Effects of the Exxon Valdez Oil Spill on Black-legged Kittiwakes in Prince William Sound.

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Black-legged kittiwakes are the most abundant colonially nesting seabird in Prince William Sound. Approximately 40,000 kittiwakes nest at 27 colonies in the Sound. The number of breeding pairs and reproductive success were monitored at 24 of these colonies from 1984 to 1992. Of these 24 colonies, 10 colonies or the area adjacent to them were oiled by the Exxon Valdez oil spill and 14 colonies were not oiled. The unoiled colonies were used as a control to determine if the oil spill had negative effects on the birds at the oiled colonies.

The number of breeding pairs did not decline at colonies in the oiled area after the Exxon Valdez oil spill when compared to the pre-spill years. Reproductive success of kittiwakes (number chicks fledged/nests built) in 1989 at the oiled colonies was about one half of what was expected based on previous years and the reproductive success of birds at the unoiled colonies (P = 0.04). From zero percent to 37 percent of birds at oiled colonies were observed during June or July of 1989 with oil on their breast feathers and no birds at unoiled colonies had oiled breast feathers.

Reproductive success of kittiwakes at all colonies in Prince William Sound declined in the post-spill years (1990, 1991, and 1992), compared to the 5 previous years. The brood size of fledglings also decreased in the post-spill years which suggests that there was less food available during these years (Irons 1992).

Results from contaminant analysis demonstrated that in 1989 one of ten birds from oiled colonies had livers that were contaminated by petroleum hydrocarbons and a single egg collected in the oiled area had a contaminated shell. In 1990 none of the five birds collected in the oiled area were contaminated, but two of the five had contaminated stomach contents. If this contamination resulted from the oil spill, it suggests that oil may have persisted at least a year in the food chain. Only 25 percent of the birds collected for contaminant analysis have been analyzed.

References
Marine Bird Populations of Prince William Sound, Alaska, Before and After the Exxon Valdez Oil Spill
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We estimated the abundance of marine birds in Prince William Sound following the Exxon Valdez oil spill, examined population changes between pre-spill and post-spill surveys, and compared population trends in oiled zones of the Sound to trends in unoiled areas. Data from pre-oil spill boat-based surveys for birds in winter and summer 1972 and 1973 and a summer shoreline survey in 1984 were compared to data from this study collected in the winters and summers of 1989, 1990 and 1991 (Laing and Klosiewski, in prep.).

We counted approximately 100 bird species on surveys. Population estimates of 11 species or species groups declined between 1972/1973 and the years after the oil spill, including large declines for loons (Gavia spp.) (>36%), scoters (Melanitta spp.) (>54%), arctic terns (Sterna paradisaea) (>78%) and murrelets (Brachyramphus spp.) (>65%). None of the species had population estimates increase significantly. Using one-tailed t-tests, we detected a net population loss (p<0.05) in the oiled zone relative to population trends in the unoiled zone for pigeon guillemot (Cepphus columba) in March and northwestern crow (Corvus caurinus) in July, and marginally insignificant (p<0.10) losses for cormorants (Phalacrocorax spp.), harlequin duck (Histrionicus histrionicus) and black oystercatcher (Haematopus bachmani).

In shoreline habitats, using the 1984 survey as a baseline, we estimated net loss in the oiled zone relative to the unoiled zone; if the 95% confidence interval excluded zero, we concluded a loss occurred. Out of 18 species or groups examined in shoreline habitats, oiled zone losses were estimated to have occurred for loons, harlequin duck, scoters, black oystercatcher, Arctic tern, and mew gull (Larus canus). These effects were observed in 1990 and 1991, but not in 1989.

We conclude that oiled zone populations of nearshore species such as harlequin duck, black oystercatcher, pigeon guillemot and northwestern crow, as well as several offshore species, declined. Individual studies on harlequin duck, black oystercatcher and pigeon guillemot documented direct effects of oiling which may explain the population declines shown here (Andres et al., in prep.; Oakley and Kuletz, in prep.; Patton in prep.). In addition, overall population declines since 1973 of 11 species or species groups cause concern.

Statistically rigorous sampling design has rarely been used to estimate marine bird populations, and this study served to demonstrate its feasibility. However, the real value of using sampling to estimate populations is to illuminate long-term trends through repeated surveys. The lack of power of statistical tests in this study occurred because there were few baseline or post-spill surveys conducted, and because the baseline surveys occurred years before the oil spill. We hope that this study will provide scientists and policy makers with the impetus to survey populations at frequent intervals using rigorous sampling design.
References


Bioavailability of Residual PAHs From the Exxon Valdez Oil Spill

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Smith Island, located 25 miles southwest of the Bligh Reef grounding site, had one of the more heavily impacted shorelines from the Exxon Valdez spill. The coast of Smith Island is exposed and rocky, with boulder/cobble pocket beaches. Many of these beaches overlie beds of sand and gravel, and when oil came ashore it penetrated deeply into the substrate. Despite large-scale removal efforts through such means as high-pressure, hot-water washing, chemical agents, and excavation with heavy equipment, oil remains buried in portions of some beaches. At these locations, oil sheens have been observed to leach out from the substrate each year since the spill. The purpose of this study was to assess the extent to which residual polynuclear aromatic hydrocarbons (PAHs) were available for accumulation by intertidal organisms living at the site, and to evaluate the physical means by which organisms were exposed.

Since 1990, National Oceanic and Atmospheric Administration staff have sponsored a monitoring effort in Prince William Sound to evaluate the effects of both oiling and treatment at selected sites. An integral part of the program has been chemical hydrocarbon analysis of both sediments and tissues of intertidal invertebrates found at the sites. In 1990, mussels collected at the Smith Island site contained the highest concentration of PAHs of the 23 sites sampled for the National Oceanic and Atmospheric Administration Prince William Sound monitoring study, 84 parts per million (dry weight). Mussel tissue samples from nearly all other sites contained less than 10 parts per million. The comparatively high value encountered for Smith Island mussels was not reflected in results of hydrocarbon analyses of surface sediment samples from the site, although very high concentrations of both total petroleum hydrocarbons and PAHs have been consistently found in subsurface sediments over the period between 1989 to 1992 (Michel and Hayes, 1991; Michel and Hayes, in preparation).

These conditions at the Smith Island site resulted in a more detailed study being implemented at that location for the 1991 field season. In addition to standard monitoring analyses of native mussels and composite sediment samples, as had been done in 1990, mussels from a nearby site in Eshamy Bay not impacted by the oil spill were collected and subsequently transplanted to two portions of the Smith Island study area. These were collected two months later and analyzed. The results suggested a rapid and substantial bioaccumulation of PAHs, with mussels increasing their body burden from 0.7 parts per million in the transplant stock, to 5 and 20 parts per million after two months. In contrast, mussels at the Eshamy Bay donor site showed a slight decrease in total hydrocarbon loading over the same period of time, to 0.3 parts per million.

In 1992, study activities at Smith Island were further expanded and refined. Bioavailability and transport of residual oil were evaluated through analysis of
PAH concentrations and distributions in several matrices, including mussels, an artificial mussel surrogate, sediment, water, and oil sheen. "Clean" mussels were collected from an unimpacted location in Prince William Sound (Barnes Cove, Drier Bay) and transplanted to an oiled site on the north side of Smith Island, as well as to a site with similar physical substrate characteristics on the relatively unimpacted south side. Most of the mussels were transplanted to the beaches in small cages placed directly on the pebble substrate underlying the large rounded cobbles, with cobbles replaced after deployment. Additionally, anchored and buoyed cages were located just offshore of the intertidal study area in an attempt to evaluate differences between intertidal and subtidal conditions. Half of the deployed mussels were collected after 14 days in the field, and the remaining half after 52 days.

A new monitoring tool developed by the National Fisheries Contaminant Research Center of the U.S. Fish and Wildlife Service was also deployed with the mussels. These simple devices—semipermeable membrane devices (SPMDs)—are essentially polyethylene envelopes containing triolein lipid, and are designed to act as bioaccumulation surrogates through passive uptake of lipophilic contaminants such as PAHs (Huckins et al., 1990). SPMDs, also referred to as "lipid bags", were paired with groups of mussels to evaluate route of exposure to the mussels, as SPMDs are thought to selectively sample the dissolved fraction of hydrocarbons. In a broader perspective, it was hoped that the experiment would permit some insight as to whether these devices might realistically be considered for monitoring effects of oil spills.

As was the case with mussels, SPMDs were placed in the middle intertidal zones of the target beaches as well as offshore from the beaches on buoyed deployments. In addition, to account for possible atmospheric contribution of PAHs to the SPMDs in the intertidal zone, SPMDs were placed in the supratidal berm where they were exposed only to air. Half of the deployed SPMDs were collected after 14 days in the field, and the remaining half after 52 days.

Some problems were encountered with physical stability of the SPMD and mussel deployments over the 52-day term of the study. Dynamic conditions on the beaches resulted in movement of some of the cages in which the mussels and SPMDs were housed, and loss of some SPMDs. However, stations were essentially intact for the 14-day recovery, and good results were obtained for those samples.

Analysis of the 14-day SPMDs showed a statistically significant uptake in lipid bags deployed in the intertidal zone of the oiled north side of the island, relative to the unoiled south side. Absolute levels of hydrocarbons accumulated in the intertidal SPMDs were, however, low relative to values measured in mussels in 1990 and 1991: the maximum summed concentration for target PAHs was about 1.4 parts per million. Interestingly, the highest accumulation of PAHs (about double that of the highest intertidal concentration measured) occurred in the SPMDs deployed on the supratidal berm on the north side of Smith Island, while supratidal SPMDs on the south side were only slightly elevated above blanks. Because it is known that SPMDs are also efficient air samplers, this suggests that volatilization of PAHs continues to be a pathway for loss of hydrocar-
bons from the buried residual oil that remains on the north side of the island.

PAH analyses were also performed on nearshore water, beach sediment in the immediate vicinity of the mussel and SPMD deployments, and on sheen observed leaching from the substrate on falling tides. This information was used to characterize PAHs in the Smith Island environment and as a basis for comparison to PAH profiles found in the mussels and SPMDs.

Chemical results were used to evaluate the extent to which PAHs had accumulated in mussels and SPMDs, and to give information on uptake rates. Patterns of PAHs in sediments, water, and visible sheen provided a basis for proposing a model of biological exposure. We hope that by providing fundamental information on residual oil fate, bioavailability and mechanisms of exposure, this study may facilitate improved operational guidance on response and clean-up for future spills.

References

Impact of the Exxon Valdez Oil Spill on Intertidal Invertebrates Throughout the Oil Spill Region
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The Coastal Habitat Injury Assessment Study was initiated to document and quantify injury to biological resources in shallow subtidal, intertidal and supratidal (immediately above the high tide level) habitats impacted by the Exxon Valdez oil spill. The coast was divided into three major regions: Prince William Sound (PWS), Cook Inlet - Kenai Peninsula (CIK), and Kodiak Island - Alaska Peninsula (KAP). This report deals with some of the impacts documented by the intertidal invertebrate component. Results for intertidal algae are presented separately.

The coastline in the above three regions was surveyed by the Alaska Dept. of Environmental Conservation to determine the degree of oiling relative to a GIS map of shoreline habitat types. The sites were classified as heavily, moderately, lightly or non-oiled; habitat classifications included sheltered rocky, coarse textured, exposed rocky, fine textured and estuarine. Oiled sites were randomly selected from each habitat type. A list of potential control sites for each oiled site was randomly generated and, through a ground truthing (verification) process, a control site was selected from the list as a matched site for each oiled site. Control sites included lightly oiled and non-oiled locations. Experimental sites included heavily and moderately oiled locations. This presentation deals only with sheltered rocky and coarse textured habitats.

At each site samples were collected along six transects (straight lines) perpendicular to the water line and evenly spaced along the beaches. Samples were collected within each of the first three meters of vertical drop (MVD) below mean higher high water along each transect. Quadrats (sampling squares), 0.1 m², were randomly positioned within each meter drop and all organisms within the quadrat were removed. New transect lines were established three meters to the left of the preceding transects on subsequent sampling visits; sites were sampled three to four times between fall of 1989 and summer of 1991. In the laboratory, organisms were sorted, identified to the lowest possible taxonomic category, counted and weighed. Two-tailed t-tests or two-sample randomized tests were used to compare abundance and biomass between corresponding matched pairs of oiled and control sites for each taxonomic category at each MVD. Statistical comparisons of abundance and biomass were expanded to all sampled sites within each region, habitat type and MVD using Fisher's method (Sokal and Rohlf, 1982); statistical inferences were further extended to all possible locations within the universe of a given region, habitat type and MVD using Stouffer's method (Folks, 1984). Potential impact to the entire community at individual sites was examined using k-dominance curves, Shannon Wiener and Brillouin diversity.
indices, and species richness and dominance Simpson indices. Some of the more noticeable trends are reported here.

