of tidal heights within mussel beds. This last is important because often only the upper portion of a mussel bed was available for sampling owing to the constraints imposed by sampling areas accessibly by aircraft.

The four-fold interannual range spanned by the ΣPAI index (Table 4.2) reflects substantial variation in the availability of *Neocalanus* copepods as prey for near-shore zooplanktivores, especially juvenile pink salmon. Hatchery production of pink salmon insures a large and fairly constant abundance of juvenile pink salmon introduced into PWS during the seven-year study period (Table 4.2), and these juveniles remain very close to shorelines during the initial phase of their marine residence (Healey 1980, Cooney et al. 1981, Willette 2001). The interannual variability of pristane in mussels therefore probably reflects variation in the abundance of *Neocalanus* and *Calanus* copepods rather than variation in the abundance of near-shore zooplanktivores. The variation in the abundance of *Neocalanus* and *Calanus* copepods may reflect variation in contemporaneous primary production, or variation in the composition in the springtime zooplankton community, with *Neocalanus* and *Calanus* copepods accounting for a smaller proportion of the community in 2000 or 2001 compared with 1998 or 1999. Whatever the reason, the lower pristane accumulation by mussels in 2000 and 2001 probably reflects a substantial change in the nature of the zooplankton prey field compared with prior years.

The lack of intercorrelation among the marine survivals of hatchery pink salmon indicates that any relation between these survivals and pristane in mussels should at least be considered at two spatial scales. Conditions favorable for pink salmon clearly fluctuate from year to year in PWS, and these changes are related to interannual variation in environmental conditions such as sea surface temperature that affect PWS as a whole

(Wertheimer et al. 2004). The absence of intercorrelation among the marine survivals of hatchery pink salmon indicates that effects on survivals associated with localized conditions within the sound are comparable with effects that affect PWS as a whole, for otherwise the hatchery survivals would be intercorrelated. Thus, the survival of pink salmon released from individual hatcheries may have a large random component, comparable with the variability of the combined survival. Field sampling indicates that juvenile pink salmon released from hatcheries remain poorly mixed within PWS, and that the condition of these juveniles remains strongly dependent on sampling location, during their first three months of marine residence (Boldt and Haldorson 2004). The survival performance of individual hatcheries should therefore also be evaluated to determine the importance of conditions in their vicinities. The absence of correlation of marine survivals among the four hatcheries suggests that the marine survival may in large part be determined by events initially following release of the pink salmon, before these hatchery fish have time to mix within PWS.

The nonsignificant but suggestive correlation between the aggregate marine survival of hatchery pink salmon and either the ΣPAI index or the PC1 from the PCA of pristane concentrations in mussels suggests that it may yet be possible to construct a monitoring program that would have some value for predicting the marine survival of these fish for PWS as a whole. If the low significance is merely the result of inadequate statistical power resulting from too few years monitored, the strength of the correlation might improve if the monitoring were extended to include more years, but it is also possible that the correlation might become even less significant. In comparison, the lower correlations observed between marine survivals for a specific hatchery and the ΣPAI^r index for stations adjacent to it (CCH excepted) suggests that while the ΣPAI index may have some questionable potential to reflect favorable conditions for recruitment in PWS as a whole, it may be inadequate at smaller spatial scales. Note that the broadly similar correlations between marine survivals and either the ΣPAI index or the PC1 follow from the fact that contributions from most stations to PC1 are similar. Hence, inclusion of results that are occasionally anomalous from some stations does not have a large effect on the ΣPAI index because they are averaged over the more numerous remaining stations, which tend to vary coherently.

Two advantages of the ΣPAI as a monitoring index are that it tends to integrate both favorable forage conditions for pink salmon as well as predation impacts, and it is relatively inexpensive, making coverage of broad geographic areas practical. The large increases of pristane in mussels during spring require the simultaneous presence of abundant *Neocalanus* copepods, and of abundant zooplanktivores such as pink salmon. Years of low primary productivity will constrain the abundance of the former, and intense predation will constrain the latter. High abundances of *Neocalanus* copepods provide more readily available prey for juvenile pink salmon, and might correlate with increased abundances of other zooplankton species as well, which would favor salmon survival. High abundances of *Neocalanus* copepods may also provide a predation

shelter for juvenile pink salmon, wherein piscine predators of juvenile pink salmon target *Neocalanus* copepods instead during years of high copepod abundances (Willette 2001, Willette et al. 2001).

However, predation impacts on juvenile pink salmon are imperfectly reflected by the ΣPAI index, because ingestion of pristane inhibits the growth of salmonid predators of *Neocalanus* or *Calanus* (Luquet et al. 1983, 1984, and Ch. 2, this report), and it seems likely that growth of other zooplanktivorous fishes that prey on these copepods would be inhibited also. Secondary consumers that prey selectively on these copepods may consequently suffer impaired growth and hence increased vulnerability to size-selective predation subsequently, and such subsequent mortality would not be reflected by the ΣPAI index. Hence, it is possible that large increases of pristane concentrations in mussels during spring may at once reflect (1) favorable foraging conditions for juvenile pink salmon in general, with a variety of abundant prey alternatives available, (2) a more effective predation shelter for juvenile pink salmon, through provision of abundant alternative prey for their predators such as pollock (*Theragra chalcogramma*), but also (3) a higher size-selective predation risk for juvenile pink salmon, if these juveniles experience slow growth rates as a result of ingesting pristane contained in *Neocalanus* and *Calanus* copepods. The inability to distinguish which is more important, and the possibility that their importance may fluctuate from year to year, may largely account for why the index is not more closely associated with the overall marine survival of pink salmon in PWS. The correlation between the ΣPAI index and survival of hatchery pink salmon would have improved considerably had survivals been lower for the releases in 2000 and 2001, and the higher survivals realized despite the low ΣPAI index values for those years might simply reflect high abundances of zooplankton prey other than *Neocalanus* or *Calanus* copepods during spring of those years, but no data are available to support this conjecture.

