*Exxon Valdez* Oil Spill
Restoration Project Final Report

# Ecological Effects to Benthic Infauna from Lingering Oil 15 Years after the *Exxon Valdez* Oil Spill

Restoration Project 040772
Final Report

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**Study History:** Restoration Project 040772 originated from the need to understand potential ecological effects resulting from the presence of lingering oil in intertidal subsurface sediments. Earlier studies documented aspects of the recovery of benthic infauna following the *Exxon Valdez* oil spill. In 2001, studies conducted by the National Marine Fisheries Service demonstrated the presence of lingering oil buried beneath the sediment surface. Information concerning the potential ecological effects of this buried oil was identified as a data gap by the *Exxon Valdez* Oil Spill Trustee Council. Field sampling was conducted in 2004, and samples were analyzed in 2004 and 2005. Annual project status reports were prepared for 2004 and 2005. Results will be published in a peer-reviewed journal.

**Abstract:** In 2001, Short et al. (2004) identified residual oil from the 1989 *Exxon Valdez* oil spill in intertidal sediments. In the current study, five pairs of intertidal stations, each composed of one oiled and one nearby unoiled station (as identified by concurrent National Marine Fisheries Service studies), were evaluated for potential ecological impacts due to subsurface lingering oil. Oiled sediments were not toxic in the larval mussel bioassay using *Mytilus edulis galloprovincialis,* where development and survival in the 100% elutriate were equivalent to the unoiled stations and the controls. Significant toxicity was observed in the 28-day amphipod bioassay using *Leptocheirus plumulosus,* where survival at the oiled stations was 0% while survival in the unoiled stations ranged from 37 to 84%. Survival was inversely related to sediment PAH and positively related to percent fines ($p<0.05$). However, decreased survival in the unoiled sediments suggests that this test may have been influenced by the predominance of very coarse sediments and a lack of fine-grained sediments. Benthic community structure was found to be similar between most oiled and unoiled stations; however, the power of statistical tests was low due to replicate variability. Study results indicate that residual oil sequestered in intertidal sediments is not causing community-wide effects though populations of sensitive species, such as amphipods, may be impaired.

**Key Words:** Amphipod bioassay, benthic infauna, *Exxon Valdez*, larval mussel bioassay, *Leptocheirus plumulosus*, *Mytilus edulis galloprovincialis*, Prince William Sound, sediment quality, sediment toxicity bioassay.

**Project Data:** *Description of data*—chemical analyses were run on sediment samples collected from intertidal beaches. Two laboratory bioassays (larval mussel test and amphipod growth and survival test) were conducted. Benthic infauna samples were collected and enumerated to the lowest taxonomic level. *Format*—All data were entered as Excel spreadsheets. *Custodian*—contact Betsy Day, Integral Consulting Inc., 7900 SE 28th Street, Suite 300, Mercer Island, Washington 98040.

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# 1. INTRODUCTION[1]

The *Exxon Valdez* oil spill (EVOS) in March 1989 resulted in the oiling of approximately 1,500 km of south-central Alaska's coastline, with heavy oiling affecting approximately 350 km of this area. This event had major impacts on the intertidal communities, particularly in the upper intertidal zone (EVOSTC 2002). The initial spreading of *Exxon Valdez* oil in open water was exacerbated by a series of significant storm events, resulting in oil washing ashore across Prince William Sound over the course of a 2-month period. Beach cleaning that occurred within the first few months after the event, particularly high-pressure, high-temperature washing, also caused mortality of intertidal biota, with long-term repercussions on community composition in some cases (Houghton et al. 1996, Highsmith et al. 1996).