Sites showed varying degrees of impact, probably due to such variables as the amount of oil, duration of exposure and amount of cleaning or bioremediation. Several sheltered rocky and coarse textured sites showed abnormal k-dominance curves, suggestive of stressed communities or communities in transition. Communities with abnormal k-dominance curves also had a higher dominance index, lower diversity index and lower evenness values. Examination of individual taxa revealed the following trends.

The most consistent differences in abundance and biomass between oiled and control sites in sheltered rocky habitat were observed for mussels, barnacles and limpets. The mussel, *Mytilus edulis*, had significantly higher abundance and biomass (P < 0.05) on control sites at MVD 1 and 3 in PWS and CIK during 1990. Comparisons at individual sites indicate that higher biomass and abundance resulted from unusually strong recruitment of mussels on control sites. Differences in *Mytilus* biomass and abundance were absent from PWS and limited to MVD 3 in CIK by 1991, possibly due to higher mortality of recruits on control sites, as indicated by the presence of larger individuals on control and oiled sites in both regions in 1991.

The barnacle *Chthamalus dalli* had higher abundance and biomass on oiled sites in PWS and CIK, particularly at MVD 2 and 3 in 1991 (P < 0.05). Abundance and biomass data indicate unusually high settlement on oiled sites, possibly due to greater amounts of free space created by cleaning and oil-related mortality of space competitors. Abundance and biomass of the limpet *Tectura persona* was significantly higher on control sites at MVD 1 in PWS during both 1990 and 1991 (P < 0.05). Limpets can impact algae and barnacle recruitment by grazing down or bulldozing newly settled individuals.

Barnacle populations were much lower in coarse textured habitats; the dominant organisms included mussels, littorines (organisms living between high- and low-water marks) and oligochaetes(worms). The major trends are the following:

Mussel abundance and biomass showed similar trends in coarse textured habitats in PWS as occurred in sheltered rocky habitats. Significantly lower abundance and biomass on oiled sites at MVD 1 in 1990 (p < 0.001) apparently resulted from substantial mortality. Recruitment to MVD 1 on oiled sites in 1991 reduced the differences between oiled and control sites. Significantly higher mussel abundance on control sites at MVD 2 and 3 was apparently due to higher recruitment on control sites in both years. As in sheltered rocky habitats, elevated abundance on control sites was not accompanied by elevated biomass in 1991; these trends were apparently caused by higher growth rates on oiled sites and somewhat high loss rates of larger mussels on control sites. Mussels were present in low abundance on most coarse-textured matched pairs in CIK; statistical trends were moderate to weak or absent.

Littorine abundance was significantly higher on control sites at all MVD in PWS during 1990, but biomass was significantly higher on control sites only at MVD 1 (p < 0.01). Littorine populations increased by 3-5 times between 1990 and 1991 on three of the four oiled beaches, thus eliminating statistical differences at
MVD 1 and 3. Substantial increases in abundance of both *Littorina sitkana* and *L. scutulata* occurred, however, recruitment of *L. scutulata* was higher, particularly at MVD 3. Elevated abundance on control sites at MVD 2 in 1991 was due primarily to small individuals; biomass was significantly higher at MVD 2 on oiled sites (p < 0.01). Littorine populations were much lower in CIK and distinct trends were absent.

Oligochaete abundance was similar on control sites in both PWS and CIK, however, abundance was up to ten times higher on oiled sites in CIK than on control sites in 1990. There were substantial increases in abundance between 1990 and 1991 on oiled sites, thus suggesting continued impact as oligochaetes appear to benefit from oiling, particularly at MVD 3 in both regions.

Two of the most heavily stressed coarse-textured sites were Chugach Bay on the Kenai Peninsula and Snug Harbor in Prince William Sound. Both sites had an unusually high dominance by one or two taxa. The total abundance of all animals in Chugach Bay at MVD 2 and 3 was 3752 - 4450 individuals per quadrat, over 90% of which were oligochaetes in 1990. The population at MVD 2 was still dominated by oligochaetes in 1991; the population at MVD 3 was dominated by oligochaetes and platyhelminths (flattened worms). The population in Snug Harbor was dominated by amphipods (crustaceans) and oligochaetes during 1990; oligochaetes continued to dominate the population at MVD 1 in 1991, but more diverse populations, similar to control sites, had begun to develop at MVD 2 and MVD 3. Thus, some areas were stressed or still undergoing recovery by the last sampling period in 1991.

**References:**


Coastal Habitat Injury Assessment: Intertidal Algal Communities
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The Coastal Habitat Injury Assessment program, part of the Natural Resource Damage Assessment plan, investigated the effects of the Exxon Valdez oil spill on the biota of the subtidal, intertidal, and supratidal habitats from Prince William Sound to the Alaskan Peninsula. Here we report the results of a study on the effects of the Exxon Valdez oil spill on the intertidal algal communities.

A random stratified experimental design was used to compare data from matched pairs of oiled and control sites in several different habitats in the three main areas of the spill: Prince William Sound, the Cook Inlet and Kenai Peninsula area (CIK), and the area including Kodiak Island and the Alaskan Peninsula (KAP). Each area was stratified by beach type: sheltered rocky, coarse textured, exposed rocky, and estuarine. Oiled sites were selected randomly from information made available from other agencies (Sundberg et al., 1993). Control sites were selected to match each oiled site using physical criteria such as beach slope, aspect, beach texture, and wave exposure. The overall statistical design was to compare data sets within site pairs and then to compare across all sites of one habitat from an area.

Sites were sampled during the field seasons 1989 (late summer), 1990 (early summer, late summer) and 1991 (early summer, mid summer). Most sites had six randomly selected vertical transects with 3 quadrats (either 20 cm x 50 or 40 cm x 50 cm) located randomly within the first, second, and third meter drop (MVD) from the high water mark. Percent cover of algal taxa was determined in 1991 from a 40- or 80- point grid in undisturbed (control) quadrats, quadrats cleared of all overstory algae, and quadrats scraped bare. Algae from scraped quadrats at all sampling times were preserved in formalin then sorted and weighed in the laboratory.

Because in all areas the most common alga, especially in the oil-affected upper intertidal, was the rockweed Fucus gardneri Silva, we performed several measurements on collected Fucus specimens to better ascertain potential damage to the population. In the field fertile receptacles were collected from randomly selected Fucus plants to determine the viability of released eggs. Fucus plants collected in cleared quadrats were measured for a variety of attributes including plant length, number and maturity of receptacles, and occurrence of damaged fronds, epiphytes, and regeneration.

Each data set was analyzed by site pair comparisons. Percent data were arcsine-square root transformed and tested for differences by Student's t-test. Fucus attributes were tested using either t-tests or a randomization test developed by WEST, Inc., Cheyenne, WY, from the two-sample randomization test algorithms by Manly (1990). Plate data were tested with a Fisher's exact test (Sokal and Rohlf, 1981). Overall tests of significance were tested with both a Fisher's combined test (Sokal and Rohlf, 1981) and by using Stouffer's consensus test
(Rice, 1990). In the discussion below all pairs of values are reported with the control value first, followed by the value from the oiled sites, and all p-values were calculated from the Fisher's combined test.

An overview of the data shows that in most habitats in the 3 areas Fucus showed significant differences between the control and oiled sites. However, each meter drop, each habitat, and each area had different patterns.

For the Prince William Sound area Fucus percent cover was significantly less at oiled sites in all habitats in 1991, more than 2 years after the spill. This pattern was found in the upper MVD in exposed rocky sites, in the upper 2 MVD's in both sheltered rocky and estuarine sites and at all 3 MVD's in coarse textured beaches. For example, in sheltered rocky sites Fucus covered 28.4% of the area in controls, but only 11.3% in the oiled sites (p < 0.01) in late summer 1991.

In the CII area Fucus coverage generally showed more differences between oiled and control sites later in the summer in 1991. In sheltered rocky sites there was greater coverage of Fucus in controls (30.7% vs. 14.0%, p < 0.01) in the upper intertidal (1 MVD) but less coverage (16.4 vs. 37.2%, p < 0.01) 2 m lower (3 MVD). In coarse textured beaches Fucus coverage was not significantly different at any tide level in 1991. In estuarine sites there was greater coverage of Fucus in the upper 2 meters, but less coverage in the lower intertidal at control beaches.

The general pattern of more coverage by Fucus in control sites was repeated in the KAP area in the upper intertidal in sheltered rocky sites, and at all tide levels in coarse textured sites.

The lower coverage of Fucus in oiled sheltered rocky and coarse textured habitats was complemented by the lower biomass of Fucus in the oiled sites in 1991. Fucus biomass averaged 1.2 kg m⁻² in controls and only 0.36 kg m⁻² (p < 0.05) at oiled sites in the upper intertidal sheltered rocky habitats in Prince William Sound. In coarse textured sites the differences were significant in the lower intertidal (3 MVD) zone (0.62 vs. 0.28 kg m⁻², p < 0.05).

Data from oiled sites indicate that the Fucus plants present in those sites were not as reproductive as those in control sites and suffered from a higher level of epiphyte infestation. The average Fucus plant at oiled sites at the second MVD was longer (2.5 vs. 4.4 cm, p < 0.05) due to the absence of smaller plants at the oiled sites. In the upper intertidal in oiled sites the Fucus plants had fewer receptacles (860 vs. 36 m⁻², p < 0.01), fewer receptacles per mature plant (16.5 vs. 9.2, p < 0.05), and a lower reproductive index. However, egg viability was not significantly different between plants from control and oiled sites. Fucus from oiled areas had more adult plants with attached epiphytes (10% vs. 54%, p < 0.01) with a greater percentage of the area of each plant covered.

Because of the dominance of Fucus in the upper intertidal zones, the values for coverage of total algae and of perennials co-varies with the values for Fucus. The patterns for annuals and ephemerals varied with respect to the habitat and tide level. In Prince William Sound annuals and ephemerals were significantly greater in oiled sites with respect to both biomass and percent cover in rocky habitats in the upper intertidal in 1990 and early in 1991. By the end of the summer in 1991, there were no significant differences in these variables. In coarse tex-
tured sites there were initially no differences in annual and ephemeral coverage, except at the 3 MVD where coverage was higher in control sites. This pattern was retained through the end of the field season in 1991 in Prince William Sound. Estuaries in Prince William Sound had greater coverage of annuals and ephemerals in controls in early summer of 1991, but there were no differences by late summer.

The percent cover by ephemeral and annual algae in CIK in sheltered rocky and estuarine sites was similar to the trend in Prince William Sound but the differences between oiled and control sites persisted to the last visit in 1991. In coarse textured sites there were no differences in the cover of annuals and ephemerals in CIK between oiled and control sites, except for the last visit in 1991 at the 3 MVD where there was a slight trend for more annuals at oiled sites.

The KAP area had no significant differences between control and oiled sites with respect to coverage by annuals and ephemerals at any MVD at any habitat in 1991.

Other algae had varying responses to the oiling. Green algae that appeared to be adversely affected by the oiling and/or subsequent beach treatment (that is, those with percent cover values significantly lower in oiled sites) were bladed greens in all estuarine beaches, Cladophora in sheltered rocky sites in Prince William Sound, and Acrosiphonia in sheltered rocky sites in CIK. Coverage by filamentous browns was less in oiled coarse textured beaches in Prince William Sound and in oiled estuarine sites in Prince William Sound and CIK. Plants identified as Myelophycus/Scytosiphon had lower coverage in Prince William Sound sheltered rocky oiled sites. Red algae that had lower coverage in oiled sites were Halosaccion, Endocladia/Caulocanthus, Odonthalia, Palmaria, and Polysiphonia in sheltered rocky sites in CIK. Gloiopeltis in sheltered rocky sites in Prince William Sound, Cryptosiphonia in exposed rocky habitats in Prince William Sound, and Neorhodomena in both exposed rocky sites in Prince William Sound and sheltered rocky sites in CIK.

A few algal taxa had greater coverage in the oiled sites than the controls. Members of the Gigartinaceae family had enhanced coverage in exposed rocky sites in Prince William Sound and in sheltered rocky sites in CIK and KAP. Brown algae that followed a similar pattern were Myelophycus/Scytosiphon in exposed rocky sites in Prince William Sound and filamentous browns in coarse textured beaches in CIK. In the red algae, Gloiopeltis had more coverage in oiled sheltered rocky beaches in CIK as did Palmaria in oiled exposed rocky beaches in Prince William Sound. Cryptosiphonia and Odonthalia had higher percent cover in oiled sheltered rocky habitats in KAP.

The Cook Inlet/Kenai area had the highest number of significant differences between oiled and control site pairs in the algal percent cover data. Most of these differences occurred at the sheltered rocky beaches. The Kodiak/Alaska Peninsula area had few significant differences, but many of the taxa that did show differences exhibited higher coverage in oiled sites. All areas had significant differences in the amount of uncolonized substrate. There was significantly more bare rock at oiled sites in all habitats at most tide levels in the three areas.