A number of other factors would tend to weaken the correlation of the ΣPAI index and the marine survival of pink salmon in PWS, especially as the spatial scale of the index is reduced. The release strategy used by hatcheries was not consistent during the period monitored – ranging from releases of a few millions in each of up to 14 release groups throughout the spring, to several tens of millions in one or two release groups, as hatchery managers sought strategies to reduce initial predation losses. These changes in release strategy could affect the relation between the ΣPAI index and marine survival, especially with regard for the ΣPAI^r index applied to stations nearest a hatchery and survival of salmon released by the hatchery. Another important factor is variability in marine survivals after juvenile pink salmon leave the near shore a few weeks after beginning their marine residence. While most marine mortality probably occurs within the first few weeks of marine residence (Parker 1962, Parker 1968, Ricker 1976, Hartt 1980, Peterman 1987, Karpenko 1998, Willette et al. 2001), any variability in subsequent mortality would not be related to the ΣPAI index. Also, other near-shore zooplanktivores preying on *Neocalanus* or *Calanus* copepods may serve to obscure the relation of

pristane accumulation by mussels and survival of juvenile pink salmon, the overall association between the spatial distributions of near-shore zooplanktivores and the monitored mussel beds may vary interannually, and interannual variation in storms and wind-driven currents may lead to differences in the advection of fecal material produced by zooplanktivores toward or away from monitored mussel beds. Given this range of possibilities, it is doubtful that the ΣPAI index would explain most of the variation in survival of hatchery pink salmon, but with refinements it might yet have some utility for explaining a useful portion of it.

Results from an intensive multidisciplinary investigation of the factors affecting the marine survival of pink salmon confirm earlier work emphasizing the importance of both "bottom-up" (i.e. production) and "top-down" (i.e. predation) processes (Willette et al. 2001, Willette 2001) in PWS. As noted above, an advantage of the ΣPAI index is that it provides an inexpensive (if imperfect) indication of zooplankton consumption by near-shore zooplanktivores over a broad geographic area, and might be useful for characterizing interannual changes in zooplankton forage abundance interannually for PWS as a whole. If this index were combined with data on zooplankton composition and predator abundance, especially near hatcheries during spring, it might be possible to construct a composite index incorporating data from these three sources that has some value for forecasting recruitment of pink salmon to the PWS fishery. This could potentially lead to a more precise identification of the factors affecting the early marine survival of pink salmon, which may help improve management of this important fishery in PWS.

Conclusions

Concentrations of pristane increase dramatically in mussels of PWS during spring, especially in the western portion of the sound at monitoring stations near deep-water overwintering and reproductive habitat for *Neocalanus* and *Calanus* copepods, the source of the pristane. Mussels accumulate pristane through ingestion of feces produced by near-shore zooplanktivores that prey on *Neocalanus* or *Calanus* copepods. Factors determining stations where large increases of pristane concentrations occur appear to contain substantial random components, which were probably more important than site-specific characteristics of the stations monitored. Comparison of increases of pristane in mussels at monitoring stations occupied from 1995 through 2001 shows that production of pristane-laden feces changed little from 1995 through 1997, increased by 50% in 1998 and 1999, and then declined by a factor of 3 – 4 in 2000 and 2001, which probably reflects substantial interannual differences in the abundances of *Neocalanus* or *Calanus* copepods near shore during spring.

An index of pristane concentration increases in mussels was weakly correlated ($r = 0.667$) with the marine survival of juvenile pink salmon released by large hatcheries in PWS at a significance level of $\forall = 0.10$, suggesting that production of pristane might be related to marine survival in PWS considered as a whole, but more monitoring years are

necessary to determine whether this relationship can be established more clearly. Attempts to find consistent correlations between the index and marine survival of hatchery pink salmon at smaller spatial scales within PWS generally failed. However, a combination of pristane monitoring, zooplankton composition monitoring and predator monitoring near hatcheries may enable construction of a more robust index for forecasting recruitment of pink salmon to the PWS fishery through assessment of factors favoring survival during the initial phase of marine residence.

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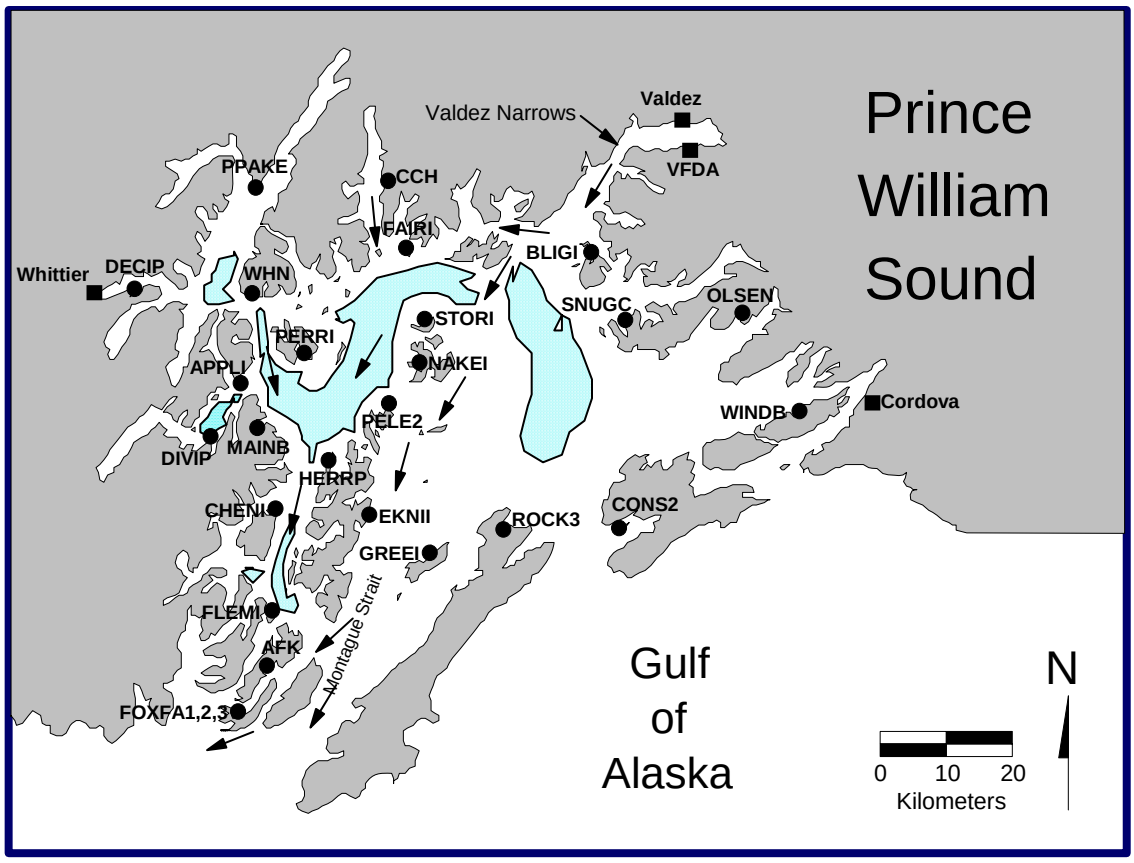

