Northwest Bay (0 & 6 m), Herring Bay (0 & 6 m), Disk Island (0 & 20 m), and Bay of Isles (6 m). Subtidal (6 m) sediments were generally as toxic as intertidal sediments. However, significant toxicity to amphipods was also found at two of the six reference sites sampled: Drier Bay (0, 6 & 20 m) and Mooselips Bay (0 m). Significant mortalities of oyster larvae were also detected with shallow sediments from oiled [Sleepy Bay (0 m), Bay of Isles (0 m), Chenega Island (0 & 6 m) and Block Island (6 & 20 m)] and reference sites [Drier Bay (0 m), Mooselips Bay (6 m), MacLeod Harbor (6 m) and Rocky Bay (6 m)]. As a result, the mean toxicities for the oiled and reference sites in 1991 were not significantly different for either amphipods or oyster larvae. The observed patterns of toxicity are consistent with the general decline of hydrocarbons in the intertidal zone over the period 1989-1991, and with the concomitant transfer of hydrocarbons into shallow subtidal sediments. These results suggest a significant decline in oil-related toxicity between 1990 and 1991, concurrent with the removal and disappearance of the lower molecular weight aromatic compounds.

To explore which fractions of petroleum were potentially most toxic, large samples of intertidal sediments (4 kg) and interstitial porewater samples (114-170 liters) were collected in September 1990 from a heavily oiled site (Bay of Isles on Knight Island) and an unoiled site (Mooselips Bay on Montague Island). The pore waters and sediments were extracted exhaustively with a mixture of methylene chloride and ethyl acetate, and the extracts were subsequently fractionated by liquid column chromatography into aliphatic, aromatic and polar components. Analysis by gas chromatography showed that the water and sediment samples from Bay of Isles contained substantial quantities of petroleum hydrocarbons representing moderately weathered petroleum (as evidenced by absence of n-alkanes below C12 and absence or depletion of mono- and di-aromatic compounds in the aromatic fraction). The pore water sample from Mooselips Bay was essentially free of petroleum hydrocarbons.

The aromatic and polar fractions from both sites were systematically tested with a variety of toxicity tests (Microtox®, SOS Chromotest®, bivalve larval survival and normal development, anaphase aberrations and sister chromatid exchange in developing bivalve larvae, and teratogenicity and anaphase aberrations in salmon embryos) to determine the relative toxicities of the two chemical fractions. Both the aromatic and polar fractions from Bay of Isles sediment samples were consistently more toxic than analogous fractions from Mooselips Bay based on Microtox® and SOS Chromotest®, and abnormality, anaphase aberration, and sister chromatid exchange responses in bivalve larvae. The polar fraction from the Bay of Isles porewater samples also exhibited greater toxicity for most endpoints than the analogous samples from Mooselips Bay. For the aromatic fractions from pore water, however, the differences in test results between the Bay of Isles and Mooselips samples were generally insignificant, and the toxicities approximated that of accompanying method blanks.

In the Bay of Isles samples, the polar and aromatic fractions elicited approximately equivalent toxic responses in the sediment extracts, while the polar fraction was usually slightly more toxic for the porewater extracts. There were no
consistent patterns, however, to distinguish responses to water and sediment samples. For example, EC-50's for the Microtox® response were 10g and 23g (dry weight) of sediment for the aromatic fractions from the two Bay of Isles samples, while those for the polar fractions from the same samples were 8.9g and 65g, respectively. Analogous EC-50's for the two water samples from Bay of Isles were 1150 ml and 445 ml for the aromatic fractions and 154 ml and 393 ml for the polar fractions, respectively. The toxicity test results with the fractionated extracts showed greater differences between Bay of Isles and Mooselips than were found in the 1990 field survey, in which Bay of Isla sediments were not significantly more toxic either to amphipods or to oyster larvae than those from Mooselips Bay. This difference probably reflects the heterogeneous distribution of oil in beach sediments. Overall, the results of these toxicity tests on these highly concentrated fractions indicate very low sediment toxicity compared to sediments from industrial or urban areas, where Microtox® EC-50's may be 2-3 orders of magnitude lower.

These toxicity measurements tend to confirm previous observations and conclusions that the acute toxicity of crude oil is caused primarily by low-molecular weight aromatic constituents. In short-term exposures, molar toxicity appears to increase with increasing concentration of aromatic rings (i.e., benzene < naphthalene < phenanthrene), at least up through 3-ring compounds, and also with the extent of substitution (i.e., benzene < toluene < xylene < ethylbenzene, etc.) (Rice et al., 1977). All of these more toxic constituents are lost during earlier stages of petroleum weathering, and were significantly depleted in Prince William Sound sediments by the time our samples were collected in fall 1990.

Oxidation products of aromatic compounds in petroleum are produced through microbial metabolism and photooxidation, and intermediary metabolites of polynuclear aromatic compounds are known to be genotoxic. Very little work has been published on these compounds, but some of them could undergo bioaccumulation and exert toxicity to marine organisms. The present studies show, however, that very low genotoxic responses were associated with petroleum in the sediments and pore water from Bay of Isles.

Previous studies also suggested that oxidation products were unlikely to exert significant short-term effects under ambient conditions. For example, although the toxicities of phenol (and p-cresol) were found to be intermediate between those of naphthalene and toluene (naphthalene < toluene < pentane), Korn et al. (1985) concluded that the phenols were not major contributors to the toxicity of water-soluble fractions (WSF) of oil, because the concentrations of toluene and naphthalene were respectively about 50x and 2x-7x higher than that of phenolic compounds in the WSF. Similarly, Malins et al. (1985) identified oxidized products of phenanthrene, including carbonyl, quinone, and carboxylic acid derivatives, in seawater after UV irradiation of a phenanthrene "slick" for 120 hours.

About half of the oxidation products of phenanthrene in the seawater after this UV irradiation were not extractable with methylene chloride, indicating oxidation to highly water-soluble products. UV irradiation of No. 2 fuel oil in a flow-through, agitated system caused less than a 2-fold increase in total extractable or-
ganic materials (compared to an unirradiated SWAF), and no differences were observed in mortalities of English sole embryos exposed for 48 hours. Irradiation under static conditions, however, enhanced the extractable organic material in the SWAF about 23-fold (to 161 ppm), and substantial mortality occurred, with an apparent EC-50 of about 25 ppm.

Preparation of SWAFs from Prudhoe Bay crude oil under identical conditions, however, produced no differences between flow-through and static conditions either in levels of total extractable organic materials or in mortalities of English sole embryos. Malins et al. (1985) concluded that these studies provided no evidence that photooxidation would under most conditions significantly enhance the toxicity of petroleum in the marine environment.

Along with the results presented here, previous studies suggest that polar constituents, whether present in the parent oil or formed as a result of degradation in the environment, do not pose a significant additional risk of toxicity or mutagenicity to marine organisms.

References
Microbial Activity in Sediments following the T/V Exxon Valdez Oil Spill
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Shortly after the grounding of the Exxon Valdez on 24 March 1989, the National Oceanic and Atmospheric Administration (NOAA) organized a multi-investigator cruise to document the extent of oil contamination of coastal habitats in Alaska. This first survey cruise was followed by five seasonal cruises over the next 2 years organized as a joint effort of NOAA and the Alaska Department of Environmental Conservation. The purpose of these survey cruises was to document oil concentration distributions and assess the relative ecological impacts of the spill to intertidal and subtidal areas.

Assessment of microbial populations was an important component of the surveys since a major fate of petroleum contaminants in marine environments depends on the ability of microorganisms to use hydrocarbons as a source of carbon and energy (Leahy and Colwell, 1990). Additionally, patterns of hydrocarbon mineralization activity and distribution of hydrocarbon-degrading microorganisms can be used as an indication of in situ biodegradation of petroleum (Madsen et al., 1991). Measurements of total numbers of hydrocarbon-degrading microorganisms and assays for the mineralization potential of hydrocarbon fractions by these populations provide evidence of the presence of hydrocarbons that can be utilized by microorganisms. When sediments from a pristine environment are perturbed with oil, this distribution reflects the extent, movement and persistence of the contamination.

We sampled 38 sites within Prince William Sound throughout a three year period following the oil spill. In these samples we measured numbers of hydrocarbon-degrading microorganisms and mineralization potentials of radiolabelled hydrocarbon fractions in shoreline sediments and subtidal surface sediments at depths to 100 m. Depending on the cruise, up to 6 isobaths were sampled for each site; intertidal (0 m), 3 m, 6 m, 20 m, 40 m, and 100 m. At the 0 m, 3 m, 6 m, and 20 m isobaths sediment samples were made up of subsamples collected from eight random locations along a 30 m transect parallel to shore by shore party or SCUBA divers. The 40 m and 100 m samples were collected by Van Veen or Smith-Machntyre grabs and subsampled from the surface of the sediment. The number of hydrocarbon-degraders in each sample was estimated by using the SheenScreen most probable number technique which uses disruption of an oil film to indicate the presence of hydrocarbon-metabolizing microorganisms (Brown and Braddock, 1990). Radiorespirometry was used to assay the hydrocarbon-oxidation potential of microorganisms in sediment slurries (Brown et al., 1991). The compounds [1-¹⁴C]-
hexadecane, [1,4,5,8-14C]-naphthalene and [9-14C]-phenanthrene were used as paradigms of aliphatic and polycyclic aromatic hydrocarbons. Hexadecane potentials were determined after 2-day incubations. Two-day naphthalene and phenanthrene potentials were universally very low and reference sites (sites known not to have been oiled by the Exxon Valdez oil spill) were generally 0 or near 0 after 10 days for all cruises. For these reasons, 8- or 10-day incubations were used for naphthalene and phenanthrene data. Significant differences (at the 95% confidence level) for numbers of hydrocarbon-degraders or mineralization potentials at a site compared to the reference sites were determined by the Mann-Whitney U Test (Zar, 1984).

The numbers of hydrocarbon-degrading bacteria vary by several orders of magnitude among sites and dates sampled after the Exxon Valdez oil spill. Ranges for numbers of hydrocarbon-utilizing bacteria during 1989 in this study were similar to those found for the Amoco Cadiz oil spill (Ward et al., 1980). Microbial studies in Alaskan coastal sediments conducted before the Exxon Valdez oil spill are limited in number. A 1975-1977 survey of Cook Inlet and the Gulf of Alaska found the highest mean numbers of hydrocarbon-oxidizing bacteria determined by a plate count method to be 8.4 X 10^5 cells/g dry weight of sediment at a site in upper Cook Inlet near several oil wells (Roubaud and Atlas, 1978). These authors hypothesized that sediments containing 10^3 to 10^5 oil-degrading bacteria/g dry weight probably had a previous history of oil exposure from either biogenic or polluting sources.

In the summer of 1989, eleven shoreline sites in Prince William Sound exceeded the maximum value for hydrocarbon-degraders found in 1978 (Roubaud and Atlas, 1978). In fall 1989 all 14 shoreline sites sampled in this study had high numbers of hydrocarbon-degraders, ranging from 3.6 x 10^9 to 5.5 x 10^9 cells/g dry weight sediment; reference sites had a median of 38 cells/g dry weight sediment. Statistically significant higher numbers of hydrocarbon-degraders were observed at these oiled sites than at the reference sites. Median numbers of hydrocarbon-degrading microorganisms on the shorelines in Prince William Sound decreased from 1989 through 1991. However, there were still several shorelines in the summer of 1991 that had > 10^9 hydrocarbon-oxidizing bacteria/g dry weight sediment. In the summer of 1989, numbers of hydrocarbon-degraders in subtidal surface sediments at depths greater than 6 m were below the detection limits of the assay (<13/g dry weight sediment). However, at some sites by the summer of 1990, there were measurable numbers of hydrocarbon-degraders at all depths (beach through 100 m). Data from the summer of 1991 show a trend toward much lower total numbers of hydrocarbon-degraders for all sites and depths, implying that conditions are no longer favorable for biodegradation or that biodegradable hydrocarbons are no longer present.

The median 2-day hexadecane mineralization potentials maintained a fairly constant level through the fall of 1990 and then dropped dramatically by the summer of 1991. In spring and summer of 1990 many sites, even at depth, had potentials for hexadecane mineralization significantly greater than the reference sites. However, in the fall of 1990 only a few sites had potentials significantly greater than the reference sites. By the summer of 1991 potentials of hexadecane
mineralization were low at all sites. The reduction of the hexadecane mineralization potentials may be due to a decrease in numbers of microorganisms acclimated to hydrocarbon biodegradation or a decrease in the hexadecane remaining in the sediment, or some combination of the two factors.

Median potentials of polycyclic aromatic hydrocarbons (PAH) oxidation increased with time from the summer of 1989 reaching a maximum in 1990 and then dropping to much lower levels in 1991. The potentials for phenanthrene mineralization were slightly greater when mineralization potentials for naphthalene and phenanthrene were run on the same sediment samples (Fall, 1989). This finding is supported by a previous study of polluted sediments in Boston Harbor, Massachusetts, where naphthalene turnover times in the Harbor were found to exceed those for phenanthrene (Shiari, 1989). The difference in potentials between phenanthrene and naphthalene seen in Prince William Sound is unlikely to exclusively account for the increase in mineralization potentials of PAH between summer of 1989 and summer of 1990. Mineralization potentials for phenanthrene remained high through the fall of 1990 but declined substantially by the summer of 1991. The data for the summer of 1991 show that there were still many sites with high phenanthrene oxidation potentials relative to the reference sites. However, the absolute values for mineralization potential were much lower than for previous cruises.

The objective of our study was to document the impact of the Exxon Valdez oil spill on the population and activity of hydrocarbon-degrading microorganisms in sediments in Prince William Sound. The numbers and activity of these microorganisms are good indicators of exposure of sediments in Prince William Sound to hydrocarbons and may be useful indicators of the mobilization of hydrocarbons with time. The increase of numbers of hydrocarbon-degraders compared to likely pre-spill values, coupled with high mineralization potentials for hexadecane and phenanthrene, also provide evidence of rapid acclimation of naturally occurring microbial populations for biodegradation of these compounds in most sediments.

References
Contamination of Subtidal Sediments by Oil From the Exxon Valdez in Prince William Sound, Alaska

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The purpose of this project was to assess the degree of petroleum hydrocarbon contamination of subtidal sediments from 32 locations in Prince William Sound resulting from the Exxon Valdez oil spill. In this paper we summarize some geographical, bathymetric and temporal trends resulting from analysis of data collected during the first 2 years following the oil spill.

We sampled sediments intertidally and at five subtidal depths in the range 0-100 m in summer and 0-20 m in spring and fall. Shallow sediments (0-20 m) were collected by beach teams or divers on 30 m transects laid along the appropriate isobath. Deep sediments (40 and 100 m) were collected with grabs. Triplicate composite sediment samples were collected at each station. Sediments were sampled in May, July, September and November 1989 and in June, July and September 1990. Oil concentrations, reported in mg/g (ppt), are estimates of equivalent concentrations in sediments of original (fresh) Exxon Valdez oil and are based on a weathering model developed in conjunction with principal components analysis (PCA; Short and Heintz, these proceedings). Exxon Valdez oil concentrations less than 25 µg/g sediment are not detected using this model because corresponding concentrations of individual polynuclear aromatic hydrocarbons on which the model is based approach analytical method detection limits of about 1 ng/g.

Geographical distribution:

Petroleum hydrocarbons were found to have contaminated subtidal sediments over a broad geographic range in Prince William Sound from the north end of Eleanor Island to southern Elrington Island. Lower intertidal (0 m) sediments were contaminated by Exxon Valdez oil at no fewer than nine locations in 1989 and 12 locations in 1990. Subtidal sediments were contaminated by Exxon Valdez oil at no fewer than 12 locations where oil had come ashore (oiled locations) in 1989 and 1990. Those locations constituted 67% of oiled locations studied in 1989 and 86% of those oiled locations studied in 1990. Contamination of subtidal sediments by Exxon Valdez oil at oiled locations reached a depth of at least 20 m at seven sites in 1989 and at 14 sites in 1990.