The objective of this research project was to investigate potential ecological impacts associated with the continued presence of residual oil in subsurface intertidal sediments approximately 15 years after the spill. Earlier studies documented impacts to algae and invertebrates due to direct physical smothering or toxicity in the first few years following the spill (Boehm et al. 1995, de Vogelaere and Foster 1994, Houghton et al. 1996, Driskell et al. 1996, Highsmith et al. 1996, Hooten and Highsmith 1996, Wolfe et al. 1996). Both Boehm et al. (1995) and Wolfe et al. (1996) concluded that sediment toxicity to invertebrates decreased rapidly in the first few years following the spill. Sublethal effects were also documented, including higher proportions of unhealthy *Fucus gardneri* at oiled sites relative to reference sites (Stekoll et al. 1996, Stekoll and Deysher 2000). Long-term persistence of *Exxon Valdez* oil in intertidal sediments was described by Hayes and Michel (1999) as primarily occurring on well-armored beaches as well as those with flat slopes along the middle beach and where a thick sediment veneer occurs over a bedrock platform. More recent field investigations by Short et al. (2004) identified residual oil at 78 of the 91 beach segments sampled in 2001. Of these 78 beach segments, oil was found in subsurface sediments at 43 beaches. In 2003, Short et al. (2006) conducted additional field studies in the areas first impacted by the spill and found a greater proportion of heavily oiled subsurface sediments than in 2001.

The EVOS Trustee Council (EVOSTC) defined recovery objectives for intertidal biota in a number of ways, including the lack of differences in community composition and organism abundance between oiled and unoiled shorelines. In 2002, EVOSTC identified the status of the intertidal community as recovering based on substantial progress in recovery, but also acknowledged that recovery was not complete based on a several factors including the continued presence of residual oil in the intertidal zone (EVOSTC 2002).

[1] The research described in this paper was supported by the *Exxon Valdez* Oil Spill Trustee Council. However, the findings and conclusions presented by the author are her own and do not necessarily reflect the views or position of the Trustee Council.

Subsurface lingering oil consists of relatively unweathered residual oil buried beneath the sediment surface. The level of exposure of benthic invertebrates to subsurface lingering oil and the consequent ecological impacts to those invertebrates depend on a number of factors that are expected to vary in importance at differing locations, including the depth in the sediment column at which the organisms live (i.e., the biologically active zone) versus the depth at which the oil exists, the presence/absence of transport mechanisms to move the oil upward into the biologically active zone, the degree of weathering of the residual oil, and the degradation processes affecting the oil.

The approach for evaluating the benthic effects of lingering subsurface oil focused on assessing benthic recovery on beaches with subsurface lingering oil relative to nearby beaches that either had been oiled, but contained little or no oil by 2001, or that were not oiled following the spill. Oiled beaches were paired with unoiled beaches that were considered to have similar physical environments to attempt to control for physical processes that could affect both oil movement within the sediment and general benthic community structure. The available funding limited this investigation to five pairs of stations. The investigation of factors affecting exposure of benthic organisms to lingering oil, such as mechanisms that may move oil through the intertidal sediments to surficial layers where polycyclic aromatic hydrocarbon (PAH) photoactivation and its associated enhancement of toxicity of PAHs may occur, could not be included in the study.

It is recognized that beach sediments were also subjected to a range of physical cleanup processes following the spill that had the potential to affect benthic community structure, including manual raking and tilling, sediment removal, trenching, and treatment (Table 1). However, the experimental design attempts to address this issue by including physical measurements to characterize the current physical nature of the sediments.

Benthic effects were evaluated at the population level through the assessment of benthic community structure, and at the individual level through the use of chronic bioassays (i.e., larval mussel test and 28-day amphipod growth and survival test). Differences in measures of benthic community structure and bioassay endpoints between oiled and unoiled stations would indicate continued impacts due to residual oil that were severe enough to alter infaunal community structure as well as impact individuals. Differences in benthic endpoints without concurrent differences in bioassay response would suggest that physical factors, such as sediment grain size, were affecting community structure, or that the bioassay test organisms were not sensitive to oil. Differences in bioassay response between oiled and unoiled stations, without concurrent differences in benthic measures, would suggest potential impacts at the organism level that were not severe enough to be expressed at the community level, or that the statistical power associated with the benthic tests was not sufficient to detect a change.

