Exxon Valdez Oil Spill
State/Federal Natural Resource Damage Assessment
Final Report

Impact of the Oil Spill on Juvenile Pink and Chum Salmon and Their Prey in Critical Nearshore Habitats

Fish/Shellfish Study Number 4
NMFS Component

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Study History: Natural Resources Damage Assessment (NRDA) Study Fish/Shellfish Number 4, NMFS Component, was initiated in 1989 as part of the 1989 NRDA Plan. The project was continued in the 1990 and 1991 NRDA Plans. Annual status reports were submitted to the Trustee Council for each of these years. The project was completed as part of the 1992 NRDA Plan, resulting in the production of this Final Report.

A total of nine peer reviewed papers have been published or are in press as a result of this work:

1. Wertheimer, A. C., and A. G. Celewycz. In Press. Abundance and Growth of Juvenile Pink Salmon in Oiled and Non-oiled Locations of Western Prince William Sound After the *Exxon Valdez* Oil Spill. *In* S.D. Rice, R.B. Spies, D.A. Wolfe, and B.A. Wright (Eds.). *Exxon Valdez* Oil Spill Symposium Proceedings. American Fisheries Society Symposium Number 18.
2. Carls, M. G., A. C. Wertheimer, J. W. Short, R. M. Smolowitz, and J. J. Stegeman. Contamination of Juvenile Pink and Chum Salmon by Hydrocarbons in Prince William Sound after the *Exxon Valdez* Oil Spill. *In* S.D. Rice, R.B. Spies, D.A. Wolfe, and B.A. Wright (Eds.). *Exxon Valdez* Oil Spill Symposium Proceedings. American Fisheries Society Symposium Number 18.
3. Sturdevant, M. V., A. C. Wertheimer, and J. L. Lum. In Press. Diet of Juvenile Pink and Chum Salmon in Oiled and Non-Oiled Nearshore Habitats in Prince William Sound, 1989 and 1990. *In* S.D. Rice, R.B. Spies, D.A. Wolfe, and B.A. Wright (Eds.). *Exxon Valdez* Oil Spill Symposium Proceedings. American Fisheries Society Symposium Number 18.
4. Celewycz, A. G., and A. C. Wertheimer. In Press. Prey Availability to Juvenile Salmon After the *Exxon Valdez* Oil Spill. *In* S.D. Rice, R.B. Spies, D.A. Wolfe, and B.A. Wright (Eds.). *Exxon Valdez* Oil Spill Symposium Proceedings. American Fisheries Society Symposium Number 18.
5. Wertheimer, A. C., N. J. Bax, A. G. Celewycz, M. G. Carls, and J. H. Landingham. Harpacticoid Copepod Abundance and Population Structure in Prince William Sound, 1 Year After the *Exxon Valdez* Oil Spill. *In* S. D. Rice, R.B. Spies, D.A. Wolfe, and B.A. Wright (Eds.). *Exxon Valdez* Oil Spill Symposium Proceedings. American Fisheries Society Symposium Number 18.
6. Fleeger, J. W., T. C. Shirley, M. G. Carls, and M. A. Todaro. In Press. Meiofaunal recolonization experiment with oiled sediments. *In* S. D. Rice, R.B. Spies, D.A. Wolfe, and B.A. Wright (Eds.). *Exxon Valdez* Oil Spill Symposium Proceedings. American Fisheries Society Symposium Number 18.
7. Carls, M. G., L. Holland, M. Larsen, J. L. Lum,, D. G. Mortensen, S. Y. Wang, and A. C. Wertheimer. In Press. Growth, feeding, and survival of pink salmon fry exposed to food

contaminated with crude oil. In Press. Growth and survival of pink salmon fry exposed to food contaminated with crude oil. *In* S. D. Rice, R.B. Spies, D.A. Wolfe, and B.A. Wright (Eds.). *Exxon Valdez* Oil Spill Symposium Proceedings. American Fisheries Society Symposium Number 18.

8. Wang, S. Y., J. L. Lum, M. G. Carls, and S. D. Rice. 1993. The relationship between growth and total nucleic acids in juvenile pink salmon *Oncorhynchus gorbuscha* fed crude oil-contaminated food. Canadian Journal of Fisheries and Aquatic Sciences 50: 996-1001.

9. Mortensen, D. M., and M. G. Carls. 1993. Effects of crude oil on growth and microstructure of juvenile pink salmon (*Oncorhynchus gorbuscha*) otoliths. *In* D. H. Secor and J. M. Dean (eds). Proceedings of the Fish Otolith Research and Application Symposium. Belle W. Barusch Institute for Marine Biology and Coastal Research. Columbia, South Carolina.

Abstract: The objective of this study was to determine the impact of the *Exxon Valdez* oil spill on juvenile pink and chum salmon during their initial period of residency in nearshore marine habitats of western Prince William Sound. In oiled locations, both pink and chum salmon fry in the nearshore marine environment were contaminated by hydrocarbons in 1989, but not in 1990. Field observations and laboratory experiments indicated that ingestion of whole oil or oil-contaminated prey was an important route of contamination. In 1989, juvenile pink salmon grew significantly slower and were significantly smaller in oiled locations than non-oiled locations, although neither their feeding nor availability of their prey was reduced in oiled locations. In 1990, size and growth of juvenile pink salmon were similar in oiled and non-oiled locations. We concluded that oil contamination from the *Exxon Valdez* reduced the growth of juvenile pink salmon in western Prince William in 1989. Because survival of pink salmon is directly related to growth during their early marine residency, survival and productivity of affected salmon populations were probably also reduced.

Key Words: Salmon, pink salmon, chum salmon, *Exxon Valdez*, oil exposure, growth, diet, feeding, zooplankton, harpacticoid copepods.

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Executive Summary

The objectives of this study were to determine the impact of the *Exxon Valdez* oil spill on juvenile pink and chum salmon during their initial period of residency in nearshore marine habitats. Field research in 1989 and 1990 compared (1) distribution, abundance, size, and nominal growth rates; (2) exposure to and contamination by hydrocarbons; (3) feeding and diet; and (4) prey abundance for these fish between oiled and non-oiled locations in western Prince William Sound. In 1991, a laboratory experiment examined the effects of ingestion of oil-contaminated food on survival and growth of juvenile pink salmon.

Pink and chum fry were less abundant in oiled than non-oiled locations in both 1989 and 1990. Because this pattern of abundance did not change between years as oil contamination diminished, we attributed these differences to geographic variation in local production of salmon fry and their migration pathways to the Gulf of Alaska.

Pink salmon fry in oiled locations were significantly smaller and had significantly lower apparent growth rates than in non-oiled locations in 1989; size and growth were similar in oiled and non-oiled locations in 1990. Because hydrocarbon contamination in the oiled locations also diminished from 1989 to 1990, we conclude that the slower growth observed in 1989 in oiled locations was caused by exposure to *Exxon Valdez* crude oil.

Chum salmon fry in the oiled locations were significantly larger than those in the non-oiled locations in both 1989 and 1990. The size difference probably was an artifact of migration timing of chum salmon in the oiled and non-oiled locations. In the non-oiled locations, many of the chum salmon were recent migrants to seawater, while in oiled locations most had been in seawater for some time. Too few chum salmon were captured in oiled locations to compare apparent growth rate between oiled and non-oiled locations. Contamination of fry in oiled locations in 1989 was shown by elevated cytochrome P4501A activity in pink and chum salmon, and by the presence of hydrocarbons in tissues from pink salmon. There was no evidence of continued contamination of either pink or chum salmon in 1990. The cell types where P4501A activity was induced in 1989 indicated that ingestion of whole oil or oil-contaminated prey was an important route of contamination. Contamination via ingestion was also

supported by the observation of oil in the stomachs of 1-4% of pink and chum salmon from oiled sites in 1989.

Feeding of pink and chum salmon fry was not reduced in the presence of oil. Pelagic zooplankton, primarily calanoid copepods, dominated the diet of juvenile pink and chum salmon in both 1989 and 1990 in both oiled and non-oiled locations. The proportion of zooplankton in the diet declined in oiled areas from 1989 to 1990. The proportion of epibenthic crustaceans (principally harpacticoid copepods) in the diet was less in oiled locations in 1989, and greater in oiled locations in 1990. Oil could have caused the diet shifts by fish avoiding contaminated epibenthic prey in 1989, or by increased feeding on oil-enhanced epibenthic harpacticoid copepods in 1990. We could not, however, definitively attribute the changes in diet as an effect of the oil spill.

Prey availability in the oiled locations was also not reduced relative to the non-oiled locations. Pelagic zooplankton did not differ significantly between oiled and non-oiled locations in 1989 or 1990. Biomass of total epibenthic crustaceans and of epibenthic harpacticoid copepods was significantly higher in oiled locations than non-oiled locations in 1989. Density of some taxa of harpacticoid copepods was depressed in experimentally-oiled sediments for 2 days post-oiling, but no oil effects were observed by 28 days post-oiling. Comparison of abundance of epibenthic harpacticoid copepods between heavily oiled and lightly oiled beaches in 1990 demonstrated that 1 year after the oil spill, harpacticoids that are important as prey to salmon fry maintained or increased their numbers in response to the direct and indirect impacts of the spill.

Results of the laboratory experiment supported the hypotheses that juvenile pink salmon were contaminated by oil via ingestion, and that oil contamination was responsible for reduced growth of pink salmon in oiled habitats. Length, weight, RNA/DNA ratios, and otolith growth declined with increasing oil concentrations. The highest dosage also reduced feeding and survival.

We conclude that pink salmon fry were contaminated by oil from the *Exxon Valdez* in the nearshore marine environment in 1989, and that this contamination caused reduced growth. Because survival of pink salmon is directly related to growth during their early marine residency, survival and productivity of affected salmon populations probably were also reduced.

Chapter 1

Introduction

When the *Exxon Valdez* ran aground on Bligh Reef on 24 March, 1989, the living marine resources of a large, pristine marine ecosystem were placed in jeopardy from direct and indirect impacts of the resulting oil spill. Eleven million gallons of crude oil moved across 10,000 square miles of water in Prince William Sound and the Gulf of Alaska, and oiled over 1,000 miles of shoreline (Maki 1991). The well-publicized destruction of large numbers of sea birds and marine mammals underscored the destructive potential of a large oil spill in a subarctic marine ecosystem; this spill killed more wildlife than any in history (Kelso and Kendziorek 1991).

The effects of the oil on the salmon resources of the region were of particular concern because of their enormous economic, recreational, and cultural value. The salmon harvest is the most valuable commercial fishery in Prince William Sound. In 1988, salmon had an ex-vessel value of $76 million dollars, over 80% of the total for all fisheries in the Sound (ADFG 1989). Salmon also represent the largest harvested biomass of the fisheries resources in the Sound. Most of the salmon landed are pink salmon (*Oncorhynchus gorbuscha*), with chum salmon (*O. keta*) the second-most abundant species (Rigby et al. 1991).

The importance of the salmon resource was reflected in the effort the NRDA process allocated towards studying the effects of the oil spill on these fish. Nine separate Fish/Shellfish studies were authorized by the Trustee Council; five of these (F/S 1,2,3,4, and 28) were directed at determining the impacts of the spill on the salmon resources of Prince William Sound. This report summarizes the NMFS component of F/S 4, Early Marine Salmon Injury Assessment in Prince William Sound.

We focused this study on juvenile pink and chum salmon because the early marine residency is a critical phase in the life history of salmon that significantly affects year-class strength (Parker 1968; Walters et al. 1978; Bax 1983; Nichelson 1986). Pink and chum salmon fry generally occupy nearshore estuarine waters in depths of 1 m or less in spring (Healey 1980). Growth during the early marine phase of pink and chum salmon is extremely rapid (LeBrasseur and Parker 1964; Healey 1980), and is important to escape mortality from size-selective predation (Parker 1971; Hargreaves and LeBrasseur 1985; Mortensen et al. 1991).

Food resources must be abundant to sustain rapid growth; food organisms must have high standing crops and local production (Bailey et al. 1975) or must be delivered to rearing areas at a high rate by currents (Cooney et al. 1978). Pink and chum salmon feed primarily on zooplankton and epibenthic crustaceans during early marine residency (Cooney et al. 1981; Heard 1991; Salo 1991). The subarctic marine ecosystem has a highly seasonal production cycle, characterized by high levels of primary and secondary production in spring (Goering et al. 1973; Larrance 1977; Smetacek et al. 1984). The timing of pink and chum salmon emigration to marine waters has presumably evolved to exploit this period of high productivity (Murphy et al. 1988; Holtby et al. 1989). Growth and mortality of juvenile fish may be coupled with the magnitude or timing of spring primary and secondary production (Cushing 1975; D'Amours 1987).

Oil in the marine environment can affect juvenile salmon in a variety of ways. Oil can be directly toxic; juvenile salmon are especially susceptible when first in seawater (Rice et al. 1975; Rice et al. 1984). Sublethal levels of hydrocarbons can affect metabolism and reduce growth (Thomas and Rice 1979; Moles and Rice 1983). Sublethal levels of water-soluble hydrocarbons can also damage olfactory lamellar surfaces, conceivably impacting migratory behavior and feeding (Babcock 1985). Contamination of prey with water-soluble fraction of crude oil has been shown to reduce feeding and growth of juvenile salmon (Schwartz 1985).

Oil pollution also can impact the prey resources of juvenile salmon. Crude oil can cause mortality and reduce abundance of zooplankton (Corner 1978; Samain et al. 1980; Johnston 1984). Epibenthic crustaceans, such as harpacticoid copepods, have been shown to be severely reduced by oiling (Giere 1979; Elmgren et al. 1983; Bodin 1991). If prey abundance were reduced due to oiling from the *Exxon Valdez*, growth and survival of juvenile salmon could also be reduced as a consequence.

Juvenile salmon in Prince William Sound were at risk of exposure and contamination by the *Exxon Valdez* oil spill because of their time of entry into the marine environment, their habitat use, and their migration route to the Gulf of Alaska. The oil spill occurred just prior to the entry of juvenile pink and chum salmon from freshwater habitats and hatcheries into the marine environment. In Prince William Sound, wild pink and chum salmon fry typically leave their natal streams in April and May (Kirkwood 1972). Hatchery releases of fry are synchronized with the timing of the spring plankton bloom and the natural emigration timing; hundreds of millions of juvenile salmon, predominately pink salmon, are released into the Sound from area hatcheries in

late April and May (Olsen 1991). In 1989, the fry in the spill area encountered large quantities of oil in both the water and the sediments of the nearshore habitats that these fish utilize. The primary migration route of juvenile salmon from Prince William Sound to the Gulf of Alaska is thought to be through the western Sound and the southwest passages, the same route the oil travelled as it dispersed into the Gulf of Alaska (ADFG 1989). Thus, large numbers of juvenile salmon originating from areas of the Sound not directly impacted by oil were also potentially exposed as they migrated seaward.

The purpose of this study was to determine the impact of the oil spill on juvenile pink and chum salmon during initial marine residency in nearshore habitats. We took a broad-based approach, examining the effects of the oil spill on the prey resources and feeding ecology of juvenile salmon, as well as the direct impacts on the salmon. Our objectives in the field research in 1989 and 1990 were to compare (1) distribution, abundance, size and apparent growth rates; (2) exposure to and contamination by hydrocarbons; (3) feeding and diet; and (4) prey abundance for these fish between oiled and non-oiled locations in western Prince William Sound. In 1991, a laboratory experiment examined the effects of ingestion of oil-contaminated food on survival and growth of juvenile pink salmon.

Because of the diversity and scope of the objectives, we have divided this report into separate chapters. Chapter 2 compares the distribution, abundance, size, and growth of pink and chum salmon fry between oiled and non-oiled locations in western Prince William Sound. Chapter 3 reports on the exposure and contamination of the fry to hydrocarbons. Chapter 4 compares juvenile salmon feeding habits between oiled and non-oiled habitats. Chapter 5 examines the impacts of the oil spill on pelagic and epibenthic prey resources. Chapter 6 details the laboratory experiment on the dose response of pink salmon fry to food contaminated with *Exxon Valdez* crude oil. Chapter 7 summarizes the results and conclusions for the entire study.

We intend to publish the information presented in this report in peer-reviewed scientific outlets. Chapters 2, 3A, 4, 5, and 6A will be reformatted and submitted for final publication in the Proceedings of the *Exxon Valdez* Oil Spill Symposium. Chapters 3B, 6B, and 6C will be reformatted and published in scientific journals or the NOAA Technical Memorandum series.

Objectives

1. Compare the abundance of juvenile pink and chum salmon between oiled and non-oiled areas.

2. Compare distribution and habitat utilization by juvenile salmon between 1989 and 1990.

3. Compare the size and growth of juvenile salmon between oiled and non-oiled areas in 1989 and 1990.

4. Capture coded-wire tagged juvenile pink salmon to determine migratory behavior and growth of fry released from Prince William Sound hatcheries.

5. Determine degree of environmental contamination at sampling locations by comparing hydrocarbon concentrations in mussels and sediments.

6. Compare hydrocarbon tissue contamination in juvenile pink salmon between oiled and non-oiled areas.

7. Compare mixed-function oxidase induction in juvenile pink and chum salmon between oiled and non-oiled areas.

8. Quantify the feeding habits of juvenile pink and chum salmon and test if indices of feeding effectiveness differed between oiled and non-oiled areas.

9. Compare the prey available to juvenile pink and chum salmon in the littoral and pelagic water column between oiled and non-oiled areas.

10. Compare the 1990 abundance of epibenthic prey species of juvenile salmon between heavily oiled and lightly oiled beaches.

11. Determine if the colonization of sediments by epibenthic prey species of juvenile salmon, such as harpacticoid copepods, was affected by the presence of oil in the sediments.

12. Determine in a laboratory experiment the effects of ingestion of oil-contaminated food on growth and survival of juvenile pink salmon.

CHAPTER 2. Distribution, Abundance, Size, and Growth of Juvenile Pink and Chum Salmon in Western Prince William Sound after the *Exxon Valdez* Oil Spill

A. G. Celewycz and A. C. Wertheimer

Abstract

The impact of the *Exxon Valdez* oil spill on distribution, abundance, size, and growth of juvenile pink and chum salmon during their initial marine residency in the nearshore waters of western Prince William Sound was studied from April to June, 1989 and 1990. A total of eight sampling locations were selected: two oiled and two non-oiled bays and two oiled and two non-oiled corridors. At each location, three nearshore habitat types (low-, medium-, and steep-gradient beaches) were sampled with a beach seine.

Pink and chum salmon fry were less abundant in oiled than non-oiled locations in both 1989 and 1990. Of 33,290 (1989) and 81,869 (1990) pink salmon captured in systematic sampling, only 18% (1989) and 14% (1990) were captured in oiled locations. Of the 7,532 (1989) and 12,857 (1990) chum salmon captured in systematic sampling, only 2% (1989) and 1% (1990) were captured in oiled locations. Because the pattern of abundance did not change between years as the degree of oil contamination diminished, these differences were attributed to geographic variation in local production of salmon fry and their migration pathways to the Gulf of Alaska, rather than exposure to oil.

Pink and chum salmon had different patterns of habitat use. Pink salmon moved rapidly from sheltered bays to the more exposed and steep beaches in corridors. In contrast, chum salmon used lower-gradient beaches and were distributed more evenly between bays and corridors. Exposure to oil-contaminated sediments may have been greater for chum salmon in oiled locations because of their habitat use.

Chum salmon fry in oiled locations were significantly larger than those in non-oiled locations in both 1989 and 1990. Too few chum salmon were captured in oiled locations to compare apparent growth rates between oiled and non-oiled locations.

Pink salmon fry in oiled locations were significantly smaller and had significantly lower apparent growth than in non-oiled locations in 1989. These differences were not observed in 1990. Because hydrocarbon contamination in the oiled locations also diminished from 1989 to 1990, we conclude that the lower growth observed in 1989 in oiled locations was caused by exposure to *Exxon Valdez* crude oil.

Introduction

The *Exxon Valdez* oil spill occurred just before the entry of juvenile pink and chum salmon from freshwater habitats and hatcheries into the marine environment. In 1989, hundreds of millions of juvenile salmon, predominately pink salmon, encountered large quantities of oil in both the water and sediments of the nearshore habitats that these fish utilize. The oil contamination posed a wide range of potential effects on juvenile salmon, ranging from direct impacts, such as mortality or physiological impairment of growth, to indirect impacts, such as disrupted migration and habitat utilization or reduced available prey resources.

Our objective in this component of the field research in 1989 and 1990 was to examine effects of the oil spill on distribution, abundance, size, and apparent growth rate of juvenile pink and chum salmon (Objectives 1-4, Chapter 1). Our basic approach was to compare these parameters between oiled and non-oiled locations in western Prince William Sound, and to examine how these comparisons changed between 1989, the year of acute contamination, and 1990, when the degree of oil pollution had greatly diminished.

Chapter 2 will be reformatted and submitted to the *Exxon Valdez Oil Spill Symposium Proceedings* for formal publication.

Methods

Sample Collection and Processing

The general sampling design incorporated eight locations: four oiled and four non-oiled (Figure 2.1). For both the oiled and non-oiled locations, two sites each were selected in bays and corridors. The study locations were paired *a priori* for pair-wise comparisons between oiled and non-oiled locations. These pairings were (non-oiled first) McClure Bay-Herring Bay; Long Bay-Snug Harbor; Culross Passage-Prince of Wales Passage; Wells Passage-Knight Island Passage.

Three habitat types (low-, medium-, and steep-gradient beaches) were sampled at each location. Low-gradient beaches were <10% grade, with granule-pebble substrate; medium-gradient beaches were 12-25% grade, with pebble-cobble substrate; and steep-gradient beaches were >50% grade, with bedrock or boulder substrate. Surface substrate composition was visually classified according to the Wentworth scale (Holme and McIntyre 1984) into four categories: boulder (>256 mm); cobble (64-256 mm); pebble (4-64 mm); and granule (2-4 mm). Any substrate <2 mm, such as sand or mud, was also included in the granule category. Particular sample sites within paired oiled and non-oiled locations were selected for similarity in wave exposure, macrophyte coverage, and substrate.

In 1989, one beach of each habitat type was sampled at each location, for a total of 24 systematically sampled sites; in 1990, two beaches of each habitat type were sampled at each location, for a total of 48 systematically sampled sites (Appendix 2.1). There were five sampling trips between 10 April and 26 June 1989, and four sampling trips between 16 April and 14 June 1990. Temperature and salinity at 1-m and 4-m depths were measured in triplicate at each location and sampling period with a conductivity-temperature meter.

In addition to these systematically sampled sites, juvenile salmon were also sampled from 3-5 km of adjacent shoreline at each location. This supplemental sampling was intended to provide additional coded-wire tags, as well as to supplement samples for hydrocarbon and otolith analyses when insufficient fish were collected at the systematically sampled beaches. The fish were typically collected during the supplemental sampling by surveying the shoreline to locate congregations of juvenile salmon, then capturing fish from these congregations with either beach seines or dip nets.

Beach seine hauls at the systematic sites were restricted to tide levels between -0.3 and +0.9 m to minimize tidal effects between sites. Fish were captured with 37-m beach seines at low- and medium-gradient sites. At the steep-gradient sites, we used a 37-m seine modified with a floor of 6-mm green mesh formed by a 9-m lead line connecting the bottom intersections of the wings with the bunt.

Catches were sorted by species and enumerated; all salmon were checked for the presence of coded-wire tags. Samples of juvenile pink and chum salmon (up to 60 of each species from each haul) were preserved in buffered 10% formalin for later fork length (FL) and weight measurements. Samples of 50 pink salmon from each bay were also preserved for analysis of otolith growth patterns. Fish collected for size analysis were retained in formalin for at least 45 d to assure uniform shrinkage. Coded-wire tagged fish were stored frozen until processed for tags by the ADFG Tag Processing Laboratory in Juneau.

Otolith samples were collected in both 1989 and 1990. In 1989, otoliths were taken from frozen fish originally collected for hydrocarbon analysis. The heads were removed from the fish and placed in 100% ethanol when the samples were prepped for the hydrocarbon processing. In 1990, fish were subsampled in the field specifically for otolith samples; the fish were measured, and the heads were removed immediately and placed in 100% ethanol. Samples were processed from fish captured in the first half of May. The heads were sent to the Washington Department of Fisheries Calcified Tissues Laboratory, where the sagittal otoliths were removed, mounted in epoxy resin, ground, and examined to determine the number of increments subsequent to the hatching and saltwater entry check; width of these increments along a standard axis in the posterodorsal quadrant of the otolith; and the mean increment width and associated error term for each group.

Statistical analysis

The univariate approach to analysis of variance (ANOVA) of a repeated measures design (Frane 1980) was used to analyze the systematic catch data (CPUE, no. of fish per haul) for pink salmon and chum salmon. In the overall analysis for each species for 1989 and 1990 combined, the factors were oil, (dichotomous scale: oiled or non-oiled), year, time period, bay/corridor, location (nested within oil and bay/corridor), and habitat. This analysis included only the same periods and sites that were sampled in both years.

Because the data for the overall ANOVA for each species were comprised of only a subset of all the systematic seine hauls from each year, ANOVAs were also run on the complete data sets of systematic catches from each year separately. For chum salmon, the factors for each of the separate ANOVAs for 1989 and 1990 were oil, time period, bay/corridor, location, and habitat, with location nested within oil and bay/corridor. For pink salmon, because of the results of the overall ANOVA, the data were subdivided further, and analyzed separately for: 1) bays in 1989, 2) corridors in 1989, 3) bays in 1990, and 4) corridors in 1990. For each of these analyses, the factors were oil, time period, location (nested within oil), and habitat.

Statistical distributions of CPUE were highly skewed because of a high number of zero catches. Transformations were not effective at eliminating this skewness. Thus, ranks of the CPUE were used in each of the analyses mentioned above. An ANOVA on ranks is conditionally distribution free, usually has good efficiency, and the approximate level of significance used in the test is usually fairly close to the true level of significance, no matter what the underlying population distribution may be (Conover 1980).

To corroborate the results of the ANOVA on ranked CPUE, a nonparametric analysis was also used to test the hypothesis of no difference in abundance of juvenile pink and chum salmon between oiled and non-oiled locations. Differences in CPUE between matched cells of the *a priori* pairs of oiled and non-oiled locations were compared with the nonparametric Wilcoxon matched-pairs signed-ranks test. Cells of the paired locations were matched by time period, bay/corridor, and habitat. For each species, 56 such comparisons were possible in 1989, and 95 were possible in 1990. For pink salmon, differences in CPUE were also tested separately in bays and corridors.

Frequency of occurrence was used as a measure of the presence of juvenile salmon and the density of their aggregations. In both 1989 and 1990, frequency of occurrence was examined in terms of: 1) catch > 0 fish, 2) catch > 100 fish, and 3) catch > 1,000 fish. Frequency of occurrence data were analyzed with a Chi-square test.

The proportion of hatchery fish in the catch was calculated for each sampling location. Because not all fish released from a hatchery are tagged, the number of hatchery fish was estimated by multiplying the number of each tag code captured by the appropriate expansion factor (the total number of fish in a release group divided by the number of tagged fish from

that release group), and then summing the expanded numbers for each tag code.

Effects of the oil spill on size, growth, and condition of juvenile salmon were examined by comparing mean FL, apparent growth rate (the change in mean FL over time), and weight/length relationship between oiled and non-oiled locations. Mean FL of pink salmon was analyzed by the two statistical methods: ANOVA (1990 only) and the Wilcoxon test (both years). An ANOVA could not be used for analysis of the 1989 FL data because of the large number of empty cells due to zero catches. The ANOVA used for the analysis of FL in 1990 included the factors oil, time period, bay/corridor, location (nested within oil and bay/corridor), and habitat. The Wilcoxon test tested only the difference in FL between oiled and non-oiled locations. It preserved possible location and habitat differences by comparing samples from the same time period and habitat type for the *a priori* pairs of oiled and non-oiled locations. For chum salmon, ANOVA of FL was not possible because of the large number of empty cells, especially from oiled sites. Only the Wilcoxon test was used to compare FL of chum salmon between oiled and non-oiled locations.

Apparent growth rate of pink salmon at each habitat type within a location was calculated as the slope of the regression of natural logarithm of weight as a function of time in days. Analysis of covariance was used to determine if data could be pooled over habitats within a sampling location. Pooling was rejected. ANOVA was used to compare the apparent growth rates of pink salmon in corridors; factors considered were oil, year, location (nested within oil), and habitat. Bays were not included in the ANOVA because there were too many empty cells to calculate valid growth rates in bays in 1989.

Fish condition was examined by comparing least-squares regression parameters between oiled and non-oiled locations, as recommended by Cone (1989). The relationship between fish weight and length was described by:

$$\ln(w) = a + b(\ln(FL)),$$

where w is weight, a is the intercept, FL is fork length, and b is the slope, the exponential rate of increase of weight with length. In this analysis, condition of the fish is measured by the weight at a given length, and is determined both by the slope and the relative elevation of the regression line.

Analysis of covariance was used to test for homogeneity of slopes and equality of adjusted means between bays and corridors for non-oiled locations and for oiled locations. If the slopes and adjusted means were not significantly ($P > 0.1$) different, data were pooled as to bay/corridor and tested between oiled and non-oiled locations. If slopes or adjusted means were significantly different between bays and corridors, tests between oiled and non-oiled locations were made separately for bays and corridors.

Repeated measures ANOVA was applied to temperature and salinity data. The factors in each ANOVA were time, oil, bay/corridor, and location, with location nested in the factors oil and bay/corridor.

Results

Abundance and Habitat Utilization

Pink Salmon

More pink salmon were captured in non-oiled than oiled locations in the systematic hauls in both 1989 and 1990. In 1989, a total of 33,290 pink salmon were captured in 120 systematic hauls, with 43% zero catches and a high catch of over 8,000. More than four times as many pink salmon were captured in non-oiled (27,200 fish) than oiled locations (6,090 fish). In 1990, a total of 81,869 pink salmon were captured in 191 hauls, with 28% zero catches and a high catch of 22,977. More than six times as many pink salmon were captured in non-oiled (70,496 fish) than oiled locations (11,373 fish). Pink salmon CPUE was significantly greater in non-oiled locations for both years combined ($P = 0.03$, Table 2.1). The non-significant year-oil interaction ($P > 0.1$) indicated that this trend was consistent in both years. Over both years, over five times more pink salmon were captured in non-oiled than oiled locations (Figure 2.2). Additionally, the Wilcoxon test for matched pairs of hauls indicated higher CPUE in non-oiled locations in each year ($P = 0.043$, 0.092 in 1989 and 1990, respectively; Table 2.2).

Pink salmon CPUE was significantly greater in corridors than bays for both years combined ($P = 0.004$, Table 2.1). Over the two years, 94% of the pink salmon were captured in corridors and only 6% were captured in bays (Figure 2.2). Because of the dramatic difference in CPUE between bays and corridors, separate analyses were run on bays and corridors for each year.

When pink salmon CPUE in bays was examined separately by year, data showed two consistent patterns. First, CPUE in both years was similar ($P > 0.1$) in oiled and non-oiled bays in both the ANOVA (Table 2.3) and the Wilcoxon test (Table 2.2). In both years, pink salmon occurred just as frequently ($P > 0.1$) in oiled and non-oiled bays (Table 2.4).

The second pattern for bays was that medium-gradient beach was the most important habitat overall for pink salmon. In 1990, pink salmon CPUE differed significantly between habitats ($P = 0.050$, Table 2.3); CPUE was greatest in the medium-gradient habitat (Figure 2.2). In 1989, habitat was not significant ($P > 0.1$), but CPUE in bays exhibited the same pattern in 1989 as in 1990: the sample mean CPUE was highest in the medium-gradient habitat (Figure 2.2, Table 2.5). In 1989, time ($P = 0.023$) and the time-habitat interaction ($P = 0.055$) were also significant in explaining

differences in CPUE in bays (Table 2.3). CPUE peaked in early May at the medium-gradient habitat, in late May in the low-gradient habitat, and early June in the steep-gradient habitat.

When pink salmon CPUE in corridors was examined separately by year, different factors were significant in explaining variations each year. In 1989, CPUE was higher in non-oiled than oiled corridors in the ANOVA ($P = 0.035$, Table 2.3), and the Wilcoxon test ($P = 0.013$, Table 2.2). Pink salmon occurred more frequently in non-oiled than oiled corridors in 1989 ($P < 0.05$, Table 2.4). In 1990, although more than six times as many pink salmon were captured in non-oiled than oiled corridors (Figure 2.2), this difference was not significant ($P > 0.1$, Tables 2.2, 2.3, and 2.4). Pink salmon occurred just as frequently in oiled as in non-oiled corridors in 1990 ($P > 0.1$, Table 2.4). In corridors in 1990, oil was significant only in the context of the time-oil interaction ($P = 0.026$, Table 2.3). In non-oiled corridors, CPUE peaked in early May; in oiled corridors, CPUE peaked in late May.

In corridors, pink salmon schools were larger but occurred less frequently in the steep-gradient habitat than other habitats. Although 74% of the pink salmon in corridors were captured in the steep-gradient habitat over both years, in neither year was the difference in CPUE between habitats significant ($P > 0.1$, Table 2.5). The highest CPUE and greatest variability in CPUE was in the steep-gradient habitat. Over both years, 25% of the hauls at steep-gradient sites in corridors captured more than 1,000 pink salmon, and 40% captured no pink salmon. In corridors, pink salmon were captured most frequently in the medium-gradient habitat and least frequently in the steep-gradient habitat in each year, although this trend was significant only in 1990 ($P < 0.05$, Table 2.5).

Chum Salmon

More chum salmon were captured in non-oiled than oiled locations in the systematic hauls in both 1989 and 1990 (Figure 2.2). In 1989, a total of 7,532 chum salmon were captured in 120 systematic beach seine hauls, with 47% zero catches and a high catch of 764. More than 41 times as many chum salmon were captured in non-oiled (7,353 fish) than oiled locations (179 fish). In 1990, a total of 19,879 chum salmon were captured in 191 beach seine hauls, with 50% zero catches and a high catch of 4,380. More than 96 times as many chum salmon were captured in non-oiled (19,675 fish) than oiled locations (204 fish). Over both years, over 70

times more chum salmon were captured in non-oiled locations. Chum salmon CPUE was significantly greater in non-oiled than oiled locations for both years combined ($P = 0.004$, Table 2.1). The non-significant year-oil interaction ($P > 0.1$) indicated that this trend was consistent in both years. The Wilcoxon test indicated significantly ($P < 0.001$) higher CPUE of chum salmon in non-oiled than oiled locations in both 1989 and 1990, with an estimated median difference in CPUE of 64.5 and 53.0 fish per haul, respectively (Table 2.2).

In 1990, habitat was also significant ($P = 0.026$) in explaining variance in CPUE of chum salmon (Table 2.6). Chum salmon CPUE was similar in low- and medium-gradient habitats, but CPUE was much greater in these habitats than in steep-gradient habitat (Figure 2.2).

Coded-Wire Tag Recoveries

Pink Salmon

A total of 424 coded-wire tagged juvenile pink salmon were recovered in 1989 and 1990 to analyze growth and migration behavior. These tag recoveries are included in the tag database analyzed by ADFG (Willette 1991). A cursory description of the tag recoveries from our study is given here.

The total number of pink salmon examined for coded-wire tags, including both systematic and supplementary catches, was 232,136 in 1989 and 202,793 in 1990. Large aggregations of fish were located and sampled in both oiled and non-oiled locations. Most of the pink salmon were caught in the corridor sites in both oiled and non-oiled areas; relatively few were captured in bays (Table 2.7). An exception was in the outer portion of Snug Harbor, where large numbers of pink salmon were observed and captured. This portion of Snug Harbor was outside the area of systematic sampling for bays because of its broad, exposed opening to Prince William Sound. Within the inner bay, both systematic and supplemental hauls caught few fish.

In 1989, 143 tagged pink salmon were recovered, predominantly in corridors (Table 2.7). Most of these fish (77%) were recovered at Wells Passage on the north end of Culross Island, across Wells Passage from the Wally Noerenberg Hatchery on Ester Island. The number of tag recoveries in other corridors ranged from 4 to 13. Tag recoveries were rare in bays in 1989. No tagged pink salmon were recovered in the non-oiled bays. In oiled bays, one tagged pink salmon was recovered in Herring Bay, none were recovered in the

inner bay of Snug Harbor, and five were recovered in the outer portion of Snug Harbor.

In 1990, 281 tagged pink salmon were recovered, again predominantly in corridors (Table 2.7). Once again, more tagged pink salmon (42%) were recovered in Wells Passage than in any other location. However, many of the tagged pink salmon in 1990 were recovered in bays. A large proportion of the recoveries in 1990 was from a wild-stock tagging program that did not occur in 1989. In 1990, 111 (39%) tagged pink salmon were recovered in Herring Bay. Of these, 106 tags were from wild-stock tagging operations at Herring Creek in Herring Bay (102 tags), Loomis Creek (3 tags), and Totemoff Creek (1 tag). No other tags were recovered in bays in 1990.

Hatchery fish comprised a substantial proportion of the total fish captured at several locations (Table 2.7). Hatchery fish comprised 66% and 100% of the catch in Wells Passage in 1989 and 1990, respectively. Hatchery fish also comprised 44% and 58% of the catch in Herring Bay in 1989 and 1990, respectively. An additional 1% of the catch at Herring Bay in 1990 could be attributed to wild stocks originating outside of Herring Bay itself. The proportion of hatchery fish at Knight Island Passage increased from 16% in 1989 to 43% in 1990. The proportion captured at Prince of Wales declined from 14% in 1989 to 3% in 1990. In all other locations, including outer Snug Harbor, hatchery fish comprised 7% or less of the catch; no hatchery fish were recovered in Long Bay, McClure Bay, or inside Snug Harbor.

Chum Salmon

A total 15 coded-wire tagged chum salmon fry were recovered, all in 1990 (Table 2.8). The number of chum salmon checked for tags was 30,195 in 1989 and 32,737 in 1990. Large aggregations of juvenile salmon that included chum salmon were located only in non-oiled locations; in both years, 99% of the total catch was in non-oiled locations. All 15 tags captured had been released from the Wally Noerenberg Hatchery and were recovered at the non-oiled corridor location in Wells Passage.

Based on the code-wire tag recoveries, hatchery fish composed 100% of the catch of chum salmon at Wells Passage in 1990 and were not detected at any of the other locations (Table 2.8). Because large numbers of chum salmon were released from the Wally Noerenberg Hatchery without coded-wire tags in representative groups, we could not estimate the proportion of hatchery fish in the catch in 1989.

Timing of Catch

The pattern of the total catch (systematic and supplemental) of pink salmon over time differed between bays and corridors in both 1989 and 1990 (Figure 2.3). Catch in bays was generally lower than in corridors, except for the early (April) sampling period. Catch in bays was small and stable in April and May, although in 1990 there was a pronounced increase in catch in May. Catch in corridors was small in April, increased rapidly to peak in May, and declined in early June. In 1989, when sampling extended to late June, catch continued to decline in the non-oiled corridors, but increased somewhat in the oiled corridors.

The pattern of the total catch of chum salmon over time did not differ between bays and corridors; the major difference in catch over time was between oiled and non-oiled locations (Figure 2.3). In general, catches were small in April, peaked in May, and declined in June. Timing was similar for non-oiled bays and corridors and for oiled bays and corridors, with the non-oiled locations consistently an order of magnitude or more greater than the oiled locations.

Size, Growth, and Condition

Pink Salmon

Pink salmon were larger in non-oiled than oiled locations in 1989, but not in 1990. Based on the matched-pairs comparisons, mean FL was significantly ($P = 0.066$) greater in non-oiled than oiled locations in 1989 (Table 2.9, Figure 2.4). Mean FL was significantly ($P = 0.026$) greater in non-oiled than oiled corridors, but did not differ ($P > 0.1$) between oiled and non-oiled bays. In 1990, pink salmon mean FL did not differ ($P > 0.1$) between oiled and non-oiled locations overall, and also between bays and corridors considered separately. Pink salmon mean FL did not differ ($P > 0.1$) between oiled and non-oiled locations in 1990, whether mean FL was analyzed with the matched-pairs comparisons (Table 2.9) or with ANOVA (Table 2.10). Mean FL increased significantly with time in 1990 ($P < 0.001$). The time-bay/corridor interaction was also significant ($P = 0.047$) in explaining variation in FL in 1990, indicating that the mean FL divergence between bays and corridors in May (Figure 2.5) was significant.

Mean FL of pink salmon in 1989 and 1990 was similar in oiled and non-oiled locations in April, before diverging in May, with higher FL in non-oiled than in oiled locations (Figures 2.4 and 2.5). The same temporal pattern of divergence was

apparent for fish from bays and corridors, with fish from corridors having higher mean FL after early May.

Frequency distributions of pink salmon FL differed between bays and corridors in both years (Figures 2.6 and 2.7). In bays, FL had a mode of 32-33 mm in April and May, which indicated that the fish were predominantly recent migrants from fresh water. The FL distributions of the few fish captured in bays in June showed no distinct peak. In corridors, the mode of the FL distribution shifted from 31-32 mm in April to 40 mm in late May and 45 mm in June. The distribution in corridors generally shifted towards larger FL and widened until June, when the tails of the distribution began to truncate.

Analysis of otoliths indicated that most juvenile pink salmon from oiled and non-oiled bays in May 1990 were recent migrants from fresh water. The percentage of fish with discernable early marine growth increments on the otoliths was 17% and 27% in Long Bay and McClure Bay, respectively, and 23% in Herring Bay (Table 2.11). Most fish (64%) from Snug Harbor, however, had discernable early marine growth increments. For fish with measurable marine growth increments, mean increment width did not differ significantly ($P > 0.1$) between bays.

Otoliths from the 1989 collections were not usable for increment analysis. The edges of the otoliths were eroded to the extent that the early marine zone could not be discriminated. This erosion of the otoliths was probably due to extensive frozen storage prior to preservation in ethanol.

Apparent growth of pink salmon was lower in oiled than non-oiled corridors in 1989 but not in 1990 (Table 2.12). The year-oil interaction was significant ($P = 0.054$), and growth was significantly ($P = 0.035$) higher in 1990 than in 1989 (Table 2.13). This interaction was significant because of lower growth in oiled than non-oiled corridors in 1989, and a similar rate in oiled corridors in 1990 (Figure 2.8). At each habitat in 1989, mean growth of pink salmon was lower in oiled than non-oiled corridors (Table 2.12). Over all habitat types, growth in 1989 was 1.5% body weight per day in oiled corridors, significantly ($P < 0.05$) lower than the 2.9% body weight per day in non-oiled corridors. In 1990, in contrast, growth at both low- and medium-gradient habitats tended to be higher in oiled than non-oiled corridors (Table 2.12). Growth over all habitat types in 1990 was slightly higher in oiled than non-oiled corridors, 3.5% and 3.1% body weight per day, respectively; these rates were not significantly ($P > 0.1$) different. The significant

difference between years was due to the large change in growth in oiled locations from 1989 to 1990.

Apparent growth of pink salmon in corridors by habitat also differed between years, as indicated by the significant (P = 0.008) year-habitat interaction (Table 2.13). This interaction was caused by higher growth in steep-gradient habitats of both oiled and non-oiled corridors in 1990 compared to 1989. Growth in low- and medium-gradient habitats of both oiled and non-oiled corridors was similar in 1989 and 1990.

In 1989, condition (weight at a given length) of pink salmon was higher in oiled than in non-oiled locations. The logarithmic weight/length regressions did not differ significantly ($P > 0.100$) between bays and corridors in 1989. Therefore, data from bays and corridors were pooled to compare condition between oiled and non-oiled locations. The resulting regression equations did not differ significantly in slope; however, the adjusted means were significantly different ($P < 0.001$; Table 2.14). The difference in condition, although significant, was small: adjusted mean weights were 0.439 g in the oiled locations and 0.431 g in the non-oiled locations.

In 1990, pink salmon were again generally heavier at a given length in oiled than non-oiled locations over the range of sizes occurring in the habitats sampled. Bays and corridors were analyzed separately in 1990 because there was a significant difference ($P < 0.001$) in the weight/length regressions between bays and corridors in non-oiled locations. Regression slopes differed significantly ($P < 0.001$) between oiled and non-oiled bays, and between oiled and non-oiled corridors (Table 2.14). For both bays and corridors, slopes were steeper and y-intercepts were smaller for pink salmon in non-oiled locations; the lines intersected at 40 mm in bays and 65 mm in corridors (Figure 2.9). Below the intersections, pink salmon were heavier at a given length in oiled locations; above the intersections, pink salmon were heavier at a given length in non-oiled locations. In bays, most (83%) of the pink salmon captured were smaller than the intersection point of 40 mm, and in corridors most (98%) were smaller than the intersection point of 65 mm. Because these intersection points occur at sizes at which most pink salmon have left the nearshore environment in bays and corridors (Figures 2.6 and 2.7), the interpretation is the same in 1990 as in 1989: pink salmon generally had higher condition in the oiled locations.

Chum Salmon

Chum salmon were larger in oiled than non-oiled locations in both years. Based on the nonparametric matched-pairs comparison, chum salmon were significantly larger in oiled locations in both 1989 ($P = 0.021$) and 1990 ($P = 0.081$, Table 2.9). The estimated median difference between sizes in oiled and non-oiled locations was 5.8 mm in 1989 and 4.2 mm in 1990. Because so few chum salmon were caught in oiled locations, ANOVA could not be used to test for differences in size or growth rates between oiled and non-oiled locations in either year.

Size distributions of chum salmon differed between oiled and non-oiled locations in both 1989 and 1990 (Figure 2.10). In non-oiled locations, 46% of the fish were less than 40 mm FL, and only 27% were larger than 44 mm. In the oiled locations, only 17% of the fish were less than 40 mm, and 69% were larger than 44 mm.

Chum salmon condition was examined separately for bays and corridors because the weight/length regressions differed significantly between bays and corridors. Condition of chum salmon was similar in oiled and non-oiled corridors in 1989 and in 1990; neither slopes nor adjusted means of the weight/length regression differed significantly ($P > 0.1$; Table 2.14).

In bays in 1989, condition of chum salmon was generally higher in oiled than non-oiled bays. Weight/length regressions differed significantly ($P < 0.001$) between oiled and non-oiled bays in 1989 (Table 2.14). The slope of the regression line was steeper and the y-intercept smaller in non-oiled bays; the regression lines intersected at 57 mm. Below the intersection, chum salmon were heavier at a given length in oiled bays; above the intersection, chum salmon were heavier at a given length in non-oiled bays. Most chum salmon (85% in oiled bays and 99% in non-oiled bays) were smaller than 57 mm (Figure 2.10).

In 1990, comparison of condition of chum salmon between oiled and non-oiled bays had no clear interpretation. As in 1989, weight/length regressions differed significantly ($P < 0.001$) between oiled and non-oiled bays in 1990 (Table 2.14), and the slope of the regression line was steeper and the y-intercept smaller in non-oiled bays (Figure 2.10). The regression lines intersected at 44 mm. In non-oiled bays, 94% of the chum salmon sizes were smaller than the intersection, while in oiled bays, only 18% of the fish were smaller than the intersection. Because size distributions of chum salmon from oiled and non-oiled bays were distinct and

virtually isolated on either side of the intersection point, no direct comparison could be made for condition that would be valid across the size range sampled.

Temperature and Salinity

Water temperature was similar in oiled and non-oiled locations in both 1989 and 1990. Temperature generally increased from April to June at all locations in both years at both 1-m and 4-m depths (Figure 2.11). At both the 1-m and 4-m depths, there were no significant differences ($P > 0.1$) between oiled and non-oiled locations or between bays and corridors (Table 2.15). However, there were significant time-bay/corridor ($P = 0.001$) and time-oil-bay/corridor ($P = 0.056$) interactions at the 4-m depth in 1989 due to different patterns in the change in temperature over time in the bays.

Salinity was generally higher at oiled than non-oiled locations at both 1-m and 4-m depths in both years (Figure 2.11). Salinity was significantly higher at oiled than non-oiled locations at 1-m in both 1989 ($P = 0.002$) and 1990 ($P = 0.022$), and at 4-m in 1989 ($P < 0.001$, Table 2.16). Salinity decreased from April to June. In 1989, salinity at 4-m depth declined to a lesser extent over time at oiled than non-oiled locations, resulting in a significant time-oil interaction ($P = 0.002$). Also in 1989, there was significant interaction between bay/corridor and oil at the 1-m depth ($P = 0.036$) due to extremely low salinity in the non-oiled bays. These interactions reflect differences in the degree to which oiled and non-oiled locations differed, but do not contradict the conclusion that salinity was higher overall in the oiled locations. No significant interactions were seen in the salinity comparisons in 1990.

Discussion

Juvenile pink and chum salmon were less abundant in oiled than non-oiled locations in both 1989 and 1990. Avoidance of oiled habitats or direct mortality are possible explanations of the differences in abundance. However, no evidence of direct mortality was observed in oiled locations. In both years, large schools of juvenile pink salmon were observed and sampled in both oiled and non-oiled locations. Pink salmon fry did not appear to avoid oil. Schools of pink salmon were observed under large expanses of mousse accumulated along booms in outer Snug Harbor in 1989; the fish may actually have been using the mousse for cover.

Because the pattern of salmon abundance did not change between years as the degree of oil contamination diminished (Chapter 3, this report), and because pink salmon did not appear to be avoiding oil, we conclude that the differences observed in abundance between oiled and non-oiled locations were more likely due to geographic differences in the localized production of salmon fry and their migration pathways to the Gulf of Alaska, rather than to exposure to oil. The main criterion in selecting sampling locations in this study was the categorization as to "oiled" and "non-oiled." Because of the distribution of the spill, non-oiled study locations were clustered in the northwest region of Prince William Sound, on or close to the mainland, while oiled locations were generally more southerly and on islands (Figure 2.1). These geographic differences were reflected in physical differences between the locations. Although water temperature did not differ between oiled and non-oiled locations, salinity was lower in non-oiled locations, especially at the surface. Spawning populations of pink and chum salmon in the non-oiled portion of western Prince William Sound are substantially larger than in the oiled section (Pirtle 1977). Most of the hatchery production for these species is also located out of the main spill area; only one (Armin Koernig Hatchery) of the four major pink salmon facilities in Prince William Sound was directly in the path of the spill (PWSRPT 1983).

After entering the nearshore marine waters of Prince William Sound in early spring, juvenile pink salmon moved rapidly from sheltered bays to the more exposed and steep beaches in corridors. Salmon fry in bays originated mostly from nearby streams, whereas fish in corridors were comprised of multiple stocks of salmon fry that had left the bays and were migrating toward the Gulf of Alaska. There were exceptions to this generalization, such as the aggregations of fish observed in the outer portion of Snug Harbor, and the recovery of tags from hatchery and non-local wild stocks

inside Herring Bay, but the abundance, timing, size distribution, and otolith data indicated that most of the juvenile fish leave the bays rapidly, and aggregate in large schools in steep-gradient habitats in the corridors. Previous work in Sawmill Bay in the southwestern Sound also showed that juvenile pink salmon moved rapidly from that bay to adjacent corridors (Cooney et al. 1981). Our observations of this behavior over a wider geographic range reinforces Cooney's (1990) conclusion that the oil-deflection boom in Port San Juan in 1989 disrupted the normal migration behavior of fish released from the Armin F. Koernig Hatchery into Sawmill Bay.

Exposure to oil-contaminated sediments may have been greater for chum salmon than pink salmon because of their habitat preferences. Abundance of chum salmon was similar in bays and corridors, and chum salmon primarily utilized low- and medium-gradient beaches. These beaches were predominately cobble and finer substrates. Crude oil typically penetrates and accumulates in such fine substrates to a greater degree than the boulder or bedrock substrate that predominated in the steep-gradient habitat. Because of their utilization of habitats with substrates in which oil tended to accumulate and persist, chum salmon were probably exposed to higher concentrations of hydrocarbons than pink salmon. In fact, for fish from oiled locations in 1989, higher median levels of mixed-function oxidases were found in chum salmon than in pink salmon (Chapter 3, this report).

We did not detect any impact of the oil spill on either size or growth of pink salmon in bays. Pink salmon size was similar in oiled and non-oiled bays in both 1989 and 1990. The distribution, size, and otolith data all indicated short residence time and, thus, little opportunity for growth in both oiled and non-oiled bays before the fish moved into corridors. Apparent growth rates of pink salmon in bays could not be calculated because of insufficient data. The limited data available from otoliths indicated that growth was not reduced in oiled bays in 1990. Unfortunately, we could not recover this information from otoliths from fish collected in bays in 1989.

Juvenile pink salmon were smaller and grew less in oiled than non-oiled corridors of western Prince William Sound in 1989, but not in 1990. Because growth is based on change in size of unmarked fish over time, interpretation is complicated by recruitment of newly emerged fry into the marine environment and by size-specific movement from nearshore to offshore water (LeBrasseur and Parker 1964; Healey 1980) and between different nearshore habitats (Celewycz 1990). In this study, we did account for habitat differences in comparing apparent growth rate. Furthermore, our analysis of apparent growth

rate of unmarked pink salmon is consistent with research on tagged fish which showed that pink salmon from the same tag groups were significantly smaller when recovered in heavily-oiled than in lightly-oiled or non-oiled locations in 1989, but not in 1990 (Willette 1991).

We attribute the reduced growth of juvenile pink salmon observed in 1989 to hydrocarbon contamination in oiled locations. This is in contrast to Neff's (1991) conclusion: "It is extremely unlikely that hydrocarbon concentrations resulting from the spilled oil have had or will have any adverse effects on plants and animals living in the water column of Prince William Sound and the western Gulf of Alaska, including commercial fishery species." Hydrocarbon contamination was observed in tissues of juvenile pink salmon in 1989 (Chapter 3, this report), in spite of the low concentrations of oil in the water column reported by Neff (1991). The low concentrations of oil in the water do not exclude other routes of contamination; ingestion of oil, either directly or via contaminated prey, was probably an important route of exposure for juvenile pink salmon (Chapters 3 and 4, this report). Recent laboratory studies have shown that ingestion of crude oil reduces growth rates of juvenile pink salmon (Chapter 6, this report). Exposure and contamination of juvenile salmon diminished from 1989 to 1990 (Chapter 3, this report). Because the differences in size and growth of juvenile pink salmon between oiled and non-oiled corridors also diminished in 1990 as exposure levels and contamination decreased, we conclude that the presence of oil inhibited the growth of pink salmon in 1989.

In both 1989 and 1990, juvenile chum salmon were significantly larger in oiled than non-oiled locations. Because the size difference was consistent over years, we do not attribute this to oiling. Chum salmon fry average 38-39 mm FL at emergence from spawning gravel and are generally less than 40 mm FL when they emigrate from fresh water to seawater (Koski 1975; Helle 1980; Salo 1991). Many of the chum salmon captured in non-oiled locations were less than 40 mm, indicating they were recent migrants from their natal streams. In contrast, chum salmon were extremely rare in oiled locations, and their size distribution indicated that few were recent migrants and that most had been in salt water for some time. Thus, the size differences we observed for chum salmon may have been an artifact of migration timing of different stocks in the oiled and non-oiled locations.

The relationship between weight and length of fish is frequently used a measure of the effect of biotic or abiotic factors on the health or well-being of the fish. This condition measure did not differ consistently for chum salmon between oiled and non-oiled locations. In contrast, pink

salmon had consistently higher condition in oiled locations. Other authors have argued that condition should be a sensitive indicator of effects of the oil spill on feeding and growth of juvenile pink salmon (Brannon et al., in press). In fact, condition does not appear to be a sensitive measure of growth reductions caused by exposure to oil. Carls et al. (Chapter 6, this report) found that pink salmon juveniles ingesting low and moderate dosages of crude oil had similar or higher condition than control fish, even though growth was reduced at those dosages.

Although growth of pink salmon was reduced in the oiled locations in 1989, pink salmon were still feeding and growing there. Feeding effectiveness was not reduced in oiled locations (Chapter 4, this report). Growth was observed in heavily oiled locations in this study and by Willette (1991). The fish in oiled locations were not emaciated; condition was actually higher in the oiled locations. Pink salmon fry can metabolize and depurate hydrocarbons (Thomas and Rice 1979). Depuration of hydrocarbons, however, has a metabolic cost, which can reduce food conversion efficiency in juvenile salmon (Moles and Rice 1983). For example, juvenile Atlantic salmon (*Salmo salar*) exposed to sublethal concentrations of crude oil had reduced growth which coincided with reduced food conversion efficiency rather than reduced food intake (Vignier et al. 1992). The oil contamination in Prince William Sound was apparently within the depuration capability of juvenile salmon, but the physiological cost of exposure and depuration was sufficient to reduce growth of pink salmon in oiled locations.

Table 2.1. ANOVA table: ranked CPUE of juvenile pink salmon and chum salmon in Prince William Sound, 1989 and 1990. Each factor without an associated probability was used as the error term in the significance test for the factors listed above it; df = degrees of freedom, o = oil, b = bay/corridor, l = location, (ob) indicates nesting within oil and bay/corridor, y = year, t = time, and h = habitat.

		Pink salmon		Chum salmon	
Source	df	F	P	F	P
o	1	10.54	0.03	33.56	0.004
b	1	33.33	0.004	0.07	0.81
ob	1	12.03	0.03	0.00	0.95
l(ob)	4				
y	1	0.76	>0.40	0.00	0.95
yo	1	0.89	0.40	1.53	0.28
yb	1	0.20	0.68	0.16	0.71
yob	1	1.63	0.27	1.34	0.31
yl(ob)	4				
t	2	0.97	0.42	0.40	>0.63
to	2	4.12	0.06	1.32	0.32
tb	2	2.09	0.19	0.20	0.82
tob	2	0.21	0.82	0.07	0.93
tl(ob)	8				
yt	2	2.28	>0.27	1.08	>0.37
yto	2	1.72	0.24	0.73	0.73
ytb	2	0.96	0.42	0.15	0.86
ytob	2	0.43	0.67	0.49	0.63
ytl(ob)	8				
h	2	4.20	0.06	8.18	0.01
ho	2	0.22	0.81	2.25	0.17
hb	2	0.27	0.77	1.12	0.37
hob	2	0.21	0.82	1.03	0.40
hl(ob)	8				
yh	2	0.30	0.75	1.14	0.37
yho	2	0.19	0.83	0.37	0.70
yhb	2	0.08	0.93	0.00	0.95
yhob	2	0.02	0.98	0.12	0.89
yhl(ob)	8				
th	4	7.58	0.001	2.55	0.08
tho	4	0.18	0.94	0.81	0.54
thb	4	0.97	0.45	0.42	0.79
thob	4	1.19	0.35	0.27	0.89
thl(ob)	16				
yth	4	0.96	0.46	0.75	0.58
ytho	4	0.20	0.94	0.22	0.92
ythb	4	0.46	0.76	0.80	0.54
ythob	4	2.20	0.12	0.55	0.70
ythl(ob)	16				

Table 2.2. Summary table of Wilcoxon matched-pairs signed-ranks tests for CPUE of juvenile pink and chum salmon in Prince William Sound in 1989 and 1990. A negative value for the median difference indicates higher CPUE in non-oiled than oiled locations.

Species/year	Pairs	Wilcoxon Statistic	*P*	Median Difference	95% CI of Median Difference
Pink Salmon/1989					
All	56	289	0.043	-24	(-107, 0)
Bays	28	75	0.737	0	(-2, 8)
Corridors	28	77	0.013	-114	(-655, -4)
Pink Salmon/1990					
All	95	925	0.092	-4	(-32, -0)
Bays	48	328	0.273	-2	(-14, 1)
Corridors	7	260	0.112	-52	(-290, 3)
Chum Salmon/1989	56	27	0.000	-64	(-130, -42)
Chum Salmon/1990	95	56	0.000	-53	(-92, -36)

Table 2.3. ANOVA table: ranks of CPUE of pink salmon in bays and corridors of Prince William Sound in 1989 and 1990. Each factor without an associated probability was used as the error term in the significance test for the factors listed above it; df = degrees of freedom, o = oil, l = location, (o) indicates nesting within oil, t = time, and h = habitat.

		Bays		Corridors	
Source	df	F	*P*	F	*P*
			1989		
o	1	0.20	0.698	27.10	0.035
l(o)	2				
t	3	6.80	0.023	1.81	0.245
to	3	1.41	0.329	2.99	0.117
tl(o)	6				
h	2	1.10	0.416	0.06	0.943
oh	2	0.36	0.715	0.05	0.951
hl(o)	4				
th	6	2.90	0.055	1.71	0.202
toh	6	0.47	0.815	0.44	0.838
thl(o)	12				
			1990		
o	1	3.67	0.196	3.94	0.186
l(o)	2				
t	3	0.35	0.790	5.34	0.040
to	3	0.39	0.768	6.52	0.026
tl(o)	6				
h	2	6.90	0.050	1.00	0.445
oh	2	0.01	0.988	0.02	0.982
hl(o)	4				
th	6	1.64	0.218	1.61	0.226
toh	6	0.31	0.918	0.95	0.499
thl(o)	12				

Table 2.4. Abundance of pink salmon in Prince William Sound in 1989 and 1990, as measured by five different parameters broken down by bays and corridors and by oiled and non-oiled locations. Significance was based on ANOVAs for rank of CPUE and on chi-square tests for frequency of occurrence.

	Bays			Corridors		
	Non-Oiled	Oiled	Significance	Non-Oiled	Oiled	Significance
			1989			
CPUE	23	8	n.t.	1,057	225	n.t.
Mean rank	24	25	n.s.	29	20	**
Frequency of occurrence (%)	37	42	n.s.	83	54	**
Frequency of >100 fish (%)	1	0	--	37	21	n.s.
Frequency of >1000 fish (%)	0	0	--	25	8	--
			1990			
CPUE	70	34	n.t.	1,429	203	n.t.
Mean rank	53	44	n.s.	55	41	n.s.
Frequency of occurrence (%)	75	62	n.s.	83	69	n.s.
Frequency of >100 fish (%)	10	8	--	43	25	*
Frequency of >1000 fish (%)	2	0	--	17	6	n.s.

n.t. not tested.
n.s. not significant.
* $0.050 < P < 0.100$.
** $0.010 < P < 0.050$.
*** $P < 0.010$.
-- not enough samples for valid test.

Table 2.5. Abundance of pink salmon in Prince William Sound in 1989 and 1990, as measured by five different parameters broken down by habitats separately in bays and corridors; LG = low-gradient habitat, MG = medium-gradient habitat, and SG = steep-gradient habitat. Significance was based on ANOVAs for rank of CPUE and on chi-square tests for frequency of occurrence.

	Bays				Corridors			
	LG	MG	SG	Significance	LG	MG	SG	Significance
				1989				
CPUE	5	32	11	n.t.	230	166	1526	n.t.
Mean rank	23	28	22	n.s.	24	25	25	n.s.
Frequency of occurrence (%)	31	56	31	n.s.	69	75	62	n.s.
Frequency of >100 fish (%)	0	6	0	--	31	31	31	n.s.
Frequency of >1000 fish (%)	0	0	0	--	12	6	31	--
				1990				
CPUE	20	100	35	n.t.	259	449	1793	n.t.
Mean rank	80	104	75	**	102	125	93	n.s.
Frequency of occurrence (%)	69	87	50	***	81	87	59	**
Frequency of >100 fish (%)	3	19	6	--	28	45	28	n.s.
Frequency of >1000 fish (%)	0	3	0	--	3	9	22	--

n.t. not tested.
n.s. not significant.
• $0.050 < P < 0.100$.
** $0.010 < P < 0.050$.
*** $P < 0.010$.
-- not enough samples for valid test.

Table 2.6. ANOVA table: ranks of CPUE of chum salmon in Prince William Sound in 1989 and 1990. Each factor without an associated probability was used as the error term in the significance test for the factors listed above it; df = degrees of freedom, o = oil, b = bay/corridor, l = location, (ob) indicates nesting within oil and bay/corridor, t = time, and h = habitat.

		1989		1990	
Source	df	F	*P*	F	*P*
o	1	54.63	0.002	0.10	0.770
b	1	0.06	0.818	0.16	0.706
ob	1	0.47	0.530	0.12	0.742
l(o)	4				
t	3	1.43	0.282	1.20	0.352
to	3	1.72	0.216	0.67	0.586
tb	3	0.60	0.628	0.44	0.729
tob	3	0.51	0.681	0.58	0.638
tl(ob)	12				
h	2	1.31	0.322	5.97	0.026
oh	2	0.13	0.877	0.62	0.563
bh	2	1.11	0.374	0.93	0.434
obh	2	1.09	0.382	0.47	0.642
hl(ob)	8				
th	6	1.42	0.249	0.93	0.489
toh	6	0.38	0.883	1.26	0.314
tbh	6	0.46	0.832	0.53	0.783
tobh	6	0.71	0.648	0.42	0.859
thl(ob)	24				

Table 2.7. Total catch, number of observed coded-wire tags, expanded catch of hatchery fish, and the percent hatchery composition of juvenile pink salmon in oiled and non-oiled locations of Prince William Sound in 1989 and 1990.

Location	Total Catch	Observed Tags	Total Hatchery Catch	Percent Hatchery Fish
		1989		
Oiled Bays				
Herring Bay	1,108	1	484	44
Snug Harbor (Inside)	949	0	0	0
Snug Harbor (Outside)	48,026	5	1,445	3
Non-oiled Bays				
McClure Bay	1,010	0	0	0
Long Bay	611	0	0	0
Oiled Corridors				
Knight Island Psg.	15,909	8	2,497	16
Prince of Wales Psg.	29,638	13	4,259	14
Non-oiled Corridors				
Culross Psg.	45,899	4	1,936	4
Wells Psg.	88,976	110	58,906	66
		1990		
Oiled Bays				
Herring Bay	5,061	111[1]	2,959	58
Snug Harbor (Inside)	3,514	0	0	0
Snug Harbor (Outside)	37,255	0	0	0
Non-oiled Bays				
McClure Bay	1,722	0	0	0
Long Bay	2,640	0	0	0
Oiled Corridors				
Knight Island Psg.	8,072	10[2]	3,495	43
Prince of Wales Psg.	68,436	40[3]	1,179	3
Non-oiled Corridors				
Culross Psg.	16,745	2	1,182	7
Wells Psg.	59,643	118	68,616	100

[1]Includes 106 tags from wild stocks.
[2]Includes 4 tags from wild stocks.
[3]Includes 37 tags from wild stocks.

Table 2.8. Total catch, number of observed coded-wire tags, expanded catch of hatchery fish, and the percent hatchery composition of juvenile chum salmon in oiled and non-oiled locations of Prince William Sound in 1989 and 1990. Because chum salmon released from some hatcheries in 1989 were not marked representatively, the lack of tag recoveries in 1989 does not necessarily mean that no hatchery fish were captured.

Location	Total Catch	Observed Tags	Total Hatchery Catch	Percent Hatchery Fish
		1989		
Oiled Bays				
Herring Bay	216	0	--	--
Snug Harbor (Inside)	0	0	--	--
Snug Harbor (Outside)	0	0	--	--
Non-oiled Bays				
McClure Bay	10,850	0	--	--
Long Bay	1,299	0	--	--
Oiled Corridors				
Knight Island Psg.	117	0	--	--
Prince of Wales Psg.	8	0	--	--
Non-oiled Corridors				
Culross Psg.	6,471	0	--	--
Wells Psg.	11,234	0	--	--
		1990		
Oiled Bays				
Herring Bay	109	0	0	0
Snug Harbor (Inside)	0	0	0	0
Snug Harbor (Outside)	1	0	0	0
Non-oiled Bays				
McClure Bay	19,250	0	0	0
Long Bay	2,478	0	0	0
Oiled Corridors				
Knight Island Psg.	142	0	0	0
Prince of Wales Psg.	1	0	0	0
Non-oiled Corridors				
Culross Psg.	7,595	0	0	0
Wells Psg.	3,161	15	5,769	100

Table 2.9. Summary table of Wilcoxon matched-pair signed-rank tests for FL of juvenile pink and chum salmon in Prince William Sound in 1989 and 1990. A negative value for the median difference indicates larger FL in non-oiled than oiled locations; median values are in mm.

Species	Pairs	Wilcoxon Statistic	*P*	Median Difference	95% CI of Median Difference
			1989		
Pink salmon					
All	29	132	0.066	-1	(-4, 0)
Bays	10	30	0.838	1	(-6, 3)
Corridors	19	39	0.026	-2	(-6, -1)
Chum Salmon					
All	18	139	0.021	6	(1, 12)
			1990		
Pink salmon					
All	39	346	0.544	0	(-2, 1)
Bays	18	80	0.828	0	(-1, 2)
Corridors	21	93	0.444	-1	(-3, 2)
Chum Salmon					
All	13	71	0.081	4	(-1, 11)

Table 2.10. ANOVA table: mean FL of juvenile pink salmon in Prince William Sound in 1990. Each factor without an associated probability was used as the error term in the significance test for the factors listed above it; df = degrees of freedom, o = oil, b = bay/corridor, l = location, (ob) indicates nesting within oil and bay/corridor, t = time, and h = habitat.

Source	df	F	*P*
o	1	0.00	0.967
b	1	2.31	0.203
ob	1	0.38	0.570
l(ob)	4		
t	3	16.76	0.001
to	3	1.21	0.350
tb	3	3.58	0.047
tob	3	0.01	0.998
l(ob)	12		
h	2	0.16	0.851
oh	2	0.26	0.775
bh	2	0.21	0.818
obh	2	0.01	0.991
hl(ob)	8		
th	6	0.50	0.795
toh	6	0.27	0.941
tbh	6	0.10	0.995
thl(ob)	13		

Table 2.11. Number of fish processed for otolith increments, number and percent of otolith samples with discernable marine zone (DMZ), mean and SE of number of otolith increments in DMZ, and mean, SE, and 95% confidence interval (CI) of otolith increment width in DMZ for juvenile pink salmon sampled in four bays in Prince William Sound, May 1990.

	Non-oiled		Oiled	
	McClure Bay	Long Bay	Herring Bay	Snug Harbor
Sample Date	5/04/90	5/05/90	5/03/90	5/05/90
Number Processed	44	46	35	44
Number w/ DMZ	12	8	8	28
Percent w/ DMZ	27	17	23	64
Mean No. Increments	11.4	9.8	11.0	14.1
SE, Mean No. Increments	0.8	2.00	1.4	1.1
Mean Increment Width	1.034	1.072	1.278	1.104
SE, Mean Increment Width	0.045	0.111	0.089	0.038
95% CI, Mean Increment Width	(0.914-1.154)	(0.925-1.219)	(1.130-1.425)	(1.051-1.168)

Table 2.12. Apparent daily growth of juvenile pink salmon by habitat type at oiled and non-oiled corridors in Prince William Sound in 1989 and 1990. Growth was assumed to be exponential over time and was determined as the slope of the regression of the natural logarithm of weight on time in days. Numbers in table are % increase in body weight per day, with standard deviation in parentheses; LG = low gradient; MG = medium gradient; SG = steep gradient.

Location	Habitat		
	LG	MG	SG
1989 Oiled Corridors			
Knight Island Passage	0.94 (0.17)	2.61 (0.17)	2.08 (0.34)
Prince of Wales Passage	2.18 (0.20)	0.68 (0.39)	0.45 (0.42)
Mean	1.56	1.64	1.26
1989 Non-oiled Corridors			
Wells Passage	2.46 (0.26)	3.86 (0.35)	1.81 (0.40)
Culross Passage	2.05 (0.18)	2.61 (0.15)	4.53 (0.30)
Mean	2.25	3.23	3.17
1990 Oiled Corridors			
Knight Island Passage	2.37 (0.20)	4.14 (0.32)	5.03 (0.24)
Prince of Wales Passage	2.36 (0.16)	2.87 (0.18)	3.86 (0.29)
Mean	2.36	3.50	4.44
1990 Non-oiled Corridors			
Wells Passage	2.09 (0.11)	1.99 (0.11)	3.48 (0.17)
Culross Passage	1.68 (0.31)	1.74 (0.18)	7.69 (0.44)
Mean	1.88	1.86	5.58

Table 2.13. ANOVA table: apparent daily growth of juvenile pink salmon in oiled and non-oiled corridors of Prince William Sound in 1989 and 1990. Each factor without an associated probability was used as the error term in the significance test for the factors listed above it; df = degrees of freedom, o = oil, l = location, (o) indicates nesting within oil, y = year, and h = habitat.

Source	df	F	*P*
o	1	0.94	0.435
l(o)	2		
y	1	27.35	0.035
yo	1	17.14	0.054
yl(o)	2		
h	2	1.51	0.324
oh	2	0.42	0.681
hl(o)	4		
yh	2	21.00	0.008
yoh	2	4.19	0.104
yhl(o)	4		

Table 2.14. Comparison of weight/length relationship of juvenile pink and chum salmon between oiled and non-oiled areas of Prince William Sound in 1989 and 1990; n = number of samples, a = y-intercept, b = slope, *P*,b is the probability value for the tests of homogeneity of slopes for the pairs of regression lines shown, *P*,am is the probability value for differences in adjusted means, and intersect is the length (mm) at which non-parallel regression lines intersect.

Location	n	a	b	R^2	*P*,b	*P*,am	Intersect
			1989 Pink Salmon				
Non-oiled bays	236	-13.1693	3.3245	85.8	0.129	0.425	Parallel
Non-oiled corridors	899	-13.5611	3.4386	97.8			
Oiled bays	245	-13.7751	3.5010	93.1	0.276	0.239	Parallel
Oiled corridors	850	-13.5479	3.4408	97.1			
Non-oiled pooled bays and corridors	1,136	-13.5620	3.4385	97.7	0.343	0.000	Parallel
Oiled pooled bays and corridors	1,096	-13.6256	3.4610	97.1			
			1990 Pink Salmon				
Non-oiled bays	791	-14.2775	3.6312	92.7	0.000	--	40
Oiled bays	796	-13.5057	3.4208	95.3			
Non-oiled corridors	1,464	-13.6495	3.4518	97.2	0.000	--	65
Oiled corridors	1,067	-13.3544	3.3813	97.6			
			1989 Chum Salmon				
Non-oiled bays	832	-13.2737	3.3904	91.2	0.000	0.000	57
Oiled bays	92	-11.4371	2.9179	93.2			
Non-oiled corridors	957	-13.7470	3.5232	96.7	0.257	0.132	Parallel
Oiled corridors	45	-14.1126	3.6272	98.6			
			1990 Chum Salmon				
Non-oiled bays	1,476	-14.9194	3.8274	90.5	0.000	--	44
Oiled bays	83	-13.2117	3.3748	97.9			
Non-oiled corridors	1,122	-13.7671	3.5204	96.1	0.423	0.426	Parallel
Oiled corridors	129	-13.9401	3.5649	97.5			

Table 2.15. ANOVA table: temperature at 1-m and 4-m depths in Prince William Sound, 1989 and 1990. Each factor without an associated probability was used as the error term in the significance test for the factors above it; df = degrees of freedom, o = oil, b = bay/corridor, l = location, (ob) indicates nesting within oil and bay/corridor, and t = time.

Source	1989 df	1989 F	1989 *P*	1990 df	1990 F	1990 *P*
			1-m Depth			
o	1	0.71	0.446	1	0.01	0.911
b	1	4.17	0.111	1	0.15	0.718
ob	1	0.24	0.651	1	0.03	0.863
l(ob)	4			4		
t	4	18.42	0.000	3	69.49	0.000
to	4	0.60	0.668	3	0.46	0.713
tb	4	0.69	0.607	3	0.20	0.896
tob	4	0.26	0.901	3	0.87	0.483
tl(ob)	16			12		
Error	72			63		
Total	111			94		
			4-m Depth			
o	1	3.72	0.126	1	0.30	0.615
b	1	2.10	0.221	1	0.01	0.944
ob	1	0.01	0.923	1	0.00	0.964
l(ob)	4			4		
t	4	438.12	0.000	3	189.80	0.000
to	4	1.05	0.415	3	2.38	0.121
tb	4	7.60	0.001	3	0.30	0.825
tob	4	2.90	0.056	3	0.32	0.812
tl(ob)	16			12		
Error	72			63		
Total	111			94		

Table 2.16. ANOVA table: salinity at 1-m and 4-m depths in Prince William Sound, 1989 and 1990. Each factor without an associated probability was used as the error term in the significance test for the factors above it; df = degrees of freedom, o = oil, b = bay/corridor, l = location, (ob) indicates nesting within oil and bay/corridor, and t = time.

	1989			1990		
Source	df	F	*P*	df	F	*P*
			1-m Depth			
o	1	53.51	0.002	1	100.45	0.022
b	1	5.82	0.073	1	2.60	0.182
ob	1	9.60	0.036	1	1.26	0.325
l(ob)	4			4		
t	4	3.47	0.032	3	4.64	0.022
to	4	1.50	0.249	3	0.29	0.829
tb	4	0.40	0.804	3	0.29	0.896
tob	4	0.40	0.809	3	0.29	0.832
tl(ob)	16			12		
Error	72			63		
Total	111			94		
			4-m Depth			
o	1	144.14	0.000	1	1.60	0.274
b	1	23.26	0.009	1	2.00	0.230
ob	1	0.03	0.871	1	0.64	0.467
l(ob)	4			4		
t	4	49.13	0.000	3	6.80	0.006
to	4	7.11	0.002	3	0.46	0.713
tb	4	4.49	0.013	3	0.25	0.857
tob	4	0.31	0.866	3	0.91	0.463
tl(ob)	16			12		
Error	72			63		
Total	111			94		

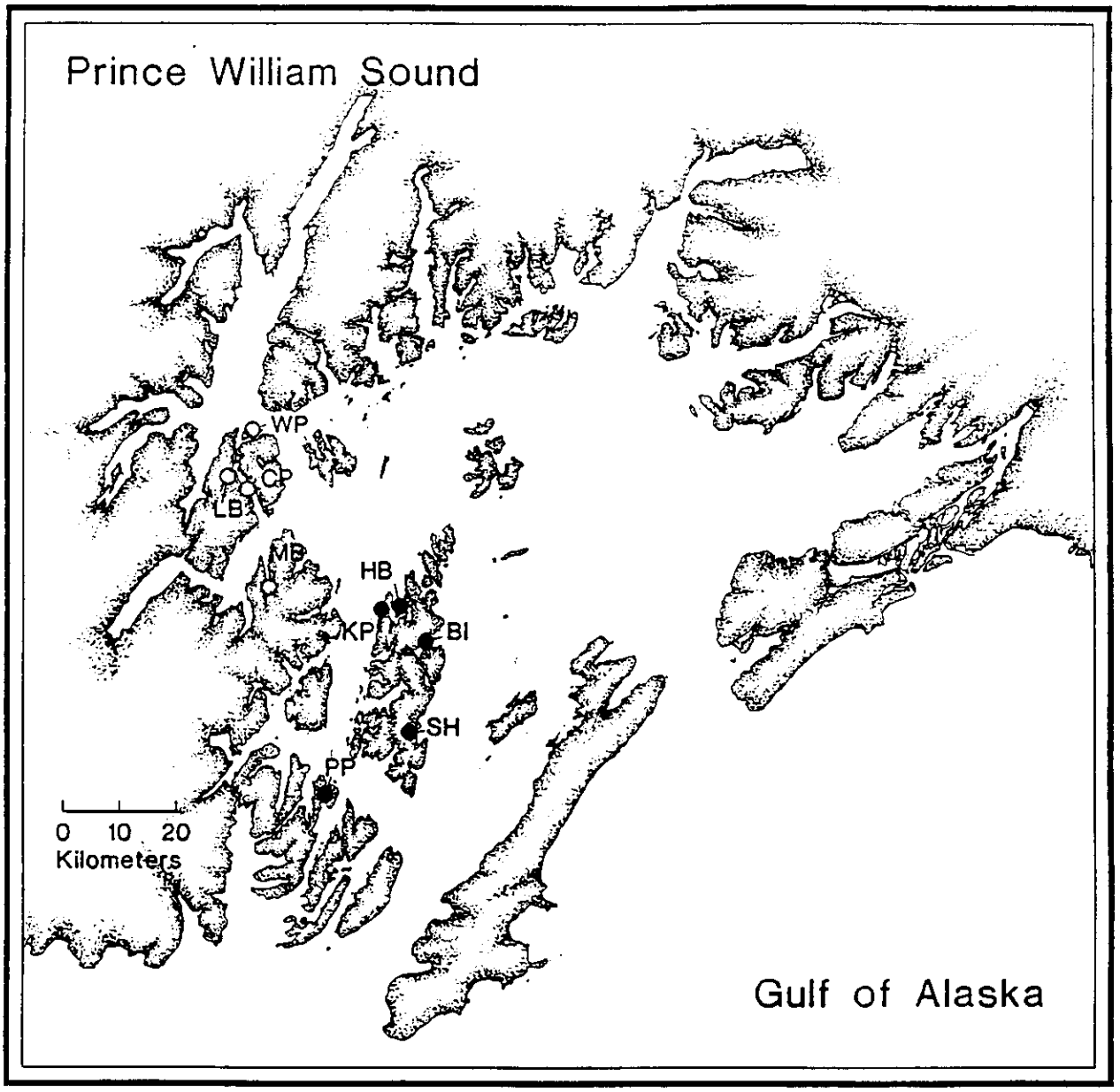


Figure 2.1. Sampling locations in western Prince William Sound for NMFS component of NRDA study F/S-4. Oiled locations, Herring Bay (HB), Snug Harbor (SH), Bay of Isles (BI), Knight Island Passage (KP), and Prince of Wales Passage (PP), are marked by a filled circle; and non-oiled sampling locations, McClure Bay (MB), Long Bay (LB), Wells Passage (WP), and Culross Passage (CP), are marked by an unfilled circle.

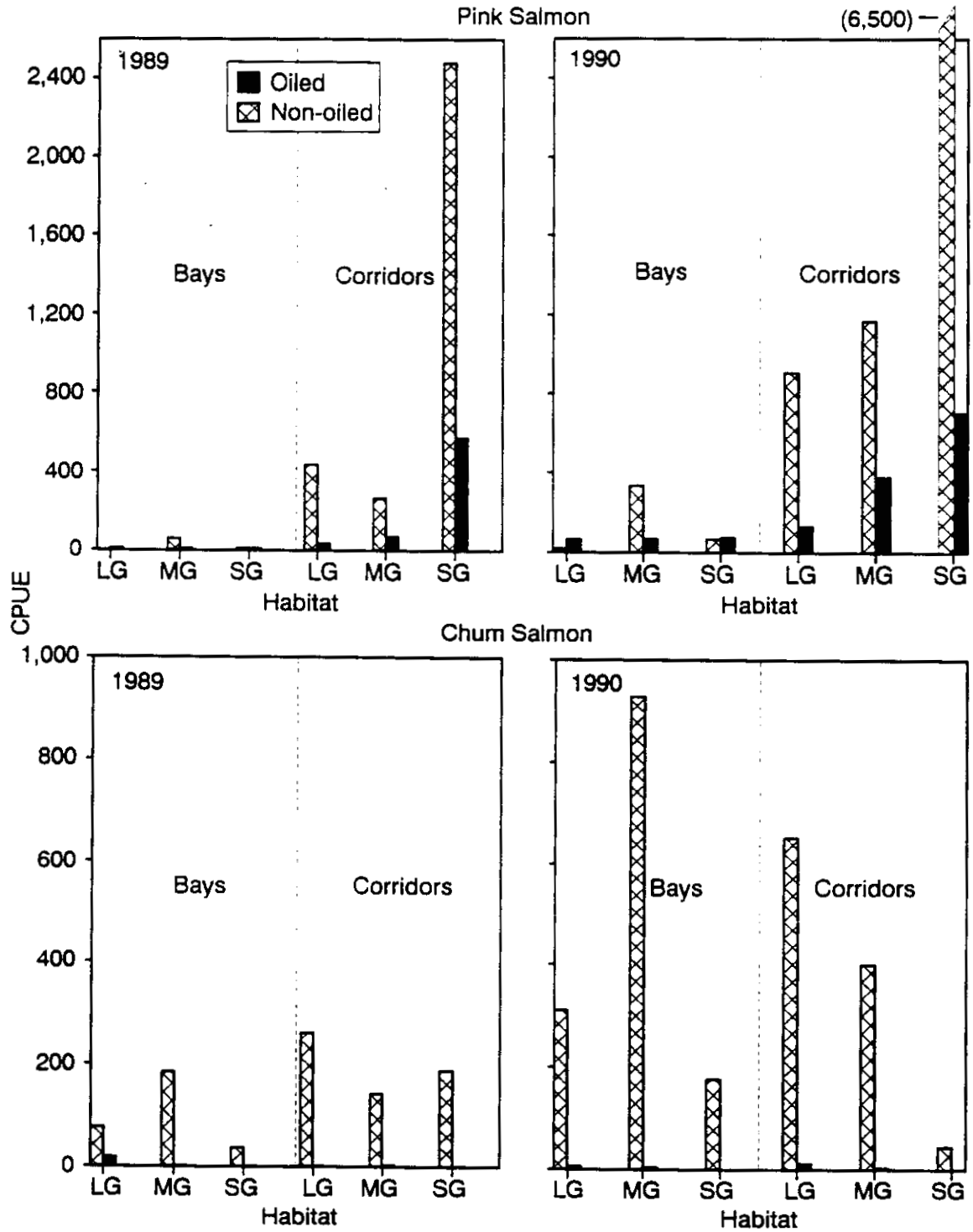


Figure 2.2. Mean CPUE of juvenile pink and chum salmon by habitat type in oiled and non-oiled bays and corridors of Prince William Sound in 1989 and 1990; LG = low gradient; MG = medium gradient; and SG = steep gradient habitat.

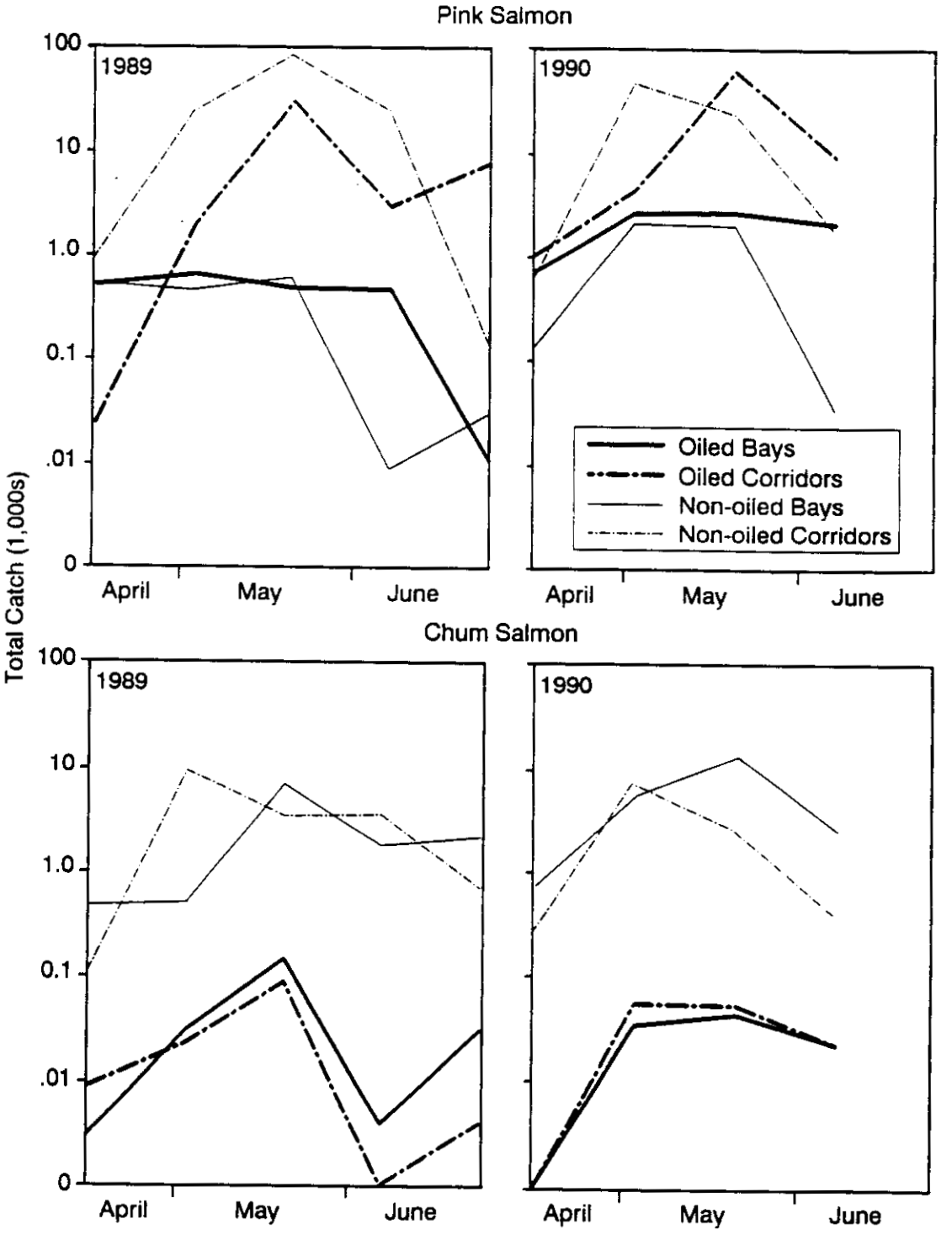


Figure 2.3. Total catch (in logarithmic scale) of juvenile pink and chum salmon in Prince William Sound in April-June, 1989 and 1990. Fish captured in outer Snug Harbor are not included.

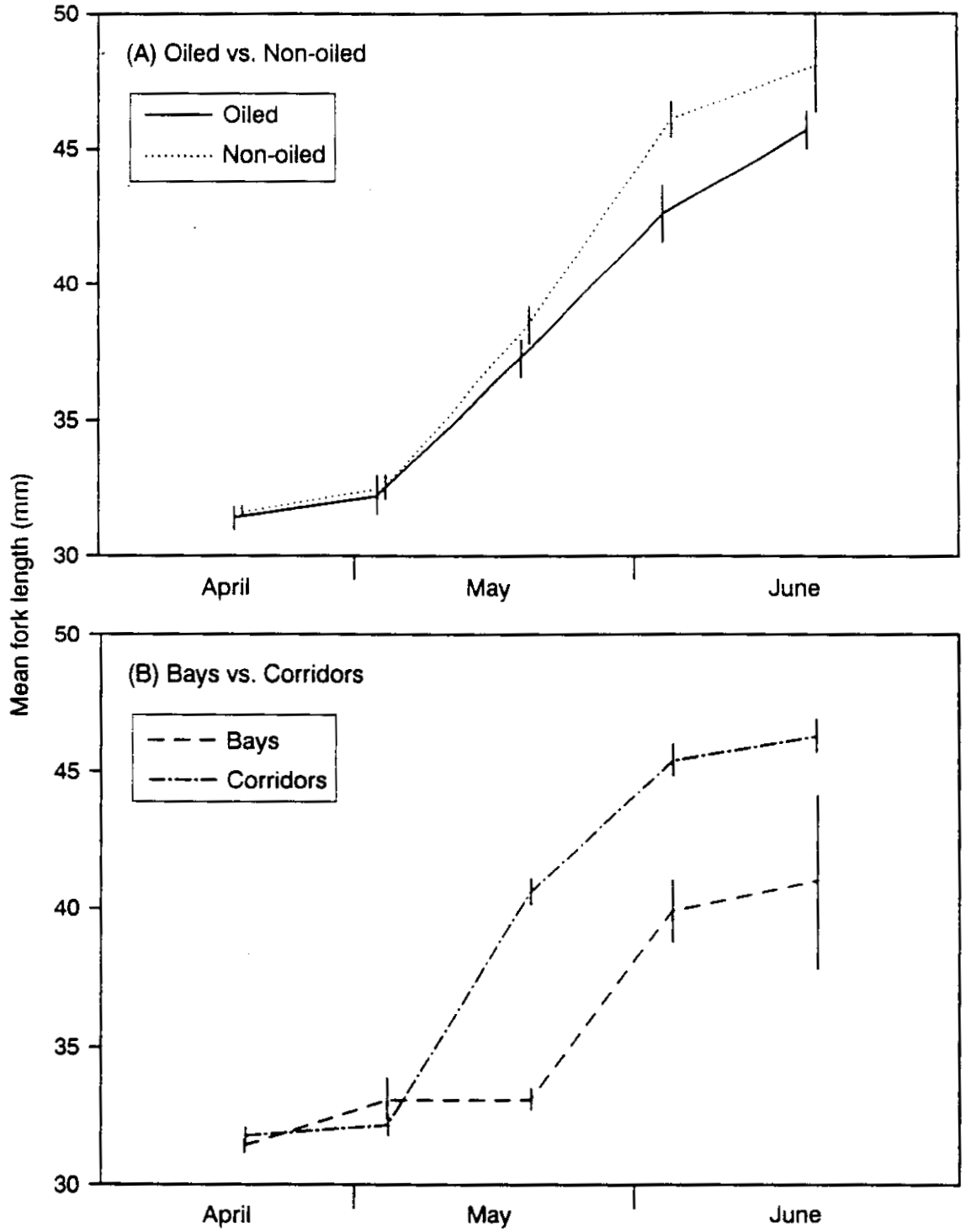


Figure 2.4. Mean FL of juvenile pink salmon captured in Prince William Sound in 1989, pooled by oiled and non-oiled locations (A) and by bays and corridors (B). Vertical bars are 95% confidence intervals of the means.

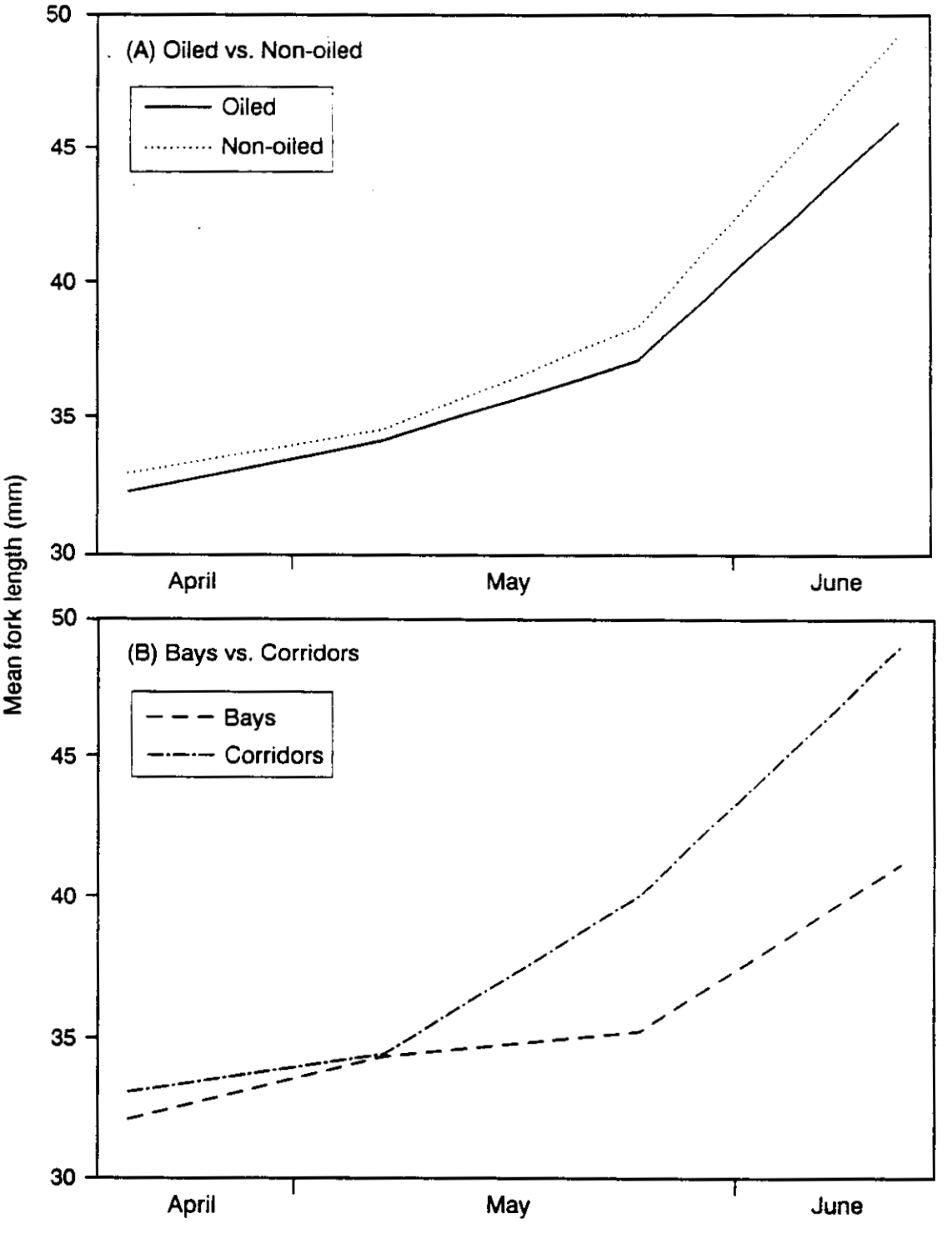


Figure 2.5. Mean FL of pink salmon captured in Prince William Sound in 1990 in (A) oiled vs. non-oiled areas and (B) bays vs. corridors; 95% confidence intervals were all within 0.5 mm of the indicated means.