Bathymetric distribution:

The greatest concentrations of Exxon Valdez oil in benthic sediments were found in the lower intertidal region (0 m). An average concentration (n=3) as high as 24.7 mg/g was found at 0 m on Disk Island in July 1989. The greatest concentrations of Exxon Valdez oil in subtidal sediments were found at the shallow depths. The highest concentration recorded was 5.2 mg/g in a sediment sample collected at about 3 m at Sleepy Bay in September 1989. Concentrations of Exxon Valdez oil exceeding 1.0 mg/g in subtidal sediments occurred at nine locations in 1989 and reached a depth of 20 m at Eshamy Bay in July 1989. How-
ever, no significant difference was found in the concentration of oil between depths at oiled sites in July (when all depths were sampled). In 1990 the highest concentration of oil in subtidal sediments recorded was 4.3 mg/g at 20 m at Fox Farm in September. Concentrations of oil exceeding 1.0 mg/g were found at two locations (Herring Bay and Fox Farm) reaching a depth of 20 m at both sites.

In July 1990 when all depths were again sampled oil concentrations in sediments at oiled sites were greater (p<0.01) at 3 and 6 m than at greater depths. The majority of sediments from 40 and 100 m were found not to be contaminated with Exxon Valdez oil. Where contamination was found it was at relatively low concentrations (≤0.43 mg/g, 1989; ≤0.45 mg/g, 1990) of oil.

**Temporal distribution:**

Examination of temporal changes in the contamination of sediments by oil revealed that detectable quantities of Exxon Valdez oil moved over time to shallower subtidal depths at locations with heavily oiled shorelines. At Sleepy Bay no significant trend was seen in the concentration of oil in sediments at mean lower low water (0 m) between May 1989 and September 1990. Nevertheless, over the same period of time subtidal sediments at 3, 6 and 20 m in Sleepy Bay showed increasing oil concentrations to a peak concentration followed by a decline to levels comparable to early post-spill levels or less. At the 3 m depth the peak (p<0.05) occurred in September 1989. The peak occurred in November 1989 at 6 m (p<0.005) and 20 m (p<0.01) and persisted at 6 m until June 1990.

At Northwest Bay and Herring Bay also there was some evidence that oil moved to greater depths over time. Northwest Bay and Herring Bay showed significant decreases (p<0.01 and p<0.001 respectively) in the concentration of oil in sediments at mean lower low water (0 m) between May 1989 and September 1990. At both sites, the oil concentrations in sediments collected at 3 m did not change significantly between May 1989 and September 1990. At Northwest Bay the concentration of oil in sediments at 6 m peaked (≈0.95 mg/g, p<0.05) in September 1989, whereas oil concentrations in sediments at 20 m showed no significant change during the study period. At Herring Bay no significant change occurred in oil concentrations in sediments at 6 m during the study period, but concentrations changed at 20 m reaching a maximum (≈0.55 mg/g, p<0.05) in September 1990.

Oil was detected in subtidal sediments at a number of locations in Prince William Sound but concentrations were markedly less than in sediment samples from heavily oiled intertidal sites. Oil became broadly distributed in subtidal sediments during the first 2 years following the Exxon Valdez oil spill. Oil concentrations attained their highest values in the low intertidal and shallow subtidal (0-20 m) regions.

Sediments collected at 40 and 100 m were for the most part not contaminated with Exxon Valdez oil. There was some indication that some movement of oil down slope took place at heavily contaminated sites. Although oil concentrations in subtidal sediments were probably not acutely toxic to most organisms, the low-level oil concentrations were widespread, persistent over the 2-year period, and would be a source of chronic exposure to subtidal communities.
Determination of Petroleum-Derived Hydrocarbons in Seawater Following the Exxon Valdez Oil Spill: Analysis of Seawater Extracts

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We analyzed samples of subsurface seawater within Prince William Sound following the Exxon Valdez oil spill, to evaluate the extent of water contamination by petroleum hydrocarbons. These samples were collected in three groups beginning 1, 3, and 5 weeks following the oil spill. Triplicate samples were collected from depths of 1 and 5 meters at the 30 locations sampled, which ranged from heavily oiled locations to control locations that were not affected by the spill. All three of the triplicate samples were analyzed from the first group of samples collected, but only one of the three triplicate samples was analyzed from each of the subsequent two groups. Each 900 ml seawater sample was extracted twice with a total volume of 75 ml dichloromethane within 5 minutes of initial collection, then stored at -20°C until analysis.

These samples were analyzed using single ion mode gas chromatography-mass spectrometry (GCMS/SIM) for the most abundant 2 to 5 ring polynuclear aromatic hydrocarbons (PAHs) 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 (C10 to C30).

During the first sampling period, summed PAHs were highest at sampling stations adjacent to beaches that were heavily contaminated by the spilled oil. Summed PAH concentrations ranged up to 6.60 ± 0.62 μg/L seawater (95% confidence interval, n = 3) at Snug Harbor, and ranged from 1.92 ± 0.40 μg/L to 5.23 ± 1.27 μg/L at sampling stations near heavily oiled beaches of Northwest Bay, Herring Bay, southeast Eleanor island, north Smith island, and the Bay of Isles. These summed PAH concentrations include PAHs from any source, and do not distinguish PAHs associated with particulate oil and dissolved PAHs. Elevated PAH concentrations were also detected at several more open-waters sites between Knight and Montague islands. At all these sites, summed PAH concentrations were slightly higher at the 1 m depths than at the 5 m depths.

In contrast, summed PAHs were lowest at sampling stations that were near the margin or else were distant from the path of the spilled oil through the Sound. PAH concentrations typically ranged from 0.4 ± 0.2 to 1.5 ± 0.6 μg/L seawater at these locations.

The relative concentrations of individual PAHs differed markedly among the sampling sites. At sites near the margin or distant from the path of the spilled oil, naphthalene was the predominant PAH compound detected, accounting for 40% to 100% of the summed PAHs found. Although naphthalene was consistently detected at both 1 m and 5 m at these sites, other PAHs were only sporadically detected at concentrations near detection limits.

However, at sites near heavily oiled beaches, or at the more open-water sites between Knight and Montague islands.
where elevated PAH concentrations were found, numerous PAH's were simultaneously detected at concentrations substantially above detection limits. Naphthalene accounted for generally less than 40% of the PAH's found at these sites, and the proportion decreased with increasing summed PAH concentrations.

At sites near heavily oiled beaches, or at the more open-water sites between Knight and Montague islands where elevated PAH concentrations were found, relative concentrations of detected PAH's are very highly correlated with corresponding relative PAH concentrations of Exxon Valdez crude oil. Product-moment correlation coefficients of PAH's in Exxon Valdez crude oil and in samples from these sites generally range from 0.85 to 0.95 (p < 0.001) with 14 to 18 PAH's included in the correlation (but with naphthalene excluded). Also, PAH's that are absent or present at low relative concentrations in Exxon Valdez crude oil were not detected in these samples.

We conclude from these results (1) that Exxon Valdez crude oil is the proximate source of PAH's in samples where measured PAH's are elevated and strongly correlated with Exxon Valdez crude oil PAH's, and (2) that an additional source of naphthalene is present in all samples, suggesting an unknown sampling contamination source for naphthalene only. The first conclusion derives from the close association of samples containing elevated PAH concentrations with areas directly impacted by the spilled oil, the strong correlation of relative PAH concentrations in these samples and in the spilled oil, the general absence of these PAH's in samples from areas marginal or distant from the path of the spilled oil, and the absence of a plausible alternative explanation of these observed results. The second conclusion derives from the ubiquity of naphthalene at a minimum apparent concentration of about 0.4 µg/L seawater; a similar pattern of naphthalene detection persisted in the second and third sampling periods.

We estimated total PAH's attributable to Exxon Valdez crude oil in samples where PAH's are strongly correlated. This estimate is the sum of measured PAH's except naphthalene; plus an amount of naphthalene proportional with measured amounts of 1- and 2-methyl-naphthalene, consistent with this same proportion in Exxon Valdez crude oil. In every case, the naphthalene calculated in this manner as attributable to Exxon Valdez crude oil is less than the measured amount of naphthalene in the sample.

Exxon Valdez oil PAH's (EVO-PAH) are quantitatively parallel with summed PAH's: EVO-PAH concentrations ranged up to 6.24 ± 0.63 µg/L seawater at Snug Harbor, and ranged from 1.26 ± 0.40 µg/L to 4.72 ± 1.18 µg/L at sampling stations near heavily oiled beaches of Northwest Bay, Herring Bay, southeast Eleanor island, north Smith island, and the Bay of Isles. Elevated EVO-PAH concentrations were also detected at several more open-water sites between Knight and Montague Islands. At all these sites, EVO-PAH concentrations were generally somewhat higher at the 1 m depths than at the 5 m depths.

EVO-PAH concentrations generally declined with time. At most sites, EVO-PAH concentrations declined by a factor of 2 or more from the first to the second sampling period, and by more than a factor of 2 from the second to the third sampling period. Exceptions included sites where oiled beach clean-up activities had commenced, such as at Herring
Bay or Snug Harbor, where EVO-PAH concentrations increased slightly by the second sampling period at some depths. EVO-PAH’s were not evident at any open-water site after the first sampling period, and were generally less than 1 µg/L seawater at any site by the third sampling period.

Comparison of EVO-PAH concentrations and C_{19} through C_{27} n-alkane concentrations suggests the presence of at least some particulate oil in the samples that contained EVO-PAH’s. To estimate relative proportions of dissolved and particulate EVO-PAH’s, we calculated an aromatic hydrocarbon enrichment factor (AHEF) as the ratio of EVO-PAH measured in a sample, and the minimum expected EVO-PAH based on measured C_{19} through C_{27} n-alkane hydrocarbons. At sites where PAH’s are strongly correlated with Exxon Valdez PAH’s, this AHEF ranged from 0.69 to 5.99. Values of this AHEF near 1 are consistent with particulate oil, whereas values substantially above 1 indicate dissolved PAH. However, this AHEF index is not rigorous indicator of particulate oil, because values near 1 may by chance be due to dissolved EVO-PAH’s present with odd carbon-numbered alkanes derived from natural sources.

The relative concentrations of dissolved EVO-PAH’s suggests they are determined by dissolution kinetics, and not by solubility of individual PAH’s. Relative dissolved PAH concentrations that correlate strongly with those of Exxon Valdez crude oil, suggests that the composition of dissolved PAH’s matches that of the oil. The initial relative rates of dissolution of individual PAH’s are determined by differences among individual PAH’s of molecular attractive forces to the seawater phase and to the remaining oil phase, and by the relative concentrations of the PAH’s in the oil. Differences of these attractive forces among PAH’s are approximately proportional with molecular surface area, which varies by less than a factor of 2 among the EVO-PAH’s (naphthalene through C-4 phenanthrene), whereas the relative concentrations of these compounds in the oil vary more than 30-fold. Initial dissolution rates are therefore mainly determined by relative concentrations in the oil. In contrast, solubility is not an important factor because concentrations attained by dissolved PAH’s are well below solubility limits, and the volume of affected seawater in Prince William Sound is much greater than the volume of the spilled oil, thereby guaranteeing that solubility limits of EVO-PAH’s are never approached.

Although readily detectable, these concentrations of EVO-PAH’s are well below levels that are acutely toxic to marine fauna. On the other hand, these data demonstrate that PAH’s from Exxon Valdez crude oil were available to subsurface marine fauna the first few weeks following the oil spill, especially in nearshore, near-surface waters that are particularly productive areas biologically. In addition, if mononuclear aromatic hydrocarbons had been measured in addition to the PAH’s that were measured, total aromatic hydrocarbon concentrations in the seawater column derived from spilled Exxon Valdez oil would almost certainly have been higher, possibly exceeding the State of Alaska water quality standard of 10 µg/L seawater, because mononuclear aromatic hydrocarbons are much more abundant than PAH’s in crude oil.
Methods for Determining Crude Oil Contamination in Sediments and Biota After the *Exxon Valdez* Oil Spill

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The grounding of the *Exxon Valdez* on March 24, 1989 spilled almost 11 million gallons of Prudhoe Bay crude oil (PBCO) into the waters of Prince William Sound, Alaska. As part of the Natural Resource Damage Assessment effort, thousands of samples of sediment and biota were collected to determine the distribution of the spilled crude oil and the exposure of the marine animals. Therefore, the use of rapid, low-cost analytical methods, generally known as screening methods, to estimate concentrations of petroleum-related aromatic compounds (ACs) in samples was vitally important to the production of timely information in the emergency response. Screening methods can rapidly process large numbers of samples to provide a semiquantitative estimate of contaminant concentrations and thus, allow ranking of samples by degree of contamination. Accordingly, the most contaminated samples can be located by screening; then, detailed analyses, e.g., gas chromatography/mass spectrometry (GC/MS), can be focused on the selected samples to confirm the presence of contaminants. Screening for metabolites of ACs in fish and marine mammals

Thousands of samples of fish and marine mammals were collected from the *Exxon Valdez* spill area to determine the exposure of these animals to PBCO. Because fish and marine mammals extensively metabolize most ACs in their livers and the metabolites are transferred to bile for excretion, AC metabolites must be measured in these animals to establish their exposure to PBCO. Concentrations of metabolites were estimated in bile of fish and marine mammals using a reverse-phase high-performance liquid chromatographic (HPLC) screening method that measured fluorescence at wavelength pairs specific for 2- and 3-ring petroleum-related ACs (Krahm et al. 1992). Then, GC/MS was used to validate the HPLC screening results by measuring concentrations of individual metabolites of petroleum-related ACs, e.g., alkyated naphthols and phenanthrols, in these animals. Because the concentrations of metabolites measured by HPLC screening and sums of AC metabolites from GC/MS were highly correlated, the bile screening method was validated as an effective tool for estimating concentrations of AC metabolites. Screening for crude oil in sediments

Following the *Exxon Valdez* spill, thousands of sediment samples were collected to determine the degree and distribution of the oiling. Because analyzing all these samples by GC/MS would be excessively expensive and time-consuming, priorities for analyses needed to be set. Therefore, a size-exclusion HPLC method used previously to measure AC contaminants in urban sediments (Krahm et al. 1991) was employed to determine concentrations of PBCO in more than 400 sediment samples. Sediments from a large number of sites in the spill area...
were surveyed and many were found to be contaminated by PBCO (Krahn et al. submitted). Similar to the results for bile, summed concentrations of individual ACs in the sediments determined by GC/MS were found to be highly correlated with the concentrations of ACs measured by HPLC screening method. Thus, the utility of the rapid HPLC screening method has been extended to analyzing sediment samples for the ACs characteristic of crude oil, thereby directing priorities for GC/MS analyses. As a result, the overall costs of the analyses have been reduced, while still providing the necessary detailed data in a timely fashion.

Establishing source of contamination by HPLC and GC/MS analyses

When fish or marine mammals were exposed to PBCO in the field or injected with PBCO in the laboratory, the chromatographic patterns were similar, but some differences were also apparent. Variations in bile chromatographic patterns can occur because of variations in the degree of exposure of individual animals to the oil or to species-specific differences in metabolism of the petroleum ACs.

In addition, physical factors from the chromatographic process itself, such as the chromatography column used, the condition of the column or the acidity of the mobile phase, can affect the appearance of a chromatogram in reverse-phase HPLC. Therefore, due to both the variability of the metabolic process in various fish species and to the variability of the reverse-phase chromatography of the metabolites, the HPLC chromatographic pattern of bile can only suggest the type of contamination. However, the source of contamination can often be established by examining GC/MS results. For example, evidence of PBCO contamination in fish and marine mammals was provided by identifying high proportions of certain bile metabolites (i.e., the alkylated naphthols, phenanthrols and dibenzothiophenols) that result from the metabolic conversion of ACs that are characteristic of PBCO (Krahn et al. 1992).