The perennial alga Fucus was the species most obviously affected by the Exxon
Valdez oil spill, especially in the upper intertidal. As of the end of the summer in 1991 coverage by Fucus was still significantly less in most oiled beaches. Our data indicate that recovery will be limited by the few mature plants in the area since the dispersal of Fucus zygotes is restricted to an effective diameter of 1 to 2 m (McConnaughey, 1985). The presence of a protective canopy is probably also a factor in enhancing the survival of Fucus germlings (van Tamelen and Stekoll, 1993). The length of time for the upper intertidal populations of Fucus to recover to the level of the control sites is unknown at present, but will be a function of the rate of dispersal from nearby Fucus beds.

References
Sundberg, K. A., L. Deysher, and L. McDonald. 1993. Intertidal site selection utilizing a geographic information system. This symposium proceedings
van Tamelen, P. G. and M. S. Stekoll. 1993. Damage and recovery rates of Fucus in Herrng Bay, Knight Island. This symposium proceedings.
Damage and Recovery Rates of *Fucus* in Herring Bay, Knight Island
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The brown alga *Fucus gardneri* is the most abundant intertidal seaweed in Herring Bay, Knight Island, Prince William Sound, comprising up to 90% of the total algal biomass. The abundance of this plant and its simple and observable life cycle allowed detailed observations and experiments to be carried out regarding the consequences of the *Exxon Valdez* oil spill. Much of Herring Bay was heavily oiled, and a variety of clean-up technologies were performed throughout the bay. However, the southeast arm of the bay received little or no oil, allowing comparisons of oiled and unoiled areas within the same bay. Over three field seasons, 1990-92, we were able to quantify the damage done by the oil spill and associated clean-up to *Fucus* populations in Herring Bay. Using a variety of experimental techniques we also determined some of the factors influencing recovery of *Fucus* populations.

Five pairs of oiled and control sites were monitored, but for brevity and clarity we will report only the results from one representative pair of sites. The pair we will consider are two sheltered rocky sites. The oiled site was heavily oiled and probably received high pressure-hot water clean-up treatment. To assess damage to *Fucus* populations and assess recovery rates, we monitored 18 permanent plots (20x50 cm) at each oiled and control site. There were six randomly located plots in each of three tidal levels. Sampling consisted of estimating percent cover of all sessile organisms using a systematic point contact method. All *Fucus* plants were then measured, and, if they were reproductive, the number of receptacles and stage of development were recorded.

In the upper intertidal, *Fucus* covered about 50% of the area at the control site, while at the oiled site *Fucus* cover was initially about 10%, increasing to about 20% in 1992. None of these differences were statistically significant due to the high variability in the data. In the mid intertidal, initially 80% of the area was occupied by *Fucus* at the control site, compared to 20% at the oiled site. By 1992 these significant differences had converged and were no longer statistically different. The percent cover of ephemeral algae was greater at the oiled site compared to the control. In the mid intertidal, about 20% of the area was initially covered by ephemeral algae, declining to almost none in 1991. Ephemeral algae were always scarcer at the control site in the mid intertidal. In the low intertidal, ephemeral algae were initially more than twice as abundant at the oiled site compared to the control site, but this difference disappeared in 1991.

The differences in the percent cover of *Fucus* can be attributed to lower densities of large plants (>10 cm) and reproductive plants at the oiled site. In 1990 and 1991 large plants were almost nonexistent at the oiled site in the upper and mid intertidal. At the control site there were about 5 large plants per quadrat in the upper intertidal and 5-20 in the mid intertidal. In 1992, there were no significant differences between the oiled and
controls sites in the number of large plants, but the upper intertidal recovery is not apparent. There were also few reproductive plants at the oiled site in the upper and mid intertidal. At the control site there were about 5 reproductive plants per quadrat at the same two tidal levels.

At the oiled site in the mid intertidal there were more germlings in late 1990 and early 1991. In 1991, there were more small plants and in 1992 there were more medium plants at the oiled site. This year class of plants probably settled the summer after the oil spill in 1989 and have grown into new size classes over the years, increasing the number of plants in subsequently larger size classes. It seems that recovery of Fucus in the mid intertidal is proceeding.

In order to assess the ability of Fucus to recolonize damaged sites the settlement rate of Fucus eggs was estimated. The relative number of Fucus eggs landing on oiled and control beaches was assessed by placing four Plexiglass plates designed to trap Fucus eggs at each of three tidal levels. By scoring the plates with a utility knife, grooves just wider than the diameter of a Fucus egg were made, Fucus eggs settle on the plates, concentrating in the grooves. These plates were left in the field for 24 hours after which the number of eggs on each plate was counted under a dissecting microscope. The 24-hour cycle was repeated for three consecutive days. These observations were performed three times in the summer of 1992. The distance to the nearest fertile Fucus plant was measured during all three sampling periods

There were almost no eggs collected by the egg catcher plates at the oiled sites throughout the summer. At the control sites egg counts averaged up to 800 eggs per plate per day. This difference in settlement rate can be explained in part by the low density of reproductive plants at oiled sites. The distance to the nearest fertile Fucus plant, which is inversely related to density, was about four times as great in oiled areas.

To assess Fucus recruitment and germling survival, unglazed ceramic plates were made and deployed in the field. These plates were made with grooves of three widths and two depths. Half of the plates were seeded in the lab with Fucus eggs, and the germlings were grown for a month before deployment. Four plates were placed at each of two tidal levels at three oiled and three control sites. After 2 months the number of germlings in each groove was recorded. Areas not in grooves on the plates were also monitored.

Seeded germlings only survived in the grooves of the ceramic tiles, and recruitment only occurred in the grooves. The widest, shallow grooves provided little protection for germlings from detrimental conditions. The narrowest grooves were slightly smaller than Fucus eggs and Fucus did not recruit well to these grooves. Medium-width grooves and deep, wide grooves provided good habitats for young Fucus plants.

Experiments performed in 1991 using petri dishes instead of ceramic plates showed that germlings usually did not survive more than 1 month on flat surfaces. To determine possible mechanisms of this high mortality, the survival, estimated percent cover, of these germlings was compared to the desiccation rate at the site of the petri dishes. Also, four of these dishes were experimentally exposed to whiplash from adult plants. Desiccation rates were greater at oiled sites, and desiccation was negatively cor-
related with percent cover of seeded germlings in petri dishes. Where desiccation was greater, Fucus germlings were less abundant due to higher mortality. Adult Fucus canopy can lower desiccation stress, potentially enhancing germling survival. However, when germlings on plates were placed under Fucus canopy without herbivores and in constant contact with the ocean, very few germlings survived after 2 weeks. Whiplash from the adult plants removed the germlings from the substrate. By providing a refuge from desiccation and whiplash from adult plants, cracks and crevices in the substrate seem to greatly enhance germling survival.

The growth rates of established Fucus plants were determined by marking randomly chosen individual plants in each of three size categories at three tidal levels. The size categories were 2.0-4.5 cm, 5.0-9.5 cm, and >10 cm. These plants were measured to the nearest 0.5 cm in spring 1991, fall 1991, and summer 1992. New plants were tagged when mortality or loss of tags occurred.

In the upper intertidal in oiled areas Fucus plants in all size classes grew about twice as fast as plants in control sites over a period of 1 year. In the mid intertidal only large plants grew faster in the oiled areas. This indicates that once Fucus plants become established in oiled areas recovery may proceed rapidly.

Results from our experiments and observations showed that large Fucus plants in the upper and mid intertidal showed lower densities in oiled sites compared to control sites. Fewer large plants created less cover of Fucus and more open space for ephemeral algae to colonize. Ephemeral algae showed greater abundances up to 2 years after the spill at oiled sites. Since reproductive Fucus plants are usually at least 10 cm in length, fewer large plants meant lower densities of reproductive Fucus at oiled sites. Since Fucus eggs have limited dispersal, the lack of reproductive plants has led to observed lower Fucus egg settlement at oiled sites. Heterogeneity in settlement substrata was required at both oiled and control sites for recruitment of settled eggs into the Fucus populations. At oiled sites, cracks and crevices reduce the effects of heat and desiccation, while at control sites, whiplash from adult plants was ameliorated by cracks. Thus, surface heterogeneity is almost essential for Fucus to recruit into damaged and unoiled areas. New recruits in upper zones of oiled areas were confined to relatively deep cracks in the rock surface, and casual observations revealed that almost all Fucus plants are found to be attached in cracks.

Due to low settlement rates and severe environmental conditions recruitment of Fucus into areas severely damaged by the oil spill and associated clean-up efforts, particularly the upper intertidal, has been minimal. In areas with less harsh conditions, mid and low intertidal zones, Fucus has recruited abundantly. Once recruited to a damaged area Fucus plants grow faster due to reduced intraspecific competition since few plants remain at oiled sites, especially if those sites were heavily cleaned. Thus, where recruitment has occurred, recovery of Fucus populations is proceeding rapidly. However, where Fucus recruitment is low, recovery has been slow.
Meiofaunal Recolonization Experiment with Oiled Sediments; The Harpacticoid Copepod Assemblage

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To gain insight into the effects of the Exxon Valdez oil spill on the meioobenthos (small organisms living on the ocean floor) of Prince William Sound, Prudhoe Bay crude oil was used in a colonization study initiated in 1990. Sediment, collected near Juneau, Alaska, was repeatedly frozen and thawed, washed with freshwater and sieved through 2 and 0.417 mm screens to kill and remove meiofauna. Prudhoe Bay crude oil was added and mixed into this azoic sediment to reach concentrations of 0.5 and 1.7% crude oil. The resulting mixture was added to replicated colonization trays (13 x 28 x 33 cm). Non-oiled azoic sediments were added to additional trays. Triplicate trays of all treatments were placed flush with the sediment surface on beach transects in a randomized block design near mean low water (-0.6 m) in Herring Bay (a cove that was heavily oiled from the Exxon Valdez spill), Prince William Sound. Trays were sampled by coring on days 0, 1, 2 and 29. Cores were also collected from nearby undisturbed ambient sediments on each collection date, including Day 0, to quantify the colonizing source pool. Here, we report on an analysis of the harpacticoid copepod species from these collections.

Collections of meiofauna taken from azoic sediment prior to placement in the field (Day 0) suggested that although meiofauna were killed, decomposition was incomplete. Copepods, with chitinuous cuticles, were especially resistant. Residual copepods in our colonization samples were identified by observation of 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. All copepods were recorded as "dead" or "alive" at the time of collection based on this observation. Generally, the number of "dead" copepods was roughly equal in all experimental treatments (high, low and control) on all collection dates. An ANOVA conducted on data from Day 29 did not identify an oiled treatment effect suggesting that dead copepods were residuals in all treatments including the controls, and that the density of "dead" copepods was not related to oil-induced mortality. Dead copepods were rare in ambient sediments.

Harpacticoids were diverse with > 40 species encountered. The density of copepods increased in ambient sediments throughout the collection period. Highest densities of copepods in colonization trays were also observed in Day 29 collections. Species analysis indicated that sediments and colonization trays were occupied primarily by copepods assoc-
ated with surrounding eel-grass and algal-mat habitats. Most species displayed strongly prehensile first legs, belonging to families associated with a phytal lifestyle. Day 1 and 2 collections demonstrated that colonization was generally rapid (mean densities in trays were similar to those from ambient sediment collections). No single 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.

A two-way ANOVA was conducted as a randomized block (tray replication was blocked) design. ANOVA treatments were collection day (1, 2 or 29) and oil treatment (control, 0% oil, low, 0.5% oil or high, 1.7% oil). Tests were performed on the total living copepods, unidentified copepodites, and the five most abundant species, two of which were in the Family Diosaccidae, and one each in the Families Canthocamptidae, Laophontidae and Ectinosomatidae. Collection day effects were significant in all taxa tested reflecting the increase in abundances from Days 1-29 (density in ambient collections increased for the same time). A treatment effect was observed in total copepods, and species designated Canthocamptus sp. and Ectinosomatidae sp. 1. For total copepods and Canthocamptus sp., Tukey’s Studentized Range Test indicated that densities in control and low-oiled sediments did not differ, but that densities in high-oiled sediments were significantly lower than other treatments. For Ectinosomatidae sp. 1, means were significantly different in all experimental treatments, but densities in control sediments were significantly higher than in low-oiled treatments and, in turn, densities in low-oiled were significantly higher than in high-oiled treatments. Our data suggest that differences in colonization rate (either immigration or emigration), rather than oil-induced mortality, were the cause of this difference.

Detrended correspondence analysis was conducted to determine if an oiling effect influenced the copepod assemblage. Results indicate that two axes comprised >70% of the variance. Axis one contained relatively high levels of variation, with values approaching two standard deviations overall (four indicates complete faunal turnover). All natural samples separated from experiments on axis one and clustered near to each other indicating that experimental trays could be distinguished from the surrounding sediments in species composition on all collections. Other collections were separated on axes 2 with only 1 standard deviation of faunal turnover. Day 1 and 2 low- and high-oiled treatments clustered together as did control, low- and high-oiled sediments from Day 29. Control Day 1 collections were intermediate. Results therefore indicate that an oiling effect was present on Days 1 and 2, but no difference between control and oiled sediments was apparent by Day 29. Species diversity and evenness were similar in all treatments, suggesting that were no oil-related effect on the number of species colonizing the experimental trays.