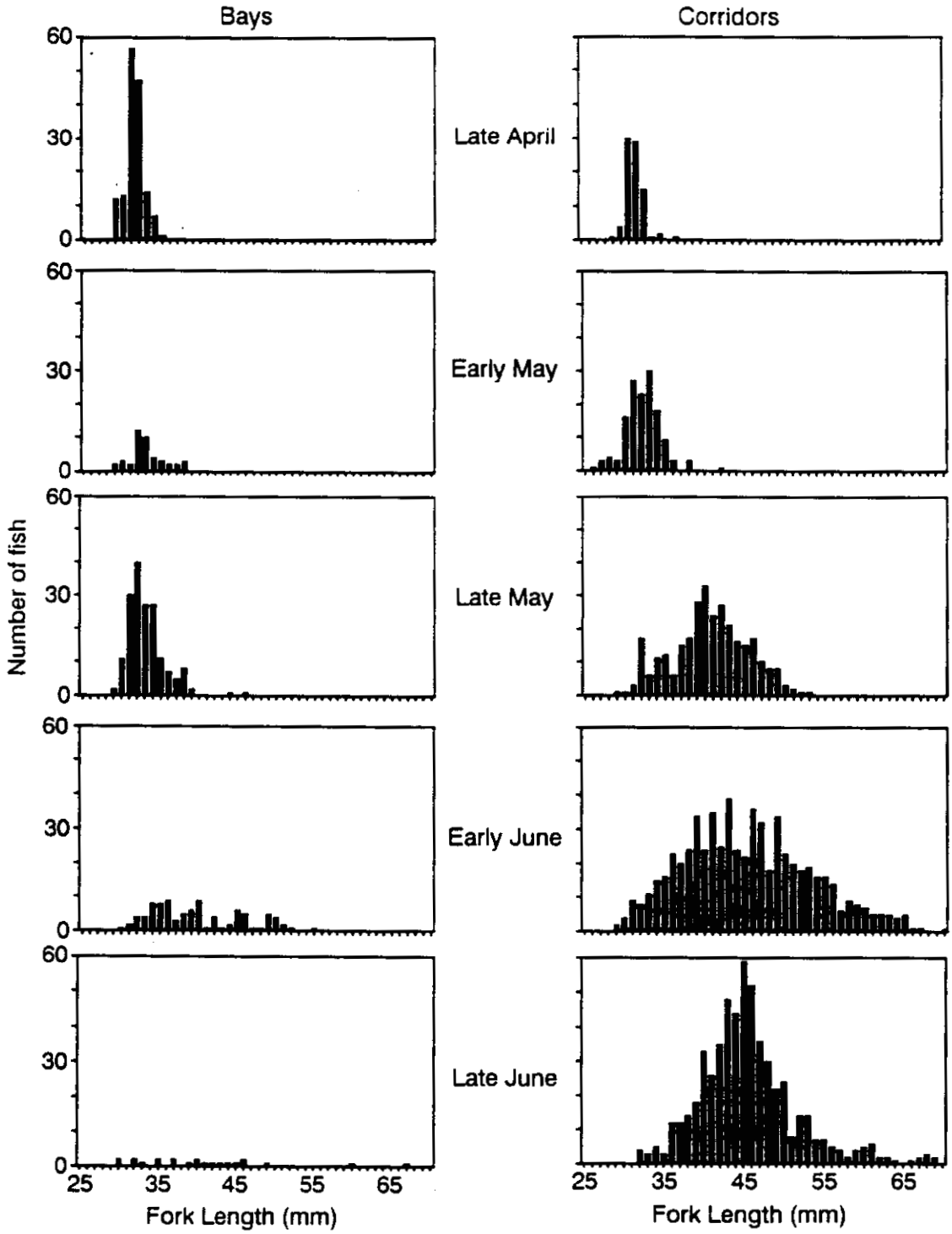


Figure 2.6. Histograms of FL of juvenile pink salmon captured in Prince William Sound in 1989.

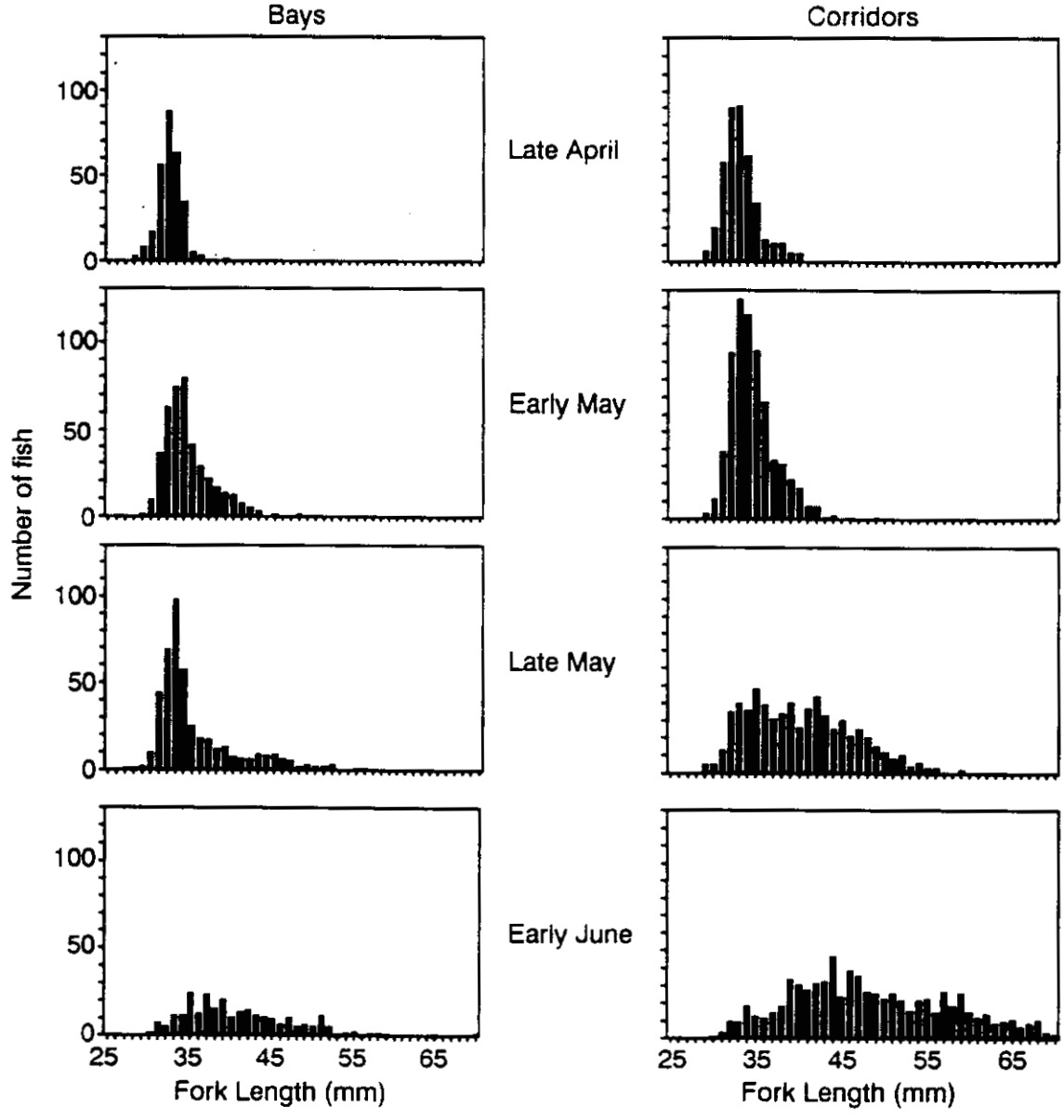


Figure 2.7. Histograms of FL of juvenile pink salmon captured in Prince William Sound in 1990.

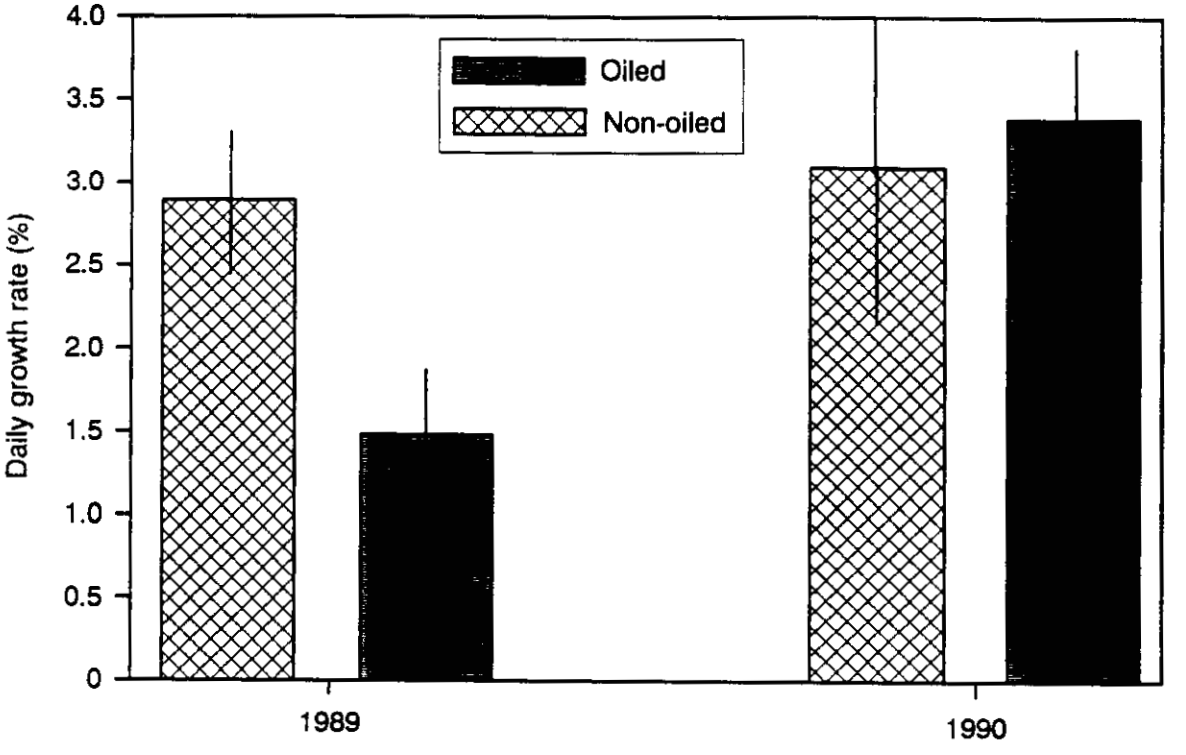


Figure 2.8. Apparent daily growth (±1 SE) of juvenile pink salmon in corridors of Prince William Sound in 1989 and 1990.

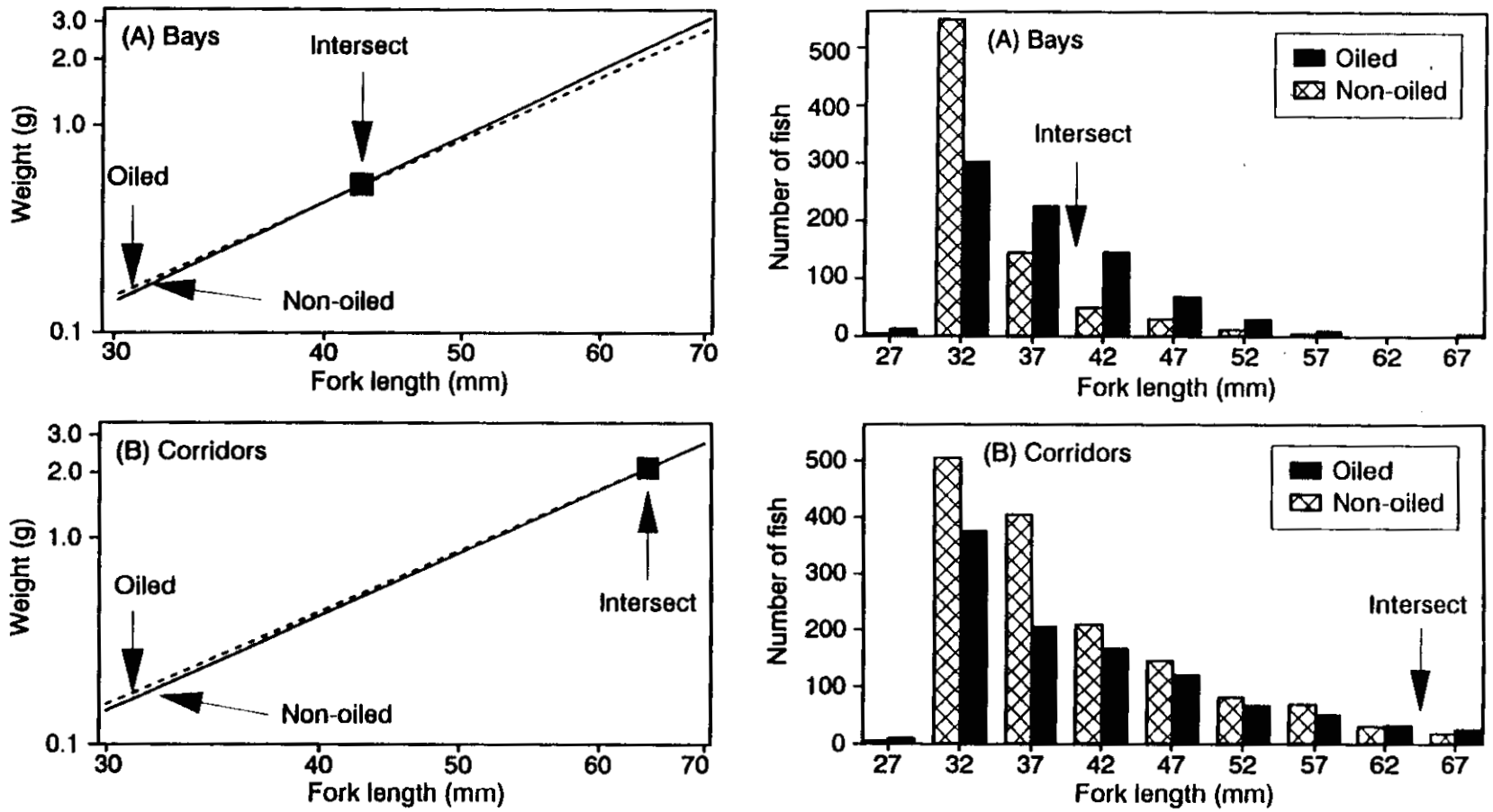


Figure 2.9. Weight/length relationships (ln-ln scale) and histograms of FL in 5-mm increments of pink salmon in oiled and non-oiled bays (A) and corridors (B) in Prince William Sound, April-June, 1990. The intersection points of the weight/length regression lines are indicated on all graphs.

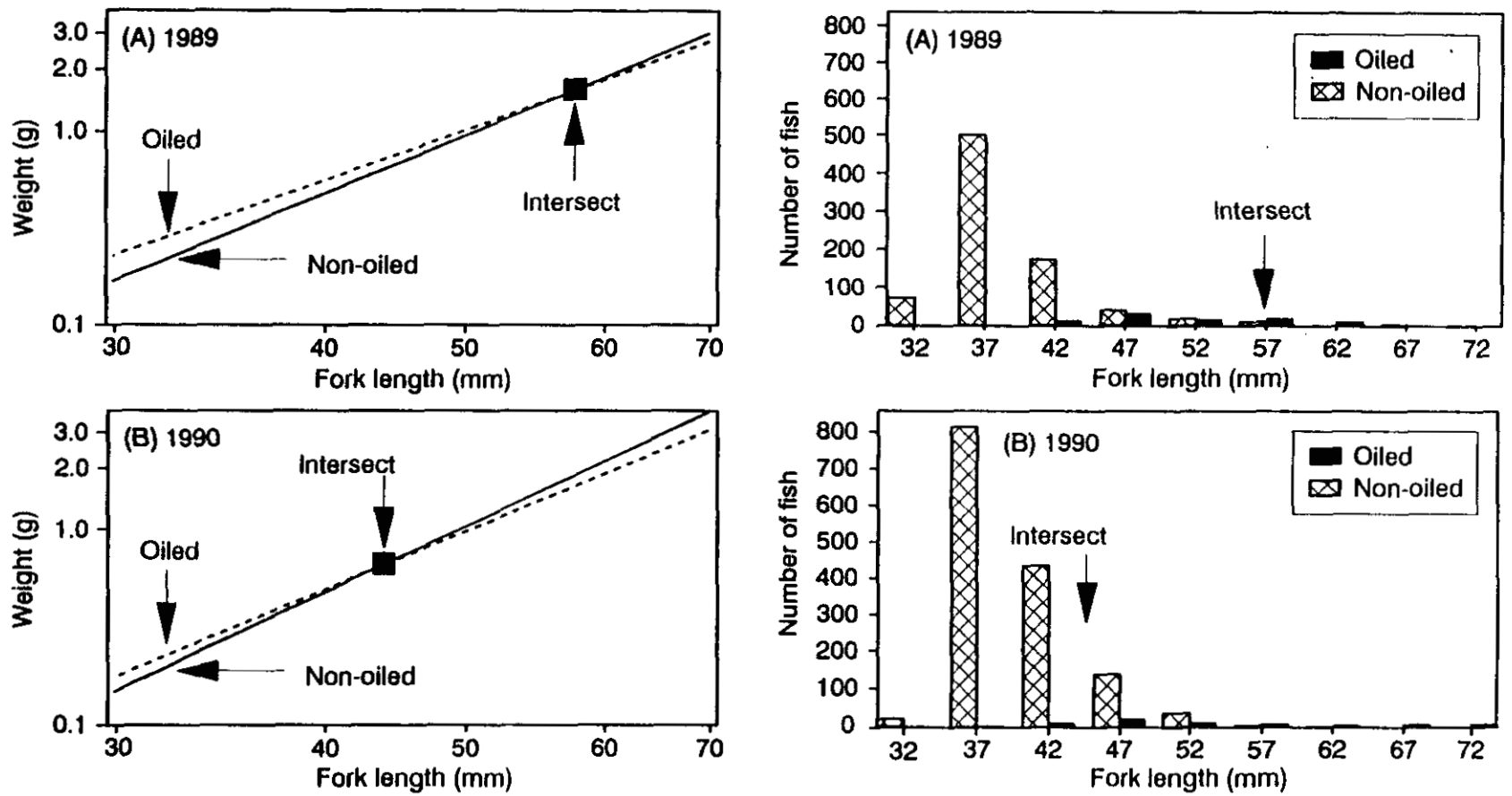


Figure 2.10. Weight/length relationships (ln-ln scale) and histograms of FL in 5-mm increments of chum salmon in oiled and non-oiled bays in Prince William Sound in 1989 (A) and 1990 (B). The intersection points of the weight/length regression lines are indicated on all graphs.

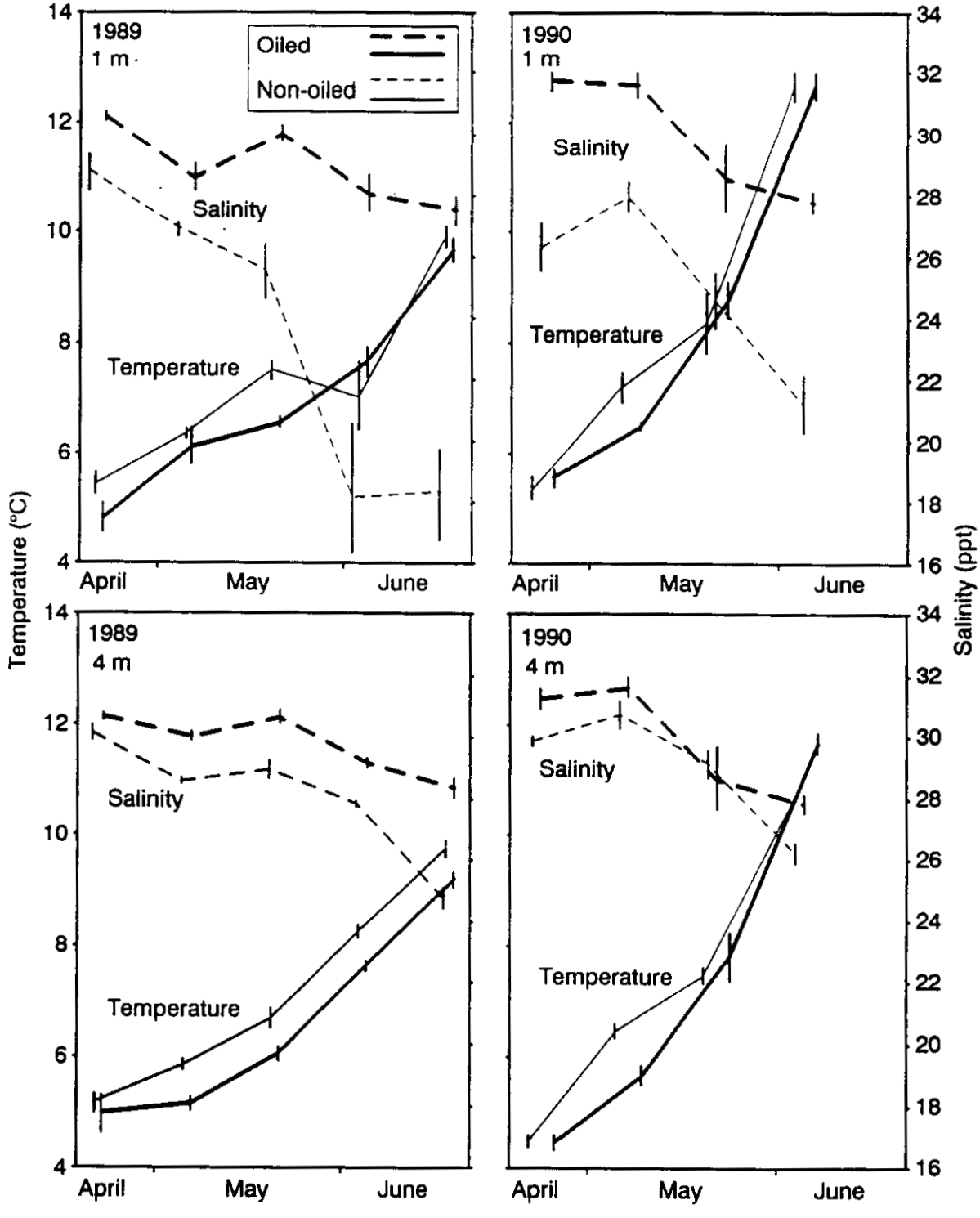
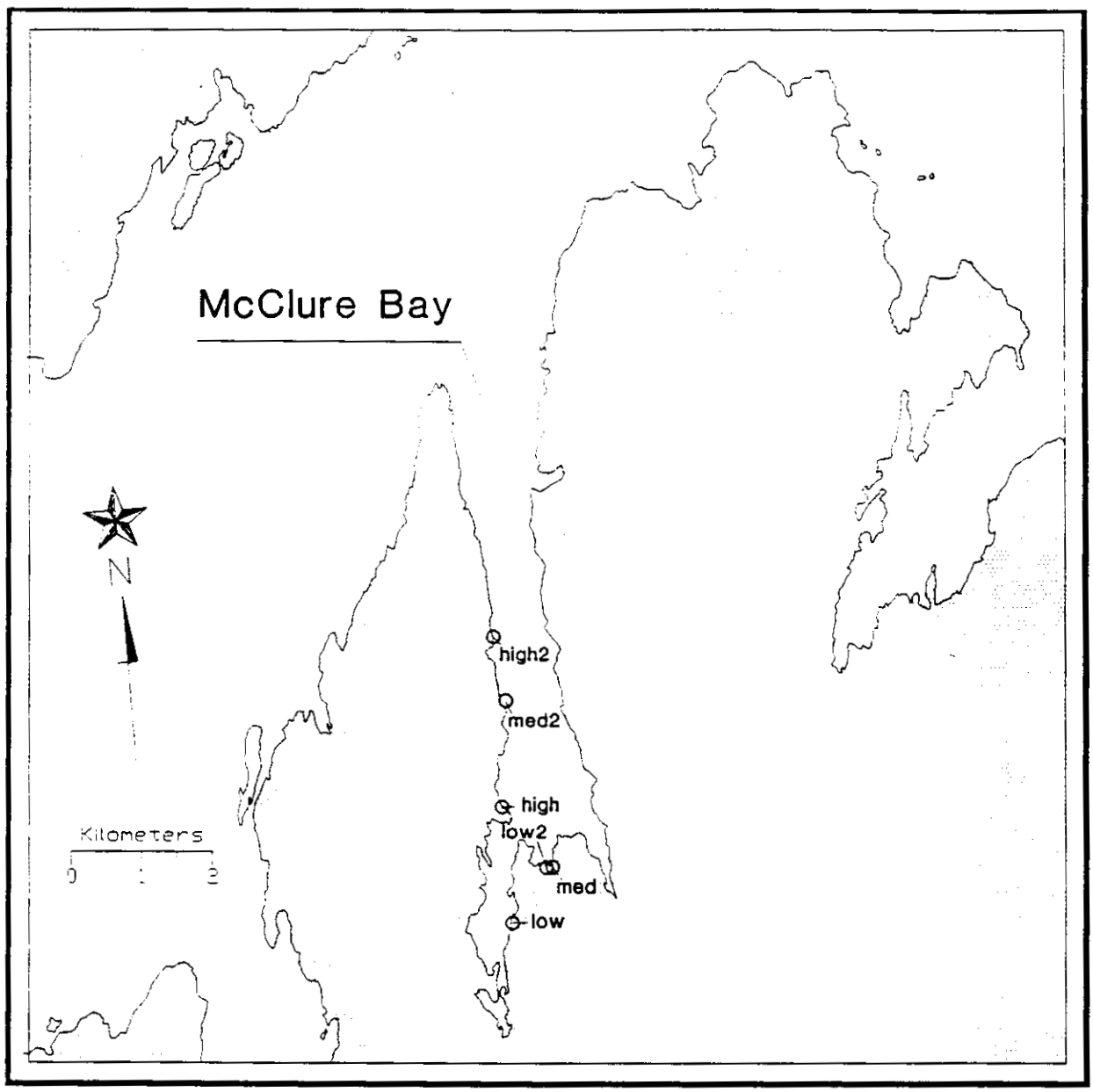
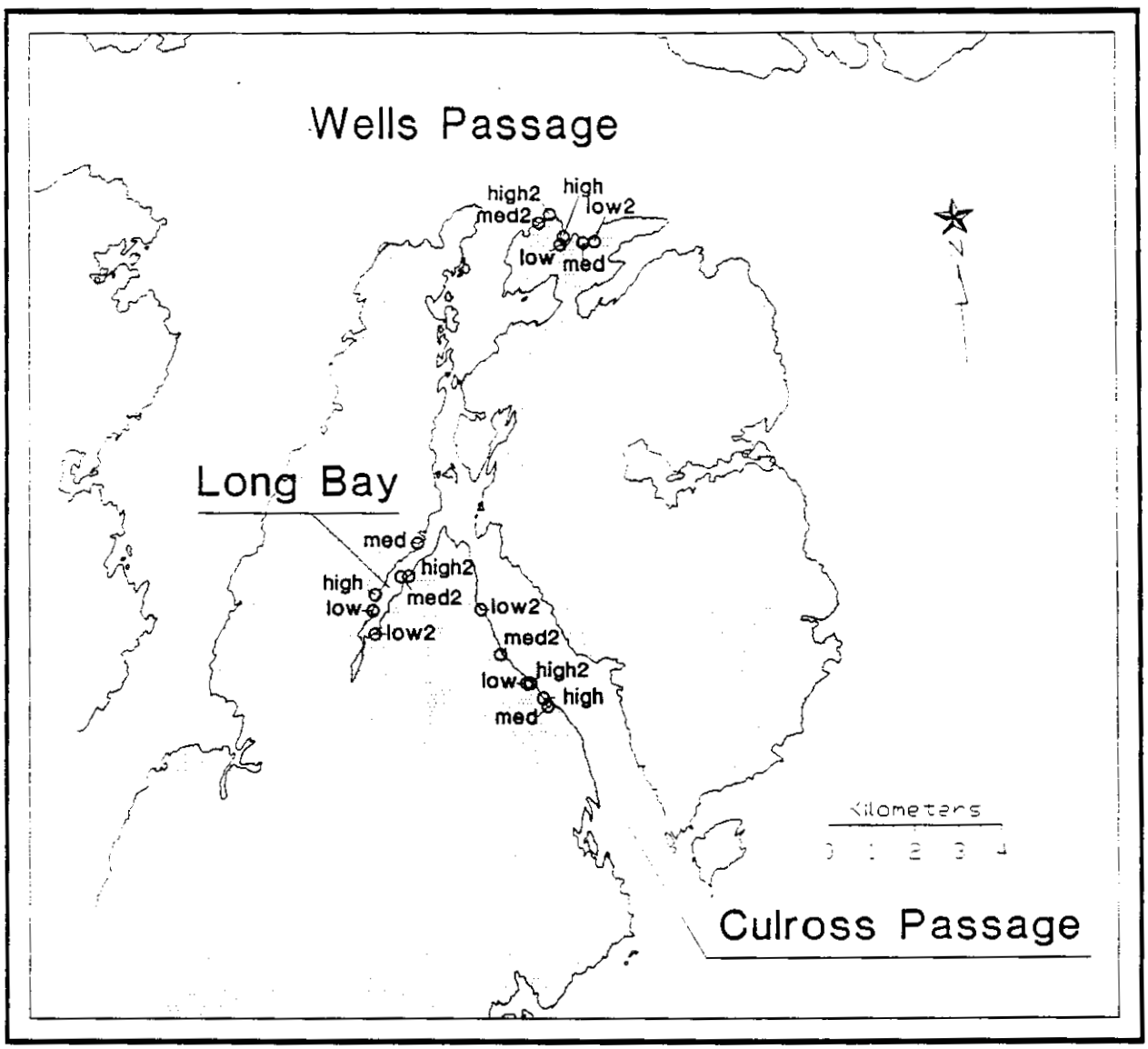


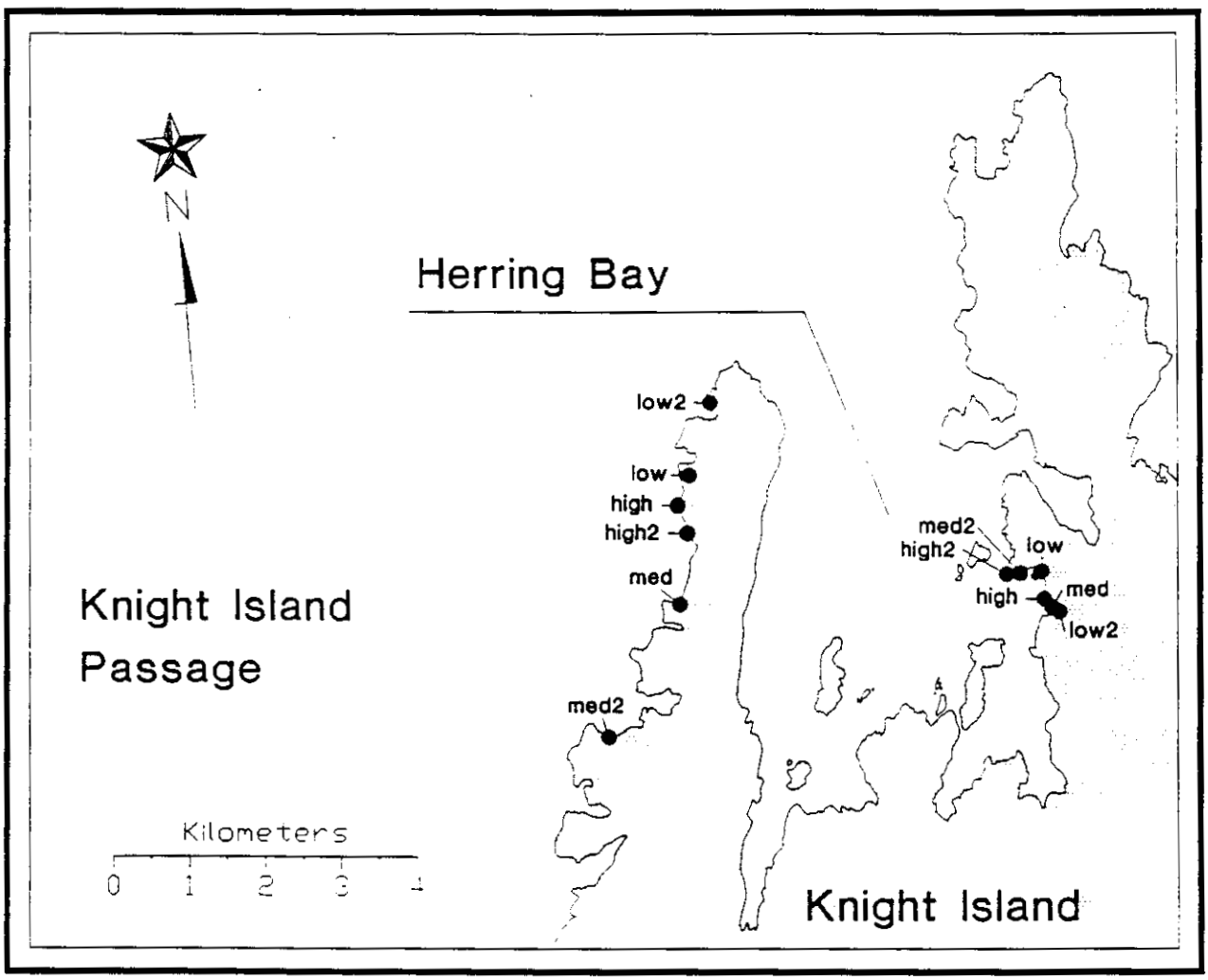
Figure 2.11. Water temperature and salinity (±1 SE) at 1-m and 4-m depths in Prince William Sound from April to June 1989 and 1990.



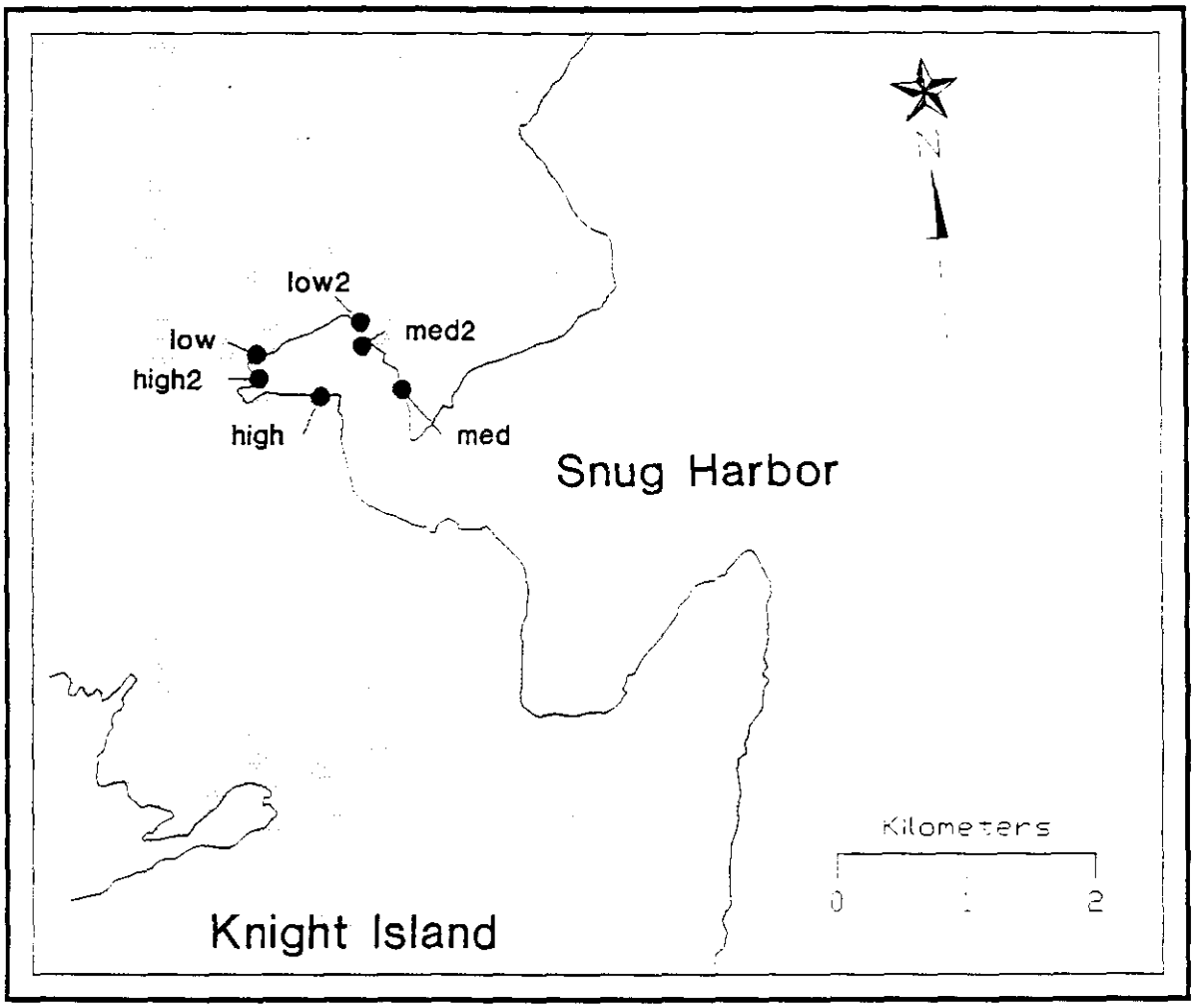
Appendix 2.1A. Systematic sampling sites at McClure Bay; low = low gradient, med = medium gradient, and high = steep gradient. Sites followed by the number 2 were sampled only in 1990; all other sites were sampled both in 1989 and 1990.



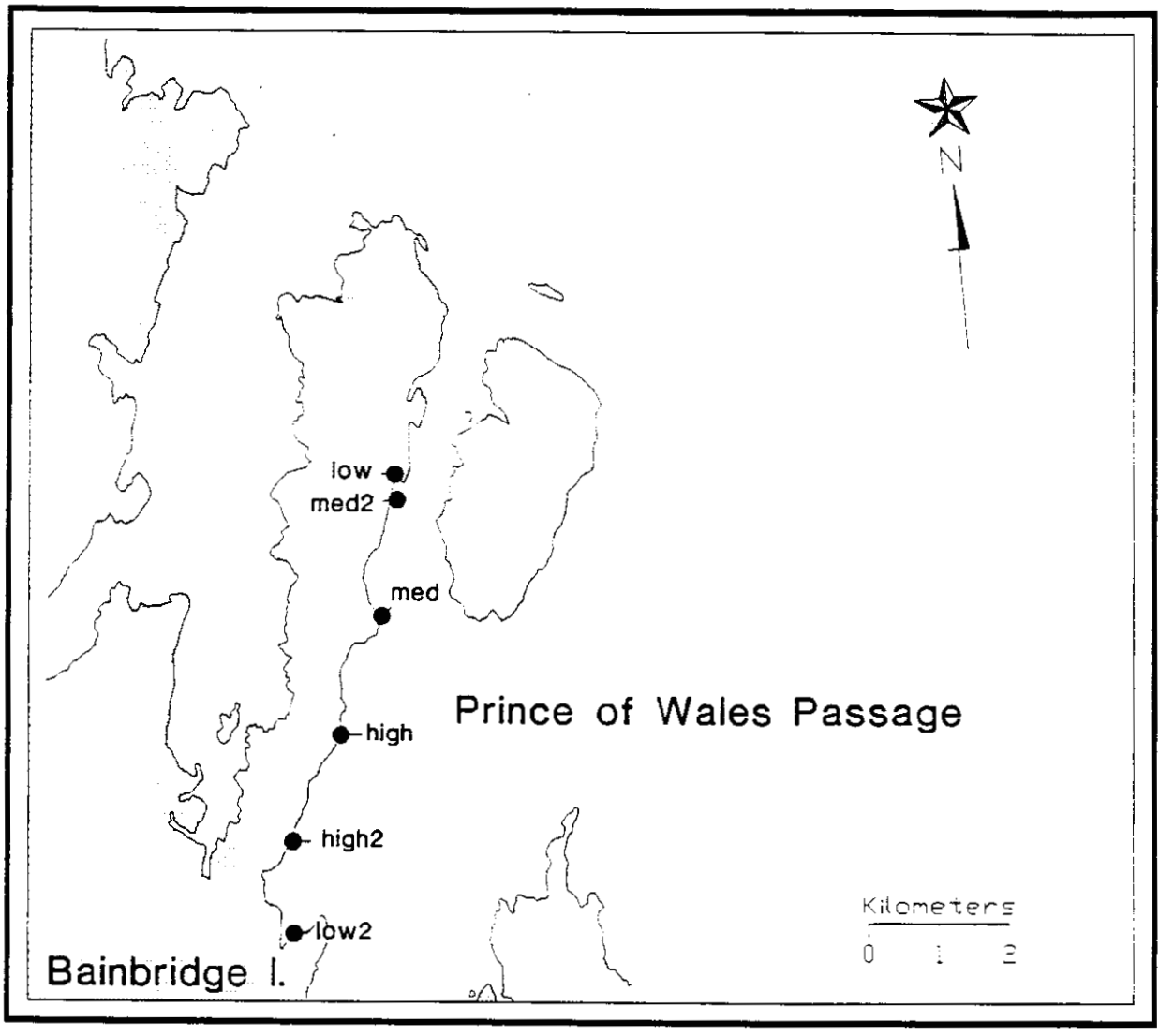
Appendix 2.1B. Systematic sampling sites at Long Bay, Wells Passage, and Culross Passage; low = low gradient, med = medium gradient, and high = steep gradient. Sites followed by the number 2 were sampled only in 1990; all other sites were sampled both in 1989 and 1990.



Appendix 2.1C. Systematic sampling sites in Herring Bay and Knight Island Passage; low = low gradient, med = medium gradient, and high = steep gradient. Sites followed by the number 2 were sampled only in 1990; all other sites were sampled both in 1989 and 1990.



Appendix 2.1D. Systematic sampling sites in Snug Harbor; low = low gradient, med = medium gradient, and high = steep gradient. Sites followed by the number 2 were sampled only in 1990; all other sites were sampled both in 1989 and 1990.



Appendix 2.1E. Systematic sampling sites in Prince of Wales Passage; low = low gradient, med = medium gradient, and high = steep gradient. Sites followed by the number 2 were sampled only in 1990; all other sites were sampled both in 1989 and 1990.

CHAPTER 3

Exposure and Contamination of Juvenile Salmon, Mussels, and Sediments in Prince William Sound following the *Exxon Valdez* Oil Spill.

Preface

This chapter is composed of two sections: A) Exposure and contamination of juvenile pink and chum salmon by hydrocarbons in Prince William Sound following the *Exxon Valdez* oil spill, and B) Contamination of surficial sediments by *Exxon Valdez* crude oil in nearshore habitats of juvenile salmon in Prince William Sound. We have avoided reference to sediment data in section 3A because of the rate at which data have returned from the analytical laboratories and difficulties in interpretation. The last 37% of the sediment samples arrived in October 1992, thus precluding completion of final analysis for this report deadline.

CHAPTER 3A. Exposure and Contamination of Juvenile Pink and Chum Salmon by Hydrocarbons in Prince William Sound following the *Exxon Valdez* Oil Spill.

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R. M. Smolowitz, and J. J. Stegeman

Abstract

Juvenile pink and chum salmon were collected from oiled nearshore marine habitats in Prince William Sound in 1989 and 1990 to determine if they were contaminated by exposure to *Exxon Valdez* crude oil (*EVC*). For comparison, fish were also collected from non-oiled, control areas located as closely as possible to oiled areas. Mussels were routinely sampled to obtain site-specific measures of biological availability of hydrocarbons. Mussels were contaminated with polynuclear aromatic hydrocarbons (PAH) in oiled areas in 1989 (6,942 ± 1,427 ng/g); much lower concentrations were observed in control locations (25 ± 13 ng/g). Contamination of mussel tissues persisted into 1990, but at greatly reduced concentrations (24 ± 5 and 6 ± 1 ng/g for oiled and non-oiled locations, respectively). In 1989, PAH concentrations were significantly greater in pink salmon tissues from oiled areas (181 ± 28 ng/g) than from non-oiled areas (54 ± 8 ng/g). In 1990, PAH concentrations (51 ± 7 ng/g) were not significantly different in oiled and non-oiled locations. Most of the PAHs detected in pink salmon and mussel tissues were also found in *Exxon Valdez* crude oil (97.0% ± 0.5). As may be expected after a single perturbation, PAH concentrations in mussels and pink salmon increased to peak levels after the spill, and then declined. Cytochrome P4501A was significantly induced in juvenile pink and chum salmon from oiled locations in 1989 but not in 1990. Based on these observations, we conclude that *Exxon Valdez* crude oil caused these differential changes in polynuclear aromatic hydrocarbon concentrations and induced cytochrome P4501A enzyme activity. The degree of contamination of visceral tissues and the frequency of tissue types staining for P4501A indicated that ingestion of whole oil was an important route of contamination for juvenile pink and chum salmon. We consider that contamination of juvenile salmon was physiologically significant and contributed to the reduced growth observed in junvenile pink salmon from oiled areas of Prince William Sound in 1989.

Introduction

Juvenile salmon in Prince William Sound (PWS) were at risk of exposure and contamination by the *Exxon Valdez* (*EV*) oil spill because of their time of entry into the marine environment, their habitat use, and their migration route to the Gulf of Alaska. Oil in the marine environment can affect salmon in a variety of ways. It can be directly toxic; juvenile salmon are especially susceptible when first in seawater (Rice et al. 1975; Rice et al. 1984). Sublethal levels of petroleum hydrocarbons in the water column can affect metabolism and reduce growth of fry (Rice et al. 1975). Sublethal levels of water-soluble-fractions (WSF) of petroleum hydrocarbons can also damage olfactory lamellar surfaces, thus conceivably impacting migratory behavior and feeding patterns (Babcock 1985). Reduction in growth of juvenile salmon can be caused by contamination of prey by WSF (Schwartz 1985) and by direct ingestion of crude oil (Chapter 6 of this report).

Our objective was to determine if hydrocarbon concentrations in juvenile pink salmon and induction of cytochrome P4501A enzymes in juvenile pink and chum salmon differed between oiled and non-oiled areas (objectives 5-7 in Chapter 1).

Chapter 3A will be reformatted and submitted to the *Exxon Valdez Oil Spill Symposium Proceedings* for formal publication.

Methods

Eight locations in western PWS were chosen for study based on the distribution of *ECV* in early April 1989. Four of these locations were oiled (Herring Bay, Snug Harbor, Knight Island Passage, and Prince of Wales Passage) and four were non-oiled controls (McClure Bay, Long Bay, Culross Passage, and Wells Passage) (Figure 3A.1). We chose non-oiled locations as near as possible to oiled locations and attempted to match site characteristics. Non-oiled locations were clustered in the northwest region of PWS, on or close to the mainland, while oiled locations were generally more southerly and on islands (Figure 3A.1). Samples for hydrocarbon analysis were collected during five trips between 10 April and 26 June in 1989, and during four trips between 16 April and 14 June in 1990. Field blanks were regularly collected during sampling and frozen for quality control of jars, methods, storage, and processing.

To evaluate the degree of hydrocarbon contamination at each location, mussel (*Mytilus trossulus*) samples were collected throughout the sampling period. Sufficient quantities of whole mussels required to yield at least 10 g of tissue were collected by hand, placed in hydrocarbon-free glass jars, and frozen until analysis for hydrocarbon content. Mussel shell lengths ranged from 17.2 to 49.9 mm.

Juvenile pink salmon were collected in nearshore habitats by beach seine or dip net. Because sheen and tar balls were often trapped in the seine at the oiled locations in 1989, care was taken to herd the fry away from such sources of external contamination. Contaminated nets were not used to collect fish in non-oiled areas. Our intent was to collect sufficient numbers for triplicate samples of approximately 10 g of fry at each location on each sampling trip. When numbers of fry collected by seine at routinely sampled beaches were insufficient, additional fry were collected by seine or dip net from other littoral sites within the sampling location as available. Live fry were maintained in buckets of seawater, transported back to the ship, placed in hydrocarbon-free glass jars, and frozen until analysis for hydrocarbon content. Juvenile chum salmon were not collected for hydrocarbon analysis. Because a freezer was not available on the first sampling trip (April 1989), samples were packed in ice until they could be frozen. Samples of juvenile pink and chum salmon were also preserved in 10% buffered formalin for analysis of cytochrome P4501A enzyme induction as an indicator of exposure to hydrocarbons. Pink salmon lengths ranged from a minimum of 28 mm in April to a maximum of 94 mm in June; mean lengths increased from 32.4 ± 0.1 mm in April to 47.1 ± 0.2 mm in June. Chum salmon lengths ranged from a minimum of 30 mm in April to a maximum of 85 mm in June; mean lengths increased from 36.1 ± 0.1 mm in April to 45.0 ± 0.2 mm in June.

All captured salmon were screened for the presence of coded-wire tags (Jefferts et al. 1963) to identify fry originating from specific hatchery locations. Tagged fry were placed live into hydrocarbon-free glass vials and frozen individually. The heads were subsequently removed at the laboratory with hydrocarbon-free equipment, and the tags were recovered and decoded. These fry were identified by hatchery of origin and release group. There were sufficient tag recoveries in Knight Island Passage (oiled) and in Wells Passage (non-oiled) that tagged carcasses could be pooled by release group (from Ester Hatchery, PWS) for hydrocarbon analysis.

To ensure that hydrocarbons detected in pink salmon tissues were not due to external contamination, a subset of pink salmon samples collected from oiled locations in 1989 (n = 17, involving approximately 797 fry) were dissected with hydrocarbon-free equipment and separated into carcass and viscera subsamples. Heads were removed during dissection and preserved for otolith analysis; carcass samples therefore contained integument and muscle tissues *sans* heads. We hypothesized that if observed hydrocarbon contamination were merely an external artifact of sampling in polluted water where sheen and mousse were often present, viscera would show little or no contamination relative to the carcass. Separated samples spanned the period 15 April through 21 May 1989 (22-58 days after the spill). One viscera subsample was destroyed during analytical procedures.

Hydrocarbon concentrations in sediments were determined by gas chromatography followed by mass spectrometry for aromatics (Larsen et al. 1992, Krahn et al. 1993) or flame ionization detection for aliphatics (GERG 1989, Larsen et al. 1992). Samples were analyzed at either of two laboratories, the Geochemical and Environmental Research Group (GERG) at Texas A&M University or the Auke Bay Laboratory (ABL), Juneau, Alaska, and reported via the PWSOIL database (Manen et al. in prep). The majority of our samples were analyzed by GERG (GERG 1989, Krahn et al. 1993). Data for an unweathered *EVC* sample were supplied by ABL. In each sample, hydrocarbon concentrations were accepted only if corresponding deuterated internal standards ranged from 30 to 150%; records with concentrations outside these limits were deleted (7% of the data). Machine detection limits (MDL) for each hydrocarbon were reported by the analytical labs; concentrations below the MDL were set to zero.

Polynuclear aromatic hydrocarbons (PAH) were chosen as the primary hydrocarbon measurement in this study because PAHs are not normally present intertidally in PWS and because natural sources of alkanes are present in PWS (Karinen et al. 1993). All aromatic hydrocarbons reported by the analytical laboratories were included in the filtered PAH concentration; of these, only 1.5% ± 0.8 (mussels, N = 53) or 3.8% ± 0.6 (pink salmon, N = 95) did not occur in *EVC*. Reported conclusions were not changed when unfiltered PAH or filtered concentrations restricted to only

those aromatics present in EVC were used as the hydrocarbon measurement. Concentration changes in various subcomponents of PAH (naphthalenes, fluorenes, dibenzothiophenes, phenanthrenes, and chrysenes) were similar to changes in PAH, thus the more generalized PAH measurement was used to summarize the data. Concentration of PAH in sample blanks filtered by deuterated recovery and MDL was zero (N=11).

Concentrations of hydrocarbons in mussel and pink salmon samples were analyzed with single factor (oil code) ANOVA. Because variance was proportional to the means, data were log transformed before analysis (ln(concentration + 1)). Outcomes of all statistical tests were judged "significant" where $P \leq 0.05$. Numerical results are reported as means ± SE.

Hydrocarbons detected in the carcass and viscera tissues of pink salmon were analyzed with two-factor (oil code and tissue type) ANOVA. Data were log transformed before analysis. Significance of differences between treatment means was determined with a least-significant difference option (SAS 1989). To verify that concentration differences between carcass and viscera tissues were not simply sampling artifacts, data from oiled locations were reanalyzed with single-factor (tissue type) ANOVA after subtracting mean concentrations at non-oiled locations from the concentrations observed at oiled locations (by analyte and tissue type, as appropriate). One of the non-oiled viscera subsamples was apparently contaminated, possibly due to procedural errors, and was not included in the analysis. Concentrations of individual PAH compounds in fry tissues were compared to those in EVC oil by normalizing all concentrations in each respective sample to the total PAH concentration in that sample.

Samples of juvenile pink and chum salmon preserved in formalin were analyzed for cytochrome P4501A induction at Woods Hole Oceanographic Institute. Approximately six fish per sample group were processed. No background information on samples and collection locations was provided to researchers at Woods Hole prior to the analysis. Fry were sectioned with standard histologic techniques and stained with monoclonal antibody 1-12-3 and a peroxidase-labeled second antibody (Smolowitz et al. 1991). Slides were stained in duplicate for both specific antibody and control antibody. Staining intensity and occurrence were determined for tissues from each fry. Staining intensity is the density of the peroxide-stained cells within a tissue, ranked from 0 (negative) to 5 (heavy). Occurrence is the relative frequency of stained cells in a tissue, ranked from 0 (no staining) to 3 (diffuse). Each sample was assigned an overall induction rank, based on the frequency of induction of fish in the sample, staining intensity and staining occurrence. Induction ranks were 0 = negative; 1 = very mild; 2 = mild; 3 = mild/moderate; 4 = moderate; 5 = moderate/strong; and 6 = strong.

Induction ranks were 0 = negative; 1 = very mild; 2 = mild; 3 = mild/moderate; 4 = moderate; 5 = moderate/strong; and 6 = strong.

Additional comparisons among tissues processed for cytochrome P4501A were necessary due to procedural differences in the preservation of fry samples. In 1989, all fry remained in formalin until analysis, but in 1990, fry were transferred to isopropanol after 6-8 weeks preservation in formalin. Four comparisons were possible where subsamples of pink salmon from the same seine set in 1989 had been preserved in both formalin and isopropanol. On average, preservation in isopropanol reduced the sensitivity of the immunohistochemical assay by one overall rank, with a range of -2 levels to equal.

Results

Site Contamination

Mussels from locations that were not visibly oiled (McClure Bay, Culross Passage, Long Bay, and Wells Passage) contained relatively few hydrocarbons compared to mussels from visibly oiled locations (Herring Bay, Knight Island Passage, Snug Harbor, and Prince of Wales Passage) (Fig. 3A.2). An exception occurred in Culross Passage, where slightly elevated concentrations were observed in tissues collected on May 4, 1989. Small amounts of mousse were observed on beaches in the vicinity of the May 4 mussel collection, but because the contamination was low and did not persist, we continued to consider Culross Passage as a reference location.

In 1989, alkane and PAH concentrations in mussel tissues from oiled locations were significantly greater than concentrations in tissues from non-oiled locations (Fig. 3A.2). Results were not altered by including or excluding the contaminated Culross Passage sample. In non-oiled locations, only 6 ± 2% of the PAH were above minimum detection limits (MDL), but 69 ± 3% of the PAH were above MDL in oiled locations.

Concentrations of PAH in mussels increased over time at all oiled sites except Snug Harbor, peaked above 20,000 ng/g 22 to 73 days after the spill, then declined to less than 2,001 ng/g by day 131. Peak hydrocarbon concentrations may have occurred before our first collection in Snug Harbor (22 d). At control sites, PAH concentrations were $\leq$ 52 ng/g and did not exhibit any trends, except (as noted previously) there was a minor peak (205 ng/g) in Culross Passage 41 days after the spill.

Contamination of mussel tissues at oiled locations persisted, at much reduced levels, into 1990, while non-oiled locations remained clean. Differences in PAH concentrations in mussels were significant between non-oiled and oiled locations in 1990 (Fig. 3A.2).

Salmon Fry

In 1989, PAH concentrations in pink salmon fry from oiled locations were significantly greater than in fry from non-oiled locations (Fig. 3A.3). Alkane concentrations did not differ significantly between oiled and non-oiled locations (Fig. 3A.3).

Concentrations of PAH in pink salmon fry at oiled sites either increased to a peak and then declined, or generally declined after the first sampling. Mean PAH concentrations in pink salmon fry increased to a maximum (510 and 263 ng/g) 46 and 58 d after the spill in Snug Harbor and Herring Bay, respectively, and then declined. In Prince of Wales Passage and Knight Island Passage,

maximum concentrations (314 ± 5 and 675 ng/g) were observed in the first samples collected (39 and 40 d after the spill), and generally declined thereafter. Mean PAH concentrations at reference sites remained below 74 ng/g, except concentrations rose to a minor peak (154 ± 2 ng/g) in Culross Passage 57 d after the spill. In 1990, there were no significant differences in alkane and PAH concentrations in pink salmon between oiled and non-oiled locations (Fig. 3A.3).

Concentrations of PAHs in the tissues of CWT fry collected in 1989 did not differ significantly between oiled and non-oiled locations (Knight Island Passage versus Wells Passage). However, Mean unfiltered PAH concentrations were higher in tissues of fish collected from the oiled site. This trend suggests that tagged fry, released from known sites, did accumulate hydrocarbons.

Our previous reports contained results of unfiltered hydrocarbon concentrations in fry marked with coded wire tags (CWT). Mean unfiltered PAH concentrations were higher in tissues of fish collected from the oiled site (156 ± 135 ng/g, N = 3, in Knight Island Passage) than those from the non-oiled site (313 ± 87, N = 4, in Wells Passage), and differences were marginally significant ($P = 0.089$). This small data set was the only one lost during the hydrocarbon filtration process; all 7 samples were eliminated due to errors in deuterated surrogate recoveries.

In 1989, PAH concentrations in pink salmon carcass and viscera tissues were significantly greater in tissues from oiled locations than in corresponding tissues from non-oiled locations (Fig. 3A.4). In fry collected from oiled locations, concentrations of PAH were significantly greater in viscera than carcass tissues; PAH concentrations in viscera remained significantly greater than in carcasses when concentrations were corrected by mean non-oiled concentrations. Alkane concentrations in corresponding tissues did not differ significantly between oiled and non-oiled locations, but were significantly greater in viscera tissues than in carcass tissues (Fig. 3A.4).

Relative PAH concentrations in fry carcass and viscera tissues were consistent with the relative PAH concentrations in *EVC* (Fig. 3A.5). Naphthalenes, biphenyl, fluorenes, dibenzothiophenes, and chrysenes were important components in *EVC*. These compounds were also present in carcass and viscera tissue. Concentrations of fluorenes through C-4 phenanthrenes were proportionately higher in tissue samples than in the parent oil, especially in the viscera. Chrysenes were present in both types of tissue. Similarly, relative PAH concentrations in whole pink salmon fry and in mussels were also consistent with relative PAH concentrations in *EVC*.

Cytochrome P4501A induction

Pink salmon fry from oiled locations in 1989 generally had higher staining ranks and showed staining in more cell types than fry from non-oiled locations (Table 3A.1). In 1989, the median rank for samples from oiled locations was 4 (range was 2 to 6); the median rank from non-oiled locations was 1 (range was 1 to 2) (Fig. 3A.6). The median rank was significantly greater in oiled locations than non-oiled locations ($P < 0.01$, Mann-Whitney test). We infer, therefore, that the minimum hydrocarbon induction rank was 3.

Chum salmon were rare in the oiled locations sampled; samples were collected only in Herring Bay. These samples had higher ranks and more tissue types stained than did samples of chum salmon from non-oiled locations (Table 3A.2). In 1989, the median rank for samples from oiled locations was 6 (range was 5 to 6); the median rank from non-oiled locations was 1 (range was 0 to 1) (Fig. 3A.6). The median rank was significantly greater in oiled locations than non-oiled locations ($P < 0.05$, Mann-Whitney test).

Among pink or chum salmon in each sample with an overall staining rank of 3 or higher, frequency, intensity, and diffuseness (occurrence) of staining were compared for gill epithelial tissue, gill endothelial tissue (pillar cells), and cecal epithelial tissue (Table 3A.3). In pink salmon, the median frequency of P4501A staining was 0.8 for gill pillar cells and cecal epithelial cells, and 0.3 for gill epithelial cells. Median intensity and occurrence was highest in gill pillar cells, and similar for gill and cecal epithelial cells. In chum salmon, staining was observed in gill pillar cells and cecal epithelial cells in all fish analyzed from the oiled location (median frequency = 1.0), while median frequency of staining for gill epithelial cells was 0.5. Median intensity and occurrence of staining for these tissues in chum salmon was highest in cecal epithelial cells, intermediate in gill pillar cells, and lowest in gill epithelial cells.

Differential staining of P4501A was not detected in pink and chum tissues in nearshore habitats in 1990. All samples ranked either 1 or 0, and the number of tissue types staining was greatly reduced in the oiled locations compared to 1989 (Tables 3A.1 and 3A.2). Although the median rank for pink salmon in the oiled locations was 1 in 1990, compared to 0 in the non-oiled locations, ranks were not significantly different ($P > 0.3$, Mann-Whitney test) (Fig. 3A.6). In 1990, the median rank for chum salmon was 0 for both oiled and non-oiled locations (Fig. 3A.6). Assuming preservation in isopropanol reduced staining by 1 rank, no samples would be promoted to the previously defined hydrocarbon induction range (3-6) if activity level were increased by 1.

Discussion

Bivalve molluscs are generally considered an excellent measure of the biological availability of petroleum hydrocarbons at specific sites because of their sessile nature and low capacity to metabolize hydrocarbons (Vandermeulen and Penrose 1978, Stegeman 1985, Livingstone et al. 1989). Mussels can bioaccumulate petroleum hydrocarbons by more than 1,000 times the exposure level (Fossato and Canzonier 1976). Despite the extremely low concentrations of hydrocarbons in the water column in the spill area (Short and Rounds 1993a), hydrocarbon concentrations in mussel tissues were dramatically higher in oiled locations than reference locations in 1989. As may be expected after a single spill perturbation, concentrations of PAH increased in mussels from oiled locations after the *EV* spill, peaked, and then declined. Composition of PAHs in mussels from oiled locations were characteristic of *EVC* (98.5 ± 0.8 percent *EV* PAH), and included chrysenes and substituted chrysenes. Differences observed in hydrocarbon concentrations in mussels, therefore, can generally be attributed to the differential contamination of the nearshore marine environment by *EVC*.

Concentrations of PAH were also significantly elevated in pink salmon from oiled locations, peaked after the *EV* spill and then declined. Composition of PAHs in juvenile salmon tissues from oiled locations exhibited characteristics of *EVC* (96.2 ± 0.6 percent *EV* PAH), including chrysenes, and was similar to the more clearly defined PAH composition in mussel tissues collected at the same locations. Since pink salmon fry are pelagic and are capable of actively metabolizing hydrocarbons (Rice et al. 1977), we expected greater variability in salmon tissues than in mussel tissues. Induction of P4501A in salmon was another indicator of oiling. Although other compounds, including PCBs and other halogenated hydrocarbons, are inducers of P4501A in fish (Stegeman 1990), the presence of a strong effect only in the year of the spill, and the absence of strong induction in nearby, but non-oiled reference locations, indicated that *EVC* was the inducer. Differences between oiled and reference locations in PAH concentrations and P4501A induction in salmon fry in 1989, therefore, were also due to differential contamination by *EVC*.

Not all pink salmon fry collected at contaminated locations had hydrocarbons in their tissues, and not all fry collected in control locations were hydrocarbon-free. Uncontaminated samples from oiled locations may indicate that fry had not been exposed long enough to become contaminated, or fry had been exposed for sufficient time that metabolism and depuration had reduced hydrocarbon body burden below detectable limits, or that hydrocarbon concentrations in the area had declined significantly. In one instance, salmon tissues from a non-oiled reference site (Wells Passage, May 5, 1989) showed possible

contamination. The migratory nature of pink salmon fry may also explain this observation. Alternatively, the Wells Passage locations may have been exposed to a small amount of oil for a short time; small amounts of oil were observed nearby (Culross Passage on May 4), and Applegate Island, also nearby, was heavily oiled.

The observed contamination of juvenile salmon was not superficial contamination caused by collection techniques because the viscera contained significantly higher hydrocarbon concentrations than the carcasses. Likewise, the induction of cytochrome P4501A could not have been due to superficial exposure at the time of sampling; it is clear evidence that the tissues of pink and chum fry were exposed to hydrocarbons in the oiled locations.

Fry may have been exposed directly to whole oil due to their behavior in the water column. During their nearshore phase, these salmonids often occupy shallow littoral locations (Salo 1991; Heard 1991) and feed at or just below the water surface. We frequently observed dense schools of juvenile salmon in water covered by sheen and mousse from *EVC*, with individuals jumping through the contaminated surface. At the time of these observations, total concentrations of aromatic hydrocarbons dissolved in the water column were less than 7 µg/L (Short and Rounds 1993a; Maki 1991; Neff 1991), thereby excluding water-soluble fractions (WSF) as the primary route of contamination.

Direct ingestion of oil particles by pink salmon fry is another possible route of contamination. Evidence of oil ingestion by juvenile pink and chum salmon in Prince William Sound in 1989 was found during analysis of stomach contents: oil sheen or droplets were observed in several fish from oiled locations, but not in fish from non-oiled locations (Chapter 4 of this report). Oil particles that are the same size as prey could have been mistaken for prey and ingested directly. Oil particles ranging from 0.01 to 1.0 mm diameter were observed as deep as 80 m in Chedabucto Bay following the wreck of the tanker Arrow (Forrester 1971).

A third possible route of contamination of salmon fry is through ingestion of contaminated prey. In the stomach contents of juvenile chinook salmon from Puget Sound, mean concentrations of aromatic hydrocarbons were approximately 650 times higher in samples from a contaminated estuary than from a more pristine estuary (McCain et al. 1990). Particulate crude oil may be ingested directly by zooplankton (Conover 1971). Various studies have also shown hydrocarbon uptake from the WSF of oil by crustaceans (e.g., Macek et al 1979; Schwartz 1985; Carls 1987). Epibenthic microcrustaceans, such as harpacticoid copepods, may bioaccumulate oil from sediments, and therefore, pass hydrocarbons up the food chain. Uptake of hydrocarbons by benthic organisms may be via interstitial water and is,

therefore, kinetically controlled by desorption from sediment particles and organic matter (Landrum 1989). Hydrocarbons, particularly the more strongly sorbed compounds, may also be assimilated by benthic organisms via ingestion (Landrum 1989).

Relative induction of P4501A activity in different tissues may provide insight on the route of hydrocarbon contamination. Endothelial cells, such as gill pillar cells, are strongly induced in teleosts by both intraperitoneal administration of PAHs (mimicking the ingestion route; Miller et al. 1989) and by exposure of fish to waterborne PAHs (Smolowitz et al. 1992). Induction of gill and gut epithelial cells, however, may differ because initial cell exposure depends on the route of contamination (Miller et al. 1989, Stegeman et al. 1991).

The high frequency, intensity, and occurrence of staining in the cecal epithelium of chum salmon fry indicate ingestion was an important route of contamination for these fish. Staining of cecal epithelial cells for P4501A was observed in all chum salmon fry examined from the oiled location (Herring Bay); intensity and occurrence of the staining was higher in cecal epithelial tissue than in gill endothelial cells. In contrast, gill epithelial cells were stained in 20 to 50% of the fry examined, and median occurrence and intensity of staining was lower than for the gill endothelial cells.

The pattern of P4501A staining in the tissues of pink salmon suggest that ingestion was also a route of contamination for pink salmon fry. In samples of pink salmon that were definitely induced (rank $\geq$ 3), median frequency of staining in cecal epithelial cells was similar to gill endothelial cells, and higher than in gill epithelial cells. However, median intensity and occurrence of staining was similar for gill and cecal epithelial tissues and cells in pink salmon fry, and in one sample (from Knight Island Passage) the frequency of induction in gill epithelial cells was higher than in cecal epithelial cells. These results indicate that direct exposure to waterborne oil was also a contamination route in some circumstances.

Although differences in staining between gill and cecal epithelial may be caused by disparate routes of contamination, induction of these tissues could result from direct exposure, ingestion, or by both routes, as discussed previously. Experiments in which salmon fry are exposed to petroleum hydrocarbons directly via water or diet are necessary to confirm that the relative induction of gill and gut epithelia distinguish between these routes of contamination. Such studies are in progress.

Differences in feeding behavior between pink and chum salmon in the nearshore marine environment may be important in causing differences in the degree of exposure to different routes of

differences in the degree of exposure to different routes of contamination. Pink and chum salmon fry co-occur in shallow littoral habitats, but epibenthic prey are more important as food for chum salmon than pink salmon (Kaczynski et al. 1973; Bailey et al. 1975; Cooney et al. 1981). Thus, chum salmon may be more likely to feed near the bottom in littoral habitats and ingest prey contaminated by oiled sediments. Conversely, pelagic zooplankton are more important as food for pink salmon, and therefore, pink salmon may be more likely to encounter surface contamination.

The physiological consequences of the contamination of juvenile salmon in Prince William Sound in 1989 can be inferred both from laboratory studies and empirical field evidence. Direct exposure to WSF of crude oil and the consumption of prey contaminated with WSF have been shown to reduce growth of pink salmon fry (Rice et al. 1975; Schwartz 1985). Ingestion of food contaminated with whole crude oil has also been shown to reduce growth and survival of pink salmon fry (Chapter 6 of this report). Reduced growth rates may also affect the survival of juvenile salmonids indirectly because rapid growth is important to escape size-selective predation (Parker 1971; Hargreaves and LeBrasseur 1985; Mortensen et al. 1991). In 1989, the apparent growth rate of pink salmon fry was lower in oiled locations in Prince William Sound than in non-oiled locations (Chapter 2 of this report); growth rates of specific tagged groups of pink salmon fry were also reduced in heavily oiled locations in 1989 (Willette 1991). We conclude that contamination of pink and chum salmon in PWS was physiologically significant, and affected their capacity for growth and survival.

Table 3A.1. Ranking for cytochrome P4501A staining in tissues of pink salmon fry sampled in oiled and non-oiled nearshore marine habitats in Prince William Sound in 1989 and 1990. Sample rankings ranging from 0 (negative) to 6 (strong) were based on extent of occurrence and intensity of immunochemical staining for P4501A; n_f = number of fry examined, n_t = number of tissue types stained.

Sampling location	n_f	Date	Rank	Tissues stained n_t	Tissues stained type[1]
		1989			
Non-oiled					
McClure Bay	5	May 15	1	1	L_h
Wells Passage	4	May 21	1	3	$L_h;G_p;I_c$
Culross Passage	6	May 20	2	3	$L_h;G_p;I_c$
Culross Passage	6	May 31	1	3	$L_h;K_c;I_c$
Oiled					
Herring Bay	4	May 14	2	4	$L_h;G_p;K_c;I_c$
Herring Bay	4	May 30	6	11	$L_h;G_{p,a,e};K_{t,s,g,c};I_{p,c};H$
Snug Harbor-Inner Bay	6	May 17	3	9	$L_{b,h};G_{p,e};K_{t,s,c};I_c;P$
Snug Harbor-Inner Bay	4	Jun 2	4	11	$L_h;G_{p,e};K_{t,s,c};I_{a,p,c};H;P$
Snug Harbor-Outer Bay	6	May 16	4	10	$L_h;G_{p,a,e};K_{v,s,c};I_{a,p,c}$
Snug Harbor-Outer Bay	6	Jun 8	5	6	$L_h;G_{a,p};K_c;I_c;H$
Snug Harbor-Outer Bay	6	Jun 18	2	3	$L_h;G_p;H$
Knight Island Psg	6	May 19	4	11	$L_h;G_{p,a,e};K_{s,g,c};I_{a,p,c};H$
Knight Island Psg	6	May 19	5	11	$L_h;G_{p,a,e};K_{s,g,c};I_c;H;P$
Knight Island Psg	6	Jun 18	4	6	$L_h;G_{p,e};K_c;I_c;H$
Prince of Wales Psg	6	May 17	6	5	$L_h;G_{p,e};I_c;H$
Prince of Wales Psg	6	Jun 9	4	7	$L_h;G_p;K_c;I_{a,p,c};H$
Prince of Wales Psg	6	Jun 25	2	2	$L_h;K_c$
		1990			
Non-oiled					
McClure Bay	6	Apr 22	0	0	none
Long Bay	6	May 5	0	0	none
Wells Passage	6	Apr 21	0	0	none
Culross Passage	6	May 8	1	1	I_c
Oiled					
Herring Bay	4	May 3	1	2	$L_h;I_c$
Herring Bay	6	Jun 2	0	0	none
Herring Bay	6	Jun 11	0	0	none
Snug Harbor-Inner Bay	6	Apr 19	1	3	$L_h;G_p;K_c$
Snug Harbor-Inner Bay	6	May 19	0	0	none
Knight Island Psg	6	May 7	1	5	$G_p;K_{s,c};I_{a,c}$
Prince of Wales Psg	6	Apr 20	1	0	I_c

[1]Tissue types that immunochemically stained for cytochrome P4501A: L_h = liver hepatocytes; $G_{p,a,e}$ = Gill pillar cells (endothelium), gill buds or arch endothelium, gill epithelium; $K_{c,g,s,t,v}$ = kidney collecting ducts, glomerular endothelium, sinusoidal endothelium, tubular epithelium, vascular endothelium; $I_{a,p,c}$ = anterior intestinal epithelium; H = heart endothelium; P = pharyngeal epithelium.

Table 3A.2. Ranking for cytochrome P4501A staining in tissues of chum salmon fry from oiled and non-oiled nearshore marine habitats in Prince William Sound in 1989 and 1990. Sample rankings ranging from 0 (negative) to 6 (strong) were based on extent of occurrence and intensity of immunochemical staining for P4501A in histological sections; n_f = number of fry examined, n_t = number of tissue types stained.

Sampling location	n_f	Date	Rank	Tissues stained n_t	Tissues stained type[1]
		1989			
Non-oiled					
McClure Bay	4	May 15	0	0	none
Long Bay	6	May 16	1	1	L_h
Culross Passage	6	May 20	1	1	L_h
Wells Passage	6	May 21	1	1	L_h
Culross Passage	6	Jun 23	1	1	L_h
Oiled					
Herring Bay	4	May 14	6	10	L_h;$G_{e,a,p}$;$K_{g,c}$;$I_{a,p,c}$;H
Herring Bay	6	May 30	6	11	L_h;$G_{e,p}$;$K_{g,c,s}$;$I_{a,p,c}$;H;P
Herring Bay	6	Jun 15	5	8	L_h;$G_{e,a,p}$;$I_{a,c}$;H;P
		1990			
Non-oiled					
McClure Bay	6	May 9	0	0	none
McClure Bay	4	May 17	1	2	L_h;I_c
Long Bay	6	May 5	0	0	none
Oiled					
Herring Bay	6	May 3	0	0	none
Herring Bay	4	May 16	0	1	L_h
Herring Bay	6	Jun 2	0	0	none

[1]Tissue types that immunochemically stained for cytochrome P4501A: L_h = liver hepatocytes; $G_{p,a,e}$ = Gill pillar cells (endothelium), gill buds or arch endothelium, gill epithelium; $K_{c,g,s,t,v}$ = kidney collecting ducts, glomerular endothelium, sinusoidal endothelium, tubular epithelium, vascular endothelium; $I_{a,p,c}$ = anterior intestinal epithelium, posterior intestinal epithelium, cecal epithelium; H = heart endothelium; P = pharyngeal epithelium.

Table 3A.3. Frequency, intensity, and occurrence of P4501A staining in gill pillar cells (GP); gill epithelial cells (GE); and cecal epithelial cells (CE) in pink and chum salmon fry in which P4501A activity was induced in 1989; n_f = number of fry examined. Frequency is the proportion of fish in a sample showing induction at that tissue; intensity is the median degree of staining in fish in a sample in which induction was observed, ranked from 1 (very mild) to 5 (strong); and occurrence is median of the relative frequency of stained cells within a tissue, ranked from 1 (rare) to 3 (diffuse).

Location	n_f	Date	Frequency			Intensity			Occurrence		
			GP	GE	CE	GP	GE	CE	GP	GE	CE
					CHUM SALMON						
Herring Bay	6	Jun 15	1.0	0.2	1.0	2.5	2.0	2.0	2.5	1.0	2.0
Herring Bay	6	May 30	1.0	0.5	1.0	2.0	2.0	3.5	2.0	1.0	3.0
Herring Bay	4	May 14	1.0	0.5	1.0	3.5	2.5	3.0	2.5	1.0	3.0
MEDIAN			1.0	0.5	1.0	2.5	2.0	3.0	2.5	1.0	3.0
					PINK SALMON						
Herring Bay	4	May 30	1.0	0.5	1.0	3.0	2.5	3.0	3.0	1.5	2.5
Snug Harbor	6	May 16	0.8	0.8	0.8	4.0	2.0	4.0	3.0	2.0	3.0[1]
Snug Harbor	6	May 17	0.4	0.4	0.7	2.0	2.5	2.5	2.0	2.5	2.0[1]
Snug Harbor	4	Jun 2	1.0	0.2	1.0	2.5	2.0	2.0	2.5	1.0	2.5
Snug Harbor	6	Jun 8	0.8	0.0	0.3	3.0	---	2.0	2.0	---	1.5
Knight Is. Psg.	6	May 19	1.0	1.0	1.0[1]	4.0	2.0	3.0[1]	3.0	2.0	2.0
Knight Is. Psg.	6	May 19	1.0	1.0	0.3[1]	4.0	3.0	1.0[1]	3.0	1.0	2.0
Knight Is. Psg.	6	Jun 18	0.3	0.2	0.5	1.5	2.0	1.0[1]	1.5	1.0	2.0
Prince Wales Psg.	6	May 17	0.5	0.2	0.8	3.0	3.0	2.0[1]	3.0	2.0	2.0
Prince Wales Psg.	6	Jun 9	0.3	0.0	1.0	1.0	---	1.5	2.5	---	2.0
MEDIAN			0.8	0.3	0.8	3.0	2.2	2.0	2.8	1.8	2.0

[1]Cases in which frequency, median intensity, or median occurrence of P4501A staining within a sample was greater for gill epithelial cells than for caecal epithelial cells.

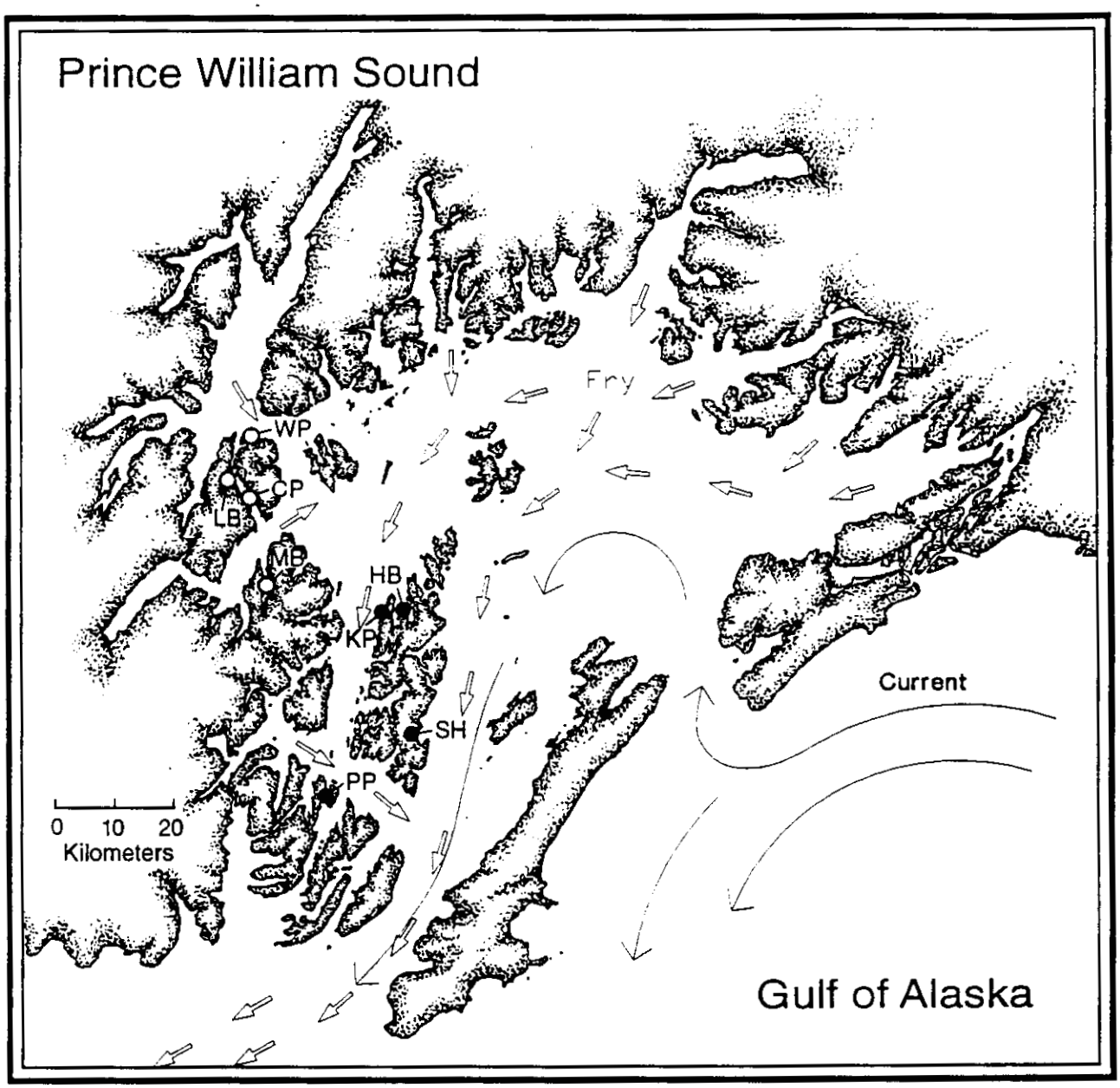

Fig. 3A.1. Prince William Sound depicting sample locations and hypothesized migratory routes of salmon fry (open arrows) from the Sound to the Gulf of Alaska. Non-oiled locations (open circles) were Culross Passage (CP), Long Bay (LB), McClure Bay (MB), and Wells Passage (WP); oiled locations (solid circles) were Herring Bay (HB), Knight Island Passage (KP), Prince of Wales Passage (PP), and Snug Harbor (SH).

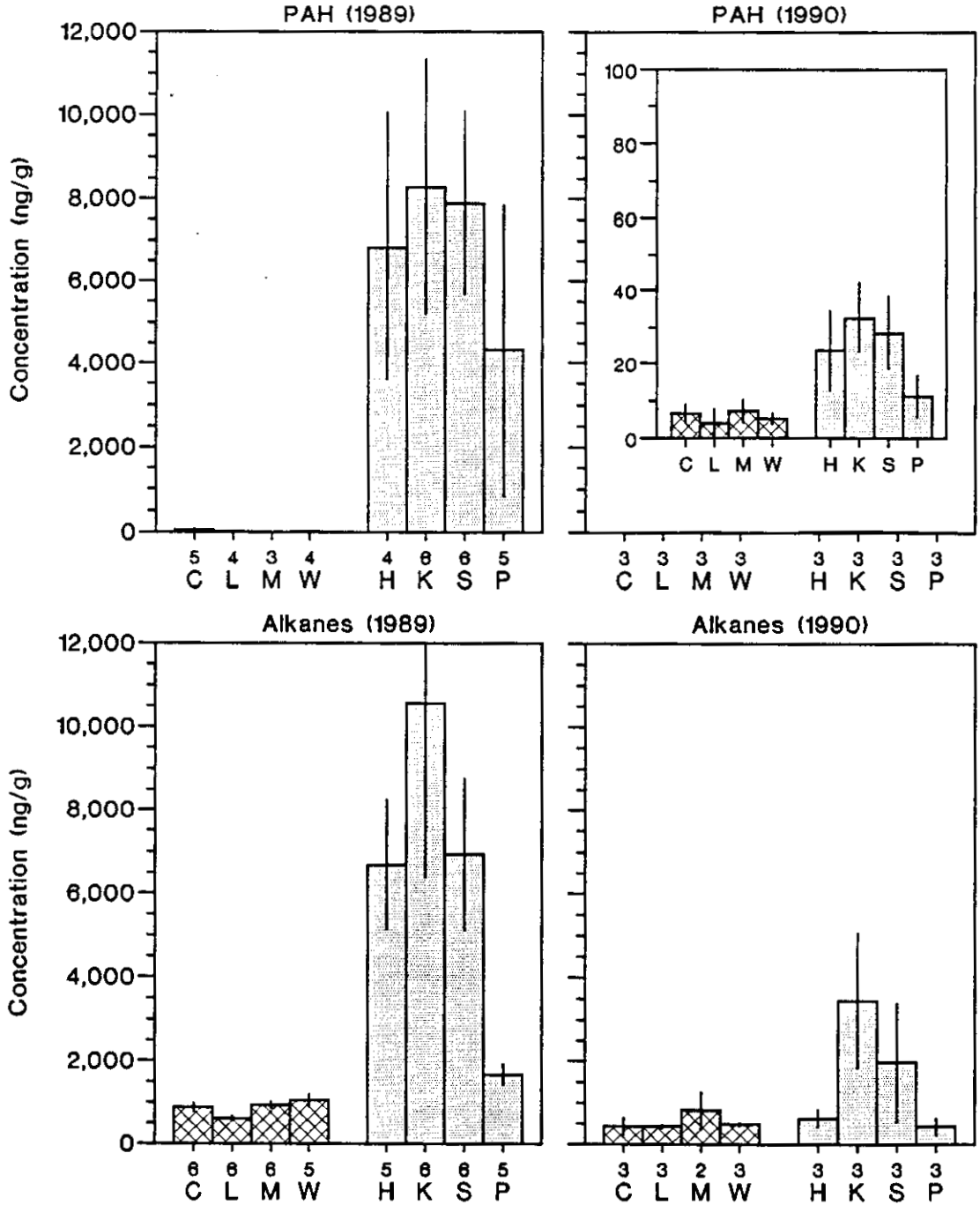

Fig. 3A.2. Polynuclear aromatic hydrocarbon (PAH) and alkane concentrations in *Mytilus trossulus* tissues from non-oiled and oiled locations in PWS in 1989 and 1990. Non-oiled locations were Culross Passage (C), Long Bay (L), McClure Bay (M), and Wells Passage (W); oiled locations were Herring Bay (H), Knight Island Passage (K), Snug Harbor (S), and Prince of Wales Passage (P). Numbers along the X-axis indicate sample size. Error bars are ±1 SE.

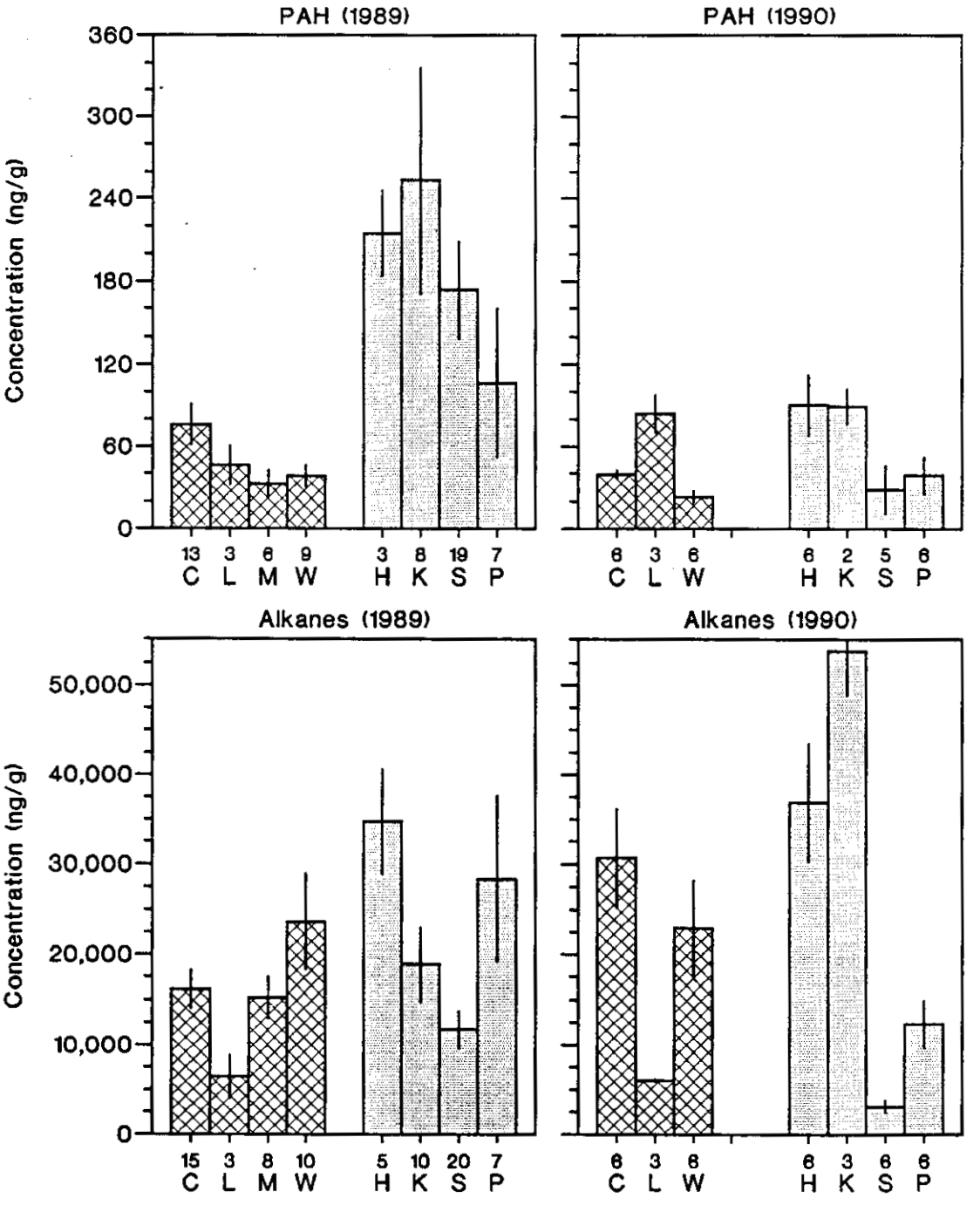

Fig. 3A.3. Hydrocarbon concentrations in pink salmon tissues from non-oiled and oiled locations in PWS in 1989 and 1990. Non-oiled locations were Culross Passage (C), Long Bay (L), McClure Bay (M), and Wells Passage (W); oiled locations were Herring Bay (H), Knight Island Passage (K), Snug Harbor (S) and Prince of Wales Passage (P). Numbers along the X-axis indicate sample size. Error bars are ±1 SE.

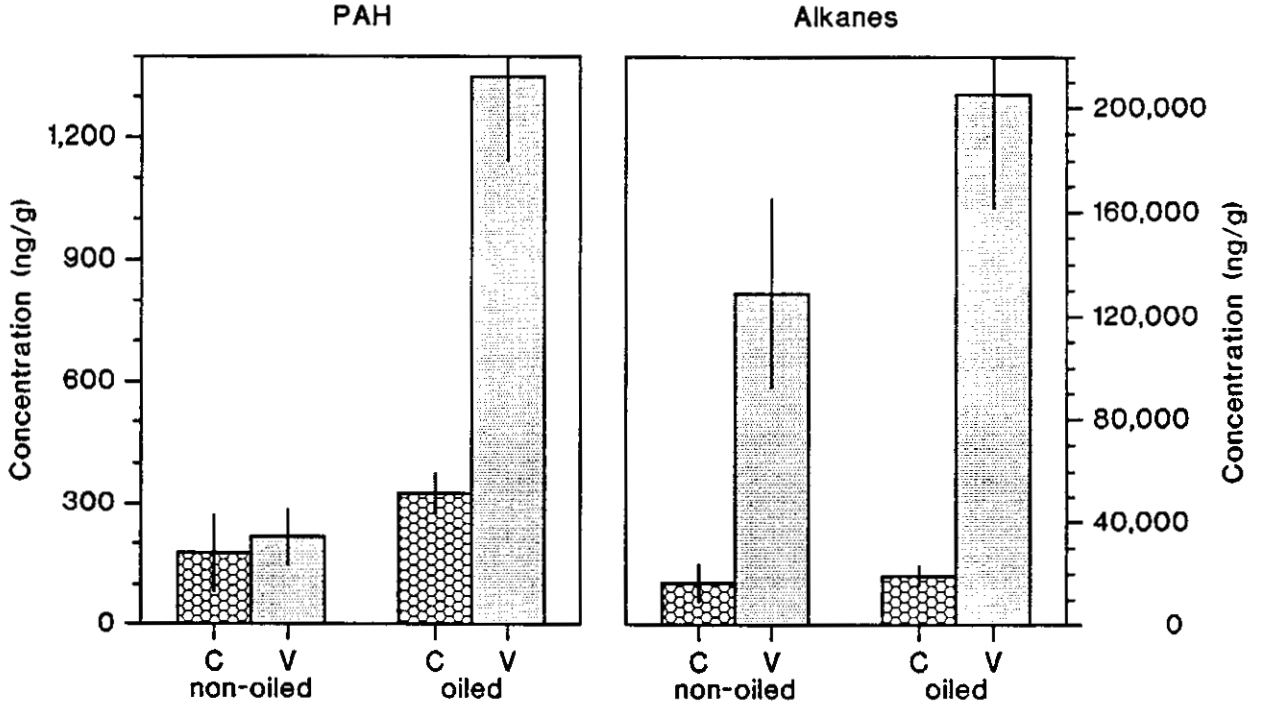

Fig. 3A.4. Concentrations of hydrocarbons in carcass (C) and viscera (V) tissues of pink salmon fry from non-oiled and oiled locations in PWS in 1989. Error bars are ±1 SE.

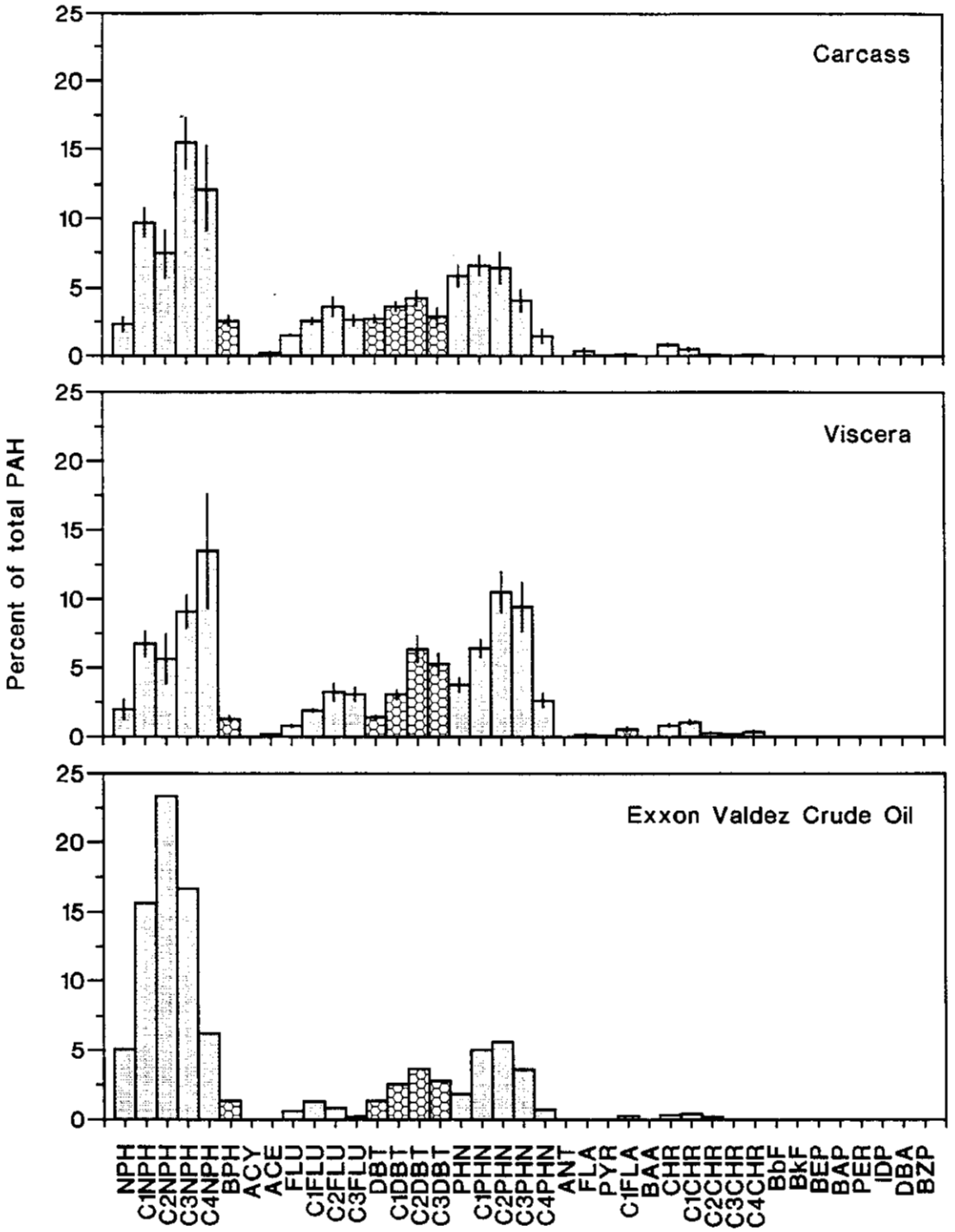

Fig. 3A.5. Composition of aromatic hydrocarbons in pink salmon carcasses and viscera from oiled locations in PWS, and in *EVC*. Error bars are ±1 SE.