The HPLC chromatograms from sediment are easier to interpret than those from bile. These chromatographic patterns are less variable than those from bile, because the size-exclusion chromatography is stable and because sediment screening measures the ACs themselves. Many of the confounding factors present in the bile chromatograms due to species-specific differences in degree of metabolism or excretion of metabolites are not found in the sediment chromatograms. However, HPLC chromatographic patterns were not consistent among all the extracted sediments from the Prince William Sound area. The differences were not due solely to the degree of weathering of the crude oil, but reflected different sources of ACs, e.g., crude oil or diesel fuel (Krahn et al. submitted).

For example, the chromatograms of Herring Bay and Knight Island sediments, two sites that were heavily oiled, were nearly superimposable with those from weathered PBCO. Furthermore, the chromatographic patterns from the Herring Bay and Knight Island sediments were very different from those of other sources of contamination (e.g., diesel fuel or marine lubrication oil) that might be found in Alaskan sediments. In contrast, results from screening sediments from MacLeod Harbor and Olsen Bay, sites not in the direct path of the
spill, revealed low concentrations of ACs and an HPLC chromatographic pattern that resembled that of diesel fuel. The contaminant source suggested by HPLC screening of these sediments could often be confirmed by comparing the identities and proportions of the ACs determined by GC/MS to similar characteristics of the probable sources. For example, evidence for PBCO as the source of contamination in many Prince William Sound sediments (e.g., those from Herring Bay and Knight Island) was provided by identifying in these samples the high proportions of the alkylated naphthalenes, phenanthrenes and dibenzoanthiphenes that are characteristic of this crude oil (Krahn et al. submitted).

The HPLC screening methods have important roles in evaluating anthropogenic contamination in samples of bile and sediment. First, samples containing AC contaminants can be rapidly ranked by degree of contamination and second, HPLC chromatographic patterns can provide a basis for suggesting possible contaminant sources. As a result, expensive GC/MS resources can be effectively allocated. This approach—combining HPLC screening for petroleum-related ACs or their metabolites in sediment and bile with confirmation of contaminant concentrations in selected samples by GC/MS—has proven useful in establishing the extent of damage to natural resources following the Exxon Valdez oil spill.

References
Qualitative and Quantitative Determination of Exxon Valdez Crude Oil in Sediment Samples Using Principal Component Analysis of Hydrocarbon Data

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We have developed a model for sediment hydrocarbon data that may be used to (1) distinguish Exxon Valdez oil contamination from other sources of hydrocarbons, (2) estimate the original amount of Exxon Valdez oil in sediments when present, and (3) determine the relative degree of weathering of a sample. The model is derived from an assumption of first-order loss kinetics of each of the hydrocarbon analytes employed, where principal component analysis is used to identify a weathering pattern that is characteristic of spilled Exxon Valdez crude oil. When applied to consistent sediment hydrocarbon data sets derived from samples that were collected from known oiled beaches, one principal component accounts for more than 96% of the data variance. First-order rate constants for hydrocarbon analyte losses were estimated using this data subset, and the relative magnitudes of these constants indicates that the weathering process is predominantly kinetically controlled, where the rate of loss of aromatic hydrocarbon analytes decreases with extent of alkyl substitution.

Use of this model as an interpretive aid and as a unifying framework will be presented, together with results of the application of the model to hydrocarbon data derived from over 2,200 Natural Resource Damage Assessment sediment hydrocarbon samples collected from 1989 through 1991.
Nearshore Subtidal Transport of Hydrocarbons and Sediments Following the Exxon Valdez Oil Spill

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From 1989-1992, subtidal sediment traps were placed in Prince William Sound to capture settling organic and mineral particulate matter offshore of oiled and unoiled shorelines. As a component of water quality, settling particulates were collected to: (1) determine if petroleum hydrocarbons were present; and (2) to learn more about subtidal sediment transport processes affecting sediments at the study sites.

Sedimentation of hydrocarbons is a rapid and important fate of spilled oil. Estimates of accumulation in the subtidal include 8-10% of the Amoco Cadiz oil off the Brittany Coast (Gundlach et al., 1983) and 10-15% of the unrecovered Texis oil in the Swedish archipelago (Johansson et al., 1980). Oil can sink by adsorption to sediment, possibly by electrostatic bonding to fine-grained clay micelles (Bassin and Ichiye, 1977), and through uptake by zooplankton and subsequent deposition in fecal pellets (Conover, 1971). Salinity, clay minerology, and the presence of organic matter (which may mask adsorption sites) can affect the adsorption (Meyers and Quinn, 1973).

Oiled beaches act as a reservoir from which hydrocarbons may be removed by erosion and deposited in offshore sediments. Ten percent of the oil stranded on untreated shorelines after the Baffin Island Oil Spill project was transported into shallow offshore subtidal sediments (3-7 m depth) (Boehm et al., 1987). While the bulk of stranded shoreline oil was removed from Prince William Sound beaches in 1989 and 1990, by a combination of treatment activities, natural physical processes and biodegradation, a number of locations have intertidal subsurface oil lenses that are still fluid (at least during summer months) and that are persistent because of protection from surface weathering processes.

Persistence and mobilization of spilled oil is related to physical and biological processes, such as wave, tide and wind energy, microbial degradation and bioturbation. Sediment grain size and oil quantity and composition are among many interacting variables (Blount, 1978; Gundlach et al., 1978). Oil eroded from contaminated shorelines and entering the water column may settle in the nearshore subtidal or move into deeper waters before settling depending on particle size and shape, settling velocity, wave energy, tidal current velocities, and longshore currents (Gundlach et al., 1978). After settling on the benthic surface, oiled sediments can continue to be moved by bottom currents, resuspended by wave-induced oscillatory currents, or be buried deeper into the benthic sediments by bioturbation.

A laboratory study by Bragg et al. (1990) using oiled sediments from Prince William Sound shorelines found that the formation of an emulsion of micron-sized
mineral particles, polar components of oil residue and seawater impeded the adhesion of oil to the larger rocks on the shorelines, allowing natural removal by waves and tidal flushing. Bragg concluded that since the emulsion floc particles were composed mainly of seawater and fine-grained sediments, they would be transported great distances before settling and would be widely dispersed. While this is supported by Stokes Law of settling velocities for individual fine sediment grains, sediments may be trapped in estuaries as physicochemical flocculation with other particles in the water column produce settling rates an order of magnitude greater than the individual grains (Drake, 1976; Kranck, 1975).

Methods

Sediment traps have been used after oil spills to monitor settling particulates for oil contamination (Tsese spill in 1977, Johansson et al., 1980) and to determine sedimentation rates in embayments (Lund-Hansen, 1991) and the open ocean (Woods Hole, 1989). For this study, base-mounted sediment traps consisting of PVC pipe, 15 cm in diameter and 1.2 meters tall were deployed at sites in Prince William Sound representing a variety of oiling and coastal conditions. The traps were placed at 10, 15, and 20 meter water depths offshore of oiled and unoiled shorelines. Divers retrieved and redeployed the traps at approximately 3 month intervals from November 1989 through mid-March 1992. Sediments were immediately filtered from the traps on the vessel and samples were frozen for later hydrocarbon chemistry and grain size analysis.

Benthic core samples were taken at each of the sediment trap locations to evaluate the sedimentary processes at work at the sites, such as erosional and depositional events, and define background hydrocarbon concentrations and depth of petroleum hydrocarbon contamination. Sediment samples from the upper 2 cm of benthic sediments around each trap were taken to evaluate grain size distributions and hydrocarbon chemistry. The relative contribution of sub-tidal transport processes (bed-load, saltation and suspension) (Visher 1969; Middleton, 1976) and delineation of erosional and depositional events (Sundborg, 1956) will be estimated by evaluation of grain size distributions and inspection of the sediment cores.

In addition, approximations of wave energy at particular sites are being calculated from hindcasts using the Automated Coastal Engineering System (U.S. Army Corps of Engineers, 1991) and wind data from the National Oceanic and Atmospheric Administration and National Weather Service stations in Prince William Sound. Results from the hindcasts will provide wave parameters from which bottom stresses can be derived (Komar, 1974). Bottom stress calculations combined with grain size data will allow estimations of the likelihood of sediment resuspension and transport by waves.

Results

Two years after the spill, elevated concentrations of petroleum hydrocarbons were consistently found in trapped suspended particles near initially heavily oiled shorelines. Sediments retrieved from the traps at five sites in August 1990 showed petroleum hydrocarbon patterns consistent with Exxon Valdez crude oil, with the highest concentrations at heavily oiled Sleepy Bay, and lowest at the unoiled control site in Port Fidalgo, indicating an association of petroleum hydrocarbons and trapped sediments with
oiled shorelines.

This association persists in sediments captured over the winter of 1990-91 at 13 sites (retrieved in March 1991). The pattern of hydrocarbons is substantially altered however, with consistent and substantial enrichment of chrysenes relative to the other aromatic hydrocarbon classes at each trap location. While the reasons for this alteration of aromatic hydrocarbon patterns is not well understood, the pattern could reflect complex weathering processes.

The highest concentrations of petroleum hydrocarbons found in March 1991 were in trapped sediments from offshore of heavily oiled locations in Northwest Bay, Sleepy Bay and Snug Harbor, while the lowest concentrations were at unoiled or lightly oiled locations in Eshamy Bay, Stockdale Harbor and Port Fidalgo. This pattern again demonstrates a clear association between concentrations of petroleum hydrocarbons in trapped sediments and degree of oil impact on the adjacent shoreline. Several sites with high concentrations in trapped sediments have subsurface oiling: a persistent lower-inertial subsurface lens of fluid oil (documented by ADEC shoreline surveys as late as June 1992) remains at Northwest Bay, and significant subsurface oil has been noted on shoreline segments in Sleepy Bay. Benthic sediment samples collected adjacent to the sediment traps in August 1990 also indicate elevated petroleum hydrocarbon concentrations at trap sites adjacent to oiled shorelines. Further conclusions await the results of remaining hydrocarbon analysis.

Hydrocarbon chemistry analysis of the remaining sediment trap, benthic and core samples will be completed by January 1993. Results of grain size, minerology and organic carbon analyses of the sediment trap and benthic samples are currently being evaluated and will be related to the hydrocarbon results for each site and deployment period to correlate sedimentology with petroleum hydrocarbons. Benthic sediment core stratigraphy will be evaluated for background chemistry and any depositional events. Grain size frequency distributions are being evaluated for an understanding of transport mechanisms at each site.

References


Short-term Biological Effects of Shoreline Treatment on Intertidal Biota Exposed to the *Exxon Valdez* Oil Spill

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A substantial amount of the 11.6 million gallons of Alaska North Slope crude oil spilled from the *T/V Exxon Valdez* on March 24, 1989, was deposited on beaches in Prince William Sound. Following the spill, biological studies were conducted on the biota of the intertidal and shallow subtidal habitats in the Sound to determine short-term effects of several shoreline treatment techniques considered for beach cleanup. Four treatment methodologies examined were high-pressure hot-water (HP-HW) and low-pressure warm-water (LP-WW) wash, and applications of a dispersant (Corexit 7664) and a beach cleaner (Corexit 9580 M2). These methods were designed to remobilize oil that coated the substrate and facilitate its removal from the beaches. Only LP-WW and HP-HW treatments were employed on a routine basis in the Sound.

The basic objective of these studies was to assess and compare the short-term biological impacts of several alternative treatment methodologies. The major elements of these studies, conducted on three islands at the north end of the Knight Island Archipelago in protected boulder/cobble habitat, were pre- and post-treatment measurements of abundance, cover, and community composition of the biota at specific levels in the test areas. These studies employed a stratified-random design with replicate quadrat sampling before and after implementation of the specific shoreline treatment methodologies. Each program compared two treatment alternatives; the programs were independent and not compared at the time.

In all test series, each test plot was exposed to a different treatment. Contrasted tests were sequential rather than simultaneous. Test plots extended from upper to lower intertidal but treatment was supposed to be restricted to mid and upper levels, where most oil came to rest during the initial stranding. We surveyed at least at mid and lower elevations in each test plot.

The purpose of this paper is to present data from several treatment effect studies and provide a general summary of short-term biological effects of treatment. Secondarily, we are presenting a qualitative assessment of the validity of the studies themselves.

The relevant questions to be addressed by these studies include: (1) Was treatment accompanied by biological damage? (2) Was the damage caused by thermal, chemical, or physical effects? (3) Which type of treatment caused the least amount of damage? and (4) Was the observed damage long-lived?

An operative assumption for these studies was that the variables examined would not change significantly over the 3 to 10 days between the pre- and post-treatment surveys except as a consequence of the treatment regime associated with the studies. We applied this
assumption to comparisons of all variables examined, ranging from oil cover and cover intensity to community descriptors (e.g., number of species and abundance of individuals) and species abundance.

Our approach was to compare pre-treatment data for variables such as algal and epifaunal cover, epifaunal density, average number of taxa per quadrat, species diversity, and density of dead epifauna, and species composition with post-treatment measurements from the same transects. Additionally, in response to thermal impacts, we measured cover by dead *Fucus gardneri* at Herring Bay following the HP-HW treatment. We applied a stratified-random design stratified on the basis of elevation. We designated mud and lower intertidal strata at all sites based on the dominant biological assemblages; an upper intertidal stratum was included in the Corexit 7664 study at Ingot Island.

Types of effects observed included reductions in density and obvious mortality due to abrasion, crushing, or thermal exposure. Generally, all treatments in the upper and mid intertidal appeared to reduce cover by live algae but HP-HW treatment at Herring Bay and LP-WW treatment in the Corexit 9580 M2 test appeared to cause the most damage (p < 0.01). The greatest decrease in algal cover occurred in the middle intertidal, where treatment and high algal cover overlapped. Changes in algal cover in the lower intertidal zone were not significant.

Epifaunal cover in the upper and middle intertidal, highest in the middle intertidal zone, was commonly 50 percent lower following treatment, but generally low cover and high small-scale variability confounded the statistical significance of the changes. Epifaunal cover in the algae-dominated lower intertidal was quite low and stable.

Epifaunal density was generally higher at lower intertidal levels. Strong decreases (100-fold) occurred in the HP-HW treatment tests in the middle intertidal (p < 0.01). A significant decline following the 165-minute HP-HW treatment at the lower level at Herring Bay reflects reduced density of a hermit crab and a periwinkle. Density changed inconsistently following the other types of treatment, suggesting that impacts from those methods were not strong. Moderate but insignificant increases in density in both tests at Ingot Island seem to represent an increase in abundance of scavengers like hermit crabs, probably in response to the increased availability of dead or damaged organisms.

The average number of taxa per quadrat tended to decline following treatment. Strongest declines occurred at both tide levels at Herring Bay following HP-HW treatment (p < 0.01).

We measured density of dead epifauna to provide an indication of mortality in animals such as crabs and mussels. Density was highest and changes were stronger at mid intertidal levels where mussels were most abundant. Large increases in dead animals observed following HP-HW treatments reflect higher numbers of gaping attached mussel shells with intact tissues, suggesting a strong impact from HP-HW treatment. Density of dead animals decreased following treatment in all other tests, probably reflecting the tendency of the wash activities to flush loose materials from the areas.

Several taxa displayed substantial changes in abundance during the tests. Significant changes for algae were mostly
declines that probably resulting from vigorous washing. In a few cases, algal cover increased significantly, possibly because of increased visibility following washing.

Many of the significant changes observed in invertebrates probably reflect removal or relocation caused by the vigorous washing. Barnacles, periwinkles, limpets, mussel, hermit crabs, and a whelk declined significantly at some sites. At other sites, density of periwinkles, a whelk, and a hermit crab increased significantly, probably as a reflection of relocation by the treatment or immigration in response to increased abundance of dislocated prey.