Figure 4.1. Mussel sampling stations in Prince William Sound, Alaska. Sampling stations denoted by filled circles (See Table 4.1 for abbreviations). Arrows indicate path of outmigrating juvenile pink salmon. Shaded areas indicate 400 m isobath (overwintering habitat for *Neocalanus spp.*) Filled squares indicate towns and VFDA hatchery. Other pink salmon hatcheries: AFK – Armin F. Koening, CCH – Cannery Creek, WHN – Wally H. Noerenburg.

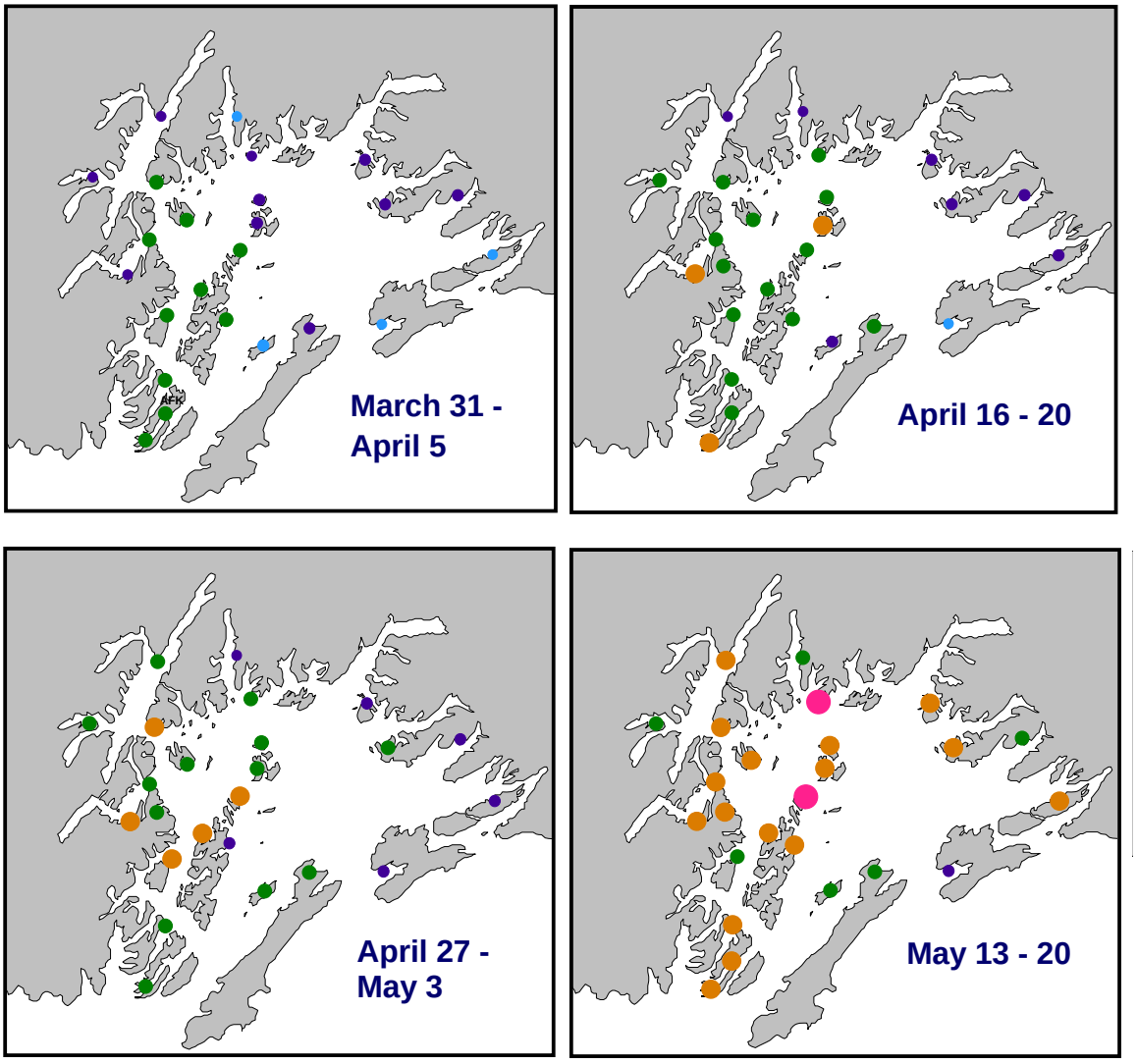

Figure 4.2. Pristane in mussels during April and May, 1995. Pristane concentrations at each station and sampling are coded according to the legend above.