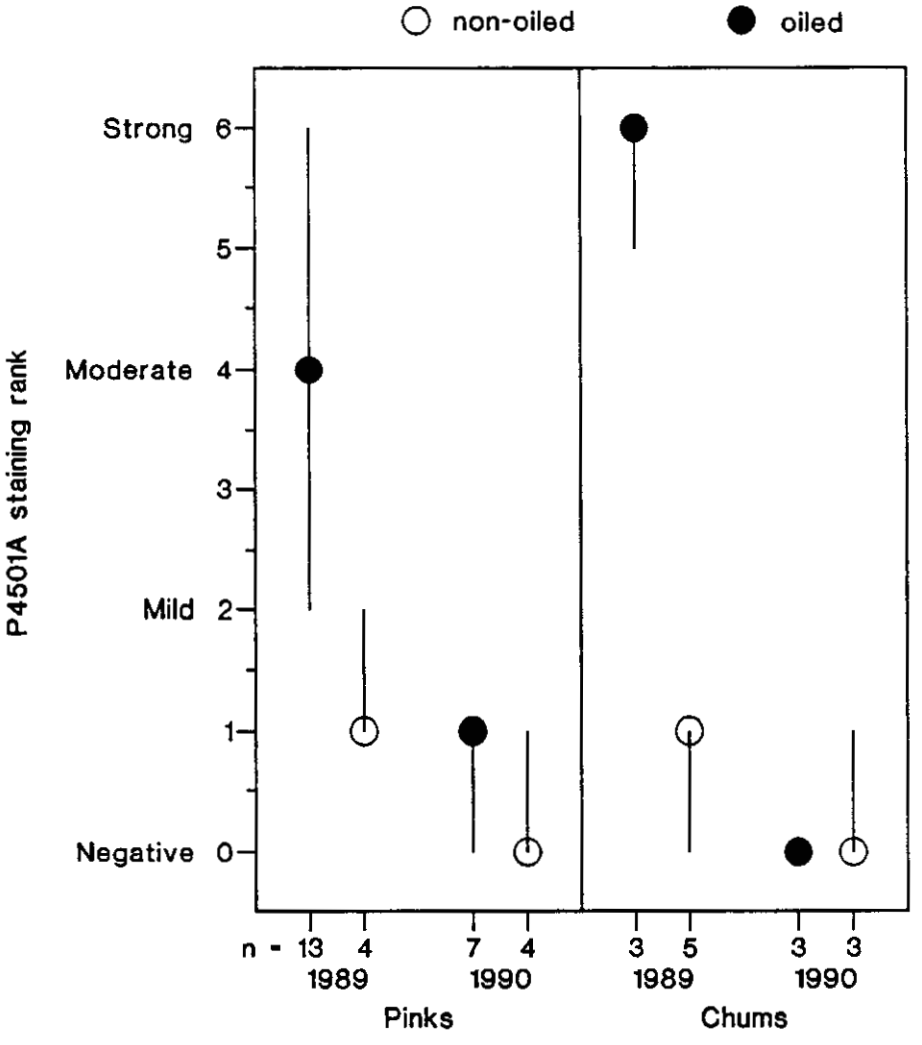

Fig. 3A.6. Medians and ranges of ranks of P4501A staining in pink and chum salmon fry sampled in nearshore waters in oiled and non-oiled areas of Prince William Sound. Sample sizes are indicated along the X-axis.

CHAPTER 3B. Contamination of Surficial Sediments by *Exxon Valdez* Crude Oil in Nearshore Habitats of Juvenile Salmon in Prince William Sound.

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Abstract

Sediment samples were collected in conjunction with biological sampling in Prince William Sound (PWS) in 1989 and 1990. Four visibly oiled locations were compared to four reference locations that did not appear oiled. In 1989, PAH and alkane concentrations were significantly greater at oiled locations than reference locations. Polycyclic aromatic hydrocarbon (PAH) concentrations peaked 22 to 27 d after the spill at visibly oiled locations and then declined; composition generally matched that of *Exxon Valdez* crude oil (EVC). At contaminated locations, PAH concentrations increased with elevation above mean lower low water, or increased to a peak, and then declined. This pattern of contamination was anticipated from visual observation; EVC was generally deposited on beaches in distinct bands, often with prominent oiling near the high water mark. Concentration of PAH in sediments at reference locations tended to remain low, but minor peaks exceeding background concentration occurred at all four locations 58 to 129 d after the spill. At three reference locations (Culross Passage, Long Bay, and McClure Bay), PAH composition at peak concentrations matched that of EVC. Similar temporal changes in PAH concentration in sediment at reference and visibly oiled locations indicated a single underlying cause. We conclude that areas outside the slick trajectory were contaminated by EVC. However, because the contamination detected at reference locations was significantly less than at visibly oiled locations, comparisons between oiled and reference locations presented elsewhere in this report are not invalidated; actual comparisons are between heavily oiled and slightly oiled locations.

Introduction

The primary objective of this study was to assess contamination of intertidal sediments in Prince William Sound by *Exxon Valdez* crude oil (Objective 5 in Chapter 1). These sediments were collected from locations sampled for juvenile salmon and their littoral prey. Secondary objectives were to describe the spatial distribution of the oiled sediment, and changes in hydrocarbon concentrations as functions of elevation and time. All sediment samples were collected in conjuction with the biological research described elsewhere in this report.

Other potential sources of background contamination, including Katalla seep oil, are considered in this chapter. Katalla oil, a low-sulfur crude, may be transported by the Alaska Coastal Current from the Katalla and Yakataga areas (Page et al. in prep). Seep oil, associated with with suspended sediments, may be deposited in Prince William Sound as current velocities fall (Page et al. in prep).

Chapter 3B will be reformatted and submitted to a refereed journal for publication.

Methods

Sediment collection

Intertidal sediment samples were collected in conjunction with biological sampling from eight locations in western Prince William Sound (PWS), chosen for study based on the distribution of *Exxon Valdez* crude oil (EVC) in early April 1989. Four of these locations were visibly oiled (Herring Bay, Knight Island Passage, Prince of Wales Passage, and Snug Harbor) and four were non-oiled reference locations (Culross Passage, Long Bay, McClure Bay, and Wells Passage) (see Figure 2.1 in Chapter 2). Reference locations were chosen as near as possible to oiled locations. All collection sites were selected for similarity in wave exposure, macrophyte coverage, and substrate (Chapter 2). Reference locations were clustered in the northwest region of PWS, on or close to the mainland; visibly oiled locations were generally more southerly and on islands. Two separate beaches, a low gradient (<10% grade) and a medium gradient (12 to 25% grade), were sampled at each location.

Sediments were collected during five trips between 10 April and 26 June in 1989, and during four trips between 16 April and 14 June in 1990. Throughout this report, time is presented as days after spill; 24 March 1989 is day 0. To characterize the distribution of oil in 1989 as a function of elevation, sediments in Long Bay, McClure Bay, Herring Bay, and Snug Harbor were collected along parallel transects placed at the water line from -0.3 to 3.3 m mean lower low water (MLLW), spaced at 0.6-m increments; these sediments were collected in association with epibenthic sled transects (Chapter 5A). Collection times were recorded and later used to calculate elevations using an algorithm that predicts tide height (Carls and Short 1992). To characterize geographic distribution of oiled sediments, samples were collected from all locations along 18-m transects within an elevation range of -0.3 to +0.9 m MLLW: these transects were placed along the water line adjacent to low- and medium-gradient pink salmon seine sites (Chapter 2).

Samples were collected from the top 2 cm of sediment with spoons and placed in 120-ml glass jars with Teflon[1] lids. Fine-grained sediments (<2 mm) were preferentially collected; coarser sediments were avoided unless finer material was not available. In 1989, one composite sample was collected per transect; exact collection spots were haphazard and covered the entire transect distance. In 1990, triplicate samples were collected, each a

[1]Reference to trade names does not imply endorsement by the National Marine Fisheries Services, NOAA.

composite of sediment from eight randomly chosen spots along the transects. Samples were frozen immediately after collection, except during the first sampling trip (10-23 April 1989) when a freezer was not available; in this case, samples were packed in ice until they could be frozen. Field blanks (air) were regularly collected during sampling and frozen for quality control.

All equipment and glassware used for hydrocarbon sampling and storage were hydrocarbon-free at the time of collection. Equipment was prewashed with soap and hot water, rinsed with hot and cold water, dried, and rinsed with acetone and then dichloromethane to remove any residual hydrocarbons. The acetone rinse was used only when equipment or glassware was moist. Hydrocarbon-free jars were purchased directly from a manufacturer, or prepared by baking at 400°C for 4 h, or washed and cleaned in the same manner as other collection equipment.

Hydrocarbon analysis

Hydrocarbon concentrations (ng/g wet weight) in sediments were determined by gas chromatography followed by mass spectrometry for aromatics or flame ionization detection for aliphatics (GERG 1989). Sediment samples were analyzed at the Geochemical and Environmental Research Group (GERG) at Texas A&M University in batchs of approximately 48 samples and reported via the PWSOIL database (Manen and Price, in prep). Data for unweathered and weathered EVC were supplied by the Auke Bay Laboratory (Short and Rounds 1993).

Of the sediment samples analyzed in this study, 20% were excluded because they were associated with samples possibly contaminated from an unknown, extraneous source (Short and Heintz 1992). Samples initially deemed suspicious (drawn from all data in the PWSOIL database, including other studies not reported here) contained much higher polycyclic aromatic hydrocarbon (PAH) concentrations than corresponding replicate samples, were often collected from reference stations where such high PAH concentrations had not been observed previously, and were concentrated in a few chemical analysis batches. To objectively identify such samples and batches, a procedure was developed to first identify samples that contained much higher hydrocarbon concentrations than corresponding replicate samples, and then to determine the probability that a batch would contain the observed number of these apparently aberrant samples on the null hypothesis that such samples would be randomly distributed among batches. All the samples of a batch were excluded if this probability was less than 0.01; most of the excluded batches had probabilities much lower than 0.01. These procedures and criteria are described more completely by Short and Heintz (1992).

In each remaining sample, hydrocarbon concentrations were accepted only if corresponding deuterated internal standards ranged from 30 to 150%; records with concentrations outside these limits were deleted (20% of the data). This prevented variations in the data from being amplified by application of large multipliers, or minimized by large divisors. After elimination of unacceptable data, 75 elevation samples, 152 geographic distribution samples, and 9 blanks remained. Method detection limits (MDL) for each hydrocarbon were experimentally determined by the analytical labs; concentrations below MDL were set to zero ($0.2 \leq MDL \leq 2.3$ ng/g, dependent on compound).

Polycyclic aromatic hydrocarbons were chosen as the primary hydrocarbon measurement in this study because most PAH are not normally present intertidally in PWS and because natural sources of alkanes are present in PWS (Karinen et al. 1993). The only two naturally occurring PAH, detected sporadically at low concentrations by Karinen et al. (1993), were phenanthrene and perylene. Composition of PAH is defined as the set of normalized concentrations of PAH compounds (Table 3B.1); concentration of each individual compound was divided by the total PAH concentration in the sample. Average composition was determined from replicate observations where appropriate. The PAH composition of samples or sample means was compared to PAH composition of EVC graphically, and scored as "matching EVC," "maybe EVC," or "not like EVC." In particular, naphthalene, fluorene, dibenzothiophene, phenanthrene, and chrysene percentages were compared: percentages in EVC peaked at the C2-substituted compounds in each homologous series except chrysenes, where C1-chrysene was peak. Some allowance was made for changes in composition due to weathering and other causes. Composition of unweathered EVC (Figure 3B.1) was 67% naphthalenes, 17% phenanthrenes, 10% dibenzothiophenes, 3% fluorenes, 1% chrysenes, and 2% other PAH (Short and Rounds 1993a). Composition of weathered EVC (Figure 3B.1) was 52% ± 2 naphthalenes, 27% ± 1 phenanthrenes, 9.8% ± 0.6 dibenzothiophenes, 6.6% ± 0.2 fluorenes, 29.9% ± 0.3 chrysenes, and 2% other PAH (Sales et al. in prep).

Comparison of the ratio of dibenzothiophene to phenanthrene allows discrimination between Katalla seep oil and EVC because Katalla oil contains little dibenzothiophene (Page et al. in prep). The C2-methyl substituted homologs were chosen to compute this ratio because they generally occur at higher concentrations than other homologous compounds in their respective families, and because they are more resistant to weathering than unsubstituted and C1-substituted compounds (Page et al. in prep). Ratio calculation was limited to samples where C2-phenanthrene concentration was greater than zero (i.e., above MDL). The expected ratio of C2-dibenzothiophene (C2DBT) to C2-phenanthrene (C2PHN) was 0.64 for unweathered EVC and 0.34 ± 0.01 for weathered EVC (Short and Rounds 1993a, Sales et al. in prep).

The C2DBT/C2PHN ratio for Katalla oil, 0.083 ± 0.003, was estimated from Constantine Harbor sediments 26 March 1989 to 8 August 1990 (Babcock et al. in prep).

Statistical analysis

As a rough comparison between sediments from oiled and reference locations, concentrations of PAH and alkanes in sediment samples were analyzed separately with single factor (oil code) ANOVA. Because variance was proportional to the means, data were log transformed before analysis (ln(concentration + 1)). Outcomes of all statistical tests were judged "significant" where $P \leq 0.05$. Numerical results (non-transformed) are reported as means ± standard error (SE).

Blank samples

The total PAH concentration in blank samples did not exceed 5.23 ng/g (1.4 ± 0.5 ng/g, N = 11). The PAH composition in blank samples did not resemble the composition of EVC (Figure 3B.1). Naphthalene and C1-naphthalene accounted for 45 to 100% of the observed blank concentration, but did not exceed 4.03 ng/g. Concentrations of C-2 through C-4 naphthalenes were less than MDL in all blanks. Phenanthrene and fluoranthene (≤0.80 and ≤0.23 ng/g, respectively) were detected in 18% of the sample blanks. Benzo-g,h,i-perylene (≤0.56 ng/g) was detected in 36% of the blank samples. Concentrations of PAH exceeding approximately 5 ng/g in field samples, therefore, should be attributable to sources other than analytical noise.

Alkane concentration in blank samples did not exceed 138 ng/g in blank samples (N = 20) except in one sample (726 ng/g). Concentrations were strongly skewed to the low end; the median concentration was 25 ng/g. The mean alkane concentration, after elimination of the apparent outlier, was 40 ± 10 ng/g (N = 19).

Results

Oil distribution as a function of elevation

At Herring Bay and Snug Harbor, two locations contaminated with EVC, PAH concentrations at low- and medium-gradient beaches increased with elevation above MLLW, or increased to a peak and then declined (Figure 3B.2). This pattern of contamination was anticipated from visual observation; EVC oil was generally deposited on beaches in distinct bands, often with prominent oiling near the high water mark. Where data were available for both visual observation and PAH concentration (days 59 and 62 for Herring Bay and Snug Harbor, respectively), quantities of oil observed in sediments and the degree of contamination estimated visually were consistent (Table 3B.2 and Figure 3B.2). Concentrations of PAH varied considerably from site to site, and over time. Although sediments were not sampled from multiple elevations at other oiled locations, visual estimates of oiling indicated oil concentrations also likely peaked in the mid to upper intertidal zone at these locations (Table 3B.3).

At Long Bay and McClure Bay, two reference locations, PAH concentrations were much lower than at visibly oiled locations, but a weak relationship between PAH and elevation was evident (Figure 3B.3). Concentrations tended to peak in the mid to upper intertidal zone; these changes in PAH concentration as functions of elevation in Long Bay (day 74) and McClure Bay (day 28) were similar to those observed for the low-gradient beach in Snug Harbor.

Unusually high and erratic PAH concentrations were observed on the medium-gradient beach in Long Bay 45 d after the *EV* spill (Figure 3B.3). These high concentrations were due almost entirely (92 to 96%) to naphthalenes; compounds characteristic of EVC, such as fluorenes, dibenzothiophenes, and phenanthrenes, were present in low quantities, but chrysenes were absent (Figure 3B.4). Peak concentrations (>1,139 ng/g) exceeded our estimated background concentration (60 ng/g) by more than an order of magnitude. Because these naphthalenes may have originated from sources other than EVC, we reanalyzed data from reference locations without naphthalenes; the relationship between concentration and elevation was slight or negligible (Figure 3B.5).

To ensure that napthalenes from sources other than EVC were not obscuring the relationship between elevation and PAH at oiled locations, we also examined PAH concentrations at these locations with napthalene removed (Figure 3B.6). Subtraction of naphthalene concentrations from total PAH also removed naphthalenes contributed by EVC, but demonstrated that the

relationship between PAH concentration and elevation did not depend on naphthalenes at oiled locations (Figure 3B.6).

Background Concentrations

The PAH background concentration in PWS exceeded the 5 ng/g analytical noise levels detected in blank samples. We consider that our earliest data (days 20 to 26) from beaches least likely to be contaminated by EVC (low-gradient reference beaches) approximate the background concentration for this study. On this basis, the expected background concentration was 60 ± 2 ng/g, N = 4 (Figure 3B.1). Some medium-gradient reference beaches, particularly in Culross Passage, may have been contaminated by EVC before first sampling; the estimated mean concentration rose to 110 ± 28 ng/g (N = 7) when all reference beaches were included. We reject the latter background estimation as too high.

Possible sources for background PAH may include natural geochemical processes and some biological processes. For example, phenanthrene and perylene were found sporadically in sediments at all sampling stations in a baseline study by Karinen et al. (1993). In the baseline study, all aromatic analytes except perylene were near or below detection limits at five non-polluted sampling stations (Bligh Island, Dayville Flats, Naked Island, Olsen Bay, and Siwash Bay); seasonal trends were not evident (Karinen et al. 1993).

The background concentration observed in 1989 was greater than the baseline concentration observed by Karinen et al. (1993) between 1977 and 1980. Pre- and post-spill concentrations were compared on a dry weight basis for PAH compounds common to both data sets (see Table 3B.1 for common compounds). The mean pre-spill baseline PAH concentration at non-polluted locations was 9 ± 2 ng/g dry weight (N = 67; range, 0-93 ng/g). The post-spill background concentration for common compounds (40 ± 10 ng/g dry weight; range, 24-68 ng/g; N = 4) was greater than the historical baseline concentration.

The primary difference between the pre-spill baseline and post-spill background data was naphthalene content; concentrations of other PAH compounds were similar. Napthalenes (naphthalene, c1-naphthalene, dimethylnaphthalene, and trimethylnaphthalene) accounted for 14% (1.2 ± 0.2 ng/g dry weight) of the pre-spill baseline concentration, but 88% (35 ± 8 ng/g dry weight) of the 1989 background concentration. Pre- and post-spill concentrations of all other PAH compounds were similar (7 ± 2 ng/g and 4 ± 2 ng/g dry weight, respectively). The percentage of naphthalenes in our background data may represent an increase in naphthalenes above the true baseline or may have

been caused by differences in pre- and post-spill analytical technique.

Geographic and temporal distribution of oil

In sediments collected between -0.3 and +0.9 m MLLW, PAH and alkane concentrations in sediments at visibly oiled locations were significantly greater than at reference locations in 1989 (P = 0.031 and P = 0.005, respectively; Figure 3B.7). The highest PAH concentrations occurred in Snug Harbor. In 1990, PAH and alkane concentrations did not differ significantly between oiled and reference locations.

Concentrations of PAH in sediments collected from locations visibly contaminated with EVC peaked 22 to 27 d after the spill except in Herring Bay, where concentrations peaked 59 d after the spill (Figure 3B.8). The highest concentrations were observed in sediments from the medium-gradient beach in Snug Harbor. After the initial peak, concentrations declined through August, (our last 1989 sampling), and remained at low levels when sampling resumed in April 1990. In 1990, a secondary peak in PAH concentration occurred in late May to mid-June (425 to 445 d post-spill) in most locations.

Composition of PAH at visibly oiled locations was generally consistent with that of EVC (Figure 3B.9), but did not match EVC composition in all cases (Figure 3B.8). For example, in Herring Bay at the time of peak PAH concentrations (59 d), the percentage of naphthalenes was high, but heavier compounds through chrysenes were present in every sample. However, PAH composition in most sediment samples from visibly oiled locations was consistent with the PAH composition of EVC (Figure 3B.8). At these locations, C2DBT/C2PHN averaged 0.72 in 1989 and 0.52 in 1990; C2DBT was below MDL in 10 of 57 samples that contained C2PHN. The minimum observed PAH concentration with EVC characteristics at oiled locations was 54 ng/g.

Concentrations of PAH in sediments at reference locations tended to remain low, but minor peaks occurred at all four locations (Figure 3B.10). These peaks occurred 58 to 129 d after the spill, later than peak concentrations at locations visibly contaminated with EVC. Composition of PAH at peak concentration was similar to that of EVC in Culross Passage, Long Bay, and McClure Bay, but not in Wells Passage. In 1989, C2DBT/C2PHN averaged 0.88 in reference samples with detectable quantities of C2PHN, and was greater than 0.77 for all samples identified as containing or maybe containing EVC; C2DBT was absent in only 1 sample of 10 that contained C2PHN. A secondary peak in PAH concentration was observed at some reference locations in 1990 about the same time secondary peaks were noted at locations

visibly oiled with EVC (Figure 3B.10). The PAH composition in samples from Long Bay and McClure Bay at these secondary peaks was similar to that of EVC, and C2DBT/C2PHN was greater than 0.46.

An example of the change in PAH composition at a reference location is presented in Figure 3B.11 for the low-gradient beach in Culross Passage. Before and after peak concentrations, observed 129 d after the spill (Figure 3B.10), naphthalenes accounted for nearly all of the PAH. At peak concentration, however, the PAH composition resembled EVC.

Concentrations of PAH at reference locations that had EVC characteristics were generally greater than or equal to 209 ng/g, except for one sample (77 ng/g). Concentration of PAH in samples exhibiting characteristics of EVC at reference locations, therefore, generally exceeded the background concentration (60 ng/g) by a factor of more than 3.

Discussion

Intertidal sediments collected between -0.3 and +0.9 m MLLW at reference and visibly oiled locations were contaminated with EVC. Contamination at reference locations was slight. Concentrations of PAH peaked 22 to 129 d after the spill and then declined in a manner consistent with perturbation by a single oil spill. The secondary PAH peak observed in 1990 may have been due to remobilization of oil as a consequence of renewed cleanup efforts. Concentrations peaked later in reference locations than at visibly oiled locations as the oil was dispersed beyond the original slick trajectory. Composition of PAH at concentration peaks was generally consistent with EVC, including sediments from three of four reference locations. The high C2DBT/C2PHN ratios (greater than 0.5) were consistent with EVC, not Katalla seep oil. We were surprised to find contamination by EVC in McClure Bay and Long Bay. Neither of these bays was visibly oiled, and both bays were protected with booms. Apparently booms did not provide complete protection against dissolved and dispersed particulate oil. Because concentrations at reference locations remained significantly lower than at visibly oiled locations, we continued to consider them as reference locations for comparisons of biological responses.

Oil was not distributed uniformly across beaches, and thus, because of sampling protocol, our geographic sampling did not compare peak concentrations between locations. Oil was generally deposited on beaches in distinct bands, often with prominent oiling near the high water mark. The relationship between concentration and elevation was slight or negligible at reference sites, but strong at visibly oiled sites. Much of our data, however, were collected between -0.3 and 0.9 m MLLW, well below the zone of maximum PAH concentrations. As a result, concentration differences between reference and visibly oiled locations was probably considerably greater than estimated by our sampling for geographic oil distribution.

The biological data presented in Chapter 3A and these sediment data are consistent. Timing of peak PAH concentrations in sediments, mussels, and pink salmon at locations visibly contaminated with EVC was similar in all cases (Figure 3B.12). Concentrations peaked 22 to 73 days after the spill, and then declined. Secondary concentration peaks were frequent in 1990. Concentrations of PAH in mussels were generally much higher than in sediments at oiled locations. Composition of PAH usually matched that of EVC in 1989 in all three sample types. Characteristics of EVC were frequently detected in 1990 at these locations in sediments and mussels, but not in pink salmon.

Concentration peaks were also detected at locations not visibly contaminated with EVC. Timing of peak PAH concentrations in sediments, mussels, and pink salmon (Chapter 3A) was also similar

at these reference locations (Figure 3B.13). Concentrations peaked between 28 and 129 days after the spill, and then generally declined. Concentrations tended to peak slightly earlier in mussels and pink salmon (28 to 72 d) than in sediments (74 to 129 d). Timing of peak concentrations in reference locations for mussels and pink salmon was similar to the timing in visibly oiled locations. Peak PAH concentrations in sediments occurred later at reference locations than at oiled locations. As at locations visibly contaminated with EVC, secondary concentration peaks were noted, particularly at Long Bay. In contrast to visibly oiled locations, concentrations of PAH at reference locations were generally higher in sediments than in mussels. Composition of PAH in these samples was occasionally similar to EVC in 1989, usually near peak concentrations. These data indicate that areas outside the slick trajectory were contaminated with EVC in 1989. In 1990, composition in samples from reference locations was rarely similar to EVC in sediments, and never in mussels or salmon.

The correspondence of PAH concentration changes in biological samples (mussels and pink salmon, Chapter 3A) and sediments is further indication that reference locations were contaminated (Figure 3B.13). Concentration changes at reference locations were similar, but smaller, than those at visibly oiled locations (Figure 3B.12). The timing of these changes indicated a single underlying cause. Although their sampling was less frequent, a baseline study conducted from 1977 to 1980 by Karinen et al. (1993) found no evidence for seasonal variation in PAH concentration, thus eliminating the alternative explanation that natural seasonal variability caused the changes in concentration that we observed. Additionally, composition of PAH in all three sample types at reference locations was occasionally similar to EVC, usually near peak concentrations, and C2DBT/C2PHN ($\geq$0.5) exceeded that of Katalla seep oil (0.1).

Seawater transported oil beyond the visible boundaries of the slick trajectory. Small amounts of oil were transported by seawater into Culross Passage; small globules of mousse were observed on intertidal rocks on May 4, 1989. This observation is consistent with the hypothesis that Culross Passage was contaminated with EVC. Long Bay opens into Culross Passage, and there is substantial water exchange between them (Figure 2.1). Thus oil may also have been transported via water into Long Bay. Our Wells Passage location was near the north end of Culross Passage on Culross Island. Beaches on the east side of Culross Island were visibly oiled about 8 km southeast of our Wells Passage sampling location (ADEC 1989), and oil on water was observed within 5 km of our sampling location (Pavia 1991). Contamination by EVC in Wells Passage at about the same time as in Culross Passage, but concentration changes tended to be smaller. McClure Bay and Culross Passage both open onto Port Nellie Juan. Applegate Island, located near the north entrance

of Port Nellie Juan, was heavily oiled, and shoreline near the south entrance was lightly oiled (ADEC 1989). Oil on water extended 14 km into Port Nellie Juan, well past the entrance of McClure Bay (Pavia, 1992). Changes in PAH concentration and composition in McClure Bay suggest a degree of contamination by EVC similar to those at other reference locations.

We conclude that our reference locations were slightly contaminated by EVC. We hypothesize that dispersed particulate oil was transported by water and generally deposited in sediments over a broader area than that encompassed by the slick. These findings are consistent with the conclusion reached by Short and Rounds (1993) that a substantial portion of the oil spilled from the *Exxon Valdez* dispersed into the water column as particulate oil. However, the contamination detected in reference locations was significantly less than in visibly oiled locations, and therefore the comparisons of oiled and reference locations presented elsewhere in this report are not invalidated; comparisons are actually between heavily oiled and slightly oiled locations.

Table 3B.1. Abbreviations of PAH compounds used in text and figures. Compounds with asterisks were common to the 1977 to 1980 baseline data reported by Karinen et al. (1993). Dimethylnaphthalene and trimethylnaphthalene were also common to both data sets.

Abbreviation	Compound
NPH*	naphthalene
C1NPH*	C-1 naphthalenes
C2NPH	C-2 naphthalenes
C3NPH	C-3 naphthalenes
C4NPH	C-4 naphthalenes
BPH*	biphenyl
ACY	acenaphthylene
ACE	acenaphthene
FLU*	fluorene
C1FLU	C-1 fluorenes
C2FLU	C-2 fluorenes
C3FLU	C-3 fluorenes
DBT*	dibenzothiophene
C1DBT	C-1 dibenzothiophenes
C2DBT	C-2 dibenzothiophenes
C3DBT	C-3 dibenzothiophenes
PHN*	phenanthrene
C1PHN	C-1 phenanthrenes/anthracenes
C2PHN	C-2 phenanthrenes/anthracenes
C3PHN	C-3 phenanthrenes/anthracenes
C4PHN	C-4 phenanthrenes/anthracenes
ANT*	anthracene
FLA*	fluoranthene
PYR*	pyrene
C1FLA	C-1 fluoranthenes/pyrenes
BAA*	benz-a-anthracene
CHR*	chrysene
C1CHR	C-1 chrysenes
C2CHR	C-2 chrysenes
C3CHR	C-3 chrysenes
C4CHR	C-4 chrysenes
BbF	benzo-b-fluoranthene
BkF	benzo-k-fluoranthene
BEP*	benzo-e-pyrene
BAP*	benzo-a-pyrene
PER*	perylene
IDP	indeno-123-cd-pyrene
DBA	dibenzo-a,h-anthracene
BZP	benzo-g,h,i-perylene

Table 3B.2. Visual estimate of oiling in 1989 at Herring Bay and Snug Harbor 59 and 62 days after the *Exxon Valdez* oil spill. Estimates were made from available photographs taken along elevation transects.

Location	Day	Gradient	Elevation	Oiling
Herring Bay	59	low	0.7	None visible
			1.8	Moderate
			2.8	Heavy
		medium	-0.1	None visible
			1.1	None visible
			2.3	Moderate
			2.7	None to moderate
Snug Harbor	62	low	-0.2	None visible
			0.5	None visible
			1.2	None visible
			1.8	Light to moderate
			2.3	Light
			2.8	None visible
		medium	-0.2	None visible
			0.4	Light
			1.1	Light to heavy
			1.6	Moderate to heavy
			2.3	Moderate to heavy
			2.8	Heavy

Table 3B.3. Visual estimation of oiling during the first sampling at locations contaminated with EVC. There was no visible oiling at any reference location (Culross Passage, Long Bay, McClure Bay, and Wells Passage).

Location	Day	Gradient	Elevation	Oiling
Herring Bay	19	low	1.2 to 4.6	Heavy
		medium	1.5 to 4.6	Moderate
Knight Is. Pass	25	low	0.3 to 0.9	Moderate
			0.9 to 3.7	Heavy
			3.7 to 4.3	Light
		medium	0.3 to 0.7	Light
			0.7 to 3.5	Heavy
			3.5 to 4.5	Moderate
Prince of Wales	27	low	0.3 to 3.7	Light to heavy
		medium	1.2 to 2.4	Heavy
			2.4 to 3.7	Moderate
Snug Harbor	22	low	0.5 to 3.0	Moderate
			3.0 to 3.7	Light
			3.7 to 4.3	Heavy
		medium	0.3 to 4.3	Heavy

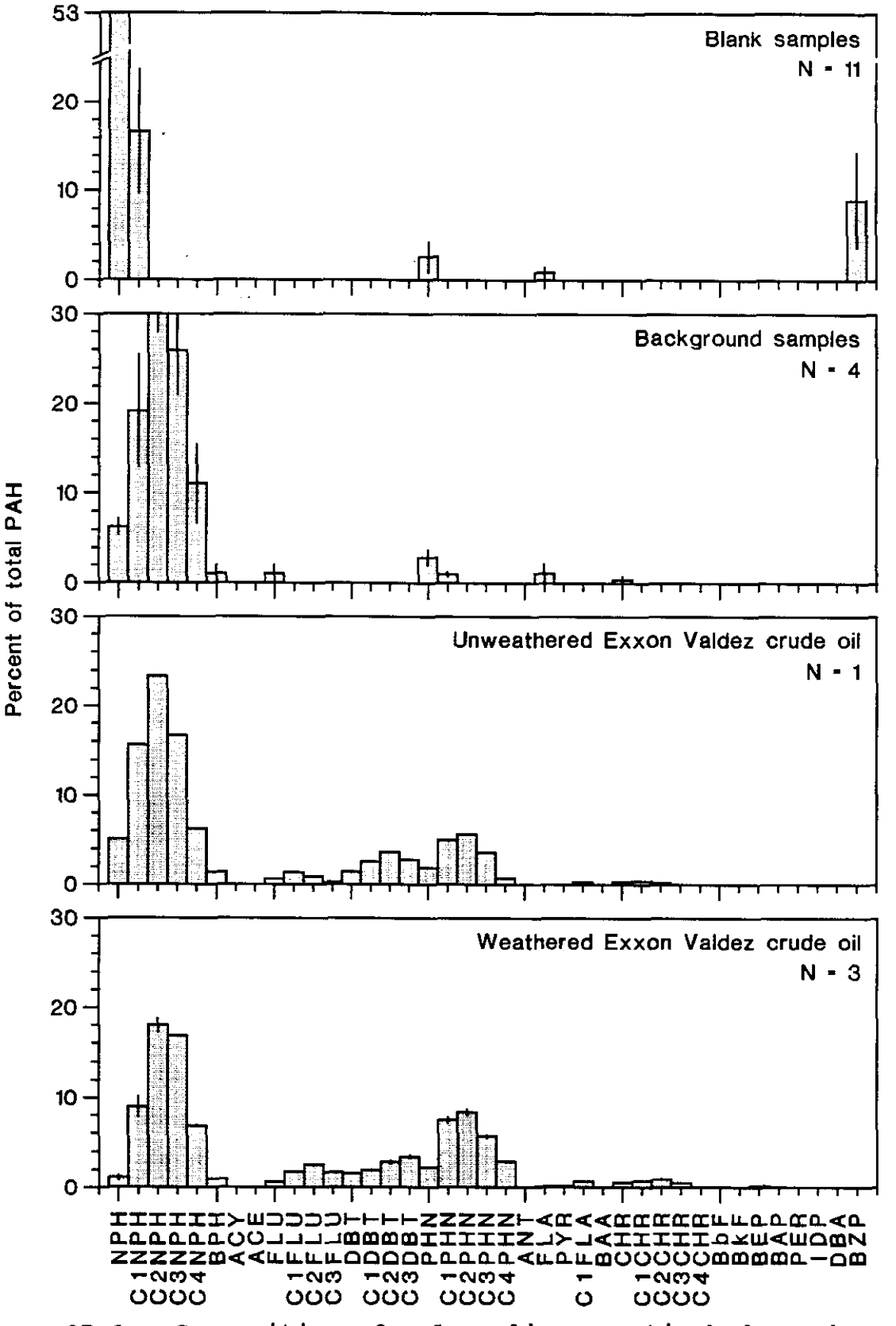

Figure 3B.1. Composition of polycyclic aromatic hydrocarbon (PAH) compounds in blank (1.4 ± 0.5 ng/g) and background (60 ± 2 ng/g) samples compared to composition of unweathered and weathered *Exxon Valdez* crude oil (EVC). Data for EVC samples were supplied by Short and Rounds (1993). Compound abbreviations are defined in Table 3B.1. Error bars are ±1 SE.

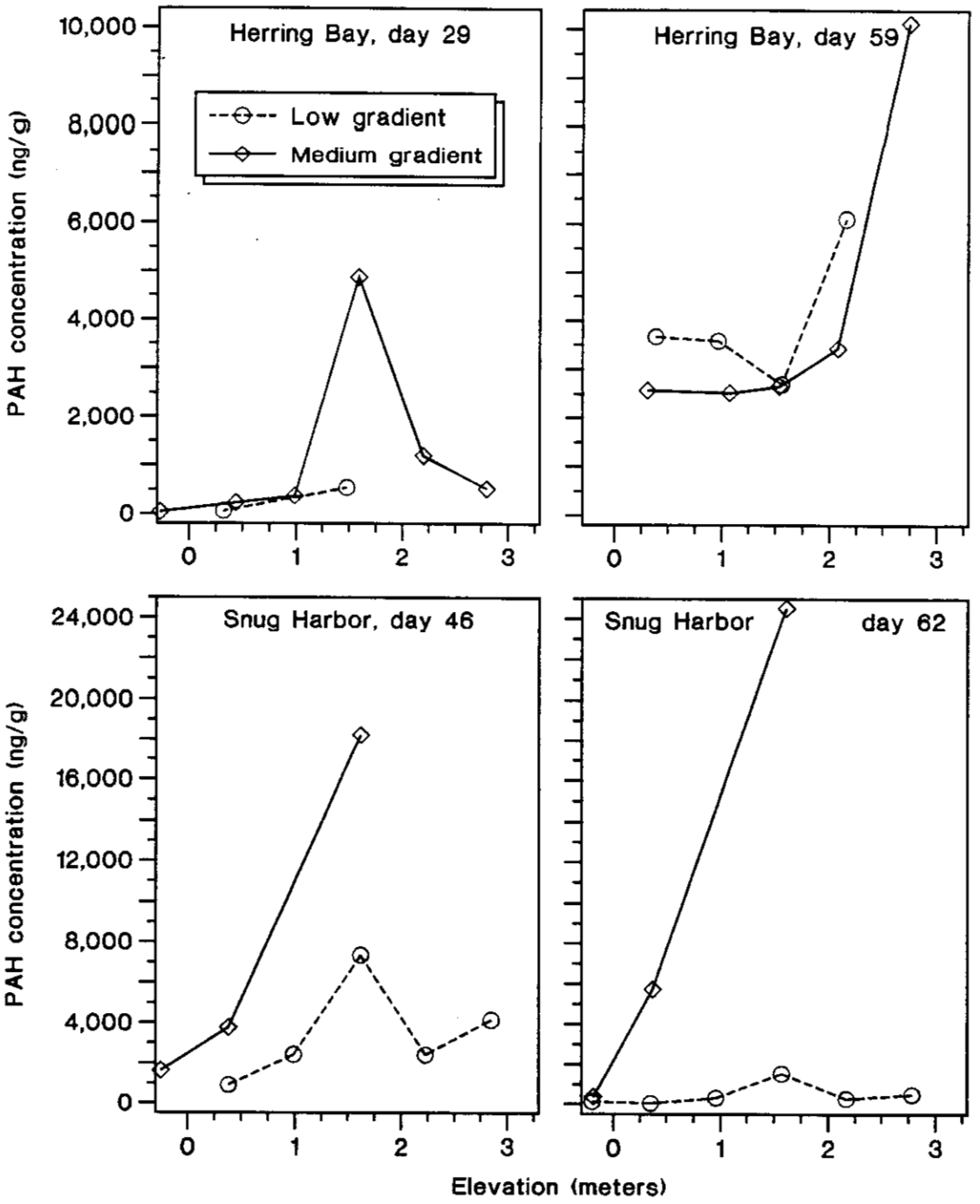

Figure 3B.2. Concentration of polycyclic aromatic hydrocarbons (PAH) as a function of elevation above mean lower low water at locations visibly oiled by *Exxon Valdez* crude oil. Two different beach gradients, low (<10% grade) and medium (12 to 25% grade), in Herring Bay and Snug Harbor were observed 29, 46, 59, or 62 d after the *Exxon Valdez* oil spill.

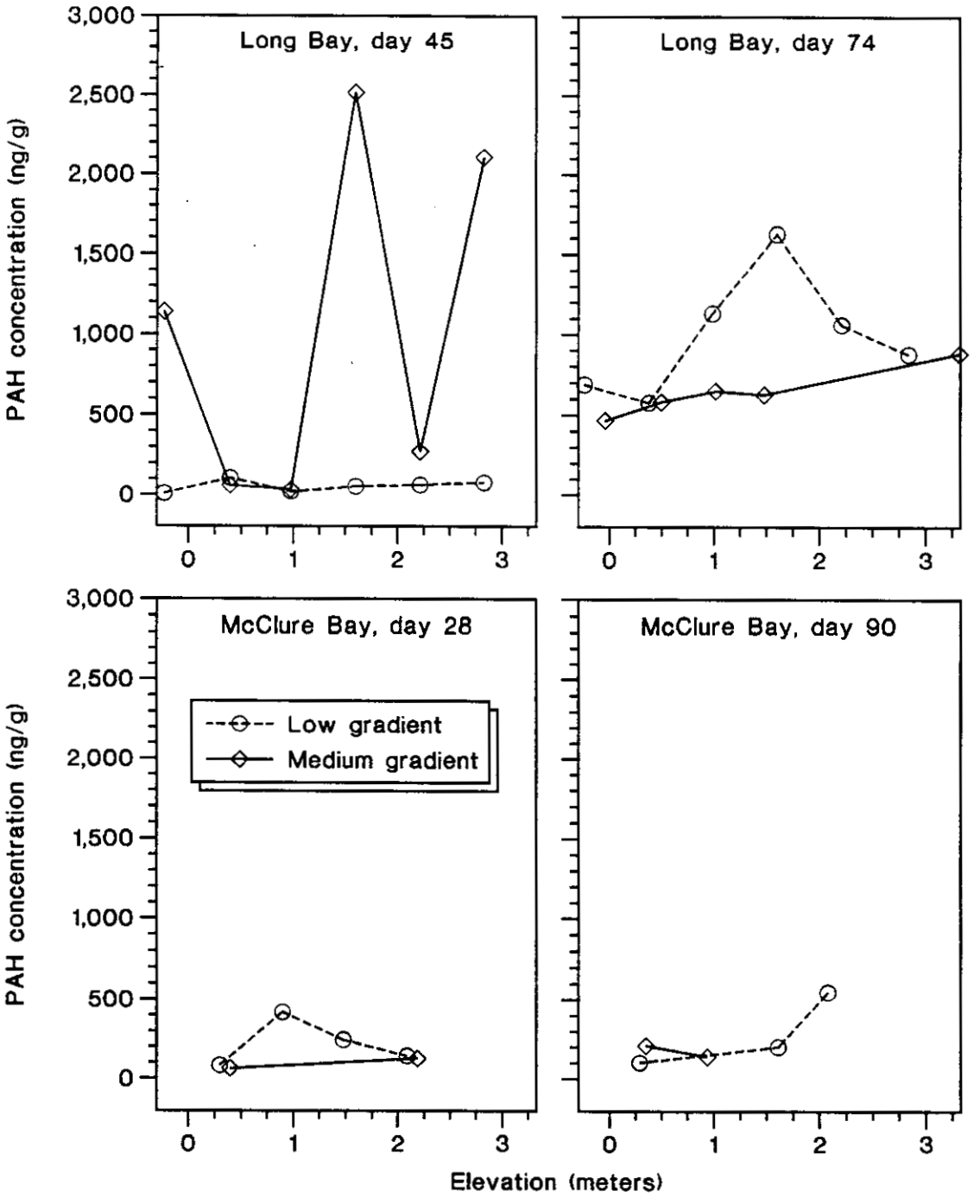

Figure 3B.3. Polycyclic aromatic hydrocarbons (PAH) concentration as a function of elevation above mean lower low water at reference locations. Two different beach gradients, low (<10% grade) and medium (12 to 25% grade), in Long Bay and McClure Bay were observed 28, 45, 74, or 90 d after the *Exxon Valdez* oil spill.

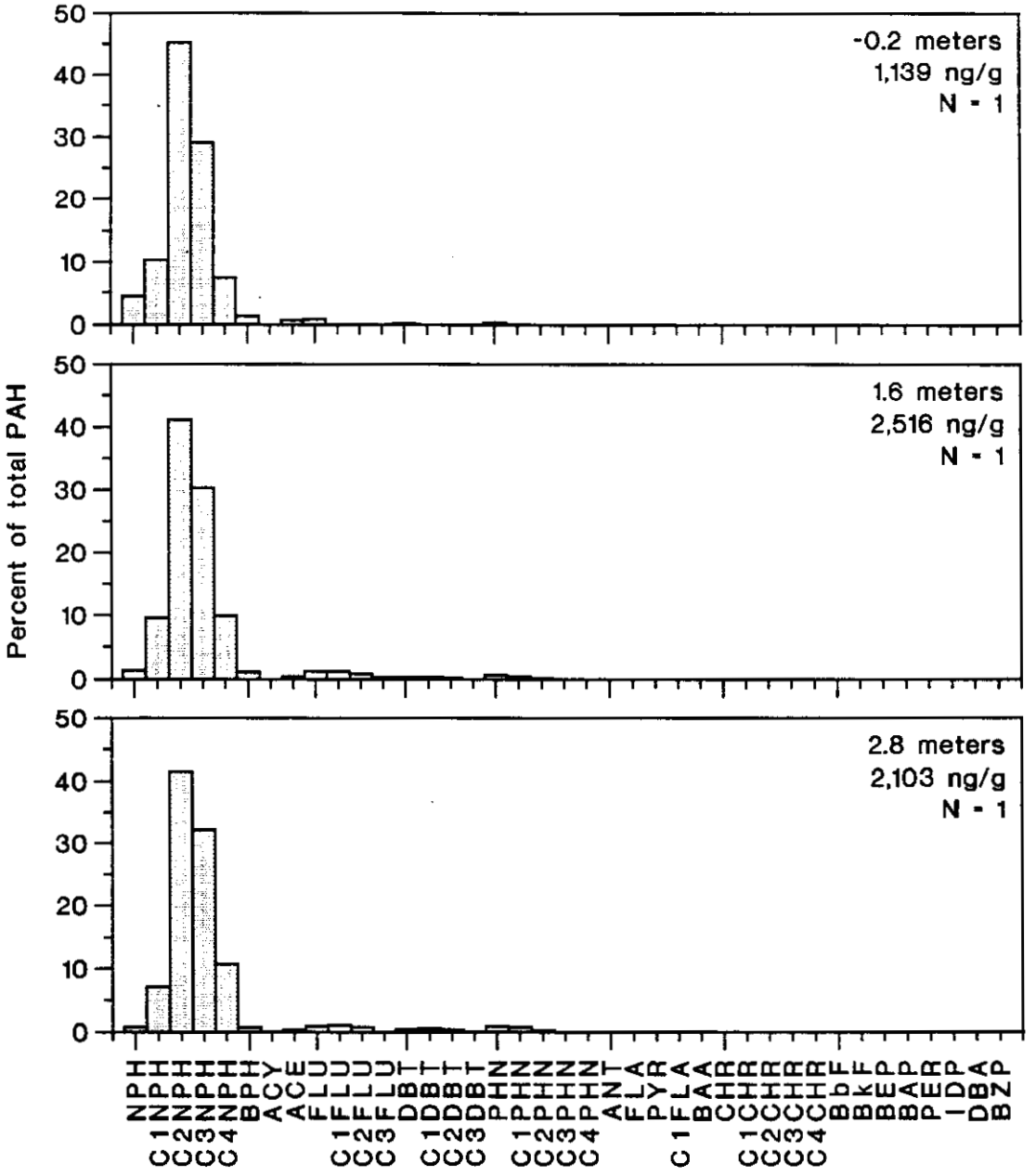

Figure 3B.4. Composition of polycyclic aromatic hydrocarbons (PAH) in Long Bay sediments at several elevations 45 d after the *Exxon Valdez* oil spill. Concentrations of PAH at the elevations presented were anomalously high, and were due almost entirely to naphthalenes. Sample elevation, concentration, and size are reported on each graph. Compound abbreviations are defined in Table 3B.1.

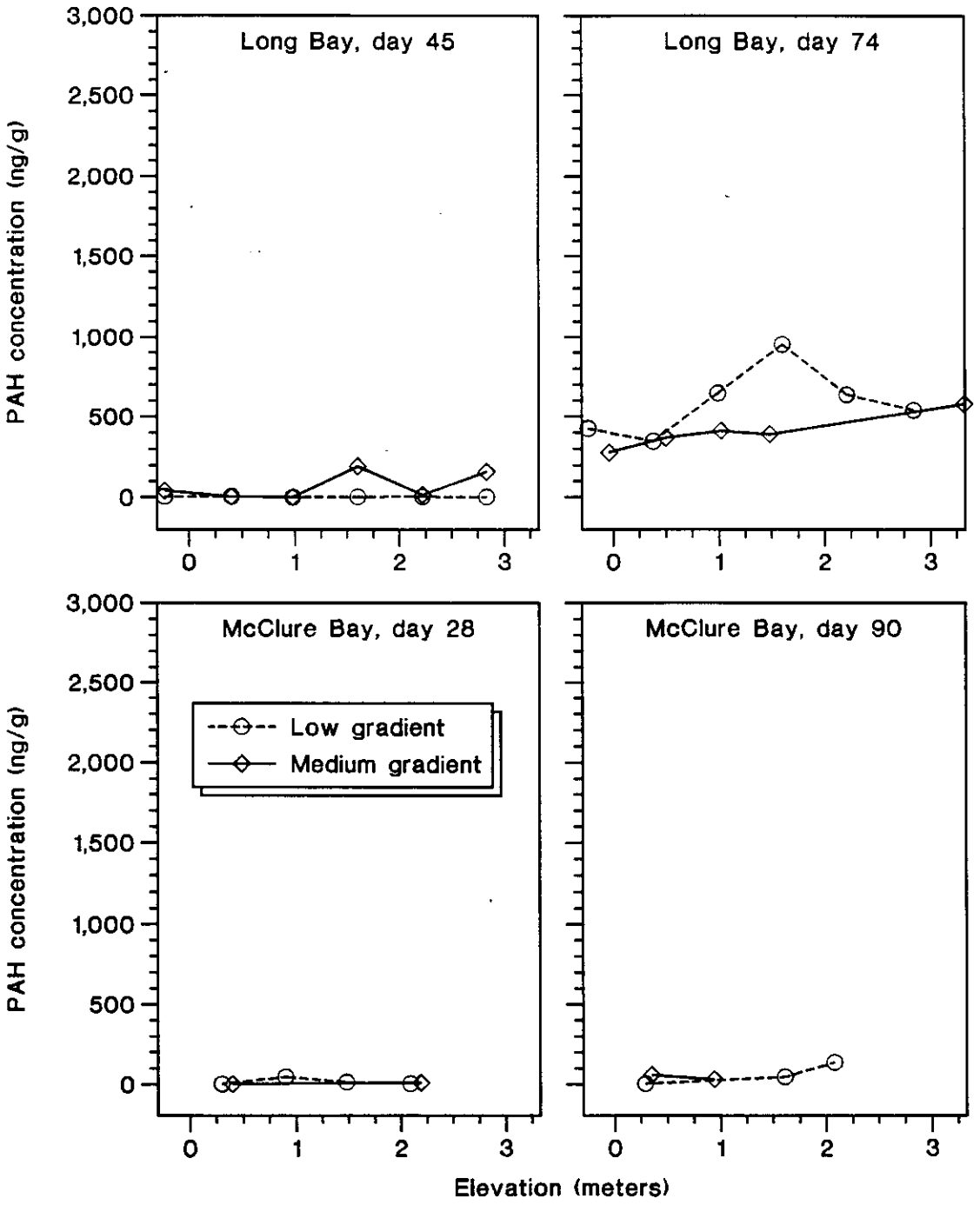

Figure 3B.5. Polycyclic aromatic hydrocarbon (PAH) concentration minus naphthalenes as a function of elevation above mean lower low water at low- and medium-gradient beaches at reference locations.

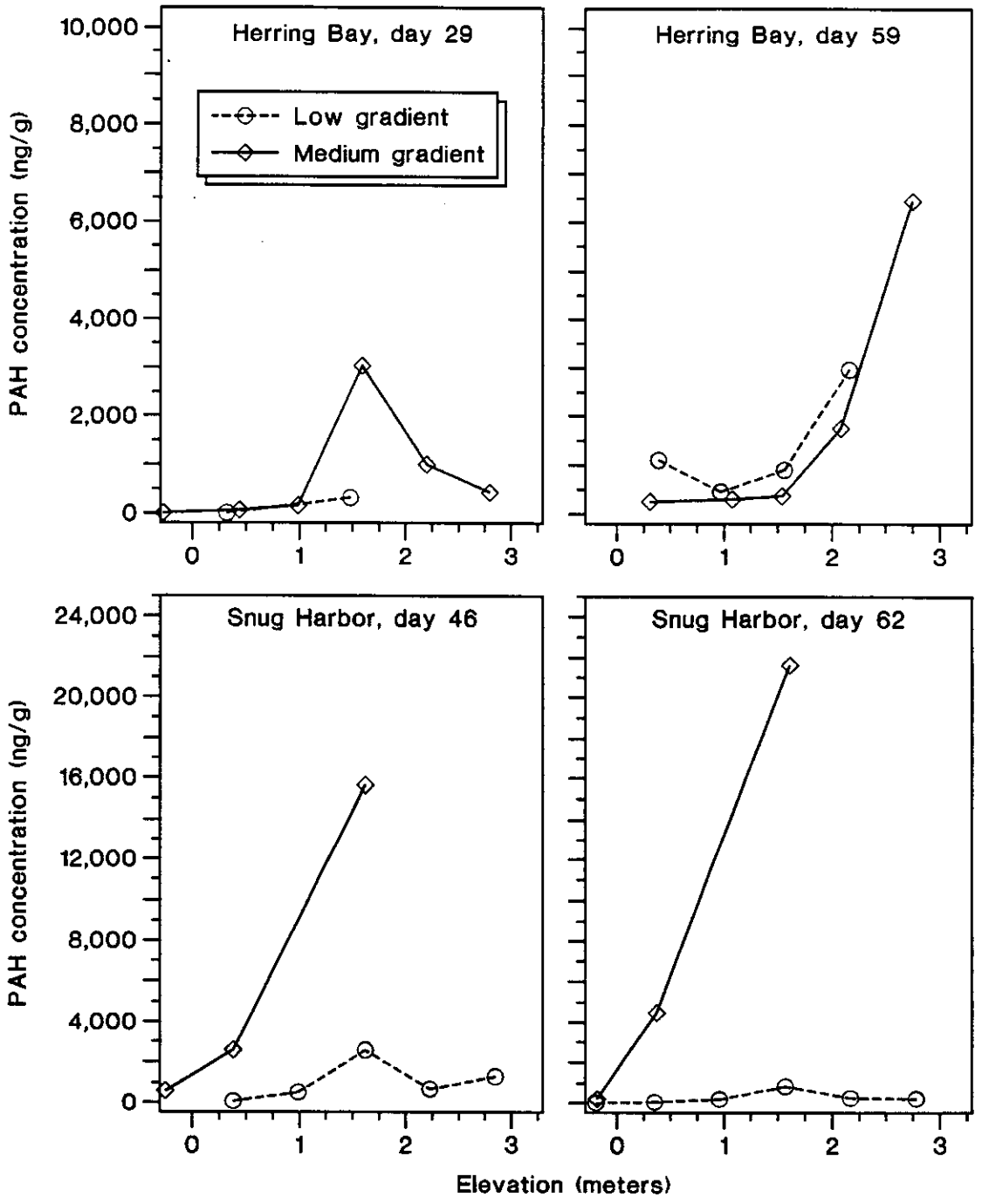

Figure 3B.6. Polycyclic aromatic hydrocarbon (PAH) concentration minus naphthalenes as a function of elevation above mean lower low water at low- and medium-gradient beaches at visibly oiled locations.

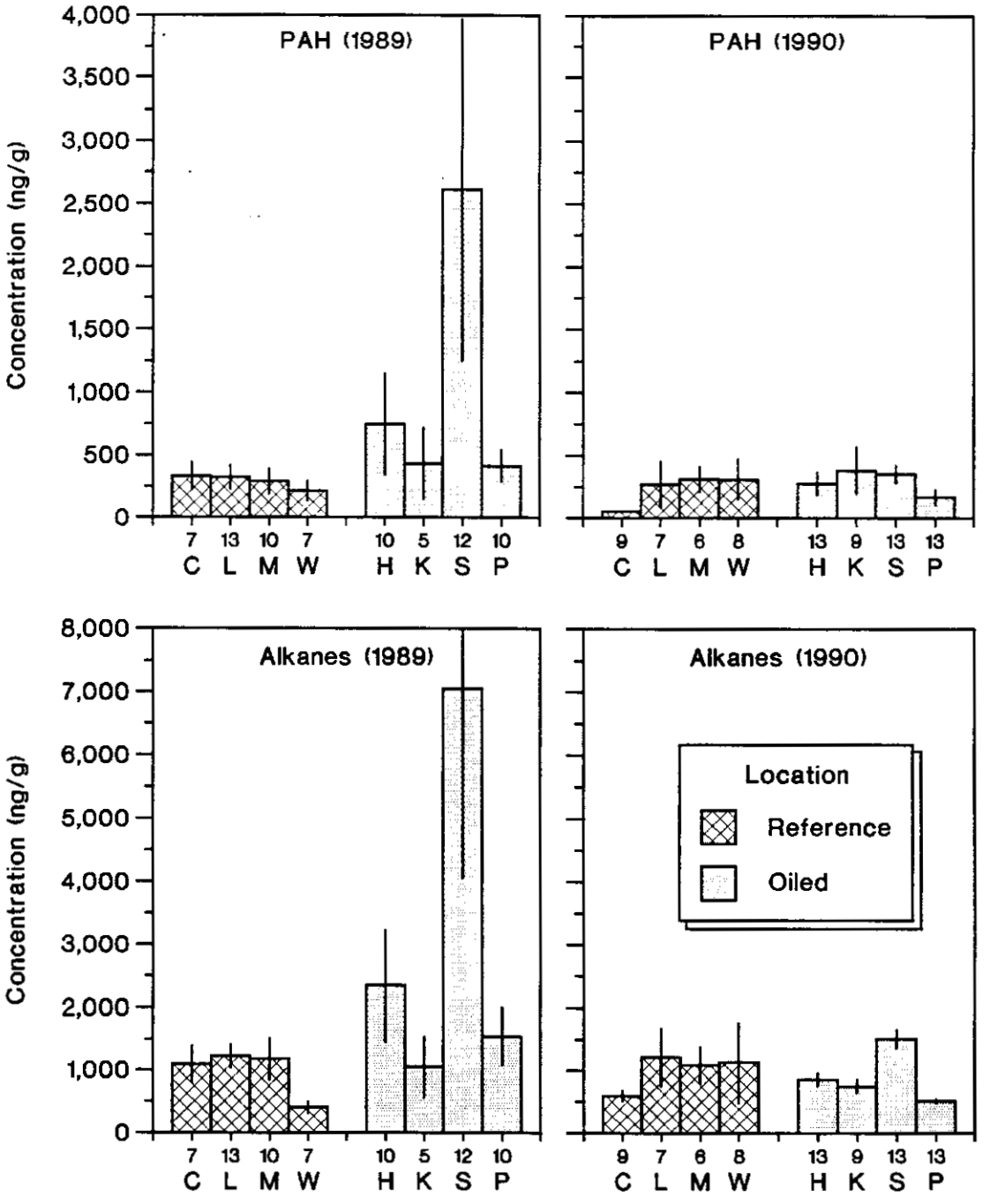

Figure 3B.7. Concentrations of polycyclic aromatic hydrocarbons (PAH) and alkanes in sediments collected between -0.3 and +0.9 m mean lower low water from reference and oiled locations in Prince William Sound in 1989 and 1990. Reference locations were Culross Passage (C), Long Bay (L), McClure Bay (M), and Wells Passage (W). Oiled locations were Herring Bay (H), Knight Island Passage (K), Snug Harbor (S), and Prince of Wales Passage (P). Numbers along the X-axis indicate sample size. Error bars are ±1 SE.

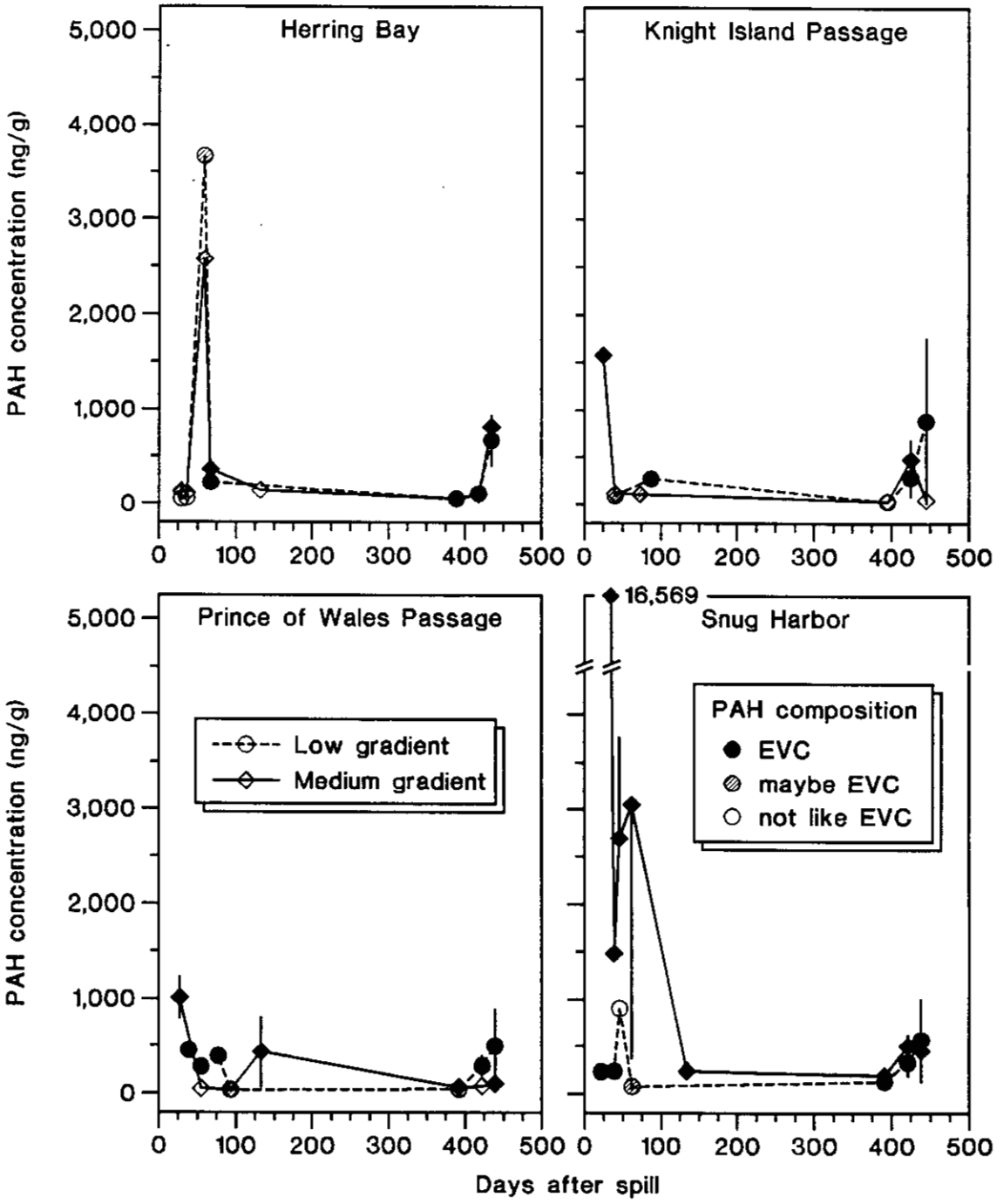

Figure 3B.8. Polycyclic aromatic hydrocarbon (PAH) concentration in sediments from low- and medium-gradient beaches visibly contaminated with *Exxon Valdez* crude oil plotted against time. Classification of PAH composition is indicated. Error bars are ±1 SE.

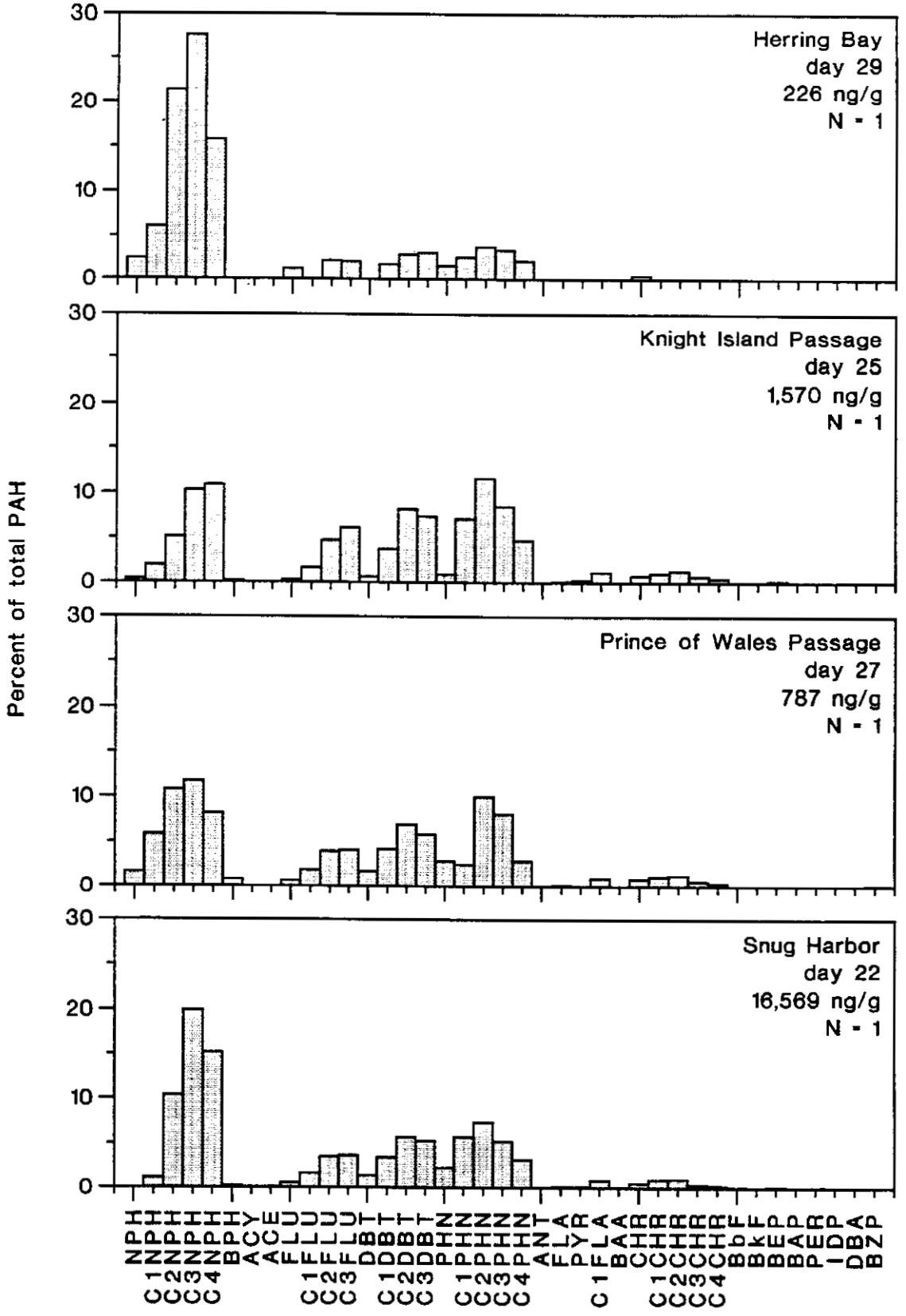

Figure 3B.9. Composition of polycyclic aromatic hydrocarbons (PAH) in sediments collected from visibly oiled locations at or near peak oiling. Sample time, concentration, and size are reported on each graph. Compound abbreviations are defined in Table 3B.1.

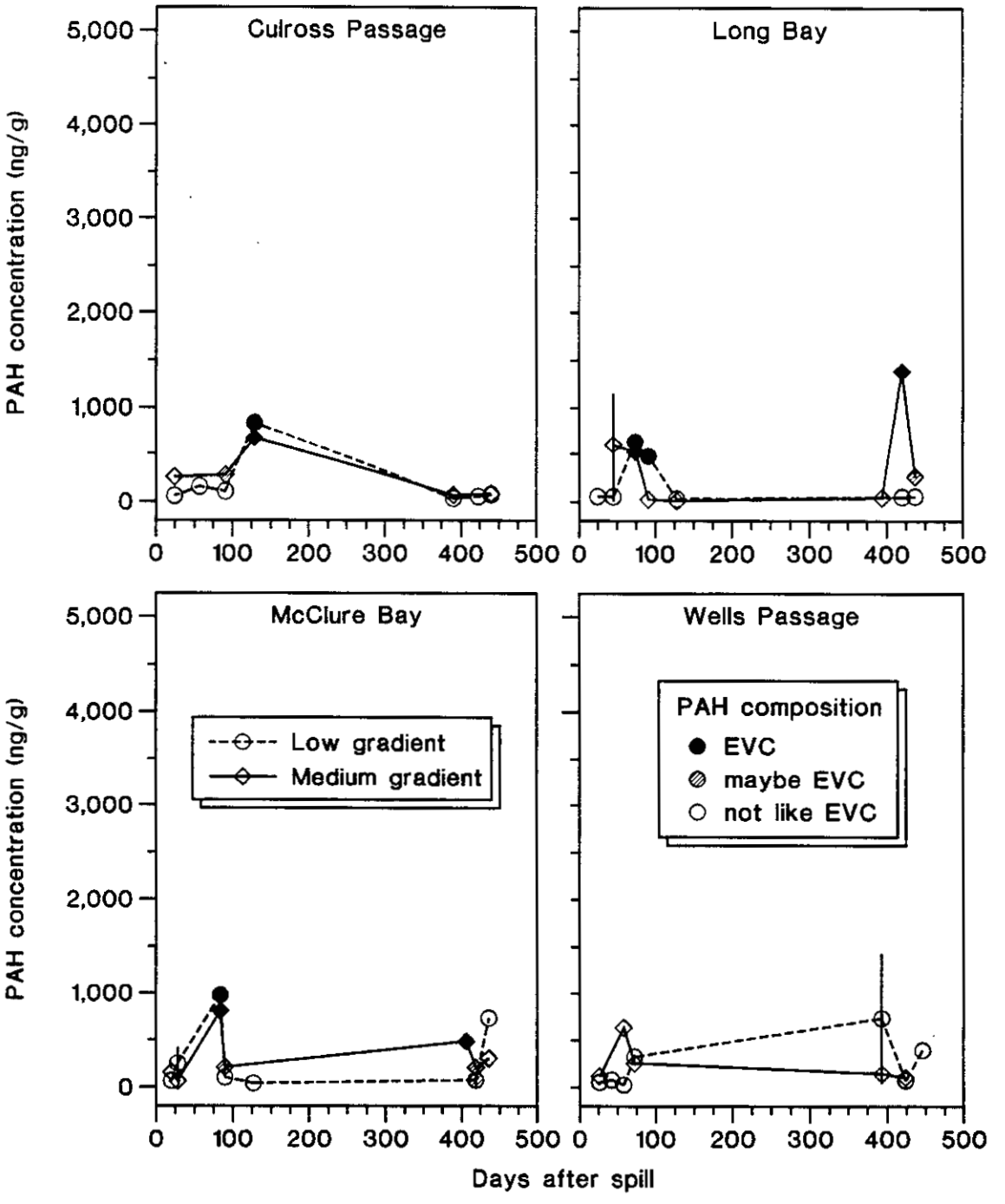

Figure 3B.10. Polycyclic aromatic hydrocarbon (PAH) concentration in sediments collected from low- and medium-gradient reference beaches. Classification of PAH composition is indicated. Error bars are ±1 SE.

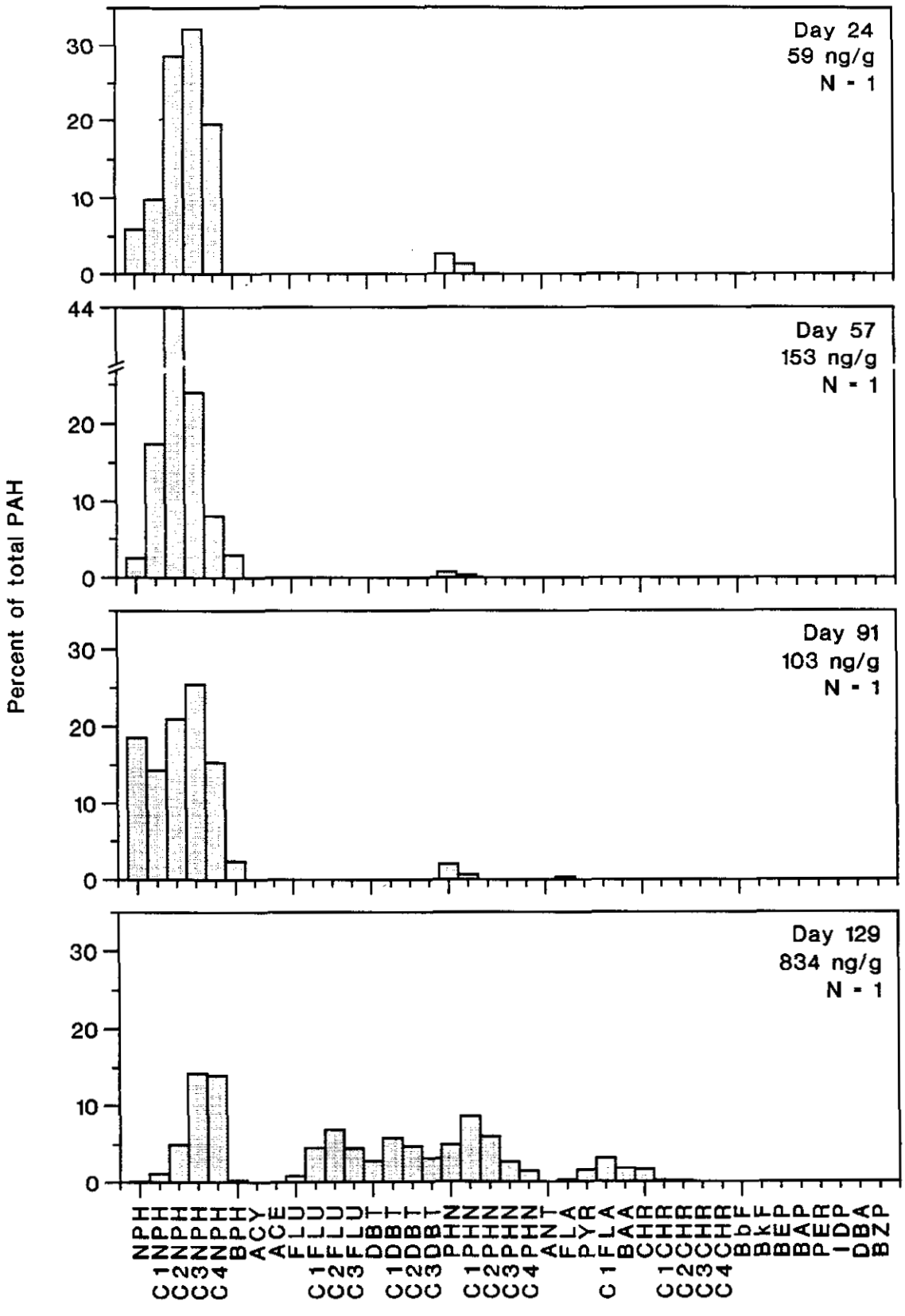

Figure 3B.11. Composition of polycyclic aromatic hydrocarbons (PAH) in sediments at a low-gradient beach in Culross Passage. Composition at the peak concentration (129 d) was similar to that of *Exxon Valdez* crude oil, but not earlier. Sample elevation, concentration, and size are reported on each graph. Compound abbreviations are defined in Table 3B.1.

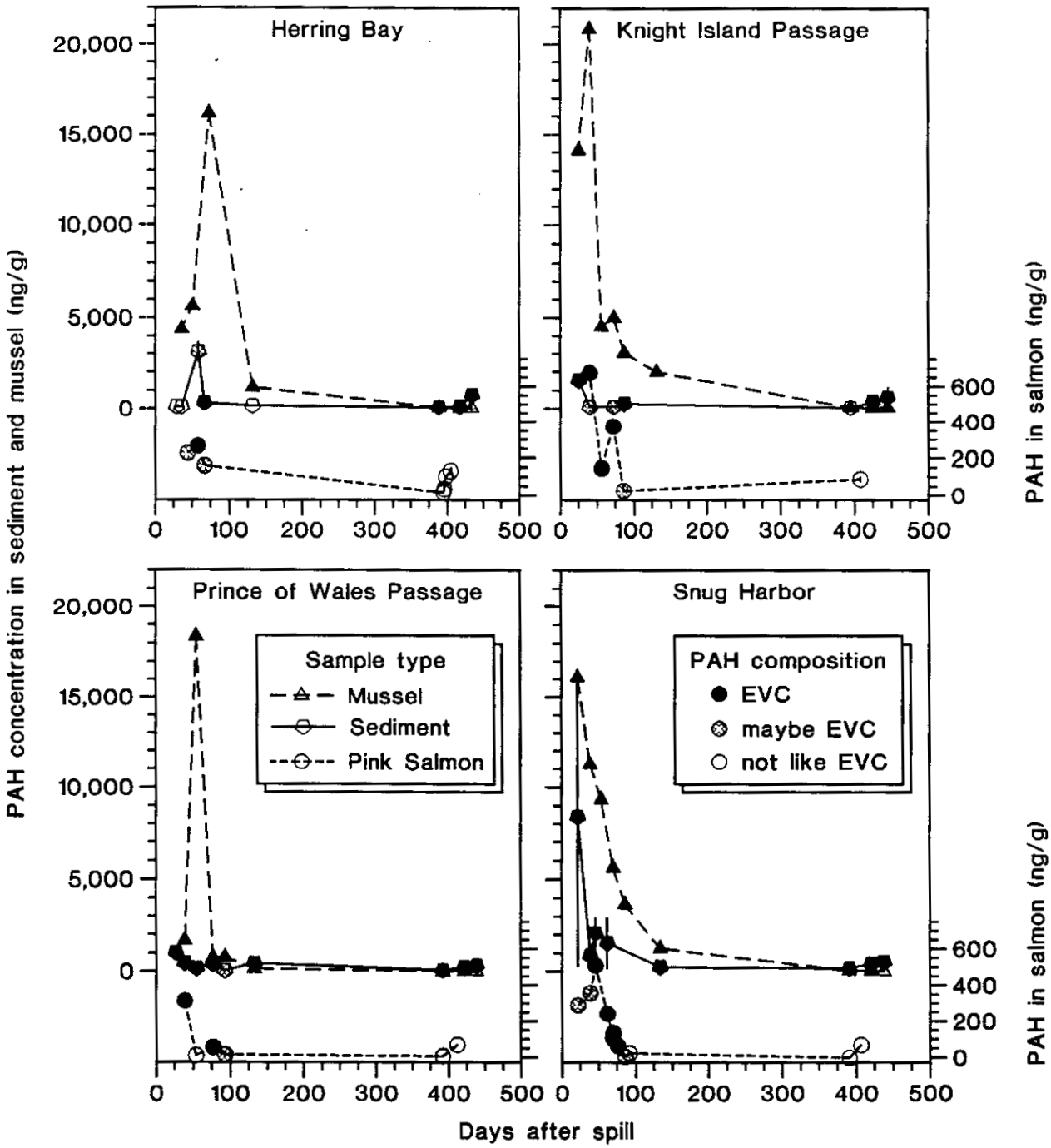

Figure 3B.12. Comparison of PAH concentration in sediments, mussels, and pink salmon collected from visibly oiled locations. Sediment concentrations from both beach gradients were combined at each location. An independent ordinate scale is used for concentrations in pink salmon. Classification of PAH composition is indicated. Mussel and pink salmon data were obtained from Chapter 3A. Error bars are ±1 SE.

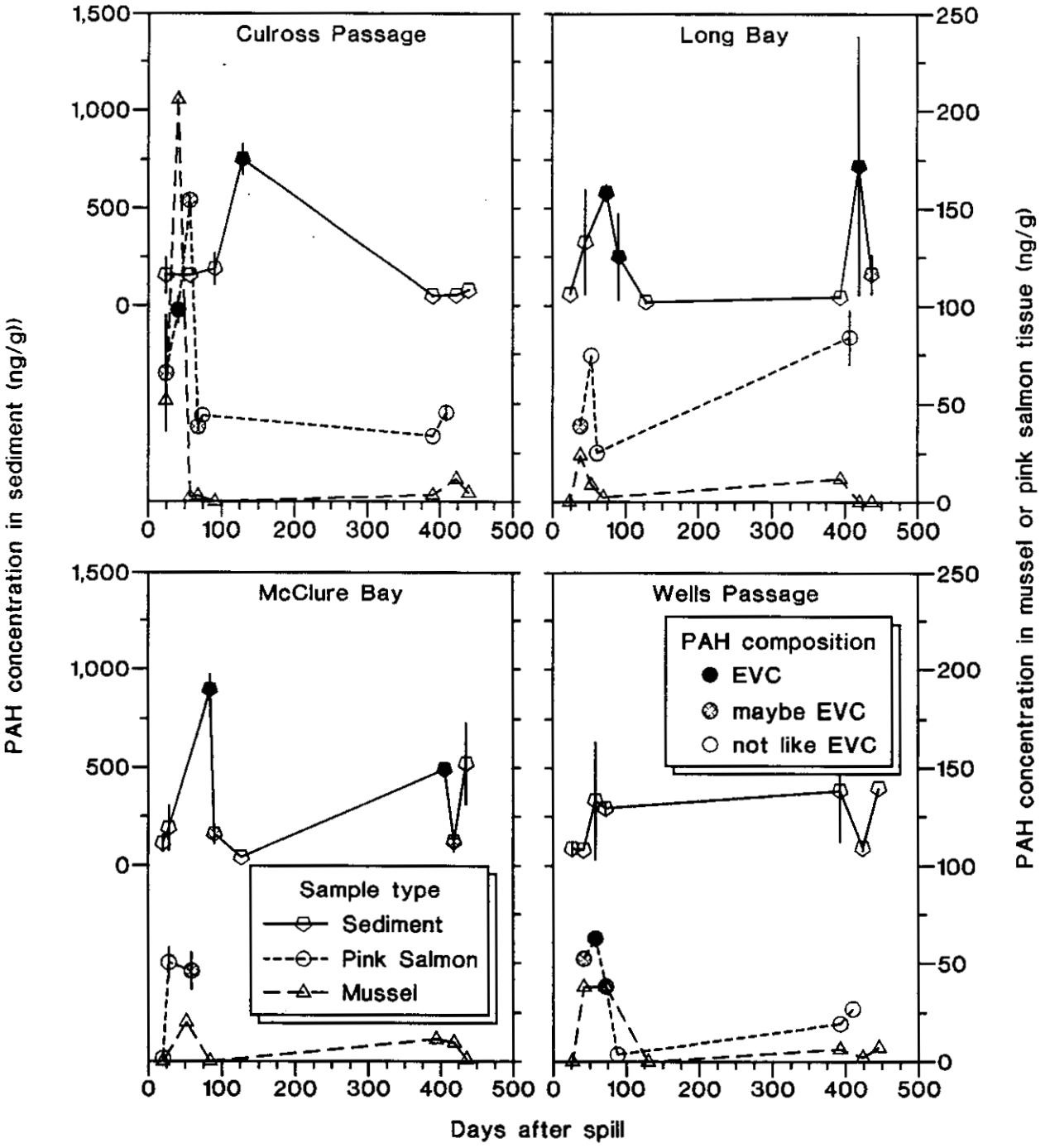

Figure 3B.13. Comparison of polycyclic aromatic hydrocarbon (PAH) concentration in sediments, mussels, and pink salmon collected from reference locations. Sediment concentrations from both beach types were combined at each location. Different ordinate scales are used for sediments and tissues. Classification of PAH composition is indicated. Mussel and pink salmon data were obtained from Chapter 3A. Error bars are ±1 SE.

CHAPTER 4. Diet of Juvenile Pink and Chum Salmon in Oiled and Non-oiled Nearshore Habitats in Prince William Sound, 1989 and 1990

M. V. Sturdevant, A. C. Wertheimer, and J. L. Lum

Abstract

The diets of juvenile pink and chum salmon were studied in nearshore habitats in oiled and non-oiled areas of Prince William Sound following the *Exxon Valdez* oil spill. Fish were captured in beach seines from April to mid-June in 1989 and 1990 at three habitat types at two oiled and two non-oiled sites in both bays and corridors. Diet was characterized in terms of biomass, numbers, and frequency of occurrence of organisms.

In 1989 and 1990, pelagic zooplankton comprised 50-94% of the diet biomass of pink salmon in bays and corridors in oiled and non-oiled areas; it comprised 39-73% of the diet of chum salmon in oiled and non-oiled areas. Small and large calanoid copepods were the primary zooplankters consumed by pink and chum salmon, respectively. Epibenthic prey were utilized most at low- and medium-gradient habitats, particularly by chum salmon.

Feeding effectiveness of pink and chum salmon was not reduced in the presence of oil. Measures of stomach fullness and food quantity were similar in oiled and non-oiled areas in both 1989 and 1990.

Salmon diet in oiled and non-oiled areas changed from 1989 to 1990. In 1989, pelagic zooplankton were consumed by pink salmon more in oiled areas than in non-oiled areas. Both pink and chum salmon fed less on zooplankton in oiled areas in 1990. Conversely, both species utilized epibenthic prey less in the oiled area in 1989, and more in the oiled area in 1990. These changes in diet between oiled and non-oiled areas could have been caused by differences in distribution of fish, distribution of prey, or effects of oil.

Oil globules or sheen were observed in 1% of pink and 4% of chum salmon stomachs from oiled sites in 1989; no oil was observed in fish stomachs from non-oiled sites in 1989 or from any sites in 1990. Thus, ingestion of oil, either directly as prey-sized globules or indirectly via contaminated prey, was an important route of hydrocarbon contamination of juvenile salmon in oiled habitats in 1989.

Introduction

This research compared the feeding habits of juvenile pink and chum salmon between oiled and non-oiled nearshore habitats in western Prince William Sound in 1989 and 1990 in the wake of the *Exxon Valdez* oil spill. The rapid growth of salmon fry under normal, undisturbed environmental conditions depends on abundant food resources (Bailey et al. 1975; Cooney et al. 1981). Diet varies according to the composition and abundance of food organisms available in different habitats, and available foraging habitats may vary in different areas (Heard 1991). In areas where low-gradient beaches support substantial epibenthic production, initial food of salmon often consists of epibenthic prey (Feller and Kaczynski 1975; Sibert et al. 1977; Cordell 1986); the importance of pelagic prey may increase with time, or fish may switch to pelagic prey as they move away from shore (Landingham and Mothershead 1988; Cooney et al. 1981; Cooney 1990). In Alaska waters, where nearshore habitats are often dominated by steep, rocky beaches with little epibenthic production, pelagic prey may be more important than epibenthic prey throughout the nearshore residency (Bailey et al. 1975; Kron and Yuen 1978; Murphy et al. 1988). Use of prey resources can also vary by species, size, location, and time of year (Heard 1991).

Natural variability in consumption from pelagic and epibenthic production systems may be impacted by oil in the water column and on beaches. Oil may affect the diet of juvenile salmon by altering abundance and composition of pelagic and epibenthic prey, by interfering with the fish's ability to feed in some habitats, or by reducing assimilation efficiency. The purpose of this study was to determine if *Exxon Valdez* oil affected feeding of pink and chum salmon in nearshore areas of Prince William Sound in the year of the spill (1989) and the year following.

This research addresses Objective 8 of Fish/Shellfish Study Number 4 (Chapter 1, this report): to quantify feeding habits of juvenile pink and chum salmon and test if indices of feeding effectiveness differed between oiled and non-oiled areas. Specific objectives were 1) to compare feeding effectiveness of juvenile salmon between oiled and non-oiled areas; 2) to compare diet composition between oiled and non-oiled areas, between bays and corridors, and between habitats; and 3) to examine stomach contents for evidence of oil ingestion.

Chapter 4 will be reformatted and submitted to the *Exxon Valdez Oil Spill Symposium Proceedings* for formal publication. *Theory and Application in Fish Food Habits*

Methods

Study Design

We selected four sampling locations in the oiled area and four in the non-oiled area of western Prince William Sound (Figure 2.1). The oiled and non-oiled areas were identified by visual inspection of the actual distribution of oil in the first 2 weeks after the spill. Within each area, two bays and two corridors leading to the Gulf of Alaska were selected. These eight locations were paired *a priori* for pairwise comparisons between oiled and non-oiled areas. The paired bays were Herring Bay-McClure Bay and Snug Harbor-Long Bay; the paired corridors were Prince of Wales Passage-Culross Passage and Knight Island Passage-Wells Passage (oiled and non-oiled areas, respectively).

In each location, we selected sites representing three different habitat types. Low-gradient habitats were <10% grade with granule-pebble substrate, medium-gradient habitats were 11-25% grade with pebble-cobble substrate; and steep-gradient habitats were >45% grade with boulder or bedrock substrates. Surface substrate composition was visually classified according to the Wentworth scale (Holme and MacIntyre 1984) into four categories: granule (2-4 mm), pebble (4-64 mm), cobble (64-256 mm), and boulder (>256 mm). Any substrate <2 mm, such as sand or mud, was also included in the granule category.

Sampling effort differed between years. In 1989, one beach of each habitat type was sampled at each location, for a total of 24 systematically sampled sites. In 1990, two beaches of each habitat type were sampled at each location, for a total of 48 systematically sampled sites (maps A-E, Appendix 2.1). Samples were also collected along 3-5 km of adjacent shoreline at each location to supplement catches at specific habitat types. In 1989, we collected fish in five trips from 10 April to 26 June; in 1990, we collected fish in four trips from 16 April to 14 June. Trips took place at 2-week intervals.

Sampling at the systematic sites was restricted to tidal levels from -1 to +3 m to minimize tidal effects between sites. We used beach seines 37 m long with 6-mm mesh to collect fish (see Chapter 2, this report). Dip nets were also used to collect fish during supplementary sampling. Sampling was in conjunction with research on pink and chum salmon abundance, distribution, size, and growth (Chapter 2, this report), exposure to and contamination by oil (Chapter 3, this report), and epibenthic and pelagic prey (Chapter 5, this report).

Sample Processing

Samples of up to 60 each of pink and chum salmon from each habitat type at each location were fixed in buffered 10% formalin. After a standard fixation period of ≥45 days to minimize variance in shrinkage (Gosho 1977), fish from each site were weighed to the nearest mg wet weight and measured to the nearest mm fork length (FL). Wet weight was converted to dry weight by a standard conversion factor of 0.21 (Volk et al. 1984). Up to 10 fish of each species were randomly subsampled and preserved in 40% isopropyl alcohol for stomach analysis.

During initial stomach processing, two measures of feeding effectiveness were obtained for each fish. First, when the stomach was excised, fullness was visually ranked on a scale from 0 to 6 as empty, containing trace amount, 25%, 50%, 75%, or 100% full, or distended, respectively. Second, the intact stomach was weighed to the nearest mg, stomach contents were removed, and the empty stomach was weighed again to estimate wet weight of stomach contents by subtraction.

We then determined prey composition. Prey organisms were identified to the lowest practical taxa (generally to the level of order) and counted. Prey items were categorized by production system: pelagic zooplankton, epibenthos, or drift insects. Pelagic zooplankton were subcategorized as small calanoid copepods (<2.5 mm metasomal length), large calanoid copepods (≥2.5 mm metasomal length), and other zooplankters. Calanoid subcategories were specified because they generally represent different biological systems: small calanoids were genera produced in neritic waters, primarily *Pseudocalanus*, while large calanoids were genera produced in the Gulf of Alaska and advected into Prince William Sound, primarily *Calanus* and *Neocalanus* (Cooney 1986). Epibenthos was divided into harpacticoid copepods and other epibenthic organisms. These subcategories were specified because of the importance of harpacticoids in the diet of juvenile salmon in other regions (e.g., Kaczynski et al. 1973; Healey 1979; Landingham 1982). Harpacticoids from 61 pink and 109 chum salmon stomachs from systematic and supplemental samples in 1989 were identified to family or genus to identify important taxa.

Total dry weight of each prey taxon in a stomach was estimated by multiplying number in the stomach by its average dry weight. Sources for these values included Cooney et al. (1981) for calanoids and J. Landingham (pers. comm.; Auke Bay

Laboratory, National Marine Fisheries Service, 11305 Glacier Hwy., Juneau, AK 99801-8626) for harpacticoids and most other taxa. When literature values were not available, mean dry weight was determined by weighing a sample of intact specimens of a taxon dried at 60°C for 24 hours.

Fish diets were described by seven measures of feeding effectiveness (quantity) and five measures of diet composition. Feeding effectiveness included stomach fullness, wet weight of contents, content wet weight as a percent of fish wet weight, total prey dry weight as a percent of fish dry weight, percent of empty stomachs in a haul of fish, total number of prey, and total dry weight of prey. Composition measures included percent occurrence, number, percent number, dry weight, and percent dry weight of each of the three production systems and four prey subcategories.

We described diets primarily by prey biomass (dry weight), because this parameter best represents the energetic importance of prey to predators (Bowen 1983); percent number and percent frequency of occurrence were used to supplement characterization of the diets (Hyslop 1980). Relative importance diagrams with axes for the three percentage components (Simenstad and Kinney 1978; McEachran et al. 1976) were constructed to compare prey composition between oiled and non-oiled areas.

Statistical Analysis

Of the 608 pink salmon and 493 chum salmon examined in 1989, 397 pinks and 112 chums could be used in comparisons of fish from *a priori* paired locations matched by time period and habitat; in 1990, all 595 pinks and 136 chums examined could be included in paired comparisons. The Wilcoxon matched-pairs signed-rank test (Conover 1980) was used to compare medians of feeding effectiveness, diet composition, and size of fish used in diet analyses between oiled and non-oiled locations. A parametric technique such as nested analysis of variance was not appropriate because of the large number of sets with zero catches (Chapter 2, this report). The Wilcoxon test computed the "estimated median difference" and its confidence interval for the ranked set of differences between matched pairs. For each set of comparisons, we tested the hypothesis that the estimated median difference in the paired parameters was not different from zero. A significant ($P < 0.1$) negative value for the difference indicated that the non-oiled parameter was greater than the oiled parameter.

We previously reported results of Wilcoxon tests on mean differences between diet parameters for oiled and non-oiled areas (Wertheimer et al. 1991). We reanalyzed the data because medians were a more accurate measure of central tendency for data that were not normally distributed. No changes resulted from reanalysis; each set of comparisons produced identical trends in diet and similar levels of significance.

The number of possible Wilcoxon comparisons varied between years for both pink and chum salmon because all habitats were not represented in all periods. Only hauls with five or more fish were used. For pink salmon, 21 paired comparisons were possible in 1989, and 31 were possible in 1990. In 1989, 13 of the 21 possible comparisons were from corridor and 8 were from bays. Systematic hauls were doubled in 1990, and bays and corridors were represented by 15 and 16 pairs, respectively. Comparisons of chum salmon diet were limited to six pairs of hauls in 1989 and seven pairs in 1990, from low-gradient corridors and low- and medium-gradient bays.

Diets of fish from oiled and non-oiled areas were further contrasted to complement results of Wilcoxon tests of differences between these areas. For these contrasts, summary statistics (median, mean, and standard error of the mean) for all diet parameters were computed from individual fish values rather than the composite groups. Because the medians and means were generated by averaging over all oiled or non-oiled fish, differences between summary values, particularly means, were not necessarily the same as the estimated median difference between paired comparisons.

Results

Feeding Effectiveness

Feeding effectiveness of pink salmon was not reduced in the oiled area compared to the non-oiled area in either year. In 1989, 16% of pink salmon stomachs were empty in both oiled and non-oiled areas (Table 4.1). Of the seven measures of feeding effectiveness, total prey dry weight and dry weight as a percent of body weight were significantly higher in the oiled area ($P = 0.046$). In 1990, empty stomachs occurred at rates of 9% in the oiled area and 10% in the non-oiled area (Table 4.1), and no measures of food quantity differed significantly between oiled and non-oiled areas.

Feeding effectiveness of chum salmon also was not reduced in oiled compared to non-oiled areas. In 1989, 3% of chum salmon stomachs were empty in the oiled area, compared to 8% in the non-oiled area; in 1990, 1% of the stomachs were empty in each area (Table 4.2). In 1989, no significant difference was detected in any of the seven measures of feeding effectiveness. In 1990, only dry weight of prey as a percent of total body weight was lower for fish from the oiled area ($P = 0.052$).

Diet Composition

Overview

Pelagic zooplankton formed the largest proportion of the diets of salmon in both years. For pink salmon, zooplankton was 66% of prey dry weight in 1989 and 71% in 1990; for chum salmon, it was 69% and 43%. Calanoid copepods made up most of the zooplankton biomass consumed by both species (Figures 4.1-4.3). Both large and small calanoids contributed important biomass components, but small calanoids were consumed by pink salmon more frequently and in greater numbers than large calanoids. In both years, median measures of large calanoids were zero even when means were large, indicating their patchy occurrence in pink salmon diet. For chum salmon, however, large calanoids were consumed more frequently than small calanoids and made up most of the calanoid biomass. Medians were often zero for small calanoid diet parameters. Large calanoids tended to be entirely present or entirely absent in hauls of chum salmon, suggesting they were not always available, but were preferred when present. The two size classes occurred together in fish diets in corridors more often than in bays, indicating greater mixing of neritic and oceanic production in corridors than in bays. Other zooplankters occurred in the diets

frequently, but were rarely an important component by weight or number. Cladocerans, polychaetes (Family Syllidae), and fish larvae were occasionally important for both species (Tables 4.3-4.6).