Abundance of dead specimens increased substantially for two species at one site. Density of dead mussels and cover by dead Fucus increased significantly at mid levels at Herring Bay following both HP-HW treatments. HP-HW treatment caused Fucus to change from a normal olive-green color to blackish orange, the color of dead Fucus in drift wrack accumulations. HP-HW treatment for 165 minutes caused twice the mortality as 95-minute exposure.

The type and number of significant changes varied considerably by elevation and type of treatment, probably reflecting the position of the zone relative to washing activities and rigor of washing. Far more of the significant changes were decreases. Significant changes were far more common at the mid and lower tide levels than at upper level but 80 percent of the significant changes at upper levels were declines. Live organisms were generally less abundant following treatment at the mid levels although periwinkles increased considerably at some locations. In contrast, changes at lower levels were more evenly distributed between decreases and increases.

The relative frequency of significant changes in abundance varied considerably by type of treatment. Corexit and LP-WW treatments were accompanied by relatively few decreases whereas nearly all changes observed following HP-HW treatment were decreases.

Several flaws in sampling design weakened the ability of the studies to evaluate treatment effects. Comparability of substrate and biota in paired test plots was weak. For example, algal cover at the Disk and Ingot Island sites and epifaunal cover, beach slope, and sediment composition at Disk Island varied considerably between the Corexit and LP-WW test plots.

While much of the protected shoreline in Prince William Sound is mixed soft substrate, systematic evaluations of treatment effects were not conducted in these habitats. In the one instance where the study area included soft substrate, density of hardshelled clams declined from about 160/sq. m. to about 40/sq. m. overnight. This 75-percent reduction in density was probably a consequence of physical effects but because similar habitat was not present in the paired test plot, the cause of mortality is unclear.

Overall, the data suggest that the effects of both chemicals were less severe than those caused by LP-WW or HP-HW treatment. The data, our observations, and a review of the study design also indicate that LP-WW wash accompanying tests of chemical efficiency was less rigorous than when performed by itself. The tests were not comparable in terms of temperature regimes, duration, or consistency of coverage.

Because of the proximity of the paired sites and timing differences in testing, the probability of cross-test interactions
is high. Such interactions could explain several declines in variables at Disk Island where two pre-treatment surveys were conducted.

The absence of reference sites to evaluate the "No-treatment" alternative constitutes a major design flaw since no data exist to support the operative assumption for the tests, i.e., changes observed during the various tests did not occur universally throughout the Sound at unoiled sites.

In summary, the various treatments were accompanied by biological damage, including reductions in density and obvious mortality due to abrasion, crushing, and thermal exposure. While the type and degree of damage varied by type of treatment, some damage accompanied all types of treatment examined. Damage was manifest as (1) a significant degree of reduction in one or more community or population attributes; or (2) increases in the percentage of dead mussels or Fucus. Severity and persistence of effects varied by type of treatment. However, the programs were not designed in a manner allowing discrimination among potential causes of damage.

The greatest damage appeared to be a response to thermal exposure or physical/mechanical disturbance. HP-HW wash, resulting in high mortality in algae and epifauna, caused the most severe and persistent effects. Effects of LP-WW washes were less severe and persistent and cursory data on temperature gradients downslope and across the test plots suggest that LP-WW treatment probably did not cause significant thermal impacts in the intertidal biota. The dispersant and beach-cleaner applications caused few apparent short-term effects. However, flaws in sampling design constrain the degree to which we can extrapolate from the conclusions.

Observations of displacement and mortality in clams at Disk Island and mussels at Ingot Island suggest that physical effects are substantial. Treatment dislodged or excavated many specimens that subsequently became crushed or moribund. Treatment with the dispersant and beach cleaner was accompanied by fewer significant changes in species abundance or community attributes than other methods. However, based on other studies (Houghton et al., 1991), the "no-treatment" alternative appears to produce fewer short- and long-term changes and faster recovery.

References
Growth and Survival of the Predatory Snail *Nucella lamellosa* in Areas Exposed to the Exxon Valdez Oil Spill

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Populations of the predatory snail *Nucella lamellosa* (frilled dogwinkle or drill) were studied in Prince William Sound to determine growth and survival at Oiled, Oiled and Cleaned, and Unooled sites. In 1991, individual tags were used to mark animals in resident stocks where possible, or, at sites where populations had been decimated, to tag animals imported from a reference population (Hogg Bay). Tagging was done during April/May and July 1991.

Samples of animals tagged in May were measured in July 1991 and samples from most sites were again measured in September 1991 and July 1992. Size changes over periods of up to 14 months were used to evaluate growth differences among treatments and recapture rates were used to estimate survival. This study provides insights regarding the efficacy of treatment following an oil spill and complications associated with possible restoration efforts for species with direct development following a major environmental perturbation.

The Brody-Bertalanffy model with seasonal adjustment was used to describe growth (Sager 1982) and recast as a difference equation using size pairs (\(S_o\) and \(S_{o+t}\)), a time interval (\(\Delta t\)) and Julian day/365 (t):

\[
S_{o+t} = S_o - (S_o - S) e^{-\frac{(1-e)K}{2\pi}} \sin 2\pi(t + t_\lambda)
\]

Eq 1

with parameters:

- \(S\) = asymptotic size
- \(K\) = the growth rate constant
- \(\epsilon\) = parameter measuring strength of the seasonal effect; equal to 1 with no effect.
- \(t_\lambda\) = parameter that adjusts the time of minimum growth.

Numbers of size pairs (\(S_o\) and \(S_{o+t}\)) at each location are: 1. Unooled sites: Bass Harbor (pairs = 295), Crab Bay (401), Eshamy Bay (360), Hogg Bay (420) and Outside Bay (484); 2. Oiled sites: Crafton Island (239 from Hogg), Herring Bay (32 local and 47 from Hogg), and Snug Harbor (72 from Hogg); and, 3. Oiled and Cleaned sites: Block Island (266 from Hogg), Northwest Bay (418 local and 255 from Hogg), and Smith Island (47 from Hogg).

Parameters in Eq. 1 were estimated by nonlinear regression. The parameters \(\epsilon\) and \(t_\lambda\) both were close to 0, indicating a strong seasonal effect with minimum growth close to January 1; therefore differences in growth among treatments were focused on just \(K\) and \(S_o\). The parameters \(K\) and \(S_o\) are highly correlated and so comparisons among treatments were made using their product \((K x S_o)\), termed \(\omega\) (Gallucci and Quinn 1979, Appeldoorn 1983), which has dimensions of cm/yr and approximates the instantaneous growth rate of a newly hatched individual; the larger the value of \(\omega\), the faster the growth.
For Unoiled sites (N=5), \( w \) ranged from 38.04 - 52.08 with a mean of 44.09. For Oiled sites (N=3) \( w \) ranged from 16.60 - 32.56 with a mean of 24.04 and at Oiled and Cleaned beaches (N=3), \( w \) ranged from 21.08 to 48.93 with a mean of 36.85. Differences among treatments was tested by ANOVA (N=11) with a resulting \( p=0.049 \). There was no overlap of \( w \) values for Unoiled and Oiled sites. Estimates of \( w \) for snails at Oiled and Cleaned sites were in between and overlapped the \( w \) values of *Nucella lamellosa* at Unoiled sites and Oiled sites.

Survival of *Nucella* was estimated from the recapture of tagged animals at all of the same sites as growth was determined with the exception of Smith Island where animals were not sampled in July 1992. Two time periods were used: April/May 1991 to June/July 1992 and July 1991 to June/July 1992. At some sites, tagged animals were released both in April/May 1991 and in July 1991. Survival (S) from tagging in either April/May or July 1991 to July 1992 was adjusted to an annual rate (\( S_A \)) by

\[
S_A = S^{\frac{\Delta t}{365}} \quad \text{Eq. 2}
\]

where \( \Delta t \) is the difference in years from tagging to July 1992, which ranged from 0.953 to 1.175.

For Unoiled sites (N=5), annual survival probability ranged from 0.123 (Hogg Bay) to 0.319 (Eshamy Bay) with a mean of 0.217. For Oiled sites (N=3), annual survival rate ranged from 0.044 (Snug Harbor) to 0.060 (Herring Bay) with a mean of 0.054. At Oiled and Cleaned sites, the two estimates were 0.108 (Block Island) and 0.114 (Northwest Bay) with a mean of 0.111. Differences among treatments were tested by ANOVA (N=10) following an arcsin transformation of survival probabilities with a resulting \( p=0.005 \). There was no overlap of annual survival rates for any of the treatments.

Estimates of annual survival must be taken as minimum values because there were probably some tagged animals living at each site that were not found in July 1992. Additional sampling would be necessary to obtain better estimates of the actual numbers present at the final census; however, there is no reason to suspect bias in the samples with respect to treatment. Given the errors associated with variable effort in collections at the sites, the trend of survival rates probably is correct: best survival at Unoiled locations, worst at Oiled sites that were not cleaned, and intermediate at Oiled and Cleaned sites.

Trends of survival are similar to trends shown in growth in the sense that *Nucella lamellosa* at Unoiled sites generally had both better growth and survival than animals at Oiled or Oiled and Cleaned sites. Growth and survival at Oiled and Cleaned sites, in general, appeared to be better than at Oiled sites that were not cleaned. However, the numbers of sites is small and clearly number of study sites for each treatment should be increased in future studies of these and other populations recovering from oil damage.

References


Recovery of Prince William Sound Intertidal Infauna from *Exxon Valdez* spill and Treatments-1990-1992

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Much of the crude oil spilled from the tanker *Exxon Valdez* on March 24, 1989, was deposited on beaches in Prince William Sound. Major beach cleanup activities began in May and continued throughout the summer of 1989. About 400 km of shoreline were treated in the sound in 1989 using various hydraulic wash and bioremediation (fertilization) techniques; additional mechanical cleanup and bioremediation occurred during the summers of 1990 and 1991.

High pressure, hot-water (HPHW) washes used on mixed gravel/sand/silt beaches in 1989 altered the nature of habitat available to infauna. Hydraulic washing of heavily oiled upper beach areas transported large quantities of silts, sands (to 4 mm diameter), and even pebbles (4 to 64 mm) down the face of the beach to the water’s edge. Coarser materials were deposited on the lower beach, while suspended oils and silts were carried from the area by currents in both surface and water column plumes. Presumably many organisms, along with a large proportion of the organic matter in the sediment column, were similarly displaced.

The overall objectives of this study were to evaluate recovery of important intertidal habitats and resources from the effects of oiling and shoreline treatment and to assess the influence of HPHW treatments on the nature and rates of recovery (Houghton et al. 1991, 1992). The study plan established was designed, in part, to document persistence of effects of 1989 hot-water washes, if they remained evident, over the broader area where hot-water treatments had been applied. Primary variables isolated in the sampling design were habitat type, tidal elevation, degree of oiling, and use of high pressure, hot-water shoreline treatments. This paper reports sampling of mixed gravel/sand/silt (mixed-soft) beaches that had been unoiled (reference or Category 1 sites), oiled but not treated with HPHW washes (Category 2), and oiled with subsequent HPHW-wash treatment (Category 3). Information on initial oiling and on shoreline treatments applied at our study sites were derived from State of Alaska and Exxon records and through contacts with on-site personnel.

Quantitative field surveys were conducted in Prince William Sound in midsummer (late June to early July) 1990, 1991, and 1992 to document environmental conditions and infaunal assemblages at 9 to 12 intertidal sites in mixed-soft habitats. Two stations were established at each site to represent intertidal elevations (zones) of biological interest. At each station (elevation), five sediment cores (0.009 m² by 15 cm deep) were randomly collected. Samples were field sieved on 1.0-mm screens and preserved.
In the laboratory, all infauna were identified to the lowest practicable taxon. Additional samples were taken at each station for sediment hydrocarbon analysis (all years), grain size determination (1991 on), and total organic carbon (TOC) and total Kjeldahl nitrogen (TKN) analysis (1992 only). Samples of littleneck clams (*Protothaca staminea*) were collected for age and growth analysis and for analyses of tissue hydrocarbon content. Little-neck clams also were tagged and transplanted between sites with differing residual sediment hydrocarbon levels in May 1991. Clams were recovered in September 1991, and survival, growth, and bioaccumulation of hydrocarbons were evaluated as a function of sediment hydrocarbon concentration.

Study results confirmed that protected sand and gravel beaches were severely affected by hydraulic treatments that greatly altered beach morphology. Coarse sands and fine gravels were flushed from upper intertidal elevations and often buried the lower beach in several centimeters of sediment. In this process, many infaunal organisms along with a high percentage of the silts and organic materials in the sediments were dislodged and transported from the site. Hydraulic treatments left the lower beaches in many areas covered with coarse sediments with a low content of fines. TOC and TKN were lowest in sediments at HPHW-washed lower stations.

Distribution patterns of polycyclic aromatic hydrocarbons (PAHs) in mixed-soft sediments in 1990 and 1991 were similar: PAHs were significantly (to three orders of magnitude) lower at unoiled (Category 1) sites than at oiled (Category 2), or oiled and HPHW-washed (Category 3) sites. PAH concentrations at Category 2 sites were lower at the lower elevations and highest at upper elevations in 1990. By 1991 substantial weathering had occurred at middle and upper elevations, and concentrations were reduced by an order of magnitude; little weathering had occurred at lower stations. In contrast, at Category 3 sites PAH concentrations were lower at upper and lower intertidal elevations and higher at mid- and subtidal elevations. By 1991 only very slight reductions in PAH concentration had occurred at Category 3 intertidal stations, but subtidal concentrations had dropped by an order of magnitude.

These patterns suggest that although shoreline HPHW treatment has resulted in an initial drop in oiling in intertidal sediments (in 1990 Category 2 sites had greater average intertidal PAH than Category 3), it has not made a dramatic difference in overall hydrocarbon concentrations. Ten-fold declines were observed at three of four elevations in Category 2 but at only one of four elevations in Category 3. Based on the degree of replication included in the averages, these changes, indicative of considerable weathering, are fairly reliable. The most prevalent constituents in 1991, in order of importance, were compounds of dibenzothiophene, phenanthrene, and naphthalene. In contrast, the most prevalent compounds in 1990 were naphthalenes, phenanthrenes, fluorenes, and dibenzothiophenes. Reduced concentrations of naphthalenes and fluorenes are another reflection of the weathering process.

Several compounds occur at sufficiently high concentrations in sediments at some stations to raise concerns about
sublethal effects of exposure. Processes that could be influenced by chronic exposures to low levels of PAH include survival, reproduction, development, and growth. In the clam transplanting experiment (carried out in 1991), there was a clear correlation between higher sediment hydrocarbon concentrations and reduced survival of littleneck clams.

In 1990, 1991, and 1992, infauna at lower mixed-soft stations appeared only moderately affected by the spill on Category 2 (oiled but untreated) beaches; significant differences between Category 1 (unooled) and Category 2 stations were few. The infauna on Category 3 (oiled and hot-water-washed) beaches, however, was fundamentally altered in comparison to both other beach categories. Number of species, number of organisms, and species diversity varied significantly among station categories; lowest values were at the HPHW-treated beaches in all 3 years. Most major taxa (gastropods, bivalves, polychaetes) had significantly lower abundances on Category 3 beaches than on Category 1 and/or 2 beaches in 1990 and 1991. In 1992 these relative abundances remained unchanged but were no longer significant indicating that some recovery is under way.

In 1991 and 1992 several dominant taxa were most abundant at the lower intertidal station at the heavily oiled Category 2 site at Block Island. This area continued to show extremely high sediment oiling yet had higher densities of the deposit-feeding bivalve *Macoma* ssp., harpacticoid copepods, and oligochaetes than any site group. These taxa may be capable of exploiting hydrocarbon-degrading bacteria in these oily sediments.