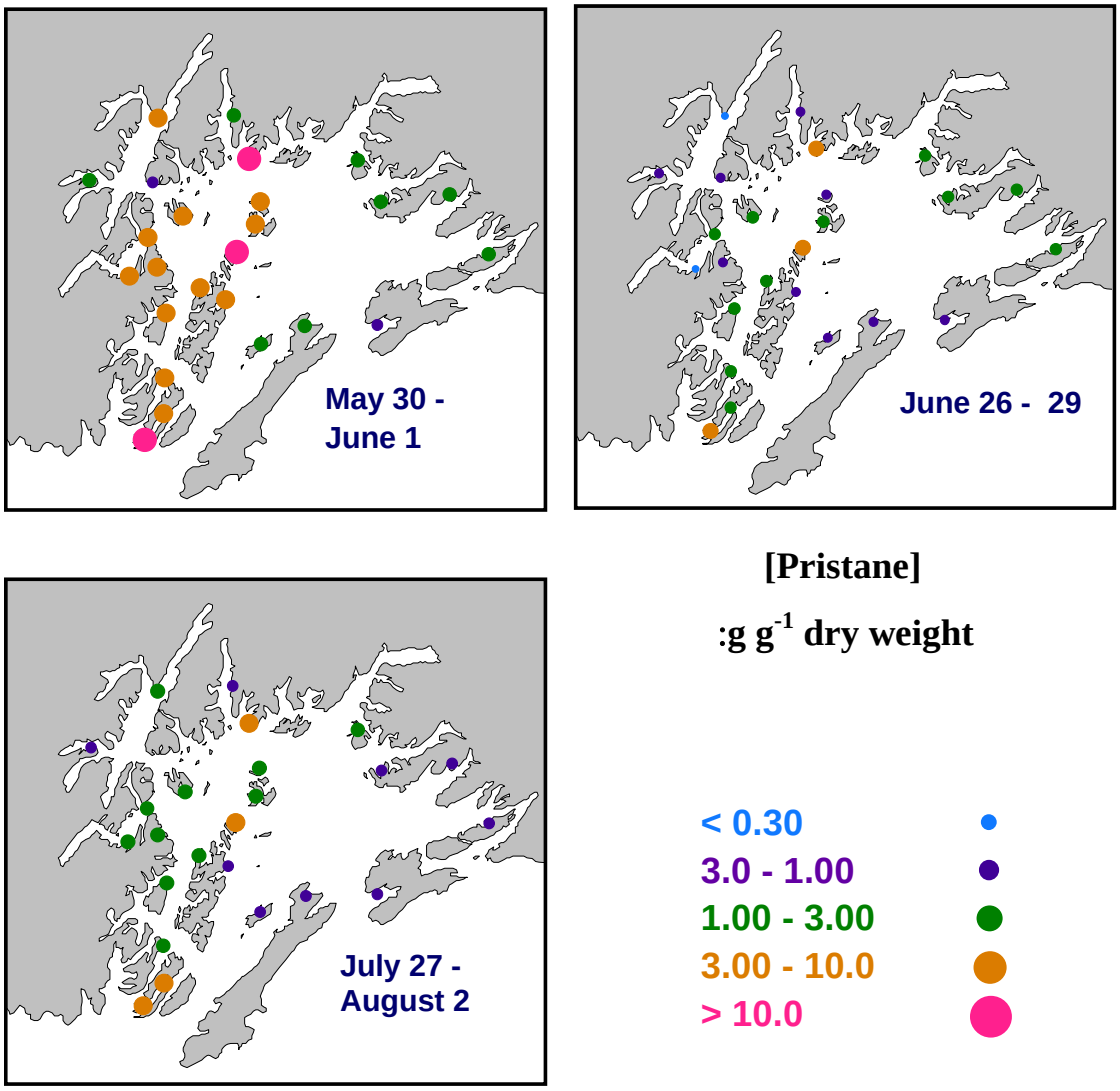

Figure 4.3. Pristane in mussels during late May through early August, 1995. Pristane concentrations at each station and sampling are coded according to the legend above.

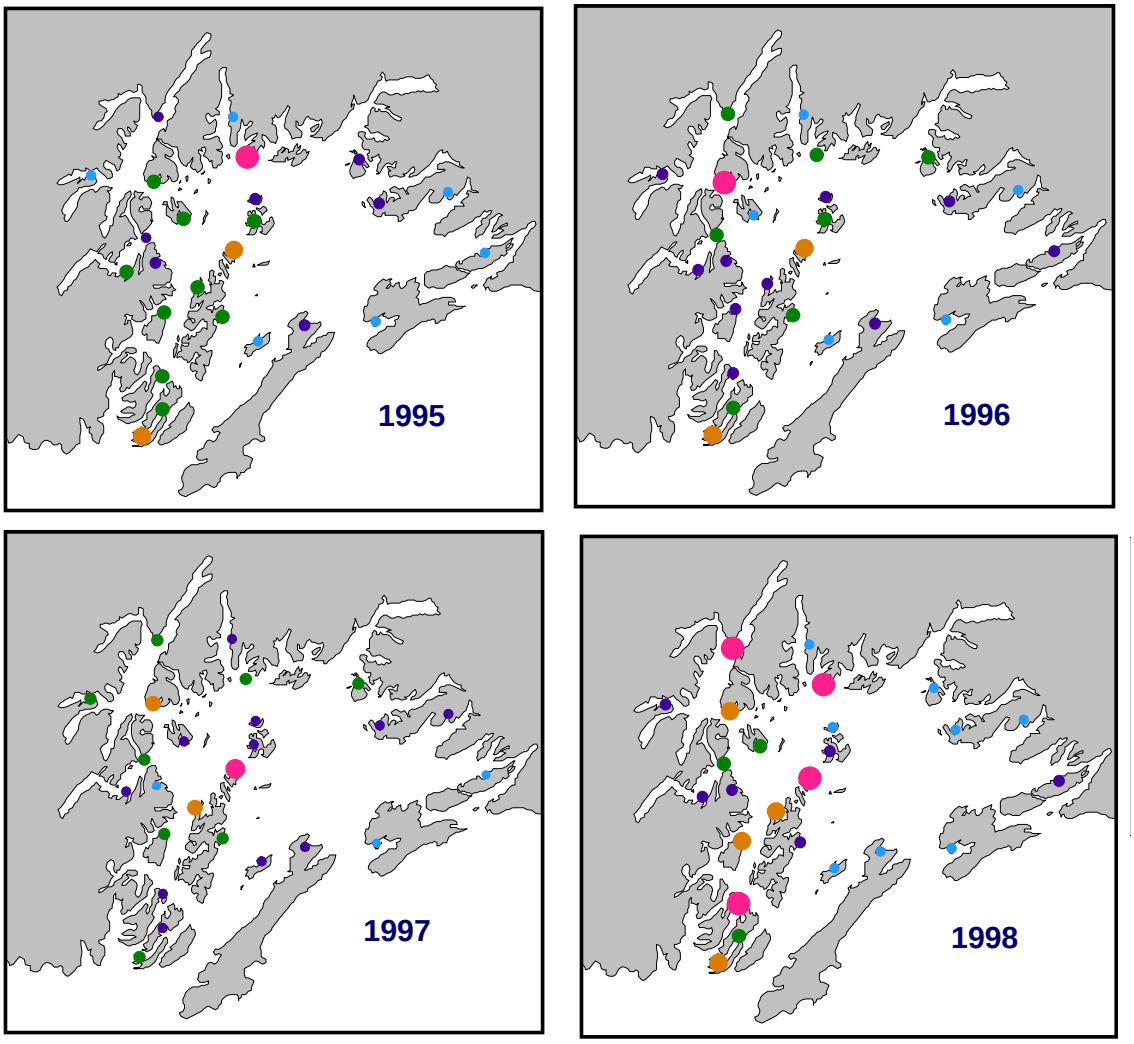

Figure 4.4. Pristane accumulation index (ΣPAI) for 1995 – 1998. Index values for each station are coded according to the legend above.