Epibenthos was the second-most important production system in salmon diets (Figures 4.1-4.3). Although harpacticoid copepods occurred frequently in diets of both species, they formed most of the epibenthic biomass in pink salmon, while they were a smaller proportion in chum salmon then other epibenthic prey. Both species consumed only epibenthic harpacticoids; interstitial forms were not eaten. *Harpacticus*, *Tisbe*, and *Dactylopodia* were the most important genera, comprising 84% and 85% of the harpacticoid biomass of pink and chum salmon, respectively. *Harpacticus* was the largest component (48% and 47%), *Tisbe* was second-largest (22% and 27%), and *Dactylopodia* was third-largest (14% and 11%) for pink and chum salmon. Juvenile mollusks, gammarid amphipods, and cumaceans contributed large proportions of epibenthic prey in both species; epibenthic larvae of intertidal chironomids were also large components in chum salmon diets (Tables 4.3-4.6).

Drift insects (primarily adult Diptera) were usually a small proportion of the diets by dry weight. However, frequency of occurrence differed for pink and chum salmon; the frequency of drift insects was 21-35% in chum salmon diets, but was usually less than 20% in pink salmon diets (Figures 4.1-4.3).

Diet composition differed between bays and corridors and between low-, medium- and steep-gradient habitats. Zooplankton was a larger proportion of pink salmon diet in bays than in corridors, averaging 78% of total prey dry weight in bays and 65% in corridors. Zooplankton was always the dominant prey consumed by pink salmon in steep-gradient habitats (Tables 4.7 and 4.8). It comprised most of the biomass consumed in low- and medium-gradient habitats in bays, but in these habitats in corridors, epibenthic prey often contributed as much as zooplankton.

Comparisons of chum salmon diets between bays and corridors and between habitats were complicated by the paucity of samples from corridors and the lack of samples from steep-gradient habitats. Zooplankton was a larger proportion of chum salmon diets than epibenthos at most low- and medium-gradient habitats (Table 4.9); however, epibenthos comprised similar or larger proportions of chum diets than zooplankton in one low- and two medium-gradient bay habitats and one low-gradient corridor habitat over the two years.

Oil Effects: Pink Salmon

Use of prey production systems by pink salmon differed between oiled and non-oiled areas in both years; however, trends were opposite in 1989 and 1990 (Figure 4.4). In 1989, zooplankton was higher in all five composition measures for the oiled area; percent number (P = 0.014), dry weight (P = 0.020), and percent dry weight of zooplankton (P = 0.042) were significantly higher in the oiled area (Table 4.10). Zooplankton averaged 71% and 53% of prey biomass in oiled and non-oiled areas. In 1990, most zooplankton composition measures were lower for the oiled area; percent number (P = 0.002), dry weight (P = 0.016), and percent dry weight of zooplankton (P = 0.004) were significantly lower for the oiled area (Table 4.11). Zooplankton averaged 58% and 77% of prey dry weight in oiled and non-oiled areas, respectively.

The interannual shift in diets from oiled and non-oiled areas was also evident when prey production systems were split into subcategories. Compared to non-oiled areas, calanoids were used more in the oiled area in 1989 (Table 4.12), and less in the oiled area in 1990 (Table 4.13). In 1989, two measures of total calanoids were higher for the oiled area: percent number (P = 0.053) and dry weight (P = 0.037). In 1990, however, measures of total calanoids (except number of organisms) were lower for the oiled area (P < 0.015).

Interannual differences in zooplankton eaten in oiled vs. non-oiled areas were driven by large calanoids in 1989 and by small calanoids in 1990. In 1989, all measures of large calanoids except percent frequency of occurrence were significantly higher (P < 0.1) in the oiled area; small calanoid diet parameters tended to be higher in the oiled area, but no differences were significant (Table 4.12). In 1990, all measures of small calanoids except number of organisms were significantly lower (P < 0.064) in the oiled area; diet parameters for large calanoids were not different (Table 4.13).

The interannual shift in use of zooplankton between oiled and non-oiled areas was different for bays and corridors. In 1989, overall proportions of zooplankton consumed in oiled bays and corridors were equal to or greater than these proportions in the non-oiled area (Figure 4.1). Zooplankton comprised 79% of the diet by weight in oiled bays and 79% in non-oiled bays; it comprised 71% in oiled corridors and 50% in non-oiled corridors. Higher zooplankton composition in the oiled area in 1989, therefore, was weighted by effects in corridors, rather than bays. In 1990, however, lower zooplankton composition in the oiled area was weighted by differential use in bays, rather than corridors. The

proportion of zooplankton was 58% in oiled bays and 94% in non-oiled bays, while it was 68% in oiled corridors and 72% in non-oiled corridors (Figure 4.2).

Differences in zooplankton use between oiled and non-oiled areas were generally consistent in different habitats. Compared to non-oiled habitats, percent biomass of total zooplankton consumed was higher in 1989 in all oiled habitats except medium-gradient habitat in bays (Table 4.7). In 1990, zooplankton was lower in all oiled habitats except steep-gradient habitats in bays and low-gradient habitats in corridors (Table 4.8).

Trends in use of epibenthos also reversed from 1989 to 1990 (Figure 4.4). In 1989, all five measures of epibenthos tended to be lower in oiled areas than non-oiled areas (Table 4.10). In 1990, however, measures for epibenthos tended to be higher in oiled areas than non-oiled areas (Table 4.11); percent number and percent dry weight were significantly higher ($P = 0.005$ and 0.011, respectively).

Interannual differences in use of epibenthos in oiled vs. non-oiled areas were driven by consumption of harpacticoid copepods. In 1989, diet parameters for harpacticoid copepods tended to be lower for the oiled area (Table 4.12), but none of these comparisons was significant. In 1990, the pattern was reversed; all diet parameters for harpacticoid copepods were significantly higher for the oiled area ($P < 0.085$; Table 4.13).

The interannual shift in use of epibenthos also differed for bays and corridors. In 1989, epibenthos formed a smaller proportion of the diets in both bays and corridors in the oiled area than the non-oiled area. Epibenthos comprised 10% of the diet in oiled bays and 27% in non-oiled bays; it comprised 17% in oiled corridors and 47% in non-oiled corridors (Figure 4.1). Lower use of epibenthos in the oiled area in 1989, therefore, was weighted by differences in corridors. In 1990, however, greater differences in epibenthic consumption between oiled and non-oiled areas existed in bays than in corridors. The proportion of epibenthos was 14% in oiled bays and 3% in non-oiled bays; it was 27% in oiled corridors and 28% in non-oiled corridors (Figure 4.2). In 1990, therefore, higher consumption of epibenthos in the oiled area resulted from differences in bays, rather than corridors.

Differences in use of epibenthos between oiled and non-oiled areas were usually consistent in different habitats. In 1989, epibenthos consumption was lower in oiled habitats except medium- and steep-gradient habitats in bays (Table

4.7). In 1990, epibenthos consumption in oiled habitats was higher or similar to that in non-oiled habitats for all except low-gradient habitat in corridors (Table 4.8).

In both years, drift insects were uncommon in diets and no significant difference existed between oiled and non-oiled areas (Tables 4.10 and 4.11). Drift insect use was similar among oiled and non-oiled habitats, except that they tended to form a higher percentage in low- and medium-gradient habitats in oiled bays than in non-oiled bays in both years (Tables 4.7 and 4.8).

Size of pink salmon did not affect comparisons of diets between oiled and non-oiled areas. Neither FL nor weight of fish used in diet studies differed between oiled and non-oiled areas in either 1989 or 1990 ($P > 1.0$). Median FL of pink salmon was 37 mm in oiled areas and 38 mm in non-oiled areas in 1989; it was 35 mm in oiled areas and 34 mm in non-oiled areas in 1990. Wet weight was also similar (Table 4.14). Pink salmon consumed both zooplankton and epibenthos throughout their size range. The proportion of zooplankton consumed tended to decrease as FL increased, while the proportion of epibenthos tended to increase as FL increased. Correlations between FL and percent dry weight of prey consumed were low, but significant ($P < 0.05$), for zooplankton in 1990 ($r = -0.19$) and percent dry weight of epibenthos in 1989 and 1990 ($r = 0.14$ and 0.18).

Oil Effects: Chum Salmon

Use of prey production systems between oiled and non-oiled areas also differed for chum salmon, but fewer differences were significant than for pink salmon in both years. In 1989, total zooplankton use in the oiled area was not significantly different from the non-oiled area (Figure 4.5; Table 4.15). Zooplankton was 62% of prey biomass in the oiled area and 59% in the non-oiled area. In 1990, zooplankton composition measures (except number of organisms) tended to be lower in the oiled area; percent number and percent dry weight of zooplankton were significantly ($P = 0.052$ and 0.076) lower in the oiled area (Figure 4.5; Table 4.16). Zooplankton averaged 41% and 69% of prey biomass in oiled and non-oiled areas, respectively.

Trends in use of prey production systems between oiled and non-oiled areas were also mirrored by prey subcategories. Although no difference in overall zooplankton use was observed in 1989, calanoids tended to be used less in the oiled area in both 1989 and 1990 (Tables 4.17 and 4.18). These differences were not significant in 1989, but in 1990,

total calanoid measures (except number of organisms) were significantly lower in the oiled area ($P < 0.076$).

Differences between oiled and non-oiled areas were driven by small calanoids in 1989 and by large calanoids in 1990 (Tables 4.17 and 4.18), the opposite of the pattern for pink salmon. Small calanoid composition measures tended to be lower in the oiled area, but only percent frequency of occurrence was significant ($P = 0.059$), and no significant difference existed for use of large calanoids. In 1990, no difference in small calanoid measures was observed; however, all large calanoid composition measures were significantly lower in the oiled area ($P < 0.059$).

Differences in use of zooplankton between oiled and non-oiled areas were not consistent in different habitats. In 1989, chum salmon consumed similar proportions of zooplankton in oiled and non-oiled low-gradient habitats in both bays and corridors; in contrast, they consumed less zooplankton in oiled medium-gradient habitat in bays (Table 4.9). In 1990, zooplankton use was substantially lower in the oiled area for both low- and medium-gradient habitats in bays, but was substantially higher in the oiled area for low-gradient habitat in corridors; the difference in the two bay habitats, therefore, accounted for significantly lower zooplankton use in the oiled area.

The pattern of use of epibenthos between oiled and non-oiled areas also reversed from 1989 to 1990 (Figure 4.5). In 1989, a weak trend for differential use of epibenthic prey was observed; four of five epibenthic composition measures tended to be lower in the oiled area, but the differences were not significant. Epibenthos was 21% and 26% of the diet by weight in the oiled and non-oiled areas, respectively. In 1990, epibenthic measures tended to be higher in the oiled area, but these differences were not significant. Epibenthos averaged 55% and 27% of prey biomass in oiled and non-oiled areas, respectively.

Interannual trends in use of epibenthos were driven by harpacticoids. Harpacticoids tended to be used less in the oiled area than the non-oiled area in 1989 (Table 4.17), but a trend for greater use of harpacticoids in the oiled area existed in 1990 (Table 4.18). In 1990, number and dry weight of harpacticoids were significantly higher in the oiled area ($P < 0.063$).

Use of epibenthos by chum salmon differed between habitats (Table 4.9). Compared to non-oiled areas, smaller proportions of epibenthos were consumed in 1989 in low-gradient habitats in both oiled bays and oiled corridors, but

larger proportions were consumed in medium-gradient habitat in oiled bays. The 1989 trend toward lower epibenthic consumption in the oiled compared to the non-oiled area, therefore, was due to differences in low-gradient habitats. In 1990, however, epibenthos was higher in the oiled area for both low- and medium-gradient habitats in bays, but was lower in low-gradient habitat in oiled corridors. Differences in bay habitats in 1990, therefore, accounted for the trend toward greater use of epibenthos in the oiled area.

No significant difference in use of drift insects was observed in 1989 or 1990. In 1989, the proportions of drift insects tended to be higher in oiled low-gradient habitats in both bays and corridors, but tended to be lower in oiled medium-gradient habitats in bays; proportions were similar in all oiled habitats compared to non-oiled habitats in 1990 (Table 4.11).

Size of chum salmon differed between oiled and non-oiled areas, but the size difference probably did not affect diet comparisons because size and diet were not strongly correlated. FL and weight of chum salmon were greater in the oiled area in both 1989 ($P = 0.036$) and 1990 ($P = 0.076$). Median FL in 1989 was 48 mm and 37 mm in oiled and non-oiled areas, respectively; in 1990, FL was 48 mm in the oiled area and 40 mm in the non-oiled area (Table 4.14). Chums ate from both production systems throughout their size range. Correlations between FL and prey composition were significant only for zooplankton in 1989; percent dry weight of zooplankton decreased with increasing chum FL ($r = -0.25$; $P < 0.05$).

Oil Ingestion

Oil sheen and globules of tarry material were observed in the stomachs of some pink and chum salmon in 1989. Out of a total of 286 pink salmon stomachs from oiled sites in 1989, one had sheen and one had tar globules (0.7% of total). Sheen was also noted in 3 of 67 (4.5%) chum salmon stomachs from oiled sites in 1989. No sheen or tar globules were observed in the 322 stomachs of pink salmon or the 426 stomachs of chum salmon from non-oiled sites. In 1990, no evidence of oil was observed in any of the 595 pink salmon stomachs or 136 chum salmon stomachs. Fish which had ingested oil were collected in both bays and corridors, from late April to mid-June; pink salmon were from steep-gradient habitats and chum salmon were from low- and medium-gradient habitats. Only one of the five fish had an empty stomach; the other four had consumed pelagic, epibenthic, and drift insect prey.

Discussion

We found no indication that feeding effectiveness was reduced in the oiled area compared to the non-oiled area for either pink or chum salmon in either year. Fish fed normally despite the presence of oil in pelagic and epibenthic foraging areas. Stomachs were as full, as much total prey was consumed, and empty stomachs occurred as frequently in oiled areas as in non-oiled areas in both 1989 and 1990. Indeed, some measures of feeding effectiveness were higher for pink salmon in oiled areas in the year of the spill.

Although the quantity of food eaten was not reduced in the presence of oil, we did find evidence that oil was ingested at all three habitat types in both bays and corridors in the oiled area in 1989. We attributed this oil to the *Exxon Valdez* spill because no oil was observed in stomachs from non-oiled areas in 1989 or from any 1990 collections. Although the incidence of oil observed in the stomachs was low, the occurrence of any observable oil supports the conclusion (Chapter 3, this report) that ingestion was a route of contamination of these fish in Prince William Sound in 1989. The rate of contamination cannot be inferred by the incidence of observation because oil may be consumed intermittently, and it may not be recognizable as it is digested or if it is contained in prey.

Ingestion of oil can directly affect both feeding and growth rates of juvenile salmon. In laboratory studies, food contaminated with water-soluble fraction and whole crude oil reduced pink salmon growth and feeding at high doses (Schwartz 1985; Chapter 6, this report). At lower doses, however, consumption of oil-contaminated food reduced growth in both pink and Atlantic salmon while food consumption remained stable or increased relative to controls (Chapter 6, this report; Vignier et al. 1992). Rice et al. (1977) speculated that energy demands remain elevated throughout the process of hydrocarbon depuration. Thus, the ingestion of oil in contaminated habitats in Prince William Sound, even at levels sufficient to cause physiological effects (e.g., reduced growth), is compatible with our observation that food consumption was not lowered in the oiled area.

We did not observe any differences in salmon diet composition that we could definitely associate with the impact of oiling. We did, however, observe a shift in diet composition between oiled and non-oiled areas from 1989 to 1990. A possible explanation for this change between years is that salmon avoided epibenthic prey in contaminated nearshore habitats in 1989, and instead, shifted their feeding toward zooplankton prey. This argument is supported by two observations.

First, the proportion of zooplankton consumed by both pink and chum salmon decreased from 1989 to 1990 in the oiled area, even though zooplankton was more abundant in 1990 than in 1989 (Chapter 2A, this report). Second, epibenthic prey was a smaller proportion of salmon diets in the oiled area in 1989, even though abundance of epibenthos was higher in the oiled area and abundance of zooplankton did not differ between oiled and non-oiled areas in 1989 (Chapter 2, this report).

Avoidance of epibenthic prey in contaminated habitats in 1989, however, is not supported by other observations in Prince William Sound or by laboratory feeding studies. Distribution of juvenile salmon in 1989 and 1990 did not indicate avoidance of oiled habitats (Chapter 5A, this report). Furthermore, although overall consumption of epibenthic prey increased from 1989 to 1990 as zooplankton consumption declined, in some oiled habitats in 1989, epibenthic prey comprised up to 45% and 54% of pink and chum salmon diets, respectively, which were greater proportions than in non-oiled habitats. Laboratory studies have shown that juvenile salmon will consume oiled food at equal or greater rates than non-oiled food at contamination doses that significantly reduce growth (Schwartz 1985; Vignier et al. 1992; Chapter 6C, this report).

An alternate explanation for the shifts in diet composition between 1989 and 1990 is that enhanced abundance of epibenthic prey influenced diet composition in 1990. In that year, both species ate proportionately more epibenthos in the oiled area than in the non-oiled area. Densities of harpacticoid copepods were shown to be higher on heavily-oiled beaches in western Prince William Sound in 1990 (Chapter 5B, this report). Unfortunately, in 1990, epibenthic prey were not sampled at the habitats where salmon diet studies were conducted, and we have no direct comparison with 1989 estimates of epibenthic prey biomass.

The diets of juvenile pink and chum salmon were dominated by pelagic zooplankton, primarily calanoid copepods, in both oiled and non-oiled nearshore areas of western Prince William Sound in 1989 and 1990. While calanoids and other zooplankters are common in diets of juvenile salmon throughout their range (Heard 1991), epibenthic prey, especially harpacticoid copepods, have been reported as the primary initial prey source of salmon in many estuaries and nearshore marine habitats (Healey 1979; Godin 1981; Landingham 1982; Volk et al. 1984). In Alaskan waters, however, pelagic prey often dominates the early diet of both pink and chum salmon. Epibenthic prey were not common in fish stomachs throughout the period of outmigration in an inlet in southeast Alaska (Bailey et al. 1975). High

utilization of zooplankton was also reported from southwestern Prince William Sound, but salmon fed more on harpacticoids during their initial bay residency than in the corridor outside it (Cooney et al. 1981; Cooney 1990). In our study, zooplankton dominated the diet of salmon in bays as well as in corridors, although diet composition in corridors reflected greater mixing of neritic and oceanic prey resources.

Few studies have compared salmon diets between different nearshore habitats. Pelagic prey usually dominated the diets in low-, medium-, and steep-gradient habitats in Prince William Sound, but some habitat specificity existed. Higher proportions of epibenthos were consumed in low- and medium-gradient habitats than in steep-gradient habitat; diets in these habitats reflected both higher diversity of epibenthic taxa in general, and higher abundance of harpacticoids specifically, than existed at steep-gradient habitat (Chapter 5, this report). Information on the relative availability of zooplankton in different habitats was not available. However, zooplankton always dominated pink salmon diets in steep-gradient habitat, where fish were most abundant (Chapter 2, this report). Conversely, chum salmon used epibenthic prey more extensively than pink salmon; fish were more abundant in lower-gradient habitats (Chapter 2, this report) and did not forage in steep-gradient habitats.

In many studies that reported initial dependence on harpacticoids in the Pacific Northwest, fish were sampled at beaches with substantial epibenthic production, habitats similar to our medium-gradient habitat (e.g., Kaczynski et al. 1973; Godin 1981). In several Alaskan studies, however, different prey were consumed in different habitats. Zooplankton (primarily calanoid copepods) dominated the diets of pink salmon at steep-gradient beaches, while epibenthos (primarily harpacticoid copepods) dominated the diets at low- and medium-gradient beaches in Tutka Bay, Alaska (Kron and Yuen 1978). Similar diet-habitat relationships have been found in Auke Bay, Alaska (M. Sturdevant and J. Landingham, unpub. data). Low-gradient habitats were not available to salmon foraging in Traitor's Cove, Alaska, and diets predominately consisted of zooplankton (Bailey et al. 1975). Similarly, in Prince William Sound, low- and medium-gradient habitats were less common than steep-gradient habitat in most areas, but zooplankton were usually the principal prey in all of these habitats.

Because of their distribution in lower-gradient habitats and their propensity to forage more heavily on the abundant epibenthos in these habitats, chum salmon were more susceptible to hydrocarbon exposure in the oiled area than

pinks. Oil deposits from the *Exxon Valdez* remained longer and natural cleaning processes occurred more slowly in protected low-energy beaches (Owens 1991), typically the lower-gradient habitats. Chum salmon in oiled habitats generally had higher levels of mixed-function oxidase induction than pink salmon (Chapter 3A, this report), which also supports the idea that they were more susceptible to hydrocarbon exposure. However, because oil was mixed in stomachs with prey from all three production systems, we could not determine which system fish were feeding on when the oil was ingested.

We conclude that oil did not interfere with feeding of pink and chum salmon in Prince William Sound, although some fish ingested oil either directly or via contaminated prey. Changes in diet composition between oiled and non-oiled areas in 1989 and 1990 may have been caused by the oil spill; oil could have caused avoidance of contaminated epibenthic prey 1989, or could have enhanced epibenthic prey populations in 1990. We could not, however, definitively attribute the changes in diet to oil.

Table 4.1. Feeding effectiveness median, mean, and mean standard error (SE) for juvenile pink salmon in oiled and non-oiled areas of Prince William Sound in 1989 (201 and 196 fish) and 1990 (299 and 296 fish). %Empty = percent of stomachs without food; WW = mg wet weight of stomach contents; WW%BW = stomach contents as percent of fish wet body weight; DW%BW = total prey dry weight as percent of fish dry body weight; Fullness = stomach fullness index; DW = mg dry weight of prey.

Feeding Effectiveness	Oiled			Non-oiled		
	Median	Mean	(SE)	Median	Mean	(SE)
			1989			
%Empty	0.0	16.3	(6.1)	10.0	16.2	(5.7)
WW	6.0	8.7	(0.7)	4.0	6.4	(0.5)
WW%BW	1.9	2.1	(0.1)	1.2	1.5	(0.1)
DW%BW	3.4	4.6	(0.6)	1.7	2.6	(0.2)
Fullness	3.0	2.6	(0.1)	2.0	2.4	(0.1)
Number	50.0	122.6	(15.4)	46.0	96.5	(10.7)
DW	2.2	3.2	(0.3)	1.1	2.2	(0.3)
			1990			
%Empty	0.0	9.0	(2.6)	0.0	10.4	(3.4)
WW	3.0	5.8	(0.6)	2.0	6.0	(0.5)
WW%BW	0.9	1.4	(0.1)	1.1	1.5	(0.1)
DW%BW	1.3	3.1	(0.3)	2.2	3.4	(0.2)
Fullness	3.0	2.9	(0.1)	3.0	2.9	(0.1)
Number	41.0	75.4	(6.3)	30.5	146.6	(17.5)
DW	1.0	2.1	(0.2)	1.2	2.6	(0.2)

Table 4.2. Feeding effectiveness median, mean, and mean standard error (SE) for juvenile chum salmon in oiled and non-oiled areas of Prince William Sound in 1989 (54 and 58 fish) and 1990 (66 and 70 fish). %Empty = percent of stomachs without food; WW = mg wet weight of stomach contents; WW%BW = stomach contents as percent of fish wet body weight; DW%BW = total prey dry weight as percent of fish dry body weight; Fullness = stomach fullness index; DW = mg dry weight of prey.

Feeding Effectiveness	Oiled			Non-oiled		
	Median	Mean	(SE)	Median	Mean	(SE)
			1989			
%Empty	0.0	3.3	(2.1)	0.0	8.3	(8.3)
WW	13.5	16.6	(1.8)	6.0	8.8	(1.4)
WW%BW	1.5	2.0	(0.2)	1.6	1.7	(0.2)
DW%BW	2.3	2.7	(0.3)	1.9	3.2	(0.6)
Full	3.3	2.8	(0.2)	2.5	2.7	(0.2)
Number	22.0	24.6	(2.6)	20.0	68.0	(18.1)
DW	2.8	4.8	(0.7)	1.3	2.9	(0.5)
			1990			
%Empty	0.0	1.4	(1.4)	0.0	1.4	(1.4)
WW	18.0	21.6	(2.0)	9.0	13.3	(1.8)
WW%BW	2.0	2.1	(0.2)	1.8	2.3	(0.2)
DW%BW	1.2	1.5	(0.2)	2.6	6.3	(2.3)
Full	5.0	4.0	(0.2)	4.0	3.6	(0.2)
Number	59.5	85.0	(11.9)	17.5	45.9	(9.7)
DW	2.1	2.7	(0.3)	2.7	5.9	(2.0)

Table 4.3. Percent frequency of occurrence (%FO), percent number (%No.), and percent dry weight (%DW) of 68 prey taxa in 169 pink salmon from oiled (OIL) and 169 pink salmon from non-oiled (N-O) nearshore habitats, in Prince William Sound, 1989. Fish with empty stomachs are not included. Subtotals do not sum to 100.0% due to rounding of data.

Prey Taxa	%FO		%No.		%DW	
	OIL	N-O	OIL	N-O	OIL	N-O
PELAGIC ZOOPLANKTON, CALANOIDS						
Arthropoda						
Crustacea						
Copepoda, Calanoida						
Large	47.3	36.7	2.0	1.3	36.4	26.9
Small	74.0	58.0	24.5	18.6	25.6	22.2
Nauplius	17.2	19.5	1.2	6.2	0.0	0.0
Subtotals			27.7	26.1	62.0	49.1
OTHER PELAGIC ZOOPLANKTON						
Annelida						
Polychaeta						
Larvae	1.2	5.3	0.0	0.1	0.0	0.1
Adult	30.2	24.3	1.9	1.0	2.4	1.4
Arthropoda						
Crustacea						
Cladocera						
Evadne	4.7	3.0	0.1	0.0	0.1	0.0
Podon	4.1	1.8	0.1	0.1	0.1	0.1
Copepoda, Cyclopodia	0.0	0.6	0.0	0.0	0.0	0.0
Copepoda, Monstrilloida	0.6	4.1	0.0	0.0	0.0	0.0
Cirripedia						
Barnacle nauplius	3.6	18.9	0.1	0.5	0.0	0.2
Barnacle molt	0.0	3.6	0.0	0.0	0.0	0.3
Malacostraca						
Amphipoda						
Caprellidea	0.0	2.4	0.0	0.0	0.0	0.7
Hyperiidea	21.3	15.4	1.1	1.0	0.8	0.9
Decapoda						
Zoea (nauplius)	1.8	0.6	0.0	0.0	0.0	0.0
Euphausiacea						
Euphausiid, general						
Adult	0.6	0.0	0.0	0.0	0.0	0.0
Calyptopis	10.7	10.7	0.2	0.6	0.0	0.0
Furcilia	22.5	10.1	0.3	0.3	0.1	0.1
Nauplius	4.1	5.9	0.2	1.4	0.0	0.0
Pycnogonida						
Sea Spider	0.0	0.6	0.0	0.0	0.0	0.1
Bryzoa						
Cyphonautes larvae	8.9	25.4	0.1	1.2	0.0	0.0
Chordata						
Urochordata						
Larvacea						
Oikopleura	13.6	11.8	1.7	0.5	0.5	0.2
Vertebrata						
Fish, general						
Egg	0.6	1.2	0.0	0.0	0.0	0.0
Larvae	8.9	4.7	0.1	0.1	1.0	0.6

Table 4.3. Continued.

Prey Taxa	%FO		%No.		%DW	
	OIL	N-O	OIL	N-O	OIL	N-O
Mollusca						
Gastropoda						
Gastropod, general						
Egg case	2.4	5.3	0.0	0.1	0.0	0.0
Veliger	25.4	26.0	21.9	4.8	5.3	1.3
Pteropoda						
Limacina	2.4	3.0	0.1	0.1	0.1	0.1
Miscellaneous						
Invertebrate eggs	4.1	7.7	0.0	0.1	0.0	0.0
Subtotals			28.0	11.9	10.4	6.0
EPIBENTHOS, HARPACTICOIDS						
Arthropoda						
Crustacea						
Copepoda, Harpacticoida						
Dactylopodia						
Adult	21.3	27.2	0.6	0.7	0.3	0.4
Gravid female	3.0	7.1	0.0	0.2	0.0	0.2
Ectinosomatidae						
Adult	17.8	25.4	0.7	0.9	0.3	0.5
Gravid female	4.1	1.2	0.1	0.0	0.0	0.0
Harpacticoid, general						
Egg packet	37.3	50.3	3.9	3.5	1.4	1.3
Nauplius	2.4	4.7	0.0	0.2	0.0	0.0
Copepodite	24.9	49.1	1.7	3.0	0.2	0.5
Adult	54.4	64.5	21.9	13.7	10.9	7.8
Gravid female	12.4	10.1	0.3	0.3	0.2	0.2
Harpacticus						
Copepodite	0.6	1.8	0.0	0.0	0.0	0.0
Adult	19.5	14.8	1.8	1.4	0.7	0.6
Adult male	28.4	36.7	1.3	2.9	0.1	0.4
Adult female	22.5	39.1	1.1	4.0	0.7	2.8
Clasping pair	0.0	4.1	0.0	0.1	0.0	0.1
Gravid female	7.1	10.7	0.1	0.1	0.1	0.1
Laophontidae						
Adult	16.6	19.5	0.7	1.3	0.4	0.7
Gravid female	3.0	7.7	0.1	0.2	0.0	0.1
Tisbe						
Copepodite	1.2	3.6	0.0	0.1	0.0	0.0
Adult	29.0	47.9	3.8	13.0	1.8	7.4
Gravid female	10.7	13.6	0.4	0.8	0.3	0.7
Zaus						
Adult	7.7	14.8	0.1	0.2	0.1	0.1
Gravid female	0.6	0.0	0.0	0.0	0.0	0.0
Subtotals			38.4	46.6	17.5	24.0

Table 4.3. Continued.

Prey Taxa	%FO		%No.		%DW	
	OIL	N-O	OIL	N-O	OIL	N-O
OTHER EPIBENTHOS						
Arthropoda						
Chelicerata						
Arachnida						
Halacaridae						
Mite	1.2	3.6	0.0	0.0	0.0	0.1
Crustacea						
Cirripedia						
Barnacle cyprid	9.5	11.2	0.2	0.2	0.0	0.1
Malacostraca						
Amphipoda						
Gammaridea						
< 2mm	1.8	8.3	0.1	0.1	0.2	0.4
2.0 - 6.9 mm	5.3	1.2	0.1	0.0	0.6	0.1
Cumacea	8.9	18.3	1.1	1.0	1.7	1.8
Ostracoda	0.6	3.0	0.0	0.0	0.0	0.0
Uniramia						
Chilopoda						
Centipede	0.0	0.6	0.0	0.0	0.0	0.3
Insecta						
Collembola	1.8	7.7	0.0	0.1	0.0	0.1
Diptera						
Chironomidea						
Larvae	0.0	7.1	0.0	0.2	0.0	0.3
Pupa	1.2	4.7	0.0	0.1	0.0	0.2
Adult	2.4	1.2	0.1	0.0	0.1	0.0
Mollusca						
Bivalvia						
Bivalve, juvenile	8.9	32.5	0.2	5.8	0.1	2.0
Gastropoda						
Gastropod, juvenile	17.2	20.7	3.1	7.0	4.6	12.1
Nematoda	0.0	1.8	0.0	0.0	0.0	0.0
Platyhelminthes						
Turbellaria	0.0	1.2	0.0	0.0	0.0	0.0
Subtotals			4.8	14.5	7.4	17.6
DRIFT INSECTS						
Arthropoda						
Uniramia						
Insecta						
General, 2.6 - 3.8 mm	8.9	16.0	0.1	0.2	1.0	2.2
Diptera, 2.6 - 3.8 mm	1.8	0.6	0.0	0.0	0.2	0.3
Diptera, 3.9 - 10.5 mm	4.1	1.8	0.0	0.0	1.5	0.7
Subtotals			0.2	0.2	2.7	3.2
UNIDENTIFIED						
Unidentified			1.0	1.0	0.0	0.0
TOTALS			100.1	100.3	100.0	99.9

Table 4.4. Percent frequency of occurrence (%FO), percent number (%No.), and percent dry weight (%DW) of 65 prey taxa in 276 and 266 pink salmon from oiled (OIL) and non-oiled (N-O) nearshore habitats in Prince William Sound, 1990. Fish with empty stomachs are not included.

Prey Taxa	%FO		%No.		%DW	
	OIL	N-O	OIL	N-O	OIL	N-O
PELAGIC ZOOPLANKTON, CALANOIDS						
Arthropoda						
Crustacea						
Copepoda, Calanoida						
Large	30.8	36.5	2.4	0.9	7.0	5.6
Small	58.3	90.2	11.7	16.5	9.0	11.2
Nauplius	37.3	34.6	12.2	5.1	4.6	4.6
Subtotals			26.3	22.5	20.6	21.4
OTHER PELAGIC ZOOPLANKTON						
Annelida						
Polychaeta						
Larvae	4.7	6.0	0.3	0.4	0.2	2.1
Adult	40.9	33.8	3.7	0.6	6.8	4.3
Arthropoda						
Crustacea						
Cladocera						
Evadne	17.0	26.7	4.3	16.0	1.8	5.7
Podon	13.0	25.9	1.0	16.7	1.6	5.0
Copepoda, Cyclopodia	1.1	0.8	0.0	0.1	0.3	0.2
Copepoda, Monstrilloida	0.7	0.8	0.0	0.0	0.1	0.1
Cirripedia						
Barnacle nauplius	7.6	13.9	0.2	0.9	1.0	3.2
Barnacle, molt	0.7	1.1	0.0	0.1	0.2	0.1
Malacostraca						
Amphipoda						
Hyperiidea	3.6	0.4	0.1	0.0	1.8	0.1
Euphausiacea						
Euphausiid, general						
Adult	0.4	0.0	0.0	0.0	0.1	0.0
Calyptopis	12.0	5.6	0.8	0.0	2.2	0.7
Furcilia	3.6	4.5	0.1	0.0	0.9	0.6
Nauplius	14.5	4.9	1.8	0.2	2.4	0.3
Bryzoa						
Cyphonautes larvae	5.8	10.9	1.0	0.9	0.8	3.3
Chaetognatha	0.4	0.4	0.0	0.0	0.0	0.1
Chordata						
Urochordata						
Larvacea						
Oikopleura	2.9	1.5	0.5	0.0	0.4	0.2
Thaliacea						
Salps	0.0	1.5	0.0	0.1	0.0	0.3
Vertebrata						
Fish, general						
Egg	1.5	3.0	0.2	0.0	0.3	0.9
Larvae	8.0	4.9	0.2	0.1	0.4	0.8

Table 4.4. Continued.

Prey Taxa	%FO		%No.		%DW	
	OIL	N-O	OIL	N-O	OIL	N-O
Mollusca						
Cephalopoda						
Teuthoidea						
Squid, juvenile	0.4	0.0	0.0	0.0	0.1	0.0
Gastropoda						
Gastropod, general						
Egg case	0.4	5.6	0.0	0.2	0.2	2.3
Veliger	0.4	1.9	0.0	0.0	0.0	0.4
Miscellaneous						
Invertebrate eggs	15.2	25.9	0.4	0.3	2.5	3.9
Subtotals			14.6	36.7	24.1	34.4
EPIBENTHOS, HARPACTICOIDS						
Arthropoda						
Crustacea						
Copepoda, Harpacticoida						
Dactylopodia						
Gravid female	2.5	0.0	0.0	0.0	0.3	0.0
Adult	4.7	2.3	0.1	0.0	0.4	0.1
Ectinosomatidae						
Adult	1.1	0.4	0.0	0.0	0.1	0.0
Harpacticoid, general						
Egg packet	40.9	18.4	3.5	2.3	6.4	2.3
Nauplius	4.4	2.6	1.0	0.1	0.7	0.1
Copepodite	44.6	25.9	10.2	8.8	6.9	3.6
Adult	63.0	47.4	20.2	7.6	9.0	6.4
Gravid female	23.9	11.7	1.2	0.4	3.6	2.0
Harpacticus						
Copepodite	0.4	0.0	0.0	0.0	0.0	0.0
Adult male	7.6	1.1	0.3	0.0	1.2	0.1
Adult female	11.2	2.6	0.5	0.0	1.6	0.1
Clasping pair	0.0	0.4	0.0	0.0	0.0	0.0
Gravid female	1.5	0.4	0.0	0.0	0.2	0.0
Tisbe						
Copepodite	1.1	0.0	0.0	0.0	0.1	0.0
Gravid female	1.0	1.9	0.0	0.0	0.1	0.1
Adult	10.1	3.0	0.5	0.0	1.1	0.2
Zaus						
Adult	2.9	0.0	0.1	0.0	0.2	0.0
Subtotals			37.6	19.2	31.8	15.0
OTHER EPIBENTHOS						
Arthropoda						
Chelicerata						
Arachnida						
Halacaridae						
Mite	0.0	0.8	0.0	0.0	0.0	0.3
Crustacea						
Cirripedia						
Barnacle cyprid	13.4	15.4	0.6	0.3	2.4	4.4

Table 4.4. Continued.

Prey Taxa	%FO		%No.		%DW	
	OIL	N-O	OIL	N-O	OIL	N-O
Malacostraca						
Amphipoda						
Gammaridea						
< 2 mm	15.6	11.3	0.4	0.3	2.8	1.8
2.0 - 6.9 mm	3.6	5.6	0.0	0.2	1.2	1.2
> 6.9 mm	0.0	0.9	0.0	0.0	0.0	0.5
Cumacean	6.2	3.4	3.4	0.0	2.1	0.5
Isopoda	1.5	1.1	0.0	0.0	0.1	0.2
Ostracoda	0.7	0.0	0.0	0.0	0.0	0.0
Uniramia						
Insecta						
Diptera						
Chironomidae						
Larvae	2.5	1.5	0.1	0.0	0.3	0.2
Pupa	5.8	2.3	0.3	0.0	1.0	0.5
Adult	5.4	0.4	0.1	0.0	0.7	0.2
General insect						
Ceratopogonidae	0.4	0.4	0.0	0.0	0.0	0.0
Mollusca						
Bivalvia						
Bivalve, juvenile	15.2	30.1	8.7	17.5	1.6	6.5
Gastropoda						
Gastropod juvenile	11.2	18.1	0.5	0.4	1.1	3.7
Nematoda	0.4	0.4	0.0	0.0	0.0	0.0
Platyhelminthes						
Turbellaria	0.7	0.0	0.0	0.0	0.0	0.0
Subtotals			14.1	18.7	13.4	20.0
DRIFT INSECTS						
Arthropoda						
Uniramia						
Insecta						
General, 2.6 - 3.8 mm	10.1	6.8	0.2	0.0	1.1	1.2
Diptera, 2.6 - 3.8 mm	0.0	0.4	0.0	0.0	0.0	0.1
Diptera, 3.9 - 10.5 mm	4.0	1.1	0.2	0.0	1.1	0.1
Stonefly	1.1	0.0	0.0	0.0	0.2	0.0
Subtotals			0.4	0.1	2.4	1.4
UNIDENTIFIED						
Unidentified	51.8	53.4	7.0	2.8	7.7	7.8
TOTALS			100.0	100.0	100.0	100.0

Table 4.5. Percent frequency of occurrence (%FO), percent number (%No.), and percent dry weight (%DW) of 54 prey taxa in 52 and 53 chum salmon from oiled (OIL) and non-oiled (N-O) nearshore habitats, respectively, in Prince William Sound, 1989. Fish with empty stomachs are not included. Subtotals do not sum to 100.0% due to rounding of data.

Prey Taxa	%FO		%No.		%DW	
	OIL	N-O	OIL	N-O	OIL	N-O
PELAGIC ZOOPLANKTON, CALANOIDS						
Arthropoda						
Crustacea						
Copepoda, Calanoida						
Large	59.6	50.9	25.1	3.9	63.4	45.1
Small	9.6	54.7	1.9	1.3	0.3	26.7
Nauplius	1.9	7.6	0.5	0.6	0.0	0.0
Subtotals			27.5	5.8	63.7	71.8
OTHER PELAGIC ZOOPLANKTON						
Annelida						
Polychaeta						
Adult	11.5	17.0	2.6	0.7	0.4	0.6
Arthropoda						
Crustacea						
Cirripedia						
Barnacle nauplius	0.0	3.8	0.0	0.1	0.0	0.0
Barnacle, molt	9.6	0.0	0.6	0.0	0.5	0.0
Malacostraca						
Amphipoda						
Hyperiidea	7.7	3.8	0.5	0.1	0.1	0.0
Euphausiacea						
Euphausiid, general						
Calyptopis	3.9	13.2	0.2	0.7	0.0	0.2
Furcilia	13.5	11.3	1.1	0.2	0.0	0.0
Nauplius	0.0	1.9	0.0	0.1	0.0	0.0
Bryzoa						
cyphonautes larvae	0.0	5.7	0.0	0.1	0.0	0.0
Chordata						
Vertebrata						
Fish, general						
Egg	5.8	0.0	0.7	0.0	0.1	0.0
Larvae	9.6	3.8	0.4	0.1	0.4	0.2
Urochordata						
Larvacea						
Oikopleura	40.2	18.9	15.5	1.8	0.6	0.3
Mollusca						
Gastropoda						
Egg case	1.9	5.7	0.1	2.7	0.0	0.0
Veliger	5.8	3.8	0.5	0.1	0.0	0.0
Miscellaneous						
Invertebrate eggs	1.9	13.2	0.1	0.2	0.0	0.0
Subtotals			22.1	6.8	2.2	1.5

Table 4.5. Continued.

Prey Taxa	%FO		%No.		%DW	
	OIL	N-O	OIL	N-O	OIL	N-O
EPIBENTHOS, HARPACTICOIDS						
Arthropoda						
Crustacea						
Copepoda, Harpacticoida						
Dactylopodia						
Adult	5.8	5.7	0.4	0.1	0.0	0.0
Gravid female	0.0	3.8	0.0	0.1	0.0	0.0
Ectinosomatidae						
Adult	5.8	11.3	0.2	0.8	0.0	0.3
Gravid female	0.0	1.9	0.0	0.0	0.0	0.0
Harpacticoid, general						
Egg packet	23.1	32.1	1.8	2.1	0.1	0.4
Copepodite	17.3	17.0	2.1	3.8	0.0	0.3
Adult	30.8	56.6	6.9	8.9	0.5	2.7
Gravid female	11.5	0.0	0.6	0.0	0.1	0.0
Harpacticus						
Copepodite	3.9	0.0	0.2	0.0	0.0	0.0
Adult	9.6	9.4	1.5	0.5	0.1	0.1
Adult male	15.4	26.4	2.6	10.4	0.0	0.7
Adult female	30.8	24.5	5.8	9.2	0.5	3.5
Clasping pair	0.0	7.6	0.0	0.6	0.0	0.3
Gravid female	1.9	9.4	0.1	0.5	0.0	0.3
Laophontidae						
Adult	3.9	9.4	0.2	1.7	0.0	0.5
Gravid female	0.0	3.8	0.0	0.1	0.0	0.0
Tisbe						
Copepodite	1.9	0.0	0.2	0.0	0.0	0.0
Adult	9.6	20.8	0.8	1.4	0.1	0.4
Gravid female	3.9	13.2	0.2	0.4	0.0	0.2
Zaus						
Adult	0.0	5.7	0.0	0.1	0.0	0.0
Gravid female	0.0	1.9	0.0	0.0	0.0	0.0
Subtotals			23.5	40.5	1.4	9.8
OTHER EPIBENTHOS						
Arthropoda						
Chelicerata						
Arachnida						
Halacaridae						
Mite	0.0	1.9	0.0	0.0	0.0	0.0
Crustacea						
Cirripedia						
Barnacle cyprid	7.7	11.3	0.3	0.7	0.0	0.2
Malacostraca						
Amphipoda						
Gammaridea						
< 2 mm	3.9	15.1	0.6	0.4	0.3	0.8
2.0 - 6.9 mm	9.6	1.9	1.0	0.1	0.9	0.4
> 6.9 mm	5.8	3.8	0.4	0.1	3.4	5.2
Cumacea	11.5	9.4	4.6	0.2	1.0	0.2

Table 4.5. Continued.

Prey Taxa	%FO		%No.		%DW	
	OIL	N-O	OIL	N-O	OIL	N-O
Uniramia						
Insecta						
Collembola	0.0	7.6	0.0	0.1	0.0	0.1
Diptera						
Chironomidae						
Larvae	1.9	20.8	0.1	2.9	0.0	3.3
Pupa	13.5	26.4	11.5	0.7	3.3	0.9
Adult	5.8	15.1	0.4	0.4	0.1	0.3
Mollusca						
Bivalvia						
Bivalve, juvenile	0.0	3.8	0.0	0.1	0.0	0.0
Gastropoda						
Gastropod juvenile	1.9	3.8	0.1	0.1	0.0	0.1
Subtotals			18.9	5.7	9.0	11.5
DRIFT INSECTS						
Arthropoda						
Uniramia						
Insecta						
General, 2.6 - 3.8 mm	9.6	22.6	1.1	0.5	1.6	3.1
Diptera, 3.9 - 10.5 mm	25.0	7.6	3.8	0.1	19.2	2.4
Stonefly	1.9	0.0	0.6	0.0	3.1	0.0
Subtotals			5.4	0.6	23.8	5.4
UNIDENTIFIED						
Unidentified	34.6	20.8	2.6	0.7	0.0	0.0
TOTALS			100.0	100.0	100.0	100.0

Table 4.6. Percent frequency of occurrence (%FO), percent number (%No.), and percent dry weight of 45 prey taxa in 65 and 69 chum salmon from oiled (OIL) and non-oiled (N-O) nearshore habitats, respectively, in Prince William Sound, 1990. Fish with empty stomachs are not included. Subtotals do not sum to 100.0% due to rounding.

Prey Taxa	%FO		%No.		%DW	
	OIL	N-O	OIL	N-O	OIL	N-O
PELAGIC ZOOPLANKTON, CALANOIDS						
Arthropoda						
Crustacea						
Copepoda, Calanoida						
Large	21.5	65.2	1.1	8.8	16.4	33.5
Small	27.7	39.1	2.8	7.1	2.5	1.5
Nauplius	1.5	0.0	0.0	0.0	0.0	0.0
Subtotals			3.9	15.9	18.9	35.0
OTHER PELAGIC ZOOPLANKTON						
Annelida						
Polychaeta						
Larvae	1.5	0.0	0.2	0.0	0.2	0.0
Adult	49.2	31.9	28.8	7.1	30.2	1.8
Arthropoda						
Crustacea						
Cladocera						
Evadne	1.5	4.3	0.0	2.0	0.0	0.2
Copepoda, Cyclopoida	1.5	5.8	0.1	0.5	0.0	0.1
Copepoda, Monstrilloida	1.5	0.0	0.0	0.0	0.0	0.0
Cirripedia						
Barnacle nauplius	1.5	2.9	0.0	0.5	0.0	0.0
Barnacle molt	1.5	7.2	0.0	1.4	0.1	1.7
Malacostraca						
Amphipoda						
Hyperiidea	13.8	0.0	2.4	0.0	1.6	0.0
Decapoda						
Zoea (nauplius)	3.1	0.0	0.5	0.0	0.2	0.0
Euphausiacea						
Euphausiid, general						
Egg	1.5	0.0	0.0	0.0	0.0	0.0
Nauplius	1.5	1.4	0.0	0.1	0.0	0.0
Furcilia	4.6	1.4	0.1	0.1	0.0	0.0
Bryozoa						
Cyphonautes larvae	4.6	4.3	0.1	2.8	0.0	0.0
Chordata						
Urochordata						
Larvacea						
Oikopleura	1.5	8.7	0.0	1.8	0.0	0.1
Thaliacea						
Salps	4.6	8.7	0.1	2.8	0.0	0.2
Mollusca						
Gastropoda						
Gastropod, general						
Egg case	1.5	4.3	0.0	0.1	0.0	0.0
Veliger	1.5	8.7	0.0	0.3	0.0	0.0

Table 4.6. Continued.

Prey Taxa	%FO		%No.		%DW	
	OIL	N-O	OIL	N-O	OIL	N-O
Miscellaneous						
Invertebrate eggs	24.6	24.6	0.9	2.1	0.0	0.0
Subtotals			33.2	21.6	32.3	4.1
EPIBENTHOS, HARPACTICOIDS						
Arthropoda						
Crustacea						
Copepoda, Harpacticoida						
Ectinosomatidae						
Adult	1.5	0.0	0.0	0.0	0.0	0.0
Harpacticoid, general						
Egg packet	10.8	8.7	1.4	1.4	0.0	0.1
Copepodite	12.3	8.7	3.9	8.0	0.4	0.2
Adult	63.1	39.1	16.9	11.9	7.1	1.2
Gravid female	9.2	15.9	0.9	1.3	0.6	0.2
Subtotals			23.1	22.6	8.1	1.7
OTHER EPIBENTHOS						
Arthropoda						
Crustacea						
Cirripedia						
Barnacle cyprid	10.8	4.3	0.1	0.4	0.1	0.0
Malacostraca						
Amphipoda						
Gammaridea						
< 2 mm	20.0	31.9	0.7	6.0	2.3	4.5
2.0 - 6.9 mm	7.7	18.8	0.2	15.1	1.1	19.8
> 6.9 mm	0.0	11.6	0.0	2.3	0.0	31.4
Cumacea	20.0	14.5	1.6	2.8	2.0	0.9
Decapoda						
Crab megalops	0.0	1.4	0.0	0.0	0.0	0.1
Ostracoda	1.5	0.0	0.2	0.0	0.0	0.0
Insecta						
Diptera						
Chironomidae						
Larvae	7.7	8.7	0.3	0.2	0.4	0.1
Pupa	33.8	14.5	9.2	1.0	16.4	0.4
Adult	52.3	29.0	15.0	2.3	15.8	0.6
Collembola						
sp. 1	1.5	5.8	0.0	0.2	0.0	0.0
sp. 2	0.0	1.4	0.0	0.1	0.0	0.0
Mollusca						
Bivalvia						
Bivalve, juvenile	4.6	5.8	0.1	1.1	0.0	0.1
Gastropoda						
Gastropod juvenile	4.6	0.0	0.2	0.0	0.3	0.0
Subtotals			27.6	31.5	38.4	57.9

Table 4.6. Continued.

Prey Taxa	%FO		%No.		%DW	
	OIL	N-O	OIL	N-O	OIL	N-O
DRIFT INSECTS						
Arthropoda						
Insecta						
General, 2.6 - 3.8 mm	9.2	17.4	0.1	0.4	1.0	1.0
Diptera, 2.6 - 3.8 mm	0.0	1.4	0.0	0.1	0.0	0.1
Diptera, 3.9 - 10.5 mm	1.5	0.0	0.0	0.0	0.6	0.0
Subtotals			0.1	0.5	1.6	1.1
UNIDENTIFIED						
Unidentified	80.0	46.4	11.5	7.8	0.4	0.1
TOTALS			99.4	99.9	99.7	99.9

Table 4.7. Percent dry weight of prey in the diet of 397 pink salmon fry collected in oiled and non-oiled areas of Prince William Sound, Alaska, April to June 1989, by low-, medium-, and steep-gradient habitats in bays and corridors.

Prey Category	Oiled			Non-oiled		
	Low	Medium	Steep	Low	Medium	Steep
			Bays			
Large Calanoids	91.6	26.6	51.4	41.0	8.7	69.3
Small Calanoids	5.8	8.7	37.0	46.5	62.1	10.5
Other Zooplankton	1.6	28.8	0.0	0.2	7.2	4.5
Total Zooplankton	99.0	64.2	88.3	87.8	78.0	84.3
Harpacticoids	0.9	1.8	0.8	4.7	17.4	0.2
Other Epibenthos	0.0	19.6	1.0	1.4	2.2	1.5
Total Epibenthos	0.9	21.5	1.8	6.2	19.6	1.7
Drift Insects	0.0	14.3	9.9	6.0	2.3	14.0
			Corridors			
Large Calanoids	31.6	24.5	50.9	22.4	28.0	41.7
Small Calanoids	13.8	43.8	23.3	13.5	3.5	30.2
Other Zooplankton	7.9	12.1	10.5	4.1	8.2	6.7
Total Zooplankton	53.4	80.4	84.7	40.1	39.8	78.6
Harpacticoids	35.4	14.3	6.6	26.5	44.2	6.1
Other Epibenthos	10.0	3.9	7.1	31.7	11.8	10.6
Total Epibenthos	45.4	18.2	13.8	58.2	56.0	16.7
Drift Insects	1.1	1.5	1.5	1.6	4.1	4.6

Table 4.8. Percent dry weight of prey categories in the diet of 595 pink salmon fry collected in oiled and non-oiled areas of Prince William Sound, Alaska, April to June 1990, by low-, medium-, and steep-gradient habitats in bays and corridors.

Prey Category	Oiled			Non-oiled		
	Low	Medium	Steep	Low	Medium	Steep
Bays						
Large Calanoids	0.0	18.1	17.8	57.6	70.2	21.5
Small Calanoids	18.0	11.4	65.1	30.2	20.9	39.5
Other Zooplankton	44.6	13.5	9.1	6.5	21.8	21.8
Total Zooplankton	62.6	42.9	92.0	94.3	82.8	82.8
Harpacticoids	14.0	10.5	5.5	4.5	0.9	1.5
Other Epibenthos	4.4	5.0	1.6	1.0	0.9	5.9
Total Epibenthos	18.4	15.5	7.1	5.5	1.8	7.5
Drift Insects	18.5	41.5	0.8	0.0	3.3	9.3
Corridors						
Large Calanoids	49.8	57.8	45.8	7.8	27.9	12.6
Small Calanoids	4.4	11.6	7.5	24.8	37.5	11.9
Other Zooplankton	5.6	9.2	12.7	17.1	17.2	51.9
Total Zooplankton	59.7	78.5	66.0	49.7	82.5	76.2
Harpacticoids	19.2	13.9	5.3	27.3	6.6	1.0
Other Epibenthos	18.5	2.3	23.6	21.5	10.1	21.8
Total Epibenthos	37.7	16.2	28.9	48.8	16.7	22.8
Drift Insects	2.3	5.1	4.5	1.3	0.7	0.6

Table 4.9. Percent dry weight of prey in the diets of 112 and 136 juvenile chum salmon in oiled and non-oiled areas of Prince William Sound, Alaska, April to June 1989 and 1990, by low- and medium-gradient habitats in bays and corridors (Corr.).

	Oiled			Non-oiled		
	Bays		Corr.	Bays		Corr.
Prey Category	Low	Medium	Low	Low	Medium	Low
			1989			
Large Calanoids	74.3	36.4	63.6	0.0	64.7	65.0
Small Calanoids	0.0	0.8	0.3	78.1	13.4	0.5
Other Zooplankton	1.6	6.2	1.4	0.0	2.8	1.9
Total Zooplankton	75.9	43.4	65.3	78.1	80.9	67.4
Harpacticoids	0.7	6.1	0.6	3.3	1.7	16.9
Other Epibenthos	6.5	47.8	0.1	13.6	1.4	14.1
Total Epibenthos	7.2	53.9	0.6	16.9	3.1	30.9
Drift Insects	16.9	2.7	34.1	5.0	16.0	1.7
			1990			
Large Calanoids	3.0	0.0	36.9	69.4	92.8	19.3
Small Calanoids	0.6	0.0	5.4	4.9	4.5	0.5
Other Zooplankton	48.4	0.0	24.8	12.3	0.0	3.1
Total Zooplankton	52.0	0.0	67.1	86.6	97.4	22.9
Harpacticoids	1.1	1.1	19.1	1.6	0.2	2.0
Other Epibenthos	46.0	98.8	10.2	9.8	0.3	74.2
Total Epibenthos	47.2	99.9	29.3	11.3	0.5	76.2
Drift Insects	0.4	0.0	3.4	1.9	2.2	0.9

Table 4.10. Diet composition by prey production system for juvenile pink salmon from oiled (201) and non-oiled (196) areas in 1989. FO = frequency of occurrence; DW = mg dry weight.

Diet Composition	Oiled			Non-oiled		
	Median	Mean	(SE)	Median	Mean	(SE)
			Pelagic Zooplankton			
%FO	100.0	78.9	(7.6)	90.0	75.8	(6.5)
Number	23.0	81.2	(12.7)	17.0	42.4	(4.6)
%Number	77.8	64.9	(2.8)	42.8	44.9	(2.7)
DW	1.8	2.8	(0.3)	0.7	1.4	(0.1)
%DW	91.7	71.0	(2.8)	58.4	53.0	(2.9)
			Epibenthos			
%FO	60.0	63.1	(7.1)	88.8	71.6	(6.5)
Number	5.0	63.0	(10.7)	21.0	68.2	(11.2)
%Number	15.0	31.3	(2.7)	44.4	48.1	(2.7)
DW	0.1	1.0	(0.2)	0.3	1.1	(0.3)
%DW	3.4	24.5	(2.7)	26.4	37.5	(2.8)
			Drift Insects			
%FO	10.0	12.6	(3.0)	20.0	24.0	(6.1)
Number	0.0	0.2	(0.1)	0.0	0.2	(0.0)
%Number	0.0	1.1	(0.6)	0.0	1.8	(0.5)
DW	0.0	0.1	(0.0)	0.0	0.1	(0.0)
%DW	0.0	3.8	(1.1)	0.0	7.0	(1.5)

Table 4.11. Diet composition by prey production system for juvenile pink salmon from oiled (299) and non-oiled (296) areas in 1990. FO = frequency of occurrence; DW = mg dry weight.

Diet Composition	Oiled			Non-oiled		
	Median	Mean	(SE)	Median	Mean	(SE)
			Pelagic Zooplankton			
%FO	100.0	82.3	(3.4)	100.0	82.2	(4.3)
Number	14.0	33.3	(3.3)	20.0	96.5	(14.4)
%Number	46.6	48.7	(2.2)	81.8	67.9	(2.1)
DW	0.4	1.5	(0.2)	0.8	2.2	(0.2)
%DW	71.8	57.7	(2.4)	95.6	76.9	(2.0)
			Epibenthos			
%FO	90.0	75.9	(4.1)	77.5	61.1	(5.2)
Number	9.5	42.3	(4.9)	2.0	61.9	(9.4)
%Number	41.0	43.3	(2.2)	8.3	25.1	(2.0)
DW	0.1	0.6	(0.1)	0.0	0.7	(0.1)
%DW	17.6	34.5	(2.2)	3.3	20.4	(1.9)
			Drift Insects			
%FO	0.0	14.6	(3.2)	0.0	10.0	(3.3)
Number	0.0	0.3	(0.1)	0.0	0.1	(0.0)
%Number	0.0	2.3	(0.7)	0.0	0.7	(0.4)
DW	0.0	0.2	(0.1)	0.0	0.0	(0.0)
%DW	0.0	7.1	(1.3)	0.0	2.4	(0.7)

Table 4.12. Diet composition by prey subcategory for juvenile pink salmon from oiled (201) and non-oiled (196) areas in 1989. FO = frequency of occurrence; DW = mg dry weight.

Diet Composition	Oiled			Non-oiled		
	Median	Mean	(SE)	Median	Mean	(SE)
			Total Calanoids			
%FO	70.0	64.9	(7.8)	70.0	59.0	(8.1)
Number	11.0	40.5	(4.7)	5.0	29.1	(3.9)
%Number	41.1	43.2	(3.0)	7.6	28.7	(2.6)
DW	0.7	2.4	(0.3)	0.4	1.3	(0.1)
%DW	66.3	53.0	(3.3)	30.5	40.8	(3.1)
			Small Calanoids			
%FO	70.0	59.7	(8.3)	40.0	52.5	(8.2)
Number	9.0	35.7	(4.4)	2.0	20.8	(9.0)
%Number	19.6	31.9	(2.6)	2.7	21.5	(2.3)
DW	0.2	1.0	(0.1)	0.1	0.6	(0.1)
%DW	8.0	25.3	(2.4)	4.1	21.4	(2.3)
			Large Calanoids			
%FO	30.0	39.3	(8.6)	11.3	26.5	(6.8)
Number	0.0	1.4	(0.2)	0.0	2.9	(0.6)
%Number	0.0	3.3	(0.7)	0.0	10.6	(1.8)
DW	0.0	0.7	(0.1)	0.0	1.4	(0.3)
%DW	0.0	19.3	(2.3)	0.0	28.0	(2.8)
			Harpacticoids			
%FO	60.0	58.9	(6.8)	70.0	63.3	(6.9)
Number	2.0	56.0	(10.3)	10.0	52.0	(8.7)
%Number	6.8	25.5	(2.6)	22.4	35.0	(2.7)
DW	0.0	0.7	(0.1)	0.1	0.6	(0.1)
%DW	1.0	1.8	(2.4)	7.3	23.3	(2.3)

Table 4.13. Diet composition by prey subcategory for juvenile pink salmon from oiled (299) and non-oiled (296) areas in 1990. FO = frequency of occurrence; DW = mg dry weight.

Diet Composition	Oiled			Non-oiled		
	Median	Mean	(SE)	Median	Mean	(SE)
			Total Calanoids			
%FO	66.7	57.3	(5.3)	100.0	76.6	(4.4)
Number	3.0	21.5	(2.9)	10.0	36.7	(4.4)
%Number	8.2	29.9	(2.2)	50.0	47.6	(2.3)
DW	0.1	1.2	(0.2)	0.5	1.5	(0.1)
%DW	15.0	37.7	(2.5)	71.5	58.5	(2.4)
			Small Calanoids			
%FO	50.0	53.4	(5.4)	100.0	75.8	(4.4)
Number	2.0	19.5	(2.9)	9.0	35.3	(4.3)
%Number	5.5	25.9	(2.0)	34.2	41.5	(2.2)
DW	0.0	0.3	(0.0)	0.2	0.7	(0.1)
%DW	2.0	17.8	(1.7)	18.4	35.9	(2.2)
			Large Calanoids			
%FO	0.0	29.2	(5.0)	0.0	30.8	(5.2)
Number	0.0	2.0	(0.3)	0.0	1.5	(0.2)
%Number	0.0	4.0	(0.7)	0.0	6.2	(0.9)
DW	0.0	1.0	(0.2)	0.0	0.7	(0.1)
%DW	0.0	19.9	(2.0)	0.0	22.6	(2.1)
			Harpacticoids			
%FO	77.8	68.1	(4.5)	52.8	51.4	(5.2)
Number	6.0	30.7	(3.7)	1.0	31.3	(7.2)
%Number	16.7	34.1	(2.2)	0.4	16.5	(1.8)
DW	0.1	0.3	(0.0)	0.0	0.3	(0.1)
%DW	6.2	22.4	(1.8)	0.3	10.6	(1.3)

Table 4.14. Size of juvenile pink and chum salmon (median, mean, and mean standard error (SE)) in oiled and non-oiled areas of Prince William Sound in 1989 and 1990. FL = mm fork length; WW = mg wet body weight.

Size	Oiled			Non-oiled		
	Median	Mean	(SE)	Median	Mean	(SE)
			Pinks, 1989			
FL	37.0	38.0	(0.5)	37.5	39.1	(0.6)
WW	320.0	417.5	(24.7)	339.5	458.5	(26.3)
			Pinks, 1990			
FL	35.0	37.7	(0.5)	34.0	36.9	(0.4)
WW	257.0	420.0	(27.6)	222.0	368.7	(20.5)
			Chums, 1989			
FL	48.0	47.8	(1.0)	37.0	38.7	(0.7)
WW	876.5	926.5	(57.7)	328.5	446.4	(35.8)
			Chums, 1990			
FL	48.0	51.5	(1.4)	40.0	41.8	(0.7)
WW	893.0	1329.0	(131.0)	489.0	583.3	(34.7)

Table 4.15. Diet composition by prey production system for juvenile chum salmon from oiled (54) and non-oiled (58) areas in 1989. FO = frequency of occurrence; DW = mg dry weight.

Diet Composition	Oiled			Non-oiled		
	Median	Mean	(SE)	Median	Mean	(SE)
			Pelagic Zooplankton			
%FO	100.0	90.7	(6.0)	100.0	90.0	(6.8)
Number	8.0	12.7	(1.8)	8.0	39.1	(15.3)
%Number	47.1	49.1	(5.0)	66.7	56.0	(5.1)
DW	1.5	3.3	(0.5)	1.2	2.3	(0.5)
%DW	68.3	58.6	(5.6)	79.6	62.0	(5.3)
			Epibenthos			
%FO	73.9	67.4	(11.4)	87.5	82.5	(8.1)
Number	2.0	10.7	(2.1)	4.0	31.6	(11.4)
%Number	19.4	35.0	(5.0)	33.3	37.4	(4.9)
DW	0.0	0.5	(0.2)	0.1	0.7	(0.2)
%DW	1.0	21.1	(4.4)	8.4	25.6	(4.5)
			Drift Insects			
%FO	10.6	29.6	(15.8)	38.8	31.3	(8.7)
Number	0.0	1.4	(0.4)	0.0	0.4	(0.1)
%Number	0.0	7.4	(2.3)	0.0	3.6	(1.4)
DW	0.0	1.2	(0.4)	0.0	0.2	(0.0)
%DW	0.0	18.3	(4.3)	0.0	12.3	(3.8)

Table 4.16. Diet composition by prey production system for juvenile chum salmon from oiled (66) and non-oiled (70) areas in 1990. FO = frequency of occurrence; DW = mg dry weight.

Diet Composition	Oiled			Non-oiled		
	Median	Mean	(SE)	Median	Mean	(SE)
			Pelagic Zooplankton			
%FO	78.3	70.0	(10.8)	100.0	90.8	(4.1)
Number	5.0	32.6	(7.1)	11.0	17.4	(2.7)
%Number	17.7	30.4	(3.9)	61.6	57.8	(4.2)
DW	0.5	1.4	(0.3)	2.0	2.4	(0.3)
%DW	32.1	40.8	(4.8)	91.4	69.0	(4.8)
			Epibenthos			
%FO	100.0	93.3	(4.5)	77.5	73.5	(8.2)
Number	30.0	43.7	(8.0)	3.0	25.3	(9.3)
%Number	53.9	53.8	(4.4)	15.4	29.9	(4.1)
DW	1.0	1.3	(0.1)	0.1	3.6	(2.1)
%DW	61.8	54.8	(4.7)	4.8	27.2	(4.6)
			Drift Insects			
%FO	100.0	21.2	(7.0)	25.0	35.1	(11.9)
Number	0.0	0.1	(0.0)	0.0	0.3	(0.1)
%Number	0.0	1.0	(0.8)	0.0	1.7	(0.7)
DW	0.0	0.0	(0.0)	0.0	0.1	(0.0)
%DW	0.0	3.7	(1.9)	0.0	2.2	(0.8)

Table 4.17. Diet composition by prey subcategory for juvenile chum salmon from oiled (54) and non-oiled (58) areas in 1989. FO = frequency of occurrence; DW = mg dry weight.

Diet Composition	Oiled			Non-oiled		
	Median	Mean	(SE)	Median	Mean	(SE)
			Total Calanoids			
%FO	95.0	66.9	(19.5)	83.8	84.6	(6.1)
Number	4.0	7.0	(1.0)	6.0	34.1	(15.4)
%Number	21.1	30.1	(4.4)	33.3	40.2	(5.0)
DW	1.5	3.2	(0.5)	1.0	2.3	(0.5)
%DW	51.1	49.7	(5.9)	74.9	59.8	(5.3)
			Small Calanoids			
%FO	0.0	10.2	(8.2)	55.0	56.7	(8.4)
Number	0.0	0.5	(0.3)	1.0	30.7	(15.5)
%Number	0.0	3.2	(1.5)	2.2	19.9	(4.5)
DW	0.0	0.0	(0.0)	0.0	0.8	(0.4)
%DW	0.0	3.7	(2.5)	0.7	19.7	(4.7)
			Large Calanoids			
%FO	82.5	60.8	(19.6)	43.8	47.9	(21.5)
Number	3.0	6.4	(1.0)	1.0	2.9	(0.5)
%Number	10.1	26.2	(4.2)	0.8	18.1	(4.0)
DW	1.5	3.1	(0.5)	0.5	1.4	(0.3)
%DW	42.1	46.0	(6.0)	27.5	40.0	(5.9)
			Harpacticoids			
%FO	58.3	53.7	(9.0)	66.3	63.8	(8.8)
Number	1.5	6.0	(1.3)	1.0	30.1	(12.5)
%Number	9.1	22.3	(4.0)	4.6	26.4	(4.5)
DW	0.0	0.1	(0.0)	0.0	0.3	(0.1)
%DW	0.3	6.1	(2.0)	1.1	12.2	(3.1)

Table 4.18. Diet composition by prey subcategory for juvenile chum salmon from oiled (66) and non-oiled (70) areas in 1990. FO = frequency of occurrence; DW = mg dry weight.

Diet Composition	Oiled			Non-oiled		
	Median	Mean	(SE)	Median	Mean	(SE)
Total Calanoids						
%FO	35.0	49.2	(13.5)	92.9	78.8	(9.0)
Number	0.0	3.4	(1.2)	5.0	7.4	(1.0)
%Number	0.0	5.6	(1.5)	27.0	36.5	(4.5)
DW	0.0	0.5	(0.2)	1.5	2.1	(0.3)
%DW	0.0	17.0	(3.8)	73.3	56.9	(5.2)
Small Calanoids						
%FO	34.3	47.1	(18.1)	48.8	50.6	(10.7)
Number	0.0	2.5	(1.0)	0.0	3.3	(0.9)
%Number	0.0	2.4	(0.9)	0.0	11.0	(2.4)
DW	0.0	0.1	(0.0)	0.0	0.1	(0.0)
%DW	0.0	2.5	(0.9)	0.0	5.1	(1.8)
Large Calanoids						
%FO	28.3	36.7	(15.8)	85.7	79.8	(9.6)
Number	0.0	0.9	(0.3)	3.0	4.1	(0.6)
%Number	0.0	3.2	(1.2)	8.6	25.5	(3.9)
DW	0.0	0.4	(0.2)	1.5	2.0	(0.3)
%DW	0.0	14.5	(3.7)	64.6	51.7	(5.1)
Harpacticoids						
%FO	73.3	69.5	(7.9)	40.0	45.9	(7.6)
Number	3.0	19.9	(8.0)	0.0	10.6	(4.8)
%Number	4.3	20.5	(3.9)	0.0	12.6	(2.7)
DW	0.0	0.2	(0.1)	0.0	0.1	(0.0)
%DW	1.3	12.1	(3.0)	0.0	5.0	(1.7)

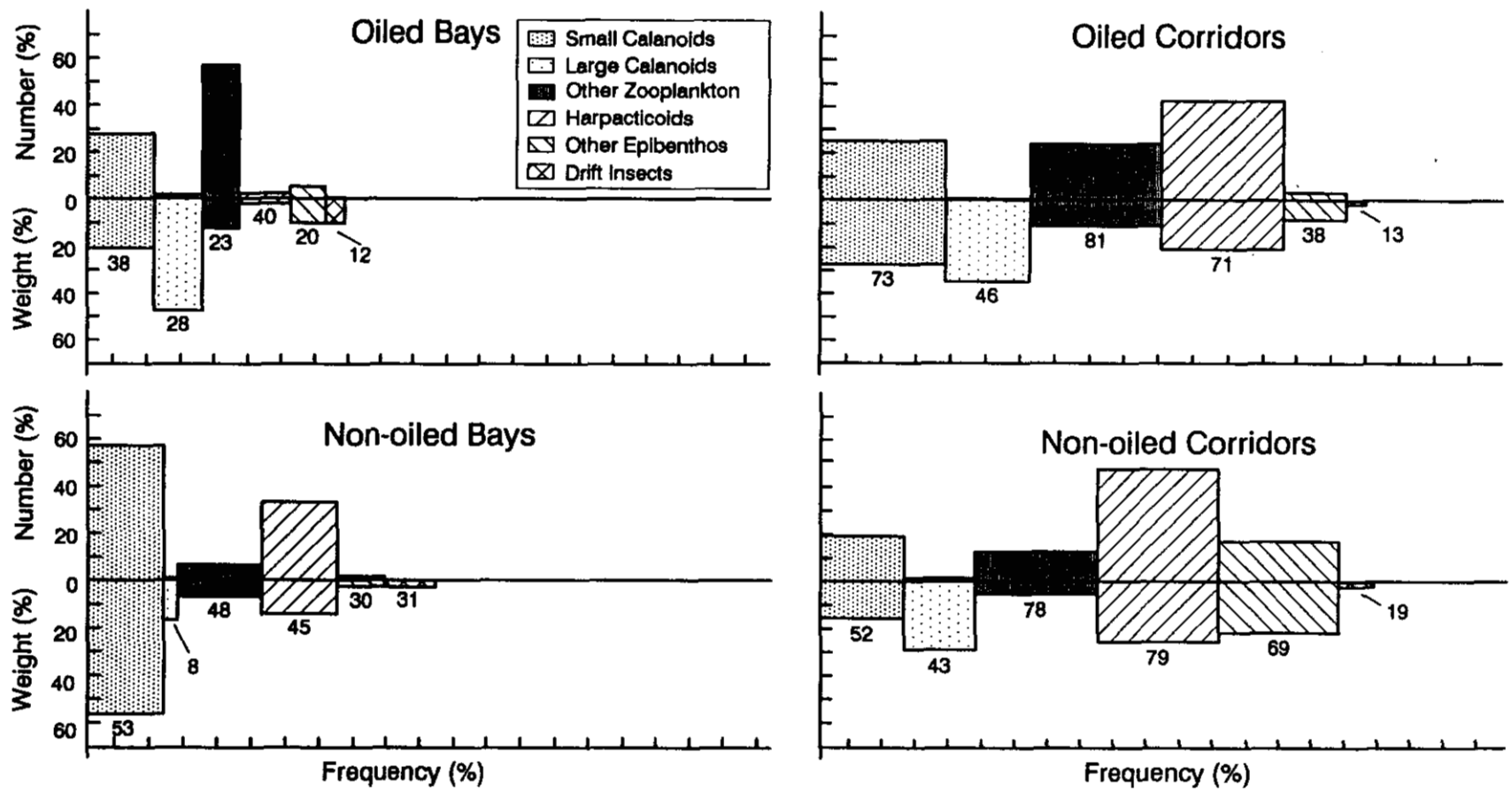

Figure 4.1. Prey composition of 397 pink salmon from oiled and non-oiled bays and corridors in Prince William Sound, 1989. Height of bar above line indicates percent number, height of bar below line indicates percent dry weight, and width of bar indicates percent frequency of occurrence (magnitude indicated below each bar). Each division on the x-axis represents 20%.

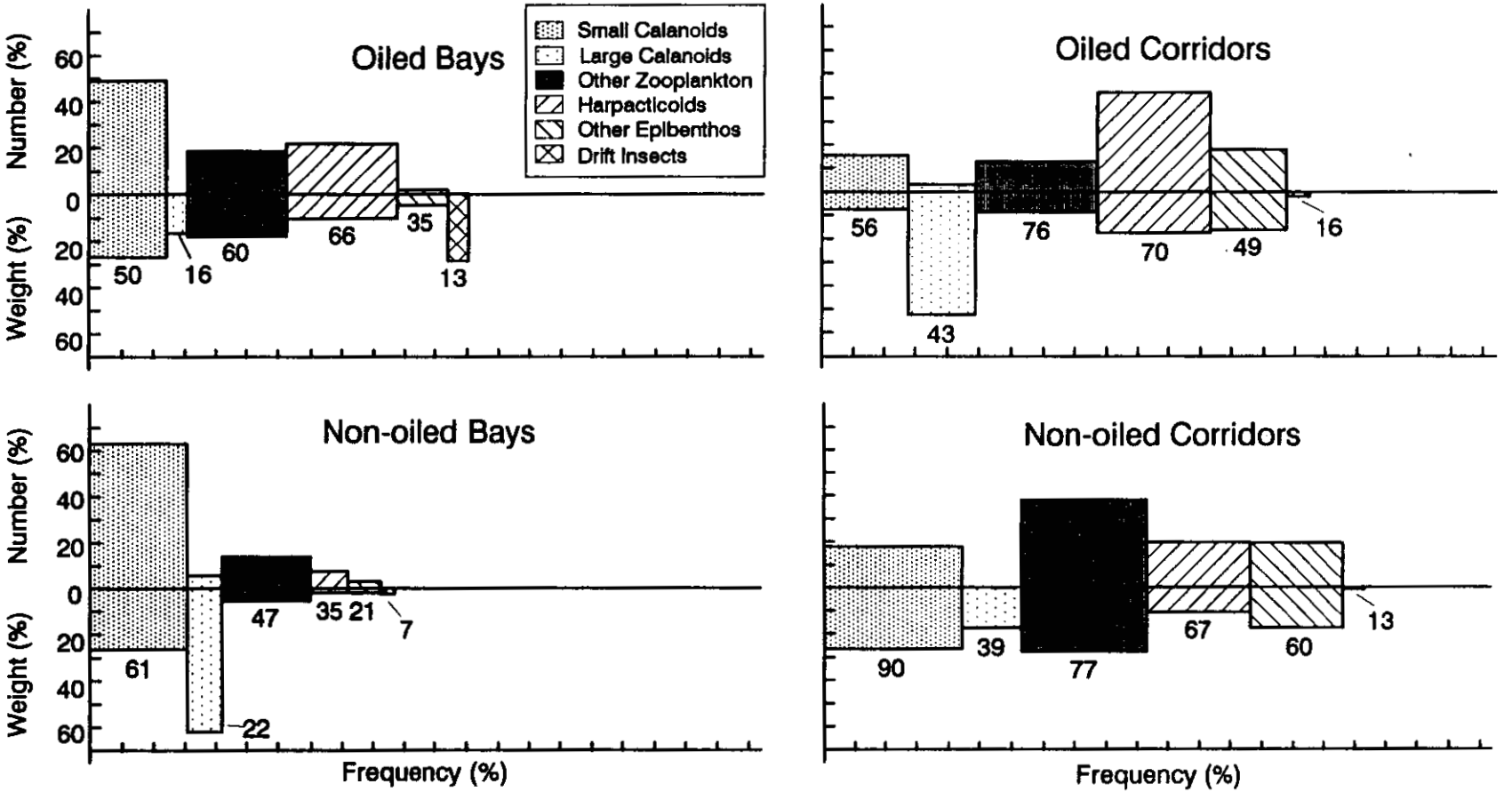

Figure 4.2. Prey composition of 595 pink salmon from oiled and non-oiled bays and corridors in Prince William Sound, 1990. Height of bar above line indicates percent number, height of bar below line indicates percent dry weight, and width of bar indicates percent frequency of occurrence (magnitude indicated below each bar). Each division on the x-axis represents 20%.

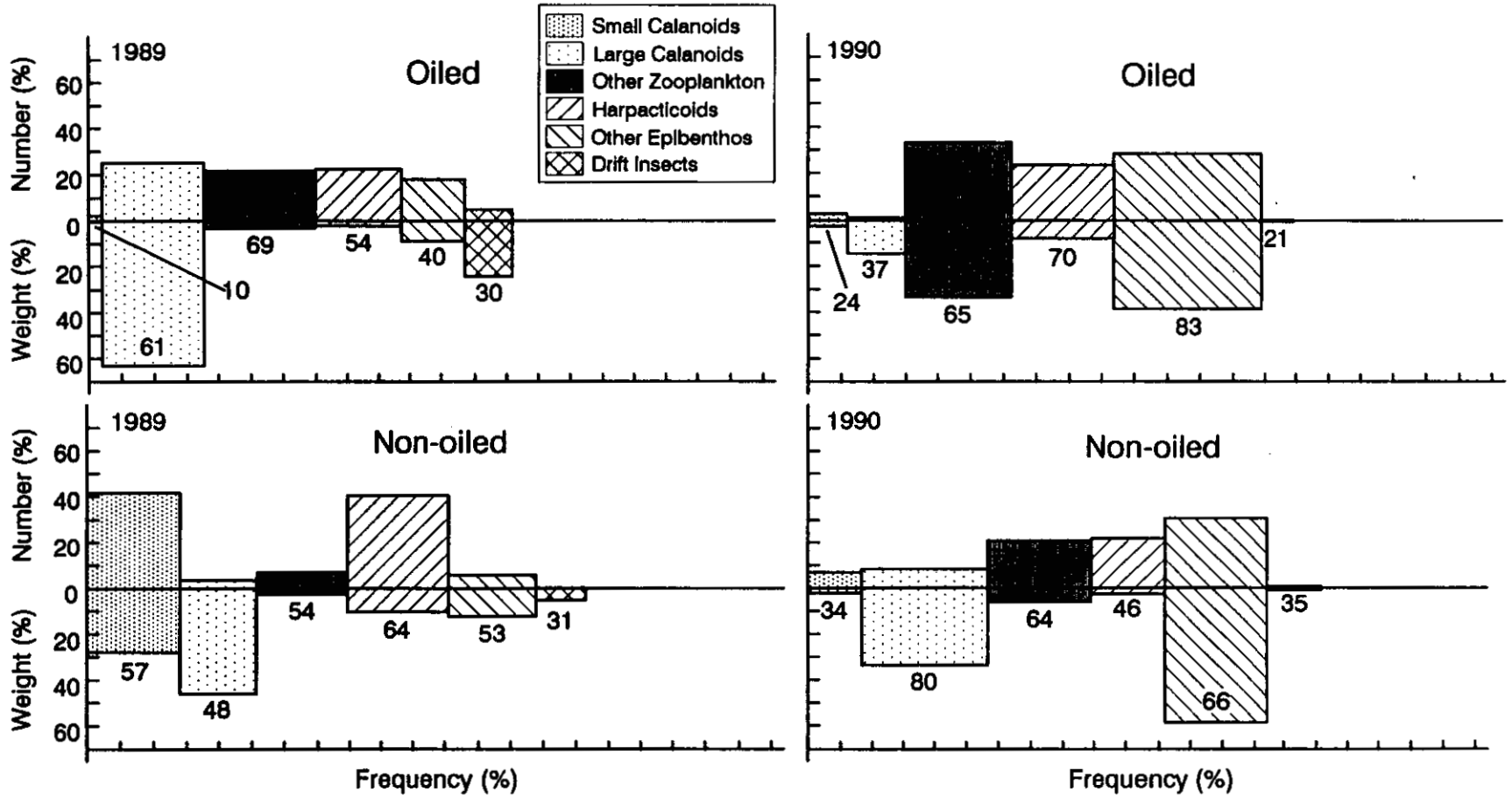

Figure 4.3. Prey composition of 112 and 136 chum salmon from oiled and non-oiled areas in Prince William Sound, 1989 and 1990. Height of bar above line indicates percent number, height of bar below line indicates percent dry weight, and width of bar indicates percent frequency of occurrence (magnitude indicated below each bar). Each division on the x-axis represents 20%.

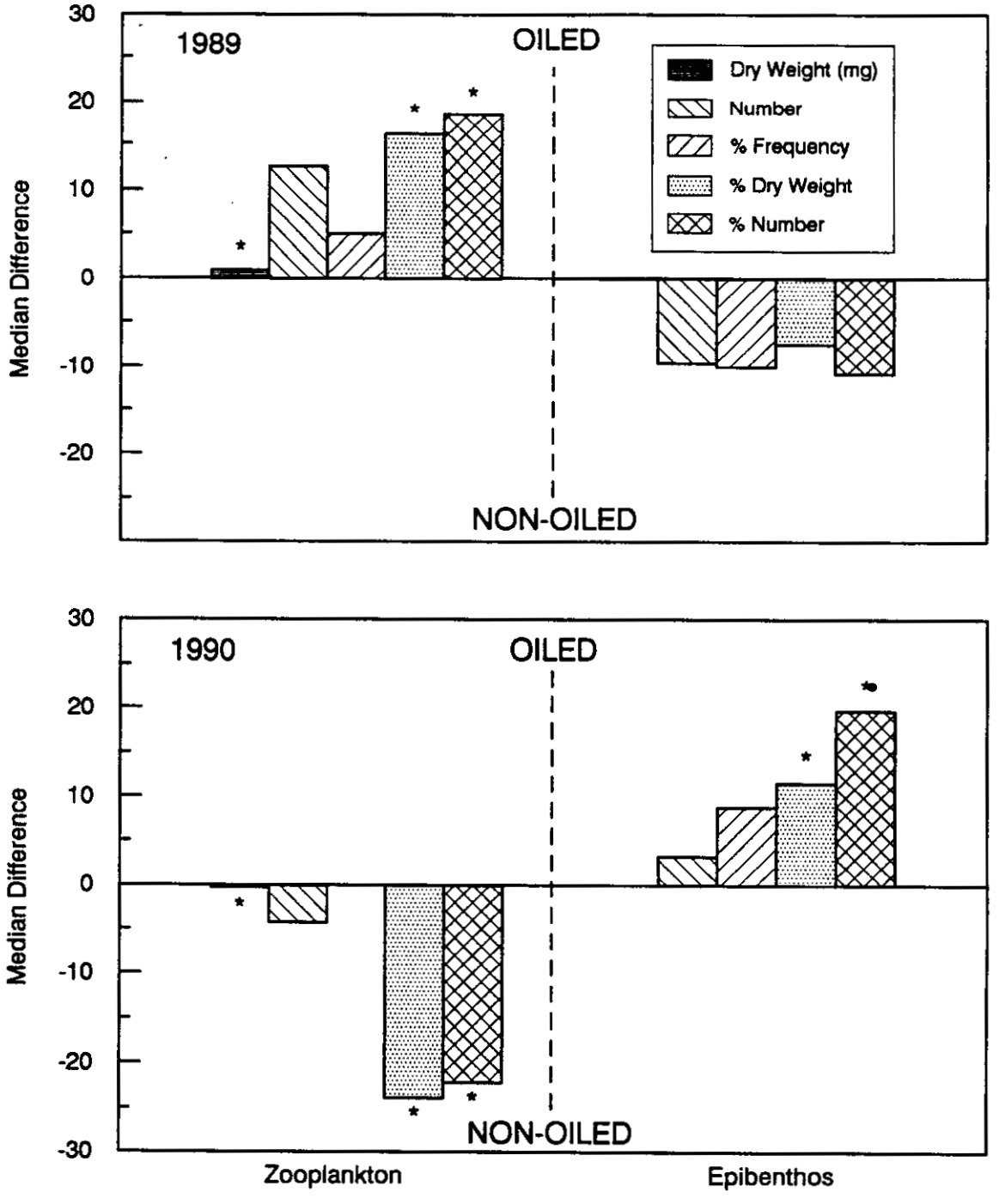

Figure 4.4. Interannual shift in prey composition for pink salmon between oiled and non-oiled areas of Prince William Sound, 1989 and 1990. A negative Wilcoxon median difference indicates non-oiled value > oiled value. An asterisk indicates a significant difference ($P < 0.1$).

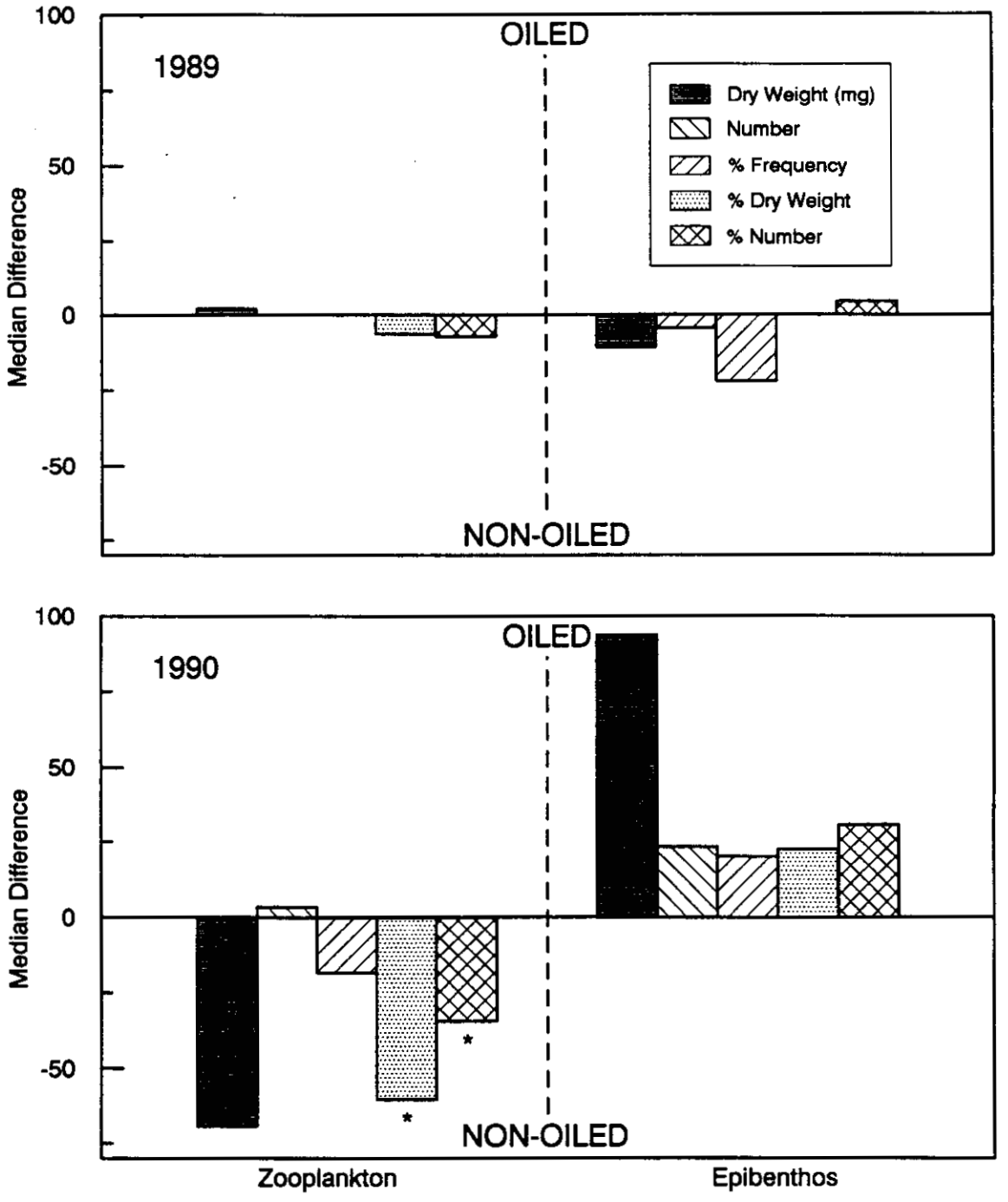

Figure 4.5. Interannual shift in prey composition for chum salmon between oiled and non-oiled areas of Prince William Sound, 1989 and 1990. A negative Wilcoxon median difference indicates non-oiled value > oiled value. An asterisk indicates a significant difference ($P < 0.1$).

CHAPTER 5

Impacts of the *Exxon Valdez* Oil Spill on Pelagic and Epibenthic Prey Resources of Juvenile Salmon in Western Prince William Sound

Preface

Prey resources of juvenile salmon, such as zooplankton and epibenthic crustaceans, must be plentiful to sustain the high growth rates characteristic of these fish during their initial marine residency. Oil in the marine environment can cause mortality and reduce the abundance of these prey resources (e.g., Samain et al. 1980; Bodin 1991). If prey abundance in Prince William Sound were reduced due to oiling from the *Exxon Valdez*, the growth and survival of juvenile salmon could also be reduced as a consequence.

In this chapter, we examine the impact of the *Exxon Valdez* oil spill on the prey resources of juvenile salmon in western Prince William Sound. This chapter is composed of three separate sections, which address sequentially Objectives 9-11 listed in Chapter 1. Chapter 5A examines distribution, abundance, and composition of both pelagic (1989 and 1990) and epibenthic (1989 only) prey resources in oiled and non-oiled locations in western Prince William Sound following the oil spill. Chapter 5B takes a more detailed look at the abundance and population structure of harpacticoid copepods at lightly oiled and heavily oiled beaches 1 year after the oil spill. Chapter 5C examines meiofaunal recolonization and community structure after experimental contamination of sediments with North Slope crude oil from the *Exxon Valdez*. This manipulative field experiment was designed to study the effects of contamination of sediments on meiofauna without confounding hydrographic or physical intersite differences.

CHAPTER 5A. Distribution, Abundance, and Composition of Prey Resources of Juvenile Salmon

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Abstract

The impact of the *Exxon Valdez* oil spill on potential prey resources of juvenile salmon was studied from April to June 1989 (pelagic zooplankton and epibenthic crustaceans) and 1990 (pelagic zooplankton only). A total of eight locations were sampled: two each in oiled and non-oiled bays, and two each in oiled and non-oiled corridors in western Prince William Sound. Pelagic zooplankton were sampled at each location with 20-m vertical hauls. Epibenthic crustaceans were sampled with a 10-m haul with an epibenthic sled at three nearshore habitat types: low-, medium-, and steep-gradient beaches.

We did not detect any reduction in abundance of either pelagic zooplankton or epibenthic crustaceans important in the diet of juvenile salmon resulting from the *Exxon Valdez* oil spill. Density, biomass, and diversity (number of taxa) of pelagic zooplankton did not differ significantly between oiled and non-oiled locations in either 1989 or 1990. Biomass of total epibenthic crustaceans and biomass of harpacticoid copepods were significantly greater in oiled than non-oiled locations. Diversity of epibenthic crustaceans, however, was similar in oiled and non-oiled locations. We conclude that the *Exxon Valdez* oil spill did not reduce the available prey resources of juvenile salmon in western Prince William Sound.

Introduction

Juvenile salmon must have abundant prey resources to sustain the high growth rates characteristic of their initial marine residency. During their first weeks of marine residency, pink and chum salmon fry remain near shore, feeding on zooplankton and epibenthic organisms (Heard 1991; Salo 1991). Epibenthic prey, such as harpacticoid copepods, are the main food items in some areas (Kaczynski et al. 1973; Landingham 1982; Volk et al. 1984), whereas zooplankton, such as calanoid copepods and euphasiid eggs and larvae, are the predominant prey in other areas (Bailey et al. 1975; Healey 1980; Cooney et al. 1981). The subarctic marine ecosystem has a highly seasonal production cycle, characterized by high levels of primary and secondary production in spring (Goering et al. 1973; Larrance 1977; Smetacek et al. 1984). The timing of pink and chum salmon emigration to seawater has presumably evolved to exploit this period of high productivity (Murphy et al. 1988; Holtby et al. 1989).

In the spring of 1989, much of the surface water and the littoral sediments of western Prince William Sound were contaminated by oil from the *Exxon Valdez*. Oil has been shown to have detrimental impacts on both pelagic zooplankton and epibenthic crustaceans (e.g., Samain et al. 1980; Bodin 1991). Concern for catastrophic disruption of the food webs supporting salmon populations increased as the oil spread.

Our objectives in this component of the field research in 1989 and 1990 were to assess the impact of the oil spill on available prey resources of juvenile salmon in western Prince William Sound (Objective 9, Chapter 1). Our approach was to compare density, biomass, and diversity of pelagic zooplankton and epibenthic crustaceans between oiled and non-oiled locations in western Prince William Sound in 1989, to determine if prey resources were significantly lower in oiled habitats, and to examine how these comparisons changed for pelagic zooplankton between 1989, the year of acute contamination, and 1990, when the degree of oil pollution had greatly diminished.

Chapter 5A will be reformatted and submitted to the *Exxon Valdez Oil Spill Symposium Proceedings* for formal publication.

Methods

We selected four sampling locations in the oiled area and four in the non-oiled area of western Prince William Sound (Figure 2.1). The oiled and non-oiled areas were identified by visual inspection of the actual distribution of oil in the first two weeks after the spill. Within each area, two bays and two migration corridors leading to the Gulf of Alaska were selected for a total of eight sampling locations. The non-oiled locations were in McClure Bay and Long Bay, and in Culross Passage and Wells Passage. The oiled locations were in Herring Bay and Snug Harbor, and in Prince of Wales Passage and Knight Island Passage. Prey were sampled in five trips between 10 April and 26 June 1989, and four trips between 16 April and 14 June 1990. Confirmation of our designation of oiled vs. non-oiled was provided by hydrocarbon analysis of mussel and sediment samples from each sampling location (Chapter 3, this report).

Pelagic Zooplankton

Triplicate samples of pelagic zooplankton were taken at each location on each sampling trip with a 20-m vertical haul of a 0.5-m diameter 243-micron-mesh net. Water of sufficient depth for the haul was located with a depth-sounder or sounding line. After a haul, a seawater pump was used to wash down the outside of the sampling net. The contents were then rinsed with filtered seawater and preserved in 5% formalin.

Zooplankton samples were analyzed by the Alaska Department of Fish and Game (ADFG) Limnology Laboratory. Organisms in a subsample from each sample were identified and counted. Identification was generally to order level, except for calanoid and harpacticoid copepods, which were identified to genus or species level. Sex and life stages (copepodite, adult, egg-carrying females, clasping pairs) were also determined. A total number for the sample was computed by dividing the subsample count by the subsample fraction. Density (no/m^3) of an organism within a sample was computed by dividing the number of organisms by the volume of water sampled. In 1989, wet weights of organisms were determined for each sample by weighing up to 100 individuals from each taxon. Biomass (g/m^3) of an organism within a sample was computed by multiplying the mean weight of the organism by the number in the sample and then dividing by the volume of water sampled. In 1990, only counts were made. Mean weights of the taxa across all samples processed in 1989 were used to estimate biomass of taxa in 1990.