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 hydrocarbons 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.
Intertidal: Meiofaunal Recolonization

Our data suggest that copepods have the ability to recolonize azoic sediments over small spatial scales (many cm²) quickly following the addition of hydrocarbons (see also Alongi et al., 1983; Decker and Fleeger, 1984). Meiofauna are known to be active colonizers through the water column, especially in muddy sediments (Chandler and Fleeger, 1983; Palmer, 1988), and many individuals colonized our experimental trays after one day, even in highly-oil sediments.

However, hydrocarbons, especially at high concentrations, altered colonizing ability. The abundance of two individual species and total copepods was significantly depressed at high-oil dosages. The effect was short-lived however, as a influence of high-oil dosages on community structure was apparent on Days 1 and 2 of the experiment, but not identifiable after 29 days.

We could identify no strong evidence for oil-induced mortality in our colonization trays; the number of “dead” copepods was not different in oiled-com pared to non-oiled trays on any collection. Copepods may have avoided the oil-enriched trays or they may have emigrated from the trays at faster rates. Recent data suggest that copepods that have entered the water column have the ability to select specific locations to colonize (Fegley, 1988; Sun and Fleeger unpublished data), 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.

In summary, abundance data collected after an oil spill cannot alone determine if migration or birth and death processes are responsible for changes in density. Our data suggest that meiofauna are rapid colonizers, but at high doses of oil, colonization rates are significantly reduced. Better information on the source pool of immigrating copepods is needed to help interpret density changes in a given locale after a spill of the magnitude of the Exxon Valdez.

References
Influence of the Exxon Valdez Oil Spill on Intertidal Algae: Tests of the Effect of Residual Oil on Algal Colonization

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Following the Exxon Valdez oil spill in 1989, we began to study the effects of stranded oil on algal colonization in rocky intertidal communities in Prince William Sound. This knowledge is critical because (1) algae are extremely important structurally and functionally to intertidal communities; (2) there appears to be a predictable succession of organisms following a spill, and enhancement of the early phases could reduce the time to recovery; (3) there is evidence that oil inhibits algal growth (Stranghan, 1971); and (4) there is evidence that cleanup measures on rocky shores might even delay recovery, if their biotic effects are as harmful as those of the oil (Thomas, 1978; Foster et al., 1990). As part of recovery from a spill, succession on oiled substrates can differ from natural succession, due to residual toxicity of the oil, large scale mortality that reduces available propagules, and loss of herbivores (Southward and Southward, 1978). We were particularly interested in whether normal mechanisms of colonization were affected by the stranded Exxon Valdez oil, so we examined the initial stages of a succession, the settlement, growth, and early survivorship of algae.

Rocks or tiles were used as substrates for colonization. In 1989, oiled rocks of a similar size were collected from beaches on Knight and Eleanor Islands. In 1990, oiled rocks were collected from the western arm of Herring Bay. In addition, clean rocks were collected from the supratidal zone in southeast Herring Bay, coated with fresh Prudhoe Bay oil, and allowed to weather for a month until the oil was a tarred consistency. Ceramic tiles were similarly prepared with Prudhoe Bay crude oil.

To account for inherent patchiness in algal colonization, we paired oiled and unoiled substrates in each experiment. In addition, the Herring Bay experiments were set up on carefully matched oiled and unoiled beaches. The oiled rocks had one half of the upper surface area cleaned with methylene chloride and oiled tiles were paired with unoiled tiles. All substrates were placed in the rocky intertidal. Prepared rocks from Knight and Eleanor Islands were placed on Gull Island, an unoiled site southeast of the grounding site, and retrieved in 1990. The tiles and the Herring Bay rocks were placed on 6 beaches (3 oiled, 3 unoiled) in Herring Bay and retrieved in 1991. Also in 1991, new oiled and unoiled tiles (unglazed) were placed, ridged side up, on the 6 Herring Bay beaches and a new treatment was added. Half the tile pairs were in cages designed to exclude grazers and half were unprotected. Settlement on these tiles was measured 1992.

In most cases, the oiled sides of rocks and oiled tiles had been colonized by significantly fewer algae than the clean surfaces. On average, algal coverage on oiled sides was less than one third the
coverage on the clean sides. In 1990, for example, the half-cleaned rocks on Gull Island had approximately 70% less algal cover on the oiled sides than the cleaned sides (paired t-test; p=0.001). In Herring Bay in 1991, many of the rocks had been lost, so only the tile data are presented here. For the six beaches, paired t-tests revealed consistently lower algal cover and fewer Fucus germlings on oiled tiles than on unoiled tiles, but at p levels that were generally 0.1 to 0.2. These results prompted the caging experiment in 1991. The 1992 results for the caged tiles also showed a reduction in algal cover, with significant differences (paired t-test; p<0.05) detected at several beaches. For the other beaches, p ranged from 0.06 to 0.17 for Fucus germlings. Testing the combined probabilities from the separate tests (Sokal and Rohlf, 1981) revealed that oil significantly reduced the settlement of Fucus germlings (p<0.001; c² test with 12 d.f.). The effect of oil on settlement on the exposed tiles was not as consistent.

Excluding grazers enhanced the ability to detect statistically significant effects of the oil in Herring Bay. Grazers were not controlled on Gull Island or the 1990 studies in Herring Bay. Grazers feeding preferentially on the oiled sides of rocks and tiles could have accounted for our results. However, we hypothesize that given the susceptibility of grazers to stranded oil (Peltier et al., 1976; Crapp, 1971), the grazers would probably feed preferentially on the unoiled substrates, which would mean we may have underestimated the effect of the oil. Indeed, in our 1991 study, we saw significantly more limpets and littorinids on unoiled tiles than oiled tiles (p<0.001; combined probabilities from separate paired t-tests, c² test with 48 d.f.; Sokal and Rohlf, 1981).

The effect of oil on algal colonization was very pronounced and consistent across a variety of locations in Prince William Sound and from year to year as well as across methods. The two-thirds reduction in percent cover indicates how sensitive this stage of the natural recovery process was to even residual levels of oil. The paired design helped reduce variability due to differences in surface texture of rocks and tiles, and heterogeneity of microenvironments on Gull Island and in Herring Bay. The reduced colonization on oiled substrates in our experiments is similar to that previously described (Nelson, 1981; Notini, 1978; Southward and Southward, 1978), suggesting that inhibition of initial recovery reported from other spills was operating at our sites in Prince William Sound.

References


Variability of Exxon Valdez Hydrocarbon Concentrations in Mussel Bed Sediments

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Concern for mussel beds contaminated by the Exxon Valdez oil spill increased rapidly in 1991 when poor recovery in several predator species was thought to be linked to oiled mussels. A pilot survey confirmed the persistence of Exxon Valdez oil at relatively high concentrations in mussels and underlying sediments in several beds (Babcock et al., these proceedings). The uneven distribution of oil within beds observed during the survey prompted us to sample several beds intensively in 1992 to determine the within-bed variation in sediment hydrocarbon concentrations. In this paper we document concentrations and distribution of hydrocarbons within one bed and examine the effects of several variables.

The study site, on the northern tip of Chenega Island, is fairly typical of highly oiled beds. It is small (approximately 50 m²) and on a low angle beach (4.4% slope) protected from intense wave action by bedrock headlands. Tidal range occupied by the bed is approximately 1.43 m to 1.77 m above mean lower low water. Mussel densities average 1900 animals per m² on sediments ranging from small pea gravel to fine silt.

At each of the 15 subsites within this bed, mussel density was determined and sediments entrained in the mussel byssal mat (threadlike mass holding mussels together), and sediments under this mat to a depth of 2 cm were sampled for hydrocarbons. All sediment samples were extracted and analyzed by UV spectrophotometry at the Auke Bay Laboratory. This method, adapted from Krahm (1991), approximates total oil concentrations based on the concentrations of two and three ring aromatic compounds that fluoresce at the phenanthrene wavelengths (260/380 nm). Although analytical results are not strictly quantitative and can not be compared with results produced by GC-MS analysis, they are extremely useful in comparing large numbers of samples.

Oil concentrations in sediments underlying the mussel bed were highly variable, ranging from 20 µg/g wet weight to 40,498 µg/g with a mean of 13,662 µg/g (S.D. = 13,326 µg/g). The effects of position on the beach and depth were tested with a three way ANOVA (beach position has two components: y axis represents tidal height, x axis is at right angles to y and represents distance from the bed's central axis). Oil concentrations were significantly greater (P<.05) at the two lower tidal heights, 1.50 m and 1.59 m; mean oil concentrations were 25,778 µg/g and 26,403 µg/g respectively. At the upper tidal height, 1.73 m, mean oil concentration was 1515 µg/g. A two way split plot analysis, treating the two sediment depths sampled at a subsite as two treatments applied to one plot, determined that at each subsite oil concentrations in byssal mat sediments (mean=9383 µg/g) were significantly lower than in sediments collected below
Mussel densities ranged from 15 to 244 animals per sample quadrat (625 cm²) with a mean of 120 mussels per quadrat (1900 per m²) (S.D. = 72). The ANOVA found no significant relationship between mussel density and oil concentrations in either byssal or underlying sediments. This finding is not at odds with the idea that mussels insulate underlying sediment hydrocarbons from tidal flushing. The stability provided by even a sparse mussel cover may be sufficient to allow high levels of hydrocarbons to persist in sediments.

Sediments appear to be a complex reservoir of oil that continues to contaminate mussels. The UV analyses of mussel bed sediments have confirmed visual observations that oil in the study bed is unevenly distributed and that concentrations are higher at lower tidal heights and below the byssal mat. Given that the physical distances are minimal between the lower and upper tidal heights and between sediment depths, the apparent effects of both depth and tidal height on oil concentrations may be related to a third factor, sediment grain size. Further analysis will examine these relationships. The variability of mussel hydrocarbon concentrations within a bed and the relationship to contamination in directly underlying sediments will be clarified when GC-MS mussel analyses become available.

Given the current level of contamination three years after the Exxon Valdez spill, the decline in hydrocarbon concentrations in mussel beds to pre-spill levels will be a prolonged process. In monitoring that recovery, sampling must account for high within-bed variability.

References
Babeck, M., G. Irvine, S. Rice, P. Rounds, J. Cusick, and C. Brodersen. Oiled mussel beds two and three years after the Exxon Valdez oil spill. This symposium.
Oiled Mussel Beds Two and Three Years after the Exxon Valdez Oil Spill
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In 1991, two years after the Exxon Valdez oil spill, scientists observed crude oil associated with some mussel beds that still smelled of fresh aromatic hydrocarbons. Coincidentally, biologists observed continued reproductive failure among harlequin ducks and oystercatchers in the spill area, and possible reduced survival among young sea otters and river otters. All of these higher order consumers are dependent on mussels (Mytilus trossulus) for a large portion of their diets.

We conducted surveys to determine the geographic distribution oiled mussel beds and the concentrations of oil in the beds inside Prince William Sound and along the northwestern shoreline of the Gulf of Alaska. Thirteen mussel beds with evidence of oil present were located in Prince William Sound in 1991, and samples of mussels and underlying sediments were taken from each for analysis of aromatic hydrocarbon content.

In 1992, we resampled most of the 1991 sites and located and sampled 46 additional oiled mussel beds in Prince William Sound. At some sites we sampled more than one mussel bed. Cooperating in the survey and sampling were the Alaska Department of Fish & Game, Alaska Department of Environmental Conservation and the U.S. Fish & Wildlife Service.

Sampled oiled mussel beds within Prince William Sound encompassed primarily the Knight Island group but were actually bound by Green Island on the eastern side, Naked Island on the north, Applegate Island on the northwest, and the Fox Farm site on Elrington Island on the south.

Five control sites were also sampled (Barnes Cove on Knight Island, Olsen Bay, Crab Bay on Evans Island, and West Bay on Bligh Island); extensive histories of petroleum hydrocarbon concentrations in mussels and sediments exist for all these control sites.

Forty mussel beds were evaluated on the Kenai Peninsula, Kodiak Island, and in Katmai National Park and Preserve; oil was observed and mussels and sediments sampled at 13 of these sites. Sampled sites ranged from Tonsina Bay on the Kenai Peninsula, to Cape Nukshak in the Katmai National Park and Preserve.

Criteria for sampling mussels and underlying sediments were the appearance and smell of crude oil in the sediments immediately underlying a moderate to dense mussel bed. Triplicate sediment samples (each consisting of a composite of 8-10 subsamples) were taken along a 15-50 m transect line laid through the densest part of the mussel bed, generally 0-2 cm immediately underneath the mussels. Triplicate, pooled mussel samples (20 mussels each) were collected from the same areas as the sediment samples.

The 1991 mussel and sediment samples were analyzed using gas chromatography/mass spectroscopy (GC/MS) and units are reported as μg/g total.
aromatic hydrocarbons. Sediment samples collected in 1992 were analyzed using a UV fluorescence screening procedure adapted from Krahn et al. (1991). Excitation/emission spectra of the extracts were read at the phenanthrene wavelength (260/380 nm), and values reported are µg/g wet weight total oil equivalents (OE). This procedure does not measure individual analytes within a sample, but does approximate total oil concentration and allows comparison of relative oil concentrations between samples. The UV screening permits the rapid analyses of more samples than by the costly GC/MS procedure.