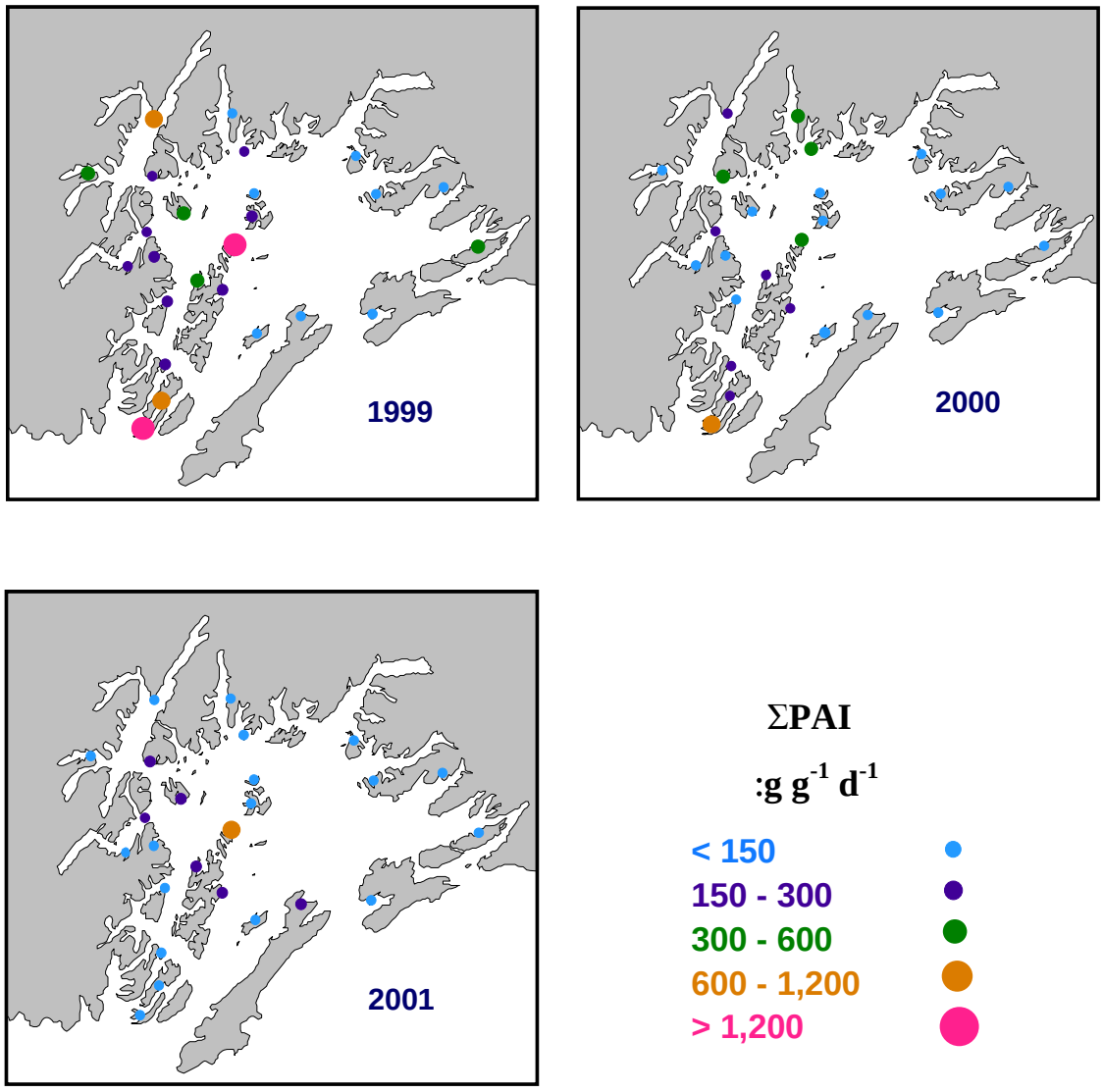

Figure 4.5. Pristane accumulation index (ΣPAI) for 1999 – 2001. Index values for each station are coded according to the legend above.