Three different sets of the pelagic zooplankton data were statistically analyzed. Because juvenile salmon prey on virtually all of the taxa we captured (Cooney et al. 1981; Brodeur and Pearcy 1990; Landingham 1993), the first set

consisted of all pelagic zooplankton sampled. Further analysis focused on two size categories of calanoid copepods, small (<2.5 mm metasomal length) and large (≥2.5 mm metasomal length). These categories parallel the analysis of feeding habits of juvenile salmon (Chapter 4, this report). Large calanoids included species such as *Neocalanus plumchrus, Neocalanus christatus, Calanus* spp., and *Eucalanus bungii*, while small calanoids included species such as *Pseudocalanus* spp., *Acartia longiremis*, and *Centropages abdominalis*.

The univariate approach to analysis of variance (ANOVA) of a repeated measures design (Frane 1980) was used to analyze zooplankton data. Measures of abundance analyzed with ANOVA were biomass and density of total zooplankton, and biomass of small and large calanoid copepods. Factors in the ANOVA were time period, oil (dichotomous scale: oiled or non-oiled), bay/corridor, and location (nested in oil and bay/corridor). Each cell had three observations, except in one case where a replicate sample was lost because of improper preservation. Based on Box-Cox diagnostic plots (Dixon et al. 1988), density and biomass data were transformed to natural logarithms (ln) to normalize distribution and maximize variance homogeneity. The number of species or species groups was used as a measure of diversity (Pielou 1975), and was also analyzed by ANOVA.

Coefficients of variation (CV) were calculated to compare variations in biomass and density of pelagic zooplanktonbetween different locations and time periods independent of their means. The formula for CV, corrected for bias, was:

$$CV = (1+1/4N)(100\bar{SD}/Y)$$

where N is the number of samples, $\bar{Y}$ is the mean density or biomass, and SD is the standard deviation (Sokal and Rohlf 1981).

Epibenthic Crustaceans

Three habitat types (low-, medium-, and steep-gradient beaches) were sampled for epibenthic crustaceans at each location. Low-gradient beaches were <10% grade, with granule-pebble substrate; medium-gradient beaches were 12-25% grade, with pebble-cobble substrate; and steep-gradient beaches were >50% grade, with bedrock or boulder substrate. Surface substrate composition was visually classified according to the Wentworth scale (Holme and McIntyre 1984): boulder (>256 mm); cobble (64-256 mm); pebble (4-64 mm); and granule (2-4 mm). Any substrate <2 mm, such as sand or mud, was also included in the granule category. Sampling sites were selected for similarity in wave exposure, macrophyte coverage, and substrate. Locations of each site are shown on Maps A-E, Appendix 2.1.

Epibenthic crustaceans were sampled in 1989 with a 10-m horizontal haul of an epibenthic sled with an attached 0.3-m diameter, 243-micron-mesh net. Runners on the sled were 11 cm high; thus, the bottom of the net was towed 11 cm above the substrate. Epibenthic sled samples were taken at 0.5-m water depth. After a haul, the outside of the sampling net was washed down with a seawater pump. The contents of the cup were then rinsed with filtered seawater and preserved in 5% formalin.

Two sets of epibenthic sled samples were collected in 1989. The first set, "systematic" samples, were taken adjacent to each systematic beach seining site immediately after the seine set (Chapter 2, this report). Systematic sled samples were collected from -0.3-m to +0.9-m tide levels. The second set, "tidal transect" samples, were taken at 0.6-m tide intervals from -0.3-m to +2.7-m tide levels (actual water depth sampled was 0.5 m deeper than the nominal tide levels). Tidal transects were sampled at each bay on each sampling trip in 1989 except Trip 1, when they were sampled only at McClure Bay and Herring Bay.

Epibenthic sled samples were analyzed by the Fisheries Research Institute, University of Washington. Crustaceans in a subsample of each sample were identified, counted, and weighed, as for pelagic zooplankton. A total number for the sample was computed by dividing the subsample count by the subsample fraction. Density (no/m^3) of an organism within a sample was computed by dividing the number of organisms in a sample by the volume of water sampled. Biomass (g/m^3) of an organism within a sample was computed by multiplying the mean weight of the organism by the number in the sample and dividing by the volume of water sampled.

The epibenthic sled sampled organisms of both pelagic and epibenthic origin, but analysis was limited to those epibenthic crustaceans that are prey of juvenile salmon (Kaczynski et al. 1973; Bailey et al. 1975; Barnard 1981; Celewycz and Cordell 1988; Landingham and Mothershead 1988). Data were analyzed by repeated measures ANOVA. Measures of abundance analyzed with ANOVA included total biomass of epibenthic crustaceans and biomass of harpacticoid copepods. Based on Box-Cox diagnostic plots, biomass data were transformed to natural logarithms to normalize distribution and maximize variance homogeneity. For the systematic epibenthic samples, the factors were time period, oil, bay/corridor, habitat, and location (nested in oil and bay/corridor). Each cell had only one observation; six cells were empty because of samples destroyed in shipping. For the tidal transect samples, the factors were time period, oil, location, habitat, and tide level, with location nested in oil. Time period 1 was excluded from the ANOVA because all four bays were not sampled on the first sampling trip. The number of species or species groups of epibenthic crustaceans was used as a measure of diversity (Pielou 1975) and analyzed by ANOVA.

Results

We did not detect any reduction in abundance of either pelagic zooplankton or epibenthic crustaceans important in the diet of juvenile salmon resulting from the *Exxon Valdez* oil spill. Neither density nor biomass of pelagic zooplankton differed significantly between oiled and non-oiled locations in either 1989 or 1990. Density and biomass of epibenthic crustaceans were significantly greater in oiled than non-oiled locations in 1989.

Pelagic Zooplankton

Density and biomass of pelagic zooplankton fluctuated widely between time periods and locations in both 1989 (Tables 5A.1, 5A.2) and 1990 (Tables 5A.3, 5A.4). Variation between replicates at the same time and location was also large, with CV ranging from 5 to 66% for density and 5 to 85% for biomass. Biomass of pelagic zooplankton differed significantly between time periods in both 1989 ($P < 0.001$, Table 5A.5) and 1990 ($P = 0.051$, Table 5A.6). Biomass peaked at different times at the different locations (Tables 5A.2, 5A.4). Biomass fluctuated up to 200-fold between different time periods at the same location, and peak biomass varied 10-fold between locations.

Density of zooplankton varied less between time periods than biomass did. Density of zooplankton differed significantly between time periods only in 1989 ($P < 0.001$, Table 5A.5), not in 1990 ($P > 0.1$, Table 5A.6). In 1989, density of zooplankton fluctuated 18-fold between different time periods at the same location, and peak numbers varied 5-fold between locations (Table 5A.1). In 1990, numbers of zooplankton fluctuated less than 9-fold between different time periods at the same location, and peak density varied 15-fold between different locations (Table 5A.3).

Calanoid copepods dominated the biomass and density of pelagic zooplankton in both 1989 and 1990. In 1989, 52 taxa of pelagic zooplankton were identified (Table 5A.7). The dominant organisms by biomass were *Calanus* spp. and *Pseudocalanus* spp. in both bays and corridors. *Pseudocalanus* spp. was most numerous overall in both bays and corridors, followed by *Calanus* spp. in corridors and Ectoprocta (Cyphonautes) in bays. At each location, calanoid copepods comprised over one-half of the density (Figure 5A.1) and biomass (Figure 5A.2) of pelagic zooplankton in 1989. Large calanoids were less numerous than small calanoids at each of the eight locations (Figure 5A.1), but comprised a higher proportion of the total biomass at all four corridor locations and two of the bays (Figure 5A.2). In 1990, 43 taxa of pelagic zooplankton were identified (Table 5A.8). In overall biomass, the dominant

organisms in corridors were *Neocalanus plumchrus* and *Calanus* spp., whereas the dominant organisms in bays were *Calanus* spp. and *Calanus marshallae*. *Pseudocalanus* spp. was again most numerous in both bays and corridors, followed by *Calanus* spp. in corridors and *Acartia longiremis* in bays. In 1990, calanoid copepods comprised over 75% of the density of pelagic zooplankton at all locations except the two oiled bays, Herring Bay and Snug Harbor (Figure 5A.3). At these sites, the most numerous organisms (excluding calanoid copepods) were euphausids and *Fritillaria* spp., respectively, in Herring Bay, and *Podon* spp. and euphausids, respectively, in Snug Harbor. Calanoid copepods comprised over 85% of the biomass of pelagic zooplankton at each site in 1990 (Figure 5A.4). At each of the eight locations, large calanoids were less numerous than small calanoids (Figure 5A.3), but comprised a much higher proportion of the total biomass than small calanoids (Figure 5A.4).

Neither zooplankton density nor biomass differed significantly ($P > 0.1$) between oiled and non-oiled locations in 1989 or 1990 (Tables 5A.5, 5A.6). In 1989, mean biomass was similar in oiled and non-oiled locations in spring, peaking in early May, and declining to low levels by early June (Figure 5A.5). In 1989, density of zooplankton in oiled locations also peaked in early May, whereas in non-oiled locations, density peaked in mid-April (Figure 5A.6). Throughout May, zooplankton density declined in both oiled and non-oiled locations. Density increased slightly from early to late June in both oiled and non-oiled locations. In contrast to 1989, sample mean biomass of zooplankton in 1990 declined more rapidly in oiled than non-oiled locations following the seasonal peak in early May (Figure 5A.5). Biomass in oiled locations continued to decline to seasonal lows through June, whereas in the non-oiled locations, sample mean biomass actually increased in June. Density of zooplankton in oiled locations also peaked in early May, but density in non-oiled locations peaked in early June (Figure 5A.6). In both oiled and non-oiled locations, mean zooplankton density increased from late May to early June. While peak density of zooplankton in non-oiled locations was similar between years, peak density in oiled locations was two times higher in 1989 than in 1990. Peak total biomass in both oiled and non-oiled locations was about two times higher in 1990 than 1989.

In 1989, bay/corridor was significant ($P = 0.042$) in explaining variation in biomass ($P = 0.042$) and density ($P = 0.021$) of pelagic zooplankton (Table 5A.5). Mean biomass was 3.4 times higher and mean density was 1.9 times higher overall in corridors than in bays. When examined over time (Figure 5A.7), the biggest difference in biomass of pelagic zooplankton between bays and corridors was in April and May. Overall, pelagic zooplankton peaked in early May in both bays and corridors. By June, the

biomass of pelagic zooplankton had declined sharply to similar levels in both bays and corridors.

In 1990, neither biomass nor density of pelagic zooplankton was significantly ($P > 0.1$) different overall between bays and corridors (Table 5A.6), although the sample mean biomass was 2.5 times higher and the sample mean density was 1.4 times higher in corridors than bays. During April and May, the sample mean biomass was higher in corridors than bays (Figure 5A.7); at the overall peak of zooplankton abundance in early May, the ln-transformed mean biomass differed between bays and corridors by more than 4 SE. In early June, however, while biomass declined sharply in corridors, it increased to the highest level observed in bays in 1990.

When calanoid copepods were examined separately by size class, the biomass of large calanoid and of small calanoid copepods did not differ significantly ($P > 0.1$) between oiled and non-oiled locations in either 1989 or 1990 (Tables 5A.9, 5A.10). Only time period was significant in explaining variation in biomass in all four of these analyses. In general, both large and small calanoids closely tracked the pattern of total zooplankton biomass in both years (Figures 5A.5, 5A.8, 5A.9). The biomass of large calanoids peaked in May in oiled and non-oiled locations in both 1989 and 1990 (Figure 5A.8). The biomass of small calanoids peaked in early May in oiled locations in both 1989 and 1990, but in non-oiled locations, biomass of small calanoids peaked in mid-April in 1989 and early June in 1990 (Figure 5A.9). Biomass of both small and large calanoids was generally higher in corridors than bays, especially during seasonal peaks in May in both years (Figures 5A.10, 5A.11), although differences over the entire spring were marginally significant ($P = 0.053$) only for small calanoids in 1989 (Table 5A.9). *Pseudocalanus* spp. showed a similar seasonal abundance pattern as other small calanoids in both 1989 and 1990 (Figure 5A.12).

Diversity of pelagic zooplankton, as measured by the number of identified taxa, did not differ significantly ($P > 0.1$) between oiled and non-oiled locations in 1989 or 1990 (Table 5A.11). The number of taxa peaked at different times at different locations, but generally increased over time (Table 5A.12). Diversity was significantly higher ($P = 0.082$) in bays than corridors in 1989; the mean number of taxa was 15.9 and 14.3 in bays and corridors, respectively. The sample mean number per haul was also higher in bays (17.7) than in corridors (17.2) in 1990, although this difference was not significant ($P > 0.1$).

Epibenthic Crustaceans

Density and biomass of organisms in the systematic samples fluctuated widely between time periods, habitats, and locations (Tables 5A.13, 5A.14). Harpacticoid copepods were the dominant epibenthic organisms at each location in terms of both density and biomass (Figures 5A.13, 5A.14). A total of 84 taxa of epibenthic origin and 42 taxa of pelagic origin were captured in the epibenthic sled in 1989; over 70% of these organisms were considered to be prey of juvenile salmon (Table 5A.15). Epibenthic organisms comprised less than one-half of the total density and biomass of organisms sampled by the sled; pelagic zooplankton in the water column were also sampled by the sled. In the epibenthic sled samples, harpacticoid copepods comprised 87% of the biomass of epibenthic crustaceans important in the diets of juvenile pink salmon. The most abundant harpacticoid copepods sampled by the sled were *Harpacticus* spp. and *Tisbe* spp., respectively. Because of the dominance in both the epibenthic samples and in the epibenthic crustaceans utilized by juvenile salmon as prey (Chapter 4, this report), further analysis of epibenthic prey focused on harpacticoid copepods.

Biomass of harpacticoid copepods was greater in oiled than non-oiled locations at each habitat type in 1989 (Figure 5A.15). In the systematic samples, oil ($P = 0.025$) and habitat ($P = 0.025$) were each significant in explaining variation in biomass of harpacticoid copepods (Table 5A.16). Mean biomass of harpacticoids was 2.5 times higher overall in oiled than non-oiled locations. Harpacticoid biomass was much lower in the steep-gradient than in either the low- or medium-gradient habitats.

The biomass of the two major genera of harpacticoids, *Harpacticus* spp. and *Tisbe* spp., showed different patterns over time in oiled and non-oiled locations (Figure 5A.16). *Harpacticus* spp. in oiled locations peaked in mid-May, whereas the peak in non-oiled locations was in mid-April. *Tisbe* spp. in oiled locations peaked twice, once in mid-April and once in mid-May, whereas the peak in non-oiled locations was in mid-May. For neither taxon was biomass significantly different between oiled and non-oiled locations ($P > 0.1$).

Time ($P = 0.066$) and bay/corridor ($P = 0.057$) were marginally significant in explaining variation in biomass of harpacticoid copepods in the systematic samples (Table 5A.16). Biomass was three times higher overall in corridors than bays, mainly because of the large peak in biomass in late May (Figure 5A.17). By June, the biomass of harpacticoids was uniformly low in both bays and corridors.

In the tidal transect samples from bays in 1989, oil ($P = 0.021$), tide level ($P < 0.001$), and the oil-tide interaction ($P = 0.001$) were significant in explaining variation in biomass of harpacticoid copepods (Table 5A.17). Both density and biomass of harpacticoid copepods were higher overall in the oiled than non-oiled locations, and both measures decreased with increasing tide level (Figure 5A.18). The biomass of both major species groups of harpacticoids, *Harpacticus* spp. and *Tisbe* spp., showed similar patterns with respect to the factors oil and tide level (Figure 5A.19). The time period-habitat-tide level interaction was also significant ($P = 0.016$) in the analysis of biomass of harpacticoid copepods (Table 5A.17), but this third-order interaction was difficult to interpret.

Diversity of epibenthic crustaceans did not differ significantly ($P > 0.1$) between oiled and non-oiled locations in the systematic samples (Table 5A.18). Time ($P < 0.001$), habitat ($P = 0.015$), and the interaction of time-oil ($P = 0.047$) were significant in explaining the variation in number of taxa. Although the number of taxa exhibited a different pattern over time at oiled and non-oiled locations, in general, the number of taxa increased over time (Figure 5A.20). The number of taxa was highest in the low-gradient and lowest in the steep-gradient habitat (Figure 5A.21). In the tidal transect sled samples, the number of taxa at both oiled and non-oiled locations decreased the further up the beach we sampled (Figure 5A.22).

Discussion

Pelagic Zooplankton

This study could demonstrate no detrimental effect of the *Exxon Valdez* oil spill on pelagic zooplankton in Prince William Sound. We observed no significant differences in either the density or biomass of pelagic zooplankton in general, or large and small calanoid copepods in particular, between oiled and non-oiled areas in either 1989 or 1990. In addition, diversity of pelagic zooplankton did not differ between oiled and non-oiled locations.

Other studies have shown that crude oil and its derivatives can negatively impact zooplankton. Lee and Nicol (1977) found zooplankton to be susceptible to the water-soluble fraction of fuel oil, especially oceanic zooplankton compared to the more neritic forms. Samain et al. (1980) concluded that there were short-term (15-30 days) detrimental effects on zooplankton following the *Amoco Cadiz* spill off Brittany. However, Conover (1971) observed no apparent effect of bunker C oil on zooplanktors in Chedabucto Bay, even though these organisms were consuming large amounts of oil. Lee (1975) and Corner et al. (1976) demonstrated that certain species of calanoids will metabolize hydrocarbons with no apparent ill effect.

One possible reason why oil did not reduce zooplankton abundance is that the concentration of oil in the water column did not reach sufficient levels to cause direct mortality. Lee (1975) observed some mortality of zooplankton at hydrocarbon concentrations above 500 ppb. Capuzzo (1987) noted that acutely lethal concentrations of crude oil to zooplankton ranged from 200 to greater than 43,000 ppb total petroleum hydrocarbons. Corner (1978) and Johnston (1984) reported acutely lethal concentrations of crude oil in the range of 100 to 10,000 ppb for zooplankton. Sublethal responses, such as decreases in growth rate, were observed at exposure concentrations that ranged from slightly lower than lethal concentration to about one-tenth lethal concentration (Anderson et al. 1987; Neff 1987). In western Prince William Sound, however, the concentration of polynuclear aromatics in the water column did not exceed 6.2 ppb following the spill (Maki 1991; Neff 1991; Short and Rounds 1991).

A second explanation for no reduction of zooplankton is that advection of zooplankton from deeper waters of Prince William Sound and the Gulf of Alaska obscured any localized impacts of oil on secondary productivity in the upper water column. Physical transport of zooplankton can substantially modify populations in bays and inlets (Lewis and Thomas 1986; Lindahl and Perissinotto 1987; Lindahl and Hernroth 1988; Aksnes et al. 1989). The shelf and coastal food webs in the northern Gulf of Alaska are supplemented by an immense amount of biomass from the

bordering ocean region (Cooney 1984), which was outside the area affected by the *Exxon Valdez* oil spill. Zooplankton abundance in the nearshore waters of Prince William Sound is strongly influenced by water circulation both within the Sound and from the Gulf of Alaska (Cooney 1986). Large calanoids, such as *Neocalanus plumchrus* and *Neocalanus cristatus*, overwinter and reproduce at depths below 300 m, migrate to the surface as copepodids, and are advected into shallow (<20 m) nearshore waters of Prince William Sound in late winter and early spring (Cooney et al. 1981; Cooney 1986; Cooney and Willette 1991). The migration of large copepods into shallow nearshore waters occurs before the beginning of seasonal phytoplankton growth rather than in response to it (Cooney 1986).

The advection of large calanoid copepods into shallow bays from deeper waters used for overwintering has been observed in other areas. Coyle et al. (1990) found that advection of zooplankton from deep adjacent waters altered species composition and biomass of the zooplankton community in Auke Bay, Alaska. Advected zooplankton included large calanoid copepods, such as *Neocalanus plumchrus* and *Calanus marshallae*, which overwintered primarily in deeper fjords nearby and were absent from shallow bays, such as Auke Bay, unless they were advected in (Coyle et al. 1990). Smaller copepods, such as *Pseudocalanus* spp., were present both inside and outside Auke Bay, so that their distribution was not markedly altered by advection (Coyle et al. 1990).

Was there a period of high initial mortality on pelagic zooplankton? As noted above, the production of large calanoids occurs in deep offshore waters that were outside the area of the oil spill. However, small neritic copepods, such as *Pseudocalanus* spp., which increase in abundance in response to localized production of the spring phytoplankton bloom (Cooney 1986), had slightly lower biomass in oiled than non-oiled locations in mid-April 1989. If a period of initial mortality occurred on small calanoids, it was extremely short-lived; by early May, the biomass of small calanoids was higher in oiled than non-oiled locations. Whether juvenile salmon were affected by this slightly lower biomass of small calanoids in oiled locations in April is doubtful; the total biomass of large and small calanoids together was no different between oiled and non-oiled locations in April, and few salmon fry were present in the nearshore marine environment at that time (Chapter 2, this report).

Corridors may be more conducive to zooplankton production than bays. The peak biomass of both large and small calanoid copepods, including *Pseudocalanus* spp., was always higher in corridors than bays in May of both 1989 and 1990. Corridor sites are impacted more than bays by circulation patterns advecting larger species of calanoid copepods from deeper waters both from

inside the Sound and from the Gulf of Alaska to nearshore waters. The smaller species of calanoids, however, are more affected by differences in localized production of phytoplankton. Higher peak abundance of small calanoid copepods, especially *Pseudocalanus* spp., in corridors may be an indication of higher production in corridors.

Epibenthic Crustaceans

Neither abundance nor diversity of epibenthic prey available to juvenile salmon was reduced in western Prince William Sound as a result of the *Exxon Valdez* oil spill. On the contrary, in both the systematic and tidal transect samples, the biomass of total prey epibenthic crustaceans in general and harpacticoid copepods in particular was significantly greater in oiled than non-oiled locations. Assemblages of epibenthic crustaceans were equally diverse at oiled and non-oiled locations.

The presence of petroleum hydrocarbons in intertidal areas has been shown to affect populations of harpacticoid copepods in different ways. Some researchers have observed dramatic declines in harpacticoid copepod populations as a result of oil: an "almost total kill within 4 days" following the Picnic Bay fuel oil spill (Wormald 1976); a clear reduction in abundance of harpacticoids within 16 days of the *Tsesis* fuel oil spill (Elmgren et al. 1983); a "phase of direct toxicity" which resulted in drastic decreases in harpacticoids within the first few weeks following the *Amoco Cadiz* crude oil spill (Bodin 1991); and an almost complete disappearance of harpacticoids within 6 weeks of the La Coruna crude oil spill (Giere 1979). Other researchers, however, have observed increases in harpacticoid copepods and other meiofauna in association with oil contamination (Naidu et al. 1978; Fricke et al. 1981; Fleeger and Chandler 1983; Feder et al. 1990). Stacey and Marcotte (1987) found that oil contamination had a detrimental effect on some species of harpacticoids, no effect on others, and a beneficial effect on yet another species.

Harpacticoid copepods are a diverse taxon; different species exploit different habitats and respond differently to oil spills. For example, 4 months after the *Amoco Cadiz* oil spill, numbers of free-living harpacticoids living on the surface of algae were 20 times higher in polluted areas than comparable unpolluted areas (Chasse 1978). At the same time, epipsammic harpacticoids in nearby sediments were virtually absent, perhaps due to high concentrations of hydrocarbons in interstitial water (Chasse 1978). Bodin (1988) also observed that after the *Amoco Cadiz* oil spill, interstitial taxa of harpacticoids declined, whereas plant-associated taxa of harpacticoids increased.

The epibenthic crustaceans sampled with the epibenthic sled were dominated by the free-living harpacticoid copepods, *Harpacticus* spp. and *Tisbe* spp. These taxa are often associated with plants above the sediment interface (Hicks and Coull 1983). We did not sample the sediments for the interstitial taxa of harpacticoids, because the harpacticoid copepods most important in the diets of juvenile salmon in Prince William Sound are the free-living forms which are available in the water column. *Harpacticus uniremis* has been identified as the most important species of harpacticoid copepod in the diet of salmon fry in Prince William Sound (Cooney et al. 1981) and in Auke Bay, Alaska (Landingham 1982; Cordell 1986). In this study, *Harpacticus* spp. and *Tisbe* spp. were the most important harpacticoid copepods in the diets of pink and chum salmon fry (Chapter 4, this report), as well as the dominant component of epibenthic prey sampled by the sled.

Reasons for increased abundance of epibenthic crustaceans that we observed in oiled locations are not clear. Increased abundance could have been directly or indirectly caused by oil, or could have been unrelated to the spill. In a simulated oil spill in Port Valdez, Alaska, Feder et al.(1990) observed an increase in abundance of harpacticoid copepods, including *Harpacticus uniremis*, and speculated that the availability of a new food resource (bacteria associated with the oil) may have led to improved survivorship of harpacticoids. Bacterial mats and high meiofauna densities are associated with natural petroleum seeps (Spies *et al.* 1980); carbon isotope analysis has shown that oil is a major carbon source at natural seeps (Bauer *et al.* 1990). After the *Exxon Valdez* oil spill, bacteria numbers in subtidal sediments were significantly greater in oiled locations than control sites (Braddock et al. *in prep*). The abundance of macroinvertebrate grazers commonly decreases after an oil spill, leading to blooms of ephemeral algae (Foster et al. 1988), which may also have led to the increased abundance of the plant-associated harpacticoids that we observed.

We cannot, however, exclude geographic variation as an explanation of the higher abundance of epibenthic crustaceans in the oiled locations. Abundance of epibenthic crustaceans may have been affected by the higher nearshore salinity at oiled than non-oiled locations (Chapter 2, this report). Abundance of juvenile salmon was also significantly lower in oiled than the non-oiled locations (Chapter 2, this report); reduced cropping by juvenile salmon could have increased the abundance of epibenthic crustaceans. However, even when juvenile salmon are utilizing epibenthic crustaceans as their primary prey, densities of the prey populations have not been affected to a significant degree (Webb 1991). At our study locations, pelagic zooplankton, rather than epibenthic crustaceans, were the primary prey eaten by pink and chum salmon fry (Chapter 4, this report).

We found that biomass of harpacticoid copepods and diversity of epibenthic taxa was highest on low-gradient beaches and lowest on steep-gradient beaches. Cooney et al. (1981) also found harpacticoid copepods to be more abundant at lower- rather than higher-gradient beaches in Prince William Sound. Pink salmon fry utilized more epibenthic crustaceans at the low- and medium-gradient habitats than at the steep-gradient habitats (Chapter 4, this report). Chum salmon fry fed even more extensively on epibenthic crustaceans than did pink salmon, possibly a reflection of their higher preference for lower-gradient beaches than pink salmon fry (Chapter 2, this report).

Although oil contamination has been associated with increased abundance of some harpacticoid copepods, the most frequently observed immediate response of harpacticoid copepod populations to an oil spill is a decline in numbers (Wormald 1976; Giere 1979; Elmgren et al. 1983). However, we found no evidence of high initial mortality of epibenthic harpacticoid copepods in response to the *Exxon Valdez* oil spill. During our first sampling trip in 1989 from 12 April to 20 April (19-27 days after the spill), the biomass of *Harpacticus* spp. was not lower in oiled than in non-oiled locations. The biomass of *Harpacticus* spp. did peak at a later time in the oiled area (mid-May) than the non-oiled area (mid-April), a possible indication of suppressed production in oiled locations immediately after the spill. However, the biomass of *Tisbe* spp. was higher in oiled than non-oiled locations in all time periods that we sampled throughout the spring.

While we found no short-term reduction of epibenthic prey in oiled locations in 1989, longer-term effects could have been caused by contamination of sediments that persisted in western Prince William Sound into 1990. However, previous research has shown that even when the presence of oil has had a detrimental effect on harpacticoid copepod populations, recovery has been rapid. After the Picnic Bay oil spill, harpacticoids recolonized sediments 8 months after the spill, and abundance returned to normal population size within 10 to 15 months after the spill (Wormald 1976). After the *Tsesis* spill, harpacticoids showed a clear recovery within 2 years (Elmgren et al. 1983), and after the La Coruna spill, harpacticoids returned to prespill levels within 1 year (Giere 1979). After the *Amoco Cadiz* spill, six species of harpacticoids recovered completely after 2 years, while full recovery of two other species took 3 years (Bodin 1991). In Prince William Sound in 1990, the abundance of epibenthic harpacticoid copepods was higher at heavily oiled than lightly oiled beaches, indicating that the epibenthic prey resources available to juvenile salmon in 1990 were not directly reduced by any continuing impact of the *Exxon Valdez* oil spill (Chapter 5B, this report).

Table 5A.1. Mean density (no./m^3) and standard deviation (s) of pelagic zooplankton from four pairs of non-oiled and oiled locations in Prince William Sound, April-June 1989.

Time	Non-oiled locations Mean	(s)	Oiled locations Mean	(s)
		Bays		
McClure Bay-Herring Bay				
Late April	2,866	(203)	5,342	(957)
Early May	4,674	(2,087)	1,580	(125)
Late May	1,008	(160)	2,423	(166)
Early June	240	(45)	1,017	(261)
Late June	1,170	(404)	1,193	(101)
Mean	1,992		2,311	
Long Bay-Snug Harbor				
Late April	5,975	(2,542)	2,493	(1,190)
Early May	337	(65)	1,332	(308)
Late May	1,081	(350)	1,303	(388)
Early June	565	(237)	318	(84)
Late June	1,732	(481)	716	(359)
Mean	1,938		1,232	
		Corridors		
Culross Passage-Prince of Wales Passage				
Late April	10,036	(1,127)	4,021	(818)
Early May	3,657	(694)	4,902	(1,715)
Late May	2,098	(460)	1,745	(442)
Early June	1,536	(206)	914	(232)
Late June	3,100	(586)	1,523	(260)
Mean	4,085		2,621	
Wells Passage-Knight Island Passage				
Late April	6,830	(1,255)	2,364	(585)
Early May	3,812	(469)	13,313	(1,438)
Late May	2,796	(859)	3,974	(199)
Early June	959	(199)	756	(293)
Late June	1,428	(178)	1,156	(106)
Mean	3,165		4,313	

Table 5A.2. Mean biomass (g/m^3) and standard deviation (s) of pelagic zooplankton from four pairs of non-oiled and oiled locations in Prince William Sound, Alaska, April-June 1989.

Time	Non-oiled locations Mean	(s)	Oiled locations Mean	(s)
		Bays		
McClure Bay-Herring Bay				
Late April	0.2315	(0.0615)	0.5127	(0.1118)
Early May	1.4426	(0.9533)	0.5615	(0.0964)
Late May	0.1395	(0.0415)	0.7463	(0.0803)
Early June	0.0074	(0.0025)	0.1322	(0.0262)
Late June	0.0393	(0.0163)	0.0184	(0.0025)
Mean	0.3720		0.3942	
Long Bay-Snug Harbor				
Late April	0.4054	(0.2960)	0.2669	(0.1534)
Early May	0.0080	(0.0014)	0.1046	(0.0544)
Late May	0.0192	(0.0064)	0.1183	(0.0544)
Early June	0.0156	(0.0081)	0.0048	(0.0013)
Late June	0.0339	(0.0094)	0.0085	(0.0037)
Mean	0.0964		0.1003	
		Corridors		
Culross Passage-Prince of Wales Passage				
Late April	1.3188	(0.2832)	1.5952	(0.8344)
Early May	1.0819	(0.4486)	1.2065	(0.3507)
Late May	0.4850	(0.0492)	0.7147	(0.3524)
Early June	0.1046	(0.0211)	0.0452	(0.0223)
Late June	0.1091	(0.0235)	0.0468	(0.0098)
Mean	0.6199		0.7699	
Wells Passage-Knight Island Passage				
Late April	1.0491	(0.5192)	0.9543	(0.4006)
Early May	1.6763	(0.6447)	2.5263	(0.4503)
Late May	0.5795	(0.2642)	2.1638	(0.7042)
Early June	0.1587	(0.0396)	0.0625	(0.0384)
Late June	0.0249	(0.0017)	0.0416	(0.0064)
Mean	0.6977		1.1497	

Table 5A.3. Mean density (no./m^3) and standard deviation (s) of pelagic zooplankton from four pairs of non-oiled and oiled locations in Prince William Sound, Alaska, April-June 1990.

Time	Non-oiled locations Mean	(s)	Oiled locations Mean	(s)
		Bays		
McClure Bay-Herring Bay				
Late April	363	(34)	2,312	(433)
Early May	180	(110)	1,838	(606)
Late May	978	(355)	1,175	(150)
Early June	541	(202)	931	(276)
Mean	515		1,564	
Long Bay-Snug Harbor				
Late April	3,057	(70)	1,243	(310)
Early May	7,095	(967)	2,583	(487)
Late May	2,330	(1,373)	1,630	(331)
Early June	14,254	(2,071)	3,750	(1,013)
Mean	6,684		2,302	
		Corridors		
Culross Passage-Prince of Wales Passage				
Late April	4,630	(593)	1,163	(438)
Early May	14,407	(7,112)	2,071	(56)
Late May	3,986	(896)	1,782	(314)
Early June	12,416	(4,879)	1,668	(179)
Mean	8,860		1,671	
Wells Passage-Knight Island Passage				
Late April	2,105	(404)	1,582	(135)
Early May	2,932	(602)	5,212	(160)
Late May	2,638	(1,316)	612	(34)
Early June	2,474	(509)	2,623	(637)
Mean	2,537		2,507	

Table 5A.4. Mean biomass (g/m^3) and standard deviation (s) of pelagic zooplankton from four pairs of non-oiled and oiled locations in Prince William Sound, Alaska, April-June 1990.

Time	Non-oiled locations Mean	(s)	Oiled locations Mean	(s)
		Bays		
McClure Bay-Herring Bay				
Late April	0.1913	(0.0376)	0.1734	(0.0168)
Early May	0.2449	(0.0508)	0.3294	(0.1303)
Late May	0.6099	(0.2200)	0.2624	(0.1279)
Early June	0.0859	(0.0437)	0.0532	(0.0130)
Mean	0.2830		0.2046	
Long Bay-Snug Harbor				
Late April	0.5382	(0.0531)	0.0660	(0.0129)
Early May	2.3013	(0.3341)	0.3108	(0.1033)
Late May	0.2345	(0.1837)	0.1962	(0.0399)
Early June	4.1551	(5.3142)	0.1579	(0.0484)
Mean	1.8073		0.1827	
		Corridors		
Culross Passage-Prince of Wales Passage				
Late April	0.7314	(0.0469)	0.1729	(0.0313)
Early May	5.6138	(2.7484)	0.7149	(0.1362)
Late May	1.4010	(0.6287)	0.3794	(0.1039)
Early June	1.3293	(0.4338)	0.1006	(0.0234)
Mean	2.2689		0.3419	
Wells Passage-Knight Island Passage				
Late April	0.5276	(0.1469)	1.6192	(0.3876)
Early May	1.6533	(0.1115)	6.4063	(0.6112)
Late May	3.0914	(1.8533)	0.4928	(0.1833)
Early June	0.2222	(0.0694)	0.1423	(0.0069)
Mean	1.3736		2.1651	

Table 5A.5. ANOVA table: ln biomass and ln density of pelagic zooplankton in Prince William Sound, April-June 1989. Each factor without an associated probability was used as the error term in the significance test for the factors listed above it; df = degrees of freedom, o = oil, b = bay/corridor, l = location, (ob) indicates nesting within oil and bay/corridor, and t = time.

Source	df	F	P
Biomass			
o	1	0.23	0.657
b	1	8.76	0.042
ob	1	0.25	0.642
l(ob)	4		
t	4	16.84	0.000
to	4	1.03	0.424
tb	4	0.63	0.649
tob	4	0.52	0.723
tl(ob)	16		
Error	79		
Total	118		
Density			
o	1	0.06	0.821
b	1	13.51	0.021
ob	1	0.67	0.458
l(ob)	4		
t	4	11.65	0.000
to	4	1.21	0.345
tb	4	1.13	0.377
tob	4	0.54	0.707
tl(ob)	16		
Error	79		
Total	118		

Table 5A.6. ANOVA table: ln biomass and ln density of pelagic zooplankton in Prince William Sound, April-June 1990. Each factor without an associated probability was used as the error term in the significance test for the factors listed above it; df = degrees of freedom, o = oil, b = bay/corridor, l = location, (ob) indicates nesting within oil and bay/corridor, and t = time.

Source	df	F	*P*
Biomass			
o	1	2.20	0.212
b	1	3.21	0.147
ob	1	0.02	0.897
l(ob)	4		
t	3	3.46	0.051
to	3	0.47	0.706
tb	3	1.84	0.194
tob	3	0.57	0.648
tl(ob)	12		
Error	64		
Total	95		
Density			
o	1	0.25	0.641
b	1	0.63	0.473
ob	1	0.60	0.482
l(ob)	4		
t	3	1.59	0.243
to	3	0.47	0.707
tb	3	0.65	0.599
tob	3	0.15	0.928
tl(ob)	12		
Error	64		
Total	95		

Table 5A.7. Percent density and biomass of pelagic zooplankton in bays and corridors of Prince William Sound from April to June 1989.

Taxon	Percent density		Percent biomass	
	Bay	Corridor	Bay	Corridor
Protozoa				
Radiolaria	0.0000	0.0018	0.0000	0.0001
Cnidaria				
Hydrozoa	2.8930	0.2939	0.3869	0.0130
Annelida				
Polychaeta	2.6407	0.0976	1.7318	0.0463
Mollusca				
Bivalvia	0.4761	0.9839	0.0368	0.0435
Gastropoda	2.1383	0.4585	0.2476	0.0342
Littorina sp.	0.0352	0.0000	0.0027	0.0000
Thecosomata	0.9735	2.8729	0.0837	1.0535
Egg case	0.0012	0.0000	0.0001	0.0000
Arthropoda				
Cladocera				
Evadne sp.	0.3197	0.1469	0.0387	0.0094
Podon sp.	0.5827	0.0356	0.1202	0.0016
Copepoda				
Copepod general	4.3770	1.4661	0.9399	0.2283
Calanoida				
Acartia clausi	0.0000	0.0060	0.0000	0.0027
Acartia longiremis	7.9265	5.3165	2.5340	1.1766
Acartia tumida	1.6612	0.0735	4.6416	0.0766
Calanus marshallae	0.0170	0.1067	0.2714	0.9212
Calanus sp.	7.9421	12.4219	49.2626	58.5162
Centropages abdominalis	0.8256	0.2166	0.5963	0.0839
Epilabidocera longipedata	0.0012	0.0018	0.0007	0.0044
Epilabidocera sp.	0.0000	0.0006	0.0000	0.0012
Eucalanus bungii	0.1081	0.0623	0.2234	0.1267
Eurytemora sp.	0.0061	0.0000	0.0041	0.0000
Heterorhabdus sp.	0.0000	0.0012	0.0000	0.0005
Metridia okhotensis	0.0000	0.0030	0.0000	0.0273
Metridia pacifica	0.2052	0.9238	0.3699	0.8990
Metridia sp.	0.0000	0.0012	0.0000	0.0014
Microcalanus sp.	0.0023	0.0000	0.0009	0.0000
Neocalanus cristatus	0.0000	0.0436	0.0000	2.0015
Pseudocalanus sp.	32.9174	63.3863	24.7522	28.0898
Tortanus discaudatus	0.0012	0.0000	0.0008	0.0000
Harpacticoida				
Harpacticoid general	0.0668	0.1058	0.0166	0.0379
Tisbe sp.	0.0113	0.0000	0.0088	0.0000
Zaus sp.	0.0622	0.0000	0.0176	0.0000
Cyclopoida				
Oithona similis	2.6132	1.2994	0.2281	0.0582
Oithona spinirostris	0.2272	0.0205	0.0227	0.0010
Oithona sp.	0.0523	0.0000	0.0041	0.0000
Poecilostomatoida				
Oncaea sp.	0.0012	0.0000	0.0001	0.0000
Monstrilloida				
Monstrilla sp.	0.0012	0.0000	0.0009	0.0000
Cirripedia				
Cirriped general	4.8731	0.6206	1.7404	0.0823

Table 5A.7. Continued

Taxon	Percent density		Percent biomass	
	Bay	Corridor	Bay	Corridor
Malacostraca				
Isopoda				
Cryptoniscidae	0.0037	0.0000	0.0029	0.0000
Amphipoda				
Parathemisto sp.	0.0049	0.0205	0.0060	0.0095
Hyperiidea	0.0227	0.0277	0.0335	0.0127
Euphausiacea				
Euphausid general	2.8139	0.3162	1.2829	0.3297
Decapoda				
Anomura	0.0285	0.0202	0.0470	0.0460
Brachyura	0.0450	0.0264	0.1103	0.0507
Phoronida				
Phoronid general	0.0355	0.0030	0.0099	0.0001
Bryozoa				
Cyphonautes	13.4078	4.9832	1.1472	0.2203
Echinodermata				
Bipinnaria	0.0034	0.0571	0.0003	0.0025
Pluteus	0.2508	0.0000	0.0371	0.0000
Urochordata				
Fritillaria sp.	6.0048	0.6696	0.7365	0.0296
Oikopleura sp.	3.2702	2.7490	8.1121	5.4744
Chaetognatha				
Sagitta sp.	0.1391	0.1289	0.0974	0.2259
Chordata				
Teleostei	0.0090	0.0296	0.0912	0.0601
Unknown	0.0012	0.0000	0.0001	0.0000
Total	100.0000	100.0000	100.0000	100.0000

Table 5A.8. Percent density and biomass of pelagic zooplankton in bays and corridors of Prince William Sound from April to June, 1990.

Taxon	Percent density		Percent biomass	
	Bay	Corridor	Bay	Corridor
Cnidaria				
Hydrozoa	1.5605	0.3672	0.1112	0.0146
Annelida				
Polychaeta	1.0340	0.2090	0.3942	0.0446
Mollusca				
Bivalvia	1.8911	2.8758	0.0838	0.0712
Gastropoda	0.0301	0.0136	0.0021	0.0005
Thecosomata	6.1296	1.9181	1.9557	0.3423
Arthropoda				
Cladocera				
Evadne sp.	0.6793	1.8515	0.0454	0.0692
Podon sp.	5.9617	0.7034	0.6572	0.0435
Copepoda				
Copepod general	3.8989	3.6812	0.5298	0.2772
Calanoida				
Acartia clausi	0.3216	0.0000	0.1393	0.0000
Acartia longiremis	13.3836	5.4525	2.7438	0.6137
Acartia tumida	0.1762	0.0240	0.2535	0.0239
Acartia sp.	0.8917	0.0000	0.3754	0.0000
Calanus marshallae	3.1631	1.2725	26.7848	3.7953
Calanus sp.	7.2932	12.8575	32.2616	31.8116
Centropages abdominalis	2.7225	0.9571	1.0663	0.2533
Epilabidocera sp.	0.0023	0.0033	0.0043	0.0008
Eucalanus bungii	0.1115	0.0759	0.1813	0.0690
Eurytemora sp.	0.2252	0.0016	0.0896	0.0003
Heterorhabdus sp.	0.0045	0.0000	0.0020	0.0000
Metridia pacifica	0.2839	1.7375	0.2933	0.8418
Neocalanus cristatus	0.0000	0.0016	0.0000	0.0422
Neocalanus plumchrus	0.8216	4.5194	15.1279	46.5408
Pseudocalanus sp.	28.4173	51.0185	12.7534	12.9000
Tortanus discaudatus	0.0023	0.0000	0.0204	0.0000
Harpacticoida				
Harpacticoid general	0.1092	0.0398	0.0411	0.0084
Tisbe sp.	0.0859	0.0289	0.0380	0.0072
Zaus sp.	0.0083	0.0000	0.0013	0.0000
Cyclopoida				
Oithona similis	2.2390	2.0714	0.1065	0.0552
Oithona spinirostris	0.0580	0.0726	0.0029	0.0021
Poecilostomatoida				
Oncaea sp.	0.1408	0.0704	0.0098	0.0028
Monstrilloida				
Monstrilla sp.	0.0008	0.0000	0.0003	0.0000
Cirripedia				
Cirriped general	6.4610	1.0237	1.1231	0.1054

Table 5A.8. Continued

Taxon	Percent density		Percent biomass	
	Bay	Corridor	Bay	Corridor
Malacostraca				
Amphipoda				
Parathemisto sp.	0.0000	0.0628	0.0000	0.0162
Euphausiacea				
Euphausid general	6.9799	1.7331	1.0485	0.1715
Decapoda				
Anomura	0.0241	0.0120	0.0412	0.0115
Brachyura	0.0324	0.0426	0.0543	0.0399
Bryozoa				
Cyphonautes	1.2479	1.5372	0.0586	0.0404
Echinodermata				
Bipinnaria	0.1167	0.0791	0.0052	0.0020
Pluteus	0.0030	0.0000	0.0003	0.0000
Urochordata				
Fritillaria sp.	2.6359	1.8488	0.1708	0.0670
Oikopleura sp.	0.6740	1.4526	1.1957	1.4412
Chaetognatha				
Sagitta sp.	0.1755	0.3803	0.2216	0.2687
Chordata				
Teleostei	0.0023	0.0033	0.0058	0.0047
Total	100.0000	100.0000	100.0000	100.0000

Table 5A.9. ANOVA table: ln biomass of small (<2.6 mm metasomal length) and large (>2.5 mm total length) calanoid copepods in Prince William Sound, April-June, 1989. Each factor without an associated probability was used as the error term in the significance test for the factors listed above it; df = degrees of freedom, o = oil, b = bay/corridor, l = location, (ob) indicates nesting within oil and bay/corridor, and t = time. Species classified as large were *Calanus marshallae*; *Calanus* sp.; *Eucalanus bungii*; *Heterorhabdus* sp.; *Neocalanus cristatus*; *Metridia okhotensis*; and adult female *Metridia pacifica*. All other calanoids identified in the samples were classified as small.

Source	df	F	*P*
Small Calanoid Copepods			
o	1	0.01	0.938
b	1	7.45	0.053
ob	1	0.40	0.562
l(ob)	4		
t	4	16.80	0.000
to	4	2.25	0.109
tb	4	0.93	0.471
tob	4	0.76	0.564
tl(ob)	16		
Error	79		
Total	118		
Large Calanoid Copepods			
o	1	0.54	0.504
b	1	3.63	0.129
ob	1	0.02	0.888
l(ob)	4		
t	4	22.86	0.000
to	4	0.91	0.481
tb	4	0.76	0.568
tob	4	0.48	0.750
tl(ob)	16		
Error	79		
Total	118		

Table 5A.10. ANOVA table: ln biomass of small (<2.6 mm metasomal length), and large (>2.5 mm total length) calanoid copepods in Prince William Sound, April-June 1990. Each factor without an associated probability was used as the error term in the significance test for the factors listed above it; df = degrees of freedom, o = oil, b = bay/corridor, l = location, (ob) indicates nesting within oil and bay/corridor, and t = time. Species classified as large were *Calanus marshallae*; *Calanus* sp.; *Eucalanus bungii*; *Heterorhabdus* sp.; *Neocalanus cristatus*; *Neocalanus plumchrus*; and adult female *Metridia pacifica*. All other calanoids identified in the samples were classified as small.

Source	df	F	P
Small Calanoid Copepods			
o	1	1.77	0.254
b	1	0.61	0.478
ob	1	0.62	0.445
l(ob)	4		
t	3	2.780	0.087
to	3	2.02	0.165
tb	3	0.49	0.697
tob	3	0.16	0.924
tl(ob)	12		
Error	64		
Total	95		
Large Calanoid Copepods			
o	1	1.81	0.249
b	1	3.95	0.118
ob	1	0.16	0.695
l(ob)	4		
t	3	4.62	0.023
to	3	0.29	0.830
tb	3	2.45	0.113
tob	3	0.69	0.575
tl(ob)	12		
Error	64		
Total	95		

Table 5A.11. ANOVA table: number of taxa of pelagic zooplankton in Prince William Sound, April-June 1989 and 1990. Each factor without an associated probability was used as the error term in the significance test for the factors listed above it; df = degrees of freedom, o = oil, b = bay/corridor, l = location, (ob) indicates nesting within oil and bay/corridor, and t = time.

Source	df	F	*P*
1989			
o	1	0.34	0.593
b	1	5.36	0.082
ob	1	0.35	0.587
l(ob)	4		
t	4	2.11	0.127
to	4	0.88	0.499
tb	4	1.18	0.358
tob	4	2.48	0.085
tl(ob)	16		
Error	79		
Total	118		
1990			
o	1	1.31	0.317
b	1	0.08	0.789
ob	1	3.17	0.100
l(ob)	4		
t	3	3.72	0.042
to	3	0.66	0.593
tb	3	0.09	0.966
tob	3	0.13	0.942
tl(ob)	12		
Error	64		
Total	95		

Table 5A.12. Mean number of species (No.) and standard deviation (s) of pelagic zooplankton by time period, oil, bay/corridor in Prince William Sound in 1989 and 1990.

Time Period	Bay				Corridor			
	Non-oiled		Oiled		Non-oiled		Oiled	
	No.	(s)	No.	(s)	No.	(s)	No.	(s)
1989								
Late April	16.7	(1.8)	15.5	(2.5)	13.0	(3.1)	16.3	(2.7)
Early May	12.8	(3.3)	17.7	(3.0)	13.7	(2.0)	9.8	(0.7)
Late May	16.7	(2.1)	16.3	(2.0)	14.7	(2.0)	12.2	(1.3)
Early June	13.8	(2.6)	15.8	(2.3)	14.0	(2.0)	16.7	(3.7)
Late June	17.7	(2.2)	16.0	(1.9)	16.5	(2.7)	7.0	(2.0)
1990								
Late April	13.3	(4.1)	17.3	(2.2)	14.8	(4.0)	14.2	(1.2)
Early May	13.7	(6.8)	20.3	(2.4)	15.2	(1.5)	17.5	(2.5)
Late May	17.8	(5.0)	21.2	(3.1)	19.2	(1.2)	18.0	(2.4)
Early June	18.2	(2.4)	19.7	(2.7)	19.3	(3.5)	19.3	(2.3)

Table 5A.13. Density (no/m^3) of epibenthos by habitat collected from four pairs of non-oiled and oiled locations in Prince William Sound, Alaska, April-June 1989, in the systematic epibenthic sled samples. Habitat designations are LG (low gradient), MG (medium gradient) and SG (steep gradient).

Time Period	Non-oiled locations LG	MG	SG	Oiled locations LG	MG	SG
			Bays			
McClure Bay-Herring Bay						
Late April	21	3613	127	1657	167	415
Early May	75	217	85	378	301	831
Late May	26	51	--	250	73	1093
Early June	42	18	11	725	2390	1071
Late June	613	997	9	1882	--	414
Mean	155	979	58	978	733	765
Long Bay-Snug Harbor						
Late April	1122	1333	169	361	197	663
Early May	169	1637	22	11027	815	106
Late May	311	55	6	229	237	63
Early June	4421	2999	242	1349	1162	150
Late June	713	125	146	1290	--	66
Mean	1347	1230	117	2851	603	210
			Corridors			
Culross Passage-Prince of Wales Passage						
Late April	66	666	171	1173	5573	165
Early May	278	1746	114	1642	527	119
Late May	710	6367	262	6861	4066	319
Early June	35	--	454	1397	211	54
Late June	55	--	311	2439	578	31
Mean	229	2926	262	2702	2191	138
Wells Passage-Knight Island Passage						
Late April	3066	207	253	205	725	290
Early May	853	613	170	1759	1986	95
Late May	1621	2490	71	7621	4967	1419
Early June	327	746	3	--	4816	709
Late June	1882	171	49	197	--	389
Mean	1550	845	109	2446	3124	580

Table 5A.14. Biomass (g/m^3) of epibenthos by habitat collected from four pairs of non-oiled and oiled locations in Prince William Sound, Alaska, April-June 1989, in the systematic epibenthic sled samples. Habitat designations are LG (low gradient), MG (medium gradient) and SG (steep gradient).

Time Period	Non-oiled locations LG	MG	SG	Oiled locations LG	MG	SG
			Bays			
McClure Bay-Herring Bay						
Late April	0	140	12	168	6	58
Early May	2	5	10	15	113	34
Late May	1	1	--	9	740	4957
Early June	1	1	1	237	684	253
Late June	5	21	0	842	--	83
Mean	2	33	4	254	386	1077
Long Bay-Snug Harbor						
Late April	32	43	4	74	28	115
Early May	9	37	4	422	40	38
Late May	15	1	0	214	12	4
Early June	84	55	4	43	55	4
Late June	12	1	2	39	--	16
Mean	30	27	3	158	34	35
			Corridors			
Culross Passage-Prince of Wales Passage						
Late April	1	16	4	29	344	14
Early May	17	144	7	115	32	8
Late May	31	465	23	552	541	43
Early June	1	--	8	50	7	6
Late June	1	--	4	201	28	1
Mean	10	208	9	189	190	14
Wells Passage-Knight Island Passage						
Late April	259	6	33	33	50	46
Early May	77	57	11	107	151	14
Late May	58	94	4	1120	395	142
Early June	8	22	0	--	132	40
Late June	70	7	3	6	--	31
Mean	94	37	10	317	182	55

Table 5A.15. Percent density and biomass of organisms of epibenthic and pelagic origin captured by the epibenthic sled in Prince William Sound from April to June, 1989. Whether the organism is a potential prey item of juvenile salmon is also indicated.

Organism	Percent Density	Percent Biomass	Prey Item
EPIBENTHIC ORIGIN			
Cnidaria			
Hydroida	0.4424	0.3036	no
Platyhelminthes			
Turbellaria	0.1093	0.0131	no
Nematoda			
Nematode general	2.3984	0.0961	no
Annelida			
Oligochaeta	0.0254	0.0081	no
Mollusca			
Mollusk general	0.0163	0.0519	no
Mytilus sp.	0.0280	0.0444	no
Arthropoda			
Halacaridae	0.6315	0.0432	yes
Nematocera	0.0036	0.0063	yes
Chironomidae	0.3488	0.2387	yes
Collembola	0.0066	0.0004	yes
Copepoda			
Halicyclops sp.	0.0005	0.0000	no
Harpacticoida			
Harpacticoid general	0.4149	0.0081	yes
Alteutha sp.	0.0084	0.0000	no
Amonardia sp.	0.1354	0.0224	yes
Amphiascoides sp.	0.0168	0.0000	yes
Amphiascopsis sp.	0.3020	0.0709	no
Amphiascus sp.	0.2863	0.0061	no
Danielssenia sp.	0.0076	0.0000	yes
Diosaccus sp.	5.8165	1.2962	yes
Harpacticus sp.	13.9517	4.0891	yes
Mesochra sp.	0.0331	0.0000	yes
Microarthridion sp.	0.0010	0.0000	yes
Paralteutha sp.	0.0056	0.0046	no
Paramphiascella sp.	0.1083	0.0327	no
Parastenhelia sp.	0.1088	0.0000	yes
Porcellidium sp.	0.0041	0.0000	no
Pseudonychocamptus sp.	0.0158	0.0004	no
Robertsonia sp.	0.0341	0.0010	yes
Scutellidium sp.	0.6739	0.0796	yes
Stenhelia sp.	0.0036	0.0000	yes
Tisbe sp.	11.7791	1.7109	yes
Zaus sp.	0.5030	0.0316	yes

Table 5A.15. Continued

Organism	Percent Density	Percent Biomass	Prey Item
Ameiridae	0.0557	0.0000	yes
Ameira sp.	0.0203	0.0000	no
Cletodidae	0.0005	0.0000	yes
Huntemannia sp.	0.0051	0.0000	yes
Ectinosomatidae	0.1807	0.0017	yes
Microsetella sp.	0.0010	0.0000	no
Laophontidae	0.7639	0.0183	yes
Echinolaophonte sp.	0.2464	0.0190	yes
Heterolaophonte sp.	2.6611	0.2087	yes
Laophonte sp.	0.0076	0.0006	yes
Laophontodes sp.	0.0158	0.0000	no
Paralaophonte	1.3371	0.0694	yes
Tegastidae	0.0086	0.0000	no
Tegastes sp.	0.0015	0.0000	no
Thalestridae	0.0061	0.0008	yes
Dactylopodia sp.	0.8264	0.0648	yes
Diarthrodes sp.	0.0102	0.0000	yes
Idomene sp.	0.0107	0.0000	no
Paradactylopodia sp.	0.0633	0.0002	yes
Parathalestris sp.	0.1481	0.0607	yes
Rhyncothalestris sp.	0.0010	0.0000	no
Thalestris sp.	0.0025	0.0002	no
Ostracoda			
Podocopa	1.1925	0.1581	yes
Malacostraca			
Cumella sp.	0.2609	0.1737	yes
Mysidacea	0.0020	0.0008	yes
Euphausiacea	0.1142	0.0088	yes
Isopoda			
Epicaridea	0.0168	0.0006	no
Gnorimosphaeroma sp.	0.0005	0.0006	no
Ianiropsis sp.	0.0041	0.0000	no
Munna sp.	0.0005	0.0002	no
Amphipoda			
Gammaridea			
Gammarid general	0.1141	0.0265	yes
Allorchestes sp.	0.0290	0.1113	yes
Ischyrocerus sp.	0.0651	0.0156	yes
Megamphopus sp.	0.0112	0.0029	yes
Paramoera sp.	0.0824	0.2414	yes
Pleustes sp.	0.0005	0.0021	yes
Pontogeneia sp.	0.0168	0.0605	yes
Synchelidium sp.	0.0102	0.0000	yes
Ampithoidae	0.0071	0.0158	yes
Ampithoe sp.	0.0092	0.0869	yes

Table 5A.15. Continued

Organism	Percent Density	Percent Biomass	Prey Item
Calliopiidae	0.0224	0.0129	yes
Calliopius sp.	0.0165	0.0710	yes
Paracalliopiella sp.	0.2809	0.3907	yes
Gammaridae	0.0163	0.0077	yes
Stenothoidae	0.0005	0.0000	yes
Decapoda			
Brachyura	0.0005	0.0065	yes
Cancer sp.	0.0010	0.0171	yes
Pleocyemata-Caridea	0.0112	0.0208	no
Heptacarpus sp.	0.0961	13.5913	no
Pandalus sp.	0.0005	0.7248	no
Paguridae	0.0005	0.0033	no
Echinodermata	0.0081	0.0000	no
Epibenthic subtotal	46.9859	24.3557	
PELAGIC ORIGIN			
Cnidaria			
Scyphozoa	0.0025	0.0025	no
Rotifera	0.0813	0.0000	no
Annelida			
Polychaeta	0.5598	0.3075	yes
Polynoidae	0.0439	0.0127	yes
Mollusca			
Bivalvia	0.8665	0.4829	yes
Gastropoda	0.3758	0.0934	yes
Archaeogastropoda	0.0910	0.3824	yes
Mesogastropoda	0.7292	1.1876	yes
Lacuna sp.	0.0066	0.2585	yes
Littorina sp.	4.6362	0.5765	yes
Opisthobranchia	0.0051	0.0042	yes
Gymnosomata	0.0219	0.1165	no
Thecosomata	0.1074	0.0769	yes
Limacina sp.	0.1135	1.7372	yes
Arthropoda			
Cladocera			
Evadne sp.	0.1213	0.0052	yes
Podon sp.	0.0712	0.0019	yes
Copepoda			
Calanoida			
Calanoid general	2.1776	0.2326	yes
Acartia sp.	0.8958	0.1444	yes
Centropages sp.	0.2036	0.0434	yes
Epilabidocera sp.	0.0020	0.0004	yes
Eucalanus sp.	0.0071	0.0071	yes

Table 5A.15. Continued

Organism	Percent Density	Percent Biomass	Prey Item
Eurytemora sp.	7.5643	0.7931	yes
Metridia sp.	0.1256	0.0813	yes
Neocalanus sp.	8.3888	53.3785	yes
Paracalanus sp.	0.0073	0.0000	yes
Pseudocalanus sp.	15.1919	5.1085	yes
Calanidae	0.9774	0.3524	yes
Calanus sp.	2.9014	8.2628	yes
Stephidae	0.0005	0.0000	yes
Cyclopoida			
Oithona sp.	1.4568	0.0388	no
Poecilostomatoida	0.4321	0.0207	no
Oncaea sp.	0.0005	0.0000	no
Monstrilloida	0.1541	0.0125	yes
Cirripedia			
Balanomorpha	2.5929	1.2285	yes
Malacostraca			
Amphipoda			
Caprellidea	0.0254	0.0088	yes
Decapoda			
Brachyura	0.0056	0.0044	yes
Bryozoa			
Gymnolaemata	1.5659	0.0423	yes
Urochordata			
Larvacea	0.0041	0.0000	yes
Fritillaria sp.	0.1246	0.0025	yes
Oikopleura sp.	0.3244	0.3522	yes
Chaetognatha			
Chaetognath general	0.0320	0.0113	yes
Chordata			
Teleostei	0.0193	0.2719	yes
Pelagic subtotal	53.0141	75.6443	
Total	100.0000	100.0000	

Table 5A.16. ANOVA table: ln biomass of epibenthic harpacticoid copepods captured in the systematic epibenthic sled samples in Prince William Sound, April-June, 1989. Each factor without an associated probability was used as the error term in the significance test for the factors listed above it; df = degrees of freedom, o = oil, b = bay/corridor, l = location, (ob) = nesting within oil and bay/corridor, t = time, and h = habitat.

Source	df	F	*P*
o	1	12.25	0.025
b	1	7.02	0.057
ob	1	1.25	0.325
l(ob)	4		
t	4	2.74	0.066
to	4	1.48	0.256
tb	4	6.60	0.002
tob	4	0.43	0.788
tl(ob)	16		
h	2	6.01	0.025
oh	2	1.25	0.337
bh	2	0.01	0.991
obh	2	0.55	0.595
hl(ob)	8		

Table 5A.17. ANOVA table: ln biomass of epibenthic harpacticoid copepods captured in the tidal transect epibenthic sled samples in Prince William Sound, April-June, 1989. Each factor without an associated probability was used as the error term in the significance test for the factors listed above it; df = degrees of freedom, o = oil, l = location, (o) = nesting within oil, t = time, h = habitat, and r = tide level.

Source	df	F	P
o	1	45.60	0.021
l(o)	2		
t	3	0.49	0.705
to	3	0.20	0.893
tl(o)	6		
h	2	0.59	0.596
oh	2	0.08	0.927
hl(o)	4		
th	6	1.60	0.230
toh	6	1.06	0.438
thl(o)	12		
r	4	25.97	0.000
or	4	15.99	0.001
rl(o)	8		
tr	12	0.59	0.830
tor	12	0.24	0.993
trl(o)	24		
hr	8	1.37	0.282
ohr	8	0.59	0.775
rhl(o)	16		
thr	24	2.07	0.016
tohr	24	1.24	0.255
thrl(o)	48		

Table 5A.18. ANOVA table: number of epibenthic taxa captured in the systematic epibenthic sled samples in Prince William Sound, June-April, 1989. Each factor without an associated probability was used as the error term in the significance test for the factors listed above it; df = degrees of freedom, o = oil, b = bay/corridor, l = location, (ob) = nesting within oil and bay/corridor, t = time, and h = habitat.

Source	df	F	*P*
o	1	1.34	0.312
b	1	0.06	0.824
ob	1	1.23	0.330
l(ob)	4		
t	4	9.71	0.000
to	4	3.08	0.047
tb	4	1.68	0.204
tob	4	2.15	0.122
tl(ob)	16		
h	2	7.37	0.015
oh	2	0.12	0.886
bh	2	0.43	0.665
obh	2	0.75	0.503
hl(ob)	8		

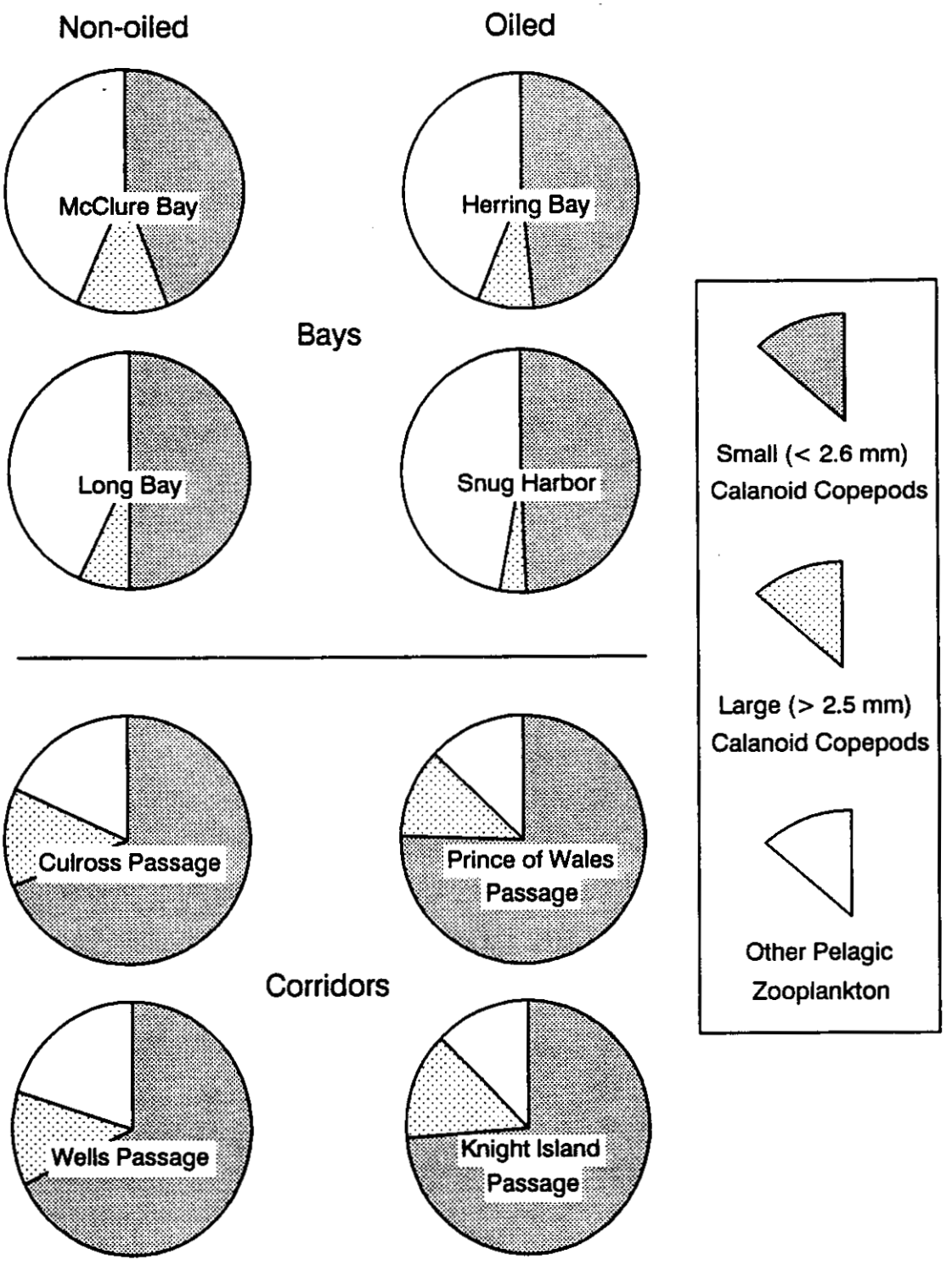

Figure 5A.1. Relative density of pelagic zooplankton from four pairs of oiled and non-oiled locations in Prince William Sound, April-June 1989.

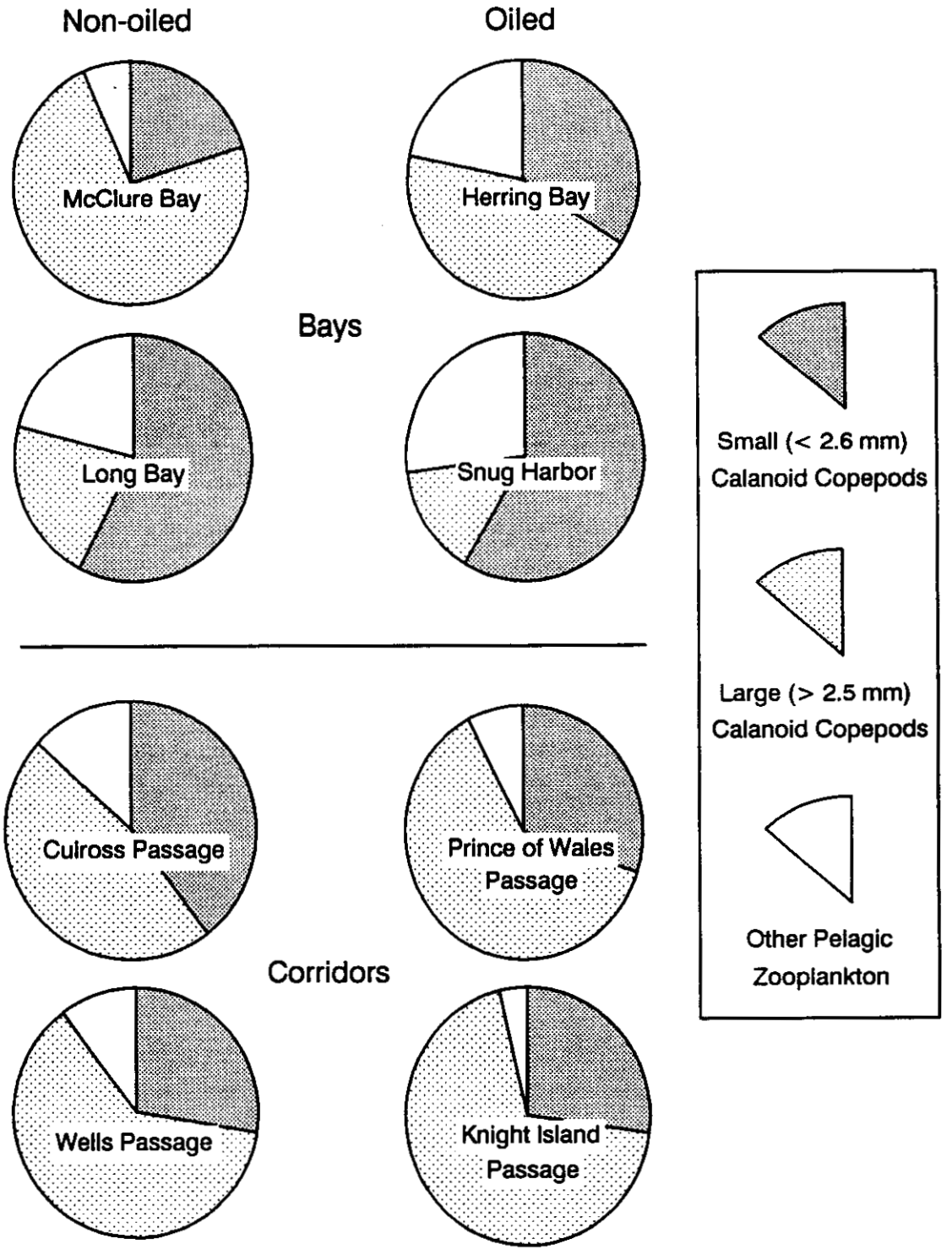

Figure 5A.2. Relative biomass of pelagic zooplankton from four pairs of oiled and non-oiled locations in Prince William Sound, April-June 1989.

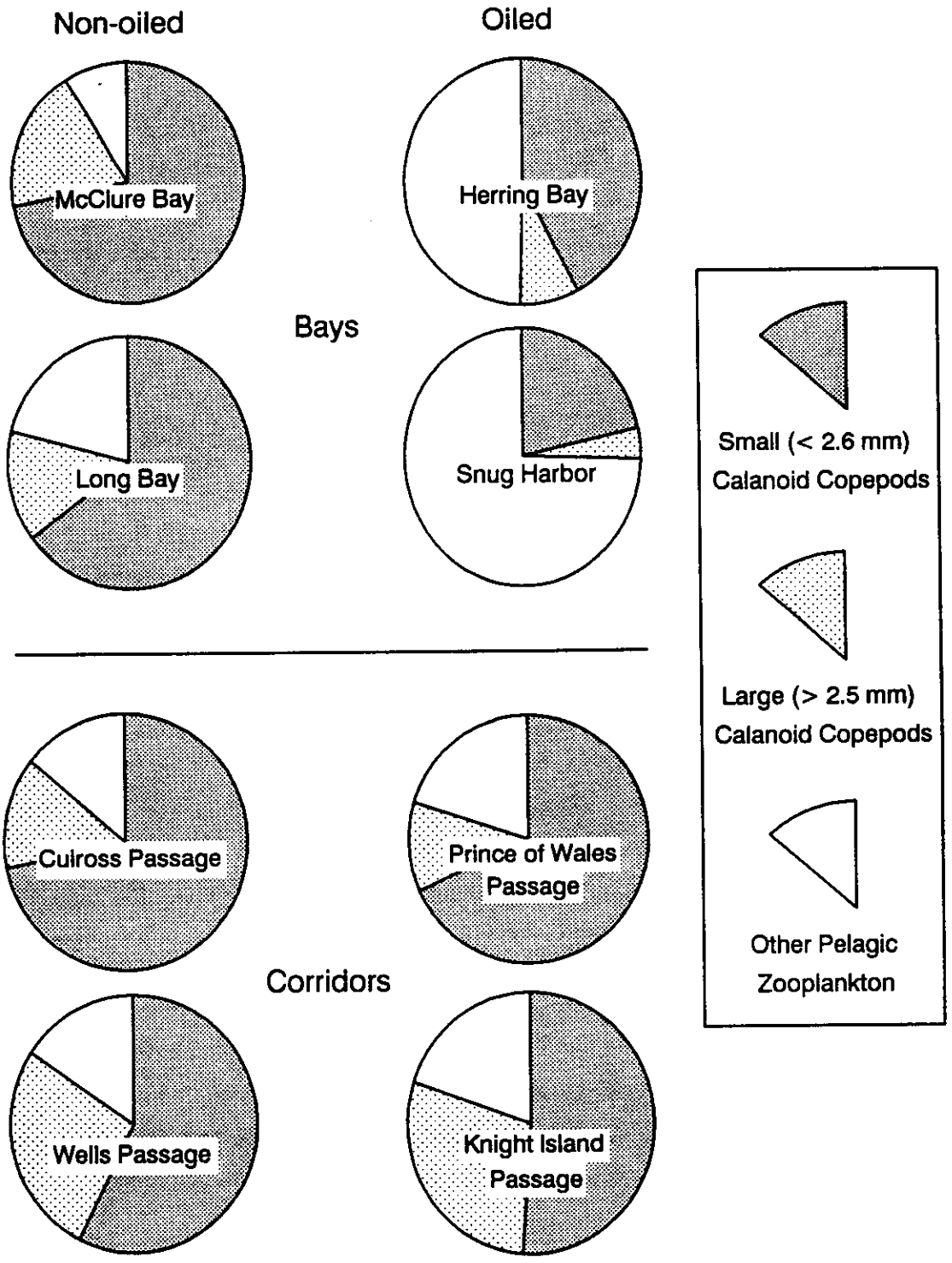

Figure 5A.3. Relative density of pelagic zooplankton from four pairs of oiled and non-oiled locations in Prince William Sound, April-June 1990.

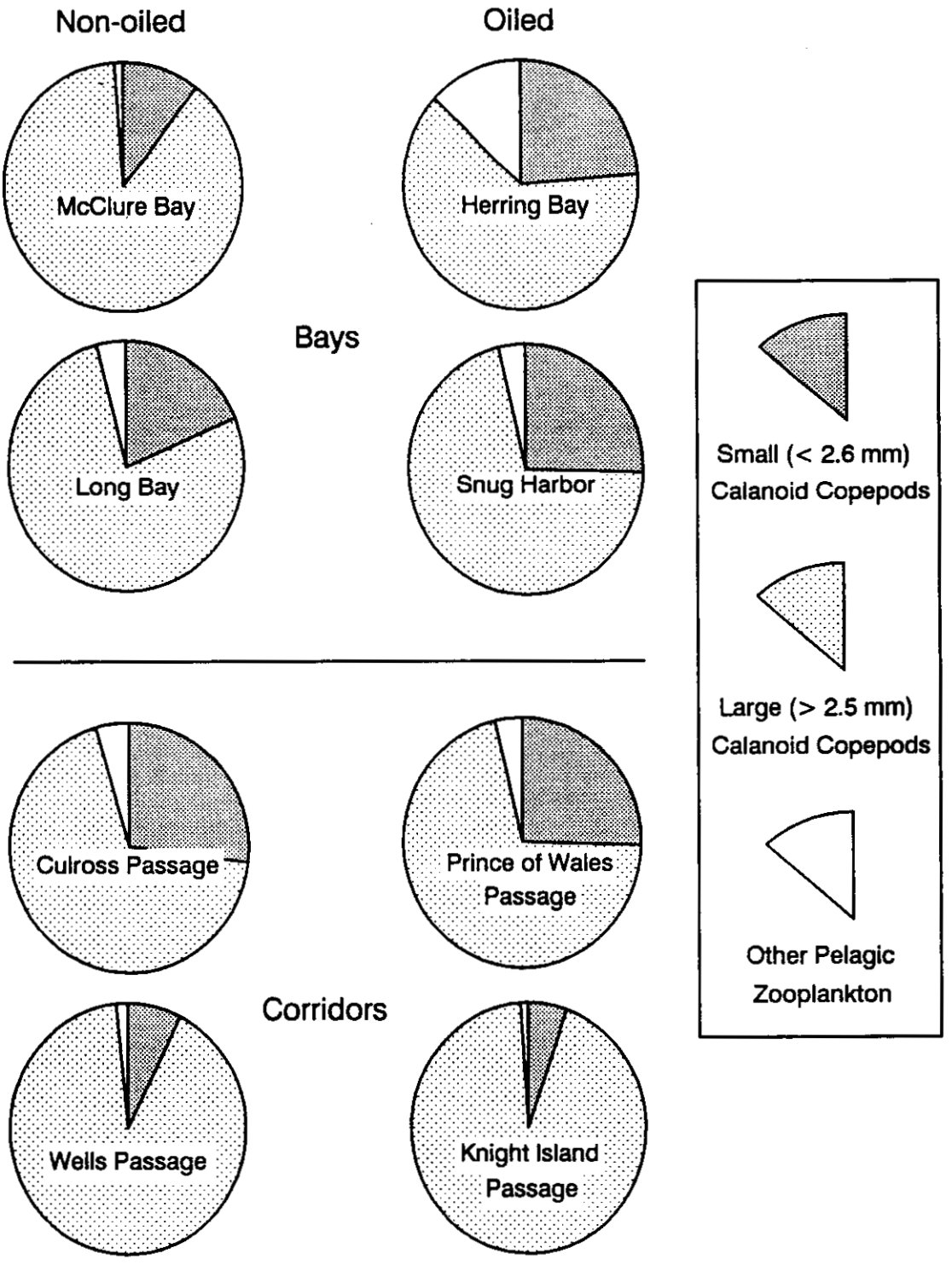

Figure 5A.4. Relative biomass of pelagic zooplankton from four pairs of oiled and non-oiled locations in Prince William Sound, April-June 1990.

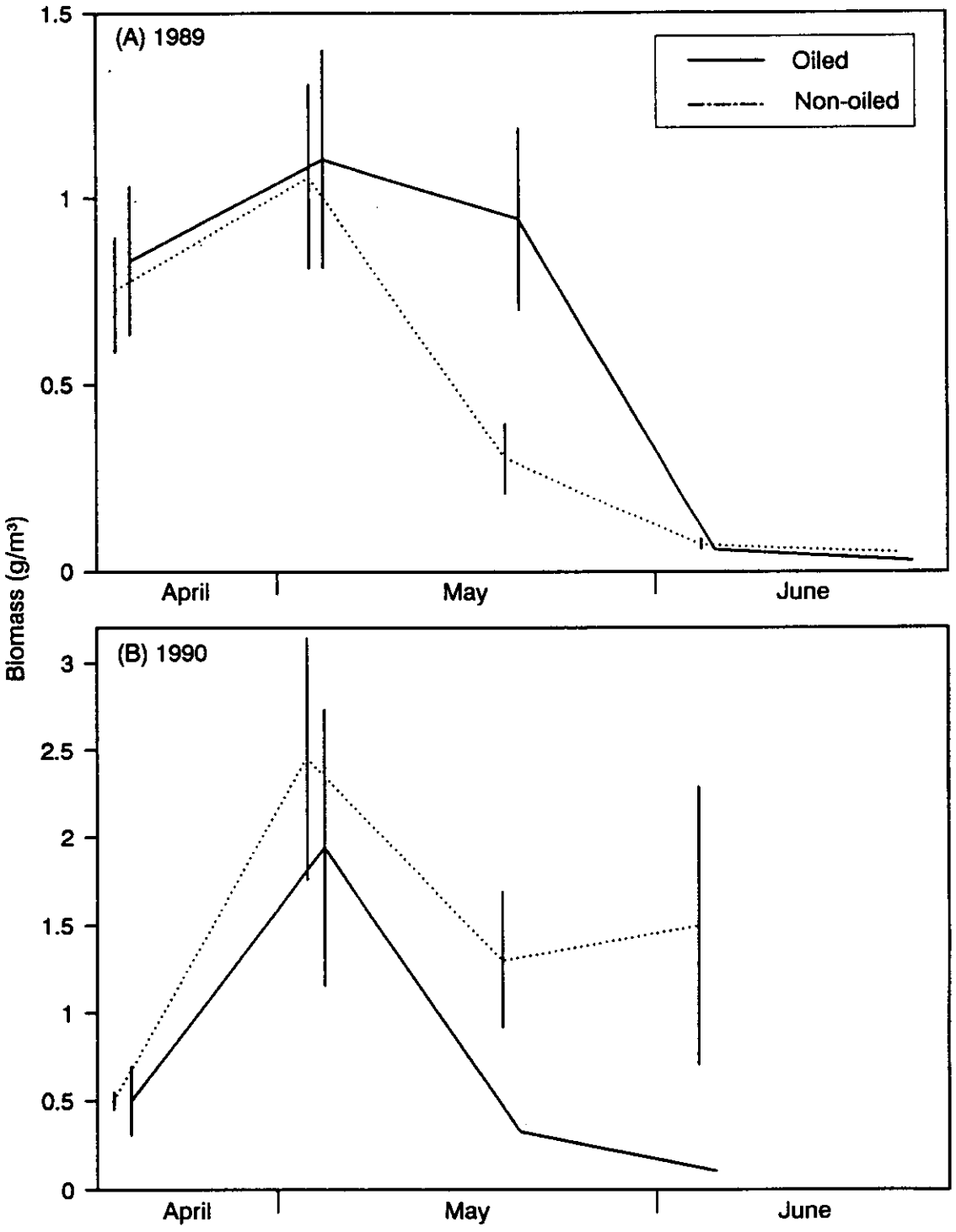

Figure 5A.5. Mean biomass (±1 SE) of pelagic zooplankton in oiled and non-oiled locations of Prince William Sound in 1989 (A) and 1990 (B).

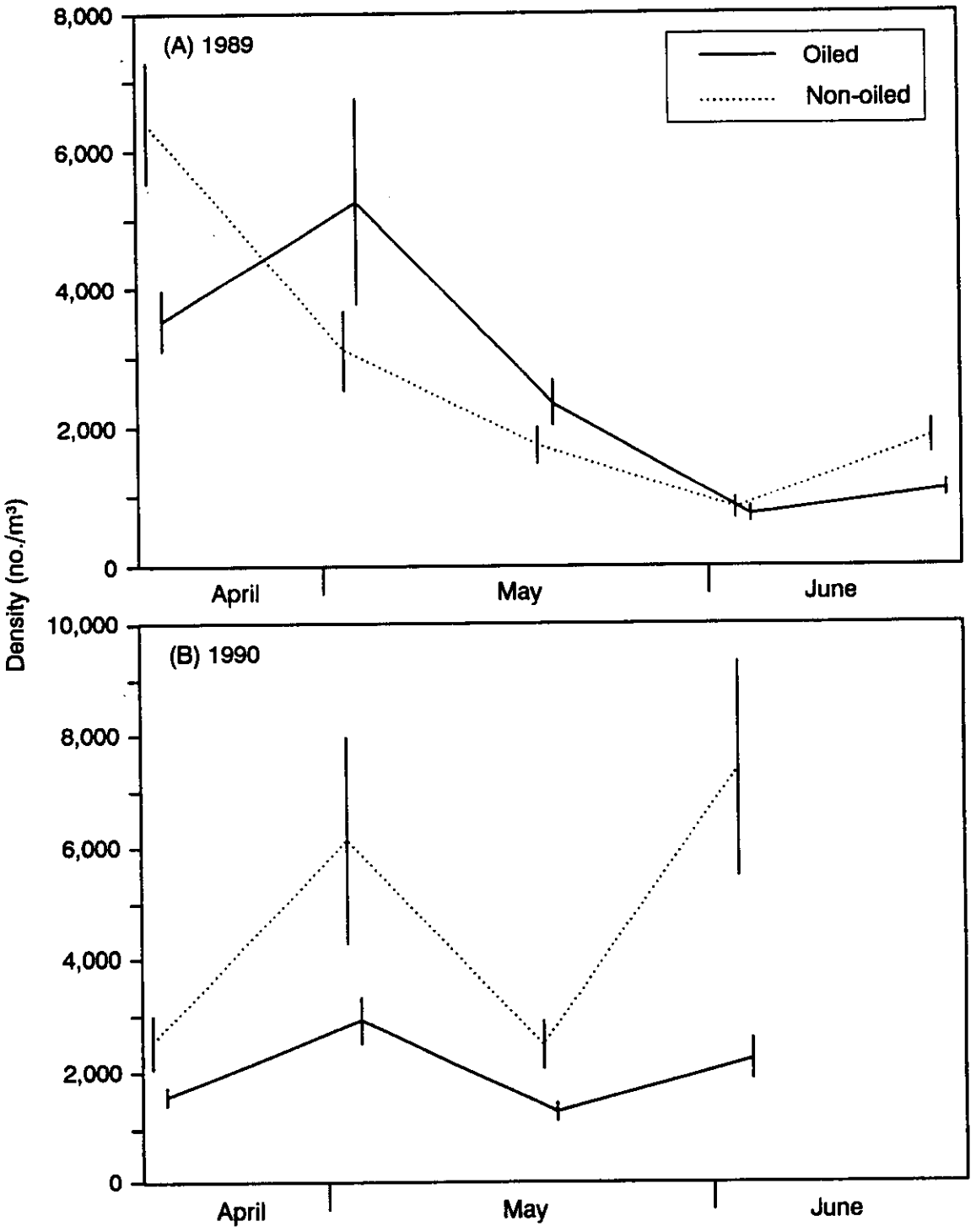

Figure 5A.6. Mean density (±1 SE) of pelagic zooplankton in oiled and non-oiled locations of Prince William Sound in 1989 (A) and 1990 (B).

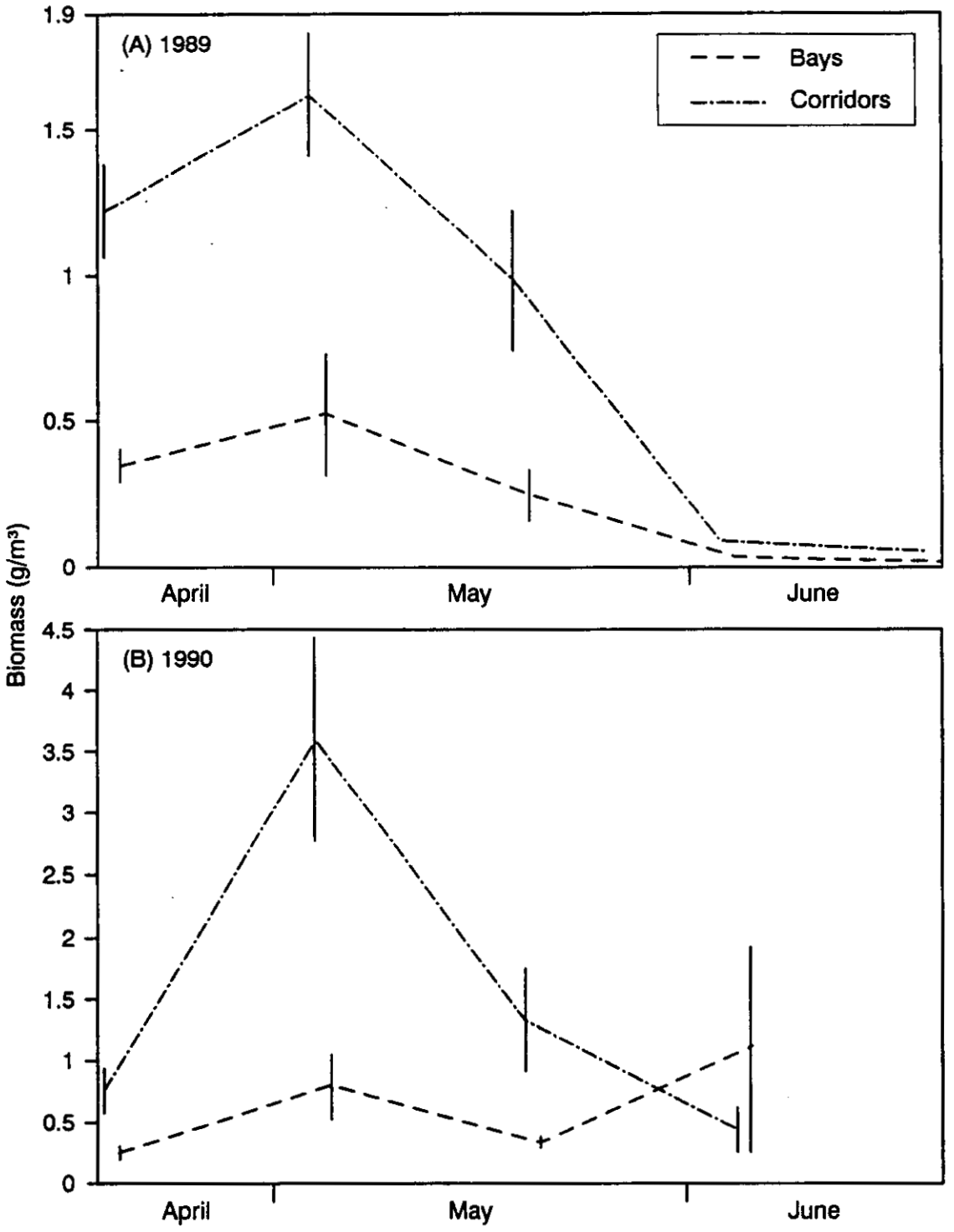

Figure 5A.7. Mean biomass (±1 SE) of pelagic zooplankton in bays and corridors of Prince William Sound in 1989(A) and 1990(B).

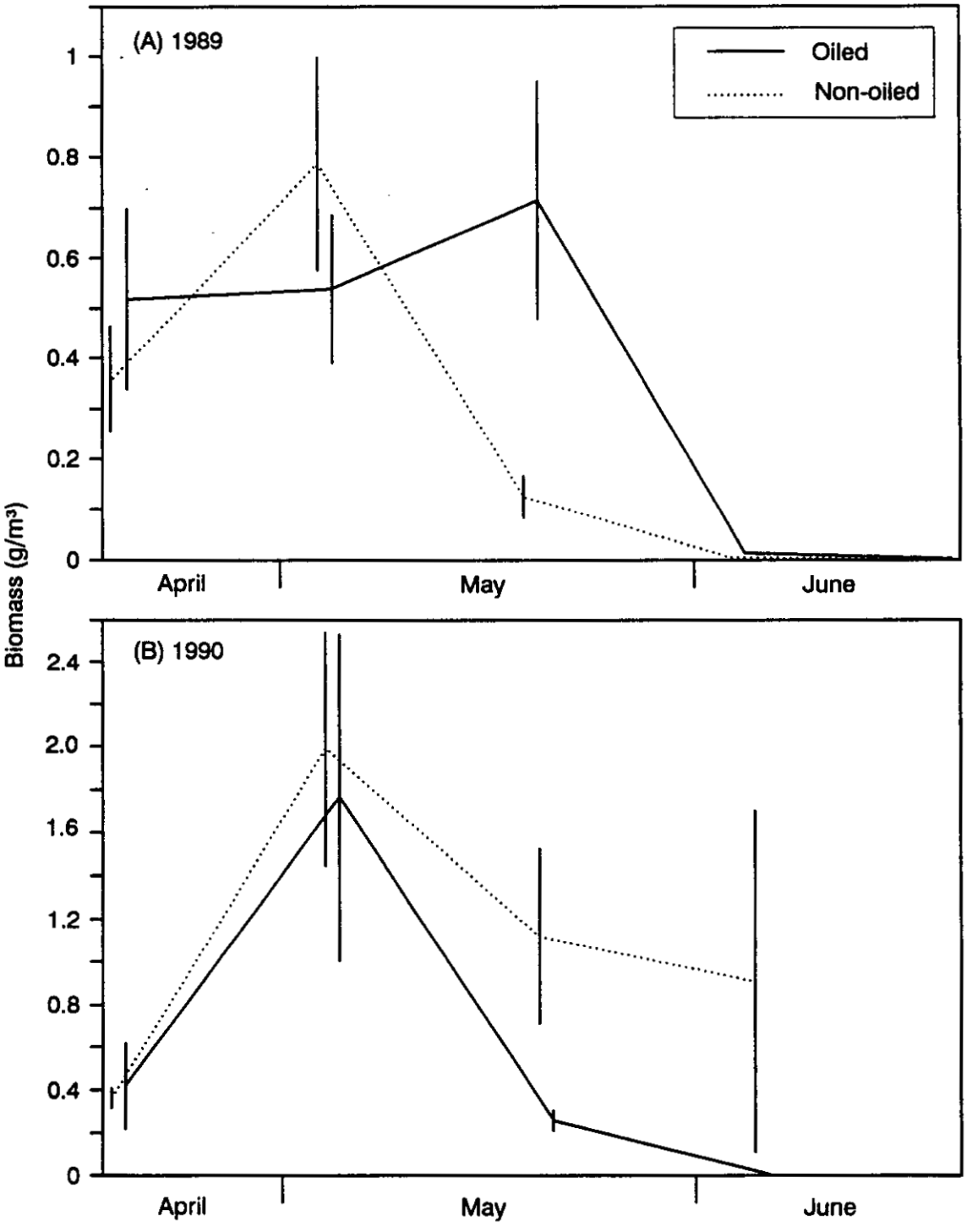

Figure 5A.8. Mean biomass (±1 SE) of large calanoid copepods in oiled and non-oiled locations of Prince William Sound in 1989 (A) and 1990 (B).

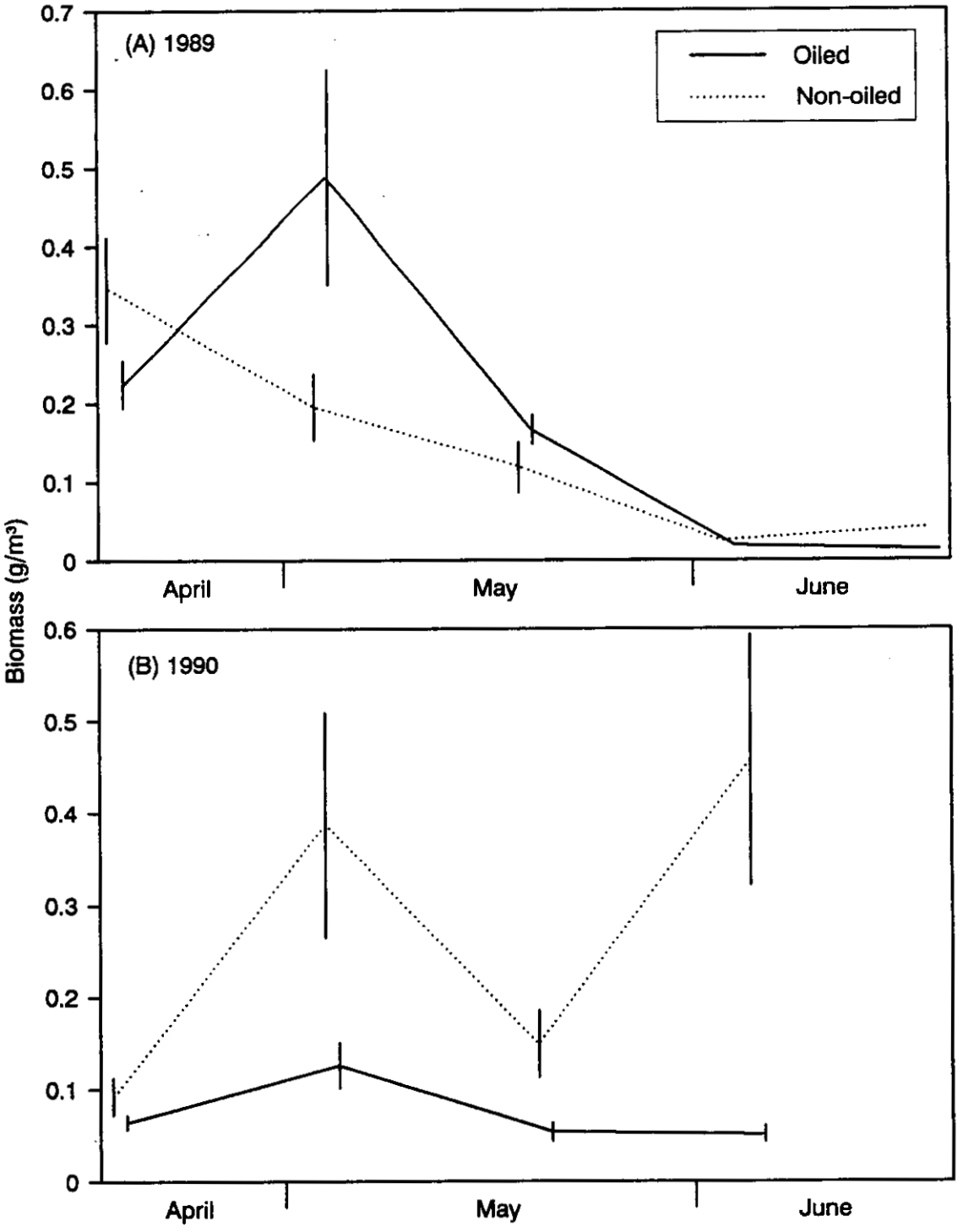

Figure 5A.9. Mean biomass (±1 SE) of small calanoid copepods in oiled and non-oiled locations of Prince William Sound in 1989 (A) and 1990 (B).