The Block Island lower station also had a high density and the highest recruitment of young-of-the-year clams despite the fact that sediment hydrocarbon concentrations were sufficient to cause significantly reduced survival and increased tissue PAH uptake in clams experimentally transplanted to this station. Interestingly, the survival of littleneck clams in the 1991 transplant experiment was highest (98%), and growth rate was greatest, at the Northwest Bay West Arm site (Category 3), which has had very clean sediments since 1990; this site has had consistently low clam recruitment compared to Block Island, however. In all three years, the Category 3 sites had the lowest overall density and lowest recruitment rates of hardshell clams (both littlenecks and butter clams, *Saxidomus giganteus*).

Analysis of infauna data confirms that the effects of shoreline treatments relate as much to physical disturbance (burial, displacement, reductions in fines and organic content) as to oiling. Infaunal assemblage variables (total organism density, diversity, richness) were negatively correlated with percentage of sands and the residual hydrocarbon levels in the sediments in 1991; total organism density was positively correlated with the percentage of fines.

The 1990-1992 data indicate that recovery of infauna on hot-water-washed beaches will take many years. Primary factors prolonging the recovery period on Category 3 beaches are the continued instability of the beach profile, reduced content of finer sediments (including organics), reduced recruitment, and destruction of the normal population (age) structure in longer lived organisms such as the hardshell clams. Residual sediment oiling may also alter the pathway to, and delay the realization of, full recovery at least one Category 2 lower
station (Block Island). Multivariate analyses confirmed patterns of category differences and trends in recovery.

References

Recovery of Prince William Sound Intertidal Epibiota From the Exxon Valdez Spill and Treatments—1990-1992

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Following the 1989 Exxon Valdez oil spill, significant concerns were raised regarding the potential effects on intertidal habitats and biota of high pressure, hot-water (HPHW) washes used to remove oil from the shorelines of Prince William Sound. The objectives of this study were to evaluate recovery of important intertidal and shallow subtidal habitats and resources from the effects of oiling and shoreline treatment and to assess the influence of HPHW treatments on the nature and rates of recovery. This study also was designed to extrapolate persistence of effects of 1989 hot-water washes over the broader area where hot-water treatments had been applied. Primary variables isolated in the sampling design were habitat type, tidal elevation, degree of oiling, and use of HPHW shoreline treatments. The status of recovery of intertidal assemblages from the oil spill and subsequent shoreline treatments was examined by repeated sampling of a suite of rocky intertidal sites during 1990, 1991, and 1992.

Studies sponsored by Exxon in 1989 demonstrated that major intertidal assemblage dominants (rockweed, mussels, limpets, snails) survived 3 to 4 months in heavily oiled habitats. Immediately following HPHW washing, however, these taxa suffered significant reductions (50 to 100 percent losses; p < 0.1; Houghton et al. 1991). Because of these identified adverse impacts, ecological effects of this type of treatment were a major focus for the present research effort.

We sampled multiple rocky shores that had been unoiled (Category 1 sites), oiled but not treated with HPHW washes (Category 2), and oiled with subsequent HPHW-wash treatment (Category 3). Information on initial oiling and on shoreline treatments applied at our study sites was derived from State of Alaska and Exxon records and through contacts with on-site personnel.

Stratified random sampling was used to assess epibiota (surface-dwelling plankton and animals) at nine to 15 intertidal rocky sites (depending on the survey) representing several exposures and degrees of disturbance in selected oiled and unoiled locations in the sound. Two to three stations were established at each site to represent intertidal elevations (zones) of biological interest. At each station, multiple 0.25-m² quadrants were randomly located, permanently marked, and sampled to document the abundance of (surface-dwelling plants) and fauna (animals). Samples of selected organisms were collected for analyses of age and growth and tissue hydrocarbon concentrations.

This sampling design allowed for monitoring long-term recovery trends at sites of known oiling and treatment history. It is also well suited (by the level of replicated sampling at each station) for comparisons, at specific points in time, between pairs of stations with similar habi-
tat but different oiling and/or treatment histories. Because of the limited number of stations that could be sampled in each habitat/oiling/treatment category, this design is less well suited to statistical inference regarding the generalized impacts of oiling and treatment over all stations with similar histories. Nevertheless, statistically significant differences among site categories were shown for some variables in all sampling years. These results have allowed us to draw conclusions regarding initial impacts and directions of recovery.

Our studies in 1990 provided strong evidence of bioaccumulation at several levels in the food web but found no evidence of biomagnification. PAH concentrations decreased from lower to higher levels of the food web and were lowest in the top predators examined. Moreover, relationships between PAH concentrations in prey and potential predators from the same site were weak. On the basis of these findings, collections of the sunstar (Pycnopodia helianthoides) and the drill (Nucella lamellosa) for PAH analysis were discontinued in 1991.

PAH analyses in 1991 focused on determining whether high concentrations of PAH in mollusk tissues at some sites were due to continued exposure to hydrocarbons or to residual hydrocarbons in the tissues from exposure during previous years. These analyses produced three important findings:

1. PAH concentration in tissues of mussels (Mytilus cf. trossulus) transplanted from reference sites to areas of high residual sediment contamination increased over the summer by an order of magnitude or more to levels of contamination as high as, or higher than, those in resident (local) animals. Levels of tissue PAHs in mussels (transplants and local animals) at Smith Island (3.7 to 20.4 ppm dry), considered one of the more highly contaminated sites remaining in the sound, were similar to the levels of PAHs in mussels from near Seward (6.2 ppm dry), a reference site.

2. Levels of contamination observed in resident mussel tissues at Smith Island in July and September 1991 had dropped more than an order of magnitude from those observed at that site in July 1990. The composition of PAHs in mussel tissues was quite similar to that seen in 1990 but reflected weathering in the source hydrocarbons. Phenanthrenes and dibenzothiophenes were dominant; naphthobenzothiophenes and fluorenes were of intermediate importance; and naphthalenes, pyrenes, and chrysenes were of low importance.

3. The most likely sources of long-term contamination of mussel tissue in the Sound are the reservoirs of subsurface oil at many sites. Large reductions in PAHs in mussel tissues from Smith Island suggest that leaching rates from such subsurface deposits of oil have declined dramatically since July 1990, however. This observation is important in consideration of the advisability of continued shoreline treatment activities, particularly in view of the fact that, by 1991, the tissue contamination at Smith Island had declined to a level similar to that observed in animals from near Seward.

Distribution and Abundance of Epibiota

In our 1990 and 1991 sampling, a high degree of variability was seen among
biota at sites subjected to varying degrees of treatment. Many of the important longer-lived dominants remained intact at some treated sites in 1990; in other areas, apparently those that had been cleaned more rigorously, these species did not survive. In 1991, recolonization of these areas was evident on most rocky shorelines. Trends in the initial impact and recovery of three key taxa (rockweed, limpets and drills) at all middle elevation rocky stations sampled illustrate that oiled but untreated (Category 2) stations were well on their way to recovery by mid-1991—that is, there were no longer significant differences in abundance between Category 1 and Category 2 stations.

Biota at HPHW-washed (Category 3) middle rocky stations, however, remained significantly depressed; mean abundances of rockweed, limpets, and drills (Nucella spp.) were very low at Category 3 rocky sites through May 1991. Mean abundance of these taxa all showed partial recovery at HPHW sites by July 1991.

By July 1992, rockweed cover and densities of limpets at HPHW middle stations exceeded those at unoiled stations. Full recovery had not yet occurred, however. Littorine snails were least abundant at Category 3 stations in 1990; in July 1991 the density of the opportunistic Littorina scutulata increased sharply at Category 3 sites and in 1992 was much more abundant at the HPHW stations. Its congener L. sikana, which lacks planktonic larval dispersal, was slower to recolonize hot-water-washed stations. Drills were virtually absent at all heavily oiled middle stations, both treated and untreated, through 1992 but remained at relatively constant low densities at lightly oiled or unoiled sites over the same period.

Lower rocky intertidal areas in Prince William Sound tend to be dominated by longer lived algae because predation by seastars and drills greatly limits the numbers of grazers. Only one HPHW-treated lower rocky station has been tracked for all 3 years of the study. In 1990 and the spring of 1991 this station had a high percent cover of ephemeral green and brown algal colonizers and limited populations of grazers. By July of 1992 this site had higher densities of limpets and littorines than the other lower rocky stations (HPHW), perhaps because of reduced numbers of seastars and drills. A primary apparent effect of HPHW washing was the reduction of longer lived red algae, especially Delessleriaceae, Gigartinaeae, Palmariaeae, and Rhodomelaceae. These groups accounted for 15.3 percent cover in 1990 and declined to 8.8 percent by 1992 at the single HPHW lower rocky site sampled in all three years. At Category 1 and 2 lower stations, this group of algae ranged between 35.1 to 48.3 percent cover over the same period.

One rocky site in Northwest Bay that was stripped bare by treatments in 1989 showed little colonization at middle and upper stations through September 1991. Films of blue-green algae and possibly other algae that developed early in 1990 and 1991 were grazed or eroded away; mostly bare rock was left. Even early successional colonization by rockweed sporelings, Fucus gardneri, or the barnacle Semibalanus balanoides, such as that seen elsewhere over broad areas, occurred only sporadically and in isolated patches. By 1992, these patches had expanded to cover portions of the middle station where crevices in the rock retain moisture; the remainder of the station,
which lies on a smooth rock bench, continued to be devoid of significant algal or animal growth. Clearly, the epibionts at this site will take many more years to recover to pre-spill conditions.

Dense stands of young rockweed that had been evident only as inconspicuous sporeling mats in some HPHW areas in 1990 were growing well in 1991 and gave a superficial appearance of a "normal" shoreline. A more detailed examination, however, revealed that the assemblage in these heavily treated areas bore little semblance to that on Category 1 (unoiled) or 2 (oiled but not hot-water-washed) shores. Longer lived, more stable components of the upper rockweed zone (rockweed; several red algae, Rhodophyta; hermit crabs, Pagurus hirsutiusculus; a limpet, Lottia pelta; and drills, Nucella lamellosa) were significantly less abundant in the HPHW-treated areas. Rockweed on many HPHW-washed sites in 1991 was composed predominantly of 2-year-old plants that were not reproductively mature and a small percentage of sporelings. In contrast, oiled sites that were not HPHW-washed included an even mix of several year classes of older and reproductively mature plants. By 1992, rockweed established as sporelings on Category 3 sites in late 1989 was reproductively mature.

Thus, oiled rocky areas not subjected to severe cleanup activity were generally indistinguishable from unoiled sites in 1990 and 1991; more heavily treated sites remained in earlier stages of recovery through 1991. By 1992, most HPHW sites were also well on their way to recovery; abundances of most taxa were similar to those on unoiled rocky beaches. Multivariate analyses confirmed patterns of category differences and trends in recovery. Qualitative examinations of other shorelines around the northern portions of the Knight Island group in July 1991 revealed many areas where early successional assemblages were present in areas where hot-water treatments were used in 1989. The broader ecological implications of resetting the successional stage over large areas of shoreline are not clear.

References
NOAA's Long-Term Ecological Recovery Monitoring Program: Overview and Implications of Recovery Trends and Treatment Effects
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NOAA's Hazardous Materials Response and Assessments Division is responsible for providing, through the Scientific Support Coordinator, guidance to the Federal On-Scene Coordinator during responses to major oil spills. The aim of that guidance is to maximize protection of marine resources. A major source of guidance comes from experiences gained during previous spill responses. Obviously, the Exxon Valdez oil spill provided many opportunities for learning about the success and failures of various containment, removal and shoreline treatment methods. This paper summarizes the rationale and approach of NOAA Hazardous Materials Response and Assessments Division shoreline treatment studies in Prince William Sound and suggests some implications for future responses and restoration.

Focus on Shoreline Treatment
One of the most controversial and poorly quantified aspects of oil spill response is the extent to which shorelines should be treated to remove oil. By May, 1989, much of the spilled crude oil was stranded on 357 miles of protected and exposed shoreline in Prince William Sound where it became an actual or perceived long-term threat to commercial and subsistence fisheries, wildlife and recreation/tourism. Acknowledging cautions expressed by the Scientific Support Coordinator and other groups, the decision was made to use any and all means practical to clean the shorelines, including high energy methods such as high-pressure hot-water washing. After considerable debate, agencies also agreed to leave a few shorelines untreated to use as reference sites provided studies be conducted to monitor impacts and recovery.

During the summer of 1989 shoreline clean-up involved unprecedented use of high-pressure (50-100 psi) hot- or warm-water (to 140F) washing. Warm-water washing became the primary method for nearly all treated shoreline in Prince William Sound (Exxon Production Research Corp., 1990). Initial tests on several types of shoreline indicated that this method removed most of the shoreline surface biota that otherwise survived oiling (Lees and Houghton, 1990 and Houghton et al, 1991). Thus a central question of the HMRAID program was the extent to which high-pressure hot water washing enhanced or delayed oil removal, return of altered shoreline integrity and especial recovery of shoreline marine communities.

Hazardous Materials Response and Assessments Division Programs
During 1989 and 1990 NOAA's Hazardous Materials Response and Assessments Division initiated chemical, geomorphological and biological monitoring programs at over 35 sites in Prince William Sound to help document the benefits and effects of various shoreline countermeasures. Primary objectives of this multifaceted effort were (1) to determine the initial effects of oiling and treatment on the nearshore environment; (2) to determine whether or not treatment enhanced
mine whether or not treatment enhanced or delayed recovery; (3) to characterize the nature of physical and biological recovery on a long-term basis; and ultimately, (4) to provide response and restoration agencies with guidance for appropriate and effective actions.

Components of the NOAA/HMRAD program include (1) biological monitoring, (2) shoreline geomorphology, and (3) oil weathering studies. The core biological program samples shorelines representing three treatment categories: unoiled and untreated; oiled but not high-pressure hot-water washed; and oiled and high-pressure hot-water washed. Oiling and treatment histories at each site were extracted from State and federal treatment records, derived from personal contacts and directly observed from historical videos and photographs.

Three shoreline types were sampled: (1) rocky, (2) cobble/gravel/mud, and (3) boulder/cobble. There is a minimum of three sites for each combination of treatment and shoreline type, and each site generally includes visual, biological and chemical surveys at each of three elevations (upper, middle and lower intertidal). Five to ten replicate measurements are made to document changes in surface oiling, epibionta and infauna at each elevation at least annually. Keystone species of mollusks are tagged, released and recovered to document improvements or impacts on growth. Composite samples of sediments, mussels and clams are sampled to document trends in concentrations of hydrocarbons. The geomorphology program (Michel et al, 1991) determines the wave exposure regime at each site and documents changes in beach profiles and trends in the abundance and character of oil both at the surface and below. In 1992, a special chemical study was undertaken to evaluate differential weathering in various microhabitats on Prince William Sound beaches and the implications for bioavailability.

The program was not part of the Natural Resource Damage Assessment (NRDA) process, but rather, an extension of response activities. Nevertheless, it provides information and insights highly relevant to any assessment of physical or biological recovery in Prince William Sound. Moreover, the program has a number of unique features that distinguish it from other research efforts in Prince William Sound. Treatment is explicitly accommodated as an analytical variable, incorporating the use of untreated shoreline segments (“set-aside” sites) as reference areas. The program also integrates observations of biology, bioaccumulation, chemical fate, shoreline geology, and coastal processes to provide a physical framework for observed biological conditions and trends. The NOAA program characterizes trends in shoreline ecosystem components not otherwise addressed in NRDA programs, e.g. intertidal infauna, and growth, recruitment and hydrocarbon contamination of clams. Finally, the program is conducted under a regimen of rigorous, open, multi-agency peer review.

Implications for Response and Restoration

Results of biological and some chemical studies through 1992 are presented in several reports at this conference (Houghton et al, 1993a and 1993b) and elsewhere (Michel and Hayes, 1992; Houghton et al, 1992). Below, we suggest some of the implications of results to date.

Did high-pressure hot-water washing enhance or impede shoreline recov-
ery? The answer depends on how we define recovery and what kind of shoreline is involved. It clearly reduced the amount of visible oil on all shoreline types. Below the surface, however, it is difficult to distinguish washed from untreated shorelines. Although the amount of subsurface oil has decreased each year, subsurface oil in various stages of weathering remained at all oiled and treated shorelines in the summer of 1992 (Michel and Hayes, 1992).