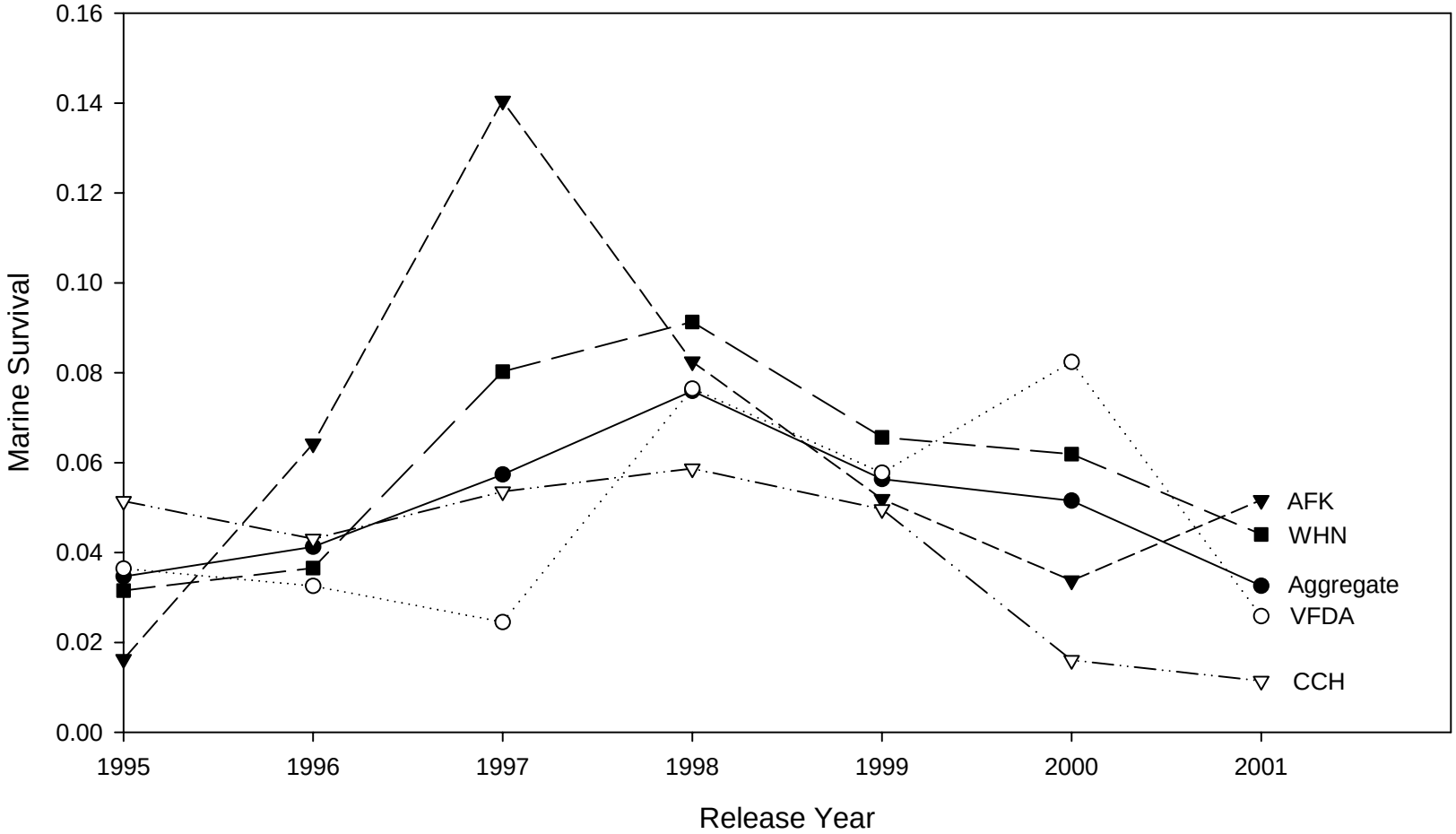

Figure 4.6. Marine survivals of pink salmon from PWS hatcheries. Salmon were released from the hatcheries on the year indicated, the year following the brood year. Hatchery abbreviations as in Figure 4.1. The aggregated marine survival of the four hatcheries combined is also indicated.

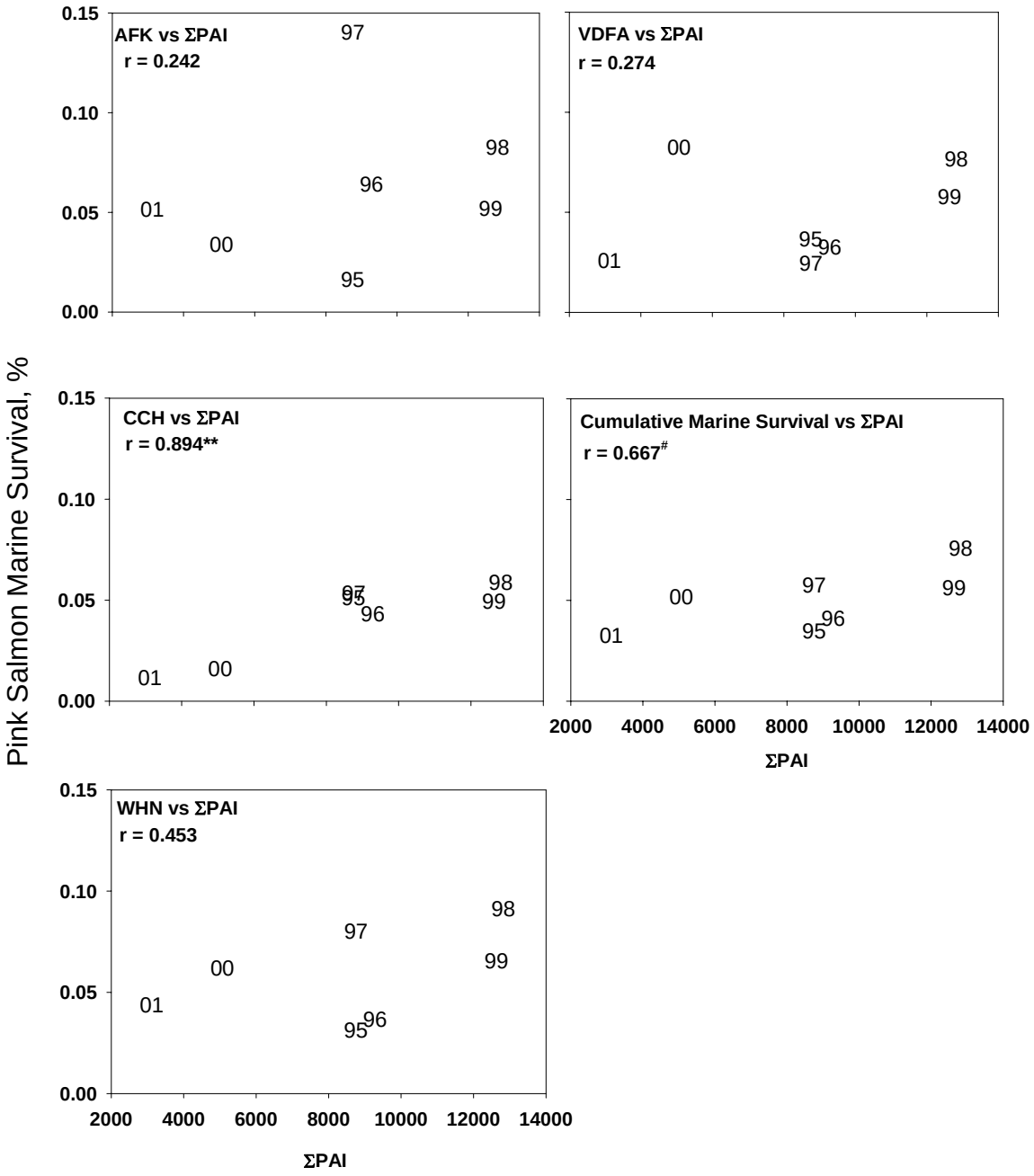

Figure 4.7. Correlation of pink salmon marine survival and pristane accumulation index (ΣPAI). The units of the ΣPAI are (:g pristane)-d. Statistical significance level of the correlation coefficient (r) is indicated by asterisks (#: P = 0.10, **: P < 0.01).