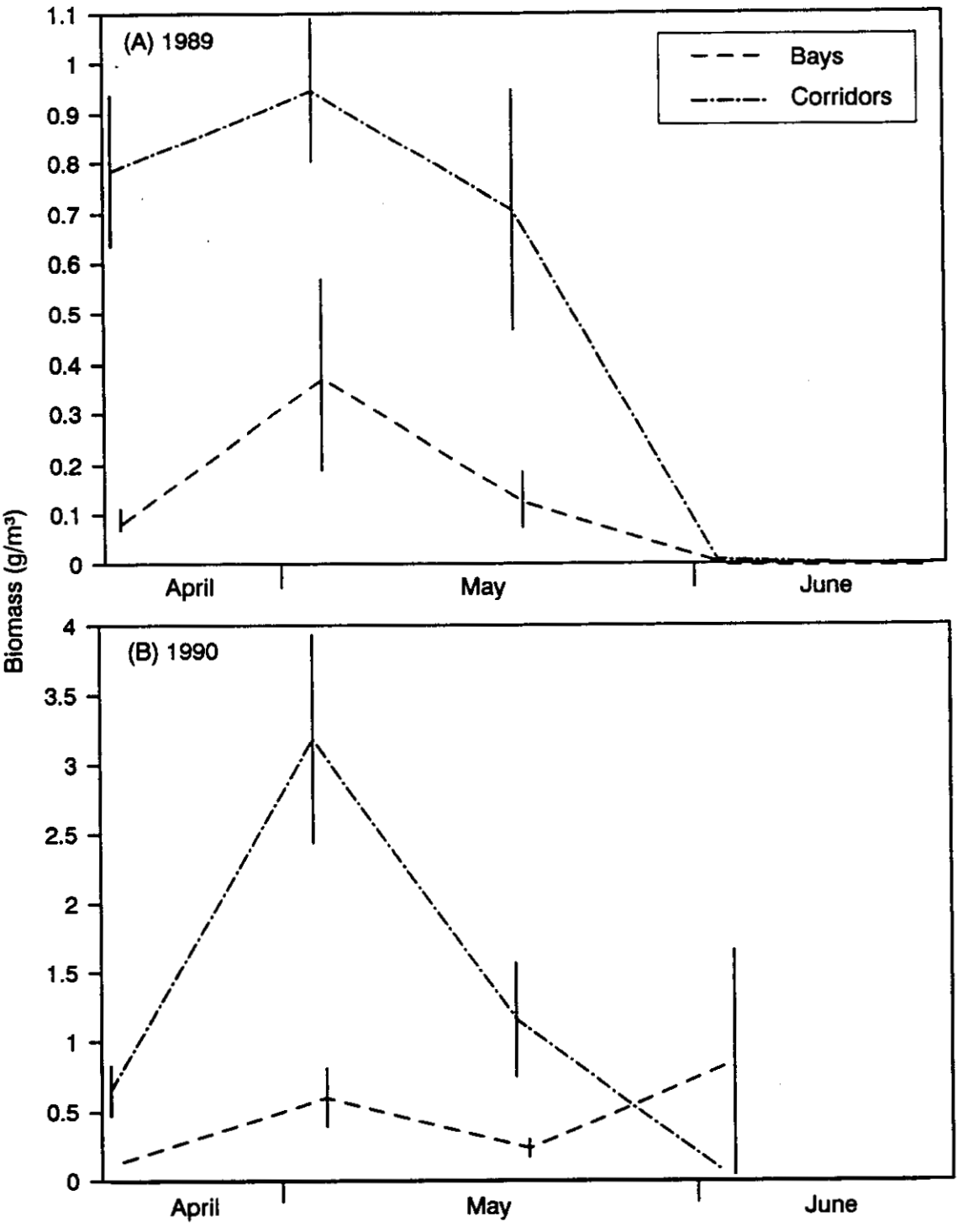

Figure 5A.10. Mean biomass (±1 SE) of large calanoid copepods in bays and corridors of Prince William Sound in 1989 (A) and 1990 (B).

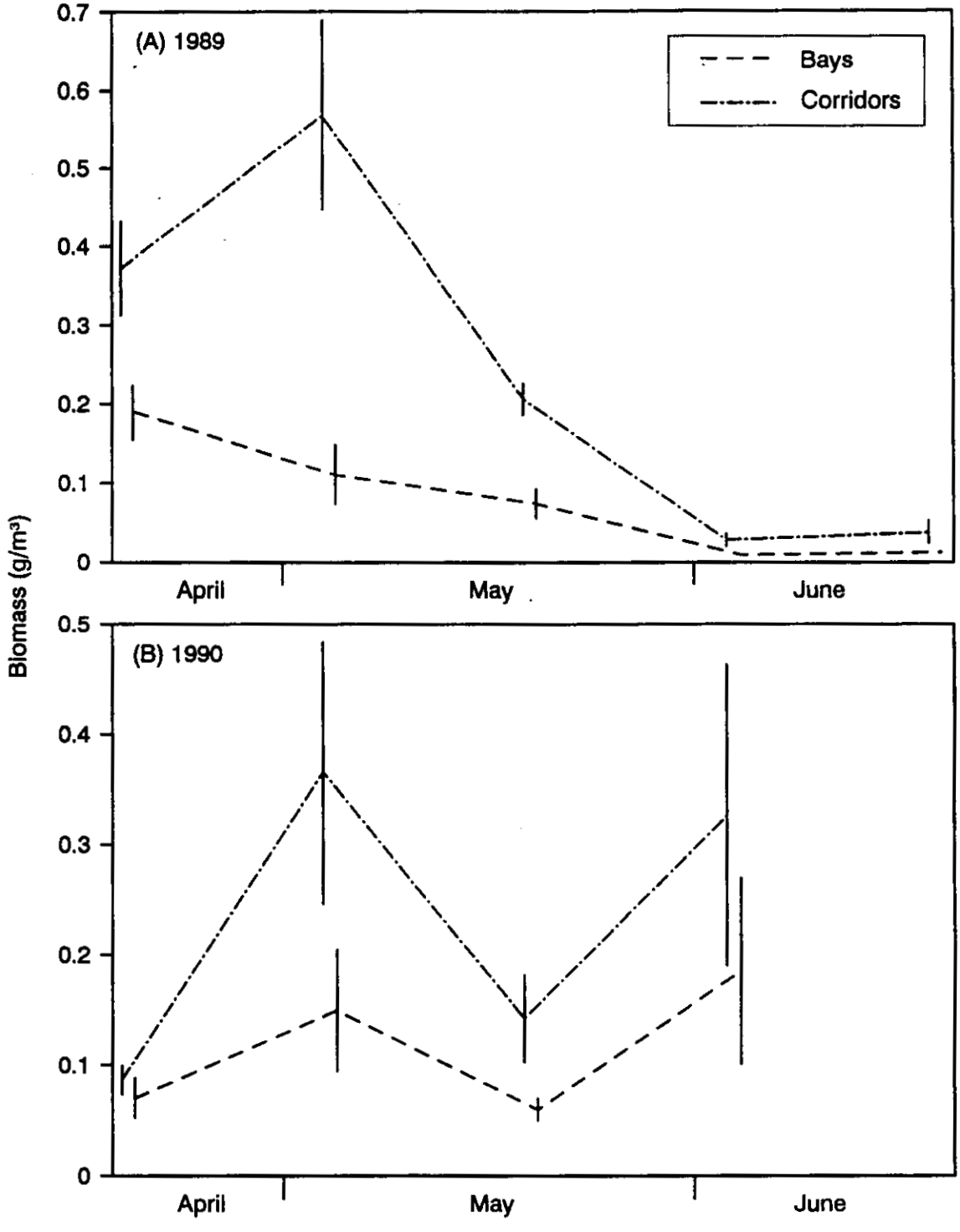

Figure 5A.11. Mean biomass (±1 SE) of small calanoid copepods in bays and corridors of Prince William Sound in 1989 (A) and 1990 (B).

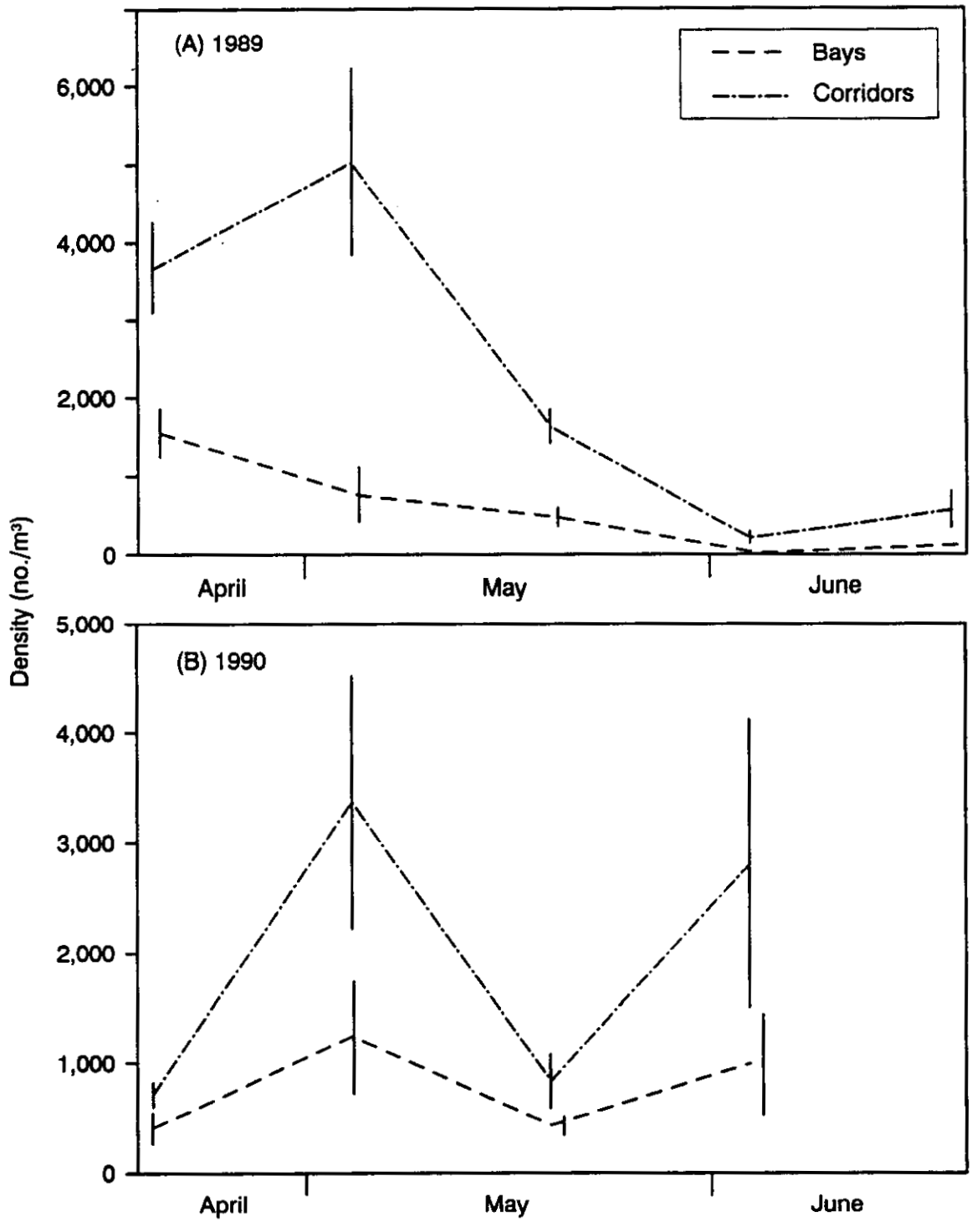

Figure 5A.12. Mean density (±1 SE) of Pseudocalanus sp. in bays and corridors of Prince William Sound in 1989 (A) and 1990(B).

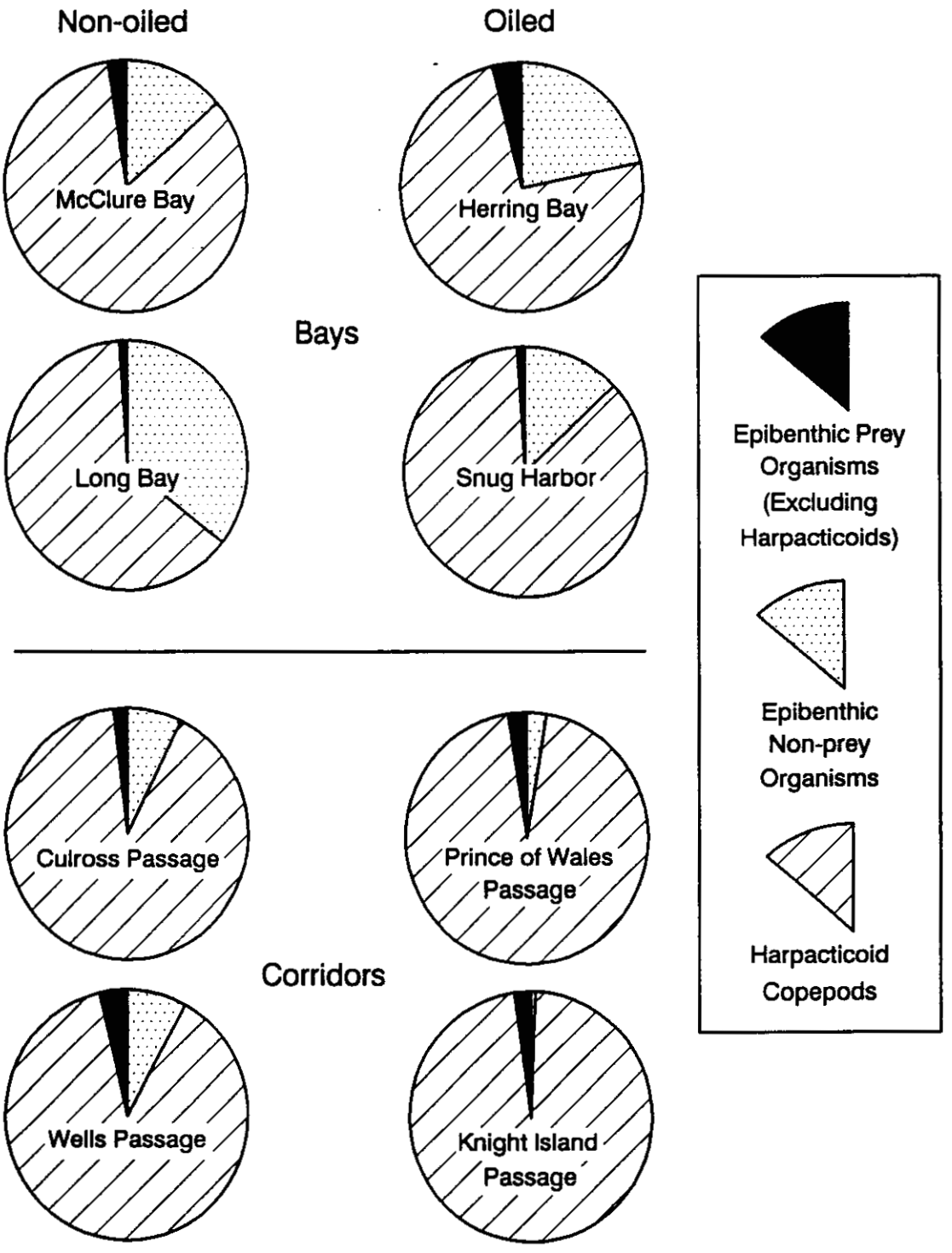

Figure 5A.13. Relative density of epibenthic crustaceans in the epibenthic sled systematic samples from four pairs of oiled and non-oiled locations in Prince William Sound, April-June 1989.

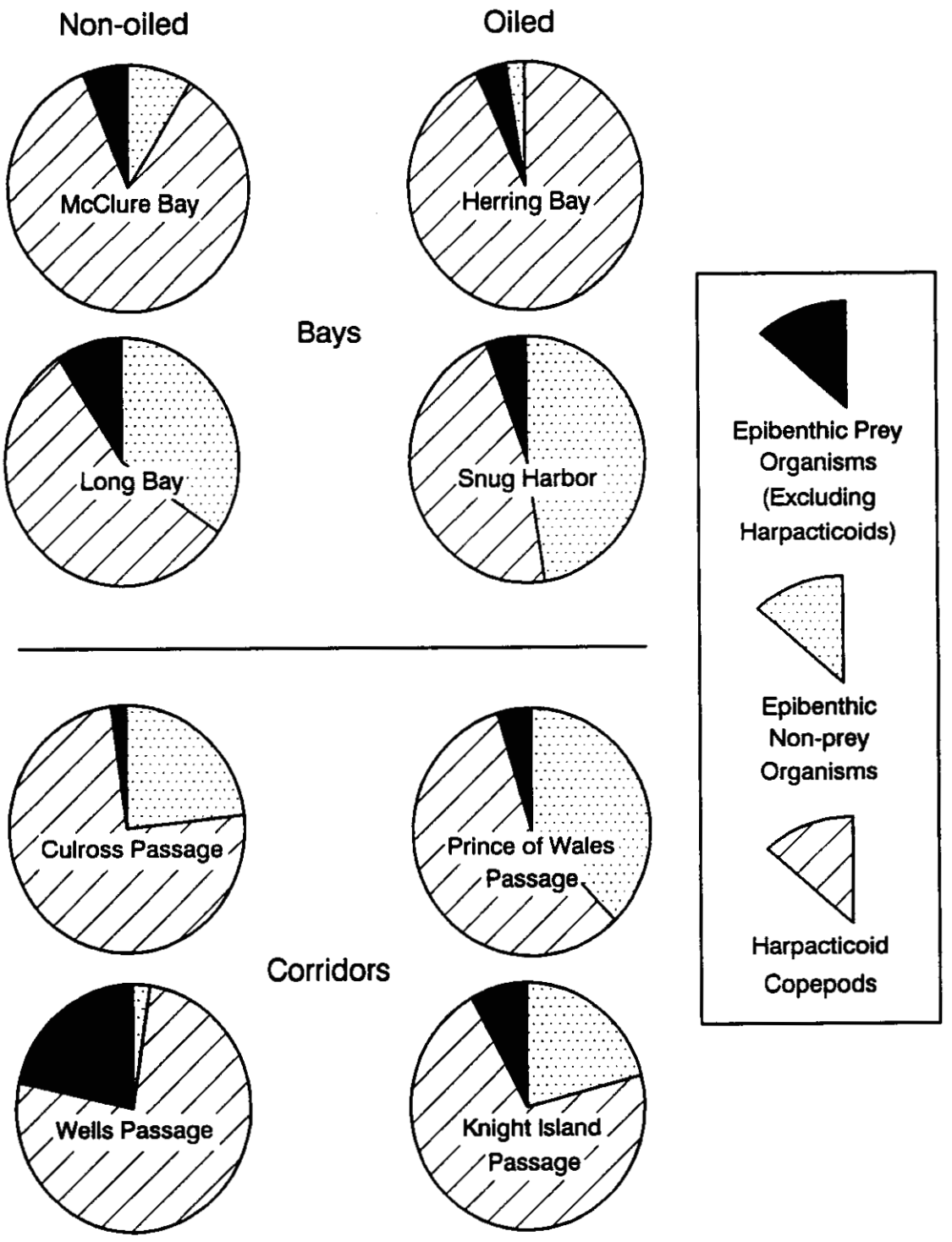

Figure 5A.14. Relative biomass of epibenthic crustaceans in the epibenthic sled systematic samples from four pairs of oiled and non-oiled locations in Prince William Sound, April-June 1989.

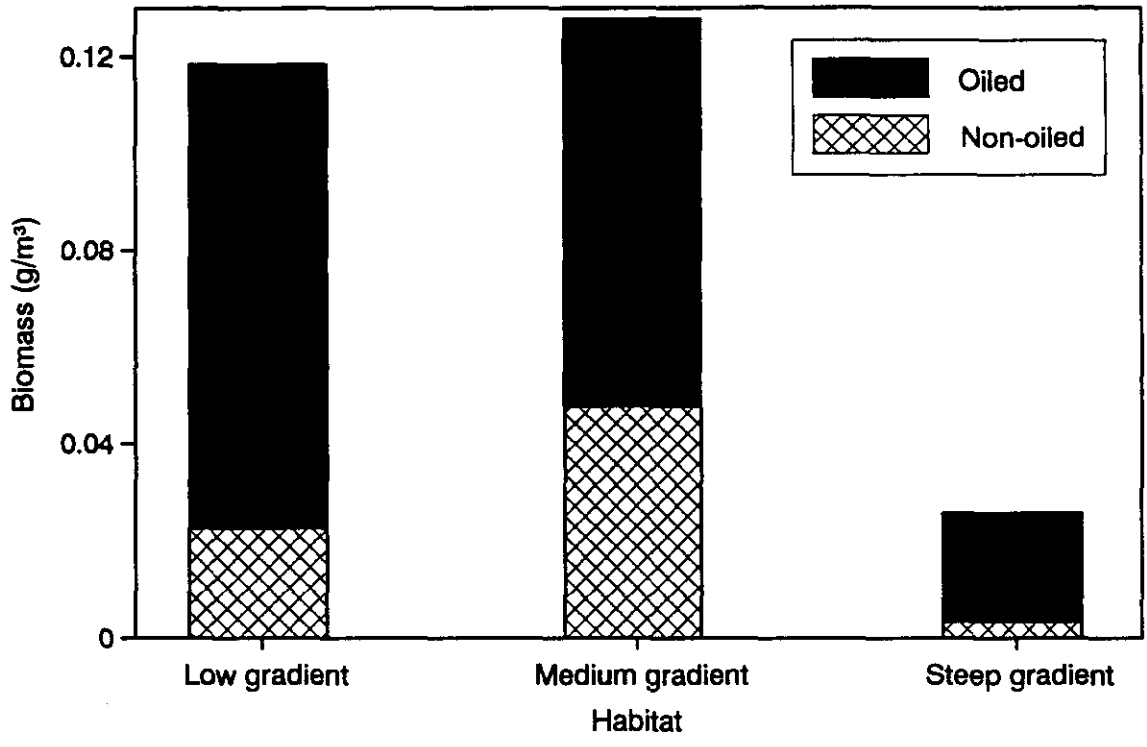

Figure 5A.15. Harpacticoid copepod mean biomass in the systematic epibenthic sled samples in oiled and non-oiled habitats in Prince William Sound, April-June 1989.

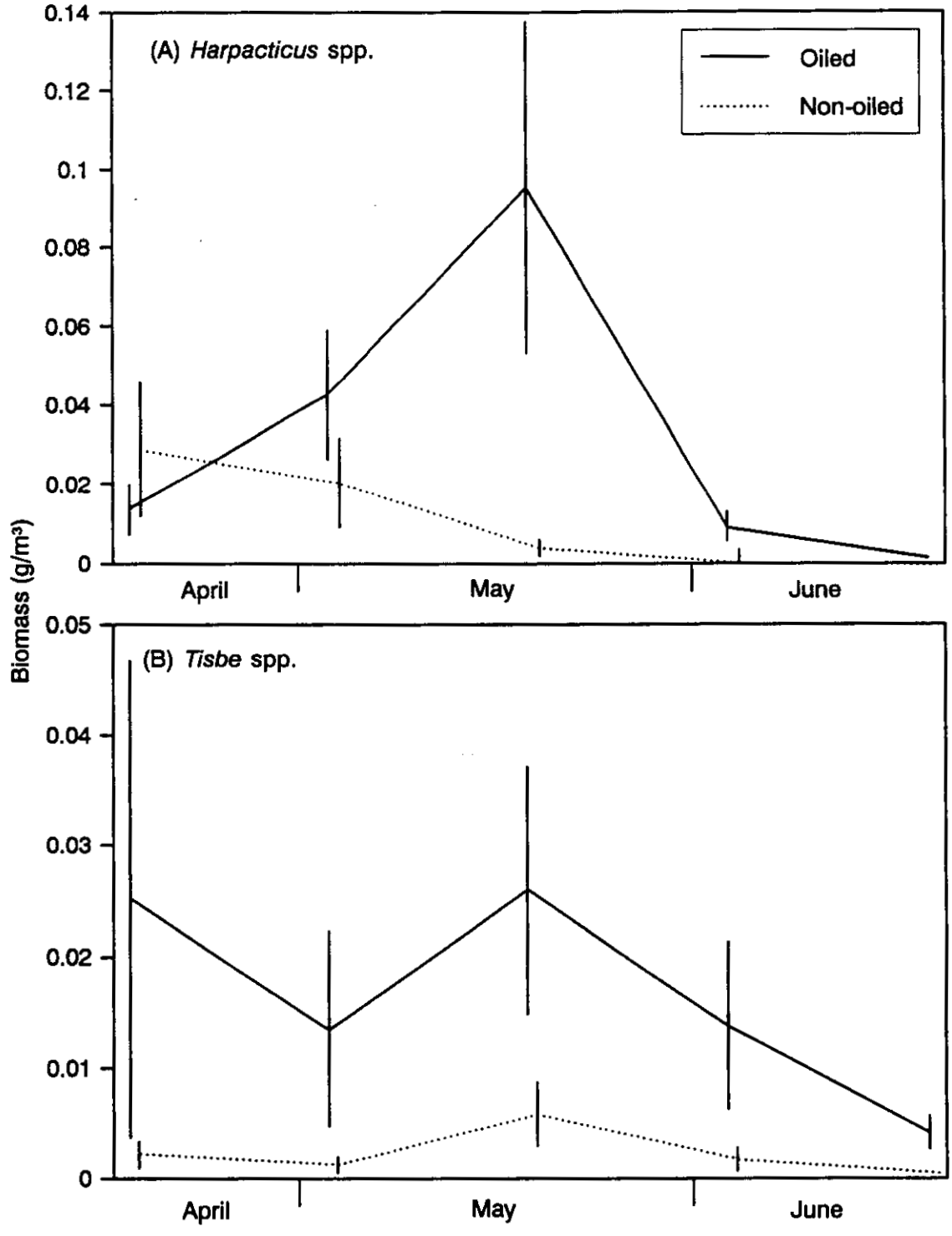

Figure 5A.16. Mean biomass (±1 SE) of Harpacticus spp. (A) and Tisbe spp. (B) in the systematic epibenthic sled samples in oiled and non-oiled locations of Prince William Sound in 1989.

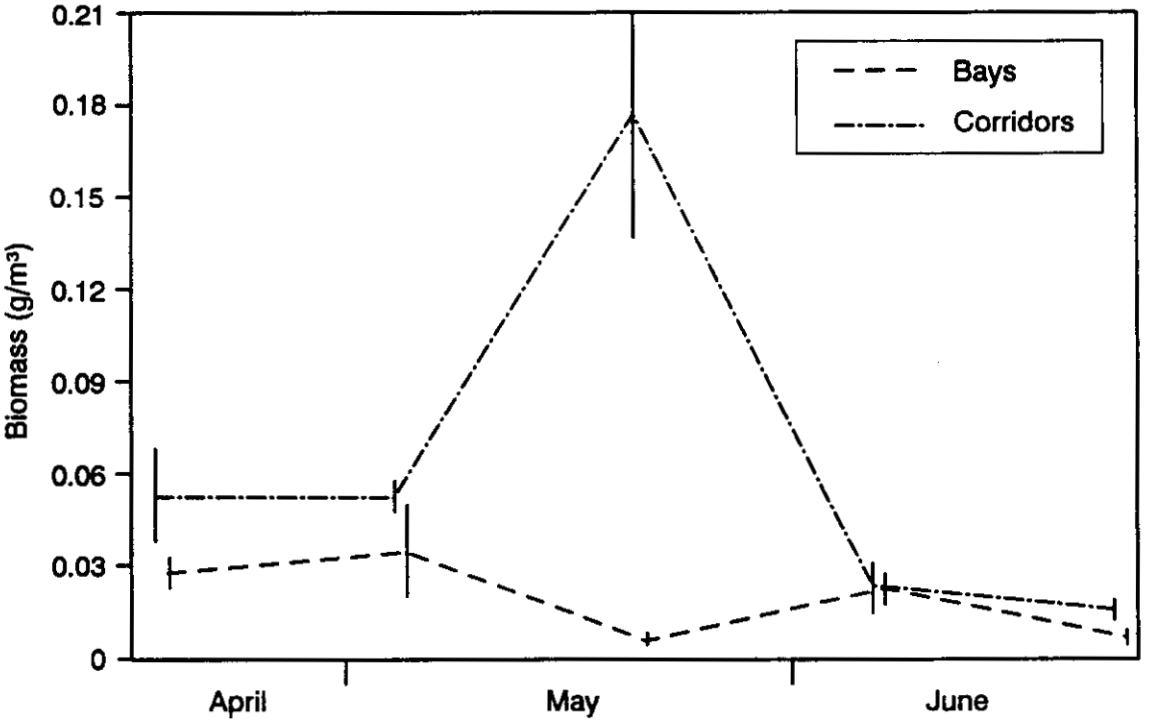

Figure 5A.17. Harpacticoid copepod mean biomass (±1 SE) in the systematic epibenthic sled samples in bays and corridors over time in Prince William Sound, April-June 1989.

CHAPTER 5B. Harpacticoid Copepod Abundance and Population Structure in Prince William Sound, 1 Year After the *Exxon Valdez* Oil Spill

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Abstract

The density and population structure of epibenthic harpacticoid copepods were compared between lightly oiled and heavily oiled beaches in two bays, Herring Bay and Bay of Isles, in Prince William Sound in 1990, 1 year after contamination from the *Exxon Valdez* oil spill. The degree of oiling was categorized according to shoreline surveys in the fall of 1989. Mean density of total epibenthic harpacticoid copepods in both bays and of *Harpacticus* spp. in Herring Bay were significantly higher on heavily oiled beaches than on lightly oiled beaches. Level of oiling in 1989 explained more of the observed variability than substrate composition, macrophyte coverage, or total organic carbon and polycyclic aromatic hydrocarbons in the sediments. For *Harpacticus* spp., *Tisbe* spp., and *Dactylopodia* spp., the proportion of juveniles or egg-bearing females did not differ between lightly and heavily oiled beaches.

The amount of oil in sediments at the sampling sites was not significantly correlated with the oiling level assigned in the 1989 shoreline survey; however, interpretation of the hydrocarbon data was complicated by probable secondary contamination at the sites. Effects of clean-up activity also could not be separated from the impacts of the oil pollution. Because density of epibenthic harpacticoids was the same or higher on beaches that had greater oil deposition compared to lightly oiled beaches, we conclude that epibenthic harpacticoids important as prey to salmon fry maintained or increased in abundance in response to direct and indirect impacts of the *Exxon Valdez* oil spill on their intertidal habitats 1 year after the spill.

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Introduction

Harpacticoid copepods are important in the diet of juvenile salmon in Alaska waters (Cooney et al. 1978, 1981; Landingham 1982; Cordell 1986; Murphy et al. 1988) and elsewhere (Mason 1974; Sibert et al. 1977; Feller and Kaczynski 1975; Godin 1981; Simenstad and Salo 1982). Harpacticoid copepods are common and abundant organisms in the intertidal zone in Prince William Sound; they were the second-most abundant component of the macrofauna and meiofauna in intertidal habitats of Port Valdez (Feder et al. 1976). Harpacticoid copepod populations can be impacted by oil pollution; their numbers have been shown to be severely reduced following shoreline oiling (Wormald 1976; Giere 1979; Elmgren et al. 1983; Bodin 1991).

The importance of harpacticoid copepods as a dominant taxon of the meiobenthos and as frequent prey of juvenile salmon led to concern regarding the possible effects of the *Exxon Valdez* spill on these animals in Prince William Sound. While the general level of oil contamination in Prince William Sound had diminished greatly from 1989 to 1990, large amounts of oil remained in the sediments of some intertidal habitats where it could potentially affect the production of epibenthic harpacticoids. Our objective in this component of the field research was to assess the impact of the oil 1 year after the spill on the abundance of harpacticoid copepods important as prey of juvenile salmon (Objective 8, Chapter 1). Our approach was to compare density and population structure between lightly oiled and heavily oiled beaches in two contaminated bays, Bay of Isles and Herring Bay.

Chapter 5B will be reformatted and submitted to a peer-reviewed scientific journal for formal publication.

Methods

Study Design and Sampling

Harpacticoid copepods were sampled in Bay of Isles and Herring Bay in the spring of 1990. Eight beaches in each bay were selected for sampling based on degree of oiling and superficial physical and biological characteristics (Figures 5B.1, 5B.2). Four beaches were located in heavily oiled areas and four in lightly oiled areas, as categorized on Alaska Department of Environmental Conservation (ADEC) survey maps for the fall of 1989 (ADEC 1989). The ADEC maps identify beaches by assigning unique alphanumeric codes to beach segments; the codes for the beach segments on which sample sites were located are shown in Table 5B.1. The number of lightly oiled beaches suitable for epibenthic sampling was limited, and random selection of beaches was not practical. After selecting lightly oiled beaches, we selected heavily oiled beaches that were superficially similar to the lightly oiled beaches in gradient, substrate, exposure, and macrophyte coverage. Some oil clean-up activity took place on all beach segments in 1989 and 1990, except for the lightly oiled beaches in Herring Bay (Table 5B.1). We could not determine whether any clean-up activity occurred in 1989 at the specific portion of the beaches we sampled for copepods; we sampled in 1990 before 1990 clean-up activity at our sample sites.

Harpacticoid copepods were sampled along a 40-m transect placed along the mean-low-tide (0-m) contour of each selected beach. The 40-m transect was divided into six sections, and four or five pump sampling sites were placed randomly at least 1 m apart within each section. Harpacticoids were sampled with a submersible pump enclosed in a housing of 15-cm diameter. Ports in the housing were covered with 0.123-mm-mesh screen. The housing was set on the substrate, and water was pumped for 30 seconds into a 0.123-mm-mesh net. Samples were then rinsed into a 500-ml bottle and preserved in buffered 5% formalin. A total of 25 pump samples was taken per transect, except at one Bay of Isles beach, where we were only able to pump 24 samples before we were flooded out by the incoming tide.

At each transect, surface substrate composition and macrophyte coverage were estimated, and samples of surface sediments were collected for total organic carbon (TOC) and hydrocarbon analysis. One sediment sampling site was located randomly within each of the six sections of the 40-m transects. A 0.25-m^2 quadrat was placed at each sediment sampling site. Surface substrate composition within the quadrat was visually classified following the Wentworth scale (Holme and McIntyre 1984) into four categories: boulder (>256 mm), cobble (64-256 mm), pebble (4-64 mm), and granule (2-4 mm). Any substrate <2 mm, such as sand or mud, was also included in the granule category. Percent

macrophyte coverage within the quadrat was also estimated visually. Fine sediment from the upper 2 cm of the substrate within the quadrat was collected into four hydrocarbon-free glass jars. Approximately equal amounts of sediments were aggregated in the jars from each of the six quadrat locations along the transects, resulting in four sediment samples from a transect. Three of the sediment samples collected from each transect were submitted for hydrocarbon analysis; the fourth sediment sample was used for TOC analysis.

Harpacticoid copepods in each sample were sorted, counted, and identified to taxa that are important dietary components of juvenile salmon. Sex and life stages (copepodite, adult, egg-carrying females, clasping pairs) were also determined. Samples were screened on sieves of 0.125-mm mesh before sorting. Densities per m^2 of total harpacticoids and of specific genera in each sample were calculated by dividing the counts by the surface area sampled (0.0177 m^2). Pentec Environmental, Inc. processed 199 pump samples taken May 24-27 in the Bay of Isles (Pentec 1991). Kinnetic Laboratories, Inc. processed pump samples taken in Herring Bay April 24-27 (KLI 1991). Because of budget constraints, only 120 of the 200 samples from Herring Bay could be processed; therefore, 15 samples were randomly chosen from the 25 taken from each transect. Contractors' methods are detailed in their final reports (Pentec 1991; KLI 1991).

Statistical analysis of the data was done on the basis of all *Harpacticus* species combined. In processing Bay of Isles data, the contractor identified *H. uniremis* and other *Harpacticus* spp. in the *Harpacticus* genus. The contractor processing Herring Bay data identified only *H. uniremis* in the genus. Subsequent review, however, showed that specimens from Herring Bay included *H. compressus* adults and *Harpacticus* spp. copepodites.

Sediment samples were processed for TOC and hydrocarbon content. Sediment TOC was determined by ignition by HUB Testing Laboratories. Hydrocarbon content was measured by the Geochemical and Environmental Research Group at Texas A&M University by gas chromatography, with mass spectrometry to determine concentrations of aromatic compounds, and with flame ionization to determine concentrations of aliphatic compounds.

Statistical Analysis

Six groups of hydrocarbons were compared between lightly oiled and heavily oiled beaches: phytane (a representative alkane); and five polycyclic aromatics (in order from light to heavy): sum naphthalenes, sum fluorenes, sum phenanthrenes, sum dibenzothiophenes, and sum chrysenes. These compounds do not occur in significant quantities in unpolluted sediments in Prince

William Sound (Karinen et al. 1993).

Analysis of variance (ANOVA) was used to test whether lightly oiled and heavily oiled beaches within a bay differed in transect characteristics and harpacticoid abundance and population structure. Transect characteristics evaluated were 1) proportion of transect covered by small (<65 mm) substrate; 2) proportion of transect covered by macrophytes; 3) TOC; and 4) hydrocarbon analytes. Abundance and population structure parameters evaluated were 1) density of combined harpacticoids; 2) density of *Harpacticus*, *Tisbe*, and *Dactylopodia*; 3) the proportion of adult females with eggs, and 4) the proportion of copepodites, for *Harpacticus*, *Tisbe*, and *Dactylopodia*.

In each ANOVA, data were transformed to normalize the distribution and maximize variance homogeneity. Harpacticoid density was transformed to natural log [ln(x+1)]. Proportional data were transformed to the arcsine of the square root of the proportion. The factors in each ANOVA were oil and beach nested within oil. The factor oil had two levels, lightly oiled and heavily oiled, and the nested factor beach had four levels. The significance of the factor oil was tested by the F ratio of the mean square of oil to the mean square of beach (nested within oil). The nested design removed the possibility of pseudoreplication (Hurlbert 1984). Because only one observation of TOC was made per beach, the ANOVA for TOC collapsed to a one-way test between levels of oiling.

If differences in total harpacticoids or specific taxa were identified by ANOVA, these parameters were then examined with multiple linear regression to determine the proportion of the variance among transects explained by the degree of oiling relative to other parameters. In each regression, the mean of the logarithmically transformed harpacticoid density by beach was the dependent variable; the independent variables were the arcsine-transformed mean proportion of substrate <65 mm, the arcsine-transformed mean proportion of macrophyte coverage, the TOC, the concentration of dibenzothiophenes and chrysenes in the sediments, and the categorical designation of heavily or lightly oiled. A stepwise regression analysis (Cruze and Weldon 1989) was used; the partial F test had to be significant at $P < 0.1$ for the variable to remain in the regression model.

Minor differences exist in some of the summary statistics presented in this report and those in the 1991 Status Report (Wertheimer et al. 1991). These discrepancies are due to clasping pairs being counted as a single organism rather than being properly counted as two (one male adult, one female copepodite) animals. Because a relatively small proportion of the copepods were clasping pairs, correction of this error did not change the conclusions of the previous analysis.

Results

Harpacticoid copepods were more abundant at the heavily oiled beaches than the lightly oiled beaches at both Herring Bay and Bay of Isles (Table 5B.2). At Herring Bay, densities of combined harpacticoids and of *Harpacticus* spp. were significantly (P = 0.039 and P = 0.046, respectively) higher at the heavily oiled beaches. Mean density over all heavily oiled beaches was 94,478/m^2 for all harpacticoids and 15,893/m^2 for *Harpacticus*, compared to 23,634/m^2 and 4,252/m^2, respectively, at lightly oiled beaches. Mean densities of *Dactylopodia* and *Tisbe* also tended to be higher at the heavily oiled beaches, although the differences were not significant. At Bay of Isles, mean density of combined harpacticoids was 92,841/m^2 at the heavily oiled beaches, compared to 68,302/m^2 at the lightly oiled beaches. This difference was marginally significant (P = 0.085). No significant difference between heavily and lightly oiled beaches was indicated for *Harpacticus*, *Tisbe*, and *Dactylopodia*.

Population structure was similar at heavily oiled and lightly oiled beaches. No significant difference (P > 0.30) was found between heavily oiled and lightly oiled beaches at Herring Bay or Bay of Isles in the proportion of adult females with eggs for *Harpacticus*, *Tisbe*, and *Dactylopodia* (Table 5B.3). Proportion of copepodites for these three taxa also did not differ (P > 0.14) between lightly and heavily oiled beaches (Table 5B.4).

No significant difference in macrophyte coverage or TOC was indicated between heavily oiled and lightly oiled beaches in Herring Bay or Bay of Isles (P > 0.36 in all instances; Table 5B.5). Macrophyte coverage averaged 87% at Bay of Isles and 71% at Herring Bay, where variability between beaches was higher. Sediment TOC varied considerably among beaches; levels may have been higher at Herring Bay (513 mg/kg) than at Bay of Isles (350 mg/kg), although this was not tested.

Substrate composition did not differ significantly between heavily and lightly oiled beaches in Bay of Isles (P = 0.253), but did differ in Herring Bay (P = 0.001; Table 5B.5). In Bay of Isles, proportion of substrate <65 mm (pebble or smaller) averaged 69% on heavily oiled and 64% on lightly oiled beaches. In Herring Bay, lightly oiled beaches had a coarser substrate, averaging 55% <65 mm compared to 77% for heavily oiled beaches.

We expected higher concentrations of hydrocarbons in the sediments from heavily oiled beaches than from lightly oiled beaches. In fact, for the six groups of hydrocarbons evaluated, we found significantly higher concentrations at the heavily oiled beaches for only dibenzothiophenes (P = 0.029) and the chrysenes (P = 0.053) at Herring Bay. None of the other analytes at Herring Bay, or for any of the analytes at Bay of Isles, differed

significantly between heavily and lightly oiled beaches.

Napthalene concentrations were anomalously high in sediments from both Herring Bay and Bay of Isles. At Herring Bay, the napthalenes had the highest concentration of the hydrocarbon compounds measured at every beach except beach 1, ranging from 0.3 to 1.0 ppm (Figure 5B.3). Napthalene concentration was highest at beach 6, on which no oil had been observed during shoreline surveys (Table 5B.1), and lowest at beach 1, a heavily oiled beach (Figure 5B.3). At Bay of Isles, the general profile of the analytes by beach for all compounds except naphthalene was for a high spike at beach 1 (heavily oiled), with beach 6 (lightly oiled) having the second-highest level (Figure 5B.4). For the napthalenes, however, the lightly oiled beaches 6 and 8 had the highest concentration, concentrations were generally elevated across all beaches, and beach 1 had the lowest concentration. Naphthalene levels at Bay of Isles ranged from 0.6 to 2.5 ppm (Figure 5B.4).

Macrophyte coverage, proportion substrate <65 mm, TOC, dibenzothiophene concentration, chrysene concentration, and level of oiling generally had no significant association with each other; the exceptions involved the polynuclear aromatic compounds. Chyrsene and dibenzothiophene concentrations were significantly ($P < 0.05$) associated in both Herring Bay ($r = 0.98$) and Bay of Isles ($r = 0.95$). These compounds were also significantly associated with level of oiling at Herring Bay ($r = 0.74$ for chrysene, $r = 0.78$ for dibenzothiophene).

The three measures of harpacticoid copepod abundance that differed significantly between levels of oil (total harpacticoids at Herring Bay and Bay of Isles, *Harpacticus* at Herring Bay) were tested against the other parameters measured at each beach to determine if any parameter other than level of oiling could have caused the observed differences. The ADEC level of oiling was significantly correlated with these harpacticoid densities; the only other parameter with a significant ($P < 0.10$) correlation with harpacticoid density was proportion substrate <65 mm at Herring Bay (Table 5B.6). The variance explained (R^2) by level of oiling was 53% and 51% for the density of total harpacticoids and *Harpacticus* in Herring Bay, and 43% for the density of total harpacticoids in Bay of Isles. When variance related to the level of oiling was removed from the data by entering level of oiling as the first variable in a stepwise multiple regression, the concentration of chrysenes in the sediments was a significant predictor of the density of total harpacticoids in Herring Bay ($P < 0.10$), increasing the variance explained by the regression from 53 to 78%. Chrysene concentration was not a significant predictor for the density of *Harpacticus* in Herring Bay or for the density of total harpacticoids in Bay of Isles. None of the other variables entered the stepwise regression models.

Discussion

The *Exxon Valdez* oil spill significantly impacted the abundance of epibenthic harpacticoid copepods on the beaches we studied in Prince William Sound. Mean densities of harpacticoid copepods in Herring Bay and Bay of Isles ranged from 23,364 to 94,478 individuals/m^2. These densities are at the high end of the range for samples taken with a similar pump in unpolluted areas in Puget Sound and comparable to a site in Auke Bay, Alaska (Cordell 1986). In all cases where a significant impact was determined, population numbers were higher on heavily oiled than on lightly oiled beaches. Not all harpacticoid copepods were equally affected. Density of total harpacticoids at both bays and *Harpacticus* at Herring Bay were higher on heavily oiled beaches, but the density of *Tisbe* and *Dactylopodia* at both bays and of *Harpacticus* at Bay of Isles did not differ significantly between lightly and heavily oiled beaches.

We cannot conclusively attribute the changes in harpacticoid density associated with the level of oiling to the oil itself. We used the level of observed oil in the ADEC survey in the fall of 1989 as representative of the degree of oil contamination. Assigning causation to differences in harpacticoid copepod density was complicated because 1) clean-up activity on the beaches could have affected harpacticoids and 2) hydrocarbon levels in the sediments were not significantly correlated with the degree of oiling assigned by ADEC.

Oil spill clean-up activity can have direct effects on the intertidal ecosystem. Houghton et al. (1991) found a reduction in species richness and infauna abundance, including the abundance of interstitial harpacticoids, the year following the *Exxon Valdez* oil spill on beaches treated with hot-water washes. We did not find epibenthic harpacticoid copepods to be reduced in association with increased clean-up activity. In Herring Bay, density of total harpacticoids and of *Harpacticus* was elevated on heavily oiled beaches, even though these beaches had been exposed to some clean-up activity, while lightly oiled beaches in this bay had no clean-up activity (Table 5B.1). However, the records of beach treatment following the spill are not sufficiently detailed to determine whether the specific transects that we sampled were treated. Because we cannot separate clean-up effects from oil effects, we consider the differences in harpacticoid density we observed to be related to the oil-spill history of the heavily oiled sites, including both contamination and clean-up.

In general, the hydrocarbons measured in the sediments at the mean-low-tide level did not correspond with the expected pattern between beaches based on the rating by ADEC. Following the

spill, oil was typically deposited in the upper intertidal zone (Owens 1991); visible oil pollution was the parameter assessed by the ADEC surveys. We expected that the oil would leach to the lower intertidal and contaminate the sediments at the mean-low-tide transects to a greater degree at beaches that had been rated as heavily oiled. We found this pattern only at Herring Bay for some of the more persistent aromatics (chrysene and dibenzothiophene). The lack of correspondence between the sediment hydrocarbon load and the ADEC rating of oiling may be due to the patchy distribution of hydrocarbons in the sediments (Owens 1991); both the deposition pattern and differences in weathering associated with tidal flushing and winter storm exposure on individual beaches contribute to the high variablity of residual hydrocarbons in the sediments.

Another explanation for the poor correspondence between the ADEC rating of oiling and the actual hydrocarbons measured in the sediments is contamination of the beaches subsequent to oiling from the *Exxon Valdez* spill. At both bays, we found high amounts of naphthalene at both lightly oiled and heavily oiled beaches. These concentrations were not consistent with the expected pattern from weathered *Exxon Valdez* crude oil. The naphthalene may have been the result of recent pollution from other sources, such as boat traffic and clean-up activity. Such contamination was probably water-borne, since even beaches in Herring Bay with no clean-up activity had elevated naphthalene levels (e.g., beach 6, Figure 5A.3). Both bays were sites of significant vessel activity in 1990 for clean-up and damage assessment. At Bay of Isles, where the highest napthalene concentrations were observed, a large clean-up fleet was present during our sampling in May 1990.

At both Bay of Isles and Herring Bay, the level of oiling in the ADEC surveys was the measure that was most highly correlated with harpacticoid density. This association could be a coincidence of water circulation conditions in these bays that resulted in both deposition of drifting crude oil and conditions that favor epibenthic harpacticoids. However, no other characteristic that we measured at the sample sites (substrate, macrophyte coverage, or TOC) was as significant as the ADEC oiling level in explaining the observed variance in epibenthic harpacticoids. Thus, increased epibenthic harpacticoid density on heavily oiled beaches in 1990 does appear to be a direct response to the degree of impact (oiling plus treatment) from the oil spill.

The presence of petroleum hydrocarbons in intertidal areas has been shown to affect populations of harpacticoid copepods in different ways. The changes caused by oil are not always predictable. The most frequently observed immediate response of harpacticoid copepod populations to an oil spill is a decline in abundance (Wormald 1976; Giere 1979; Bonsdorf 1981; Elmgren et

al. 1983; Bodin 1988). Increases in abundance of harpacticoid copepods, however, have also been observed following oil spills (Fricke et al. 1981; Bodin 1988). Increases have also been observed in experimental *in-situ* applications of hydrocarbons (Feder et al. 1990; Gilfillan et al. 1983) and following the addition of oil to marine mesocosms (Frithsen et al. 1985; Stacey and Marcotte 1987).

Some of the differences between these studies may be explained by the different ecological groups of harpacticoid copepods sampled in the different studies. A decline in harpacticoid copepod populations in the interstitial zone at the same time as an increase in the populations of free-living harpacticoids above the sediment occurred following the *Amoco Cadiz* spill (Chasse 1978). Bodin (1988) categorized species of harpacticoid copepods by their rate of colonization after the *Amoco Cadiz* spill. First to appear after the initial toxic effect of the spill were the opportunistic plant-associated species that exploit the ephemeral algal blooms that often follow a spill, and the tolerant generalist species regularly present in low densities that are stimulated by organic pollution. These groups peaked 2 years after the *Amoco Cadiz* spill. As the sediments became progressively cleaner, the opportunistic and generalist species were progressively replaced by a more sensitive group of harpacticoid copepods typical of similar sandy sites in unpolluted areas.

Although other researchers have reported effects of oil pollution on the reproductive activity of various harpacticoid species (Ustach 1977; Frithsen et al. 1985; Bodin 1991), we observed no significant differences between the lightly oiled and heavily oiled beaches in this study with respect to either the proportion of females that were carrying eggs or the proportion of copepodites in the population. One of the six populations studied (*Harpacticus* at Herring Bay) did have a significant difference in overall abundance, so differences in the proportion of copepodites or egg-carrying females might have been expected. *H. uniremis* in Prince William Sound is reported to produce only one brood per year; in Port Valdez ovigerous females were only prevalent in the population from November to May (Feder et al. 1976), with most ovigerous females occurring in February and March (Jewett and Feder 1977). This timing explains the higher density of ovigerous females in Herring Bay (sampled April 24-27) compared with the Bay of Isles (sampled May 24-27). It also suggests that the Herring Bay population was near the end of its reproductive period, which would cause the percentages of ovigerous females to be low and variable.

A single sampling date is probably not sufficient to track the reproductive dynamics of these animals. *H. uniremis* copepodites were generally most abundant in Port Valdez in March, April, and

May, although the time of hatching varies from year to year (Jewett and Feder 1977). The same study indicates that copepodites develop into adults in 1 to 1.5 months. This short and variable period of availability of copepodites complicates comparison of their abundance at different sites.

We conclude that the epibenthic prey resources available to juvenile salmon in 1990 were not reduced by continuing impacts of the *Exxon Valdez* spill. In Prince William Sound, *H. uniremis* typically dominates the diet, followed by *Tisbe* and *Dactylopodia* (Cooney et al. 1981; Chapter 4, this report). These animals are the opportunistic harpacticoid copepods (*sensu*, Bodin 1988) that are likely to increase in abundance following an oil spill. Indeed, we observed higher densities of epibenthic harpacticoids in general, and of *H. uniremis* in particular, at heavily oiled beaches. This conclusion does not exclude the possibility of an impact to these resources immediately following the spill in the spring of 1989. However, surveys of epibenthic prey of juvenile salmon in 1989 also indicated that epibenthic prey were as or more abundant in oiled locations than in non-oiled locations (Chapter 5A, this report). The epibenthic harpacticoids important as prey to salmon fry apparently maintained or increased their numbers in response to the direct and indirect impacts of the *Exxon Valdez* oil spill.

Table 5B.1. Alaska Department of Environmental Conservation (ADEC) beach segment identification, degree of oiling, and clean-up activities associated with beaches sampled for harpacticoid copepods in spring 1990. Degree of oiling was assigned in the ADEC surveys in fall 1989. HB beaches were in Herring Bay, BI beaches were in Bay of Isles.

Beach	ADEC Segment	Degree of oiling	Clean-up History[1] 1989	1990[2]
HB-1	KN118	High	B	B
HB-2	KN5001	None	NTR	NTR
HB-3	KN119	High	B,M	B,M,ME,W
HB-4	KN120	Very low	NTR	NTR
HB-5	KN126	High	B,W,T	B,M,ME,W
HB-6	KN5006	None	NTR	NTR
HB-7	KN115	High	B,M,TM	B,M,TM
HB-8	KN5004	None	NTR	NTR
BI-1	KN005	High	B,M,TM	B,M,TM
BI-2	KN006	Very low	TM	TM
BI-3	KN024	High	B,M	B,M,TM
BI-4	KN206	Very low	B,M	B,M,TM
BI-5	KN135	High	B,M,TM,W	B,M,TM
BI-6	KN006	Very low	TM	M,TM
BI-7	KN136	High	B,M,T,TM,W	B,M,TM,W
BI-8	KN205	None	B,M	M,TM

[1]B = Bioremediation; M = Manual removal; ME = Mechanical; T = Till; TM = Tarmat removal; W = Wash; NTR = No treatment.

[2]No clean-up activity occurred at the actual transect sites prior to sampling for harpacticoid copepods in 1990.

Table 5B.2. Density (no./m^2) of harpacticoid copepods sampled with an epibenthic pump in the Bay of Isles and Herring Bay in spring 1990. Number of samples at each transect were 15 for Herring Bay, and 25 for Bay of Isles (except BI-6, with 24). SE of means are shown in parentheses.

Condition	Beach	Density *Harpacticus*	*Tisbe*	*Dactylopodia*	Total Harpacticoids
Bay of Isles					
Heavily oiled	BI-1	5159 (1197)	4977 (1263)	626 (275)	82905 (12801)
	BI-3	19566 (2944)	1335 (216)	233 (110)	72060 (7881)
	BI-5	3720 (628)	9196 (2451)	1219 (331)	98762 (18848)
	BI-7	4173 (769)	4046 (614)	2268 (815)	117636 (16921)
	Mean	8154 (3816)	4889 (1630)	1086 (443)	92841 (9918)
Lightly oiled	BI-2	7336 (1356)	2290 (404)	1431 (556)	70905 (8961)
	BI-4	2600 (503)	4096 (1110)	152 (37)	35398 (5746)
	BI-6	2586 (500)	2652 (586)	881 (262)	39763 (4974)
	BI-8	5579 (1623)	22655 (5277)	3846 (1131)	127142 (19505)
	Mean	4525 (1172)	7923 (4926)	1578 (800)	68302 (21146)
ANOVA significance level		0.253	0.919	0.681	0.085
Herring Bay					
Heavily oiled	HB-1	24744 (5488)	15275 (2412)	1966 (812)	227575 (40146)
	HB-3	6979 (956)	7318 (1080)	7262 (1572)	53168 (7128)
	HB-5	13944 (3070)	18320 (3493)	4664 (1159)	60218 (8843)
	HB-7	17906 (2853)	2068 (324)	2121 (280)	36953 (7314)
	Mean	15893 (3715)	10112 (3612)	3622 (1176)	94478 (44632)
Lightly oiled	HB-2	8531 (1318)	5164 (617)	2836 (447)	22614 (2276)
	HB-4	701 (119)	5322 (744)	573 (76)	13096 (1935)
	HB-6	7126 (1756)	7085 (1088)	2237 (527)	42866 (7903)
	HB-8	652 (113)	1782 (517)	2429 (424)	15959 (2791)
	Mean	4252 (2084)	4358 (993)	1727 (481)	23634 (6714)
ANOVA significance level		0.046	0.278	0.859	0.039

Table 5B.3. Proportion of adult females with eggs for three harpacticoid copepod taxa sampled with an epibenthic pump in the Bay of Isles and Herring Bay in spring 1990.

		Proportion of adult females with eggs		
Condition	Beach	*Dactylopodia*	*Harpacticus*	*Tisbe*
Bay of Isles				
Heavily oiled	BI-1	0.34 (0.16)	0.00 (0.00)	0.17 (0.04)
	BI-3	0.49 (0.16)	0.00 (0.00)	0.13 (0.03)
	BI-5	0.50 (0.09)	0.00 (0.00)	0.20 (0.03)
	BI-7	0.16 (0.07)	0.00 (0.00)	0.11 (0.03)
	Mean	0.37 (0.08)	0.00 (0.00)	0.15 (0.02)
Lightly oiled	BI-2	0.44 (0.14)	0.01 (0.01)	0.11 (0.02)
	BI-4	0.42 (0.12)	0.03 (0.01)	0.28 (0.04)
	BI-6	0.40 (0.11)	0.00 (0.00)	0.15 (0.03)
	BI-8	0.06 (0.04)	0.00 (0.00)	0.11 (0.02)
	Mean	0.33 (0.09)	0.01 (0.01)	0.16 (0.04)
ANOVA significance level		0.580	0.334	0.562
Herring Bay				
Heavily oiled	HB-1	0.24 (0.11)	0.01 (0.01)	0.32 (0.04)
	HB-3	0.53 (0.08)	0.02 (0.01)	0.36 (0.05)
	HB-5	0.46 (0.07)	0.03 (0.01)	0.17 (0.03)
	HB-7	0.34 (0.07)	0.12 (0.02)	0.25 (0.06)
	Mean	0.39 (0.06)	0.04 (0.03)	0.27 (0.09)
Lightly oiled	HB-2	0.38 (0.06)	0.16 (0.04)	0.27 (0.04)
	HB-4	0.58 (0.09)	0.01 (0.01)	0.38 (0.02)
	HB-6	0.35 (0.10)	0.10 (0.05)	0.19 (0.03)
	HB-8	0.49 (0.06)	0.32 (0.10)	0.11 (0.05)
	Mean	0.44 (0.05)	0.15 (0.07)	0.24 (0.06)
ANOVA significance level		0.671	0.306	0.625

Table 5B.4. Proportion of copepodites for three harpacticoid copepod taxa sampled with an epibenthic pump in the Bay of Isles and Herring Bay in spring of 1990.

Condition	Beach	Proportion of copepodites		
		Dactylopodia	*Harpacticus*	*Tisbe*
Bay of Isles				
Heavily oiled	BI-1	0.05 (0.05)	0.09 (0.02)	0.44 (0.05)
	BI-3	0.07 (0.06)	0.10 (0.01)	0.40 (0.05)
	BI-5	0.09 (0.04)	0.04 (0.01)	0.24 (0.03)
	BI-7	0.00 (0.00)	0.02 (0.01)	0.24 (0.03)
	Mean	0.05 (0.02)	0.06 (0.02)	0.33 (0.05)
Lightly oiled	BI-1	0.08 (0.06)	0.28 (0.03)	0.37 (0.03)
	BI-4	0.00 (0.00)	0.21 (0.03)	0.35 (0.03)
	BI-6	0.02 (0.02)	0.05 (0.02)	0.36 (0.03)
	BI-8	0.14 (0.05)	0.11 (0.02)	0.33 (0.03)
	Mean	0.06 (0.03)	0.16 (0.05)	0.35 (0.01)
ANOVA significance level		0.728	0.139	0.640
Herring Bay				
Heavily oiled	HB-1	0.16 (0.06)	0.73 (0.03)	0.64 (0.03)
	HB-3	0.60 (0.06)	0.65 (0.04)	0.33 (0.03)
	HB-5	0.49 (0.06)	0.57 (0.04)	0.31 (0.03)
	HB-7	0.61 (0.04)	0.71 (0.03)	0.30 (0.05)
	Mean	0.46 (0.11)	0.66 (0.03)	0.40 (0.08)
Lightly oiled	HB-2	0.57 (0.07)	0.82 (0.03)	0.46 (0.04)
	HB-4	0.20 (0.08)	0.62 (0.07)	0.23 (0.04)
	HB-6	0.41 (0.05)	0.61 (0.04)	0.44 (0.05)
	HB-8	0.36 (0.04)	0.50 (0.07)	0.64 (0.05)
	Mean	0.38 (0.07)	0.63 (0.07)	0.45 (0.09)
ANOVA significance level		0.791	0.783	0.671

Table 5B.5. Physical characteristics of beaches sampled for harpacticoid copepods in the Bay of Isles and Herring Bay in spring 1990. Sample sizes per beach were 6 for substrate and macrophyte coverage and 1 for total organic carbon. Standard errors for the means are shown in parentheses.

Condition	Beach	Physical Characteristic: Substrate[1]	Macrophyte Coverage	Total Organic Carbon (mg/kg)
Bay of Isles				
Heavily oiled	BI-1	0.77 (0.08)	0.83 (0.10)	110
	BI-3	0.80 (0.04)	0.88 (0.08)	148
	BI-5	0.70 (0.05)	0.82 (0.15)	560
	BI-7	0.50 (0.06)	0.87 (0.07)	596
	Mean	0.69 (0.08)	0.85 (0.02)	354 (130)
Lightly oiled	BI-1	0.52 (0.07)	1.00 (0.0)	74
	BI-4	0.57 (0.11)	0.73 (0.11)	501
	BI-6	0.64 (0.06)	0.98 (0.02)	331
	BI-8	0.82 (0.04)	0.87 (0.08)	480
	Mean	0.64 (0.08)	0.90 (0.07)	346 (98)
ANOVA significance level		0.253	0.409	0.967
Herring Bay				
Heavily oiled	HB-1	0.90 (0.03)	1.00 (0.0)	494
	HB-3	0.82 (0.03)	0.48 (0.07)	328
	HB-5	0.65 (0.05)	0.85 (0.10)	1009
	HB-7	0.70 (0.07)	0.58 (0.18)	223
	Mean	0.77 (0.07)	0.73 (0.14)	514 (174)
Lightly oiled	HB-2	0.27 (0.10)	0.80 (0.06)	793
	HB-4	0.62 (0.08)	0.60 (0.08)	255
	HB-6	0.70 (0.07)	0.60 (0.10)	461
	HB-8	0.62 (0.10)	0.82 (0.13)	540
	Mean	0.55 (0.12)	0.70 (0.07)	512 (111)
ANOVA significance level		0.001	0.358	0.995

[1]Proportion of substrate that was pebble or smaller (<65 mm)

Table 5B.6. Correlation coefficients of harpacticoid copepod density with characteristics of beaches sampled in Bay of Isles and Herring Bay in spring 1990. DBT = dibenzothiophenes; CHRY = chrysenes.

Taxa	Substrate variable					
	Level of oiling	Macrophyte coverage	Substrate <65 mm	Total organic carbon (mg/kg)	DBT (ng/g)	CHRY (ng/g)
Bay of Isles						
Total harpacticoids	0.656*	0.014	0.117	0.092	-0.087	0.118
Herring Bay						
Total harpacticoids	0.713**	0.588	0.662*	0.154	0.274	0.212
Harpacticus	0.717**	0.272	0.249	0.226	0.558	0.515

* Significantly different from zero, $P < 0.10$
** Significantly different from zero, $P < 0.05$

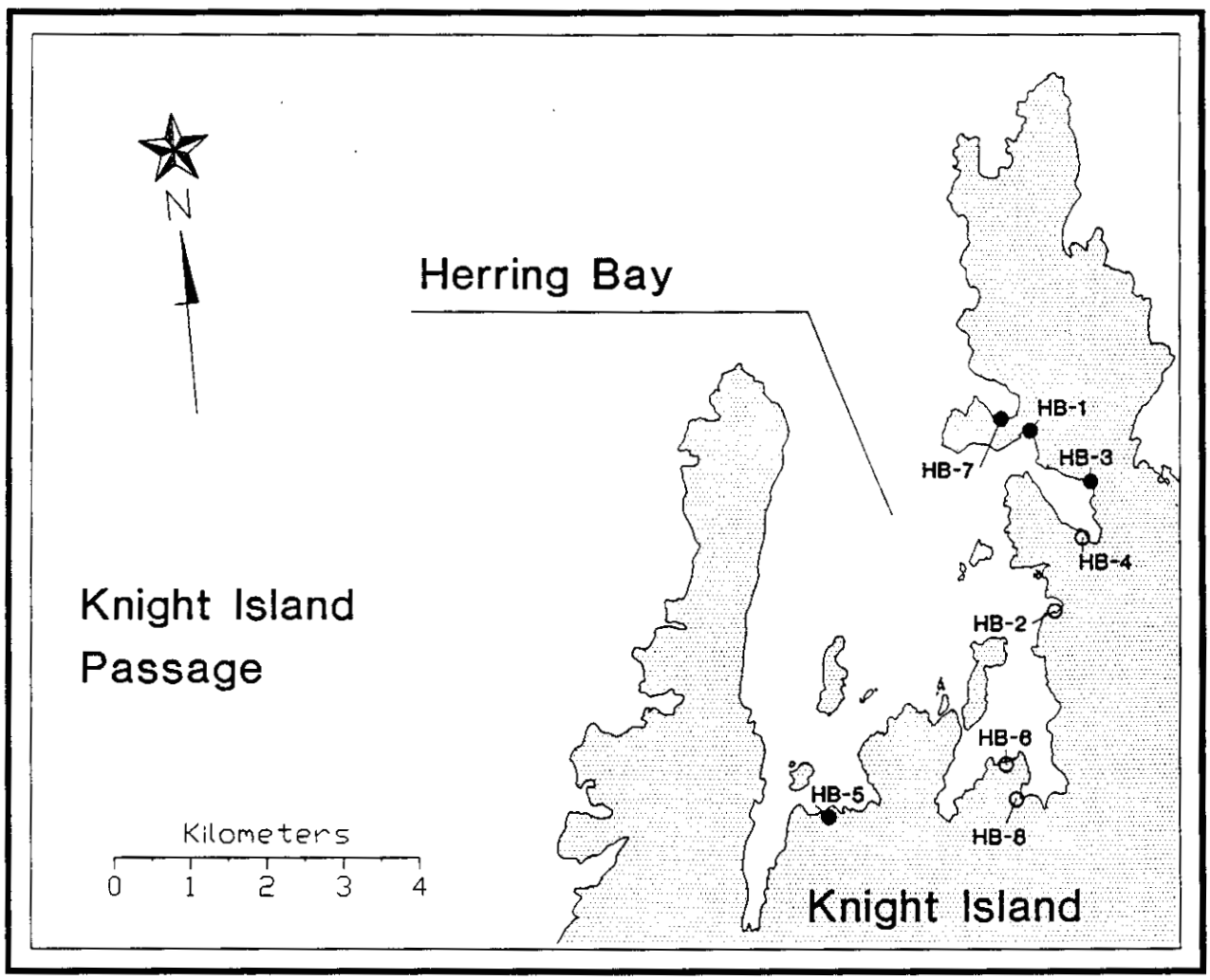

Figure 5B.1. Location of beaches in Herring Bay sampled for epibenthic harpacticoid copepods in 1990. Open circles denote lightly oiled beaches; closed circles denote heavily oiled beaches.

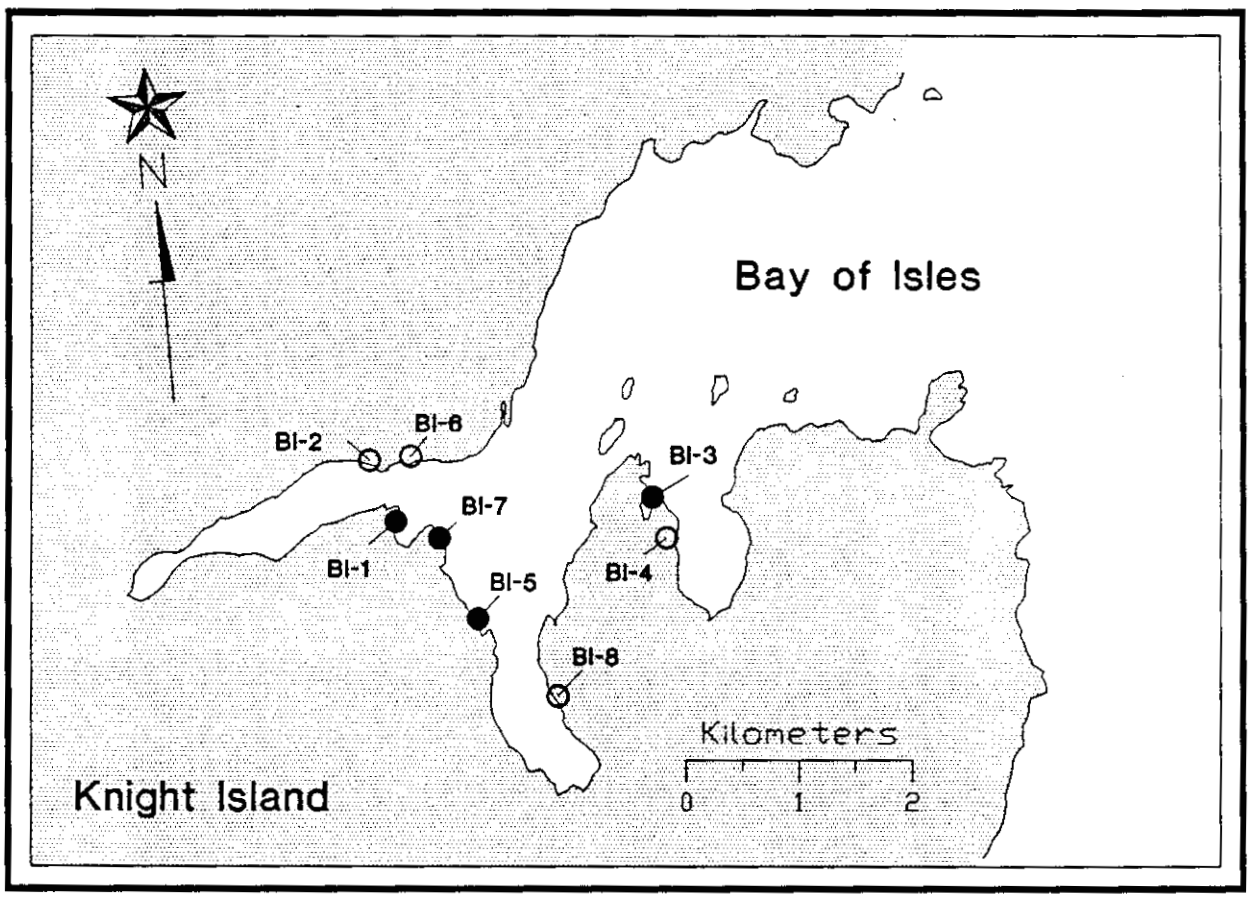

Figure 5B.2. Location of beaches in Bay of Isles sampled for epibenthic harpacticoid copepods in 1990. Open circles denote lightly oiled beaches; closed circles denote heavily oiled beaches.

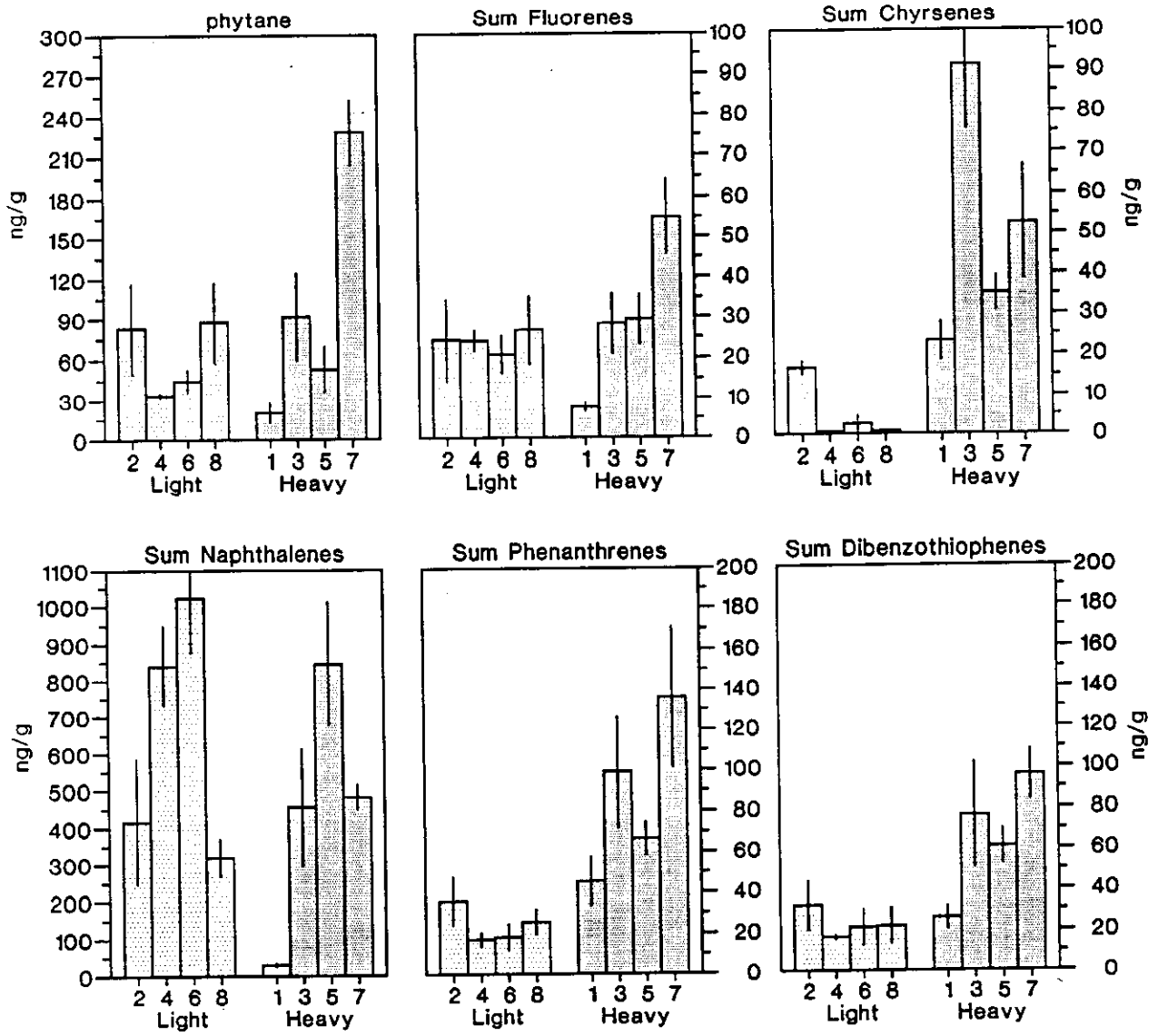

Figure 5B.3. Analyte concentrations in sediments from lightly oiled and heavily oiled beaches in Herring Bay sampled for epibenthic harpacticoid copepods in 1990. Numbers below bars refer to specific beaches listed in Table 1. Vertical lines at top of bars denote standard errors of the mean concentrations.

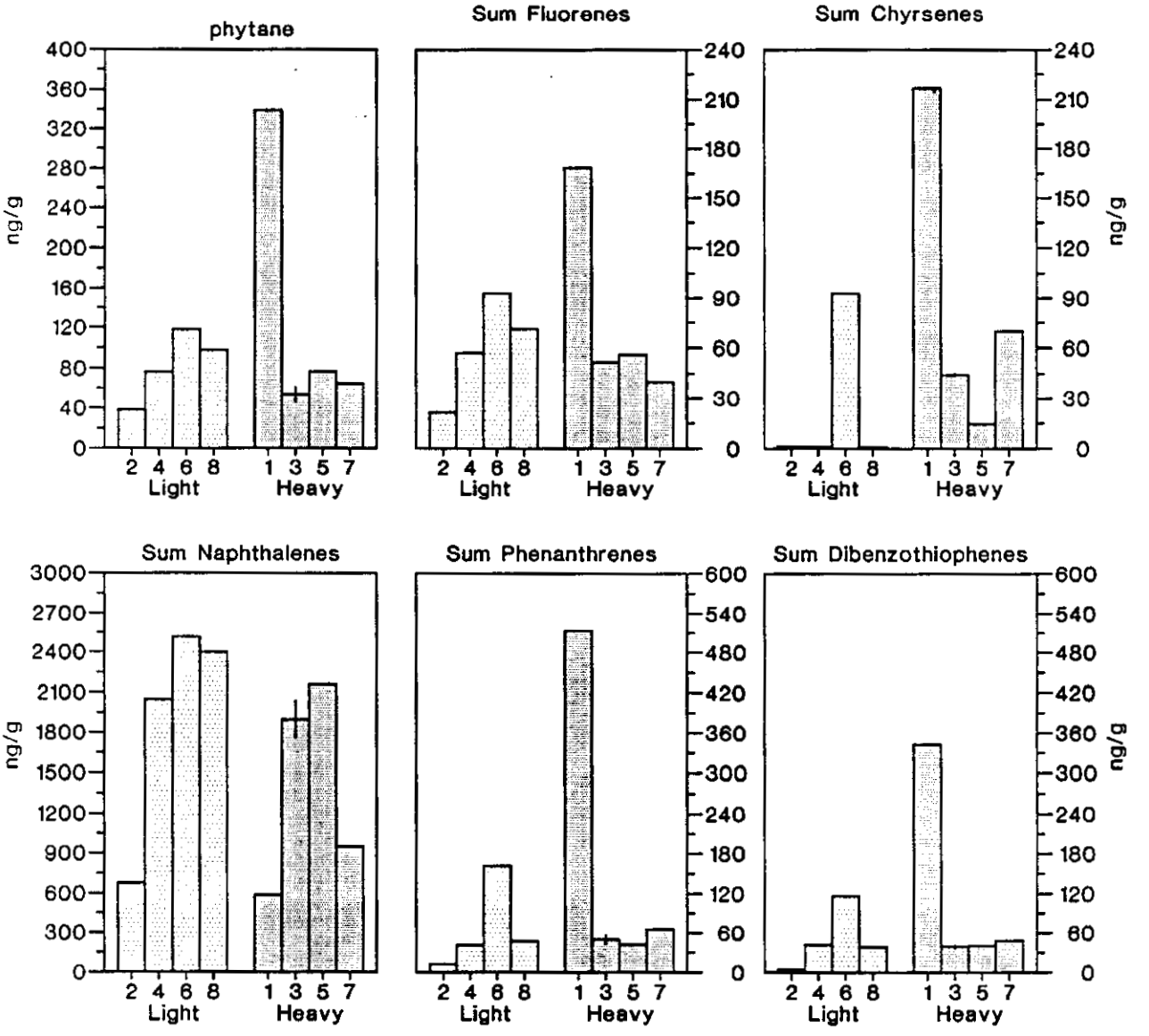

Figure 5B.4. Analyte concentrations in sediments from lightly oiled and heavily oiled beaches in Bay of Isles sampled for epibenthic harpacticoid copepods in 1990. Numbers below bars refer to specific beaches listed in Table 1. Vertical lines at top of bars for beach 3 denote standard errors of the mean concentrations; only 1 sediment sample from each of the other beaches was analyzed for hydrocarbons.

CHAPTER 5C. Recolonization of Meiofauna in Sediments Experimentally Contaminated with *Exxon Valdez* Crude Oil in Prince William Sound.

M. G. Carls, T. C. Shirley, and J. W. Fleeger

Abstract

Sediments were contaminated with *Exxon Valdez* crude oil to study the influence of oiling on meiofaunal colonization and community structure. Azoic sediments were experimentally oiled to prepare low (402 µg/g) and high (5,631 µg/g) oil treatments. Oiled sediments plus controls were placed intertidally at two sites in Prince William Sound. Meiofauna and hydrocarbon samples were collected on days 1, 2, 28 or 29, and 89. Hydrocarbon concentrations tended to decline over this time period. Colonization of sediments by meiofauna was rapid, and was completed between 2 and 28 days. Density of several taxa, including nematodes, turbellarians, and copepods, was initially depressed by oiled sediment (days 1 and 2), but treatment effects were generally not observed on day 28 and beyond. The decline in hydrocarbon concentrations may explain why depression of copepod abundance was a short-term phenomenon. Alternatively, the majority of the meiofauna may have been associated with the relatively uncontaminated surficial sediment and flocculent layer that overlaid the treated sediment and thus reducing the effects of the oiled sediment. There was no evidence for oil-induced mortality of copepods in our experiment; copepods may have avoided oil-enriched pans until surface concentrations had declined, or they may have emigrated from the trays at rates exceeding emigration from controls. Emigration of contaminated meiofauna from oiled sediments into the water column may provide a mechanism for introducing oil into the diets of other organisms, such as juvenile salmon.

Introduction

The presence of petroleum hydrocarbons in intertidal areas may affect the growth, reproduction, and survival of meiofaunal populations inhabiting impacted shoreline. The changes caused by oil are not always predictable: some researchers have observed declines in copepod populations as a result of oil (Wormald 1976; Giere 1979; Boucher 1980; and Bonsdorf 1981), but others have observed increases in meiofaunal abundance (Fricke et al. 1981; Fleeger and Chandler 1983; Stacey and Marcotte 1987; Feder et al. 1990).

Species diversity may decrease after an oil spill, and the index of dominance may increase (Bonsdorff 1981). Hydrocarbon stress does not necessarily reduce species diversity, however, even when species abundance is reduced (Oviatt et al. 1982). Oviatt et al. (1982) found the abundance of most meiofaunal groups decreased when oil-water dispersions of No. 2 fuel oil were added to microcosms; ostracods were the most sensitive group. Abundance of benthic diatoms, Foraminifera, and the polychaete *Chaetozone* sp. increased, probably because of reduced grazing pressure from oil-impacted species.

Hydrocarbons can be toxic to benthic copepods, but abundance of some species may increase. For example, the intertidal copepod *Tigriopus californicus* was asphyxiated by oil slicks and killed by the water soluble fraction (WSF) of California crude oil (Kontogiannis and Barnett 1973). Oviatt et al. (1982) observed decreased benthic respiration in oiled microcosms. Following a large spill of heavy marine diesel oil, the meiofauna of littoral sandy beaches in Picnic Bay, Hong Kong, were almost totally destroyed within 4 days (Wormald 1976). Direct toxicity and oxygen limitation were apparently the principal factors limiting recolonization (Wormald 1976). Abundances of two species of harpacticoid copepods, *Longipedia americana* and *Microarthridion littorale*, were reduced by sediment contaminated by pelagic inputs of No. 2 fuel oil, but abundance of *Enhydrosoma baruchi* increased rapidly several months after the oil was introduced (Stacey and Marcotte 1987). Fleeger and Chandler (1983), however, observed that copepod abundance was only slightly depressed by Louisiana crude oil in a *Spartina alterniflora* salt marsh, and that the abundance of *Enhydrosoma woodini* gradually increased.

When the benthos is polluted with oil, harpacticoid assemblages may change from those numerically dominated by epibenthic and demersally planktonic species, such as *L. americana* and *M. litorale*, to those dominated by inbenthic taxa, such as *Enhydrosoma baruchi*, possibly because *E. baruchi* is able to avoid hydrocarbons by remaining deeper in the sediments (Stacey and Marcotte 1987). This change in community structure may impact

fish that feed on benthic crustaceans because inbenthic prey would be harder for visual feeders to find and capture than epibenthic prey (Marcotte 1983; Marcotte and Browman 1986).

The rate of recolonization of sediments by meiofauna may range from immediate (1 d) to months (Alongi et al. 1983; Chandler and Fleeger 1983; Palmer et al. 1988). Recolonization may be particularly rapid (2 d) for copepods, but can be much longer for nematodes: >29 d (Chandler and Fleeger 1983) to nearly 90 d (Alongi et al. 1983). Although most meiofauna populations attained densities equivalent to the indigenous community within 16 d, Alongi et al. (1983) found recruited populations fluctuated more than indigenous populations, and the dominant nematode assemblage remained different than the indigenous assemblage for nearly 90 d.

The objective of this experiment was to determine if sediments contaminated by *Exxon Valdez* (*EV*) crude oil influenced meiofaunal colonization and community structure in Prince William Sound (PWS), and to gain insight on the relationship between oiled sediment, epibenthic prey, and juvenile salmonids (objective 11 in Chapter 1). It was a manipulative field experiment designed to study the effects of contamination without confounding hydrographic or other physical intersite differences. Azoic sediments were experimentally oiled with *EV* crude oil and placed, along with untreated control sediments, intertidally at two sites in Herring Bay (PWS). We hypothesized that the presence of oil in sediments would influence meiofaunal colonization and community structure.

Chapter 5C will be reformatted and submitted to the *Exxon Valdez Oil Spill Symposium Proceedings* for formal publication.

Methods

Sediment was collected in Auke Nu Cove (58°22'48" N latitude, 134°41'39" W longitude) in Auke Bay, Alaska on 1 March 1990 at -0.43 m (09:15 am) from the surface of an eelgrass (*Zostera* spp.) bed and placed in 19-L buckets. Approximately 333 L of sediment was collected and frozen.

To produce azoic sediments, the buckets were subjected to three freeze-thaw (-20°C to +20°C) cycles over 1 month. During the first thaw, the sediment was washed through 2-mm and ~0.36-mm sieves with fresh water to remove all large particles and macrofauna. Excess water was decanted off after a minimum 4 h settling period and throughout the freeze-thaw cycles. The total mud volume declined to 227 L.

High (1.7% oil by weight) and low (0.5% oil) treatments were prepared by combining sediment and *EV* crude oil in a cement mixer. The maximum oil concentration was determined by the estimated maximum amount of oil the sediment would absorb (2%) and by availability of *EV* oil. Control sediments were prepared in the same way without the addition of oil. The interior of the mixer was scrubbed with hot water and soap and rinsed thoroughly before use. Processing was sequential, starting with controls, so that preceding sediments would not be contaminated by oil. The sediment was divided randomly into three equal treatment groups (control, low oil, high oil). Two batches per treatment were processed because of limited mixer capacity. Batches of sediment, 38 L each, were tumbled in the mixer for 15 minutes, then *EV* crude oil was added. The total mixture was tumbled an additional 1.5 h, then stored in 19-L buckets with snap-top lids. Mud was mixed by hand to combine both batches in the storage buckets. Processing was completed 5 April 1990; buckets were stored at ambient air temperature. Contents of each storage bucket were turned once (17 or 18 April) to avoid possible anoxic conditions.

Oiled and control sediments were placed in separate pans arranged along two transects in Herring Bay (Figure 5C.1). Transects were placed intertidally along the upper margins of eelgrass beds in Herring Bay on consecutive days, the first in a lightly oiled cove (HBL), and the second in a heavily oiled cove (HBH) (Figure 5C.1). Oil ratings were based on ADEC shoreline data. Treatment positions along the transects were chosen randomly within three consecutive 4-m blocks. Each transect was 12 m long with nine pans (3 treatments with 3 replicate pans per treatment) positioned at 1.5-m intervals. Pans were 28 cm wide by 33 cm long by 14 cm high with straight walls; the bottoms and sides were perforated with 3-mm holes drilled at 8-mm intervals to allow water circulation and drainage. Experimental mud was distributed into pans randomly by treatment group. The pans were

buried flush with the natural substrate; excess mud was disposed well upshore from the transects. Equipment used to distribute mud and collect samples was initially hydrocarbon-free and was separated by treatment to avoid cross-contamination. Initial meiofauna samples were collected before pan burial to exclude influences of indigenous sediments.

The low-oil transect (HBL) was located at 60°27'6" N latitude, 147°42'18" W longitude in a small cove (Figure 5C.1). The entire tide flat had fresh water trickling across it at low tide, supplied by a small stream to the right of the 0-m site: water flow was greatest at the 0-m site. A rock-cobble-gravel substrate extended from the upper intertidal down nearly to the eelgrass bed where it changed to mud. The transect was positioned in the mud parallel to the water line at -0.61 m mean low water (MLW) along the upper margin of an eelgrass bed on 24 April 1990.

The high-oil transect (HBH) was located at 60°28'0" N latitude, 147°41'12" W longitude in a large cove (Figure 5C.1). A cobble-gravel beach extended from the upper intertidal zone nearly to the upper margin of an eelgrass bed. The eelgrass bed had a deep layer of soft mud. The transect was positioned in the soft substrate along the upper margin of the eelgrass bed at approximately -0.56 m MLW on 25 April 1990. Pan elevations were lower on the 0-m end of the transect (-0.70 m) than at the 12-m end (-0.46 m) to accommodate variations in the substrate. Oil remaining from the *EV* spill was obviously present; oil sheens wept out of the ground along the selected transect.

Experimental mud was added after the empty pans were buried at the HBL site, but before the pans were buried at the HBH site. At HBL, some mixing of indigenous and treatment muds began immediately because of water movements. At HBH, approximately 1 cm of non-oiled, washed gravel, obtained well above the tide line from a stream located several m to the left of the transect, was added to the bottom of each pan to fill excess volume. The HBL transect was exposed to air 18 times (1.7% of total time) during the 89 days of study; emergence time ranged from 71 to 156 minutes. The HBH transect was exposed to air 20 times (1.8% of total time) during the 89 days of study; emergence time ranged from 32 to 162 minutes. The amount and percent time the experimental pans were in air were determined with a tide calculation program written by one of us (Carls).

Seawater temperature and salinity were measured with a self-contained sensing device located at an elevation of approximately -0.1 m near the HBL transect. Temperature and salinity were 8.0 ± 0.1°C and 29.0 ± 0.1 ppt (mean and 95% confidence interval) over the first 28 days, excluding data when the sensor was exposed to air (Figure 5C.2). Temperature tended to rise from

day 46 to the end of the experiment (8.9 to 16.2°C) and salinity tended to decline (28.2 to 20.8 ppt). Air temperature ranged from 2.7 to 14.0°C, but may have been influenced by evaporative cooling. Temperature and salinity data were lost from day 28 to 46 because of battery failure. Temperature and salinity were occasionally recorded at the HBH transect, and were similar to those reported for HBL.

Meiofauna were sampled from each pan (during aerial exposure at low tide) with hand-held piston corers (modified 60-ml plastic syringes, 2.66 cm diameter); the upper 4.0 cm of each core was retained. Corers were separated by treatment to avoid cross-contamination. Initial core samples were collected in triplicate from each treatment pan (HBH) or storage bucket (HBL). Triplicate meiofauna cores were collected from each treatment pan on days 1, 2, 28 (HBH only), 29 (HBL only), and 89. Core positions were determined randomly without replacement through day 2, and completely randomly thereafter. Core placement was at least 2 cm from the margin of the pans to avoid possible edge effects. Additionally, eight samples of natural meiofauna were collected from randomly selected sites between treatment pans in each sampling period. Meiofauna samples were preserved in 10% formalin shortly after collection. Samples were collected over an 89 day period.

Hydrocarbon samples were collected with a chrome-plated brass tube 3 cm in diameter; a spoon was slipped down beside the corer to cap it off at a depth of 4-6 cm. All equipment used for hydrocarbon sampling was prewashed with soap and hot water, rinsed, dried, and rinsed with acetone and then dichloromethane. Corers were separated by treatment to avoid cross-contamination. Two initial hydrocarbon samples were collected from each treatment pan (HBH) or stock bucket (HBL). On days 2, 28 or 29, and 89, one sample was collected from each pan, plus three from randomly selected spots between the pans. Core positions were chosen randomly (as above) along with meiofauna cores to avoid conflict between sample types. Hydrocarbon samples were placed directly into hydrocarbon-free glass jars with teflon lids and frozen as soon as possible after collection.

Hydrocarbon concentrations were determined by gas chromatography followed by mass spectrometry for aromatics or flame ionization detection for aliphatics as detailed in Chapter 3b. Samples were analyzed at either of two laboratories, the Geochemical and Environmental Research Group at Texas A&M University or the Auke Bay Laboratory and reported via the PWSOIL database (Manen et al. in prep). Hydrocarbon samples were filtered by deuterated recovery and machine detection limits, and bad samples (3% of total) were excluded using the techniques presented in Chapter 3b. Data filtration eliminated the initial low- and high-oil treatment observations at the HBH site (12 samples), but because

these observations were replicates of those collected at the HBL transect, corresponding data were repeated as HBH values.

Because PAHs are not normally present intertidally in PWS (except perylene) and because there are natural sources of alkanes in PWS (Karinen et al. 1993), PAHs were chosen as the primary measure of hydrocarbons in this study. Concentration changes in various subcomponents of PAH (naphthalenes, fluorenes, dibenzothiophenes, phenanthrenes, and chrysenes) were similar to changes in PAH, thus the more generalized PAH measurement was used to summarize the data. Some alkane data, including the unresolved complex mixture (UCM), are presented.

Hydrocarbon concentrations were compared between sites for each treatment with ANOVA procedures. Because variance was proportional to the means, data were log transformed prior to analysis. To avoid pseudoreplication, multiple observations within single replicate pans were averaged before other calculations; this situation occurred on day 0 only. Outcomes were considered significant when $P \leq 0.05$.

Grain sizes, expressed as percent sand, silt, and clay, were supplied via the PWSOIL database. Sediment grain-size distributions were analyzed by regressing these percentages over time to determine if changes in grain size occurred.

In the laboratory, meiofauna passing through a 0.500-mm-mesh sieve but retained on a 0.063-mm mesh were separated from associated detritus with a sucrose flotation/centrifugation technique (Fleeger 1979). Samples were suspended in a dense sucrose solution (700 g sugar in 500 ml water) and centrifuged at 350 rpm, with the supernatant being sieved through a 0.063-mm sieve. The process was repeated at least twice; sediment pellets resulting from the centrifugation were examined for sorting efficiency. The technique was estimated to exceed 95% efficiency for nematodes and 98% for copepods in recently published studies of Alaskan meiofauna from similar habitats (Fleeger et al. 1989; Fleeger and Shirley 1990). Concentrated meiofauna were preserved in buffered 5% formalin and stained with Rose Bengal to further aid sorting.

All organisms were identified to major taxa under a stereo dissection microscope and enumerated in ruled trays. When a high density of meiofauna was present, predominant taxa (mainly nematodes) were subsampled by a technique employing a triply-balanced square design (Sherman et al. 1984) which precludes bias from edge and center effects. Copepods (adults and copepodites) were removed and retained for later identification to sex and species.

Collections of meiofauna taken from azoic sediment before placement in the field indicated that although meiofauna were killed, decomposition was incomplete. Copepods, with chitinous cuticles, were especially resistant. Residual copepods in our colonization samples were identified by observing the condition of the setae of various appendages, with particular attention given to the caudal setae; a large number of broken setae indicated to us that these individuals were dead when collected. Most copepods with broken caudal setae showed signs of decomposition including ruptured cuticles, partially decomposed flesh, and a dense detrital coating over the body. Based on this observation, all copepods were recorded as "dead" or "alive" at the time of collection.

Meiofauna results are preliminary; copepods have been analyzed in greatest detail. Processing of meiofauna samples by Tom Shirley (UAF), John Fleeger (LSU), and their staff is nearing completion (≥80%). Meiofauna data included in this report are from the HBH site only.

Abundance of copepods was tested with a two-way (day and oil treatment), randomized block ANOVA (pan replication was blocked). Significance between treatment means was tested with Tukey's Studentized Range Test. Outcomes were considered significant when $P \leq 0.05$. Detrended correspondence analysis (Hill 1979), an ordination technique similar to correspondence analysis, was conducted to determine if the oiled sediment influenced the copepod assemblage: four standard deviations of species turnover indicates a full change in species composition (Gauch 1982).

Results

Hydrocarbon Concentrations in Sediments

Hydrocarbon concentrations in the sediments (measured by TOC, PAH, and alkanes at HBL) correlated well with the percent oil added ($0.81 \leq r^2 \leq 0.94$, Figure 5C.3). Initial PAH concentrations in the sediments ranged from 4.6 μg/g (control) to 210 μg/g (high oil) (Table 5C.1). Initial PAH concentrations in indigenous sediments, measured on days 2 and 3, were 0.15 ± 0.06 and 0.27 ± 0.03 μg/g at HBL and HBH, respectively. The PAH composition in control sediments was unlike that of *EV* crude oil: the naphthalene component was relatively high and there was evidence of low temperature combustion products (benzo(b)fluoranthene and heavier compounds were present, and concentrations of increasingly methyl-substituted compounds tended to decline with respect to their unsubstituted homologs) (Figure 5C.4). Concentrations in indigenous sediments were low and essentially constant; PAH composition was characteristic of weathered *EV* crude (Figure 5C.4). In oil treatments, PAH composition matched that of *EV* crude (Figure 5C.4).