In 1991 samples, the highest total aromatic hydrocarbon concentrations in mussels were collected from Foul Bay on the Western mainland (mean = 10.31±2.9 standard error µg/g dry weight), Bay of Isles on Knight Island (mean = 6.0±1.1) and Northeastern Latouche Island (mean = 3.8±1.3). Highest concentrations of total aromatic hydrocarbons were shown in underlying sediments collected from the north end of Chenega Island (mean = 427±29.4 µg/g wet weight), a tombolo on Eleanor Island (mean = 36.1±24.3) and Bay of Isles (mean = 28.7±9.4).

In sediments samples collected in 1992, 19 mussel beds in Prince William Sound had concentrations in excess of 10,000 µg/g wet weight oil equivalents. The UV analyses of sediments collected in 1992 indicated the highest petroleum hydrocarbon concentrations from an islet in Foul Bay (mean = 62,258±1,558 µg/g), a small tombolo on an islet in Herring Bay (mean = 39,394±8,655), and the eastern shore of Applegate Island (mean = 30,394±1,081). Mean concentrations of sediments from 3 control sites were less than <3 µg/g OE.

Sediments from mussel beds in the Gulf of Alaska show highest levels from Port Dick (mean = 9,122±2,312 OE), Tonsina Bay (8,250±2,793), and Windy Bay (4,645±1,169)—all along the Kenai Peninsula.

No mussel samples collected in 1992 have been analyzed.

The limited data available to compare levels between 1991 and 1992 suggest that petroleum hydrocarbon concentrations in sediments beneath the layer created by moderately to densely packed mussels are relatively unchanged, indicating that natural processes are only slowly cleansing these beds. This means that the mussels will probably experience chronic oil exposure for years.

We have documented 31 mussel beds within Prince William Sound and 9 along the Kenai Peninsula and Alaska Peninsula showing sediment petroleum hydrocarbon levels in excess of 1700 µg/g wet weight oil equivalents. The potential continued contamination of overlying mussels which form an important food source for higher consumers needs to be closely monitored for natural recovery or for possible use of manipulative restoration measures.

References
Determination of Petroleum-Derived Hydrocarbons in Seawater Following the Exxon Valdez Oil Spill II: Analysis of Caged Mussels

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We deployed bay mussels (*Mytilus trossulus*) that were initially free of hydrocarbons in nearshore waters along the path of oil spilled by the *T/V Exxon Valdez* to determine the persistence and the biological availability of petroleum-derived hydrocarbons to living marine resources. Mussels filter substantial volumes of seawater, and may therefore accumulate petroleum hydrocarbons integrated over the transplant period.

Petroleum hydrocarbon-free mussels were collected from Admiralty Island in southeastern Alaska, and were transplanted to 12 locations inside Prince William Sound and to 18 locations outside the Sound for 2 to 6 weeks at depths of 1, 5, and 25 meters at each location. In Prince William Sound, four successive transplants were conducted in both 1989 following the spill and in 1990; two transplants were conducted during 1991.

Three successive transplants to sites along the Kenai Peninsula, Alaska Peninsula, and Kodiak Island occurred in 1989 and 1990. Mussels were retrieved at the end of each transplant period and stored frozen at -20°C for petroleum hydrocarbon analysis.

Transplanted and control mussels were analyzed using single ion mode gas chromatography-mass spectrometry (GCMS/SIM) for the most abundant 2- to 5-ring polynuclear aromatic hydrocarbons (PAH's) in the spilled oil, and using gas chromatography-flame ionization detection for alkane hydrocarbons including pristane, phytane, and the normal alkanes of 10 to 30 carbon atoms.

Results indicate that mussels transplanted along the trajectory of the oil spill accumulated particulate oil at concentrations that decreased with depth, elapsed time after the spill, and distance from heavily oiled beaches. The highest concentration of total PAH's in the transplanted mussels was 5.70 ± 0.358 standard error µg/g wet tissue weight at Herring Bay, 1 meter depth, 1 to 2 months following the spill; at the 5 and 25 meter depths, concentrations were 3.17 µg/g and 0.372 ± 0.139 µg/g, respectively. Concentrations of PAH nearly as high at the respective depths were also found at north Smith Island and at Snug Harbor.

The mussel transplant sites at each of these three locations were within 500 m of beaches that had been heavily oiled by the spill. The relative concentrations of PAH and of alkane analytes detected are generally consistent with those of Exxon Valdez oil (EVO), indicating up to 281 µg EVO/g wet tissue weight.

Lower but detectable PAH concentrations were observed at most other transplant locations within PWS, with relative concentrations of PAH and of alkane analytes that are generally consistent with those of EVO. However, the lowest PAH concentrations were found at the control site, Olsen Bay, where total PAH concentrations generally ranged from .010 to .050 µg/g, with relative concentrations that are not consistent with those of EVO.

Concentrations of PAH's consistent
with EVO inside Prince William Sound declined substantially at all locations by late summer 1989. Total PAH concentrations of up to 1.47 μg/g were observed at Herring Bay, and were at least an order of magnitude lower at north Smith Island or at Snug Harbor. Still lower concentrations of total PAH’s consistent with EVO were detected at most of the remaining locations inside Prince William Sound, although not at Olsen Bay.

Low concentrations of PAH’s were sporadically detected at locations where the transplant sites were adjacent to heavily oiled beaches in 1990 and in 1991. Total PAH concentrations of about 0.26 μg/g were detected at 1 m depth at Herring Bay and at Snug Harbor in 1990, while the highest PAH concentrations detected in 1991 were near detection limits.

Petroleum hydrocarbons were detected only sporadically in mussels deployed at locations outside Prince William Sound in 1989, and were generally below detection limits in mussels deployed during 1990 and 1991. This may have been due in part to poorer survival of the mussels transplanted to locations outside Prince William Sound, resulting from longer transport times.

The accumulation of petroleum hydrocarbons by the transplanted mussels in 1989 indicates that particulate petroleum hydrocarbons were generally available to subsurface marine fauna the summer following the spill. These results are consistent with, and support, results of a companion study where direct chemical analyses of subsurface seawater for petroleum hydrocarbon were performed on samples collected 1 to 6 weeks following the spill: both these studies found the highest concentrations of PAH’s attributable to EVO at the 1 m depths of sites adjacent to heavily oiled beaches.

However, comparison of the results of these two studies indicates that the caged mussels may accumulate petroleum hydrocarbons from much lower seawater concentrations than may be detected by direct chemical analysis.
Estimation of the Exposure Concentration of the Seawater Soluble Fraction of Crude Oil from Mussel Tissue Concentrations

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Mussels have been used for many years to monitor petroleum hydrocarbon pollution in the marine environment (Farrington, 1980). Mussels appear to be ideal living monitors as these filter-feeders process large amounts of water, rapidly accumulate oil to high concentrations without apparent toxicity and rapidly purify the accumulated body burden without appreciable metabolism of the oil hydrocarbons when placed in clean seawater. For mussels to be useful in monitoring environmental contamination, the tissue concentrations must accurately reflect the magnitude of contaminants in the water. Despite a large number of studies devoted to quantitating oil hydrocarbon concentrations in mussels as a measure of water pollution, very few laboratory or field studies have attempted to correlate oil concentration in water with tissue concentrations.

Recently, however, a careful study has documented a relationship between water and tissue concentrations (Mason, 1988, 1 & 2). The extensive laboratory data from this study using black mussels and the water soluble fraction of Quitar crude oil were carefully analyzed using a well studied mathematical uptake model which relates the oil in water concentration and the uptake and purification rate constants with tissue concentrations over time. Since the major objective for monitoring mussels is to determine the pollution concentrations in the water, there is a need for a simple method to estimate the exposure concentrations based on tissue concentrations.

The work described in the present paper extends Mason's methodology to provide a rapid simple method to estimate exposure concentrations of oil in seawater from previously determined oil concentrations in mussel tissue. The uptake period was shortened to 12 hours using small incubation volumes and frequent replacements of the water soluble fraction of Exxon Valdez crude oil to better provide a constant exposure concentration. In addition, the uptake data was analyzed using an equivalent but alternative mathematical uptake model to derive the uptake constants and provide a direct method for estimating exposure concentrations from predetermined concentrations of oil in the mussel tissue (Spacie, 1983).

Mussels (Mytilus edulis) were obtained from Hood Canal, WA, and maintained in 2.5% artificial seawater (Instant Ocean) at 11°C. The water soluble fraction (WSF) of Exxon Valdez crude oil was prepared by shaking 25 ml of oil with one liter of 2.5% artificial seawater for five minutes at 11°C. The phases were allowed to separate for 21-22 hours in the cold and the seawater phase drained from the separatory funnel into a glass stoppered bottle. The WSF was immediately diluted to 0.1%, 1%, and 10% for the mussel experiments.

Fluorescence was used to quantify oil hydrocarbons in unextracted or hexane-extracted water. The samples were excited at 280 nm and the emission read at
374 nm with no barrier filter in a Hitachi MPP-2A analytical fluorescence spectrophotometer (Mason, 1987). Fluorescence was recorded in centimeters of pen deflection. Hydrocarbon concentrations in unextracted water were determined directly by fluorescence for the time course studies on the effect of aeration of the WSF in the absence of mussels. Seawater samples taken from the flasks containing mussels were centrifuged at 1000 xg for 10 minutes at 11°C to remove debris prior to hexane extraction. The volume of water extracted with 5 ml hexane was 40 ml for the 0.1% WSF exposure; 5 ml for the 1% WSF exposure; and 0.5 ml for the 10% WSF exposure.

Hexane extraction was necessary to eliminate nonspecific fluorescence produced by the mussels and to concentrate the hydrocarbons sufficiently to be read in the fluorimeter. The hydrocarbon concentrations in the hexane fraction of the water taken from the flasks containing mussels were determined by fluorescence from a standard curve of Exxon Valdez crude oil diluted in hexane. The concentration of hydrocarbons in undiluted crude oil was determined to be 600 mg/ml by gravimetric analysis. In addition to the quantitation of hydrocarbons in the WSF by fluorescence, the concentration of aromatic hydrocarbons (AHC) in the C12-C24 range in the WSF preparations was determined by gas chromatography (GC) (Analytical Resources, Inc., Seattle, WA).

Time course experiments with mussels were performed by exposing 10 mussels/flask (approximately 5 g/mussel) to one liter volumes of 0.1%, 1%, and 10% WSF at 11°C. The flasks were gently aerated. The one liter volumes of WSF were completely replaced with fresh WSF every two hours for a total time course of 12 hours. Hydrocarbon concentrations in the water at the end of each two hour incubation were then determined. Since mussel tissue has not been analyzed at this point in time, uptake by the mussels was determined from the amount of hydrocarbons which disappeared from the water over time assuming the disappearance from the water equals uptake by the mussels. Mussels retrieved at two hour intervals were wrapped in aluminum foil and frozen at -20°C for later analysis.

Fluorescence of 2-fold dilutions of the water soluble fraction (WSF) of Exxon Valdez crude oil in 2.5% artificial seawater was directly proportional over a 100-fold range (1:8 - 1:1024 dilution). The regression was statistically significant (r = 0.996, p<0.01). This plot suggests oil hydrocarbons soluble in seawater can be reliably detected at very low concentrations. To determine the concentration of water soluble crude oil in the seawater and in the mussel tissue, a fluorescence standard curve was obtained using 10-fold dilutions of Exxon Valdez oil in hexane. The logarithm of fluorescence was linearly related to the logarithm of the hydrocarbon concentration over a 100-fold range (0.001 - 0.1 µg/ml) The regression was highly significant (r = 0.945, p<0.01). The concentration of oil in the WSF preparations used for the mussel uptake experiments determined from this standard curve were 4.27 µg/ml for the experiments using 0.1% and 10% WSF and 2.35 µg/ml for the experiments using 1% WSF.

The stability of the fluorescent hydrocarbons in the 10% WSF preparation under conditions of aeration was determined by fluorescence. Aeration of the 10% WSF in the absence of mussel resulted in a 6.4%/day loss of fluorescence (r = 0.965, p<0.01) with no statistically
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significant loss in the unaerated control over a six day period. In contrast, ten mussels in one liter aerated volumes of 0.1%, 1%, and 10% WSF removed approximately 90% of the fluorescent hydrocarbons in two hours at 11°C. The percentage loss of fluorescent hydrocarbons from the water was similar for each of the six consecutive two hour incubations which constituted an individual 12 hour time course. These results suggest that the uptake rates were rapid and uniform for 0.1%, 1%, and 10% WSF exposures throughout the 12 hour uptake period and saturation was not evident.