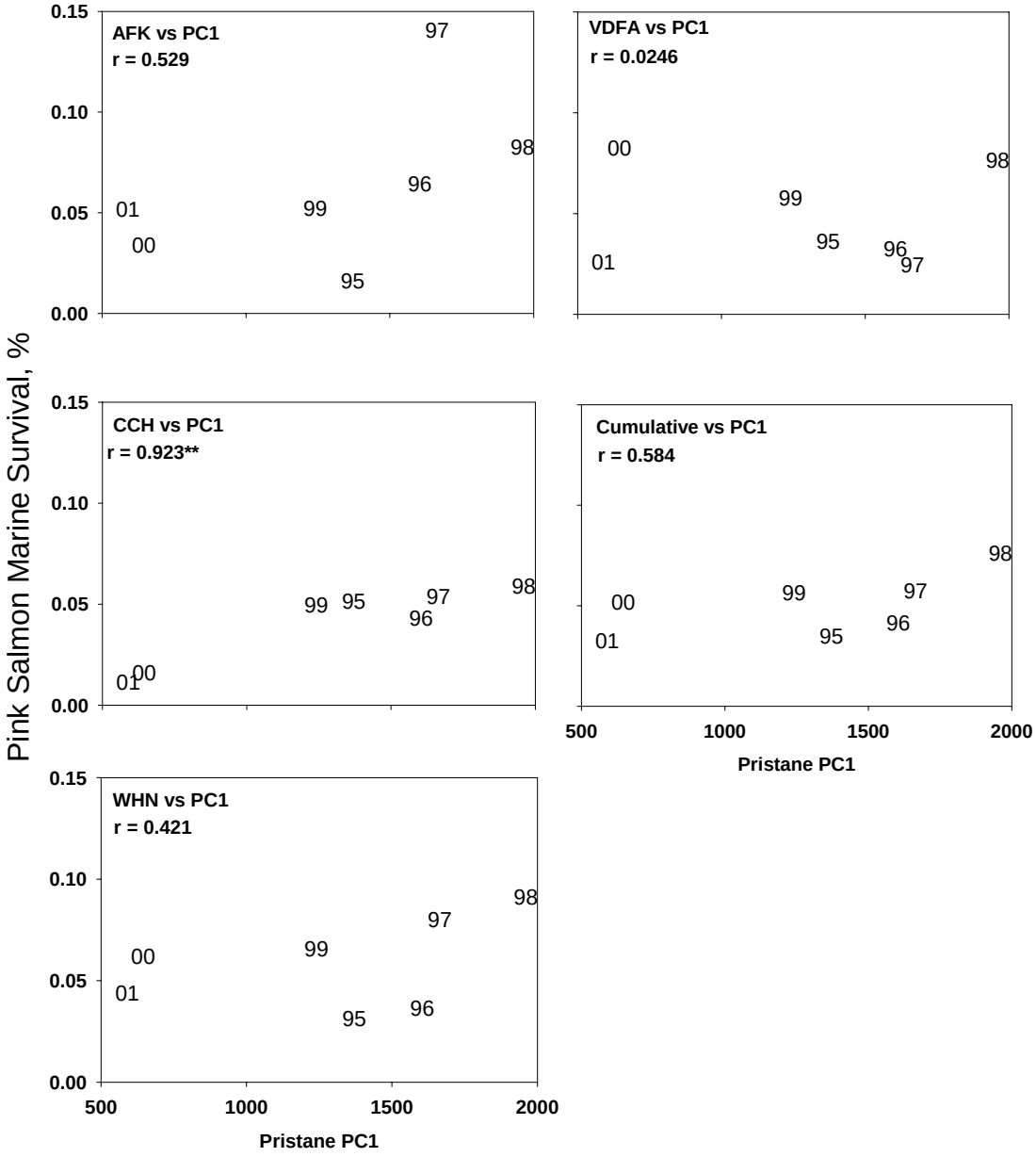

Figure 4.8. Correlation of pink salmon marine survival and the first principal component score of pristane concentrations in mussels (PC1). Statistical significance of the correlation coefficient (r) is indicated by asterisks (*: P = 0.10, **: P < 0.01).