Concentrations of PAH and alkane hydrocarbons in experimental sediments depended primarily on oil treatment and time ($P < 0.001$). At the HBL site, concentrations in oil treatments tended to decline over the first 89 days (Figure 5C.5). At the HBH site, concentrations tended to remain constant or rise slightly during the first 28 days, but declined by day 89 (Figure 5C.5). Although hydrocarbon concentrations declined during the experiment, on day 89 PAH and alkane concentrations remained significantly greater than control concentrations at HBL ($0.025 \leq P \leq 0.075$) and HBH ($P \leq 0.028$).

Sediment Grain Size

The grain size for all imported sediments was the same at both transect sites and across all treatments ($0.392 \leq P \leq 0.987$). There was weak evidence (significant regression slopes for sand and clay) that grain size changed slightly over the course of the experiment; the percentage of sand increased, and silt and clay decreased. Correlation between the grain size of experimental sediments and time was poor ($0.20 \leq r \leq 0.42$). Silt was the dominant size range in the experimental sediments (Table 5C.2). The indigenous sediments were generally finer grained than the experimental sediments, and differed between sites and over time (Table 5C.2). Over the study period, indigenous sediment tended to become coarser ($P_{sand} = 0.123$) at the HBL site, but variance between samples also increased. Indigenous sediment at the HBH site tended to become finer ($P_{clay} = 0.121$), and clay particles dominated, but again variance between samples increased.

Meiofauna

The predominate meiofauna taxa included nematodes, harpacticoid copepods, ostracods, and bivalve larvae. Other meiofauna taxa that occurred in substantial numbers on some dates included turbellarians, halocarid mites, gastropod larvae, and polychaetes. Nematodes were the numerically predominant taxon in all treatments and sampling dates (about 45% of total). Harpacticoid copepods were diverse, with more than 40 species encountered. Copepods occupying experimental sediments were primarily associated with the surrounding eelgrass and algal-mat habitats. Most species displayed strongly prehensile first legs, belonging to families associated with a phytal lifestyle. The five most abundant species of harpacticoid copepods included two species from the Family Diosaccidae, and one each from the Families Canthocamptidae, Laophonitidae, and Ectinosomatidae. Non-copepod taxa were not identified to species, but based on copepod data, we assumed that all meiofauna taxa were associated with the surrounding eelgrass and algal-mat habitats.

Pronounced seasonal changes in density occurred for all taxa in the surrounding natural sediments and in the experimental treatments. The seasonal changes were not related to treatment effects, but reflected seasonal recruitment patterns, which vary among the taxa. Changes in density of larvae of macrobenthic invertebrates (bivalves, gastropods, and polychaetes) occurred in pulses probably related to planktonic settlement.

Colonization of azoic sediments was rapid for all true meiofaunal taxa but not macrobenthic larvae. For most taxa, colonization was completed between 2 and 28 days (Figure 5C.6). For copepods, no single harpacticoid species or succession of species accounted for the rapid colonization; instead colonists belonged to a large number of taxa each found in rather low densities. Abundances of total live copepods, unidentified copepodites, and the five most abundant harpacticoid copepod species increased significantly from days 1 to 28, as did density in the indigenous sediment.

High oil concentrations reduced colonization rates of all major meiofauna taxa (Figure 5C.6). Density of total meiofauna in low- and high-oil treatments was significantly less than in controls on day 2; density was least in the high-oil treatment and intermediate in the low-oil treatment. Although not statistically significant, nematode and turbellarian densities in the high-oil treatment were also depressed on day 1.

Copepod assemblages were affected by oil on days 1 and 2, but no difference between control and oiled sediments was apparent by day 28. Densities of total copepods and *Canthocamptus* sp. were significantly lower in the high-oil treatment than in the control and low-oil treatments (days 1, 2, and 28 combined). For

Ectinosomatidae sp. 1, density in control sediments was significantly higher than in the low-oil treatment, and density in the low-oil treatment was significantly higher than in the high-oil treatment (days 1, 2, and 28 combined). Two axes, generated with detrended correspondence analysis, comprised more than 70% of the variance (Figure 5C.7). Axis one contained relatively high levels of variation, with values approaching two standard deviations overall (four indicates complete change in species composition). All indigenous samples separated from experimentals on axis one and clustered near to each other, indicating that experimental sediments could be distinguished from the surrounding sediments in species composition in all collections. Other collections were separated on axis 2 with only 1 standard deviation of faunal turnover. Day 1 and 2 low- and high-oil treatments clustered together as did control, low- and high-oil treatments from day 28. Control day 1 collections were intermediate.

Oiled sediments did not kill harpacticoid copepods. The number of dead copepods was generally equal on all collection dates in all experimental treatments, including controls. Dead copepods were rare in indigenous sediments. The dead observed in treatment sediments were, therefore, primarily those killed by freezing during sediment preparation.

Discussion

Data analysis for this study is preliminary. Meiofauna samples are now nearing completion. We expect to publish a manuscript in the proceedings of the *Exxon Valdez* Oil Spill Symposium. A portion of this research was presented at the *Exxon Valdez* Oil Spill Symposium in February (Fleeger et al. in prep).

The concentrations of *EV* oil we used in this experiment were not unrealistically high. Based on our observations, the maximum PAH concentration in the high-oil treatment (246 μg/g) was within the range of PAH concentrations observed in sediments in PWS after the *EV* oil spill (0 to 482 μg/g). The source of PAHs detected in controls was likely due to ship activity near the collection location (Auke Nu Cove).

Our data indicate that copepods have the ability to quickly recolonize azoic sediments over small spatial scales (0.1 m^2 in this experiment) following the addition of hydrocarbons (see also Alongi et al. 1983; Decker and Fleeger 1984). Meiofauna are active colonizers through the water column, especially in muddy sediments (Chandler and Fleeger 1983; Palmer 1988), and many individuals colonized our experimental sediments after 1 day, even in high-oil treatments. Hydrocarbons, however, altered colonizing ability: the densities of two individual species and total copepods were significantly depressed in high-oil treatments. The effect was short-lived, however, and oil effects were not discernable after 28 days.

Declines in hydrocarbon concentrations may explain why depression of copepod abundance was a short-term phenomenon. Alternatively, the majority of the meiofauna may have been associated with the relatively uncontaminated surficial sediment and flocculent layer that overlaid the treated sediment. In this case, little relationship between hydrocarbon concentration and meiofauna density would be expected. Although data were not collected in sufficient detail to determine the origin of meiofauna within various sediment strata, the largely phytal nature of the copepods observed tends to support this alternative hypothesis. Meiofauna in the surficial layer might be influenced by hydrocarbons migrating upward from underlying sediments, but the mobility, depth, and density of the surficial layer probably affected the rate of hydrocarbon transfer. The rate at which the surficial layer accumulated after the experiment began could also influence the amount of time treated sediments exerted direct effects on meiofauna density. Continued observation of hydrocarbon concentrations in this experiment over a longer period (2 years) indicates continued significant elevation of hydrocarbons in treated sediments, with only moderate loss in concentration.

Knowledge of the ability of organisms to recolonize an area following an oil spill is vital to understanding the impact of such spills. The response of benthic animals to hydrocarbon varies (Fleeger and Chandler 1983; Coull and Palmer 1984; Coull and Chandler 1992). A combination of effects on mortality, reproduction, and migration may all contribute to observed changes in density. Because there was no evidence for oil-induced mortality in our experiment, migration may have been more important than mortality in controlling copepod density. Copepods may have avoided oil-enriched pans, or they may have emigrated from the trays at rates exceeding emigration from controls. Recent data suggest that copepods that have entered the water column have the ability to select specific locations for colonization (Fegley 1988), but only under low-flow conditions. Emigration rates could also have been affected by hydrocarbons. Copepods under high-oil conditions may display different behaviors; they may have actively emigrated by swimming into the water column or passively emigrated by allowing themselves to be carried away by tidal currents.

Because the meiobenthos is an important link for the transfer of energy from benthic to pelagic food webs (Stacey and Marcotte 1987; Coull 1973; Marcotte 1980; Hicks and Coull 1983), the impacts of intertidal oiling may have broad implications. Harpacticoid copepods are often numerically the second-most abundant organisms in soft sediments, and are probably the most important component in the transfer of energy from the benthos to the pelagic environment (Hicks and Coull 1983). For example, juvenile pink salmon in some areas feed primarily on epibenthic meiofauna, such as harpacticoid copepods (Kaczynski et al. 1973; Landingham 1982; Volk et al. 1984). Research in PWS following the *EV* oil spill indicated that juvenile salmon were contaminated with *EV* oil, and that ingestion was the most likely mechanism (Chapter 3, this report).

Microcrustaceans, such as harpacticoid copepods, may bioaccumulate oil from sediments, and therefore pass hydrocarbons up the food chain. Various studies have shown hydrocarbon uptake from the water-soluble fraction of oil by crustaceans (e.g. Macek et al. 1979; Schwartz 1985; Carls 1987). Uptake of hydrocarbons by benthic organisms may be via interstitial water and is, therefore, kinetically controlled by desorption from sediment particles and organic matter (Landrum 1989). Amphipods (*Anonyx laticoxae*) accumulated naphthalenes from sediment contaminated with Prudhoe Bay crude oil, apparently via water, as it was slowly solubilized (Anderson et al. 1979). Naphthalene was released faster than the alkylnaphthalenes and alkylphenanthrenes; tissue levels in the benthos would probably follow the same pattern (Anderson et al. 1979). Hydrocarbons, particularly the more strongly sorbed compounds, may also be assimilated via ingestion (Landrum 1989).

In summary, oiling of intertidal sediments may influence meiofauna densities and migration rates. However, abundance data collected after an oil spill cannot alone determine if migration or birth and death processes are responsible for changes in density. Longer term information on indigenous meiofaunal populations is necessary to help interpret density changes in a given locale after an oil spill. Oil effects may be mitigated as relatively uncontaminated layers accumulate over oiled sediments. Contact of meiofauna with oiled sediments and subsequent emigration from these sediments into the water column may provide a mechanism for introducing oil into the diets of other organisms, such as juvenile pink and chum salmon. Direct measurement of hydrocarbons in meiofauna within and above oiled sediments would have provided a more rigorous test of this assertion. In associated field studies we found that juvenile pink and chum salmon were contaminated with hydrocarbons from the EV oil spill, and that ingestion was a likely route of contamination (Chapter 3 of this report). Tissues of salmon that ingested oiled food accumulated oil (Chapter 6 of this report). We infer that meiofauna were contaminated with EV hydrocarbons and may have partially mediated transfer of hydrocarbons from sediments to fish.

Table 5C.1. Initial hydrocarbon concentrations (μg/g) in treated and control sediments.

Analyte	Control		Low oil		High oil	
	Mean	(SE)	Mean	(SE)	Mean	(SE)
PAH	4.6	(0.6)	26	(3)	210	(12)
Alkanes	15.3	(4.6)	90	(11)	298	(51)
UCM	42.8	(5.7)	286	(23)	5,123	(15)
Sum	62.7		402		5,631	

Table 5C.2. Percent grain composition of experimental and indigenous sediments, measured initially and on day 89. Initial days were 0, 3, and 2 for experimental sediments, indigenous sediments at the HBL site, and indigenous sediments at the HBH site, respectively.

Particle	Size (μ)	Initial Mean	Initial (SE)	Day 89 Mean	Day 89 (SE)
		All experimental sediments			
Sand	62-2000	36.8	(1.4)	40.7	(3.2)
Silt	4-62	59.6	(1.5)	57.3	(3.3)
Clay	<4	3.6	(0.2)	2.0	(0.2)
		Indigenous sediments at HBL site			
Sand	62-2000	6.5	(1.3)	66.2	(21.7)
Silt	4-62	89.4	(3.5)	26.5	(22.5)
Clay	<4	4.1	(2.2)	7.3	(2.2)
		Indigenous sediments at HBH site			
Sand	62-2000	2.6	(0.8)	19.0	(13.1)
Silt	4-62	94.1	(1.5)	38.2	(26.1)
Clay	<4	3.4	(0.7)	42.9	(20.1)

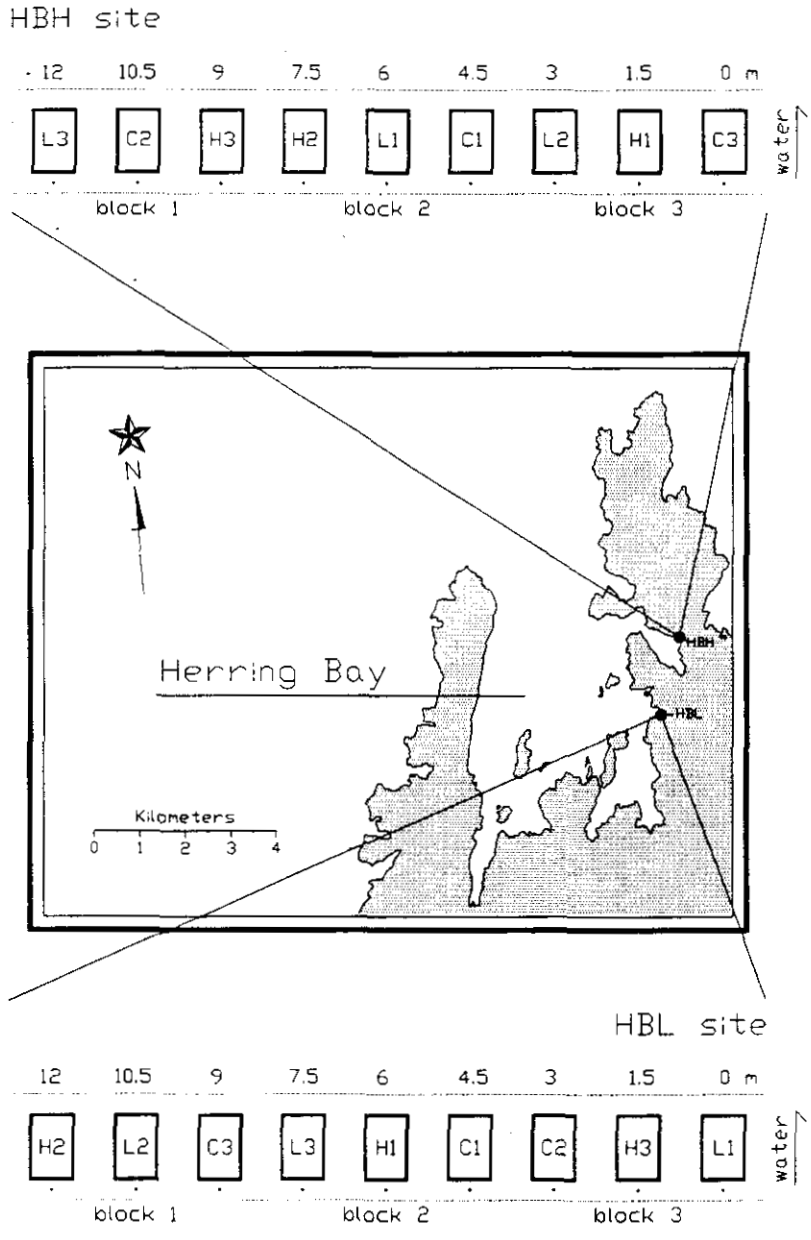

Figure 5C.1. Sites of meiofauna transects in Herring Bay, Prince William Sound, Alaska, and schematic representations of transect configurations. HBL is the low-oil transect site, and HBH is the high-oil site. Transects were 12 m long and pans were positioned as indicated: C = control, L = low oil, and H = high oil, with three replicates per treatment. Pan placement was random within three blocks at each site. Rebar was placed by each pan as a reference marker.

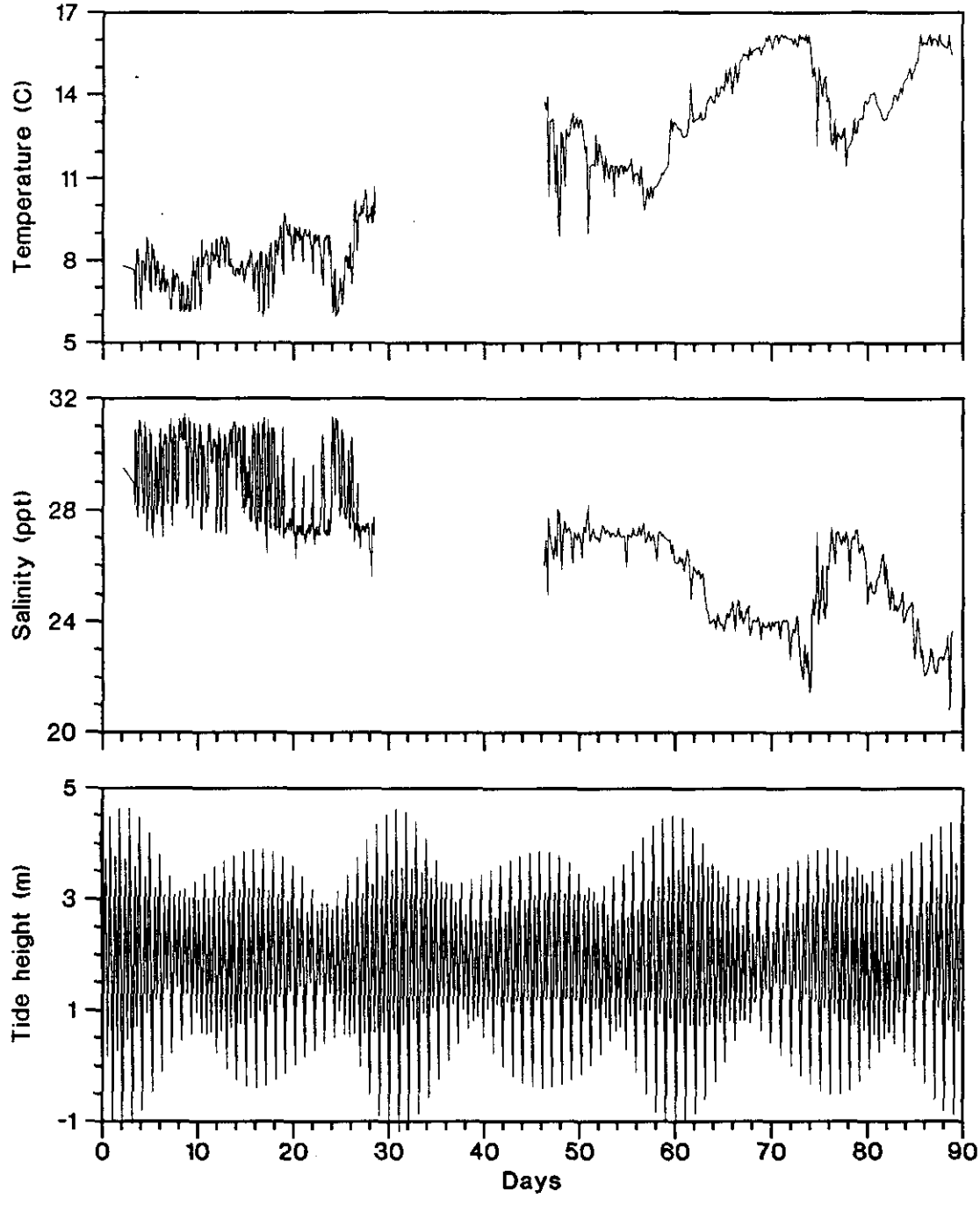

Figure 5C.2. Temperature and salinity record at the HBL site, excluding data when the sensor was exposed to air, plotted with tide cycle. The gap in the temperature and salinity records was caused by battery failure. Tide heights are recorded as daily maxima and minima.

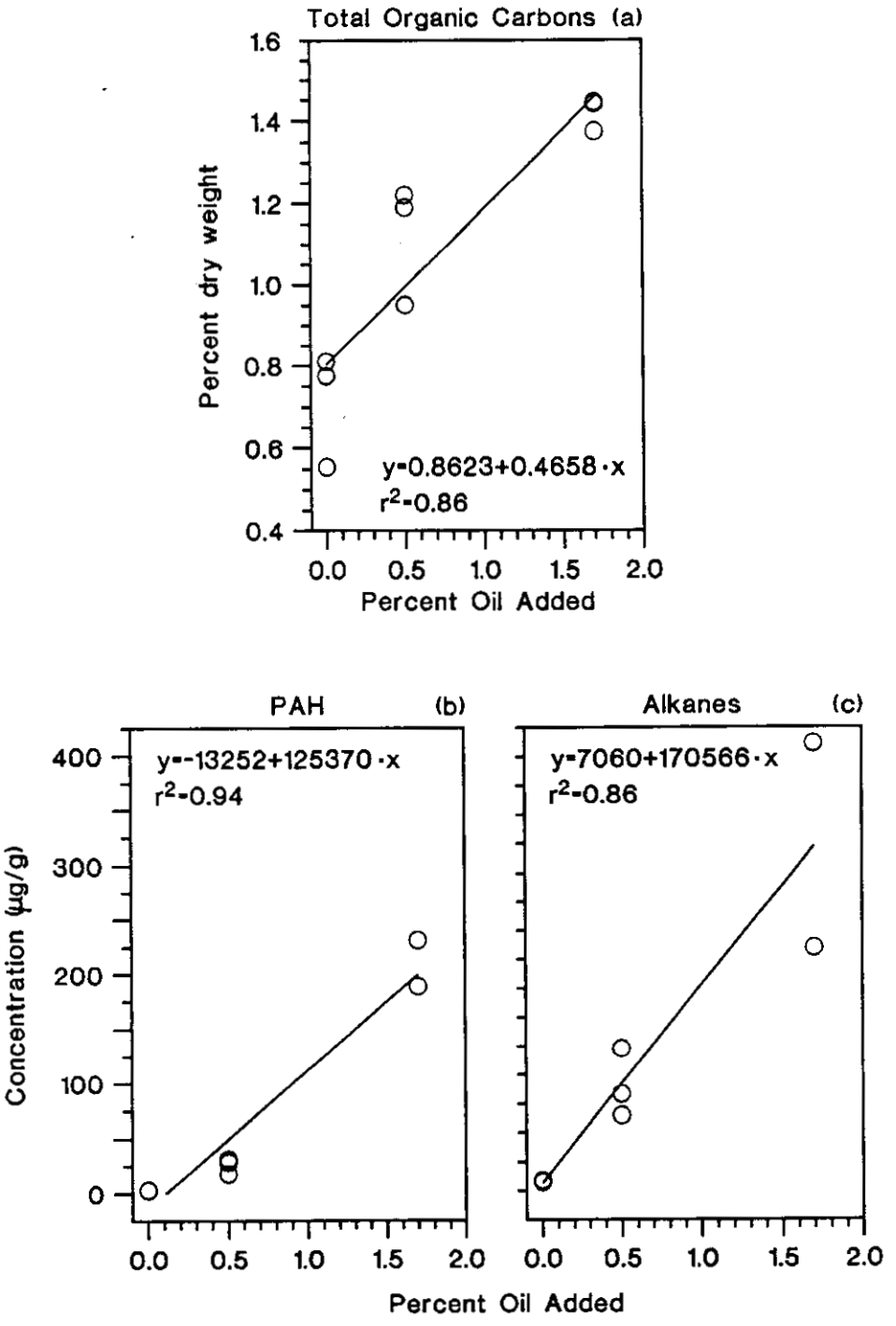

Figure 5C.3. Hydrocarbon concentration on the first day plotted against percentage of oil added. a) Total organic carbons. b) PAH. c) Alkane concentrations, excluding the unresolved complex mixture.

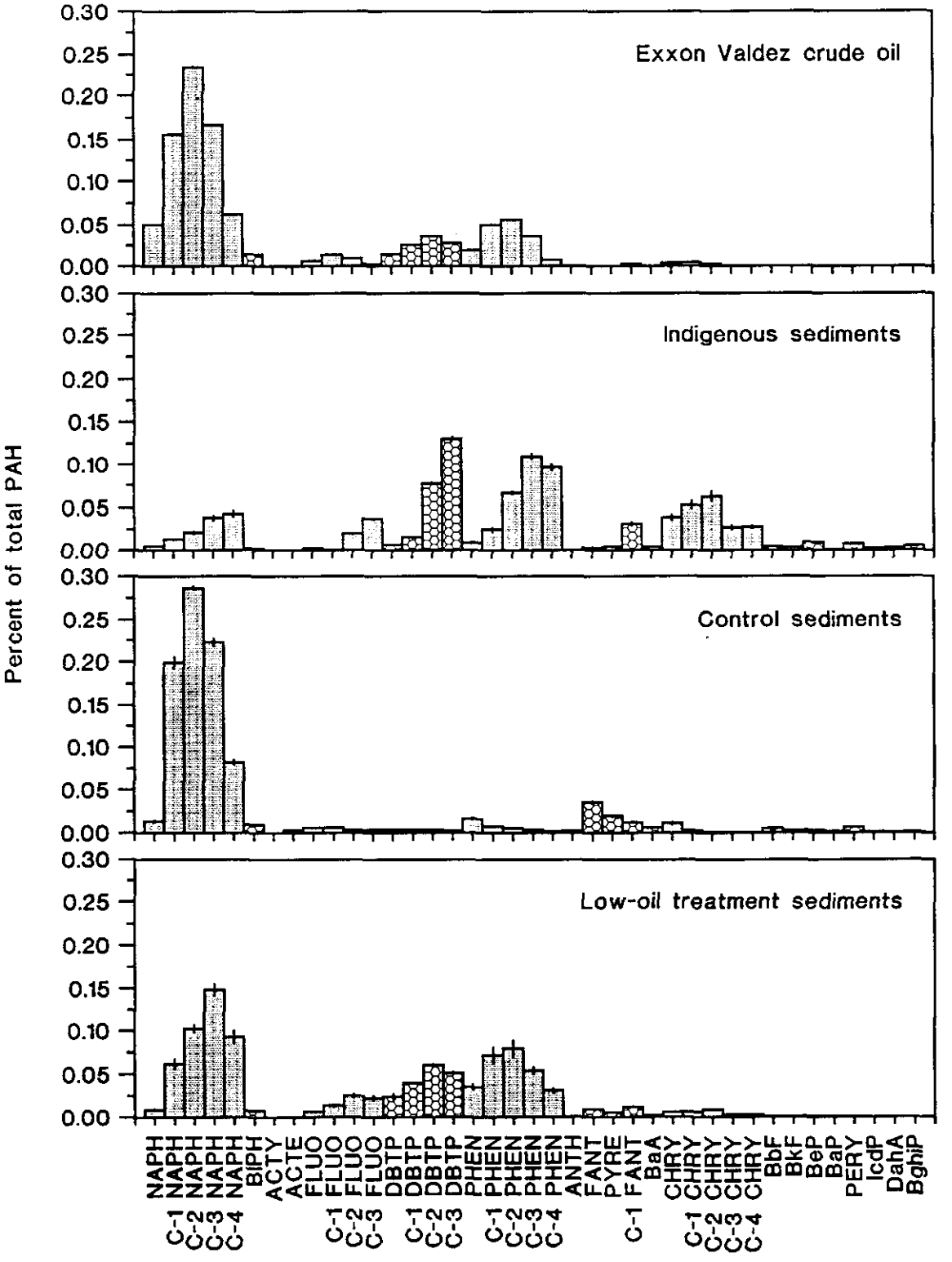

Figure 5C.4. Initial mean, normalized PAH concentrations in *EV* crude oil, experimental sediments, and indigenous sediments, measured at HBH. Concentrations in indigeous sediments were first measured on day 2.

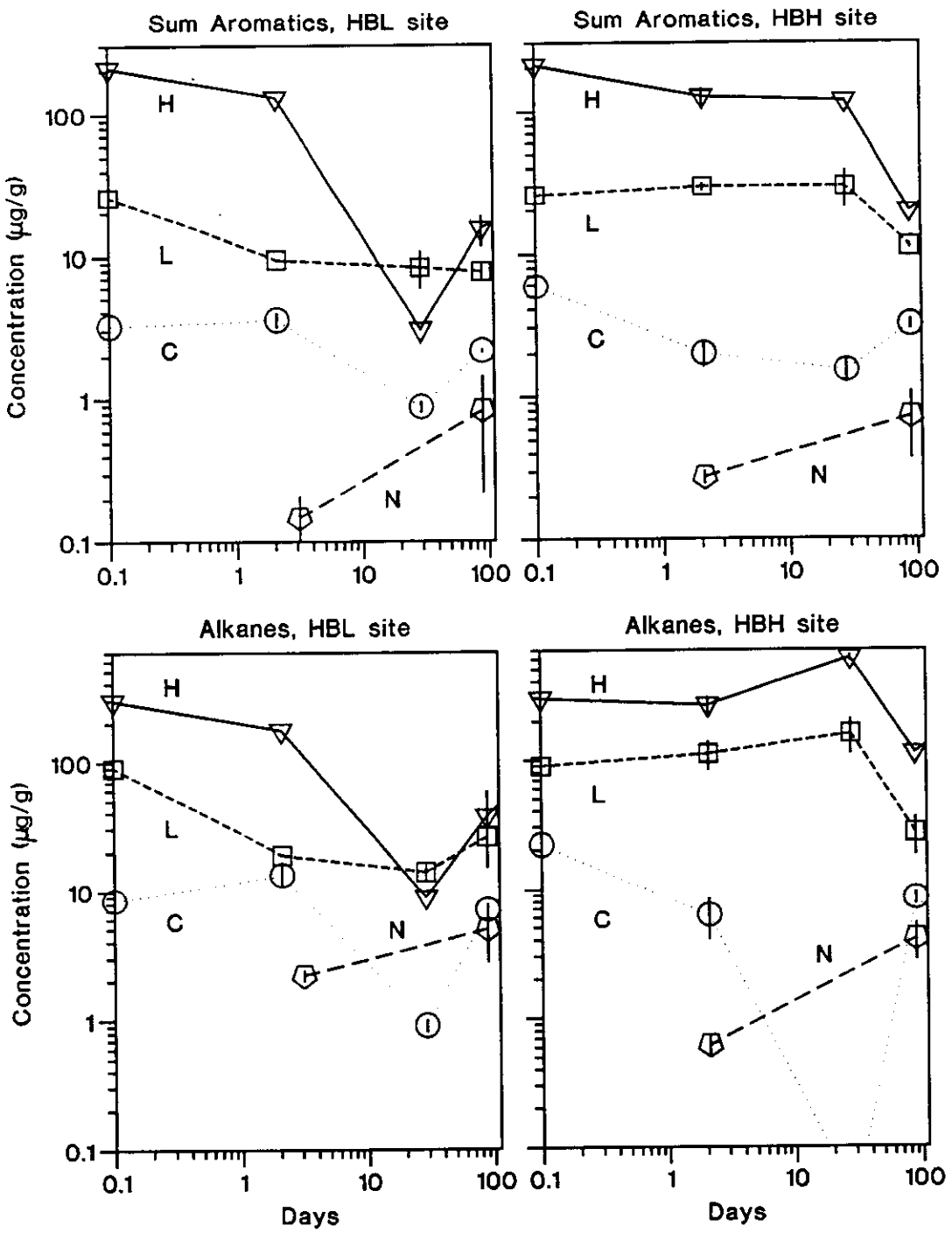

Figure 5C.5. Mean PAH and alkane concentrations at both transect sites over time for each treatment: H = High-oil, L = Low-oil, C = control, and N = indigenous. Time data were adjusted upward 0.1 d to allow log transformation. Alkane concentrations in control sediments at HBH were zero (below machine detection limits) on day 28.

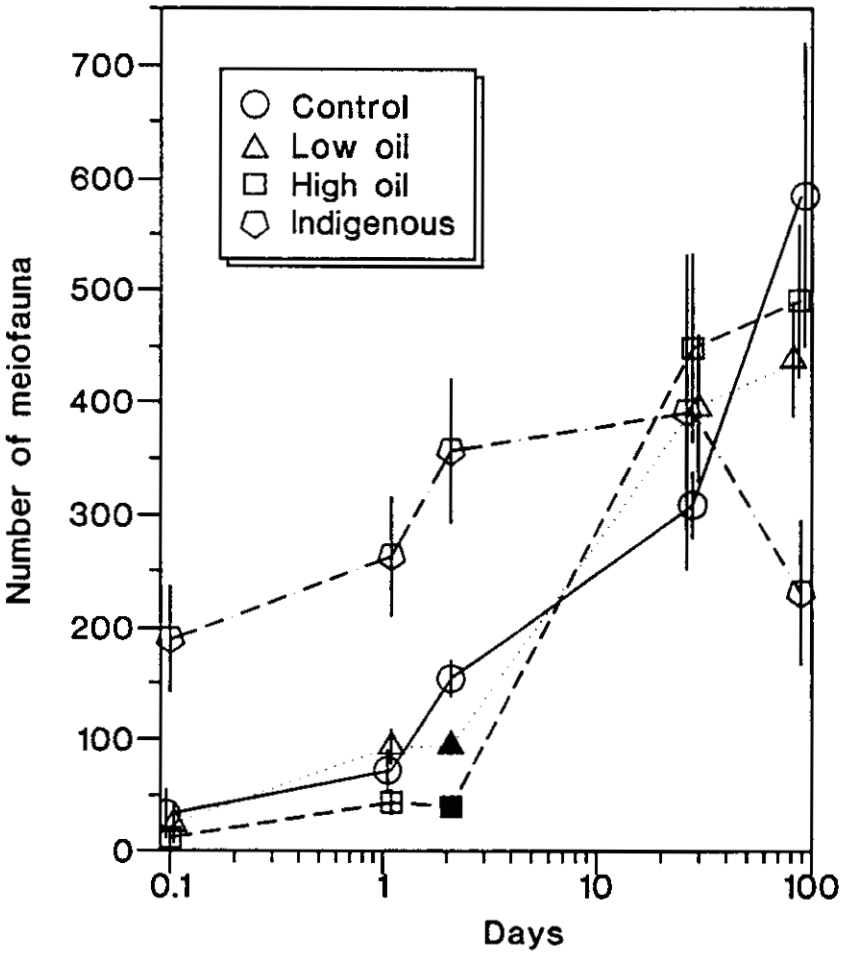

Figure 5C.6. Number of all meiofauna (collectively) in treatment pans and indigenous sediments. Error bars are ±1 standard error. Solid symbols indicate significant differences from control.

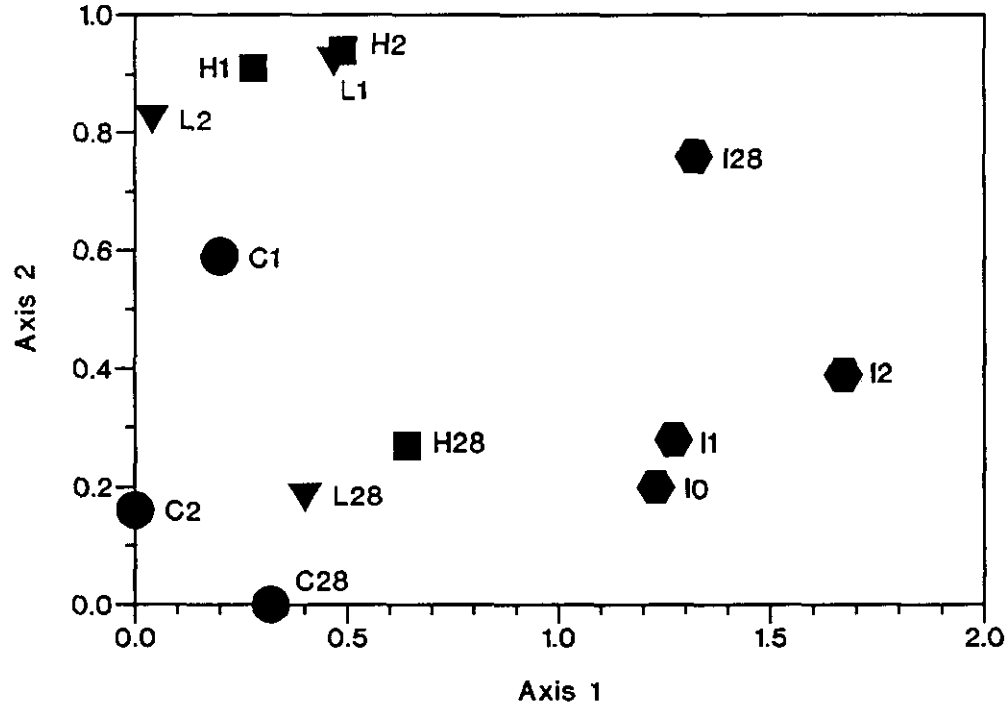

Figure 5C.7. Axis one and axis two, generated by detrended correspondence analysis, explained more than 70% of the variation in copepod density. Text indicates treatment (Control, Low-oil, High-oil, or Indigenous) and collection day (2-28).

CHAPTER 6

Effects of Oil-Contaminated Food on Growth of Juvenile Pink Salmon

Preface

To test the hypothesis that oil-contaminated food can affect growth of pink salmon fry, we conducted a laboratory experiment in 1991 (objective 12 in Chapter 1). This experiment resulted from the observation that pink salmon fry were contaminated with oil following the *Exxon Valdez* (*EV*) oil spill in Prince William Sound in 1989, and that ingestion of oiled prey or oil was the probable route of contamination (Chapter 3A). A number of different parameters were measured in this experiment, including mortality, length, weight, stomach weight, fecal weight, RNA and DNA concentrations, otolith growth (whole axis, radial distance grown during the experiment, and number of increment rings laid down during the experiment), hydrocarbon uptake, histological response, and cytochrome P4501A induction.

Results from this experiment support the hypothesis that growth is inhibited by ingestion of crude oil, and we infer that energy available for growth was reduced by increased metabolic demand in response to the oil, reduced digestive assimilation efficiency, and, at high oil concentrations, reduced feeding.

Introduction

Following the *Exxon Valdez* (*EV*) oil spill in Prince William Sound (*PWS*), juvnile pink salmon (*Oncorhynchus gorbuscha*) tissues contained axenic hydrocarbons and cytochrome P4501A enzymes were induced (Chapter 3A, this report). Because concentrations of hydrocarbons dissolved in the water column were near or below detection limits (Short and Rounds 1993; Maki 1991; Neff 1991), water soluble fractions (WSF's) were probably not the route of contamination. Intertidal substrate, however, was heavily oiled, and may have been the source of oil contamination, either by direct ingestion of particulate oil by fry, or through ingestion of prey that had ingested crude oil or been directly contaminated by it.

Following an oil spill, pelagic zooplankton and meiofauna may be contaminated by direct ingestion of particulate crude oil (Conover 1971), or by association with contaminated sediments (Landrum 1989). Oil particles ranging from 0.01 to 1.0 mm diameter were observed in water as deep as 80 m in Chedabucto Bay following the wreck of the tanker *Arrow* (Forrester 1971), providing a source of contamination for pelagic zooplankton. Uptake of hydrocarbons by harpacticoid copepods and other epibenthic microcrustaceans may be via interstitial water and may, therefore, be kinetically controlled by desorption from sediment particles and organic matter (Landrum 1989). Hydrocarbons, particularly the more strongly sorbed compounds, may also be assimilated via ingestion (Landrum 1989).

Previous research (Schwartz 1985) has shown that prey contaminated with WSF of hydrocarbons can affect juvenile salmon in a variety of ways. Feeding and growth rates of pink salmon fry fed oil-contaminated *Aretemia* nauplii declined with increasing oil concentration. Concentrations of bicyclic hydrocarbons in fry tissue peaked in 3 h, but declined by more than 50% after 12 h. Hydrocarbons were detectable in fry tissue after 10 d, but not after 23 d even though fry continued to consume oil-contaminated prey (Schwartz 1985).

The purpose of this study was to determine the effects of oil ingestion on growth and survival of juvenile pink salmon (objective 12 in Chapter 1). This study was unique because, to our knowledge, it is the first study of direct ingestion of crude oil by pink salmon.

Chapter 6A will be reformatted and submitted to a peer-reviewed scientific journal for formal publication.

Introduction

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Methods

Pink salmon fry were obtained from the Auke Creek Hatchery, Juneau, Alaska on 16 April 1991. Fry were reared in 800-L cylindrical tanks receiving approximately 20 L/min single-pass filtered seawater for 3 weeks at 4.3 ± 0.1°C. Fry were fed Biodiet[1] pellets 0.6 to 0.8 mm in diameter. Four days before treatment, an average of 1,076 fish were randomly allocated into five treatment groups, with three replicate tanks per group. Groups were untreated controls, dichloromethane (treated) control, and low-, mid-, and high-oil treatments (0.00 ± 0.03; 0.02 ± 0.04; 0.37 ± 0.03; 2.8 ± 0.2; and 35 ± 4 mg of crude oil per g food, respectively). The purpose of the treated control was to determine if dichloromethane, used as a carrier solvent to facilitate the mixing of crude oil and food pellets, affected the nutritional quality of the feed. Each 65-L tank (30 x 41 x 53 cm) received 1.4 L/min of single-pass seawater filtered through a 25-μm filter to ensure that potential prey were not introduced into the tanks. Mean water temperature was maintained at 7.8 ± 0.02°C; temperature ranged from 6 to 10°C. After 6 weeks of treatment (13 May - 26 June), all groups received untreated food for 2 weeks to investigate possible recovery.

Food pellets were contaminated by dissolving Alaska North Slope crude oil obtained from the Kenai Refinery of Chevron USA, Kenai, Alaska in dichloromethane carrier solvent and mixing it with 200-g batches of food pellets. The solvent was then removed with a rotary evaporator, followed by 1 hour of air drying. Contaminated food was prepared weekly as needed and frozen in glass jars sealed with Teflon lids until use. Food for the treated control was processed as above, except no oil was added. Control food was not modified in any way.

To measure the crude oil content in the food, samples were extracted with dichloromethane after homogenization, drying (with sodium sulfate), and addition of internal surrogate recovery standards. Due to time and budget constraints, generally only three samples (of eight possible) were processed per treatment except mid- and low-oil treatments (7 and 2 samples, respectively). Extracts were concentrated on a steambath and transferred quantitatively to a 300-ml liquid chromatography column containing, from bottom to top, alumina, silica gel, sodium sulfate, and activated copper (Larsen et al. 1992). The columns were eluted with 50 ml pentane to collect the aliphatic compounds, then with 250 ml of 1:1 pentane/dichloromethane to collect the aromatic compounds. The aromatic fraction was further purified by size exclusion, high performance liquid

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[1]Use of trade names does not imply endorsement by the National Marine Fisheries Service.

chromatography to remove residual matrix interference (Larsen et al. 1992).

The aliphatic fraction was analyzed for n-alkanes (C10-C34), total alkanes, and unresolved complex mixture (UCM) by gas chromatography/flame ionization detection. The aromatic fraction was analyzed for 25 calibrated aromatic analytes and their methylated homologs by gas chromatography/mass spectrometry in the selected ion mode, monitoring a quantitation and confirmation mass for each calibrated analyte. Final analyte concentrations were corrected for procedural losses as measured by recovery of the internal surrogate standards. Samples were analyzed at the Auke Bay Laboratory (Larsen et al. 1992).

Total alkanes, unresolved complex mixture, and total aromatics were included in the statistical analysis of the food samples. Because the food contained alkanes from natural sources, raw hydrocarbon concentrations in all samples, including controls, were greater than zero. Because controls did not contain any crude oil, mean raw control concentrations were subtracted from all concentrations to yield the concentrations of crude oil in the treatments reported above ($\bar{X}_t - \bar{X}_c \pm s_{\bar{x}_t - \bar{x}_c}$, where $\bar{X}_t$ = raw treatment concentration and $\bar{X}_c$ = raw control concentration). Treated control concentrations were not significantly different from control concentrations.

Each day, food was provided in excess at the rate of 10% of the estimated total biomass, with a minimum of 10 g per tank per day. Biomass estimates were updated weekly to compensate for fry growth, mortality, and sampling rates. Food was provided continuously with belt feeders for approximately 16 hours per day. Excess food and feces were removed twice each day, and algae was occasionally removed from the sides of the tank.

Growth, condition, and mortality were monitored for each treatment. In this chapter, we define growth as the change in size (length and weight) from day 0 to the date of observation. Initial fry size was determined on day 0 from three random samples of approximately 50 fry from all tanks. Each week, 50 fry were randomly sampled from each tank, narcotized with MS-222, measured (fork length), blotted dry, and weighed. Condition factor (CF) was calculated from length (mm) and weight (mg): $CF = weight \cdot 1000 / length^{3.576}$; the exponent was determined empirically from our data. Because fry were regularly removed for analysis, cumulative mortality (Σp) was calculated as $\Sigma p = (m_i / (n_0 - \Sigma s_i - \Sigma d_i)) \cdot 100 + p_{i-1}$, where m_i = number of dead fry removed on day i, n_0 = initial number of fry, Σs_i = cumulative number sampled for experimentation up to day i, and Σd_i = cumulative number of fry removed for disease control up to day i.

Feeding rate was measured by percent stomach weight, fecal production, and stomach fullness. Each week stomachs were excised from 15 fry and weighed to determine percentage of body weight. Stomach fullness was judged qualitatively and the percentage of fry with full stomachs was calculated by tank each week. Feces were collected from groups of approximately 50 fry isolated for 1.8 days in 4-L beakers on weeks 2, 3, 6, and 7. Feces were filtered through a 200-μm nylon net, washed onto tared glass-fiber filters, dried 24 h at 60°C, and weighed. One observation was made per replicate tank, except for week 2, when one observation was made per treatment.

Data were analyzed by week with a nested ANOVA (replicate tanks were nested in treatment), or single-factor ANOVA where insufficient data (stomach fullness and fecal mass) existed for the nested model. Percentage data were arcsine transformed before analysis. Differences between controls and treated groups were examined with the Dunnett multiple comparison test. Results were considered statistically significant where $P \leq 0.05$.

Results

Except for the high-oil treatment, mortality remained low (about 4%) in all treatments and controls (Figure 6A.1). Mortality in the high-oil group separated significantly from controls after 2 weeks and increased rapidly until fry began feeding on clean food (day 45). Mortality in the high-oil treatment did not change much after the oiled food was discontinued until the end of the experiment (day 58), but mortality in all other groups tended to rise (Figure 6A.1). The difference in survival between mid- and high-oil treatments was abrupt (Figure 6A.2); the estimated median lethal concentration (LC50), corrected for control response, was 28.6 ± 1.1 mg oil per g food at the end of the treatment period and 29.1 ± 0.6 mg/g at the end of the experiment.

Feeding rate, as estimated by percent stomach weight, increased at low doses but was depressed at high doses (Figure 6A.3). Percent stomach weight declined immediately in the high-oil treatment and remained significantly depressed until clean food was available. Fry in the high-oil treatment eventually became lethargic and struck food less frequently than controls (qualitative observation), but did continue to consume food through the 6-week treatment period. Percent stomach weight was significantly depressed during the first week in the mid-oil treatment. During weeks 2 through 6, percent stomach weight tended to be elevated in the low-oil treatment, and was significantly greater than controls in week 5. No other groups differed from the controls. After oiled food was discontinued, feeding in high-oil treatment increased rapidly and did not relate to treatment in weeks 7-8 (Figure 6A.3).

The feeding response pattern measured by percentage of fry with full stomachs was similar to the pattern measured by stomach weight. Overall (weeks 0-8), the mean percentage of control fry with full stomachs was 52 ± 4 (SE). The mean percentage of fry with full stomachs in the high-oil treatment was significantly reduced after 1 week, and remained below 10% until oiled food was discontinued (significant on weeks 1, 3, 5, and 6). Mean percentage of fry with full stomachs was also reduced in the low- and mid-oil treatments on week 1, but was not statistically different from controls on other weeks. Percent stomach weight measurements generated much higher *n* values than percentage of fry with full stomach measurements, and therefore yielded greater statistical power.

Fecal production was also affected by consumption of oiled food, and response was similar to changes in percent stomach weight (Figure 6A.2). On week 3, the fecal production in the high group was significantly depressed. No other significant response was detected, but fewer fecal data were collected than for any other

response parameter, so the statistical discrimination power was poor.

Growth of fry length and weight was inhibited by oiled food (Figures 6A.2 and 6A.4). Fry in the mid- and high-oil treatments grew significantly less than controls after 1 week of exposure and throughout the experiment. By week 2, growth was significantly depressed in all oil treatment groups, and remained significantly depressed through the end of the experiment, except for weeks 4 and 5 in the low-oil treatment. Significant reductions in growth occurred at oil concentrations as low as 1.3% of the LC50.

Pink salmon length-weight relationships (y = log(weight), x = log(length)), weeks 0-8, analyzed with analysis of covariance, were not significantly different (P_{slope} = 0.402, $P_{intercept}$ = 0.398) among treatments. For all treatments (weeks 0-8), weight = $0.001 \cdot length^{3.576}$. Except for the high-oil treatment, each treatment fit the model well; R^2 = 0.73 for the high-oil treatment, but ranged from 0.94 to 0.96 for all other treatments, including control. Because the length-weight relationship in the high-oil treatment was more variable than in other treatments, we analyzed condition factor (CF = weight * 1000 / $length^{3.576}$) on a weekly basis, reasoning that condition might change with time.

Changes in CF and percent stomach weight were similar (Figures 6A.2 and 6A.3); r = 0.43, n = 1,737. In the high-oil treatment, CF was significantly less than control by week 1, and remained depressed until oiled food was discontinued (Figure 6A.3). On week 7, CF in the high-oil treatment was still significantly depressed, but had recovered substantially. On week 8, CF in the high-oil treatment was not significantly different from control, but was more similar to the CF in the mid-oil treatment. In the mid-oil treatment, CF became significantly elevated (weeks 6 and 7); this trend began after 4 weeks.

Discussion

Mortality in the high-oil treatment may have been due to a combination of direct oil effects and starvation. Fry in the high-oil treatment continued to exhibit strike behavior and consume food throughout the 6-week treatment period, but feeding rate, measured by stomach content and fecal production, was inhibited within the first week of exposure. These fish remained small and became thin, as shown by condition factor. Fry in this high-oil treatment showed remarkable ability to recover when offered clean food; mortality ceased abruptly, swimming behavior became more normal, feeding and growth rates increased, and condition factor increased rapidly.

Percent stomach weight to total body weight provided a simple index of feeding rate in this experiment. This index relied on a constant empty stomach-weight-to-body-weight ratio for the range of fry weights observed; food content therefore increased the percent stomach weight. Sensitivity of this index to oil-contaminated food was similar to that of condition factor but sensitivity of both measurements was less than direct length and weight data. Because percent stomach weight and condition factor were correlated, CF was likely influenced by feeding, masking more subtle morphological length-weight relationships.

Physiological changes caused by the oiled food may account for the observed non-linear change in feeding rate. Although feeding rate increase in the low-oil treatment might be an example of hormesis (Smythe 1967; Laughlin et al. 1981), digestive assimilation efficiency was probably reduced by the oil, inducing increased feeding to compensate. Necrosis of the gastrointestinal tract was observed in the single low-oil treatment sample analyzed to date, so a histological mechanism for reduced assimilation efficiency may exist. Necrosis likely became more severe in the mid- and high-oil treatments; therefore, ability or desire to feed declined. In Atlantic salmon parr (*Salmo salar*) exposed to sublethal concentrations of Hibernia oil-water mixture, efficiency of food conversion was also reduced; during the first 2 weeks of exposure, growth was reduced but food intake was not (Vignier et al. 1992). Nucleic acid content of pink salmon in our study indicated that metabolic catabolism occurred at the cellular level as the result of nutritional stress or starvation (Wang et al. 1993), particularly in the high-oil treatment.

Growth was reduced as a consequence of consuming oil-contaminated food. In another study where juvenile pink salmon consumed prey contaminated with the WSF of crude oil, growth was also reduced (Schwartz 1985). Energy available for growth could have been reduced by metabolic demands, reduced feeding, or reduced digestive assimilation efficiency. Pink salmon are capable of

actively metabolizing hydrocarbons (Rice et al. 1977; Schwartz 1985). Hydrocarbons accumulated in the tissues of pink salmon in our study (preliminary results), and a single observation indicates cytochrome P4501A enzymes were induced, indicating expenditure of metabolic energy. Exposure to sublethal hydrocarbon concentrations elevates metabolism and energy demands and requires increased food intake (Rice et al. 1977). Although feeding rate did not decline in the low- and mid-oil treatments, growth was significantly depressed. This suggests that reduced growth was not simply due to starvation, but rather to increased metabolic demand or oil-induced necrosis.

Following the *EV* oil spill, there was evidence that pink salmon fry in western Prince William Sound ingested oil-contaminated prey or oil globules (Chapters 3A and 4 of this report). This intake of hydrocarbons caused metabolic demands, as demonstrated by induction of P4501A enzymes. In 1989, the apparent growth rate of pink salmon fry was lower in oiled areas than non-oiled areas in western Prince William Sound (Chapter 2, this report). Actual growth rate of specific tagged groups of pink salmon fry was lower for fish recovered in oiled areas than in non-oiled areas (Willette 1991). Decreased growth rate may prolong exposure to predation, thus leading to increased mortality (Parker 1971; Hargreaves and LeBrasseur 1985). Within a year-class, slower-growing groups of pink salmon fry have exhibited lower marine survival than their faster-growing cohorts (Mortensen et al. 1991). Thus, some reduction probably occurred in the survival of juvenile pink salmon in Prince William Sound following the *EV* oil spill.

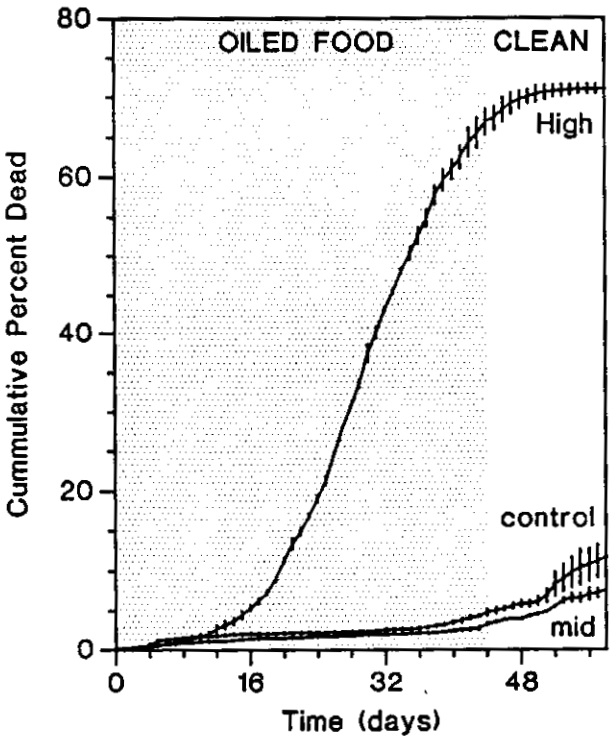

Figure 6A.1. Cumulative fry death as a function of time. The shaded region indicates the period when fry received oiled food. For clarity, only control, mid-, and high-oil treatments are displayed. Error bars are ±1 standard error.

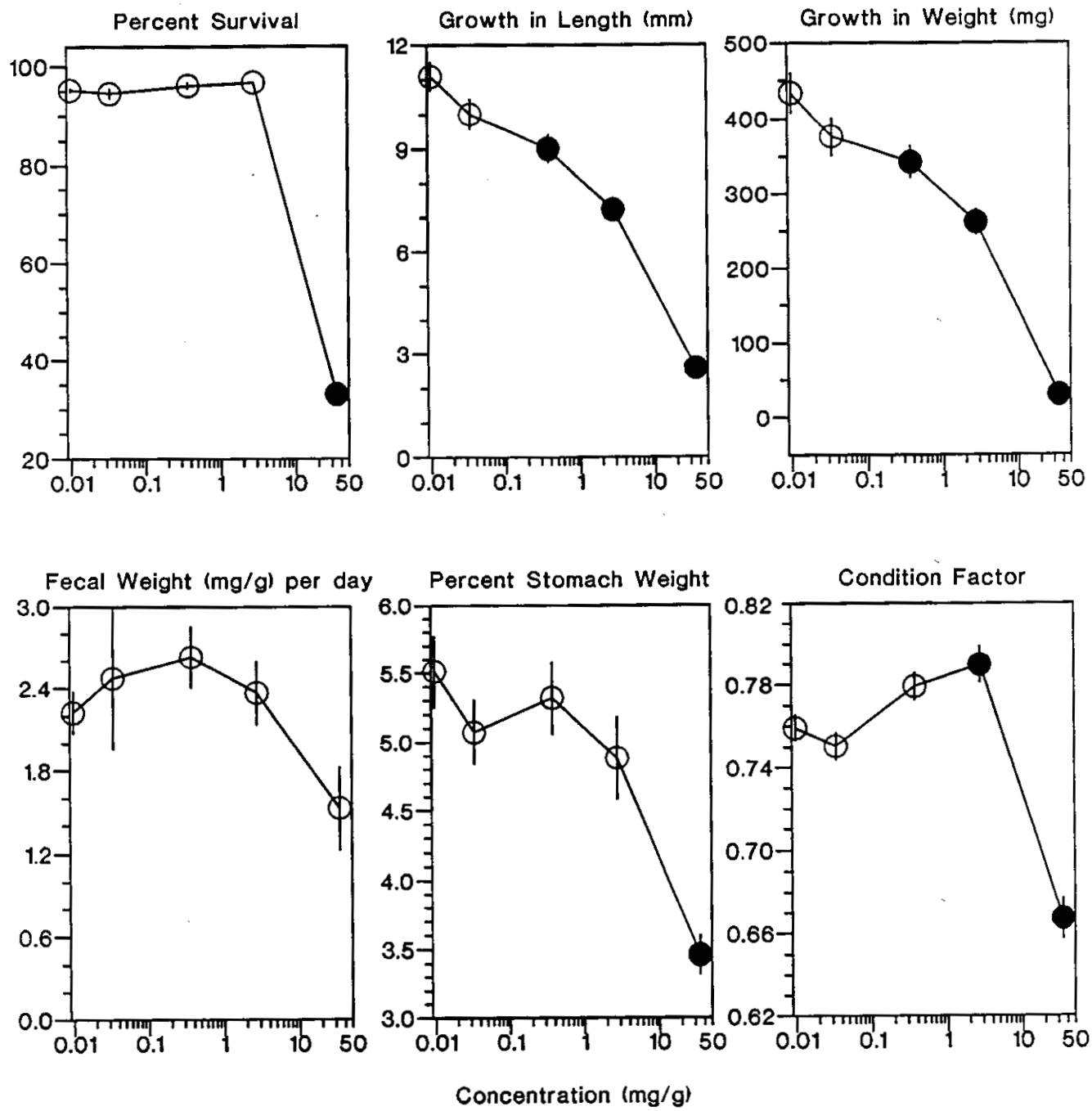

Figure 6A.2. Percent survival, growth in length and weight, fecal weight, percent stomach weight, and condition factor after 6 weeks of exposure to oiled food (log(hydrocarbon concentration + 0.01)). Solid symbols indicate significant differences from control. Error bars are ±1 standard error.

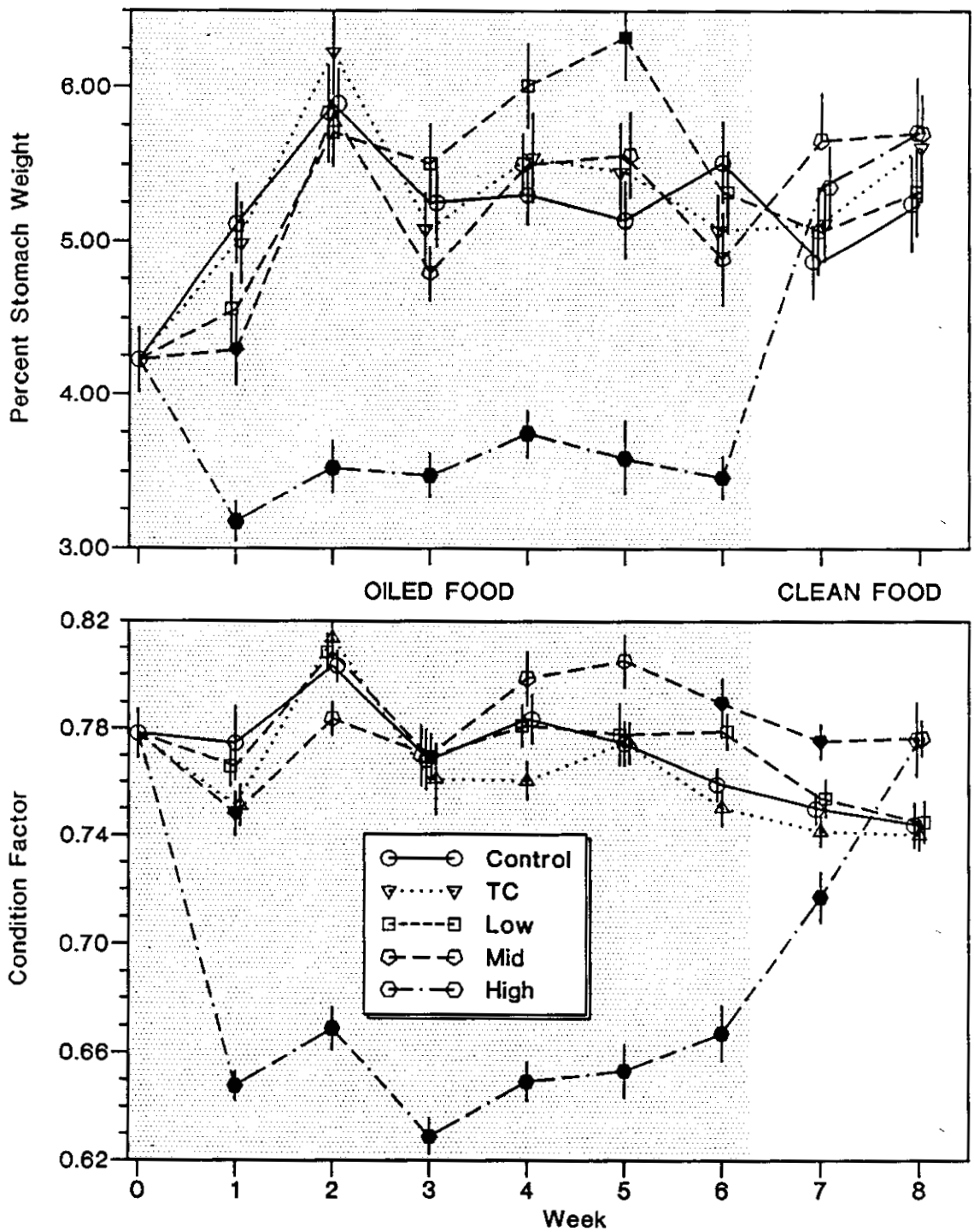

Figure 6A.3. Time series showing changes in percent stomach weight and condition factor of juvenile pink salmon. The shaded region indicates the period when fry received oiled food. Solid symbols indicate significant differences from control. Error bars are ±1 standard error.

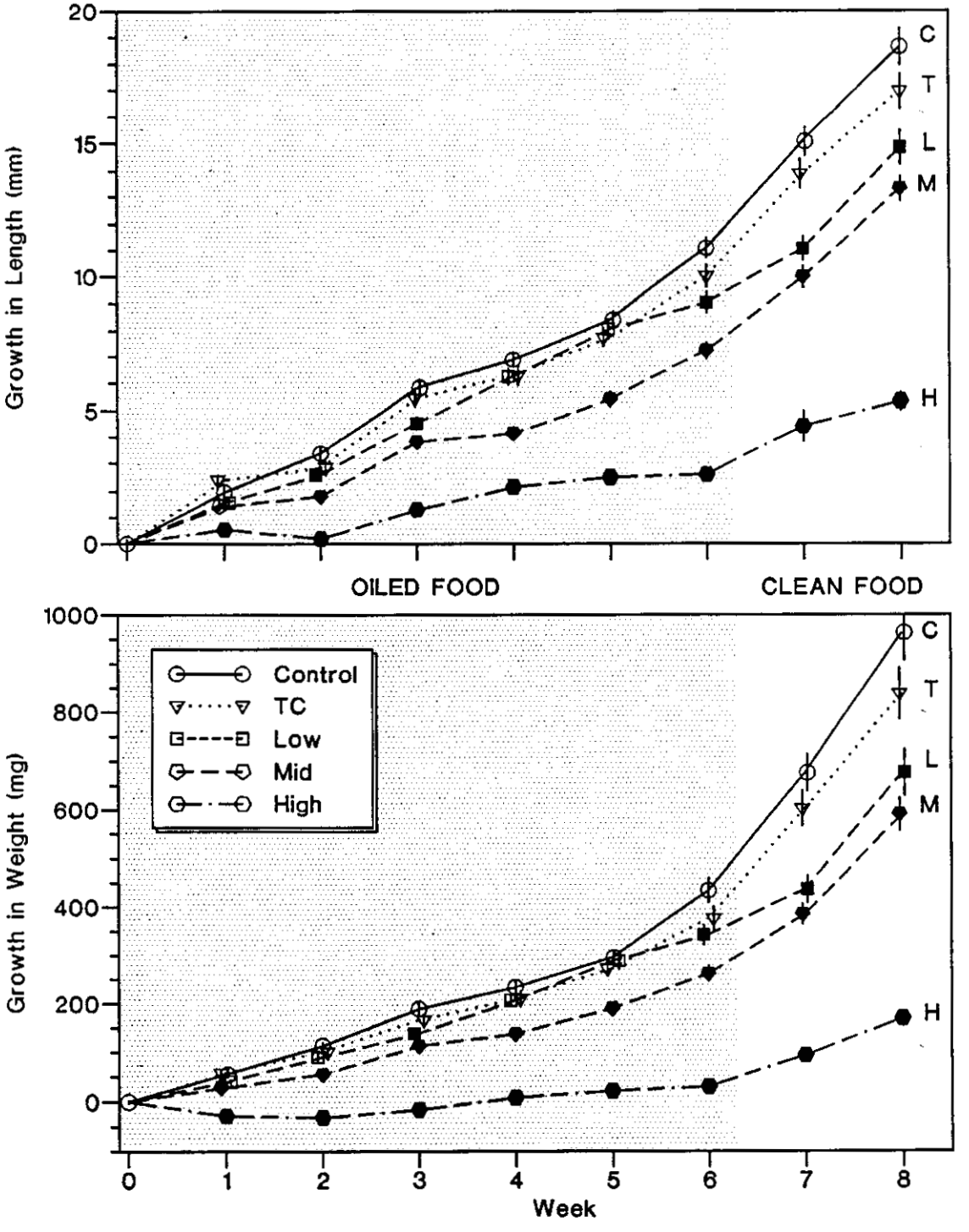

Figure 6A.4. Time series showing growth of juvenile pink salmon in length and wet weight. The shaded region indicates the period when fry received oiled food. Solid symbols indicate significant differences from control. Error bars are ±1 standard error.

CHAPTER 6B. Effects of Crude Oil Ingestion on Growth and Microstructure of Juvenile Pink Salmon (*Oncorhynchus gorbuscha*) Otoliths

D. G. Mortensen and M. G. Carls

Abstract

Juvenile pink salmon (*Oncorhynchus gorbuscha*) were fed a commercially prepared food contaminated with three levels of crude oil: 0.37 ± 0.03 mg oil per g food (low-oil treatment), 2.8 ± 0.2 mg/g (mid-oil treatment), and 35 ± 4 mg/g (high-oil treatment). Over 8 weeks, growth of sagittal otoliths and number of growth rings (increment number) in these groups were compared to untreated controls. Significant differences ($P \leq 0.05$) in otolith growth among all oil levels and the controls were evident after 1 week. Significant differences in increment number among mid- and high-oil treatments and the controls were produced by the second week. By week 6, growth of otoliths in all oil treatments was significantly less than in controls, and increment number was significantly less in mid- and high-oil treatments than in controls. All fish were fed clean food after week 6, but otoliths remained smaller and increment number less in treated groups through the remainder of the experiment. Reduced otolith growth and increment number with increasing oil concentration followed a pattern similar to that of somatic growth. Because check marks can be used as references to index the history of a fish, otolith deposits in wild fish conceivably could be used to estimate the impacts of a major environmental disaster, such as the *Exxon Valdez* oil spill. In our experiment, changes in otolith structure did not disappear when fry were returned to clean food; the otolith had faithfully recorded the event in its microstructure.

Introduction

Otoliths of fish contain a wealth of information, including a history of environmental and growth conditions (Pannella 1980; Campana and Neilson 1982; Neilson and Geen 1985; Neilson et al. 1985; Deegan and Thompson 1987) and a noticeable check mark at the time of entry into the marine environment for juvenile pink (*Oncorhynchus gorbuscha*) and chum salmon (*O. keta*) (Volk et al. 1984; Faurot et al. 1989). Otoliths, therefore, may be useful as indicators of environmental perturbations, including those caused by humans, such as the *Exxon Valdez* (*EV*) oil spill in Prince William Sound (PWS) in March 1989.

Following the *EV* oil spill, the apparent growth rate of juvenile pink salmon in oiled areas of PWS was less than that of fish in non-oiled areas (Chapter 2, this report). Clear conclusions were difficult, however, as the duration of exposure of the juvenile salmon to oil in PWS and the time and size at which they entered the marine environment were unknown. Because laboratory studies have shown that growth of juvenile pink salmon can be reduced by ingestion of oil-contaminated food (Schwartz 1985) or exposure to the water-soluble fractions (WSF) of crude oil (Moles and Rice 1983), we hypothesized that otolith growth would also be affected and that these otoliths would provide a record of the damage. Others have found that otolith and somatic growth of juvenile salmon generally correlate positively (Volk et al. 1984; Neilson and Geen 1985), but this relationship may not be linear and may become uncoupled with individual growth trajectories (Secor and Dean 1989; Campana 1990).

The objectives of this study were to quantify the effects of ingested crude oil on growth and growth ring (increment) formation of otoliths of juvenile pink salmon, and to determine if these parameters could be used as indicators of metabolic stress induced by exposure to oil from an event such as the *EV* oil spill (see Objective 12, Chapter 1).

Chapter 6B was presented at "An International Symposium on Fish Otolith Research and Application" in January 1993 and will be published in the proceedings of that symposium.

Methods

Juvenile pink salmon were collected as they emerged from incubators at the National Marine Fisheries Service Auke Creek Hatchery. Fish (about 30 mm fork length) were maintained in a 1,000-L tank (fresh water, 3-5°C) for several days to 6 weeks, depending on emergence time. The fish were then transferred to an 800-L rearing tank (seawater, 4.3 ± 0.1°C) and maintained for an additional 3 weeks before starting the experiment. The fish were fed a maintenance ration of commercial starter mash (Biodiet pellets, about 0.6 mm in diameter) at the hatchery, and starter feed (0.6-0.8 mm diameter) in seawater.

Four days before the start of the experiment, fish were randomly allocated to five treatment groups with three replicate tanks per treatment and an average of 1,076 fish per replicate. One group was fed uncontaminated food (untreated control), another was fed food mixed with dichloromethane carrier solvent (treated control), and three groups were fed food contaminated with Alaska North Slope crude oil obtained from the Kenai Refinery of Chevron USA, Kenai, Alaska. The purpose of the treated control was to determine if dichloromethane, used as a carrier solvent to facilitate the mixing of crude oil in the food pellets, significantly degraded the nutritional quality of the Biodiet feed. The three contamination levels used were 0.37 ± 0.04, 2.8 ± 0.2, and 35 ± 4 mg crude oil per g food ($\bar{X}_t - \bar{X}_c \pm s_{\bar{X}_t - \bar{X}_c}$, where $\bar{X}_t$ = mean treatment concentration and $\bar{X}_c$ = mean control concentration), hereafter referred to as low-, mid-, and high-oil treatments. Details of the technique used to measure the concentration of oil in each of the treatments are in Chapter 6C. Seawater was passed through a 25-μm filter to exclude potential prey then supplied to each 65-L tank (30 x 41 x 53 cm) at 1.4 L/min. Water temperature was maintained at 7.8 ± 0.02°C.

Food pellets were contaminated by dissolving crude oil in dichloromethane and mixing it with 200-g batches of food pellets. The solvent was then removed with a rotary evaporator followed by 1 hour of air drying. Contaminated food was prepared weekly as needed and frozen in glass jars sealed with Teflon lids until use. Treated control food was prepared as above but was mixed only with dichloromethane and received no oil. Control food was not modified.

The fish were fed contaminated food for the first 6 weeks of the experiment followed by 2 weeks of non-oiled food to observe recovery. Belt feeders presented a nearly constant supply of food to each tank 16 hours per day. Feeding rate was approximately 10% total body weight per day and was adjusted weekly based on the growth of the fish in each treatment. A minimum of 10 g food per tank per day was offered. Food was provided in excess, but the rate of ingestion was not quantified.

Excess food and feces were removed twice each day, and algae was occasionally removed from the sides of the tank.

Weekly samples of 15 fish were taken from each tank for otolith analysis, narcotized with MS-222, weighed, and measured (fork length). Heads were excised and stored in 100% ethyl alcohol until processing. Sagittal otoliths from a maximum of ten fish per sample were removed and processed by methods generally following those of Volk et al. (1984) and Secor et al. (1991). Each of the sagittae (left and right when available) were glued medial (sulcus) side down in the flat cap of a polyethylene mould. The cap was then attached to the body of the mould, and the otolith embedded in polyester casting resin. After curing, the medial side was lapped and polished to maximum diameter, after which the resin plug was thin-sectioned with a micro-cutoff saw. The polished medial side was glued to a glass petrographic slide, and the distal side was lapped and polished to remove the remaining resin, otolith overburden, and processing marks. The result was a polished thin section in which the primordial mass was still visible and growth increments could be observed at the periphery. In cases where left sagittae were lost due to breakage or over-polishing (<1%), right sagittae were substituted to provide an adequate sample size. Analysis was restricted to weeks 0, 1, 2, 6, 7, and 8 due to time and cost considerations; nearly 1,700 otoliths were processed.

Otolith thin sections were viewed under a transmitted-light compound microscope (Figure 6B.1) and the images were digitized and enhanced by a computerized imaging system. Transfers of the fish to freshwater holding after emergence, then to saltwater holding, and finally to warmer water of the experimental tanks produced a zone of opaque and dense bands (transition zone), the end of which served as the starting point for measurements and increment counts (Figure 6B.2). The transition zone varied in width from 17 to 35 μm and, as a result of poor contrast in some fish, was sometimes difficult to detect. Three transects were measured within the posterior field between the end of the transition zone and the edge of the otolith (post-emergence transects) (Figure 6B.3). The number of growth increments intersected by each transect were counted (Wilson and Larkin 1982; Deegan and Thompson 1987). Transect length measurements and increment counts were executed by the imaging software. Mean growth of the post-emergence transect, measured from time 0, and increment number intersected by the transect were used as parameters to test treatment effects. Transect growth and increment number were analyzed by week with Analysis of Variance, with tank nested in treatment. Differences between oil treatments and controls were tested with Dunnett's multiple comparison test ($P = 0.05$). Transect growth and increment number were regressed against somatic growth (change in weight and length) through week 6 of the experiment.

Major and minor axes of ground otoliths were also measured to compare growth of the whole otolith with somatic growth because the aragonite matrix was differentially deposited on the anterior rostral portions of the otoliths in some fish (Figure 6B.1). The major axis extended from the rostrum through the major primordial mass to the posterior field; the minor axis was perpendicular to the major axis and also passed through the major primordial mass. Growth of otolith axes were regressed against somatic growth (length and weight) on weeks 0, 1, 2, 6, 7, and 8; these data were restricted to untreated controls because processing of other treatments has not been completed.

Results

Post-emergence transect growth decreased with increased oil concentration. Significant reductions in transect growth occurred after a single week of exposure to oil-contaminated food (Figure 6B.4). Transect growth in low-, mid-, and high-oil treatments was always significantly less than in controls; transect growth in treated controls was occasionally less than in controls (Figure 6B.4). The size differential between otoliths in treated groups and controls did not appear to change after oiled food was discontinued on week 6; the relationships observed on week 6 persisted in weeks 7 and 8. The percent variation explained by treatment peaked at week 6 and ranged from 88 to 99%.

Fewer growth increments formed as oil concentration increased. The increment number in the mid- and high-oil treatments was less than in controls after 2 weeks exposure (Figure 6B.4). After 7 weeks, increment number was significantly less than controls for low-, mid-, and high-oil treatments, and also for treated controls in week 8 (Figure 6B.4). Relationships established after 6 weeks of exposure persisted in weeks 7 and 8. The percent variation explained by treatment generally increased over time and ranged from 22 to 89%.

Post-emergence transect growth and increment number, except for treated controls, did not correlate significantly with somatic growth (e.g., $-0.32 \leq r \leq 0.10$; $0.226 \leq P \leq 0.991$) (Table 6B.1). The negative correlation observed in treated controls was likely a type II error and not biologically significant (Table 6B.1). A clear separation of transect growth and increment number by treatment appeared by week 2 and became more prominent through week 8, but remained uncoupled from somatic growth (Figure 6B.5).

Growth of the whole otolith (major and minor axes) in controls, however, correlated significantly ($0.69 \leq r \leq 0.74$) with somatic growth (Figure 6B.6). The relationship was curvilinear for fish growth in weight and linear for growth in length (Figure 6B.6). The relationship between axis lengths remained constant as the otoliths grew; there was no significant correlation between the ratio (short axis to long axis) and somatic growth (weight; $r = 0.26$).

Discussion

Otolith growth and increment number were sensitive indicators of oil impact. Significant separation occurred between fish fed oiled and non-oiled food within the first week of the experiment. Our results are similar to the findings of experiments in which growth of juvenile chum and juvenile chinook salmon (*O. tshawytscha*) otoliths was correlated with variations in daily ration (Volk et al. 1984; Neilson and Geen 1982, 1985).

Otolith growth in our study could have been affected through several avenues: reduced feeding, metabolic cost to rid body tissues of hydrocarbons, and reduced assimilation efficiency. Measurements of nucleic acid ratios (RNA/DNA) in the somatic tissues of juvenile pink salmon from our study indicated that metabolic catabolism occurred at the cellular level as the result of nutritional stress or starvation (Wang et al. 1993), particularly in the high-oil treatment. Feeding rates declined significantly in the high-oil treatment, but increased in the low-oil treatment (Chapter 6C, in this report). Changes in otolith growth in treatments where feeding rates did not decline were, therefore, likely due to ingested oil. Metabolism of hydrocarbons may have reduced energy available for growth; preliminary indications are that cytochrome P4501A enzymes were induced in our fish. Histological changes (necrosis of the gastrointestinal tract) also may have reduced assimilation efficiency (Chapter 6C, this report). Schwartz (1985) remarked that hydrocarbons accumulated in salmon fry tissues during exposure to oil-contaminated prey were probably metabolized and excreted. Additionally, Gauldie (1990) found that otolith growth reflects somatic metabolism.

Reduction of otolith growth was not simply due to less ring deposition; the frequency of increment formation was also reduced by consumption of oil. Mugiya et al. (1981) noted that deposition of the calcified (lighter) zone and protein (dark) zone, which form an otolith increment, correlate with blood calcium levels, but the mechanisms controlling deposition frequency and width of increments in otoliths are not fully understood, and the literature on influence of environmental variables is contradictory. The frequency of increment formation is generally considered endogenous and entrained by photoperiod (Tanaka et al. 1981; Campana and Neilson 1985). However, this relationship is susceptible to alteration by water temperature, feeding periodicity, and feeding level (Neilson and Geen 1982, 1985; Campana and Neilson 1985). Feeding frequency also affects increment width (Neilson and Geen 1982; Volk et al. 1984).

Differences in increment production induced by oiled food could result from changes in nutrition, metabolism, or assimilation. Ingestion of oil-contaminated food may disrupt the neurosecretory

process and lead to deviations from the diurnal pattern of increment formation. Gauldie and Nelson (1988) determined that neurally controlled macular cells within the endolymphatic sac secrete neuroproteins which determine the growth rate and size of aragonite crystals as the otolith is formed. The process of crystal twinning controls the shape and orientation of the aragonite crystals. Because temperature was held constant in our study, it probably did not influence increment formation. Although constant water temperature can reduce the contrast of otolith increments (Campana and Neilson 1985; Neilson and Geen 1985), counting bias probably did not cause the observed treatment effects.

Although axial growth of otoliths correlated with somatic growth, post-emergence transect growth did not correlate with somatic growth at any of the treatment levels. A combination of factors may have contributed to this result. Measurements taken only on the posterior outside edge may not have reflected whole otolith growth because accretion rate on different parts of the otolith was proportional to the concentration of adjacent macular cells (Mugiya et al. 1981). Write et al. (1990) found that the anterior rostrum radius had a higher correlation with otolith weight than the posterior radius, indicating deposition differentially favored anterior portions of the otolith. Positive correlation between somatic growth and axial growth of control fish supports this argument. Otolith and somatic growth may also be uncoupled; otoliths may continue to grow despite cessation of somatic growth (Write et al. 1990). Fish with high somatic growth rates have smaller otoliths in relation to fish length than fish with lower somatic growth rates (Secor and Dean 1989). Finally, slight errors in measuring small distances on the otolith's posterior edge would have a greater effect on correlation with somatic growth than they would when correlating axial growth with somatic growth.

In summary, growth of juvenile pink salmon otoliths was sensitive to oil-contaminated food and was significantly reduced at all levels within 1 week of exposure. The number of growth increments along the post-emergence transect also proved to be a sensitive indicator of concentration by the second week of the experiment. Because check marks can be used as references to index the history of a fish, otolith deposits in wild fish conceivably could be used to estimate the impacts of a major environmental disaster, such as the *EV* oil spill. In our experiment, changes in otolith structure did not disappear when fry were returned to clean food; the otolith had faithfully recorded the event in its microstructure.

Table 6B.1. Post-emergence transect growth and increment number vs. somatic growth (mg) after 6 weeks of treatment.

Treatment	n	Slope[1]	*r*	*P*
Post-emergence transect growth vs. somatic growth				
Control	31	-0.695	-0.32	0.077
Treated control	24	-0.947	-0.62	0.001
Low oil	36	0.472	0.13	0.459
Mid oil	24	0.694	0.22	0.310
High oil	19	3.008	0.29	0.226
Increment number vs. somatic growth				
Control	31	1.026	0.09	0.621
Treated control	24	-2.935	-0.25	0.232
Low oil	36	-0.038	-0.00	0.991
Mid oil	24	0.834	0.30	0.160
High oil	19	-11.518	-0.09	0.726

[1]slope units are nm/mg for transect growth and 1,000·number/mg for increment.

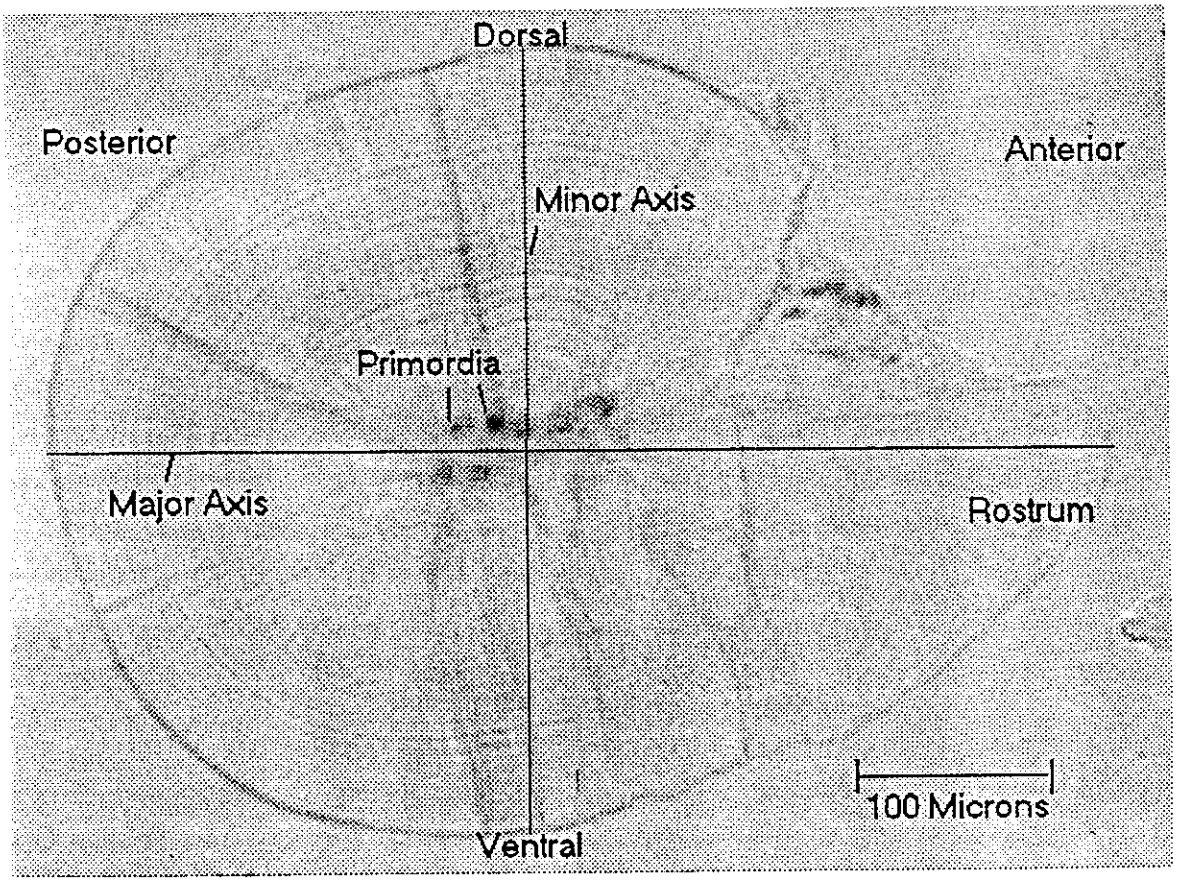

Figure 6B.1 Thin-sectioned sagitta from a control group pink salmon on week 6. The orientation and placement of major and minor axes are indicated.

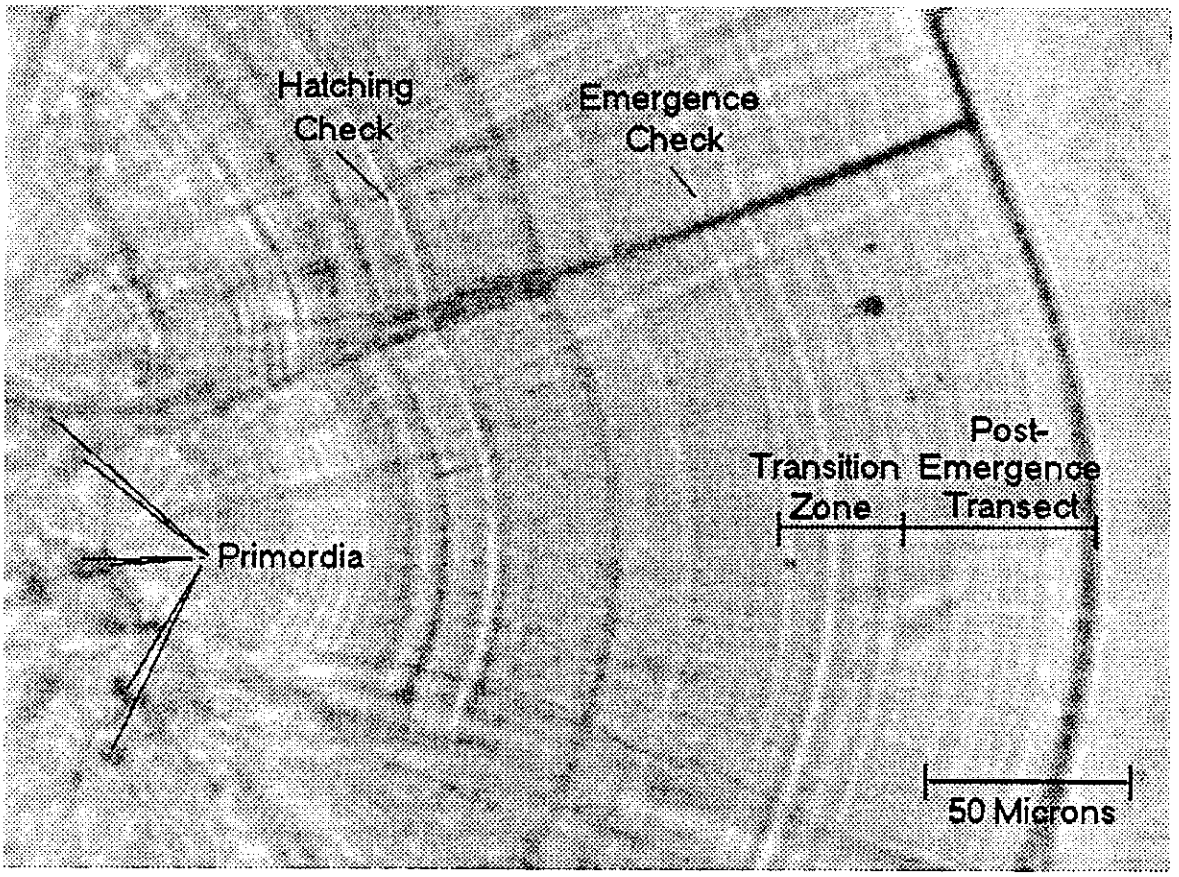

Figure 6B.2 Thin-sectioned sagitta from a control group pink salmon on week 6, indicating various landmarks, the transition zone, and the post-emergence transect.

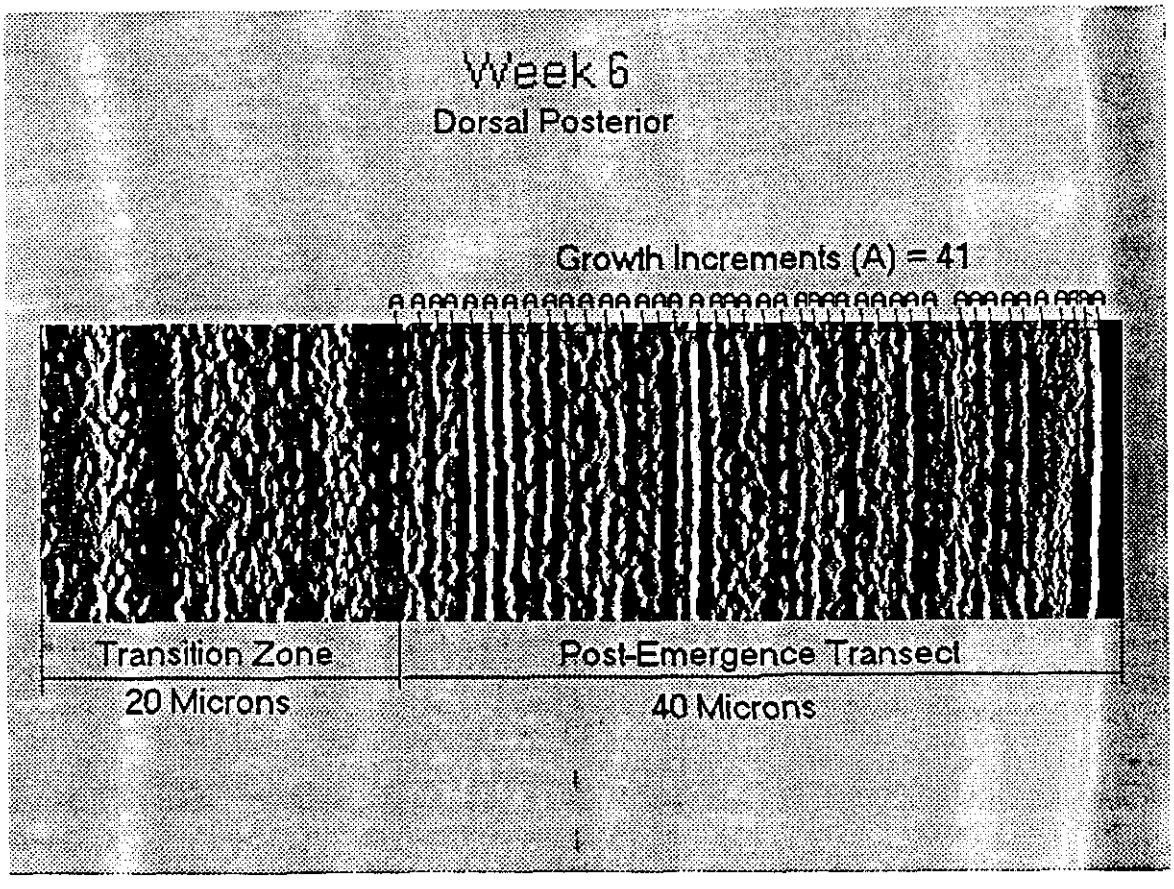

Figure 6B.3 Thin-sectioned sagitta from a control group pink salmon on week 6, showing an enhanced area in the dorsal posterior quadrant. The center portion is enhanced to show details of the transition zone and the post-emergence transect. Growth increments within the post-emergence transect are indicated by (A).

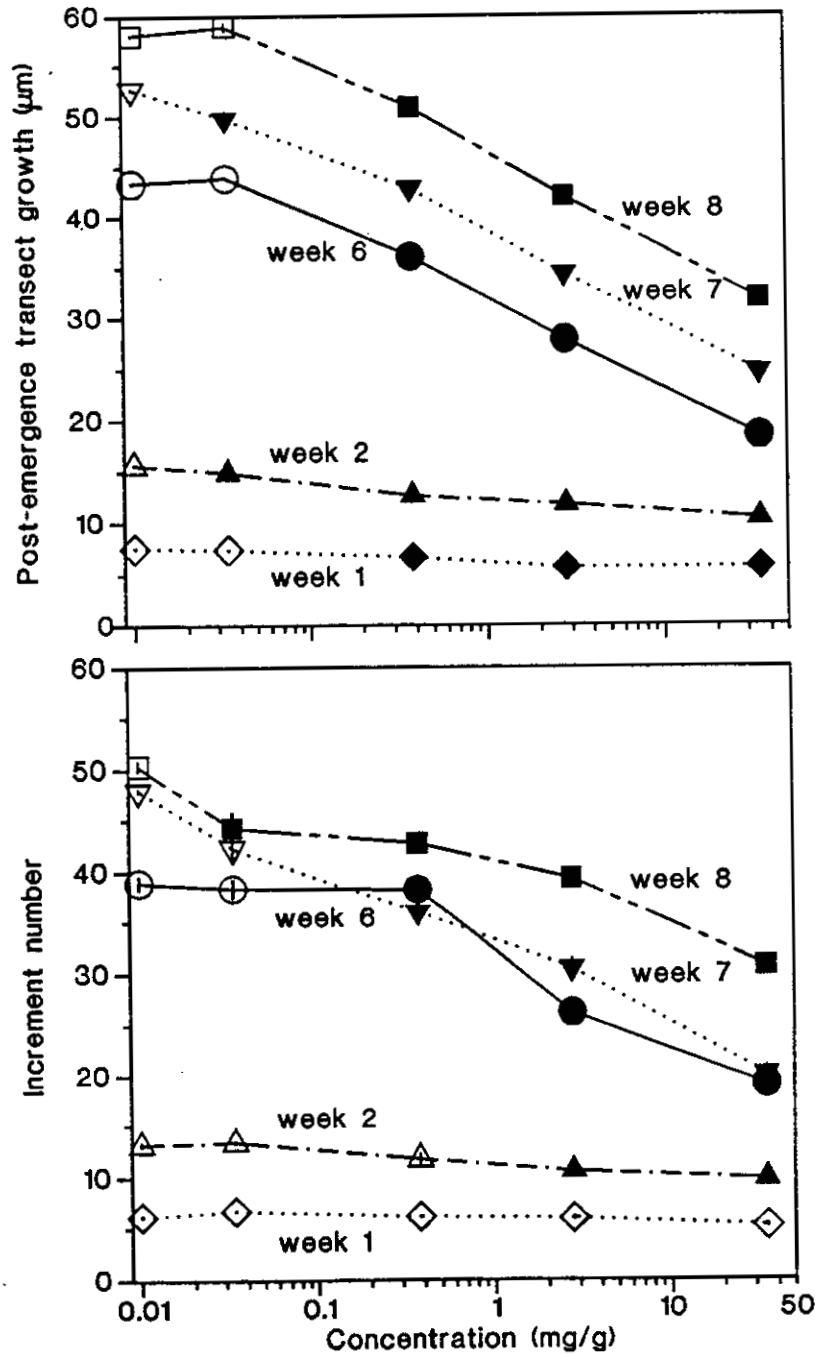

Figure 6B.4. Relationship between post-emergence transect growth, increment number, and oil concentration (log(concentration + 0.01)). Solid symbols indicate significant differences ($P = 0.05$) from controls, as tested on a weekly basis. Error bars are ±1 standard error.