The petroleum hydrocarbon concentrations remaining in the hexane extracted water were determined from the oil in hexane standard curve using fluorescence. By assuming the loss of fluorescent hydrocarbons from the water equaled the accumulation of oil hydrocarbons in the mussel tissue, uptake values for mussels could be calculated. The tissue mass was taken to be 50 grams. For the three 12 hour time courses with complete renewal of the WSF at two hour intervals, the accumulated 12 hour uptake values for 0.1%, 1%, and 10% WSF were 0.442, 2.80, and 44.7 µg oil/gm wet weight mussel tissue respectively.

Uptake constants for the three exposure concentrations were determined graphically by plotting µg oil/gm weight against the relation 1 - e^{kt} (plot 1) or by plotting µg oil/gm wet weight against c_{i} (plot 2). In plot 1, k is the base of natural logarithms; k is the depuration rate constant in day^{-1}; and t is time in days. In plot 2, c_{i} is the concentration of oil in the WSF in µg/ml and t is time in days. Both types of uptake plots have unique and valuable features. In plot 1, the slope of the least squares linear regression line is \( k_{1} / k_{2} (c_{w}) \) which can be used to calculate \( k_{2} \), the uptake rate constant knowing \( k_{2} \) and \( c_{w} \). In addition, the slope in plot 1 is equal to the maximum concentration of oil that can be accumulated by the mussels. In plot 2, the slope of the least squares regression line is \( k_{2} \), the uptake rate constant. Plot 2 differs from plot 1 as it does not depend on knowledge of \( k_{2} \), the depuration rate constant. In addition, the exposure concentration \( c_{w} \) can be estimated directly from this graph if the exposure time, t, is known.

The least squares linear regression lines were statistically significant in both plot 1 and plot 2 for each of the three exposure concentrations using the uptake values for the six time points for each plot. For plot 1, the literature value of \( k_{2} = 0.15 \) was used (Mason, 1988,1). The uptake constants were 222, 249, and 219 day^{-1} for plot 1 and 212, 238, and 209 day^{-1} for plot 2 for 0.1%, 1%, and 10% WSF exposures respectively. The maximum uptake values derived from the slopes of the linear regression lines for uptake values in µg oil/gm wet weight plotted against 1 - e^{kt} (plot 1) were 6.3, 39, and 622 µg oil/gm wet weight for 0.1%, 1%, and 10% WSF exposures respectively. These data confirm that tissue concentrations in mussels are directly related to the product of exposure concentration and time; the uptake constants are independent of exposure concentrations; and the two methods of plotting the uptake data are mathematically equivalent for determining the uptake constants.

The concentration of aromatic hydrocarbons (AHC) in the water associated with mussels taken from sites in Prince William Sound were estimated using our laboratory uptake curves. Tissue concentrations of AHC were determined in
triplicate by the Auke Bay Laboratory using GC and reported in Fish/Shellfish Study #11 (Injury to Herring). The mussels were retrieved between 4/28/89 and 5/4/89 from Fairmont Bay (5 transects); Naked Island (14 transects); Storey Island (2 transects); and Rocky Bay (4 transects).

The mean ± SD values for the tissue concentrations for the indicated number of transects for the four sites were 0.1047 ± 0.0404, 0.6955 ± 0.6227, 0.8445 ± 0.5518, and 1.256 ± 0.995 µg AHC/gm wet weight, respectively. The corresponding mean ± SD values for µg AHC/liter (PPB) using our laboratory uptake curves converted to GC values for the 0.1% WSF exposure were 0.493 ± 0.190 (FB); 3.273 ± 2.930 (NT); 3.974 ± 2.597 (SI); and 5.911 ± 4.493 (RB), respectively, for a one day exposure. The elevated values for AHC in the water or in the mussels at the oiled sites relative to Fairmont Bay values were significant by the t-test (p<0.05). Similar values were obtained from the uptake curve conducted with 1% WSF or 10% WSF.

The predicted concentrations of AHC/ml indicate that significant but low levels of oil pollution were present at selected sites in Prince William Sound 4-5 weeks after the oil spill. The estimated aromatic hydrocarbon concentrations in the water at the sites may be high as the predicted values rest on the assumption of a 24-hour site exposure. Longer exposure periods would proportionately decrease the AHD values in the water. In addition, the high fluorescence to GC conversion factor we determined (8.9) could exaggerate the predicted values. Finally, oil droplets trapped in the prepared WSF or at the environmental site would also exaggerate the values for AHC in the water. Subject to the confirmation of the oil concentrations in the mussels used for the uptake curve, the rapid and convenient laboratory assay described in this paper may provide a simple method for predicting oil exposure concentrations in water samples from determined oil concentrations in mussel tissue.

References
Impacts to Intertidal Invertebrates in Herring Bay, Prince William Sound, Following the Exxon Valdez Oil Spill

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Intertidal monitoring and experimental studies were carried out in Herring Bay, Knight Island, Prince William Sound (60°N: 147°W) as part of the Coastal Habitat Injury Assessment program (CHIA). Population densities for several species of invertebrates were compared between matched oiled and control sites from 1990 to 1992. Limpets were included because they are important intertidal grazers. The periwinkle, Littorina sitkana, the dog whelk, Nucella spp., and the six-armed starfish, Leptasterias hexactis, were studied because they lack a free-swimming larval stage, and may recover slowly after a large reduction in population over a large area.

The initial study design in Herring Bay was to select a range of sites with different oiling histories, including a non-oiled control site, and oiled sites that were mechanically treated (washed) and bioremediated. Ideally, this combination of sites would have been replicated several times to achieve statistical rigor. However, after surveys in May, 1990, review of data from the Exxon/Federal/State spring shoreline assessments, and detailed discussion with Alaska Department of Environmental Conservation monitors working in Herring Bay, treatment history of specific sites could not be determined with certainty. Therefore, the matched pair design of oiled and non-oiled study sites was adopted and treatment was not included as a variable. Sites were matched for substrate composition, slope, direction and solar aspect, wave exposure, and common biological communities. Control sites were restricted to the southeast corner of Herring Bay, where ice had prevented oil from entering in the spring of 1989. Most matched oiled sites were located in the lower-mid and western portion of Herring Bay.

In 1990, permanent 50 X 20 cm quadrats were established at five pairs of sites: three sheltered rocky and two sheltered coarse grained beaches. At each site six quadrats were located within each of three tide levels, or meters of vertical drop (MVD), below mean high water. Within each quadrat, all limpets, Nucella spp., Littorina sitkana and Leptasterias hexactis were counted. In 1991, one additional protected rocky site pair and one coarse-textured oiled beach site, for comparison to an existing control site, were added to the study. In 1992, examination of these quadrats continued and four additional protected rocky site pairs were added. There were only three quadrats per MVD for the pairs added in 1992, but they were randomly established as previously done.

Densities of the limpet, Tectura persona, have remained significantly higher at control sites during the 3-year study period, with this difference pronounced at the 1 and 2 MVD (p<0.05, at 5 of the 7 pairs at 1 MVD; p<0.01 at 3 of 7 site pairs at 2 MVD, repeated measures ANOVA). Also, the differences in density of T. per-
sona between control and oiled sites tended to increase substantially in 1991. This pattern may have continued into 1992, but is uncertain with only two sample periods for this season.

In sheltered rocky habitats, *T. persona* is common in the upper intertidal but is not abundant at the third MVD. In contrast, *T. persona* is reasonably common at the third MVD in coarse textured habitats, and the density remains significantly lower at the oiled site in only one of the three pairs at this tide level (*p*<0.04, repeated measures ANOVA). For the four site pairs added in 1992, the data support the general trends seen in the original sites. On both sample dates at the 1 MVD, *T. persona* densities were significantly lower (*p* < 0.04, t-Test) at two of the oiled sites, with weak significance (*p* < 0.17, t-Test) at the remaining pairs. A similar pattern occurred in the second and third MVD, but differences were not consistently significant.

Another limpet, *Lottia pelta*, is distributed lower on the shore, being more abundant in the second and third MVD. Similar to *T. persona*, differences in *L. pelta* densities between control and oiled sites peaked in 1991 for the second MVD at most sites. However, *L. pelta* appears to be recovering more rapidly than *T. persona*. *L. pelta* densities at the four sites established in 1992 were generally greater at control than oiled sites, but were significant in only a few cases.

The periwinkle, *Littorina sitkana*, was significantly less dense, especially at MVD 2, at four of the seven oiled sites compared to controls over the course of the study (0.000=>p<=0.03, repeated measures ANOVA). Recovery has been minimal. For the four sites added in 1992, *L. sitkana* densities tended to be lower at oiled than control sites though differences were not always significant. For example, significantly fewer individuals were found at oiled sites in only 1 of the site pairs at the 2 MVD in the spring but by summer, differences were significant at three of the four pairs (*p*<0.05 t-Test).

The dog whelk, *Nucella lamellosa*, had only sufficient densities for statistical comparison at one of the seven site pairs and there were no differences found over the course of the study. However, density at the oiled site dropped by one-half toward the latter part of the 1990 season, and a similar drop was observed in 1991. However, *N. lamellosa* densities remained higher at the oiled site compared the control on both sample dates in 1992.

The other species with direct embryological development, *Nucella lima* and *Leptasterias hexactis*, were either not present or found in very low densities, and no differences were observed between site pairs.

Reductions in densities of invertebrates, particularly intertidal grazers such as limpets and periwinkles have been reported for previous oil spills (Nelson-Smith, 1977; Mann and Clark, 1978; Southward and Southward, 1978), and our findings are consistent with these earlier results. Effects from the *Exxon Valdez* oil spill on the invertebrates in Herring Bay have been variable, but damage has been documented, and recovery remains incomplete in some cases, especially the upper intertidal zone. The loss of *Fucus* from the 1 MVD is believed to be largely responsible for the inability of limpets to survive there. However, with the exception of *T. persona*, the other af-
fected invertebrates show populations increases since the spill.

A main hypothesis of the population studies was that brooding invertebrates, such as *Littorina sitkana*, *Nucella* spp. and *Leptasterias*, would suffer greater long term consequences because of limited dispersal, and the potential effect of oil on fecundity and development. Based on the data collected to date, only *L. sitkana* appears to have been slightly affected by oil. In 1990 *L. sitkana* showed significant differences only at the coarse-textured sites, but in subsequent seasons abundances have been reduced at the 1 and 2 MVD of oiled sites, similarly to the changes seen in limpets.

References


Exxon Valdez Oil Spill: Recruitment on Oiled and Non-Oiled Substrates

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As part of the Coastal Habitat Injury Assessment program, intertidal experiments were established in Herring Bay, Knight Island, Prince William Sound (60° N; 147° W) in 1990. Two separate studies, continued through 1992, examined the effect of north slope crude oil on recruitment of barnacles, Fucus germings and filamentous algae. The effects of oil on algal recruitment are reported in a separate abstract in this symposium.

In 1990 two oiled sites and two control sites of similar character were selected to study recruitment on tarred and clean vertical rock faces. At each site, paired 10 X 10 cm plots were randomly established. One member of each pair was scraped and brushed to remove all visible tar and/or barnacles. The sites were periodically visited and numbers of barnacles and Fucus germlings were noted. In 1991 the study was expanded to include a total of five site pairs and grazing-exclusion cages were added to half of the study plots.

The 1990 data show that barnacle recruitment was initially retarded on the oiled plots at oiled sites, compared to the scraped ones (p<0.05, paired t-Test), but these differences began to fade at several sites over time (p=>0.2, paired t-Test). This trend is most evident with the caged plots. The control sites have had consistently greater densities on the control plots compared to the scraped ones. Fucus germlings began to emerge at control sites in 1990, and only in low density at the oiled sites in 1991. In 1992 Fucus was in greater abundance at oiled sites, and were greater on the unsraped plots (both control and oiled sites) compared to the scraped ones (0.2>p<0.8, paired t-Test).

Densities of grazers (limpets, Littorina sitkana, and L. scutulata), were significantly greater at control sites compared to oiled sites in 1991 (p<0.05, ANOVA for four of five site pairs).

In 1992 data analysis was further subdivided to include measurement of surviving adults of Semibalanus, Balanus, and Chthamalus dalli from the previous seasons. Fewer differences were observed in 1992 compared to the previous years for all species and ages. Adult barnacles represent those individuals which have successfully recruited onto scraped plots. Adults are found in greater density at control sites because these were nonoiled individuals alive before the spill and left as controls. However, at the oiled sites, adults have successfully recruited on both plots. Within cages, the density of surviving adults are similar on both oiled and scraped plots. However, on uncaged plots, this pattern is reversed. Chthamalus dalli were generally greater on the scraped plots compared to the oiled/control plots.

In a second recruitment study, initiated in 1990, three pairs of oiled and control sites were selected for transplanting oiled substrates. The substrates used were rocks retrieved from an oiled shoreline in Herring Bay, as well as rocks treated with fresh North Slope crude oil,
Intertidal: Recruitment on Oiled and Non-Oiled Substrate

taken from the T/J Exxon Valdez in 1989. Tarred rocks of similar size were collected from an oiled beach in Herring Bay, and represent a substrate coated with 1-year-old Exxon Valdez Prudhoe Bay crude oil (EV). One-half of each rock was cleaned with the solvent methylene chloride (MeCl₂) to remove the oil (Duncan, et al. 1992a). In addition, rocks approximating the sizes of the EV rocks were collected from a similar, but unoiled, beach. Half of each rock was dipped in fresh Prudhoe Bay Crude (PB) until a "tarred" coating was achieved. These rocks were allowed to dry for several weeks and were handled in a manner identical to the EV rocks.