**Table 1. Oiling and Cleanup History of Beaches Sampled Based on Records Gathered by National Marine Fisheries Service (Lindeberg 2005, pers. comm.).**

| Site | Station Type | Station | Oiling/Cleanup History |
|---|---|---|---|
| Disk Island | Oiled | DI067A | No records for 1989-90. Heavy equipment tilling in 1991; oiled sediment removal and trenching in 1991 and 1992 |
| | Unoiled | DI063A | Records not available |
| Northwest Bay | Oiled | EL056C | Extensive washing of sediments in 1989; no treatment in 1990 or 1991 |
| | Unoiled | EL052B | Extensive washing of sediments in 1989 and 1990 |
| Bay of Isles | Oiled | KN0136A | No cleanup in 1989-90. Manual raking and tilling in 1992 with removal of oiled sediments |
| | Unoiled | KN0205A | Records not available |
| North Herring Bay | Oiled | KN0109A | Records not available |
| | Unoiled | KN0110A | No cleanup in 1989; EPA experimental treatment site (1990?) |
| Herring Bay | Oiled | KN0114A | Records not available |
| | Unoiled | KN011A | Not oiled |

# 2. MATERIALS AND METHODS

## 2.1. EXPERIMENTAL DESIGN

The most significant challenge in developing this study design was the selection of meaningful indicators of the condition of the benthic community. Benthic infauna sampling was conducted to obtain direct measurement of the condition of benthic communities, and sampling was replicated to address the issue of natural variability. Chronic sediment toxicity tests, which evaluate sublethal endpoints such as development, growth, and reproduction, were also conducted. More than one chronic test was conducted to more fully characterize the potential effects of oil on sensitive life stages of species that represent different phyla. Potential tests were limited to those with established methodologies. The larval mussel test using *Mytilus edulis galloprovincialis* (formerly known as *Mytilus edulis*) was chosen because it is a relatively sensitive developmental test using a molluscan species that is representative of species present in Prince William Sound, including the clams that serve as the primary food source for sea otters. *Leptocheirus plumulosus* was chosen for the 28-day amphipod survival and growth test because it is the most tolerant amphipod test species for coarse grained sediments (USEPA 2001). Species from these two phyla are also prey items for sea otters (clams) and seabirds (mussels and amphipods).

Potential effects of lingering oil were assessed by evaluating differences in sediment chemistry, toxicity, and benthic community structure between oiled and unoiled station pairs. Five pairs of stations were identified (Figure 1), located in North Herring Bay, Herring Bay, Bay of Isles, Northwest Bay, and Disk Island. These stations were already being assessed for oil bioavailability by the National Marine Fisheries Service (NMFS). One station within each pair had been historically classified as heavily oiled while the other station, located nearby and containing similar geomorphology and wave exposure, lacked oil based on the 2001 sampling by Short et al. (2004). Stations classified historically as heavily oiled were purposefully selected to provide data to assess potential ecological effects under worst-case conditions. Station elevation was consistent among all stations (3-meter vertical drop from mean higher high water) to eliminate variability associated with intertidal zonation.

The sampling program focused on surface sediments (0–10 cm) because these are the sediments that typically contain the highest abundances of infauna, and the study objective was to evaluate potential impacts to intertidal benthic communities. When Short et al. (2004) investigated beaches for lingering oil, they dug pits as deep as 1–2 feet and found oil at varying depths below the sediment surface. The results of the research reported here are applicable to surface conditions above buried lingering oil but cannot be used to directly infer the concentrations or toxicity of the residual oil that is located at depth.