Furthermore, unoiled sites were not necessarily free of petroleum hydrocarbons - all reference sites had evidence of PAH’s from combustion or other sources, so there is no pristine background to return to. It also appears there are and will be some long-lasting effects of washing on the structure of some shorelines where contaminated sediment was washed from the upper to lower intertidal and even out into open water. The abundance and diversity of shoreline marine life and the abundance of specific major plants and animals was significantly reduced by high pressure hot water washing, compared to unoiled sites and sites not receiving this treatment (Houghton et al, 1992). As a result, the return of these populations to reference values has been faster at the untreated sites than at the treated sites. Sampling in 1992 showed that although recolonization and recovery are progressing at most sites, the process is far from complete (Houghton et al, 1993a and b).

How long will recovery take?
While it is very difficult and also very risky to predict the long-term course of ecological recovery based on limited temporal information, the data from 1990 - 1992 suggest that at treated sites recovery - i.e., return to abundances at unoiled sites - of various intertidal populations may take from three to over 15 years. Projections have not yet been made for complete loss of subsurface oiling or return to normal sediment structure.

Implications for Restoration
Since sub-surface oil remained at many sites in the summer of 1992 there may be a natural inclination for additional treatment. The 1992 Technical Advisory Group concluded that additional treatment would be counter productive. Michel and Hayes (in prep) conclude that subsurface oil concentrations are declining at about equal rates at treated (washed and berm relocation) sites. Mussels in some areas continued to be contaminated, but it is not clear that underlying sediments, the presumed source of contamination, are any more contaminated than sediments not underlying mussel beds. Thus it is not clear, without experimental evidence, if additional intervention will enhance reduction of contaminant loading.

We made several interesting observations that may have implications for enhancing restoration of intertidal communities. High-pressure hot water washing almost totally eliminated dogwhelks (Nucella spp.), major intertidal predators. Since they do not have planktontic larvae and cannot recolonize from planktonic settlement, they are extremely slow to return. However, transplanted stocks have survived well and grown rapidly at washed shorelines, suggesting transplanting is a feasible restoration activity. Likewise, we have observed high survival and growth of clams transplanted into areas where they were severely reduced or eliminated.

Recommendations for Future Responses
High-energy treatments, such as high-pressure hot-water washing may have a
role in the arsenal of shoreline countermeasures. However, if the clean-up strategy includes minimizing damage to shoreline resources, this method should only be used on a case-by-case basis.

Implications for monitoring

The work done to date underscores the need to continue the monitoring effort into the future in order to properly characterize the complex processes that comprise ecological recovery. Monitoring directed at evaluating treatment alternatives is an important part of responses to future spills. However, it cannot be done properly without establishing untreated set aside areas. The concepts of accurate treatment recordkeeping and set asides should be considered essential elements in any major spill response.

References


The Effects of the Exxon Valdez Oil Spill on Benthic Invertebrates in Silled Fjords in Prince William Sound

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During October 1989, when examining the effects of the Exxon Valdez oil spill on several shallow subtidal habitats in western Prince William Sound, high mortality of invertebrates and fishes was observed in a heavily oiled silled fjord. Here we present the results from the initial observations and sampling of this fjord in 1989, and subsequently in 1990 and 1991. Other silled fjords were examined in 1990. We consider the effects of the spill versus natural benthic hypoxia or anoixia.

The main silled fjord studied is a small embayment in northeastern Herring Bay which is located along the northwestern side of Knight Island. It has an area of approximately 600 km\textsuperscript{2} with the greatest depth within the basin approximately 35 m; the sill depth at the entrance of the fjord is only 4 m. At depths greater than 10 m the substrate was mainly composed of fine, flocculant silt. This fjord was considered as "heavily oiled" after two shoreline surveys were conducted during the summer and fall of 1989.

The dead and moribund animals observed in 1989 were primarily in the deeper portions (> 13 m) of the fjord. In one area extensively surveyed (approximately 70 m\textsuperscript{2}), we observed over 40 dead animals laying on the bottom, including 23 large polychaete worms and 11 starfish (all Pycnopodia helianthodes) and miscellaneous clams. Also encountered were dead mobile organisms, such as shrimp, squid and Pacific cod. In addition to the observed dead organisms, the substrate had a patchy, cobweb-like layer of the bacteria, Beggiatoa. This colorless, sulfur-dependent, hemolithotrophic bacteria is associated with decaying vegetation and low dissolved oxygen.

Immediately following these observations, we collected samples of infaunal invertebrates from three randomly placed transects along each of three depth strata (0-2, 2-8, 8-20 m) using a diver-operated suction dredge. One randomly placed 0.25 m\textsuperscript{2} quadrat was sampled to a substrate depth of 10 cm at each depth stratum. A video of each transect was made by divers and a bathymetric chart of the embayment was made using a fathometer aboard a small boat. Additional videos were taken along transects through the deeper portions of the fjord in order to document the extent of dead organisms.

Diver observations and density estimates of infauna were again made from this fjord in May and October 1990 and August 1991. In addition, two more silled fjords (one oiled and one unoiled) were examined in May 1990; four (two oiled and two unoiled) were examined in September 1990. All sites had features similar to Herring Bay. We first conducted a bathymetric survey at each site as described above. Three stations were then established at random positions along the 20 m-depth contour at each site. At each station, divers collected duplicate 0.1 m\textsuperscript{2} suction dredge samples of sediment for benthic infauna. Measurements
of temperature, salinity and dissolved oxygen were taken on all surveys, except October 1989.

Although the benthic community in Herring Bay in 1989 was obviously stressed, as noted by the dead animals, it still contained a relatively rich assemblage of infauna (e.g., 24 taxa at the family and higher taxonomic level). However, signs of disturbance were evident in the moderately low Shannon diversity index (1.7), the moderately high Simpson dominance index (0.4), and the near absence of sensitive burrowing amphipods (16/m²). The dominance index was mainly attributed to stress-resistant taxa such as the bivalves *Lucina tenuisculpta* (Lucinidae) and *Mytilus tumida* (Montacutidae), and the polychaetes *Nephtys cornuta* (Nephtyidae) and *Polydora socialis* (Spionidae).

*Lucina* and other lucines appear to be able to live where conditions are extreme and oxygen and food limited (Yonge and Thompson, 1976). *Lucina* is in the same order as the stress-tolerant *Thyasira* genus, and several species of *Thyasira* have been reported from organically enriched and polluted substrates (Pearson and Rosenberg, 1978). *Lucina* that dominated at Herring Bay in 1989 (61% of faunal abundance) were mainly older than one year; many noted were more than three years old.

Similar surveys at Herring Bay in 1990 revealed fewer dead animals than in 1989. In 90m² surveys conducted in late May and early October, we saw one dead *Runciniodia* (spring), one dead cod and three dead worms (fall) among scattered patches of *Beggiatoa*. More extensive visual searches in fall 1990 revealed some dead fishes, but there were no concentrated “dead zone” pockets as observed in 1989.

However, the infaunal community was obviously disturbed by the spring and fall of 1990. Diversity and number of taxa were extremely low in both surveys, <0.1 and <8 taxa (mainly families), respectively. Furthermore, *Lucina* was absent and the community was almost totally dominated by a single polychaete, *Nephtys cornuta*. Dissolved oxygen values in the water adjacent to the bottom averaged 5.4 mg/l in May, but were near zero in October.

Lizarraga-Partida (1974) reported *Nephtys cornuta* in semi-polluted substrates in Ensenada Bay, Mexico, in areas enriched with organic material derived from sewage-fish waste. Pearson and Rosenberg (1978) give several other examples of *Nephtys* appearing in organically enriched and polluted areas, often low in dissolved oxygen. Busdosh (1978) found *Nephtys* only in association with oiled substrate; it alone preferred oiled to clean sediment. Although *Nephtys* is mainly a predator, it also deposit feeds and thus can utilize the high organic loads associated with the decay of dead organisms.

By mid August 1991, the community in Herring Bay demonstrated dramatic signs of recovery. Diver surveys revealed no dead organisms, although *Beggiatoa* was still evident on the bottom. Almost all infaunal community parameters had recovered to or near levels observed in 1989. *Nephtys cornuta* still dominated, however, *Lucina tenuisculpta* was now present again, but in low density. The dissolved oxygen during the August sampling, one month earlier than 1989 and 1990 fall samplings, averaged 9.7 mg/l.

One group of organisms that was present in 1991 in moderate density was burrowing amphipods (132/m²). Representatives of this group included the fami-
lies Ischyroceridae, Isaeidae, Dexaminidae, Phoxocephalidae, and Lysianassidae. The low density of amphipods at Herring Bay in 1989 (16/m²) and 1990 (absent), presumably was the direct result of toxic effects of petroleum hydrocarbons. Benthic amphipods are notoriously sensitive to petroleum hydrocarbons, and massive declines in amphilicid amphipods were observed following the Amoco Cadiz oil spill (Cabioc et al., 1978; Chasse, 1978). The reoccurrence of amphipods on those previously contaminated sediments began, for some species, in one to two years (Dauvin, 1982). The density of amphipods had dramatically increased in Herring Bay by 1991, just two years since being oiled.

Hydrocarbon compounds were identified from surficial sediments from Herring Bay fjord. A gradual decline in each hydrocarbon compound was noted over the three-year period, e.g., total hydrocarbons decreased from a mean of 69.0 µg/g in 1989 to 3.7 µg/g in 1991; total polynuclear aromatic hydrocarbons decreased from a mean of 1.2 µg/g in 1989 to 0.06 µg/g in 1991; total dibenzoephenes decreased from a mean of 0.222 µg/g in 1989 to 0.006 µg/g in 1991; total alkane decreased from a mean of 2.0 µg/g in 1989 to 0.67 µg/g in 1991; and total naphthalenes decreased from a mean of 0.22 µg/g in 1989 to 0.012 µg/g in 1991. Aromatic petroleum hydrocarbons, including naphthalenes and phenanthrenes, have been reported to persist in sediments for periods in excess of six years, although in reduced concentrations (Neff and Anderson, 1981).

All four of the other silled fjord sites (two oiled and two unoiled) that were examined during late September 1990 were stressed as indicated by the low number of taxa (4-13), low Shannon diversity indices (H' < 0.1-1.3), and high Simpson dominance indices (D = 0.3-0.9). All sites were dominated by Nephtys cornuta. All sites also had bottom-water dissolved oxygen values of <1 mg/l and the sulfur-dependent bacteria, Beggiatoa, present. Dead organisms, such as juvenile Pacific herring and unidentified worms, were found in both oiled and unoiled sites. Other dead organisms on oiled sites included terebellid polychaetes, naticid snails, and brittle stars. At one unoiled site (Humpback Cove), where oxygen values were near zero, the sulfur odor could be detected by the divers at depth.

Our data from Herring Bay tends to support the notion that oil contributed to the “dead zone” observed in 1989, and the infaunal reduction in 1990. That extensive “die off” of organisms occurred in 1989, concomitantly with high concentrations of hydrocarbons in the sediment, underscores an oiling effect. This environment must have been particularly stressed, since pelagic organisms also died.

The effects of oil on subtidal benthic communities at similar or deeper depths have been observed in other studies (e.g., Cabioc et al., 1978; Msummer et al., 1989). Among the possible causes of oil-related effects are chemical toxicity of aromatic derivatives; asphyxiation or entanglement due to direct physical coating; and a variety of reproductive, behavioral, and other sublethal disorders leading ultimately to long-term population changes. In the case of the Exxon Valdez spill, there also is the possibility of indirect effects of bioremediation and other clean-up efforts. Such activities were observed, for example, at the heavily-oiled Herring
Bay site.

The organisms that inhabit silled fjords, like the fjordic portion of Herring Bay, are periodically subjected to stress caused by, or confounded by, hypoxic or anoxic conditions on the sea floor related to poor water exchange and natural cycles of organic enrichment. Similar effects on the benthos from low-oxygen stress and organic enrichment have been seen in Scandinavian and Scottish fjords (review by Pearson, 1980) and documented as the "August Effect" in New England estuaries (Rhoads and Germano, 1982).

In conclusion, it is likely that the observations made in the fjordic portion of Herring Bay during 1989 and 1990 were the result of exposure to Exxon Valdez crude oil, in conjunction with hypoxic/anoxic conditions. However, further benthic studies should be conducted in Prince William Sound fjords to provide information critical in distinguishing possible oil impacts from the natural phenomena of hypoxia and anoxia.

References


The Effects of the Exxon Valdez Oil Spill on Epibenthic Invertebrates in the Shallow Subtidal

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Studies were conducted in 1990 and 1991 to examine the effects of the Exxon Valdez oil spill on the shallow subtidal community in Prince William Sound. Here we will present results from a stratified random sampling program that compared population densities of numerically dominant species of large epibenthic invertebrates at oiled and control sites.

Sampling in 1990 was conducted within 4 different habitats within the Sound: Eelgrass habitats in shallow protected bays, Laminaria/Agarum habitats in sheltered bays (hereafter referred to as bays), Laminaria/Agarum habitats on exposed points (hereafter referred to as points), and in Nereocystis habitats on exposed coastlines. Within each habitat we sampled at 2 to 4 pairs of oiled and control sites. The oiled sites were selected from areas that had adjacent shorelines that were moderately to heavily oiled during the fall of 1989. Control sites were selected that matched the oiled site with respect to physiographic factors (e.g. exposure, slope, substrate type) but that did not have oil present on adjacent shorelines in fall 1989. We sampled within one depth stratum in eelgrass and Nereocystis habitats, and within two depth strata at bay and point habitats. Divers counted the number of large benthic invertebrates along three randomly placed 30 m x 2 m transects within each site and depth stratum.

In 1991, we sampled only in eelgrass beds and within the shallower depth stratum of bays. The sites sampled within these habitats were the same sites sampled in 1990, except that 1 additional pair of sites was sampled within the eelgrass habitat.

We will focus here on the patterns of abundance of the dominant epibenthic invertebrates within these habitats. The dominants were the helmet crab (Telmessus cheiragonus) and two species of starfish: the leather star (Dermasterias imbricata) and the sunflower seastar (Pycnopodia helianthoides). Other species of starfish were present (e.g. Echasterias troschelii, Henricia leviuscula, and Orthasterias koehleri) but these were less abundant than Dermasterias or Pycnopodia, and did not occur in all habitats. Evidence of the impacts of oiling is based primarily on the data gathered in 1990. Data collected in 1991 are used to indicate recovery of affected populations, or lack thereof.

Populations of both leather stars and helmet crabs appeared to be adversely affected by oiling and/or associated cleanup activities. In 1990, the mean density of Dermasterias was significantly greater (P<0.05) at control sites than at oiled sites in all habitats combined. On average, the starfish were about twice as abundant at oiled sites than at controls. Similarly, the helmet crab, Telmessus cheiragonus, was more abundant at control sites relative to oiled sites (P<0.01 for all habitats combined). The average population density of Telmessus was about 5 times as high at control sites than at oiled sites in 1990.
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There were no apparent adverse impacts of oil on *Pycnopodia*. Population densities of adult *Pycnopodia* were similar at oiled and control sites in both 1990 and 1991.

There was little indication of recovery of the *Dermasterias* population, as significantly more leather stars were found at control sites relative to oiled sites again in 1991 (P<0.01). There was a slight increase in the mean abundance of *Dermasterias* from 1990 to 1991, but the means for each year did not differ significantly (P=0.09 and P=0.85 in eelgrass and bay habitats respectively).

Unlike *Dermasterias*, population densities of *Telmessus* showed some signs of recovery in 1991. There were no significant differences in *Telmessus* density among oiled and control sites in either eelgrass (P=0.23) or bay habitats (P=0.27) in 1991. However, the parity noted among oiled and control sites in 1991 resulted largely from a decrease in population density at the control sites rather than an increase at the oiled sites, and may have resulted from immigration of crabs from control to oiled areas following a reduction in hydrocarbon levels in 1991.