As a control for possible effects of MeCl₂ on recruitment, half of six unoiled rocks were "cleaned" with MeCl₂ (one rock placed per site). As a control on surface heterogeneity, white clay tiles were included in the experiment as oiled and clean pairs.

At each of the six experimental sites, 12 EV, 12 PB rocks and 6 tile pairs were placed randomly at the elevation contour 2 m below MHHW (Mean Higher High Water, included in the upper intertidal zone). Control rocks for MeCl₂ were also placed at each site. Periodically, settlement by barnacles and macro algae on each surface type was recorded.

In 1991, the study was modified to include nine pairs of red clay tiles at each study site. Six of the pairs consisted of a tarred and a clean tile and half of these pairs had cages constructed around them to exclude grazers. The remaining three pairs consisted of a clean tile and a tile painted black (rather than oiled) as a control for dark coloration and possible temperature differences (Straughan, 1976). The tiles were also randomly placed at the 2-m contour.

Only 2 oiled sites were consistently colonized over the three year period (Sites 1322X & 1723X). Control sites received fewer recruits of all species (except for Fucus) compared to the oiled sites. The rocks deployed in 1990 were weathered and dislodged from the substrate over the course of the 1990-91 winter at most sites. Many of the rocks had the oil weathered completely from the sampling surface and, consequently, could not be sampled in 1991.

During 1990, the oiled halves of EV and PB rocks had lower densities of barnacle recruits in most cases. Initial settlement (early July) tended to be greater on unoiled halves but differences were only weakly significant (p=<0.18, paired t-Test). There was little recruitment in general later in the season. The freshly coated PB rocks showed greater differences, which were significant over several sample dates at both sites (p=>0.001<0.1, paired t-Test). However, the oil on the PB rocks was washed away in many cases and, by the end of the season with little recruitment occurring, no differences remained. The control rocks cleaned with methylene chloride had no differences in barnacle recruits between treated and untreated sides (0.35=>p<0.9, paired t-Test).

The six tile pairs placed in the field in 1990 had fewer barnacle recruits. Densities were significantly lower (p=0.01, paired t-Test) in early July, and weak significance (p=>0.07<0.2, paired t-Test) was found at 6 of 10 sample dates.

There was sparse settlement on the tile pairs placed at control sites in 1991. There were few differences in barnacle recruits, Fucus germlings and percent algal cover and no trends were apparent. However, the two sites (1322X and 1723X) which had good recruitment in 1990 also
had substantial recruitment activity in 1991. The caged tiles of both sites had somewhat greater numbers of barnacles on the unoiled tile compared to the oiled tile except toward the end of the recruitment season, but differences were only weakly significant (p>0.18, paired t-Test). The painted tile pairs had similar levels of barnacle and Fucus recruitment on both tiles.

In 1992, barnacle recruitment was substantially lower than in 1991 and patterns were not evident. Data on adult barnacles present on the tiles indicate very few recruits reach adult sizes. Chthamalus dalli tended to recruit better on oiled tiles in caged treatments and on unoiled tiles in uncaged treatments, and were not significantly different on painted and unpainted tiles.

In 1991, Fucus did not recruit on uncaged or painted tile pairs and only began to recruit on caged, clean tiles at the end of the season. Again in 1992, there was almost no Fucus recruitment on uncaged or painted tile pairs. For caged treatments, recruitment tended to be higher on clean tiles with a slight tendency for higher recruitment at control sites.

Recruitment, including that of algal cover, appears to play the major role in structuring invertebrate communities at the Herring Bay study sites. These studies show that oil had an initial effect on barnacle recruitment, and depending upon substrate character, may have a moderate to long-term effect on algal recruitment (Duncan et al, 1992b). Within Herring Bay rock substrate differs from site to site, and several of the study sites have a more porous substrate than others.

For barnacles it is likely that residual tar is an unstable settlement substrate and the reduced densities are a consequence of tar sloughing rather than toxicity. The sites showing the most rapid increases in invertebrate abundance are those most exposed to open water or tidal currents, which probably increases larval availability at those sites. Not surprisingly, these were also the sites hit by the floating oil. Although care was taken in matching rock sizes, the parent material (including the degree of porosity for each rock) varied greatly. This was especially true with the PB rocks selected. Many rocks were dense and non-porous and the oil quickly dissipated to the extent that comparisons between oiled and non-oiled halves could no longer be made.

The tile pairs were much less variable. Tiles placed in the field in 1990 were of a silicious clay and may have retained north slope crude more effectively than the red clay tiles used in 1991. Nonetheless, all tiles have retained a degree of oil staining not found on many of the rocks.

References
The Response Process: The Goals of the Oil Spill Health Task Force

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The day of the Exxon Valdez oil spill had special meaning for the 4,500 Alaska Natives who live on the shores of Prince William Sound and depend upon the sea and shoreline to maintain a subsistence lifestyle. Walter Meganack, the tribal chief of the native village of Port Graham, described the day as "the time when the water died." He said that the native story was different from the white man's story.

"Our values are different, how we see the water and the land, the plants and the animals are different. What the white men do for sport and recreation and money, we do for life: for the life of our bodies, for the life of our spirits, and for the life of our ancient culture. The water is sacred." (Meganack, 1989).

The sea, shoreline and inland areas also provided the majority of protein intake in the diet of Alaskan Natives in the area.

Household surveys conducted by the Subsistence Division of the Department of Fish and Game prior to the oil spill from representative Prince William Sound villages documented up to 300 pounds of protein per person per year came from wild meat, fish and fowl (ADF&G, 1984). By comparison the comparable amount of the above food purchased in the Western U.S. is 220 lb.

The Oil Spill Health Task Force was formed initially in April 1989 to respond in as timely and informative way as possible to the public's inquiry about the short and long term safety of subsistence food exposed to Exxon Valdez oil. The collective knowledge of the initial group of members (Indian Health Service, State Division of Epidemiology, Subsistence Division of the Alaska Department of Fish and Game, The North Pacific Rim Native Corporation and the Kodiak Area Native Association) on the toxicology of oil and subsistence food was nonexistent.

Calls for federal assistance were not answered. The reason for the lack of response soon became clear. In a review of the world literature on behalf of the Oil Spill Task Force, toxicologist Dr. Gary Winston of Louisiana State University found "a virtual absence of literature which investigated any human health effects, concerns or implications as a function of oil spills other than those which were related directly to primary exposure to clean up crews." (Winston, 1990). Further inquiry to the Food and Drug Administration revealed no established safety levels for hydrocarbons for food.

The Task Force (joined in the late summer of 1989 by representatives from NOAA, the Toxicology Division of the Exxon Corporation and the Alaska Department of Environmental Conservation) developed the following mission: obtain as much historical information as possible about health effects of crude oil by both direct exposure and exposure through the food chain, conduct studies of subsistence foods for contamination with oil, interpret the results of these studies, and disseminate this information to the public.

As sample data on hydrocarbon concentrations in subsistence foods became available, expert advice was needed to interpret the results. Through NOAA's
help the task force was able to gather a group of nationally known senior toxicologists and scientists in what has become known as the Oil Spill Expert Toxicology Committee (Shank, 1990). Their recommendations formed the basis of our communication to the public.

At our request the FDA did a quantitative health risk assessment of subsistence food exposed to *Exxon Valdez* oil, and in August 1990 we received an advisory opinion of the safety of aromatic hydrocarbon residues found in subsistence foods most important to Alaskan Natives.

This then is the background to the monumental effort that ensued to insure food safety and the efforts of the Task Force to communicate this information to subsistence users in the numerous villages in the oil spill area.

References


Subsistence Uses of Fish and Wildlife Resources in Areas Affected by the Exxon Valdez Oil Spill
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This paper discusses changes in subsistence uses of fish and wildlife resources in 15 predominantly Alaska Native communities whose hunting, fishing, and gathering areas were affected by the Exxon Valdez Oil Spill. It is based upon research conducted by the Division of Subsistence of the Alaska Department of Fish and Game. Study communities include Tatitlek and Chenega Bay in Prince William Sound; English Bay (Nanwalek) and Port Graham in lower Cook Inlet; Akhiok, Karluk, Larsen Bay, Old Harbor, Ouzinkie, and Port Lions in the Kodiak Island Borough; and Chignik, Chignik Lagoon, Chignik Lake, Perryville, and Ivanof Bay on the Alaska Peninsula. In 1990, the population of these 15 communities was 2,036, 82.3 percent of which was Alaska Native.

Prior to the spill, the division had conducted baseline research in all 15 villages. These studies found that subsistence harvests in these communities in the 1980s were large and diverse, ranging from about 200 pounds per person to over 600 pounds per person usable weight per year. These are substantial harvests, given that the average American family purchases about 222 pounds per person of meat, fish, and poultry annually. These subsistence harvests contained a wide variety of resources, including salmon and other fish, marine invertebrates, land mammals, marine mammals, birds and eggs, and wild plants. Virtually every household in all 15 villages used and harvested wild foods, which were widely shared within and between communities. A patterned seasonal round of subsistence harvesting structured much of economic, social, and cultural activities in each community.

In early 1990, division researchers interviewed representatives of 403 households in these 15 communities. Study findings revealed that after the spill, subsistence harvests declined markedly in the 10 communities of Prince William Sound, lower Cook Inlet, and the Kodiak Island Borough compared to pre-spill averages. Annual per capita harvests in Chenega Bay and Tatitlek were down 57 percent. In these villages, the range of resources used also dropped in the 12 months after the spill. While the average household in Tatitlek used 22.6 kinds of wild foods from April 1988 through March 1989, in the next year, this average was only 11.6 types. The change at Chenega Bay was much like that of Tatitlek. In a 12 month study year in 1985-86, the average household at Chenega Bay used 19 kinds of wild foods, compared to just 8.2 kinds in the year after the spill.

Very similar changes were documented for English Bay and Port Graham. Compared to 1987, harvest quantities were down 51 percent at English Bay and 47 percent at Port Graham. The range of resources used per household dropped at English Bay from an average of 25.0 kinds in 1987 to 13.7 kinds in 1989. At Port Graham, the household average was 21.5 in 1987 and 11.2 in 1989.

Subsistence harvests in all six Kodiak
area villages also declined in 1989 compared to pre-spill averages, although a wider range of changes was documented. Harvets as measured in pounds per person per year were down 77 percent in Ouzinkie, 60 percent in Karluk, 52 percent in Port Lions, 40 percent in Old Harbor, 31 percent in Larsen Bay, and 12 percent in Akhiok.

In contrast, subsistence harvests in the five Alaska Peninsula communities showed little change, or increased, in 1989 compared to 1984, the only pre-spill year for which comprehensive data are available. Household interviews revealed that the presence of sheen, mousse, and tar balls temporarily disrupted subsistence harvests near these communities. However, most families resumed subsistence activities within several months. Resource use diversity also remained very high in the Alaska Peninsula villages, ranging from 15.3 resources per household in Chignik Lagoon to 29.7 kinds per household in Ivanof Bay.

When asked to provide reasons for declines in subsistence harvests in the year following the spill, 33.2 percent of the sampled households attributed reductions in overall subsistence harvests to concerns about resource contamination, and 44 percent said such a concern had caused a reduction in their harvest of at least one kind of subsistence food. Levels of concern about contamination were notably higher among Prince William Sound (92.1 percent) and Lower Cook Inlet (77.8 percent) households than in the Kodiak area (29.5 percent) or Alaska Peninsula (22.8 percent) communities. Other reasons cited for lowered levels of subsistence harvests included the time harvesters spent on the oil spill cleanup and the perception that less resources were available because of spill-induced mortalities.

Regarding contamination concerns, it should be noted that little specific information was available to subsistence harvesters concerning the safety of using subsistence resources from the spill area until September 1989, by which time spring, summer, and most fall harvest opportunities had passed. Complete information from tests of fish and shellfish from subsistence foods testing programs were not available until February 1990, and information about marine mammals, birds, and deer was not available until June 1990. Interviews conducted in 1990 found that many households continued to express doubts about subsistence food safety. Respondents cited the relatively small number of samples tested in 1989, the limited sites examined, and the limited range of species tested as some reasons for their continuing questions. Also, hunters and fishermen continued to observe the presence of oil in harvest areas as well as dead and damaged wildlife which they attributed to the spill. Such signs were understood as evidence of continuing danger and many households thus acted cautiously with respect to using subsistence foods. Such behavior is culturally consistent for people whose survival has long relied upon their observations of the natural environment.

In 1991, the division conducted follow-up interviews with 221 households in seven spill-area villages pertaining to subsistence harvests during the second post-spill year. Harvest levels increased at Port Graham, Larsen Bay, and Karluk, and matched at least one pre-spill measurement. The range of resources used was also up substantially in all three communities. In two other communities, Ouzinkie and English Bay, harvest levels also increased, but
remained below pre-spill averages. This general increase in harvest levels and range of wild foods used suggests some renewed confidence in using subsistence foods during 1990.