Concurrent sampling efforts were performed for the toxicity testing and benthic community evaluation. Sediments for toxicity testing were collected to a depth of 10 cm, which generally

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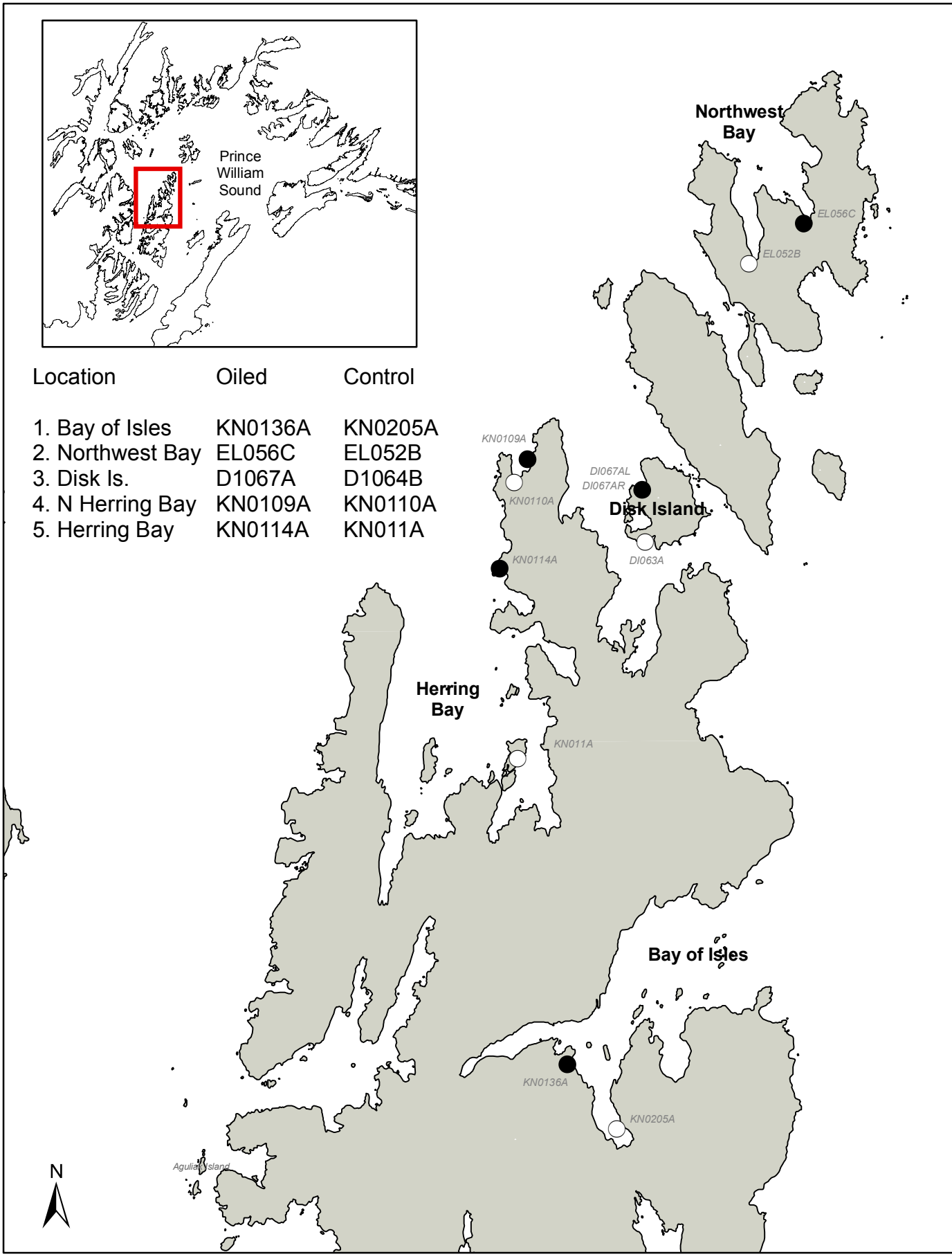


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Figure 1. Sampling Station Locations to Evaluate Benthic Effects Associated with Lingering Oil

included sediments where oil was visibly present. At each of the five station pairs, sediments were collected with a 4-in.-diameter core and analyzed for total petroleum hydrocarbons (TPH), PAHs, total organic carbon (TOC), total solids, grain-size distribution, and toxicity using the larval mussel bioassay and the 28-day amphipod survival and growth bioassay. Elutriate samples were prepared for the larval mussel bioassay and were also analyzed for PAHs.