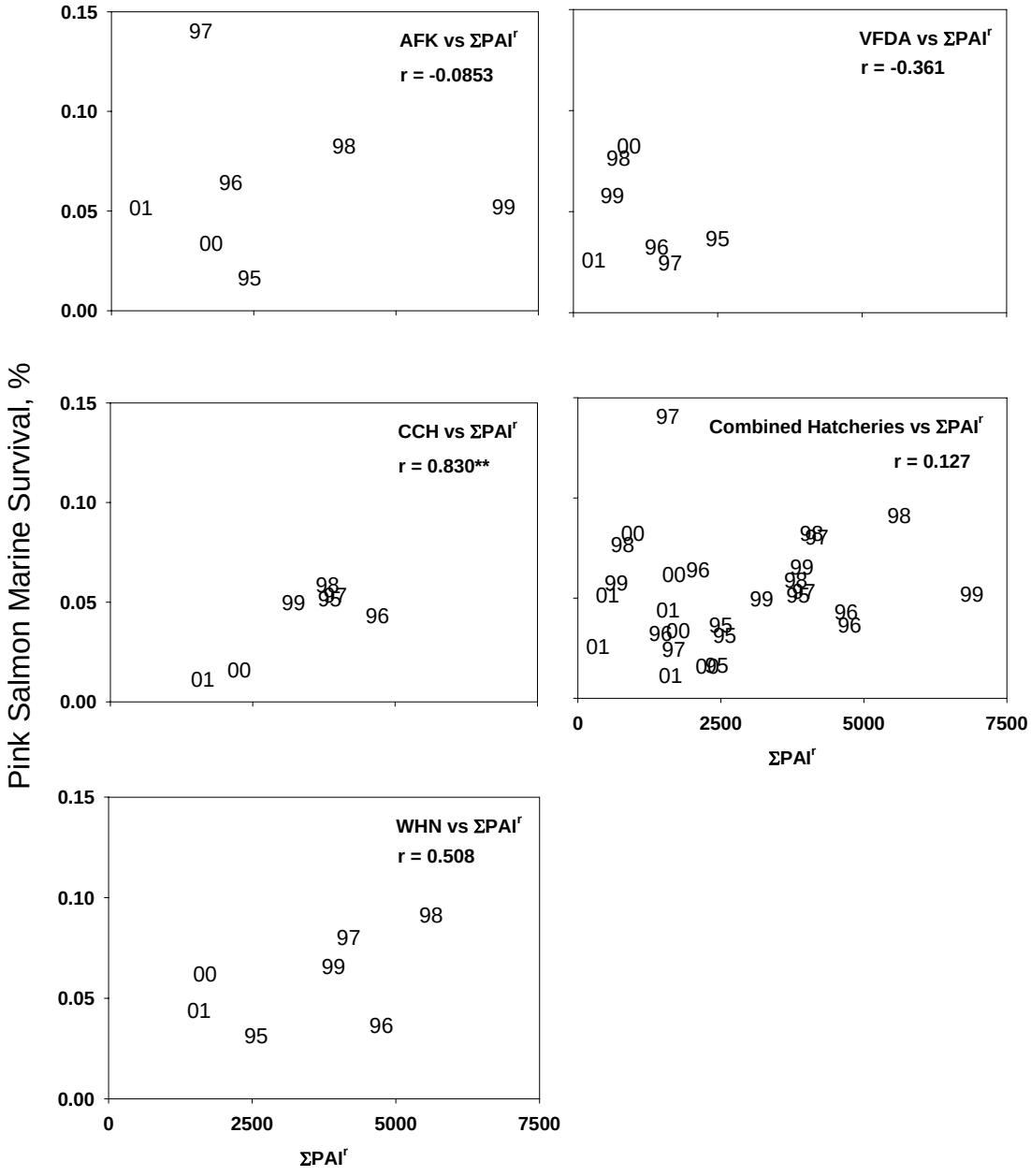

Figure 4.9. Correlation of pink salmon marine survival and the reduced pristane accumulation index (ΣPAI^r, see Methods). Statistical significance of the correlation coefficient (r) is indicated by asterisks (**: $P < 0.01$).

Table 4.l. Locations and abbreviations of mussel collection stations. The abbreviations are also used in Figure 4.1. PC1 refers to the station contributions to the first principal component of the seasonal variation of pristane concentration in mussels during the seven-year sampling period.

Station Abbreviation	Station Name	Latitude	Longitude	PC1
AFKHA	AFK Hatchery	60º 03' 08"N	148º 03' 30"W	0.098
APPLI	Applegate Island	60º 37' 30"	148º 08' 10"	0.252
BLIGI	Bligh Island	60º 52' 02"	146º 44' 59"	0.241
CANNC	Cannery Creek Hatchery	60º 59' 39"	147º 32' 19"	-0.095
CHENI	Chenega Island	60º 23' 11"	148º 00' 04"	0.182
CONSH	Constantine Harbor	60º 21' 16"	146º 40' 25"	0.256
DECIP	Decision Point	60º 48' 21"	148º 28' 35"	0.172
DIVIP	Division Point	60º 28' 55"	148º 17' 13"	0.231
EKNII	East Knight Island	60º 20' 49"	147º 38' 32"	0.308
ESTHI	Esther Is. (WN Hatchery)	60º 47' 07"	148º 03' 30"	0.223
FAIRI	Fairmont Island	60º 52' 51"	147º 26' 17"	0.094
FLEMI	Fleming Island	60º 10' 29"	148º 02' 03"	0.145
FOXF1	Fox Farm 1	59º 58' 15"	148º 08' 22"	-0.009
FOXF2	Fox Farm 2	59º 58' 07"	148º 06' 36"	-
FOXF3	Fox Farm 3	59º 58' 10"	148º 10' 22"	-
GREEI	Green Island	60º 16' 55"	147º 24' 57"	0.228
HERRP	Herring Point	60º 28' 28"	147º 47' 27"	0.233
MAINB	Main Bay	60º 32' 00"	148º 03' 30"	0.223
NAKEI	Naked Island	60º 39' 03"	147º 26' 24"	0.227
OLSEN	Olsen Bay	60º 44' 30"	146º 11' 58"	0.243
PELEA	Point Eleanor	60º 34' 33"	147º 33' 49"	0.186
PERRI	Perry Island	60º 40' 40"	147º 54' 50"	0.136
PPAKE	Point Pakenham	60º 00' 23"	148º 05' 07"	0.158
ROCKB	Rocky Bay	60º 20' 14"	147º 07' 32"	0.141
SNUGC	Snug Corner Cove	60º 44' 08"	146º 37' 32"	0.271
STORI	Storey Island	60º 43' 41"	147º 27' 02"	0.213
WINDB	Windy Bay	60º 34' 22"	145º 57' 29"	0.140

Table 4.2. Comparison of the pristane accumulation index PAI and marine survival of pink salmon.

Release Year	Number Released	Number Returning	Marine Survival	Pristane Accumulation Index (ΣPAI), :g g^{-1} d
1995	407,787,101	13,750,087	3.37%	8,670
1996	418,587,142	19,287,167	4.61%	9,200
1997	295,663,840	23,111,900	7.82%	8,670
1998	347,221,007	26,279,599	7.57%	12,600
1999	388,222,261	20,475,091	5.27%	12,600
2000	390,843,348	14,012,760	3.59%	4,990
2001	417,164,895	14,964,789	3.59%	3,130