On the other hand, lingering concerns about food safety were expressed in all five villages. Some families reported that they resumed their subsistence harvests despite misgivings because they could not afford to purchase substitutes and could no longer do without culturally important foods.

In contrast, no evidence of a recovery in subsistence uses in the second post-spill year was found for Chenega Bay and Tatitlek. At Chenega Bay, subsistence harvests from April 1990 through March 1991 were 139.2 pounds per person, virtually the same as the previous year (148.1 pounds per person) and still well below the pre-spill average of 340 pounds per person. At Tatitlek, the 1990-91 per capita harvest was 152.0 pounds, compared to 214.8 pounds per person in the first post-spill year and a pre-spill average of 497.6 pounds per person.

In these Prince William Sound communities, deep concerns about the safety of using subsistence foods from their traditional harvest areas continued. In addition, respondents from Chenega Bay and Tatitlek reported perceived declines in the numbers of some important subsistence resources, such as certain species of waterfowl, marine invertebrates, and marine mammals, which led to well below normal subsistence harvests during 1990-1991.
Overview of Subsistence Food Safety Testing Program

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The Oil Spill Health Task Force, a group chaired by a Public Health Service physician and composed of representatives from state and Federal agencies, native organizations, and Exxon, was formed soon after the Exxon Valdez oil spill to address public health concerns resulting from the spill. The Oil Spill Health Task Force served as a forum for design of the subsistence food safety testing study and the communication of the results and conclusions derived from the study to the subsistence communities.

The main objectives of the study were to determine if subsistence foods were contaminated as a result of the spill and to assess the implications for subsistence food safety. In order to put together a comprehensive study, NOAA and Exxon signed a Memorandum of Understanding in the summer of 1989 which had three principal features: (1) NOAA biologists would accompany Exxon-sponsored collection teams to the villages and participate in the sample collections; (2) tissue samples from fish and shellfish subsistence resources would be analyzed for aromatic contaminants by the NOAA National Marine Fisheries Service Environmental Conservation Division Laboratory in Seattle; and, (3) all data from the study would be made public.

These steps were taken to ensure that there were no questions about the validity of the data produced by the study and that the results and the implications for human health were made available to public health officials and the communities in a timely manner.

This paper addresses the general sampling strategy, the numbers and types of samples collected, the identification of the most contaminated sites, and issues related to the interpretation of the shellfish results. Subsequent papers in this session will address in more detail the results from chemical analysis of shellfish, fish, and marine mammal tissues, the implications of those results for human health, and an evaluation of the methods used in risk communication.

The study area included subsistence seafood collection areas in Prince William Sound (Tatitlek and Chenega Bay); Lower Cook Inlet (including the Outer Kenai Peninsula villages of Port Graham and English Bay, and Windy Bay); and Kodiak Island (Kodiak City, Chiniak, Larsen Bay, Karluk, Akhiok, Old Harbor, Ouzinkie, and Port Lions). Four subsistence areas on the Alaska Peninsula (Chignik, Perryville, Ivanof Bay, and Kashvik Bay) were sampled by ADF&G in 1990. Reference samples were collected from the village of Angoon in 1989 and Yakutat in 1990. The initial sampling plan called for approximately equal numbers of samples from each village area, although there were considerable differences in the potential degree of impact from the Exxon Valdez oil. Sampling sites that represented important subsistence use areas were selected in consultation with village representatives. The degree of oiling was not a major factor in site selection. Two important subsistence beaches for the collection of intertidal shellfish were identified in each
village area. Target species included several species of intertidal shellfish (mussels, clams, chitons), bottomfish (primarily halibut), and salmon. Other species were also collected but in much smaller numbers. Intertidal stations for the collection of shellfish did not represent exact locations on a given beach. Thus, several species (e.g., mussels, clams, and chitons) were often collected from the same station, although each species occupied a different habitat (tidal elevation, substrate) in the beach.

In 1990, the number of sample stations and the number of samples per station were increased. In 1991, only shellfish were collected. The focus in 1991 was in southwestern Prince William Sound near Chenega Bay, because that part of the Sound had potentially the greatest amount of oiled shoreline, and Windy Bay, because that was the most heavily-oiled site sampled and was a good site to examine changes in concentration over time. Both Exxon and the Alaska Department of Fish and Game sponsored sample collections and analyses in 1990 and 1991.

A total of over 1,000 shellfish samples collected from more than 65 stations were analyzed for aromatic contaminants in three years of sample collections. Five stations had one or more shellfish samples with total aromatic contaminant concentrations exceeding 1 ppm (part per million): two stations in Prince William Sound (one in Port Ashton in Sawmill Bay near the village of Chenega Bay and the other on southwestern Elrington Island); two islands in Windy Bay on the Outer Kenai Peninsula; and a station on Near Island, near the Kodiak boat harbor. The Windy Bay stations and the Elrington Island station both had obvious visual evidence of oiling.

At the most contaminated station, an island in Windy Bay, total aromatic contaminant concentrations in mussels varied by as much as three orders of magnitude from the same collection period over a relatively small beach area. The highest concentrations were found in mussels collected at a location higher in the intertidal area.
Assessment of Exposure of Subsistence Fish Species to Aromatic Compounds Following the Exxon Valdez Oil Spill

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On March 24, 1989, the Exxon Valdez ran aground on Bligh Reef spilling Prudhoe Bay crude oil (PBCO) into Prince William Sound, a relatively pristine area in Alaska. The oil spread through the Sound and into coastal areas along the Gulf of Alaska, affecting fishing grounds used by Alaska Native villages. The Alaska Natives were concerned about possible contamination by the spilled petroleum of fish and shellfish used for subsistence.

In response to this concern the National Oceanic and Atmospheric Administration (NOAA) entered into an agreement with the Exxon Corporation and the Alaska Department of Fish and Game (ADF&G) to survey native subsistence fisheries for exposure of fish and shellfish to oil. Here, we present the results from chemical analyses of several species of salmon and bottomfish collected from a number of fishing grounds used by native villagers to assess the extent of exposure of fish to spilled oil and the accumulation of petroleum-related compounds in edible flesh.

Of particular concern was the accumulation of aromatic compounds (ACs) present in oil, because some of these ACs are suspected of being toxic to humans (Dipple et al., 1984). The ACs measured included polycyclic aromatic hydrocarbons and sulfur-containing ACs. Additionally, several of the ACs (e.g., alkylated naphthalenes, phenanthrenes and dibenzothiophenes) measured were characteristic of oil and particularly PBCO (Krahn et al., 1992). The results from the analyses of edible flesh of fish, in addition to results from the analysis of shellfish (Brown et al., this volume), were then evaluated by the Alaska Oil Spill Health Task Force and the U.S. Food and Drug Administration (FDA) to assess the potential risk to Alaska Natives of eating seafood from their traditional fishing sites following the spill.

Previous studies (Varanasi et al., 1989) have shown that fish have the capacity to biotransform many ACs to polar metabolites that are readily excreted into bile, which is retained in the gall bladder. Moreover, these studies showed that the extensive biotransformation of ACs by fish greatly limits the accumulation of these compounds or their metabolites in edible tissues. Accordingly, a rapid and sensitive semiquantitative method (Krahn et al., 1986) for measuring concentrations of fluorescent aromatic compounds (FACs) in bile was used to estimate exposure of fish to ACs that are present in oil. These results were then used for prioritization of edible flesh samples for a more extensive analysis of the presence of parent ACs by gas chromatography/mass spectrometry (GC/MS).

Concentrations of FACs in the bile of five species of bottomfish and five species of salmon collected from oil-impacted sites located in Prince William Sound and along the Gulf of Alaska were
determined. Elevated concentrations of FACs in bile of fish from several sites indicated exposure to ACs. For example, in 1989, mean concentrations of FACs were highest in bile of both salmon and bottomfish from sites near Chenega Bay (6000 ± 3400 and 5100 ± 4000 ng phenanthrene equivalents per mg bile protein, respectively) and Kodiak (2800 ± 1900 and 7550 ng phenanthrene equivalents per mg bile protein, respectively), which were heavily impacted by spilled oil. Moreover, these concentrations were substantially higher than the concentrations in salmon (1100 ± 2200 ng phenanthrene equivalents per mg bile protein) and bottomfish (310 ± 120 ng phenanthrene equivalents per mg bile protein) from Angoon, a reference site.

In the following year, salmon and bottomfish from Chenega Bay showed marked decreases in biliary FAC concentrations (550 ± 250 and 1300 ± 850 ng phenanthrene equivalents per mg bile protein, respectively) suggesting that the level of exposure to ACs in salmon from the Chenega Bay site had declined. Interestingly, no temporal changes were evident in salmon sampled from sites near Kodiak in 1990 (no bottomfish were sampled from this site). The concentration (2400 ± 2000 ng phenanthrene equivalents per mg bile protein) of FACs in salmon from Kodiak was comparable to the concentration in 1989 indicating that exposure to ACs was unchanged for salmon captured at this site. These results and data from analyses of shellfish from Kodiak (Brown et al., this volume) suggest exposure to a source of ACs other than spilled oil.

The HPLC analysis of bile for FACs provides a rapid, semiquantitative assessment of exposure to ACs that are present in oil but does not allow identification of individual compounds found in bile. In a related study (Krahm et al., 1992) conducted as part of the Natural Resources Damage Assessment effort, it was shown that the bile method is a sensitive indicator of exposure of fish to specific ACs present in oil. In the study of Krahm et al. (1992), metabolites of ACs characteristic of PBCO were identified, by GC/MS, in high proportions in bile of fish injected with PBCO and in fish sampled from sites in Prince William Sound shortly after the spill. Metabolites of these ACs were not present in bile of fish sampled from a reference site distant from the spill. These findings substantiated the use of the bile method to rapidly assess exposure of fish to ACs in the present study.

As mentioned, the major use of the results on concentrations of biliary FACs was to prioritize corresponding edible flesh samples for more extensive analysis of the presence of parent ACs by GC/MS. Samples showing a range in exposure to oil were chosen for comprehensive analysis to determine if fish were accumulating significant levels of parent ACs in their edible tissue and to substantiate that low concentrations of FACs in bile accurately reflect minimal exposure to ACs and, hence, little potential for accumulation of ACs in muscle tissue.

The concentrations of biliary FACs in fish from which samples of muscle tissue were analyzed for parent ACs ranged from 10 to 18,000 ng phenanthrene equivalents per mg bile protein. In contrast, concentrations of total ACs (sum of individual selected ACs) analyzed by GC/MS in corresponding muscle tissue were low. No appreciable concentrations [<1 ng/g wet weight (ppb)] of parent ACs were detected in muscle of
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bottomfish, and although concentrations of ACs in muscle of salmon were somewhat higher they very rarely exceeded 100 ppb, and in the majority of samples the concentrations of ACs were less than 30 ppb.

These findings showed that exposure of salmon and bottomfish to ACs does not lead to any significant accumulation of parent ACs in edible muscle tissue. These results also corroborate findings from laboratory studies on the pathways of metabolism and disposition of ACs in fish, showing that polar metabolites of ACs accumulate in bile and that parent ACs do not accumulate to significant concentrations in muscle tissue (Stein et al., 1987; Varanasi et al., 1989). The consistency of the results from this field study and those of previous laboratory studies provided evidence of minimal risk of exposure of humans to parent ACs from consumption of edible flesh of fish captured at sites impacted by the spilled oil.

The extensive biotransformation by fish of ACs to polar metabolites raised the issue of whether metabolites were present at elevated concentrations in muscle tissue of fish exposed to ACs. Accordingly, a method similar to the bile screening method is being developed (Krone et al. 1992) to estimate concentrations of AC metabolites in fish tissues. This new method has not been validated as extensively as the bile method, thus the results must be considered preliminary. However, analysis of a few selected samples of muscle from salmon indicated that concentrations of AC metabolites were also quite low. This finding is consistent with previous laboratory studies (Stein et al. 1984) with ACs that showed metabolites of ACs as well as parent compounds are low in muscle of fish, and demonstrates the importance of laboratory studies, which lead to the development of techniques that provide the necessary information to respond effectively to environmental emergencies.

In summary, the results of this survey of Alaska Native subsistence seafood provided substantial evidence that fish were exposed to ACs that appeared to originate, in most cases, from the spilled oil. However, edible muscle tissue in salmon and bottomfish were found to have concentrations of ACs that rarely exceeded the relatively low concentration of 30 ppb. These data showing low concentrations of parent ACs in muscle of fish exhibiting a substantial range in exposure to ACs were used by the Alaska Oil Spill Health Task Force and the FDA in arriving at an advisory position that consumption of the flesh of fish posed minimal risk to native Alaskans.

References


Chronic exposure to an urban estuarine sediment with added 3H-benzo(a)pyrene and 14C-polychlorinated biphenyls. Marine Environmental Research 22: 123-149.