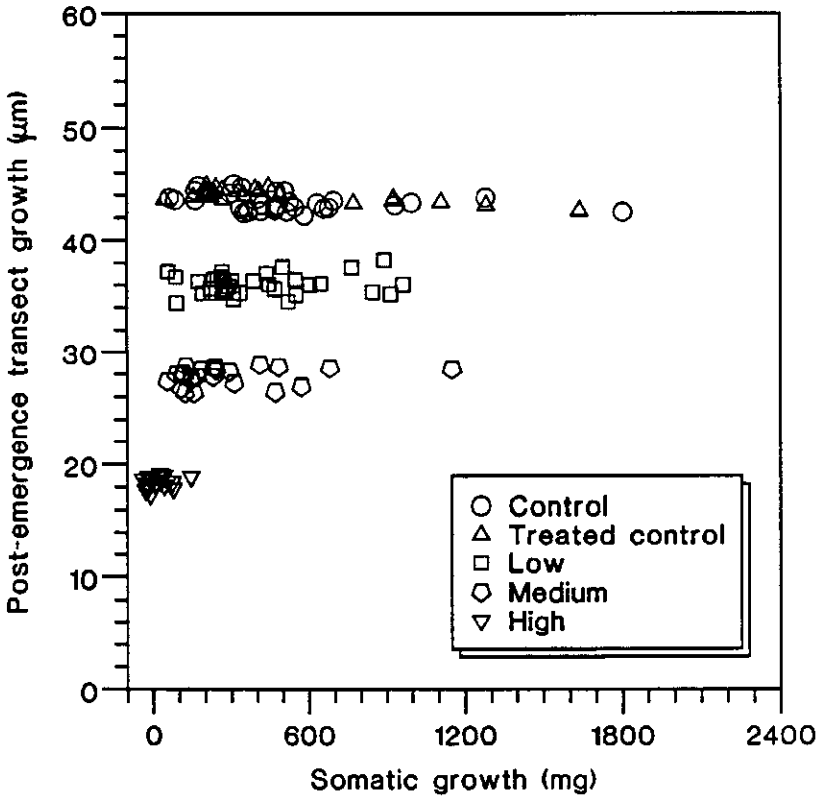

Figure 6B.5. Post-emergence transect growth and somatic growth (change in weight) were not related; data in this example are from week 6.

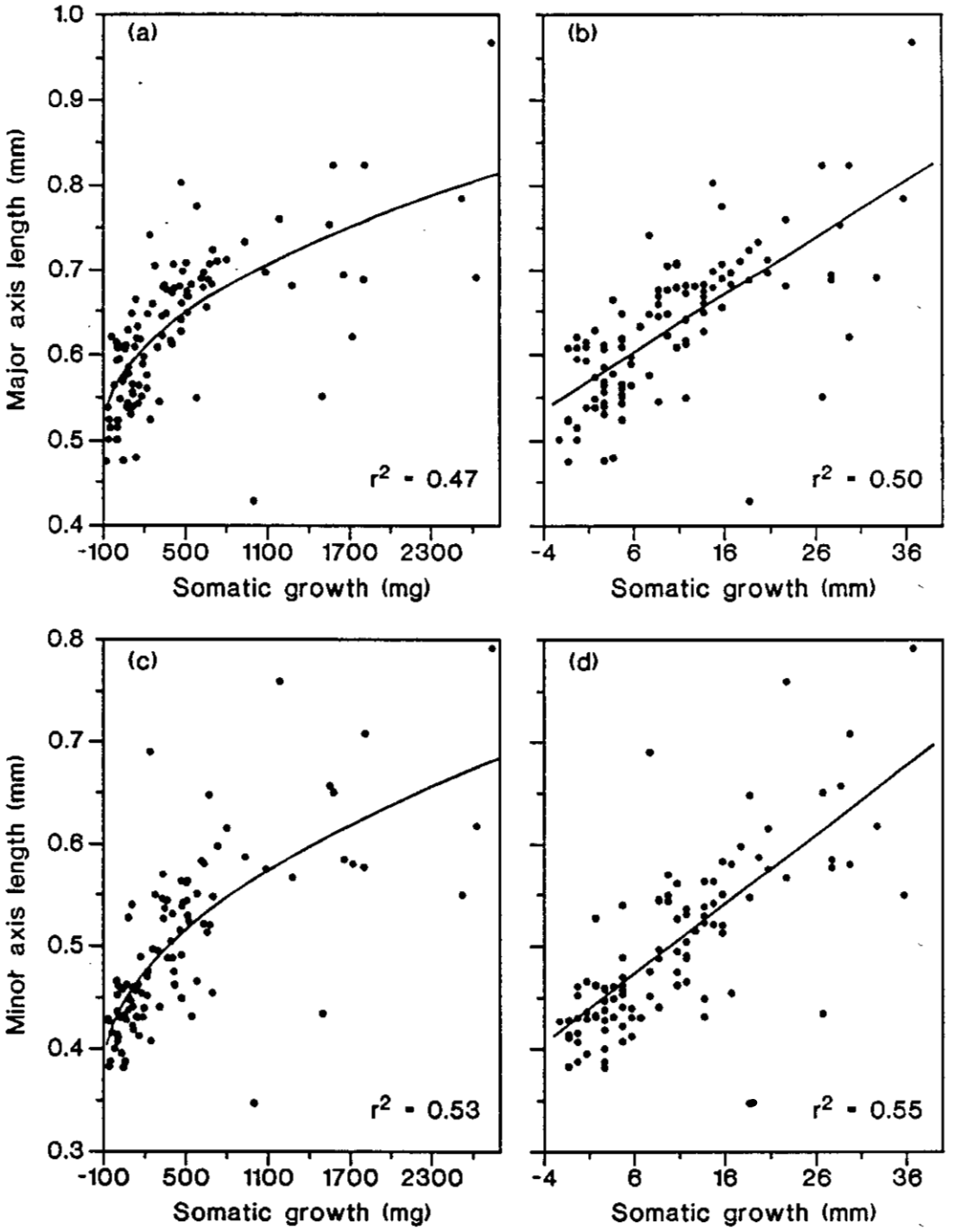

Figure 6B.6. Relationships between axial otolith length and somatic growth. Fitted curves are **a)** major axis = 0.509 + 0.00568·√(weight + 100), **b)** major axis = 0.562 + 0.00679·length, **c)** minor axis = 0.375 + 0.00574·√(weight + 100), and **d)** minor axis = 0.429 + 0.00678·length.

CHAPTER 6C. The relationship between growth and total nucleic acids in juvenile pink salmon *Oncorhynchus gorbuscha* fed crude oil-contaminated food

S. Y. Wang, J. L. Lum, M. G. Carls, and S. D. Rice.

Abstract

Total nucleic acids of juvenile pink salmon, *Oncorhynchus gorbuscha*, fed crude oil-contaminated food were analyzed to determine if nucleic acid measurements can be used to evaluate growth of fish collected at oil spill sites. In general, the nucleic acid concentration (μg/mg dry wt) of salmon fry fed food contaminated with either 0.37 or 2.8 mg crude oil per g food was not significantly affected. However, RNA concentration of fry fed food contaminated with 35 mg/g was reduced whereas DNA concentration increased. Results over 8 weeks indicate decreased protein synthesis and cell content but maintenance of cell integrity in these fish. Growth was inversely related to the level of crude oil contamination in the food. The significant correlations between measured growth and RNA/DNA ratios and RNA contents (μg RNA per mm fork length) suggest that nucleic acid measurements can be used to compare growth of fish collected from the field.

Introduction

Growth inhibition of salmonids as a result of exposure to the water-soluble fraction of crude oil (Moles and Rice 1983) or ingestion of petroleum-contaminated prey (Schwartz 1985) has been demonstrated in laboratory studies. Reduction in growth is a sensitive indicator of exposure effects because sub-lethal exposures cause metabolic changes and increased energy demands on the fish (see review by Rice 1985). After the *Exxon Valdez* oil spill in Prince William Sound, Alaska, in March of 1989, growth of pink salmon fry *in situ* was probably affected because fry caught in oiled areas were smaller and had lower apparent growth rates than fry caught in clean areas (Chapter 2, this report). However, definite conclusions on the growth of wild salmon fry were difficult to make because their time of entry into seawater, initial size, and residence time in oiled waters were not known. The need for an alternative indicator of growth in wild fish stimulated the present research.

Because growth is the result of different biochemical processes, measurements of selected biochemical markers should be useful as early indicators of perturbation in the growth process. One such measurement that is correlated with growth is the RNA/DNA ratio (see review by Bulow 1987). Since RNA is necessary for the translation of DNA, its concentration is a reflection of protein synthesis activity. The quantity of DNA per cell is usually constant, and thus DNA level is correlated with cell number. The RNA/DNA ratio then serves as an index of cellular protein synthesis activity and growth.

Changes in environmental condition that would cause decreased growth in fish have been shown to alter their RNA/DNA ratios. RNA/DNA ratios decreased as a result of exposure to various toxicants in the fathead minnow, *Pimephales promelas* (Barron and Adelman 1984) and nutritional stress in the Atlantic cod, *Gadus morhua* (Lied et al. 1983), rainbow trout *Oncorhynchus mykiss* (Jurss et al. 1986, 1987), Atlantic herring, *Clupea harengus*, and turbot, *Scophthalmus maximus* larvae (Clemmesen 1987, 1988) and carp, *Cyprinus carpio* (Bastrop et al. 1991). RNA/DNA ratios also varied seasonally in response to physiological condition and reproductive activity in bluegill, *Lepomis macrochirus* (Bulow et al. 1981). Correlation between growth and RNA/DNA ratio has been documented for the golden shiner, *Notemigonus crysoleucas* (Bulow 1970), several species of marine fish (Buckley 1984), larval striped bass, *Morone saxatilis* (Wright and Martin 1985) and saithe, *Pollachius virens* (Mathers et al. 1992). Although total nucleic acid measurements are useful for studying the effects of environmental factors on fish growth, their use in studying pollutant effects has been infrequent.

In the present study, RNA and DNA contents and concentrations of juvenile pink salmon, *Oncorhynchus gorbuscha*, fed crude oil-contaminated food for six weeks were analyzed. The goal of the study was to determine whether nucleic acid measurements can be used to evaluate the effects of crude oil on fish growth after an oil spill event. An effective biochemical indicator of growth would be one whose close correlation with measured growth can be demonstrated in the laboratory. The first objective was to determine the time course and pattern of quantitative changes in the nucleic acid of exposed fish (Objective 12 in Chapter 1). The second objective was to use results from the nucleic acid measurements to postulate cellular changes that take place in stressed fish.

Chapter 6C has been published in the Canadian Journal of Fisheries and Aquatic Sciences (Wang et *al.* 1993).

Materials And Methods

Juvenile pink salmon, approximately 30 mm fork length, were obtained from the Auke Creek Hatchery, Juneau, Alaska. The fry were maintained in an 800-L cylindrical tank for 26 days prior to the start of the experiment. The tank received approximately 20 L/min single-pass filtered seawater, and the fry were fed Biodiet #2 starter feed (0.6-0.8 mm diameter). The same feed was used later in the experiment. Four days before the start of the oil treatment, fry were randomly distributed into tanks for the five treatment groups. One group was fed uncontaminated food (untreated control), another was fed food exposed to the dichloromethane carrier solvent (treated control), and three groups were fed food contaminated with Alaska North Slope crude oil obtained from the Kenai Refinery of Chevron USA, Kenai, Alaska. The purpose of the treated control was to determine if dichloromethane, used as a carrier solvent to facilitate the mixing of crude oil in the food pellets, significantly degraded the nutritional quality of the Biodiet feed.

Three target contamination levels of 0.8, 8.0, and 80 mg crude oil per g food were chosen based on the the maximum amount of crude oil that the Biodiet feed can absorb (8% by weight). The actual amounts of crude oil in the contaminated food ($\bar{X}_t - \bar{X}_c \pm s_{\bar{x}_t - \bar{x}_c}$, where $\bar{X}_t$ = raw treatment concentration and $\bar{X}_c$ = raw control concentration) were 0.37 ± 0.03 (df = 3), 2.8 ± 0.2 (df = 8), and 35 ± 4 (df = 4) mg crude oil per g food, hereafter referred to as low-, medium-, and high-dose food, respectively. The amount of crude oil in the food was determined by gas chromatography/mass spectrometry and gas chromatography/flame ionization detection for aromatic and aliphatic hydrocarbons, respectively.

Three replicate tanks were used per treatment for a total of 15 tanks. Each of the 65-L rectangular (53 x 41 x 30 cm, L x W x H) tanks received a continuous supply of fresh seawater at a flow rate of 1.4 L/min. The seawater was filtered through a 25-μm filter to ensure that potential prey items were not introduced into the tanks. Water temperature was maintained at 7.8 ± 0.2°C.

To prepare oil-contaminated food, the appropriate amount of crude oil was dissolved in dichloromethane and added to 200-g batches of food pellets. The solvent was then removed using a rotary evaporator followed by 1 hour of air drying. Contaminated food was prepared weekly as needed and stored frozen in glass jars sealed with Teflon-lined lids. Food for the untreated control was not modified in any way.

Fry were fed contaminated food for 6 weeks. Uncontaminated food was fed for an additional 2 weeks to examine possible recovery from the effects of oil exposure. Feeding rate, approximately 10% total body weight per day, was adjusted weekly, based on the

average weekly weight of fish in each treatment. Food was provided in excess, but the rate of ingestion was not quantified and uneaten food was removed twice daily. Fry to be analyzed for nucleic acids were narcotized with MS-222, weighed, measured for fork length, decapitated, eviscerated quickly, and frozen individually at -20°C. Fifteen fry from each tank, 45 from each treatment group, were sampled weekly. Fry that were fed low dose food and those fed treated control food were analyzed on weeks 1 and 6 only. Fry that were fed control, medium, and high dose food were analyzed weekly. A total of 781 fry were analyzed.

The protocol for total DNA and RNA analysis was developed according to Munro and Fleck (1966). Frozen fry were blotted dry, weighed, and homogenized in 10 volumes of ice-cold distilled water with a Tekmar electric homogenizer. One-ml subsamples were dried at 80°C in pre-weighed aluminum pans for at least 8 h to determine the dry weight of each sample. Macromolecules including the nucleic acids were precipitated from 200 µl of the homogenate by adding 100 µl 0.6-M and 500 µl 0.2-M perchloric acid and incubating the mixture on ice for at least 10 min. The samples were then centrifuged for 10 min at 10,000 x g at 4°C. After discarding the supernatant, the pellet was washed twice with 750 µl ice-cold 0.2-M perchloric acid. DNA content of the pellet was determined by the diphenylamine procedure (Burton 1956) according to Shatkin (1969). One ml diphenylamine reagent and 0.5 ml 0.5-M perchloric acid were added to the pellet and incubated at 27 ± 1°C for 20 h. The DNA content of each sample was determined by comparing its optical density at 600 nm to those of standards prepared from salmon sperm DNA (Sigma Chemical Co.).

RNA content of the pellet was determined by a modified Schmidt-Thannhauser procedure recommended by Munro and Fleck (1966) in which ultraviolet absorption by RNA was measured. RNA was hydrolyzed by incubating the pellet in 1 ml 0.3-M potassium hydroxide at 37°C for 1 h. After cooling the digest on ice, protein and DNA were precipitated by adding 0.5 ml 1.5-M perchloric acid and incubating the mixture on ice for at least 10 min. The sample was centrifuged at 10,000 x g for 10 min at 4°C and the supernatant saved. The pellet was washed twice with 1 ml 0.2-M perchloric acid. The supernatant from each of the two washes were saved along with the original (total = 3.5 ml). The RNA content of each sample was determined by comparing the optical density of the supernatant at 260 nm to those of standards prepared from yeast RNA (Sigma Chemical Co.).

DNA and RNA assays were done in duplicates. The specificity of the diphenylamine assay and the modified Schmidt-Thannhauser procedure for DNA and RNA, respectively, were tested with spiked samples. Test samples were spiked with extra protein (bovine serum albumin) and either yeast RNA or salmon sperm DNA. The assays were found to be specific.

Nucleic acid measurements were analyzed statistically in terms of both concentration and content. Concentration, defined as μg nucleic acid per mg dry weight, refers to the amount of nucleic acid in relation to other tissue components. Content, defined as μg nucleic acid per mm fork length, refers to the amount of nucleic acid per unit length and is independent of other tissue components. The distinction is important, and their relevance to cellular changes in salmon fry that took place as a result of ingesting oil contaminated food is discussed later.

Data were analyzed with nested ANOVA techniques (replicate tanks were nested within treatment), and variance was partitioned among experimental components with the Statistical Analysis System package (SAS 1989). Outliers, less than 3%, were detected and removed using the stem-leaf technique; statistical outcomes were not affected by outlier removal. The Dunnett multiple comparison test was used to test the significance ($P < 0.05$) of differences between means of each treatment and that of control fish.

Results

Ingestion of crude oil-contaminated food had a significant effect on the nucleic acid level of salmon fry. After 1 week, fry that were fed food contaminated with 35 mg crude oil/g (high-dose food) had a significantly lower RNA concentration (total RNA per mg dry wt) but higher DNA concentration than control fish (Figure 6C.1a and 6C.1b). RNA and DNA concentrations of fry fed treated control, low-, and medium-dose food were not significantly different from those of control. After 6 weeks, the RNA concentration of fry fed high-dose food increased to a level equal to that of control fry. The RNA concentration of fry fed low-dose food remained equal to that of control, while the RNA concentration of fry fed treated control and medium-dose food was significantly lower than that of control fry. The DNA concentration of fry fed high-dose food increased and remained significantly higher than that of control fry.

Ingestion of clean food after 6 weeks of feeding on oil-contaminated food caused compensatory RNA synthesis. After 1 week of recovery during which all fry were fed clean food, the RNA concentration of those that had been fed high-dose food for 6 weeks increased from 51.5 to 62.5 μg/mg dry wt, which was significantly higher than that of control fry. Their DNA concentration decreased from 15.2 to 13.7 μg/mg dry wt but was still significantly higher than that of control fry. After 2 weeks of recovery, the DNA concentration of fry fed high-dose food remained significantly higher than that of control fry, but the difference in RNA concentration was no longer significant. The RNA/DNA ratio of fry fed contaminated food was inversely related to the level of crude oil contamination during the first 6 weeks (Figure 6C.1c). The RNA/DNA ratio of fry fed high-dose food remained consistently lower than that of control fry during the study.

Ingestion of crude oil-contaminated food had clear dose- and time-dependent effects on the nucleic acid content (amount of nucleic acid in μg per mm fork length) of salmon fry. The RNA content of control fry increased steadily during the study (Figure 6C.2a). The RNA content of fry fed medium-dose food also increased but at a slower rate, and the difference from that of control fry was generally significant after the third week. The RNA content of fry fed high-dose food was significantly lower than that of control fry and remained unchanged until week 7, after 1 week of recovery, when it increased dramatically from 20.5 to 33.6 μg/mm. The DNA content of control fry increased steadily during the study, while that of fry fed medium- and high-dose food remained essentially unchanged (Figure 6C.2b).

Growth, the increase in dry weight after 6 weeks, took place in all treatments and was inversely related to the level of crude oil contamination in the food (Figure 6C.3). Growth was not

significantly correlated with RNA concentration (Figure 6C.4a), but it was correlated with both RNA content and RNA/DNA ratio (Figure 6C.4b and 6C.4c). Although the correlation was significant for both indices, growth was more closely correlated with RNA content than with RNA/DNA ratio. RNA content accounted for 93.3% of the variability in growth, while RNA/DNA ratio accounted for only 47.8%.

Discussion

Our results allow us to postulate cellular changes in salmon fry that took place as a result of ingesting crude oil-contaminated food. The significant decrease in RNA concentration after the first week in fry that were fed food contaminated with 35 mg crude oil per g indicates a decrease in protein synthesis that was probably caused by decreased feeding. Although daily food ingestion rate was not measured, several measurements of fecal production and stomach weight/body weight ratio clearly indicated a decrease in feeding by fry provided with oil-contaminated food. Due to the decreased feeding, less energy was available for growth and the normal complement of cellular components for protein synthesis was not maintained. The level of ribosomal RNA and messenger RNA decreased relative to other cellular components, and a decrease in RNA concentration resulted. Subsequently, during the 6 weeks when oil-contaminated food was fed to salmon fry, their RNA concentration increased to a level equal to that of control fry. This was probably due to the catabolism of cellular content. The level of RNA itself did not increase; instead, the proportion of RNA relative to other cellular components increased. It should be noted that growth in terms of both length and weight took place in all treatment groups. However, this was achieved at the expense of decreased cellular content among fish fed high-dose food.

Other published results support these suggestions. The difference in RNA concentration between fish that were nutritionally stressed and control fish depended on the duration of the stress. Lied et al. (1983) found decreased RNA concentration in the white trunk muscle of Atlantic cod that starved for eight days. Bulow (1970) reported that after 15 days, golden shiner fed 2% body weight per day had a lower RNA concentration than shiner fed 6% body weight per day. When this feeding regime was continued for 45 days, the difference in RNA concentration disappeared. Jurss and Bittorf (1990) found RNA concentration in the liver of immature rainbow trout that were fed a restricted ration for 44 days to be the same as that in trout fed *ad libitum*.

Our observation that DNA concentration increased in fry that were fed oil-contaminated food further supports the suggestion found in the literature that decreased cell volume, as a result of the catabolism of cellular components, is a generalized response to long-term stress. Increased DNA concentration has been documented in the muscle and liver of bluegill in spring and summer. The decrease in cell volume was probably a result of gonad maturation, spawning, and high maintenance energy requirements associated with high summer temperature (Bulow et al. 1981). The number of cells in the liver of rainbow trout did not decrease after 4 weeks of starvation, and the DNA

concentration increased significantly (Jurss et al. 1986). In golden shiner, the difference in DNA concentration between fed and starved individuals increased over time (Bulow 1970). In general, it appears that by preserving DNA and cells, growth and recovery is favored when environmental conditions improve.

Increased growth and partial recovery were indicated in our study by the significant increase in RNA concentration and decrease in DNA concentration after fry that had been fed oil-contaminated food were fed clean food. We infer that increased RNA concentration reflects recent growth and can change significantly over a relatively short period.

We found a significant correlation between measured growth and RNA/DNA ratios in pink salmon fry that were fed food contaminated with different amounts of crude oil. The significant correlation provides support for the suggestion that the RNA/DNA ratios can serve as an index of fish condition and growth (see review by Bulow 1987). However, the amount of variation in the correlation may be a limiting factor in its usefulness as an indicator of actual growth (Bergeron et al. 1991). Miglavs and Jobling (1989) also questioned the use of RNA/DNA ratios to estimate fish growth after finding higher growth than that predicted by the RNA/DNA ratio during compensatory growth in Arctic charr, *Salvelinus alpinus*. Instead, the inverse relationship between the level of crude oil contamination in the food and RNA/DNA ratios suggests that the ratio may be more useful for making relative comparisons of the growth status or condition among fish from different environmental conditions, rather than actual growth. The high degree of correlation between measured growth and RNA content (Figure 6C.4b) implies that RNA content may be a more useful indicator of growth than the RNA/DNA ratio (Figure 6C.4c).

The conceptual basis for using RNA content as an index of fish condition and growth is simple. Fish in good health and growing rapidly are robust and have high tissue-mass-to-length ratios. Fish in poor condition are thin and have less tissue per unit length. Therefore, fish in good nutritional state and growing rapidly should have high total-RNA-to-length ratios because RNA is an absolute requirement for protein synthesis and growth. The distinction between RNA concentration and content is important. RNA concentration refers to the amount of RNA in relation to other tissue components. RNA content refers to the amount of RNA per unit length and is independent of other tissue components. Fish in poor condition have less RNA than healthy fish. However, if the quantity of other tissue components are lower also, the amount of RNA in relation to other tissue components in slow-growing fish may be indistinguishable from that of fast-growing fish. The calculation of RNA content is independent of other tissue components. Therefore, slow-growing fish, having less RNA

per unit length than fast-growing fish, would have lower RNA content, as demonstrated in the present study.

To use RNA content successfully as an index of condition and growth in fish, two precautions should be exercised. First, only eviscerated carcasses without heads should be used, or white muscle only if specimens are large enough. The white muscle is the most sensitive tissue to growth changes in terms of protein synthesis and degradation in the Atlantic cod (Houlihan et al. 1988). In addition, RNA content of liver and other internal organs can change in response to metabolic activities unrelated to growth (Bulow 1987). One possible reason for the high correlation coefficient between measured growth and RNA content in the present study was the use of fish from which the head and internal organs had been removed. The second precaution is that comparisons of RNA content should be made only among fish of the same species within a certain size range or life stage. Because both growth rate and nucleic acid composition are likely to change with age and life stage, it would not be appropriate to compare the RNA content of fishes differing widely in size or age. Because of the close correlation between measured growth and RNA content, further investigations on the use of RNA content as an indicator of condition and growth in fish are encouraged.

In conclusion, ingestion of food contaminated with 35 mg crude oil per g had significant time- and dose-dependent effects on the growth and nucleic acid level of pink salmon fry. A decrease in growth took place immediately, while catabolism of cellular content took place gradually over 6 weeks. This trend was reversed during recovery when clean food was provided. Both RNA content and RNA/DNA ratio were significantly correlated with measured growth and thus can serve as indicators of growth of fish collected from the wild. However, estimations based on RNA content may be more reliable due to its tight correlation with measured growth.

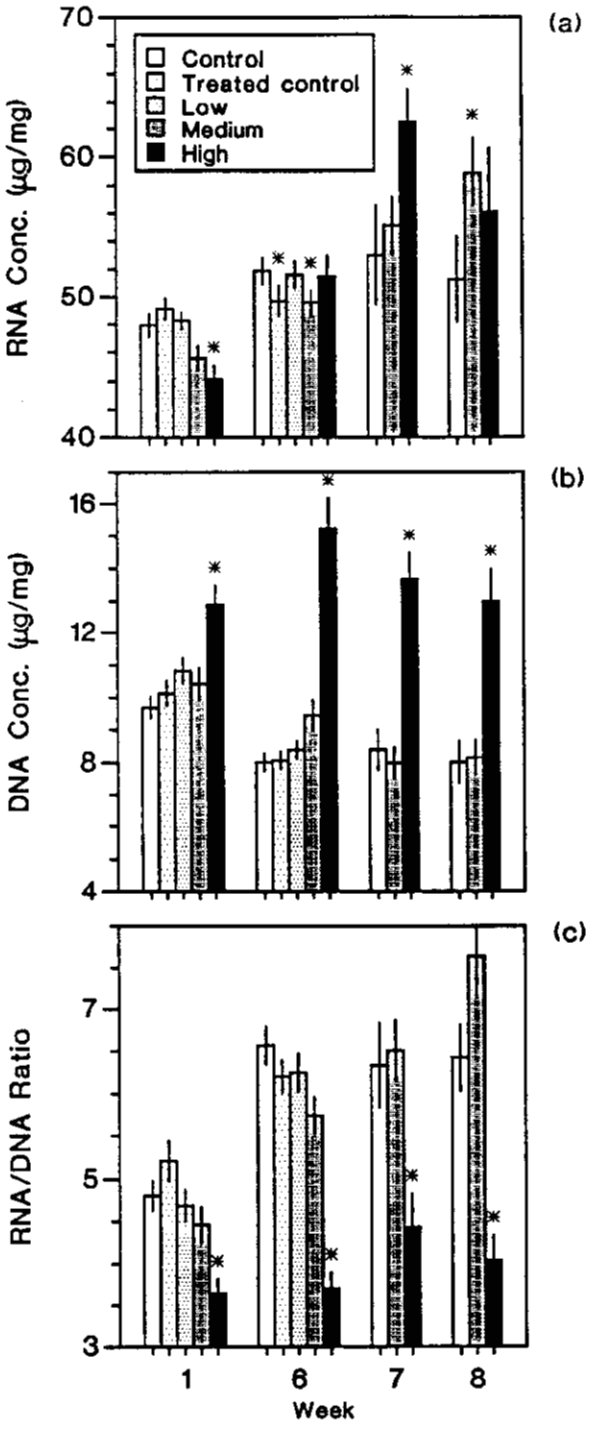

Figure 6C.1. Mean RNA (a) and DNA (b) concentrations and RNA/DNA ratios (c) of salmon fry fed crude oil-contaminated food for 6 weeks. Uncontaminated food was fed during weeks 7 and 8. Vertical lines represent standard error of the mean. Asterisks indicate significant difference from controls ($P \leq 0.05$): n = 42 for weeks 1 and 6; n = 13 for weeks 7 and 8.

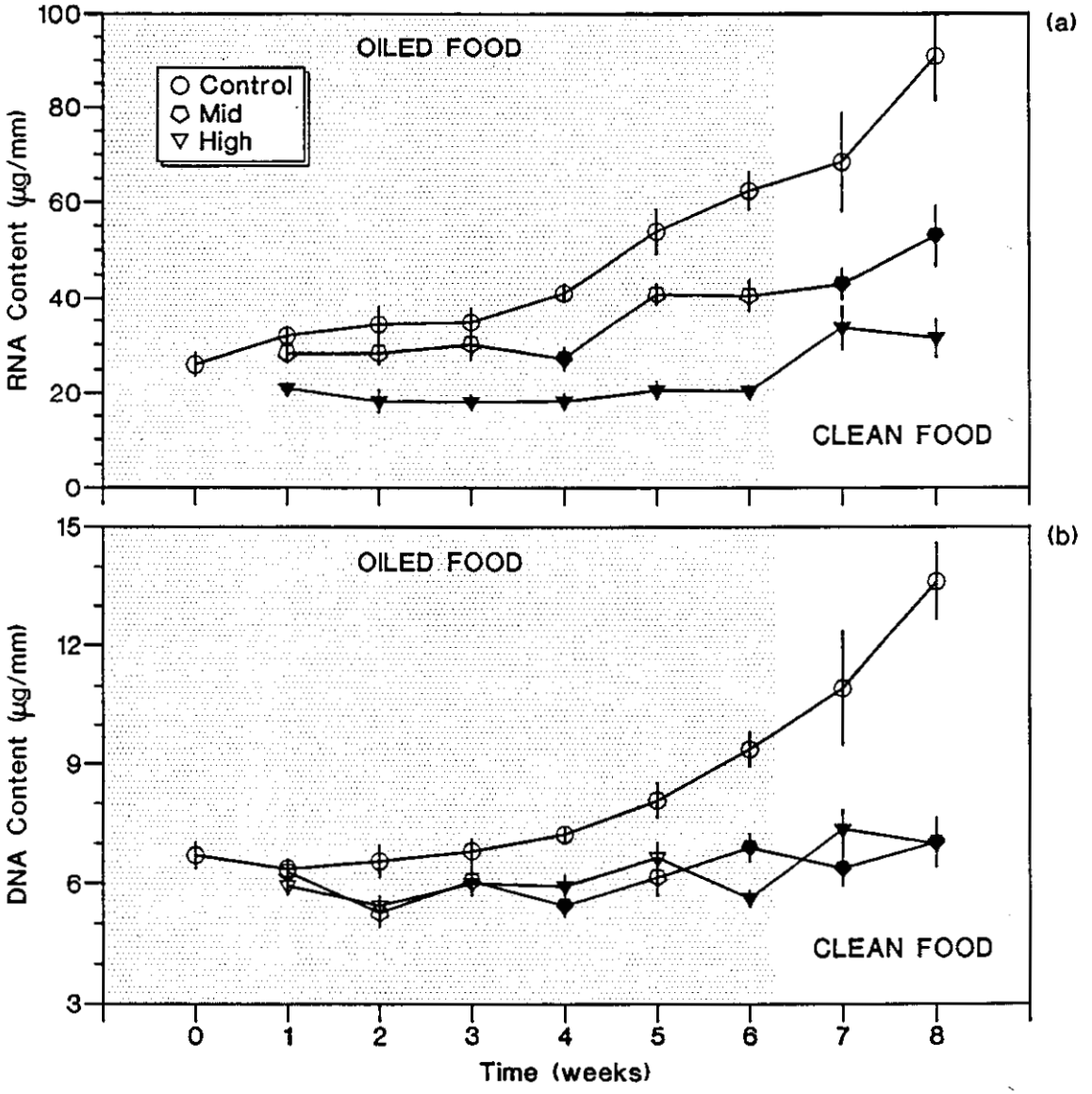

Figure 6C.2. Mean RNA (a) and DNA (b) content of salmon fry fed crude oil-contaminated food for 6 weeks. Uncontaminated food was fed during weeks 7 and 8. Vertical lines represent standard error of the mean. Solid symbols indicate significant difference from controls ($P \leq 0.05$). n = 42 for weeks 1 and 6; n = 12 for the other weeks.

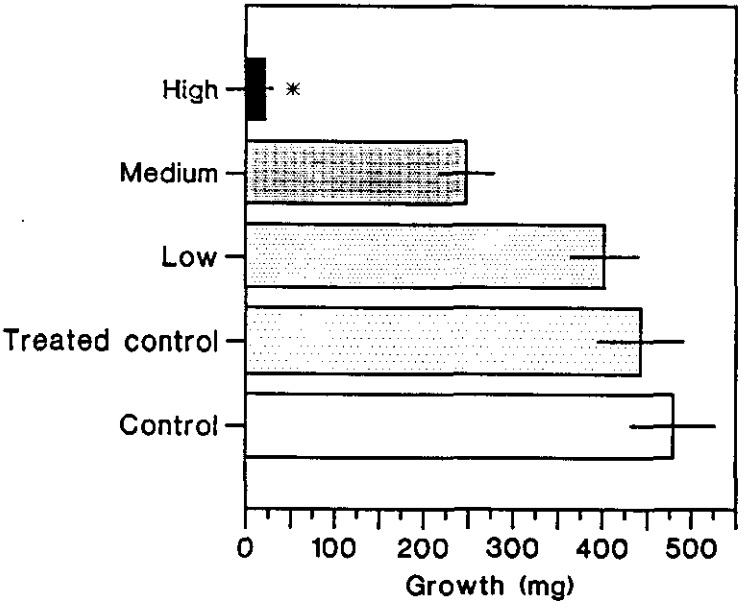

Figure 6C.3. Growth of salmon fry fed crude oil-contaminated food (increase in dry weight after 6 weeks). Asterisk indicates significant difference from controls ($P \leq 0.05$). C = control; T = treated control; L = low (0.37 mg crude oil per g food); M = medium (2.78 mg/g); H = high oil treatment (35 mg/g).

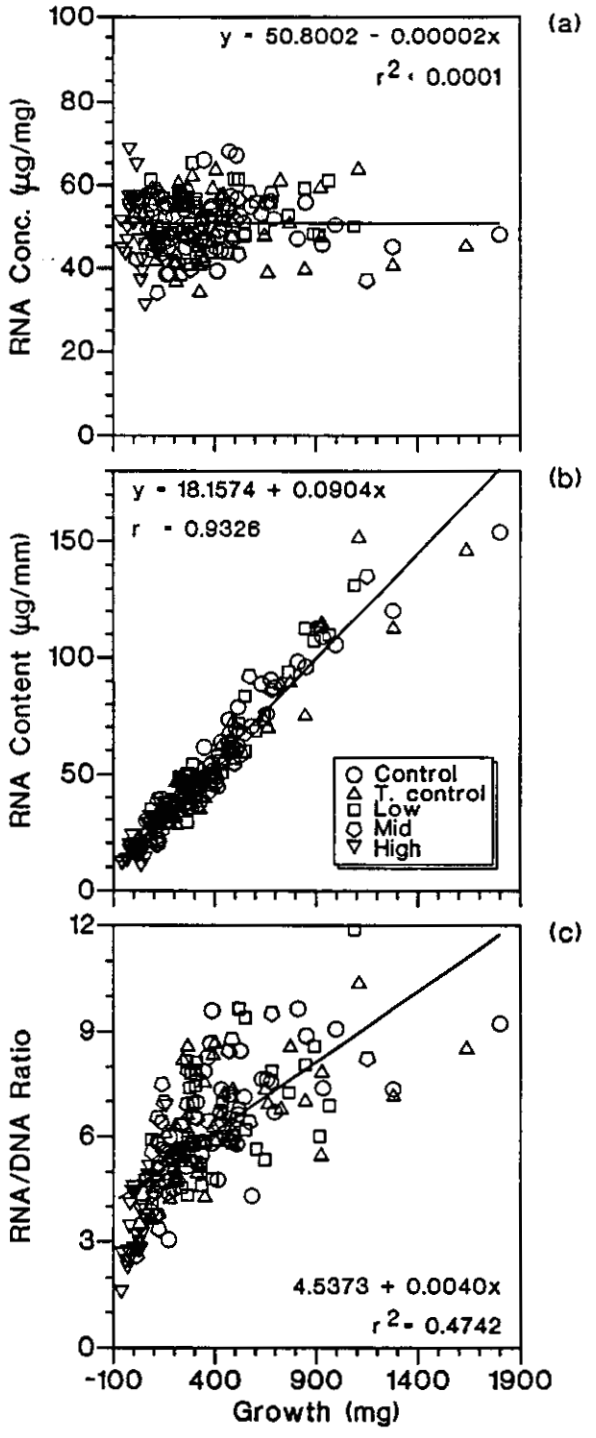

Figure 6C.4. Relationship between measured growth of salmon fry fed food contaminated with different levels of crude oil and indices of growth. a) RNA concentration (n = 202); b) RNA content (n = 202); c) RNA/DNA ratio (n = 202).

CHAPTER 7. Conclusions

In the preceding chapters, we have reviewed in detail the various research projects we undertook to determine if juvenile salmon in the nearshore marine environment of Prince William Sound had been impacted by the *Exxon Valdez* oil spill. In this chapter, we summarize the results and discuss their implications. Most of the research is now complete and will be submitted for publication in peer-reviewed journals and symposia proceedings. Some data are still incomplete; we have not received complete results for immunohistochemical and histopathological analysis of pink salmon fry from the oil-ingestion experiment (Chapter 6). These data will be important complements to the results and conclusions presented here. They will allow us to examine the association of P4501A induction with reductions in growth and hydrocarbon accumulation in pink salmon, and thus to link field observations of hydrocarbon contamination with the experimental results.

The dichotomous classification of our sampling location as "oiled" and "non-oiled" was substantiated by measurements of hydrocarbons in both mussel tissue and sediment collected in 1989; the visibly oiled locations had significantly higher levels of hydrocarbon contamination. However, low levels of hydrocarbons were also detected in sediments and mussels at our non-oiled locations, indicating that contamination from the *Exxon Valdez* extended beyond the trajectory of oil spill, as defined by visible oiling. The degree of contamination in the oiled locations had greatly diminished in 1990.

Juvenile pink and chum salmon were contaminated by exposure to *Exxon Valdez* crude oil in 1989. Petroleum hydrocarbons were detected in tissues of juvenile pink salmon collected in nearshore oiled locations in 1989. To establish that hydrocarbons detected in samples were not due to superficial external contamination, we analyzed carcasses and viscera from some fish from oiled locations separately for hydrocarbons. Both were contaminated by hydrocarbons, with higher levels in the viscera than in the carcasses. Exposure of both pink and chum salmon fry to physiologically significant levels of oil in 1989 was also indicated by elevated levels of cytochrome P4501A (also known as mixed-function oxidase or MFO) activity in fry from oiled locations. Induction of P4501A enzymes was stronger for chum salmon than for pink salmon, indicating that juvenile chum salmon were more susceptible to hydrocarbon exposure.

Ingestion of either whole oil or oil-contaminated prey was an important route of contamination of juvenile salmon. Ingestion of oil was indicated by the types of hydrocarbons in the tissues and the types of cells where P4501A activity was induced. Oil

globules or sheen were also observed in the stomachs of 1% of the pink salmon and 4% of the chum salmon examined from oiled locations in 1989.

Contamination did not continue in 1990: petroleum hydrocarbons were not found in the tissues of pink salmon fry from oiled or non-oiled locations, and P4501A induction was not observed in juvenile salmon. No oil globules or sheen were observed in stomachs of pink or chum salmon in 1990.

Juvenile pink and chum salmon were less abundant in oiled locations than in non-oiled locations in both 1989 and 1990. Because the pattern of abundance did not change as exposure levels diminished, we conclude that the difference in abundance was more likely due to geographic differences in the production and migration patterns of salmon fry rather than a response to exposure to oil.

Juvenile pink salmon moved rapidly from sheltered bays to more exposed, steep-gradient beaches in migration corridors, where they fed predominately on zooplankton. We think this rapid movement is an adaptive feeding strategy in response to the higher abundance of zooplankton in corridors than bays in Prince William Sound. The observation of this behavior over a wide geographic range reinforces the conclusion drawn in the UAF component of F/S-4 that the presence of oil-deflection booms in Port San Juan in 1989 disrupted the normal migration behavior of fish released from the Armin F. Koerning Hatchery (Cooney 1990).

Juvenile chum salmon utilized low-gradient beaches to a greater extent and consumed a higher proportion of epibenthic prey than did pink salmon. Crude oil typically penetrates and accumulates in fine substrates on lower-gradient beaches to a greater degree than the boulder or bedrock substrate that predominated in steep-gradient beaches. Because of their habitat and feeding preferences, juvenile chum salmon in oiled locations were more likely to forage over contaminated sediments than were pink salmon, which may have caused the higher P4501A activity observed for chum salmon in oiled locations in 1989.

Juvenile pink salmon grew significantly slower and were significantly smaller in oiled than in non-oiled locations in 1989. This analysis of unmarked fish is consistent with the significant reduction in growth of tagged pink salmon in oiled areas reported in the ADFG component of F/S-4 (Willette 1991). In 1990, neither size nor growth of pink salmon fry differed between oiled and non-oiled locations.

Juvenile chum salmon in the oiled locations were significantly larger than in the non-oiled locations in both 1989 and 1990. The size difference probably was a result of different migration timing of chum salmon in the oiled and non-oiled locations. In

the non-oiled locations, many of the chum salmon were recent migrants to seawater, while in oiled locations, most had been in seawater for some time. Too few chum salmon were captured in oiled locations to compare apparent growth rate between oiled and non-oiled locations.

Feeding of pink and chum salmon fry was not reduced in the presence of oil. Pelagic zooplankton, primarily calanoid copepods, dominated the diet of juvenile pink and chum salmon in both 1989 and 1990 in both oiled and non-oiled locations. The proportion of zooplankton in the diet declined in oiled locations from 1989 to 1990. The proportion of epibenthic crustaceans (principally harpacticoid copepods) in the diet was less in oiled than in non-oiled locations in 1989, and greater in oiled locations in 1990. Oil could have caused the diet shifts by fish avoiding contaminated epibenthic prey in 1989, or by increased feeding on oil-enhanced epibenthic harpacticoid copepods in 1990. We could not, however, definitively attribute the changes in diet as an effect of the oil spill.

Prey availability in the oiled locations was not reduced relative to the non-oiled locations. Abundance of zooplankton did not differ significantly between oiled and non-oiled locations in 1989 or 1990. Biomass of total epibenthic crustaceans and of epibenthic harpacticoid copepods was significantly higher in oiled locations than non-oiled locations in 1989. Density of some taxa of harpacticoid copepods was depressed in experimentally-oiled sediments for 2 days post-oiling, but no oil effects were observed by 28 days post-oiling. Comparison of abundance of epibenthic harpacticoid copepods between heavily oiled and lightly oiled beaches in 1990 demonstrated that 1 year after the oil spill, harpacticoids that are important as prey to salmon fry maintained or increased their numbers in response to the direct and indirect impacts of the spill.

We attribute the reduction in growth observed for juvenile pink salmon in 1989 to contamination from ingested oil. Temperature, prey availability, and feeding were similar or higher in oiled locations than in non-oiled locations in 1989, and therefore do not explain the reduced growth. Previous studies have shown that growth in juvenile salmon can be reduced by feeding on food contaminated with the water-soluble fraction of crude oil or with whole crude oil (Schwartz 1985; Vignier et al. 1985). Our laboratory research (Chapter 6) supports the conclusions that salmon fry were contaminated by oil via ingestion, and that this contamination was responsible for reduced growth in oiled locations. Pink salmon fry were fed food contaminated with *Exxon Valdez* crude oil; their length, weight, RNA/DNA ratios, and otolith growth declined with increasing oil concentration. At the highest dosage, feeding and survival were reduced.

Our conclusion of the impact of the oil spill on juvenile pink salmon is in contrast to Neff's (1991) conclusion that it was extremely unlikely that hydrocarbon concentrations from the *Exxon Valdez* oil spill had any adverse effect on animals living in the water column of Prince William Sound. Neff's conclusion was based on the low concentrations of oil in the water; he did not consider the possibility of exposure to and ingestion of whole oil. We frequently observed dense schools of juvenile salmon in water covered by sheen and mousse from *Exxon Valdez* oil, with individual fry jumping through the contaminated surface. Oil particles that were the same size as prey could have been ingested directly by salmon, or the fry may have consumed contaminated prey. Zooplankton may ingest particulate crude oil directly (Conover 1971), and epibenthic crustaceans may accumulate oil from contaminated sediments. Mean concentrations of aromatic hydrocarbons were 650 times higher in the stomach contents of juvenile chinook salmon from a contaminated estuary than from a more pristine estuary (McCain et al. 1990).

Pink salmon still fed and grew in oiled locations in 1989 although growth was reduced,. Feeding effectiveness was not reduced; growth was observed in heavily oiled locations in this study and by Willette (1991). The fish in oiled locations were not emaciated; condition (the relation of the weight of a fish to its length) of pink salmon was actually higher in the oiled locations. Pink salmon fry can metabolize and depurate hydrocarbons (Thomas and Rice 1979). Ingestion of hydrocarbons, however, has a metabolic cost, which can reduce food conversion efficiency in juvenile salmon. For example, juvenile Atlantic salmon exposed to crude oil had reduced growth which coincided with reduced food conversion rather than reduced food intake (Vignier et al. 1992). The oil contamination in Prince William Sound was apparently within the depuration capability of juvenile salmon, but the physiological load reduced growth of pink salmon in oiled locations.

Survival of pink salmon juveniles may have been reduced because of the effects of oil contamination on growth. Growth during the early marine period is important to escape mortality from size-selective predation (Parker 1971; Hargreaves and LeBrasseur 1985). Within a year class, slower-growing groups of pink salmon fry have lower marine survival than their faster-growing cohorts (Mortensen et al. 1991). The reduction in growth and any subsequent reduction in survival was limited to the first year of the spill. We found no measurable contamination or physiological effects (e.g., apparent growth, P4501A induction, hydrocarbon body-burden) in 1990.

Large numbers of juvenile salmon, including populations originating from outside the actual spill area, were probably exposed to hydrocarbon contamination in the marine environment.

The predominate migration route of juvenile salmon from Prince William Sound to the Gulf of Alaska is thought to be through the southwest passages (Raymond 1990). This migration route coincides with the general movement of *Exxon Valdez* oil from Prince William Sound (ADFG 1989). Willette (1991) has shown that fish originating from outside the spill area had reduced growth when recaptured in oiled sites. Such large-scale exposure to oil contamination, linked with growth reductions, could have resulted in a commensurately large-scale reduction in the overall return of pink salmon to Prince William Sound in 1990.

The record high return of pink salmon to Prince William Sound in 1990 (Rigby et al. 1991) has been used to argue that the pink salmon fishery was not harmed by the spill, and that other salmon fisheries were "likewise unharmed" (Royce et al. 1991). While the record return to the Sound clearly shows that there was not a catastrophic loss of the marine ecosystem's capacity to sustain high productivity of pink salmon, it does not preclude the possibility of damage to the resource. Conditions in the Sound in the spring of 1989 were conducive to a record return. Releases of pink salmon fry from hatcheries were close to the historic maximum (Eggers et al. in press), and spring zooplankton abundance was high in 1989, providing an excellent forage base for juvenile salmon (Cooney and Willette 1991). The record return, however, was not a function of record marine survival. Survival rates of hatchery pink salmon returning in 1990 were well within the documented range for pink salmon in Prince William Sound (Eggers et al. in press). Higher marine survival, resulting in more fish in 1990, might have occurred if a large portion of the pink salmon population had not been exposed to oil.

The effects on pink salmon observed in 1989 could have also occurred in other species. Chum salmon juveniles captured along oiled beaches showed definite P4501A induction. A wide variety of other fishes also utilize the nearshore environment of Prince William Sound and the adjacent Gulf of Alaska (Rogers et al. 1986). Many pelagic schooling fishes and larval fishes utilize zooplankton as their principal prey (Rogers et al. 1986). If ingestion of either whole oil or contaminated prey was the route of exposure for juvenile pink salmon, then a large number of other fishes with similar feeding habits also may have been contaminated.

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Literature Cited and Acknowledgements

Literature Cited

ADEC. 1989. Impact maps and summary reports of shoreline surveys of the *Exxon Valdez* spill sight. Alaska Department of Environmental Conservation Oil Spill Response Center.

ADFG. 1989. *Exxon Valdez* oil spill. Alaska Department of Fish and Game 21(4):4-37.

Aksnes, D. L., J. Aure, S. Kaartvedt, T. Magnesen, and J. Richard. 1989. Significance of advection for the carrying capacities of fjord populations. Marine Ecology Progress Series 50: 263-274.

Alongi, D. M., D. F. Boesch, and R. J. Diaz. 1983. Colonization of meiobenthos in oil-contaminated subtidal sands in the lower Chesapeake Bay. Mar. Biol. 72:325-335.

Anderson, Jack W., S. L. Kiesser, and J. W. Blaylock. 1979. Comparative uptake of naphthalenes from water and oiled sediment by benthic amphipods. p. 579-584. *In*: 1979 Oil Spill Conference (Prevention, Behavior, Control, Cleanup), Proceedings. American Petroleum Institute. Washington, D.C. 728 p.

Anderson, J. W., J. M. Neff, and P. D. Boehm. 1987. Sources, fates and effects of aromatic hydrocarbons in the Alaskan marine environment with recommendations for monitoring strategies. Report No. EPA/600/3-86/018 (NTIS No. PB 86-168291). U.S. Environmental Protection Agency, Washington, D.C. 230 p.

Babcock, M. M. 1985. Morphology of olfactory epithelium of pink salmon, *Oncorhynchus gorbuscha*, and changes following exposure to benzene: a scanning electron microscope study. p. 259-267. *In*: Marine biology of polar regions and stress on marine organisms (J. S. Gray and M. E. Christiansen (Eds)). John Wiley and Sons.

Babcock, M. M., M. G. Carls, J. W. Short, and J. F. Karinen. In prep. Pre-spill and post-spill concentrations of hydrocarbons in sediments and mussels at intertidal sites within Prince William Sound and the Gulf of Alaska. Coastal Habitat Intertidal Number 1B and Recovery Monitoring Study

Number 11 Status report. Alaska Fishery Center, NOAA/NMFS, Auke Bay Laboratory, 11305 Glacier Hwy., Juneau, AK 99801-8626.

Bailey, J. E., B. L. Wing, and C. R. Mattson. 1975. Zooplankton abundance and feeding habits of fry of pink salmon, *Oncorhynchus gorbuscha*, and chum salmon, *Oncorhynchus keta*, in Traitor's Cove, Alaska, with speculations on the carrying capacity of the area. Fisheries Bulletin 73:946-961.

Barnard, D. R. 1981. Prey relationships between juvenile pink (*Oncorhynchus gorbuscha*) and chum (*O. keta*) salmon in Prince William Sound, Alaska. MS Thesis, University of Alaska Fairbanks. 73 p.

Barron, M. G., and I. R. Adelman. 1984. Nucleic acid, protein content, and growth of larval fish sublethally exposed to various toxicants. Canadian Journal of Fisheries and Aquatic Sciences 41:141-150.

Bastrop, R., R. Spangenberg and K. Jurss. 1991. Biochemical adaptation of juvenile carp (*Cyprinus carpio* L.) to food deprivation. Comparative Biochemistry and Physiology 98A:143-149.

Bauer, J. E., R. B. Spies, J. S. Vogel, D. E. Nelson, and J. R. Southon. 1990. Radiocarbon evidence of fossil-carbon cycling in sediments of a nearshore hydrocarbon seep. Nature 348: 230-232.

Bax, N. J. 1983. Early marine mortality of marked juvenile chum salmon (*Oncorhynchus keta*) released into Hood Canal, Puget Sound, Washington, in 1980. Canadian Journal of Fisheries and Aquatic Sciences 40:426-435.

Bodin, P. 1988. Results of ecological monitoring of three beaches polluted by the *Amoco Cadiz* oil spill: development of meiofauna from 1978 to 1984. Marine Ecology Progress Series 42:105-123.

Bodin, P. 1991. Perturbations in the reproduction cycle of some harpacticoid copepod species further to the *Amoco Cadiz* spill. Hydrobiologia 209:245-257.

Bonsdorff, E. 1981. The *Antonio Gramsci* oil spill impact on the littoral and benthic ecosystems. Marine Pollution Bulletin 12(9):301-305.

Boucher, G. 1980. Impact of *Amoco Cadiz* oil spill on intertidal and sublittoral meiofauna. Mar. Pollut. Bull. 11:95-101.

Bowen, S. H. 1983. Quantitative description of the diet. p. 325-336. *In*: L. A. Nielsen and D. L. Johnson (Eds) Fisheries Techniques. American Fisheries Society, Bethesda.

Braddock, J. F., E. J. Brown, J. E. Lindstrom, T. R. Yeager, B. T. Rasley, and G. Winter. In prep. Microbial activity in sediments following the *T/V Exxon Valdez* oil spill. *Exxon Valdez* Oil Spill Symposium, Anchorage, AK.

Brannon, E. J., L. L. Moulton, L. G. Gilbertson, A. W. Maki, and J. R. Skalaki. *In Press*. An assessment of oil spill effects on pink salmon populations following the *Exxon Valdez* oil spill -- Part 1: Early life history. Proceedings of the ASTM Symposium on Environmental Toxicology and Risk Assessment. Atlanta, Georgia, 1993.

Brodeur, R. D., and W. G. Pearcy. 1990. Trophic relations of juvenile Pacific salmon off the Oregon and Washington coast. Fishery Bulletin 88:617-636.

Buckley, L. J. 1984. RNA-DNA ratio: an index of larval fish growth at the sea. Marine Biology 80:291-298.

Bulow, F. J. 1970. RNA-DNA ratios as indicators of recent growth rates of a fish. Journal of the Fisheries Research Board of Canada 27:2343-2349.

Bulow, F. J. 1987. RNA-DNA ratios as indicators of growth in fish: a review. p. 45-64. *In*: The Age and Growth of Fish. (R.C. Summerfelt and G. E. Hall, (Eds)). Iowa University Press, Ames, Iowa.

Bulow, F. J., M. E. Zeman, J. R. Winningham and W. F. Hudson. 1981. Seasonal variations in RNA-DNA ratios and in indicators of feeding, reproduction, energy storage and condition in a population of bluegill, *Lepomis macrochirus* Rafinesque. Journal of Fish Biology 18:237-244.

Burton, K. 1956. A study of the conditions and mechanism of the diphenylamine reaction for the colorimetric estimation of deoxyribose nucleic acid. Biochemical Journal 62:315-323.

Campana, S. E. 1990. How reliable are growth back-calculations based on otoliths? Canadian Journal of Fisheries and Aquatic Sciences 47:2219-2227.

Campana, S. E., and J. D. Neilson. 1982. Daily growth increments in otoliths of starry flounder (*Platichthys stellatus*) and the influences of some environmental variables in their production. Canadian Journal of Fisheries and Aquatic Sciences 39:937-942.

Campana, S. E., and J. D. Neilson. 1985. Microstructure of fish otoliths. Canadian Journal of Fisheries and Aquatic Sciences. 42:1014-1032.

Capuzzo, J. M. 1987. Biological effects of petroleum hydrocarbons: assessments from experimental results. p. 343-410. *In* D. F. Boesch and N. N. Rabalais, (Eds). Long-Term Environmental Effects of Offshore Oil and Gas Development. Elsevier Publishing Company, New York. 710 p.

Carls, M. G. 1987. Effects of direct and indirect oil exposure on larval Pacific herring (*Clupea harengus pallasi*). Marine Environmental Research 22(4):253-270.

Carls, M. G. and J. W. Short. 1992. TIDE37. Available at Alaska Fishery Center, NOAA/NMFS, Auke Bay Laboratory, 11305 Glacier Hwy., Juneau, AK 99801-8626.

Celewycz, A. G., and J. Cordell. 1988. Occurrence of potential prey items of juvenile pink salmon in nearshore waters of Auke Bay, Alaska, in 1987, as sampled with three gear types. p. 451-469. *In*: Vol. I APPRISE Annual Report, SFOS APP87-100, School of Fisheries and Ocean Sciences, University of Alaska, Fairbanks.

Celewycz, A. G. 1990. Distribution and sizes of juvenile pink and chum salmon in five habitats in Auke Bay, Alaska. p. 54-57. *In*: 14th Northeast Pacific pink and chum salmon workshop (P. A. Knutson (Eds)). Washington Department of Fisheries, Olympia.

Chandler, G.T., and J.W. Fleeger. 1983. Meiofaunal colonization of azoic estuarine sediment in Louisiana: mechanisms of dispersal. J. Exp. Mar. Biol. Ecol. 69:175-188.

Chasse, C. 1978. The ecological impact on and near shores by the *Amoco Cadiz* oil spill. Marine Pollution Bulletin 9:298-301.

Cone, R. S. 1989. The need to reconsider the use of condition indices fisheries science. Transactions of the American Fisheries Society 118:510-514.

Conover, R. J. 1971. Some relations between zooplankton and Bunker C oil in Chedabucto Bay following the wreck of the tanker *Arrow*. Journal of the Fisheries Research Board of Canada 28:1327-1330.

Conover, W. J. 1980. Practical nonparametric statistics. John Wiley and Sons, New York. 493 p.

Cooney, R. T. 1984. Some thoughts on the Alaska coastal current as a feeding habitat for juvenile salmon. p. 256-268. *In*: The Influence of Ocean Conditions on the Production of Salmonids in the North Pacific (W. C. Pearcy (Ed)). Sea Grant Program, ORESU-W-83-001, Oregon State University, Corvallis, OR.

Cooney, R. T. 1986. Zooplankton. p. 285-304. *In*: The Gulf of Alaska Physical Environment and Biological Resources (D. W. Hood and S. T. Zimmerman (Eds)). U.S. Department Interior Mining and Managing Service. OCS Study, MMS 86-0095.

Cooney, R. T. 1990. Prey fields and the feeding behavior and growth of pink salmon fry released from the Armin F. Koening Hatchery at Sawmill Bay, Evans Island, Alaska: May and June 1989. 1990 NRDA Status Report, Fish/Shellfish 4: University of Alaska, Fairbanks component.

Cooney, R. T., D. Urquhart, and D. Barnard. 1981. The behavior, feeding biology, and growth of hatchery released pink and chum salmon fry in Prince William Sound, Alaska. University of Alaska, Sea Grant Rep. 81-5. 114 p.

Cooney, R. T., D. Urquhart, R. Neve, J. Hilsinger, R. Clasby, and D. Barnard. 1978. Some aspects of the carrying capacity of Prince William Sound, Alaska, for hatchery released pink and chum salmon fry. University of Alaska, Sea Grant Rep. 78-4. IMS Report R78-3. 102 p.

Cooney, R. T., and T. M. Willette. 1991. Regional-level investigations of pink salmon production responses to interannual variations in ocean temperatures: cooperative fisheries and oceanographic studies. p. 50-62. *In*: Proceedings Fifteenth Northeast Pacific Pink and Chum Salmon Workshop (I. Guthrie and B. Wright (Eds)). Pacific Salmon Commission, Parksville, B. C., Canada.

Cordell, J. R. 1986. Structure and dynamics of an epibenthic harpacticoid assemblage and the role of predation by juvenile salmon. M.S. Thesis, University of Washington, Seattle. 134 p.

Corner, E. D. S., R. P. Harris, C. C. Kilvington, and S. C. M. O'Hara. 1976. Petroleum compounds in the marine food web: Short-term experiments on the fate of naphthalene in *Calanus*. Journal of Marine Biological Association of the United Kingdom 56:121-133.

Corner, E. D. S. 1978. Pollution studies with marine plankton. Part I. Petroleum hydrocarbons and related compounds. Advances in Marine Biology 15:289-380.

Coull, B. C. 1973. Estuarine meiofauna: A review: trophic relationships and microbial interactions. p. 499-511. *In* L. H. Stevenson and R. R. Colwel (eds). Estuarine microbial ecology. University of South Carolina Press, Columbia.

Coull, B. C., and G. T. Chandler. 1992. Pollution and meiofauna: Field, laboratory and mesocosm studies. Mar. Biol. Ann. Rev. 30:191-271.

Coull, B. C., and M. A. Palmer. 1984. Field experimentation in meiofaunal ecology. Hydrobiologia 118:1-19.

Coyle, K. O., A. J. Paul, and D. A. Ziemann. 1990. Copepod populations during the spring bloom in an Alaskan subarctic embayment. Journal Plankton Research 12: 759-797.

Cruze, E., and J. Weldon. 1989. MINITAB reference manual, release 7. Minitab Inc., State College, PA. 342 p.

Cushing, D. H. 1975. Marine ecology and fisheries. Cambridge University Press, Cambridge. 276 p.

D'Amours, D. 1987. Trophic phasing of juvenile chum salmon (*Oncorhynchus keta* Walbaum) and harpacticoid copepods in the Fraser River estuary, British Columbia. Ph.D. Thesis, University of British Columbia. 163 p.

Decker, C. M., and J. W. Fleeger. 1984. The effect of crude oil on the colonization of meiofauna into salt marsh sediments. Hydrobiologia 118:49-58.

Deegan, L. A., and B. A. Thompson. 1987. Growth rate and life history events of young-of-the-year Gulf Menhaden as determined from otoliths. Transactions of the American Fisheries Society 116:663-667.

Dixon, W. J., P. Sampson, and P. Mundle. 1988. One- and two-way analysis of variance with data screening. p. 187-208. *In*: BMDP Statistical software manual (W. J. Dixon (Ed)). University of California Press, Berkeley.

Eggers, D. M., L. R. Peltz, B. G. Bue, and T. M. Willette. In press. Trends in abundance of hatchery and wild stocks of pink salmon in Cook Inlet, Prince William Sound and Kodiak, Alaska. Proceedings of the International Symposium of Biology Interests, Enhanced and Wild Salmonids. Department of Fisheries and Oceans, Nanaimo, B. C.

Elmgren, R., S. Hansson, U. Larsson, B. Sundelin, and P. D. Boehm. 1983. The *Tsesis* oil spill: acute and long-term impact on the benthos. Marine Biology 73:51-65.

Faurot, M. W., S. W. Landino, R. R. Reisenbichler and D. G. Clark. 1989. Habitat utilization by juvenile pink and chum salmon in upper Resurrection Bay, Alaska. Technical Report EL-89-17. U.S. Army Engineer Waterways Experiment Station, Vicksburg, MS. 58 p.

Feder, H. M., L. M. Cheek, P. Flanagan, S. C. Jewitt, M. H. Johnston, A. S. Naidu, S. A. Norrell, A. J. Paul, A. Scarborough, and D. Shaw. 1976. The sediment environment of Port Valdez, Alaska: The effect of oil on this ecosystem. Environmental Protection Agency, Ecological Research Series, EPA-600/3-76-086. 321 p.

Feder, H. M., A. S. Naidu, and A. J. Paul. 1990. Trace element and biotic changes following a simulated oil spill on a mudflat in Port Valdez, Alaska. Marine Pollution Bulletin 21(3):131-137.

Fegley, S. R. 1988. A comparison of meiofaunal settlement on the sediment surface and recolonization of defaunted sandy sediment. J. Exp. Mar. Biol. Ecol. 123:97-114.

Feller, R. J., and V. W. Kaczynski. 1975. Size selective predation by juvenile chum salmon (*Oncorhynchus keta*) on epibenthic prey in Puget Sound. Journal of the Fisheries Research Board of Canada 32:1419-1429.

Fleeger, J. W. 1979. Population dynamics of three estuarine epibenthic harpacticoids (Copepoda) in South Carolina. Marine Biology 52:147-156.

Fleeger, J. W., and G. T. Chandler. 1983. Meiofauna responses to an experimental oil spill in a Louisiana salt marsh. Marine Ecology Progress Series 11:257-264.

Fleeger, J. W., T. C. Shirley, and D. A. Zimmerman. 1989. Meiofauna responses to sedimentation from an Alaska spring bloom. Marine Ecology Progress Series 57:137-145.

Fleeger, J. W., and T. C. Shirley. 1990. Meiofauna responses to sedimentation from an Alaskan spring bloom. II. Harpacticoid population dynamics. Marine Ecology Progress Series 59:239-247.

Fleeger, J. W., M. A. Todaro, T. C. Shirley, and M. G. Carls. In prep. Meiofaunal recolonization experiment with oiled sediments: the harpacticoid copepod assemblage. *Exxon Valdez* Oil Symposium, February 1993.

Forrester, W. D. 1971. Distribution of suspended oil particles following the grounding of the tanker *Arrow*. Journal of Marine Research 29:151-170.

Fossato, V. U., and W. J. Canzonier. 1976. Hydrocarbon uptake and loss by the mussel *Mytilus edulis*. Marine Biology 36:243-250.

Foster, M. S., A. P. De Vogelaere, C. Harrold, J. S. Pearse, A. B. Thum. 1988. Causes of spatial and temporal patterns in rocky intertidal communities of central and northern California. Memoir Number 9, California Academy of Sciences. 45 p.

Frane, J. 1980. The univariate approach to repeated measures--foundation, advantages,caveats. BMDP Tech. Rept 69. 34 p.

Fricke, A. H., H. F. O. Henning, and M. J. Orren. 1981. Relationship between oil pollution and psammolittoral meiofauna density of two South African beaches. Marine Environmental Research 5:59-77.

Frithsen, J. B., R. Elmgren, and D. T. Rudnick. 1985. Responses of benthic meiofauna to long-term low-level additions of No. 2 fuel oil. Marine Ecology Progress Series 23:1-14.

Gauch, H. G. 1982. Multivariate analysis in community ecology. Cambridge University Press. New York.

Gauldie, R. W., and D. G. A. Nelson. 1988. Aragonite twinning and neuroprotein secretion are the cause of daily growth rings in fish otoliths. Comparative Biochemistry and Physiology 90A(3):501-509.

Gauldie, R. W. 1990. A measure of metabolism in fish otoliths. Comparative Biochemistry and Physiology 97(4):475-480.

GERG. 1989. Standard operating procedures for NOAA/*Exxon Valdez* oil spill assessment. Geochemical and Environmental Research Group, Texas A&M University, Ten South Graham Road, College Station, Texas 77840.

Giere, O. 1979. The impact of oil pollution on intertidal meiofauna. Field studies after the *La Coruna* spill, May 1976. Cahiers de Biologie Marine 20:231-251.

Gilfillan, E. S., S. A. Hanson, D. Vallas, R. Gerber, D. S. Page, F. J. Hotham, and S. D. Pratt. 1983. Effects of spills of dispersed and non-dispersed oil on intertidal community structure. p. 457-463. *In*: Proceedings of 1983 Oil Spill Conference. American Petroleum Institute, Washington, D.C.

Godin, J. J. 1981. Daily patterns of feeding behavior, daily rations, and diets of juvenile pink salmon (*Oncorhynchus gorbuscha*) in two marine bays of British Columbia. Canadian Journal of Fisheries and Aquatic Sciences 38:10-15.

Goering, J. C., W. E. Shield, and C. J. Patton. 1973. Primary production. p. 253-271. *In*: Environmental studies of Port Valdez (D. W. Hood, W. E. Shield, and E. J. Kelley (Eds)). University Alaska Fairbanks. Institute of Marine Science and Occupation Publishers 3.

Gosho, M. E. 1977. The food and feeding habits of juvenile pink salmon in the estuaries of Kodiak Island, Alaska. M.S. Thesis, University of Washington. 87 p.

Hargreaves, N. B., and R. J. LeBrasseur. 1985. Species selective predation on juvenile pink (*Oncorhynchus gorbuscha*) and chum (*O. keta*) by coho salmon (*O. kisutch*). Can. Journal of Fisheries and Aquatic Sciences 42:659-668.

Healey, M. C. 1979. Detritus and juvenile salmon production in the Nanaimo estuary: 1. Production and feeding rates of juvenile chum salmon (*Oncorhynchus keta*). Journal of Fisheries Research Board of Canada 36:488-496.

Healey, M. C. 1980. The ecology of juvenile salmon in Georgia Strait, British Columbia. p. 203-230. *In*: Salmonid Ecosystems of the North Pacific (W. J. McNeil and D.C. Himsworth (Eds)). Oregon State University Press, Corvallis.

Heard, W. R. 1991. Life history of pink salmon (*Oncorhynchus gorbuscha*). p. 119-230. *In*: Pacific salmon life histories (C. Groot and L. Margolis (Eds)). University of British Columbia Press, Vancouver. 564 pp.

Helle, J. H. 1980. Influence of marine environment on age and size at maturity, growth, and abundance of chum salmon, *Oncorhynchus keta* (Walbaum), from Olsen Creek, Prince William Sound, Alaska. PhD Thesis, Oregon State University, Corvallis. 118 p.

Hicks, G. R. F., and B. C. Coull. 1983. The ecology of marine meiobenthic harpacticoid copepods. Oceanography of Marine Biology Annual Review 21:67-175.

Hill, M. O. 1979. DECORANA-A FORTRAN program for detrended correspondence analysis and reciprocal averaging. Cornell University Press, Ithica, N.Y.

Holme, N. A., and A. D. McIntyre. 1984. Methods for the study of marine benthos. Blackwell Scientific Publications, Boston. 387 p.

Holtby, L. B., T. E. McMahon, and J. C. Scrivener. 1989. Stream temperatures and inter-annual variability in the emigration timing of coho salmon (*Oncorhynchus kisutch*) smolts and fry and chum salmon (*O. keta*) fry from Carnation Creek, British Columbia. Canadian Journal of Fish and Aquatic Sciences 46:1396-1405.

Houghton, J. P., W. B. Driskell, D. C. Lees, and A. J. Mearns. 1991. Impacts of the *Exxon Valdez* and subsequent cleanup on intertidal biota—1 year later. Proceedings 1991 International Oil Spill Conference. American Petroleum Institute.

Houlihan, D. F., S. J. Hall, C. Gray, and B. S. Noble. 1988. Growth rates and protein turnover in Atlantic cod, *Gadus morhua*. Canadian Journal of Fisheries and Aquatic Science 45: 951-964.

Hurlbert, S. H. 1984. Pseudoreplication and the design of ecological field experiments. Ecological Monographs 54(2):187-211.

Hyslop, E. J. 1980. Stomach contents analysis--a review of methods and their application. Journal of Fish Biology 17:411-429.

Jefferts, K. B., P. K. Bergman, and H. F. Fiscus. 1963. A coded-wire identification system for macro-organisms. Nature 198:460-462.

Jewett, S. C., and H. M. Feder. 1977. Biology of the harpacticoid copepod, *Harpacticus uniremis* Kröyer on Dayville Flats, Port Valdez, Alaska. Ophelia 16:111-129.

Johnston, R. 1984. Oil pollution and its management. p. 1497-1530 *In*: Marine Ecology (O. Kinne (Ed)). Marine Ecology. Volume 5. John Wiley, New York.

Jurss, K., and T. Bittorf. 1990. The relationship between biochemical liver status and growth in immature rainbow trout (*Salmo gairdneri* Richardson) I. Effects of feeding and salinity. Zoological Journal of Physiology 94:474-485.

Jurss, K., T. Bittorf, and T. Vokler. 1986. Influence of salinity and food deprivation on growth, RNA/DNA ratio and certain enzyme activities in rainbow trout (*Salmo gairdneri*

Richardson). Comparative Biochemistry and Physiology 83B:425-433.

Jurss, K., T. Bittorf, and R. Wacke. 1987. Effects of temperature, food deprivation and salinity on growth, RNA/DNA ratio and certain enzyme activities in rainbow trout (*Salmo gairdneri* Richardson). Comparative Biochemistry and Physiology 87B:241-253.

Kaczynski, V. W., R. J. Feller, and C. Clayton. 1973. Trophic analysis of juvenile pink and chum salmon (*Oncorhynchus gorbuscha* and *O. keta*) in Puget Sound. Journal of Fisheries Research Board of Canada 30:1003-1008.

Karinen, J. F., M. M. Babcock, D. W. Brown, W. D. MacLeod, Jr., L. Scott Ramos, and J. W. Short. 1993. Hydrocarbons in intertidal sediments and mussels from Prince William Sound, Alaska, 1977-1980: characterization and probable sources. NOAA Technical Memorandum NMFS-AFSC-9. 69 p.

Kelso, D. D., and M. Kendziorek. 1991. Alaska's response to the *Exxon Valdez* oil spill. Environmental Science and Technology 25(1):16-23.

Kirkwood, J. B. 1972. Inshore-marine and freshwater life history phases of the pink salmon. University of Louisville Ph.D. Thesis. 300 p.

KLI. 1991. NOAA epibenthic pump samples—Herring Bay. Report prepared for NOAA Auke Bay Laboratories, Juneau, Alaska, by Kinnetic Laboratories Incorporated, Santa Cruz, California. 33 p.

Kontogiannis, J. E., and C. J. Barnett. 1973. The effect of oil pollution on survival of the tidal pool copepod, *Tigriopus californicus*. Environmental Pollution 4:69-79.

Koski, K. V. 1975. The survival and fitness of two stocks of chum salmon (*Oncorhynchus keta*) from egg deposion to emergence in a controlled-stream environment at Big Beef Creek. Ph.D. Thesis, University Washington, Seattle. 212 p.

Kron, T., and H. Yuen. 1978. Emigration and feeding of juvenile pink salmon *Oncorhynchus gorbuscha*, in relation to zooplankton abundance in Tutka Bay, Alaska, 1975. Alaska Department of Fish and Game, Lower Cook Inlet Data Report No. 5-5. 24 p.

Landingham, J. H. 1982. Feeding ecology of pink and chum salmon fry in the nearshore habitat of Auke Bay, Alaska. M.S. Thesis, University of Alaska, Juneau. 132 p.

Landingham, J. H., and P. D. Mothershead. 1988. Feeding habits of juvenile pink salmon in nearshore and offshore sites of Auke Bay. p. 449-469. *In*: Volume I. APPRISE Annual Report, SFOS APP87-100. School of Fisheries and Ocean Sciences, University of Alaska, Fairbanks.

Landingham, J. H. 1993. Measurement of prey of juvenile salmon: wet and dry weight, length, and volume. Processed Report, National Marine Fisheries Service, Auke Bay Laboratory, 11305 Glacier Highway, Juneau, Alaska, 99801.

Landrum, P. F. 1989. Bioavailability and toxikinetics of polycyclic aromatic hydrocarbons sorbed to sediments for the amphipod *Pontoporeia hoyi*. Environmental Science and Technology 23:588-595.

Larrance, J. D. 1977. Primary production in the mid-subarctic Pacific region 1966-68. Fisheries Bulletin 69:595-613.

Larsen, M. L., L. G. Holland, D. Fremgen, J. Lunasin, M. Wells, and J. Short. 1992. Standard Operating Procedures for the Analysis of Petroleum Hydrocarbons in Seawater, Marine Sediments, and Marine Faunal Tissue at the Auke Bay Laboratory. Unpublished manuscript. NOAA/NMFS, Auke Bay Laboratory, 11305 Glacier Highway, Juneau, Alaska 99801.

Laughlin, R. B., J. Ng, and H. E. Guard. 1981. Hormesis: A response to low environmental concentrations of petroleum hydrocarbons. Science 211:705-707.

LeBrasseur, R. J., and R. R. Parker. 1964. Growth rate of central British Columbia pink salmon (*Oncorhynchus gorbuscha*). Journal of the Fisheries Research Board of Canada 21:1101-1128.

Lee, R. F. 1975. Fate of petroleum hydrocarbons in marine zooplankton. p. 549-553. *In*: 1975 Conference on Prevention and Control of Oil Pollution, Proceedings. American Petroleum Institute. Washington, D.C. 612 p.

Lee, W. Y., and J. A. C. Nicol. 1977. The effects of the water soluble fractions of No. 2 fuel oil on the survival and behavior of coastal and oceanic zooplankton. Environmental Pollution 12:279-292.

Lewis, A. G. and A. C. Thomas. 1986. Tidal transport of planktonic copepods across the sill of a British Columbia fjord. Journal Plankton Research 8: 1079-1089.

Lied, E., G. Rosenlund, B. Lund, and A. von der Decken. 1983. Effect of starvation and refeeding on in vitro protein

synthesis in white trunk muscle of Atlantic cod (*Gadus morhua*). Comparative Biochemistry and Physiology 76B: 777-781.

Lindahl, O. and L. Hernroth. 1988. Large-scale and long-term variations in the zooplankton community of the Gullmar fjord, Sweden, in relation to advective processes. Marine Ecology Progress Series 43: 161-171.

Lindahl, O. and R. Perissinotto. 1987. Short term variations in the zooplankton community related to water exchange processes in the Gullmar fjord, Sweden. Journal Plankton Research 9: 1113-1132.

Livingstone, D. R., M. A. Kirchen, and A. Wiseman. 1989. Cytochrome P450 and oxidative metabolism in molluscs. Xenobiotica 19:1041-1062.

Macek, K. J., S. R. Petrocelli, and B. H. Sleight III. 1979. Considerations in assessing the potential for, and significance of, biomagnification of chemical residues in aquatic food chains. p. 251-268. *In*: American Society for Testing and Materials (L. L. Marking and R. A. Kimerle (Eds)). Aquatic toxicology, ASTM STP 667.

Maki, A. W. 1991. The *Exxon Valdez* oil spill: Initial environmental impact assessment. Environmental Science and Technology 25(1):24-29.

Manen, C. A., E. J. McFaul, and K. Buttleman. In prep. *Exxon Valdez* oil spill natural resource damage assessment analytical chemistry data. Available NOAA/NOS Office of Ocean Resource Conservation and Assessment, Damage Assessment Center, 6001 Executive Blvd, WSC-1 Room 323, Rockville MD 20852

Marcotte, B. M. 1980. The meiobenthos of fjords: a review and prospectus. p. 557-568. *In*: Fjord oceanography (H. J. Freeland, D. M. Farmer, and C. D. Levings (Eds)). NATO Conf. Ser. IV: Marine Sciences. Plenum Press, New York.

Marcotte, B. M. 1983. The imperatives of copepod diversity: perception, cognition, competition and predation. p. 47-72. *In*: Crustacean phylogeny (F. R. Schram (Ed)). Balkema, Rotterdam.

Marcotte, B. M., and Howard I. Browman. 1986. Foraging behavior in fishes: perspectives on variance. Environmental Biology of Fishes 16(1-3):25-33.

Mason, J. C. 1974. Behavioral ecology of chum salmon fry (*Oncorhynchus keta*) in a small estuary. Journal of the Fisheries Research Board of Canada 31:83-92.

Mathers, E. M., D. F. Houlihan, and M. J. Cunningham. 1992. Nucleic acid concentrations and enzyme activities as correlates of growth rate of the saithe *Pollachisu virens*: growth-rate estimated of open-sea fish. Marine Biology 112:363-369.

McCain, B. B., D. C. Malins, M. M. Kahn, D. W. Brown, W. D. Grolund, L. K. Moore, and S-L. Chan. 1990. Uptake of aromatic and chlorinated hydrocarbons by juvenile chinook salmon (*Oncorhynchus tshawytscha*) in an urban estuary. Archives of Environmental Contamination and Toxicology 19:10-16.

McEachran, J. D., D. F. Boesch, and J. A. Musick. 1976. Food division within two sympatric species-pairs of skates (Pisces: Rajidae). Marine Biology 35:301-317.

Michel, J. and M. O. Hayes. 1993. Evaluation of the condition of Prince William Sound shorelines following the *Exxon Valdez* oil spill and subsequent shoreline treatment. NOAA Technical Memorandum NOS ORCA 67: Volume 1. 94 p.

Miglavs, I., and M. Jobling. 1989. Effects of feeding regime on food consumption, growth rates and tissue nucleic acids in juvenile Arctic charr, *Salvelinus alpinus*, with particular respect to compensatory growth. Journal of Fish Biology 34:947-957.

Miller, M. R., D. E. Hinton, and J. J. Stegeman. 1989. Cytochrome P-450E induction and localization in gill pillar (endothelial) cells of scup and rainbow trout. Aquatic Toxicology 14:307-322.

Moles, A., and S. D. Rice. 1983. Effects of crude oil and naphthalene on growth, caloric content, and fat content of pink salmon juveniles in seawater. Transactions of the American Fisheries Society 112:205-211.

Mortensen, D. M., J. H. Landingham, A. C. Wertheimer, and S. G. Taylor. 1991. Relationship of early marine growth and survival of juvenile pink salmon to marine water temperature and secondary production in Auke Bay, Alaska. p. 38-49. *In*: Proceedings Fifteenth Northeast Pacific Pink and Chum Salmon Workshop (I. Guthrie and B. Wright (Eds)). Pacific Salmon Commission, Parksville, B. C.

Mugiya, Y., N. Watabe, J. Yamada, J. M. Dean, D. G. Dunkelberger and M. Shimizu. 1981. Diurnal rhythm in otolith formation in the goldfish, (*Carassius auratus*). Comparative Biochemistry and Physiology 68A:659-662.

Munro, H. N., and A. Fleck. 1966. Recent developments in the measurement of nucleic acids in biological materials. Analyst 19:78-88.

Murphy, M. L., J. F. Thedinga, and K. V. Koski. 1988. Size and diet of juvenile Pacific salmon during seaward migration through a small estuary in southeastern Alaska. Fisheries Bulletin 86:213-222.

Naidu, A. S., H. M. Feder, and S. A. Norrell. 1978. The effect of Prudhoe Bay crude oil on a tidal-flat ecosystem in Port Valdez, Alaska. p. 97-104. *In*: Tenth Offshore Technology Conference, Houston, Texas.

Neff, J. M. 1987. Biological effects of oil in the marine environment. Chemical Engineering Progress. November:27-33.

Neff, J. M. 1991. Water quality in Prince William Sound and the Gulf of Alaska (an update of the April, 1990 report). Arthur D. Little, 20 Acorn Park, Cambridge, Massachusetts 02140-2390. 37 p.

Nichelson, T. E. 1986. Influences of upwelling, ocean temperature, and smolt abundance on marine survival of coho salmon (*Oncorhynchus kisutch*) in the Oregon production area. Canadian Journal of Fisheries and Aquatic Science 43:527-535.

Neilson, J. D., and G. H. Geen. 1982. Otoliths of chinook salmon (*Oncorhynchus tshawytscha*): daily growth increments and factors influencing their production. Canadian Journal of Fisheries and Aquatic Sciences 39:1340-1347.

Neilson, J. D., and G. H. Geen. 1985. Effects of feeding regimes and diel temperature cycles on otolith increment formation in juvenile chinook salmon (*Oncorhynchus tshawytscha*). Fishery Bulletin 83(1):91-101.

Neilson, J. D., G. H. Geen, and D. Bottom. 1985. Estuarine growth of juvenile chinook salmon (*Oncorhynchus tshawytscha*) as inferred form otolith microstructure. Canadian Journal of Fisheries and Aquatic Sciences 42:899-908.

Olsen, J. 1991. Comparative performance of pink salmon from two hatcheries in Prince William Sound. p. 17-28. *In*: Proceedings of the 15th Northeast Pacific pink and chum

salmon workshop (B. White and I. Guthrie (Eds)). Pacific Salmon Commission, Vancouver.

Oviatt, C., J. Frithsen, J. Gearing, and P. Gearing. 1982. Low chronic additions of No. 2 fuel oil: chemical behavior, biological impact and recovery in a simulated estuarine environment. Marine Ecology Progress Series 9:121-136.

Owens, E. H. 1991. Changes in shoreline oiling conditions 1-1/2 years after the 1989 Prince William Sound spill. Woodward-Clyde, Seattle. 52 p. + appendices.

Page, D. S., P. D. Boehm, G. S. Douglas, and A. E. Bence. 1993. Identification of hydrocarbon sources in the benthic sediments of Prince William Sound and the Gulf of Alaska following the *Exxon Valdez* oil spill. Third Symposium on Environmental Toxicology and Risk Assessment, American Society for Testing and Materiels, Atlanta, Georgia, 1993.

Palmer, M. A. 1988. Dispersal of marine meiofauna: a review and conceptual model explaining passive transport and active emergence with implications for recruitment. Mar. Ecol. Prog. Ser. 48:81-91.

Palmer, M. A., P. A. Montagna, R. B. Spies, and D. Hardin. 1988. Meiofauna dispersal near natural petroleum seeps in the Santa Barbara channel: a recolonization experiment. Oil and Chemical Pollution 4:179-189.

Pannella, G. 1980. Growth patterns in fish sagittae. p. 519-560. *In* D. C. Rhodes and R. A. Lutz (eds). Skeletal Growth of Aquatic Organisms. Plenum Press, New York.

Parker, R. R. 1968. Marine mortality schedules of pink salmon of the Bella Coola River, central British Columbia. Journal of Fisheries Research Board of Canada 25:757-794.

Parker, R. R. 1971. Size selective predation among juvenile salmonid fishes in a British Columbia inlet. Journal of Fisheries Research Board of Canada 28:1503-1510.

Pavia, E., C. Crosby, P. Endres, W. Lane, and M. Siddiqui. 1991. The Prince William Sound and Homer regions evaluation group final report. Alaska Department of Environmental Conservation. 645 G Street, Suite 402, Anchorage, AK 99501.

Pentec. 1991. NOAA epibenthic pump samples—Bay of Isles. Data Report prepared for NOAA Auke Bay Laboratories, Juneau, Alaska by Pentec Environmental, Incorporated, Edmonds, Washington. 86 p.

Pielou, E. C. 1975. Ecological diversity. John Wiley and Sons: New York. 165 p.

Pirtle, R. B. 1977. Historical pink and chum salmon estimated spawning escapements from Prince William Sound, Alaska, streams, 1960-1975. Alaska Department of Fish and Game. Technical Report 35.

PWSRPT. 1983. Prince William Sound-Copper River comprehensive salmon plan. Prince William Sound Regional Fisheries Planning Team, Cordova, Alaska. 176 p.

Raymond, J. 1990. Damage to early marine salmon in Prince William Sound. 1990 NRDA Status Report, Fish/Shellfish 4: Alaska Department of Fish and Game Component. 19 p.

Rice, S. D. 1985. Effects of oil on fish. p. 157-182. *In*: Petroleum Effects in the Arctic Environment (F. R. Engelhardt (Ed)). Elsevier Applied Science Publishers, New York.

Rice, S. D., D. A. Moles, and J. W. Short. 1975. The effect of Prudhoe Bay crude oil on survival and growth of eggs, alevins, and fry of pink salmon, *Oncorhynchus gorbuscha*. p. 503-507. *In*: 1975 Conference on prevention and control of oil pollution. American Petroleum Institute, Washington, D.C.

Rice, S. D., R. E. Thomas, and J. W. Short. 1977. Effect of petroleum hydrocarbons on breathing and coughing rates and hydrocarbon uptake-depuration in pink salmon fry. p. 259-277. *In*: Physiological Responses of Marine Biota to Pollutants (F. J. Vernberg, A. Calabrese, F. P. Thurberg, and W. B. Vernberg (Eds)). Academic Press, Incorporated, New York.

Rice, S. D., D. A. Moles, J. F. Karinen, S. Korn, M. G. Carls, C. C. Brodersen, J. A. Gharrett, and M. M. Babcock. 1984. Effects of petroleum hydrocarbons on Alaskan aquatic organisms. NOAA Technical Memorandum NMFS F/NWC-67. 128 p.

Rigby, P., J. McConnaughey, and H. Savikko. 1991. Alaska commercial salmon catches, 1878-1991. Alaska Department Fish and Game Regular Information Report Number 5J91-16. 88 p.

Rogers, D. E., B. J. Rogers, and R. J. Rosenthal. 1986. The nearshore fishes. p. 399-415. *In*: The Gulf of Alaska physical environment and biological resources (D. W. Hood and S. T. Zimmerman (Eds)). U.S. Department of International Mining and Management Services. OCS Study, MMS 86-0095.

Royce, W. F., T. R. Schroeder, A. A. Olsen, and W. J. Allender. 1991. Alaska fisheries two years after the spill. *Exxon* USA. 35 p.

Sales, D., J. Gibeaut, and J. W. Short. In prep. Nearshore subtidal transport of hydrocarbons and sediments following the Exxon Valdez oil spill. Air Water 3 Final report. Alaska Fishery Center, NOAA/NMFS, Auke Bay Laboratory, 11305 Glacier Hwy., Juneau, AK 99801-8626.

Salo, E. O. 1991. Life history of chum salmon (*Oncorhynchus keta*). p. 231-310. *In*: Pacific salmon life histories (C. Groot and L. Margolis (Eds)). University of British Columbia Press, Vancouver.

Samain, J. F., J. Moal, A. Coum, J. R. Le Coz, and J. Y. Daniel. 1980. Effects of the *Amoco Cadiz* oil spill on zooplankton. Helgolander Meeresunters 33:225-235.

SAS. 1989. SAS/STAT User's Guide, Version 6, Fourth Edition, Volume 2. SAS Institute Inc., Cary, North Carolina. 846 p.

Schwartz, J. P. 1985. Effect of oil-contaminated prey on the feeding and growth rate of pink salmon fry (*Oncorhynchus gorbuscha*). p. 459-476. *In*: Pollution and Physiology: of Marine Organisms (F. Vernberg, R. Thurberg, A. Calabrese, and W. Vernberg (Eds)). University South Carolina Press, Columbia. 645 p.

Secor, D. A., and J. M. Dean. 1989. Somatic growth effects on the otolith-fish size relationship in young pond reared striped bass, (*Morone saxatilis*). Canadian Journal of Fisheries and Aquatic Sciences 46:113-121.

Secor, D. H., J. M. Dean, and E. H. Leban. 1991. Manual for otolith removal and preparation for microstructural examination. Electric Power Research Institute and the Belle W. Baruch Institute for Marine Biology and Coastal Research. 85 p.

Shatkin, A. J. 1969. Colorimetric reactions for DNA, RNA, and protein determinations. p. 231-237. *In*: Fundamental Techniques in Virology (K. Habel and N. P. Salzman (Eds)). Academic Press, New York.

Sherman, K.M., D.A. Meeter, and J.A. Reidenauer. 1984. A technique for subsampling an abundant taxon while completely sorting other taxa. Limnology and Oceanography 29(2):433-439.

Short, J. W., and R. A. Heintz. 1992. Descriptive documentation for identification of biased sediment and mussel tissue samples in the NRDA hydrocarbon database. Exxon Valdez NRDA Status Report, Subtidal 8. Available Auke Bay Laboratory, Alaska Fish. Cent., 11305 Glacier Hwy, Juneau, AK 99801.

Short, J. W. and P. Rounds. 1993a. Petroleum hydrocarbons in near-surface seawater of Prince William Sound, Alaska, following the *Exxon Valdez* oil spill I: Chemical sampling and analysis. Final report, Air/Water Study Number 3. Alaska Fishery Center, NOAA/NMFS, Auke Bay Laboratory, 11305 Glacier Hwy., Juneau, AK 99801-8626.

Short, J. W. and P. Rounds. 1993b. Petroleum hydrocarbons in near-surface seawater of Prince William Sound, Alaska, following the *Exxon Valdez* oil spill I: Analysis of caged mussels. Final report, Air/Water Study Number 3. Alaska Fishery Center, NOAA/NMFS, Auke Bay Laboratory, 11305 Glacier Hwy., Juneau, AK 99801-8626.

Sibert, J. R., T. J. Brown, M. C. Healey, B. A. Kask, and R. J. Naiman. 1977. Detritus-based food webs: exploitation by juvenile chum salmon (*Oncorhynchus keta*). Science 106:649-650.

Simenstad, C. A., and W. J. Kinney. 1978. Trophic relationships of outmigrating chum salmon in Hood Canal, Washington, 1977. Final report. University Washington, FRI-UW-7810.

Simenstad, C. A., and E. O. Salo. 1982. Foraging success as a determinant of estuarine and nearshore carrying capacity of juvenile chum salmon (*Oncorhynchus keta*) in Hood Canal, Washington. p. 21-37. *In*: Proceedings of the North Pacific Aquaculture Symposium (B. R. Melteff and R. A. Neave (Eds)). Alaska Sea Grant Rep. 82-2. University of Alaska, Fairbanks. 372 p.

Smetacek, V., B. von Bodugen, B. Knoppers, P. Peinert, F. Pollehne, P. Stegmann, and B. Zeitzschel. 1984. Seasonal stages characterizing the annual cycle of an inshore pelagic system. Rapports et Proces-Verbaux des Reunions Conseil International pour l'Exploration de la Mer 83:126-135.

Smolowitz, R. M., M. E. Hahn, and J. J. Stegeman. 1991. Immunohistochemical localization of cytochrome P-450IA1 induced by 3,3',4,4'-tetrachlorobiphenyl and by 2,3,7,8-tetrachlorodibenzo-furan in liver and extrahepatic tissues of the teleost *Stenotomus chrysops* (Scup). Drug Metabolism and Disposition 19:113-123.

Smolowitz, R. M., M. E. Hahn, and J. J. Stegeman. 1992. Cytochrome P-450IA induction in tissues, including olfactory epithelium, of top minnows (*Poeciliopsis spp.*) by waterborne benzo(a)pyrene. Carcinogenesis 13: 2395-2402.

Smythe, H. F. 1967. Sufficient challenge. Food and Cosmetics Toxicology 5:51-58.

Sokal, R. R., and F. J. Rohlf. 1981. Biometry, the principles and practice of statistics in biological research, 2nd edition. W. H. Freeman and Company, New York, New York.

Spies, R. B., P. H. Davis, and D. H. Steurmer. 1980. Ecology of a submarine petroleum seep off the California coast. p. 229-263. *In* R. A. Geyer (ed), Marine Environmental Pollution. 1. Hydrocarbons. Elsevier Press.

Stacey, B. M., and B. M. Marcotte. 1987. Chronic effect of No. 2 fuel oil on population dynamics of Harpacticoid copepods in experimental marine mesocosms. Marine Ecology Progress Series 40:61-68.

Stegeman, J. J. 1990. Detecting the biological effects of deep-sea waste disposal. Oceanus 33(2): 54-60.

Stegeman, J. J. 1985. Benzo[a]pyrene oxidation and microsomal enzyme activity in the mussel (Mytilus edulis) and other bivalve mollusc species from the western north Atlantic. Marine Biology 89:21-30.

Stegeman, J. J., R. M. Smolowitz, and M. E. Hahn. 1991. Immunohistochemical localization of environmentally induced cytochrome P450IA1 in multiple organs of the marine teleost *Stenotomus chrysops* (Scup). Toxicology and Applied Pharmocology 110:486-504.

Tanaka K., Y. Mugiya, and J. Yamada. 1981. Effects of photoperiod and feeding on daily growth patterns in otoliths of juvenile (*Tilapia nilotica*). Fishery Bulletin 79:459-466.

Thomas, R. E., and S. D. Rice. 1979. The effect of exposure temperature on oxygen consumption and opercular breathing rates of pink salmon fry exposed to toluene, napthalene, and water-soluble fractions of Cook Inlet crude oil and No. 2 fuel oil. p. 39-52. *In* W. B. Vernberg (ed), Marine pollution: functional responses. Academic Press, New York.

Ustach, J. F. 1977. Effects of sub-lethal oil levels on the reproduction of a copepod, *Nitocra affinis*. North Carolina University Sea Grant Publication No. UNC-SG-76-10.

Vandermeulen, J., and W. R. Penrose. 1978. Absence of aryl hydrocarbon hydroxylase (AHH) in three marine bivalves. Journal of Fisheries Research Board of Canada 35:643-647.

Vignier, V., J. H. Vandermeulen, and A. J. Fraser. 1992. Growth and food conversion by Atlantic salmon parr during 40 days' exposure to crude oil. Transactions of the American Fisheries Society 121:322-332.

Volk, E. C., R. C. Wissmar, C. A. Simenstad, and D. M. Eggers. 1984. Relationship between otolith microstructure and the growth of juvenile chum salmon (*Oncorhynchus keta*) under different prey rations. Canadian Journal of Fisheries and Aquatic Sciences 41:126-133.

Walters, C. J., R. Hilborn, R. M. Peterman, and M. J. Staley. 1978. Model for examining early ocean limitations of Pacific salmon. Canadian Journal of Fisheries and Aquatic Sciences 41:126-133.

Wang, S. Y., J. L. Lum, M. G. Carls, and S. D. Rice. 1993. The relationship between growth and total nucleic acids in juvenile pink salmon *Oncorhynchus gorbuscha* fed crude oil-contaminated food. Canadian Journal of Fisheries and Aquatic Sciences 50: 996-1001.

Webb, D. G. 1991. Effect of predation by juvenile Pacific salmon on marine harpacticoid copepods. II. Predator density manipulation experiments. Marine Ecology Progress Series 72:37-47.

Wertheimer, A. C., M. G. Carls, A. G. Celewycz, and M. V. Sturdevant. 1991. Impact of the oil spill on juvenile pink and chum salmon and their prey in nearsore habitats. 1991 NRDA Report F/S-4: National Marine Fisheries Component. 218 p.

Willette, T. M. 1991. Early marine salmon injury assessment in Prince William Sound. 1991 NRDA Report F/S-4: Alaska Department of Fish and Game Component. 47 p.

Wilson, K. H., and P. A. Larkin. 1982. Relationship between thickness of daily growth increments in sagittae and change in body weight of sockeye salmon (*Oncorhynchus nerka*) fry. Canadian Journal of Fisheries and Aquatic Sciences 39:1335-1339.

Wormald, A. P. 1976. Effects of a spill of marine diesel oil on the meiofauna of a sandy beach at Picnic Bay, Hong Kong. Environmental Pollution 11:117-130.

Wright, D. A., and F. D. Martin. 1985. The effect of starvation on RNA-DNA ratios and growth of larval striped bass (*Morone saxatalis*). Journal of Fish Biology 27:479-486.

Write, P. J., N. B. Metcalfe, and J. E. Thorpe. 1990. Otolith and somatic growth rates in Atlantic salmon parr, (*Salmo salar L.*): evidence against coupling. Journal of Fisheries Biology 36:241-249.

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