Also, in 1991, we noted large numbers of newly settled *Pycnopodia*, especially in the eelgrass habitats. Densities of young of the year *Pycnopodia* averaged over 30 per 100 m² in eelgrass habitats and reached densities of more than 200 per 100 m² at one site on Naked Island. Significantly more young of the year *Pycnopodia* were observed at oiled than control sites in the bay habitats (P<0.01), suggesting a possible increase in *Pycnopodia* recruitment at oiled sites. However, no significant differences were observed among oiled and control sites in eelgrass habitats (P=0.12).

We can only speculate as to the causes for the observed effects of oiling. However, we suspect that *Dermasterias* populations at oiled sites may have been reduced as a result of acute oil toxicity or by the effects of cleanup activities (especially steam cleaning). *Dermasterias* is most abundant in the shallow subtidal along rocky shorelines. They often migrate into the shallow intertidal where they feed during high tides, are exposed briefly as the tide retreats, and then drop off of rather steep rocky shores into the water. This behavior may have made these starfish especially vulnerable to oiling and to shoreline cleanup activities.

Previous studies of the toxicity of oil to asteroids suggest that low concentrations of oil may impair the feeding ability of starfish (O’Clair and Rice, 1985). However, only very high concentrations of oil are lethal to adult asteroids (reviewed in O’Clair and Rice, 1985). As a result, we suspect that losses of *Dermasterias* at oiled sites were more likely the result of cleanup activities. *Pycnopodia* can also be found in the intertidal, but unlike *Dermasterias*, seldom tend to migrate from the subtidal into the intertidal. As a result they may have been spared from direct impacts of oil and shoreline cleanup.

We suspect that lower densities of *Telmessus* at oiled sites may have resulted from losses due to the toxicity of oil. Crustaceans are especially sensitive to oil (Capuzzo, 1987) and may have been killed by high concentrations of oil associated with the spill. Alternatively, these crabs may have been able to flee the oiled areas, and migrate back to oiled sites in 1991 after hydrocarbon levels had declined.

The possible increase recruitment of *Pycnopodia* at oiled sites in 1991 may be an indirect effect of oiling. We have
noted increased recruitment at oiled sites for a number of species including *Pycnopodia*, several species of fish (Pacific cod and cottids) and small mussels (*Mytilus* sp.), and we suspect that these differences may result from enrichment of waters near sites of extensive bioremediation. However, an alternative hypothesis is that oiled sites tend to be areas where prevailing currents and winds concentrated both oil and larvae.

**References**


The Effects of the Exxon Valdez Oil Spill on Eelgrass and Subtidal Algae

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Studies were conducted in 1990 and 1991 to examine the effects of the Exxon Valdez oil spill on eelgrass (Zostera marina) and dominant subtidal algae in shallow subtidal habitats in Prince William Sound. Sampling in 1990 was conducted within 4 different habitats within the Sound: Eelgrass habitats in shallow protected bays, Laminaria/Agarum habitats in sheltered bays (hereafter referred to as bays), Laminaria/Agarum habitats on exposed points (hereafter referred to as points), and in Nereocystis habitats on exposed coastlines.

Within each habitat we sampled at two to four pairs of oiled and control sites per habitat. The oiled sites were selected from areas that had adjacent shorelines that were moderately to heavily oiled during the Fall of 1989. Control sites were selected that matched the oiled site with respect to physiographic factors (e.g. exposure, slope, substrate type) but that did not have oil present on adjacent shorelines in Fall 1989. We sampled within one depth stratum in eelgrass and Nereocystis habitats, and within two depth strata at bay and point habitats.

For eelgrass, we examined a variety of population parameters including percent cover, the density of turions (uprights protruding from the substrate) and the density of flowers. Divers counted the number of turions and estimated percent cover in each of 4 - 0.25 m² quadrats along each of three randomly placed 30 m x 2 m transects within each site.

In the bay, point and Nereocystis habitats, divers estimated the percent cover and counted the number of dominant understory algae (Eg. Agarum cribrosum, and Laminaria spp.) in each depth stratum. In addition, divers estimated the abundance of larger Nereocystis plants within a 2-m-wide band along each transect and measured the diameter of the stipe of the first 20 plants encountered. Preliminary studies indicated that stipe diameter was a good indicator of length and weight of each plant.

In 1991, we sampled only in eelgrass beds. The sites sampled within these habitats were the same sites sampled in 1990, except that 1 additional pair of sites was sampled.

The density of eelgrass turions was approximately 30% greater at control sites than at oiled sites in 1990 (P=0.08), and there were significantly fewer flowering plants at oiled sites (P=0.06). By 1991, eelgrass had apparently recovered as there were no differences noted among sites with respect to either turion density (P=0.52) or flower density (P=0.60).

The dominant plants in bay habitats were the stipate kelps Agarum cribrosum and Laminaria saccharina. The density and percent cover of Laminaria spp. (the vast majority of which were L. saccharina) were greater at the oiled sites relative to the control sites, in both deep and shal-
low depth strata (P<0.05 in all cases). *Laminaria* represented about 45%, on average of the total algal cover at oiled sites, but only 13% of the total cover at control sites.

The density of *Agarum* did not differ among sites (P=0.24 and P=0.31 at shallow and deep strata respectively). However, there were observable differences with regard to size distributions of *Agarum*. There tended to be proportionally more small plants, and proportionally fewer large plants at the oiled sites, especially in the shallower depth stratum. The size distributions differed significantly at the shallower depth stratum (P<0.01) and were nearly significant (P=0.15) in the deeper strata.

Points around the islands of the Knight Island group tended to have slightly higher algal diversity than the bays, but were still dominated by *Agarum cribosum* and *Laminaria saccharina*. There were generally higher densities of *Agarum* at the oiled than at control sites (P=0.07 shallow and P=0.02 deep). These differences were primarily attributable to significantly greater numbers of small *Agarum* (<10 cm in height) at the oiled sites (P=0.03 shallow and P=0.01 deep).

Also, the size distributions of *Agarum* (for plants larger than 10 cm) revealed a pattern similar to that observed in the bays, with proportionally fewer large individuals and more smaller plants at the oiled sites, especially in the shallower depth stratum. However, the size distributions did not differ significantly (P=0.15 shallow and P=0.32 deep).

*Nereocystis* habitats had a canopy of *Nereocystis leutkeana* with a diverse understory consisting of primarily of *Agarum cribosum*, *Pleurophycus gardneri*, and 3 *Laminaria* species (*L. saccharina*, *L. groenlandica*, and *L. yezoensis*). *Nereocystis* density was more than 7 times greater at the oiled sites relative to the control (P<0.01). Also, there were proportionally more small plants and fewer large plants at the oiled sites (P=0.10).

Eelgrass appears to have been adversely affected by the spill as evidenced by the lower densities of turions and flowers at the oiled sites. The evidence of an effect is strongly supported by similar evidence presented in a second, independent study. An evaluation of the effects of the *Exxon Valdez* oil spill on eelgrass in Prince William Sound by Teas et al. (1991) also demonstrated that there was a reduced density of flowering stalks of eelgrass at oiled sites.

In each of the three habitats on hard substrate (*Nereocystis*, *Laminaria*/*Agarum* in bays, and *Laminaria*/*Agarum* on points) we observed differences in the size distribution of the dominant alga at oiled and control sites. In all cases, there were proportionally more small algae (*Nereocystis* and *Agarum* in the *Nereocystis* habitat and *Agarum* at the other two habitats) at the oiled sites. In addition, we observed higher mean densities of one of the dominant algal species at oiled sites in each habitat: *Nereocystis* in the *Nereocystis* habitat, *Laminaria saccharina* in the bays, and small *Agarum cribosum* at points.

We interpret these differences in size distribution and density as recovery of the algal community following a loss of algae in 1989. We suspect that the spill was responsible for the loss of algae, either as a direct effect of oil or from cleanup activities, and that the free space created was quickly colonized by new recruits in 1990. An alternative hypothesis is that the differences in size distribution were the result of slower growth of algae at oiled sites.
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References
The Effects of the Exxon Valdez Oil Spill on Infaunal Invertebrates in the Eelgrass Habitat of Prince William Sound  
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Sampling was conducted in 1990 and 1991 to assess the impacts of the Exxon Valdez oil spill on infaunal invertebrates within and adjacent to shallow (<20 m) subtidal eelgrass (Zostera) beds in western Prince William Sound. We present results from a stratified random sampling program that compared measures of diversity, abundance and biomass of mainly infaunal invertebrates at paired oiled and control (unoiled) sites.

The oiled and unoiled site pairs for both years were Bay of Isles-Drier Bay, Herring Bay-Lower Herring Bay, Sleepy Bay-Moose Lips Bay, and Clammy Bay-Puffin Bay, respectively. The oiled sites were selected from areas that had adjacent shorelines moderately to heavily oiled during the fall of 1989. Control sites were selected that matched the oiled sites with respect to physiographic factors (e.g., exposure, slope, substrate type), but that did not have oil present on adjacent shorelines in fall 1989. Two depth strata (6-20 m and within the eelgrass bed [<3 m]) were sampled at each site. Three stations were established within each depth stratum and two 0.1 m² benthic samples were collected from each station with a diver-operated suction dredge. Sediment samples were concurrently collected for grain size and hydrocarbon analyses.

We tested for differences among oiled and unoiled sites using a randomization procedure (Manley, 1991). This procedure is briefly summarized as follows. (1) The blocked analysis of variance was performed. Station means were used as replicates, and replicates were blocked by oiled/unoiled pair. A sum of squares (SS) was produced for each factor. (2) Next, using the original data set, we randomly reassigned values for oil code to each station value. The ANOVA was then rerun on this new data set. (3) Step 2 was repeated 1000 times. (4) The SS from the ANOVA of the original data set was compared with SS of the 1000 randomly drawn data sets. The proportion of instances in which the SS for the randomly drawn data exceeded the SS for the original data was recorded. This value is the significance level of the test. The significance level is interpreted in the same manner as for parametric procedures. Analyses were conducted on diversity, dominance, species richness, total abundance, total biomass, total taxa, and the highest 15 ranking taxa (typically families) for abundance and biomass within each depth stratum. Separate analyses were performed that examined differences among oiled and unoiled sites within each year (1990 and 1991). In addition, 2-way analyses were performed that examined differences among oil and unoiled sites, differences among years, and their interaction.

In 1990, the health of the benthic community at 6-20 m depths, on mud/sand substrates adjacent to the eelgrass bed, was generally better at unoiled than at oiled sites. Conversely, the benthic community within the eelgrass bed was typically more robust at oiled sites. Further-
more, infaunal invertebrates were generally less abundant at the oiled sites, while epifauna tended to be more abundant at the oiled sites. In deeper waters adjacent to the bed, the biomass of three (Caecidae, Lepetidae, Veneridae) of the 15 dominant taxa was greater (p < 0.05) at the unoiled sites, while only one family (Maldanidae) was more abundant (p = 0.02) at the oiled sites. Within the eelgrass bed, several epifaunal families (e.g., spirorbids, spinids, mytilids, lacunids) were more abundant (or had greater biomass) at the oiled sites. Two dominant families (trochid snails and phoxocephalid amphipods) were more abundant (p < 0.01 for each family) at the unoiled sites.

The differences among oiled and unoiled sites appear related to oiling or cleanup activities. A preliminary analysis of hydrocarbon data suggest that in 1990, there were generally higher concentrations of hydrocarbons in the sediments from oiled sites.

There were indications of recovery of the epifaunal community in 1991. There were no differences among oiled and unoiled sites with respect to any of the diversity measures, or with respect to total biomass or total abundance. However, there were still four of the 15 dominant families that had greater biomass at the unoiled sites relative to the oiled sites.

Comparisons between years (oiled and unoiled sites combined) indicated that there were significantly greater abundance (p < 0.01) and a greater number of taxa (p < 0.05) in 1991 relative to 1990, at both depth strata. Seven families were significantly (p < 0.1) more abundant in 1991 vs. 1990, and ten families had greater (p < 0.1) biomass in 1991. None of the families displayed a significant decrease in abundance or biomass in 1991.

Comparisons between oiled vs. unoiled sites (1990 and 1991 combined) indicated that there were significantly (p < 0.1) greater diversity and total biomass at unoiled sites relative to oiled sites, at both depth strata.

The increase in the abundance of infauna in 1991 may have been related to a recovery from oiling effects at both oiled and unoiled sites. While the hydrocarbon concentrations were generally higher at oiled than unoiled sites in 1990, there was a substantial decrease in concentrations of hydrocarbons present at both oiled and unoiled sites in 1991.

Perhaps the greatest single indication of initial oil effects, followed by recovery, was the recolonization of oiled sites by sensitive burrowing amphipods. In 1990, at 6-20 m depths, the abundance of all amphipods was significantly (p < 0.01) greater at unoiled sites (unoiled mean = 47 individuals/0.1 m²; oiled mean = 19/0.1 m²). However, at this depth in 1991, no difference (p > 0.1) in total amphipod abundance or biomass was detected between oiled and unoiled sites. It is likely that the low density of amphipods at oiled sites in 1990 was the direct result of toxic effects of petroleum hydrocarbons. Benthic amphipods are notoriously sensitive to petroleum hydrocarbons, and massive declines in amphiiscid amphipods were observed following the Amoco Cadiz oil spill (Cabilch et al., 1978; Chasse, 1978). The reoccurrence of amphipods on those previously contaminated sediments began for some species in 1 to 2 years (Dauvin, 1982). Within Prince William Sound, these crustaceans had reoccupied the oiled sites in significant densities by 1991, just 2 years since being oiled.

Although the negative effects were
most apparent in this study, some increases in abundance or biomass were observed at oiled sites. The increases at the oiled sites were primarily attributable to the small epifaunal suspension feeding spiorbid and spionid polychaetes and mytilid mussels (Musculus spp.), and to a lesser extent by the surface deposit-feeding polychaetes, Nephyidae and Maldanidae. This may have been an indirect affect of oiling or cleanup, as the result of a reduction in predator abundance. However, it is possible that oiled sites tend to be areas where prevailing currents and winds concentrate both oil and larvae.

The most recent differences among oiled and unoiled sites seem to be indicative of advances in recovery of the community. We would expect the recovery to be more rapid at shallow depths. These are higher energy areas where sediments are more frequently reworked by waves, and where oil is less likely to persist. The recovery at the oiled sites may have been ameliorated by bioremediation activities that led to local enrichment of microbial and plankton communities.

Results from previous oil spills indicate that benthic communities generally represent good in situ monitors for measuring effects of oil fluxing to the bottom (e.g., Kineman et al., 1980) and that moderate amounts of oil in sediment cause impacts comparable to those we observed.

Most post-spill environmental studies elsewhere have been 3 to 5 years in duration. To date, only 2 years of monitoring has occurred, with no sampling in 1992. Collectively, our findings suggest an oiling effect on benthic invertebrates, since the components tested over the 2-year period showed that unoiled sites had the more robust community and that the community was recovering over the short period studied. However, conclusions on the effects from the spill cannot be made until the hydrocarbon data are fully analyzed.

References
Assessment of Injury to Pink Salmon Eggs and Fry

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This study is part of an integrated group of Natural Resources Damage Assessment Fish/Shellfish Studies (NRDA F/S) conducted to quantify damage to pink salmon Oncorhynchus gorbuscha as a result of the Exxon Valdez oil spill. Each study attempted to determine the injury to salmon at different stages of the life cycle. Wild pink salmon play a major role in the Prince William Sound ecosystem. Salmon are prey to a variety of terrestrial and marine mammals and birds, while also providing a pathway for nutrient transfer from marine to near-shore and terrestrial ecosystems. Wild pink salmon also contribute to the region’s commercial fisheries.

Up to 75% of the wild pink salmon which spawn in Prince William Sound use intertidal areas (Helle et al. 1964). These areas are highly susceptible to contamination from marine oil spills. Moles et al. (1987) and Rice et al. (1975) found that pink salmon eggs and pre-emergent fry were adversely affected by exposure to crude oil and that the effect was most acute in intertidal environments. The 24 March 1989 spill from the Exxon Valdez occurred just prior to the spring migration of salmon fry and contaminated many intertidal spawning areas in central and southwest Prince William Sound.

This study evaluated (1) the immediate effects of oil exposure on pre-emergent pink salmon numbers in the spring of 1989, (2) the effect of intertidal oil exposure on pink salmon egg mortality, and (3) the effect of intertidal oil exposure on pink salmon egg to pre-emergent fry survival. Samples were also collected for histopathological and mixed-function oxidase analysis. This project concentrated on southwestern Prince William Sound although streams from Montague Island and eastern Prince William Sound were sampled to provide a broader perspective.

Study streams were selected using the following criteria: (1) adult salmon returns were expected to be large enough to provide a high probability of success in egg and fry sampling, (2) egg and fry sampling had been done in past years, and (3) streams which had low to no oil impact (controls) were selected near high oil impact streams. Pink salmon fry remain in the area in the stream where they were deposited as eggs. This trait allowed oiled and control sites to be located in close proximity to each other, thus reducing any geographical effect on the findings.

Forty-eight streams were sampled for pre-emergent fry in 1990, 1991, and 1992. These included 25 streams historically sampled to forecast adult pink salmon returns and 23 additional streams from the oil impact area. Thirty-one streams were sampled for pink salmon egg mortality in 1989, 1990, and 1991. The streams sampled for egg mortality were included in the group of streams sampled for pre-emergent fry.

The methods used for both egg and pre-emergent fry sampling were similar to those described by Pirtle and McCurdy (1977). Sampling was stratified by tide zone to control for possible differences in
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egg mortality or overwinter survival due to salinity, temperature, predation, oil, or a combination of these factors. Four zones, three intertidal and one above tidal inundation were sampled, whenever possible, for each stream: 1.8 - 2.4 m, 2.4 - 3.0 m, and 3.0 - 3.7 m above mean low water, and upstream of mean high tide (3.7 m). Zone boundaries were established with a surveyor’s level and stadia rod and staked prior to sampling. No sampling was done below the 1.8 - 2.4 m zone as survival was expected to be low (Helle et al., 1964). Upstream sample areas were often within the reach of extreme high tides (3.7 - 4.6 m) since ice and snow often limit the extent of upstream sampling.

Separate linear transects were established within each zone for egg and pre-emergent fry surveys. Although most transects were 30.5 m long, some were shorter due to steep stream gradients. Transects were placed in riffle areas where spawning was observed during escapement surveys conducted by NRDA F/S Study 1. Transects ran diagonally across the stream: fry survey transects started downstream against the right bank and moved upstream to the left bank, while egg survey transects started downstream against the left bank and moved upstream to the right bank. This placement of egg and fry transects reduced sampling overlap and the influence of fall egg sampling on spring fry abundance.

Fourteen circular digs, each 0.186 m², were systematically made along each transect. The number of digs was a compromise between reducing variance and the practicality of conducting the study. Fewer digs were completed in narrow stream channels to avoid excessive sampling of the stream.

Stream oil exposure classifications were based on visual observations (NRDA F/S Studies 1 and 2) and hydrocarbon content of 1989 mussel tissue (Mytilus sp.) samples (NRDA F/S Study 1). Hydrocarbon analysis of mussel tissue and mixed-function oxidase analysis of pre-emergent fry generally agreed with visual observations of stream oil contamination. Histopathological analysis failed to detect lesions in pre-emergent fry, although results from another study (Fink, 1992) indicate the fry may have been collected too early in their life to have developed lesions.

Since the annual pre-emergent pink salmon fry density survey conducted by the Alaska Department of Fish and Game, Division of Commercial Fisheries, was underway at the time of the spill, many streams were sampled for pre-emergent fry density prior to or immediately after oil exposure. An additional session of sampling was also done approximately two weeks after the spill. This second survey allowed some streams examined during the first sampling session to be examined for immediate effects of oil contamination.

Few dead pink salmon fry were found either prior to or shortly after oil exposure. Only nine of the 52 transects examined contained more than five dead fry. No increase in fry mortality was detected between the first and second samplings, although only three of the 14 streams examined were oiled. Likewise, no difference in fry density was detected between the first and second sampling.

Egg mortality was significantly greater in oiled streams in 1989, 1990, and 1991. We believe these differences indicate an effect due to oil exposure. The 1989 investigation detected a statistically significant difference in egg mor-
tality (p=0.0001) between oiled and control streams. Examination of stream zone contrasts indicated that egg mortalities were greater in oiled streams and that statistical differences were due to elevated egg mortality in the intertidal zones. Mean mortalities for the oiled and control streams were 0.174 and 0.104, respectively.

The 1990 egg mortality study also showed a statistically significant difference (p=0.0008) between oiled and control streams. Again, examination of stream zone contrasts indicated greater mortalities in oiled streams with the statistical difference confined to the upper intertidal zone. Mean egg mortalities for the oiled and control streams were 0.295 and 0.195, respectively.

Egg mortality results were consistent with perceived oil contamination: among oiled streams, all intertidal zones were contaminated in 1989 whereas in 1990 oil remained only in the upper intertidal zone.

The 1991 evaluation demonstrated very significant egg mortality differences between oiled and control streams (p=0.0001). Inspection of stream zone contrasts indicated that egg mortalities in all zones were greater for the oiled streams. Mean mortalities for the oiled and control streams were 0.433 and 0.221, respectively. This finding was unexpected and at this time remains unexplained. We have hypothesized that the continuing and increased mortality is the result of genetic damage sustained by the eggs and alevins which incubated in oiled gravel during the fall of 1989 and spring of 1990. We are presently evaluating this hypothesis through a series of controlled rearing experiments.

No significant difference in egg-to-fry survival was detected between oiled and control streams for 1989 to 1990, 1990 to 1991, or 1991 to 1992. We feel these results were due to insufficient power in the sampling design or sampling levels to detect differences rather than a true lack of change.

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Cytochrome P450 Induction and Histopathology in Pre-emergent Pink Salmon From Oiled Streams in Prince William Sound, Alaska

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The 24 March 1989 Exxon Valdez spill in Prince William Sound and the Gulf of Alaska oiled a minimum of 213 intertidal pink salmon spawning streams. Some stream sediments remained contaminated through spring 1992. Staff from the Alaska Department of Fish and Game, Habitat Division, developed spill response recommendations and monitored the clean-up of oiled anadromous streams. After initial spill clean-up in summer 1989, the ADF&G began studies (Fink, 1992; Wiedmer, 1992) to determine if pre-emergent pink salmon eggs and fry were impacted by remaining petroleum residues. In December 1989; May, June, and September 1990; and March and May 1991, pre-emergent pink salmon eggs and fry were collected from four oiled and five control sites in Prince William Sound. A total of 23 fry samples consisting of 6 individuals each and 10 egg samples consisting of at least 6 eggs each were collected from mid to upper intertidal zones at oiled and control sites. Contaminated spawning sediments were analyzed utilizing gas chromatography with flame ionization detection (GC/FID) and gas chromatography with mass spectrometry (GC/MS).

To assess the continuing exposure of eggs and fry to hydrocarbon-contaminated spawning gravels, we evaluated the induction of cytochrome P450. Cytochrome P450 proteins are monoxygenase (MO) enzymes which mediate the metabolism of xenobiotics such as polyaromatic hydrocarbons (PAHs). Elevated levels of a specific P450 form, P450IA, have been found to indicate exposure to environmental pollutants (Stegeman and Lech, 1991). The P450IA content was evaluated by immunohistochemical analysis using monoclonal antibody 1-12-3 (Park et al., 1986; Smolowitz et al., 1991) specific for P450IA forms. Induction was ranked as negative, very mild, mild, moderate, or strong. To determine whether there were histopathological effects, Drs. Hinton and Marty, University of California, Davis, examined larval sections for the presence and severity of lesions. Lesions were ranked as none, mild, moderate, or severe.

Fry samples were stratified by time and pooled for each stream. The level of biological impact for each stream and time strata was estimated by the median of cytochrome P450IA induction and lesion scores. The Mann/Whitney (Wilcoxon) two-sample test (Conover, 1980) was used to test for statistical differences between oiled and control streams.

Cytochrome P450IA induction was observed in endothelial and epithelial cells of several organs of post-hatch larvae (13 of 16 samples) from oiled streams, and appeared to be independent of developmental stage. Organs exhibiting positive immunohistochemical staining included kidney, gill, liver, intestine, heart, brain, yolk sac, skin, peritoneal connective tissue, and pharyngeal epithelium. In 1990, induction in fry samples ranged
from negative to strong, while induction in 1991 ranged from negative to moderate. All seven fry samples from control streams were negative for induction. P450IA induction was not observed in pre-hatch egg samples, regardless of hydrocarbon exposure (December 1989 and September 1990).

P450IA content was significantly elevated in oiled streams in May and June, 1990 ($P<0.067$) and March 1991 ($P<0.10$). No statistical tests were performed on fry samples collected during December 1989 due to small sample size; however, examination of staining results indicated a difference may be present in P450IA expression between oiled and unoiled sites. No significant induction was observed in May 1991 ($P<0.20$). There was no significant change in induction intensity between 1990 and 1991.

Histopathological lesions were found in fry from six of 16 samples (35 of 93 fry, 38%) collected within the oiled zone of impacted streams in 1990-91. Lesions were found in fry from three of seven control samples (7 of 42 fry, 17%). While small sample size prevented meaningful statistical analysis, examination of median scores suggests an increase in lesions among fish collected from oiled streams in 1990, but not in 1991. Epidermal atrophy (EA) was the most frequently observed lesion. Other lesions observed included myofiber degeneration and necrosis (MDN), and individual hepatocellular degeneration and necrosis (IHN). Both MDN and IHN were absent in 1991 samples.

The occurrence of lesions appeared to correlate with the absorption of the yolk sac. Lesions were not found in post-hatch samples collected from oiled streams in December 1989, or in March 1991 (early life stages). However, lesions were found in samples collected in May/June 1990, and May 1991 (near the time of emergence).

Analyses of sediments from oiled sites indicated that polynuclear aromatic hydrocarbon constituents of Exxon Valdez crude contaminated these sites (GERG, 1990).

Immunohistochemical staining results indicate that pre-emergent pink salmon fry from oiled sites in Prince William Sound were responding to chronic exposure to PAHs by activation of the P450IA genes. While induction of P450IA can be caused by various contaminants, evidence from this study suggests that PAHs caused the observed induction. As a result of MO-mediated metabolism, toxicity of PAHs greatly increases (Wood et al., 1976). The products of MO-mediated metabolism bind to the cellular macromolecules RNA (Blobstein, et al., 1976) and DNA (Sims et al., 1974). The degree of metabolite binding is positively correlated to carcinogenic (Brookes and Lawley, 1964) and mutagenic (Jerala and Daly, 1974; Stegeman and Lech, 1991) potential. These observations indicate that elevation of cytochrome P450IA in pre-emergent pink salmon from Prince William Sound oiled streams may create an increased potential for mutagenesis and carcinogenesis.

Fish exposed to xenobiotics have demonstrated an inverse correlation between MO-activity and androgen, estrogen, and corticoid concentrations (Sivirajah et al., 1977), and testicular development (Truscott et al., 1983). Spies et al. (1988), found an inverse correlation between MO activity and parameters of female reproductive success (e.g., decreased proportion of viable eggs, decreased fertilization success, decreased embryological
success). These observations suggest that the reproductive potential of salmon developing in contaminated sediments may be diminished. The fish of this study were exposed to oil during their most dynamic developmental period; a period of rapid cellular differentiation and organogenesis. Pink salmon are semelparous, they reproduce but once. Factors with deleterious effects on the initial organogenesis of gonadal tissue will directly affect lifetime reproductive success. Changes in hormonal concentrations during the early life stages of oil-exposed fish may decrease parameters of reproductive potential such as egg number, size, and viability.

Histopathological results suggest that petroleum exposure may have caused an increase in lesion occurrence. Lesions appeared to be more severe and of greater frequency in 1990 samples as compared to 1991 samples. This might be expected if hydrocarbon concentrations within contaminated spawning substrates were reduced as a result of natural cleaning by winter storms, by other abiotic and biotic factors, and by stream treatments in the summer of 1990 at the four oiled streams.

In this study, while cytochrome P450IA induction was found in fry collected from oiled streams in December through early June, the occurrence of lesions in fry appeared to be developmental-dependent. Sac fry with abundant yolk did not exhibit lesions, while fry with minimal yolk reserves had higher probability of developing lesions, particularly epidermal atrophy. The skin is the first line of defense against pathogens and is also important in osmoregulation. Fry undergo physiological changes upon emergence, and subsequent acclimation into saltwater. Additional physiological stresses may affect an individual fry’s ability to avoid predation, thereby affecting survival.

The current study demonstrates that pre-emergent pink salmon fry in some heavily impacted streams incorporated Exxon Valdez petroleum into various tissues more than two years after the initial spill and that the hydrocarbons and their metabolites induced detectable physiological changes. These results can provide valuable links between the Exxon Valdez spill and any biological injuries in pink salmon observed in other studies.

References


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Accurate estimates of wild and hatchery pink salmon Oncorhynchus gorbuscha contributions to the commercial catch are essential to evaluate stock specific damage from the T/V Exxon Valdez oil spill. A mark is required for investigators to distinguish specific stocks during juvenile and adult life stages in order to quantify stock-specific differences in growth rates and fry to adult survival rates which may have been caused by oil damage. Coded-wire tags applied to pink salmon fry released from four Prince William Sound Hatcheries in 1989, 1990, and 1991 and to wild fry from three oiled and three unoiled streams in 1990 and 1991 provided such a mark.

Each year more than 500 million fry were enumerated and released from four hatcheries in Prince William Sound. Half-length coded wire tags were applied to approximately one out of every 560 fry released in 1989, 1990, and 1991. Approximately 2.2 million wild fry were enumerated from six streams (three oiled and three unoiled) and approximately 240,000 were tagged in both 1990 and 1991. Tags were applied to wild fry at ratios that ranged from one in three, to one in fifteen depending upon the total outmigration at each location.

Tag recovery rates vary by district, week, and processor (Peltz and Geiger, 1988). Tagging goals established in 1988, 1989, and 1990 were designed to ensure tags in subsequent adult returns could be recovered (F/S 3) in sufficient numbers to estimate the contribution of each release group to each district, week, and processor stratum in the commercial fishery. This degree of precision was required to estimate differences in production for oiled and unoiled groups of wild salmon (synthesis of F/S 1, 2, 3, and 4) and between wild and hatchery salmon. Tagging rates were held as constant as possible between and within hatcheries. An overall tagging rate of approximately 1 tag per 600 fish was chosen after analysis of the performance of previous tagging studies (Peltz and Miller, 1988; Peltz and Geiger, 1988; Geiger and Sharr, 1989).

Hatchery pink salmon fry to be tagged were randomly selected as they emerged from incubators. The fry were then anesthetized in a 1 ppm solution of Tricane Methanesulfonate (MS-222) prior to removal of adipose fins and application of tags. Tags were applied with a Northwest Marine Technology tag injector (model MKIV). A random sample of 20 clipped fish was graded for clip quality during each tagging shift. Clipped fish were tagged and passed through a quality control device (QCD) to test for tag retention. Rejected fish were retagged at least three times and if repeatedly rejected, they were destroyed to minimize the number of untagged but clipped fish in the release. Fry which retained tags were held overnight to determine short-term tagging mortality. An overnight tag retention rate was estimated by randomly selecting 200 fish and testing them with the QCD before release into saltwater rearing pens. Tag placement was checked periodically but not quantified. At Prince William Sound Aquaculture