Ten replicate 4-in.-diameter cores were collected to a depth of approximately 10 cm to assess the structure of the benthic community, and eight of the ten replicate samples were processed by a taxonomic laboratory. Immediately adjacent to each of the benthic cores, additional sediment was collected to a depth of about 2 cm for analysis of grain size, TOC, and TPH. These physical and chemical analyses were conducted on the top 2-cm interval because in many habitats a majority of benthic invertebrates are found in the upper few centimeters of surface sediment. The resulting data may provide a more meaningful evaluation of the potential exposure of benthic organisms to oil than data generated over a deeper sediment layer.

## 2.2. FIELD METHODS

All field sampling methods followed Puget Sound Estuary Program protocols (PSEP 1987). Samples were collected during low tide and transported to the sampling vessel for processing. Sediment for synoptic chemical and toxicity testing was collected using 4-in.-diameter, decontaminated, stainless-steel hand corers and placed into stainless-steel pots. Equipment was decontaminated between stations. During processing, the sediment was thoroughly homogenized until it was uniform in color and texture and then distributed to sample jars. Sediment for grain-size analysis and toxicity testing was refrigerated at approximately 4°C, while the remaining sediment samples were frozen at approximately –20°C.

Benthic samples were also collected using 4-in.-diameter cores. Upon removal of each corer from the sediment the contents of the core were placed into a labeled zip-locked bag and returned to the vessel for processing. During processing, the contents of each core were sieved using a gentle stream of site water through nested 2.5-mm and 1.0-mm benthic sieves. All organisms retained on the 2.5-mm sieve and all material retained on the 1.0-mm sieve were bagged, preserved with a solution of 10% buffered formalin, and stored in sealed buckets prior to shipment to the taxonomic laboratory.

One deviation from the experimental design occurred. Benthic samples could not be collected from North Herring Bay due to a cobble and boulder overburden that, when removed, clearly affected the population of gammaridae amphipods in surface sediments.

## 2.3. LABORATORY METHODS

### 2.3.1. Sediment Chemistry

Sediment and elutriate samples were analyzed for PAHs by the NMFS Auke Bay Laboratory (Juneau, Alaska) using their standard methodologies for samples associated with the spill (Short et al. 1996). Analytical Resources, Inc. (Seattle, Washington) analyzed both the composite and individual benthic surface sediments for TPH by EPA methods 3510, 3540/3550, and 8000, and total solids and grain size by PSEP (1986) methods. Composite samples were also analyzed for TOC by EPA method 9060.

### 2.3.2. Sediment Toxicity Bioassays

Bioassay testing was performed by MEC-Weston, Inc. (Tiburon, California). The potential toxicity of substances associated with pore water was evaluated using the 48-hour larval test with the mussel *Mytilus edulis gallioprovincialis*. Tests were conducted following methods provided in the "Inland Testing Manual" (USEPA/USACE 1998), USEPA (1995), and MEC-Weston Protocol MEC-BIO-043. A modified elutriate was prepared by mixing sediment with seawater and then removing particles >0.45 μm in diameter by settling. The initial dilution ratio of sediment to seawater targeted approximately 4.125% pore water in the final elutriate because this was the approximate average percent water content of the samples. The fraction water content of each sediment treatment (g porewater/g sediment) was determined by weighing an aliquot of sediment, drying at 80°C for 24 hours, and then reweighing. The resulting difference was then used to determine the percentage of pore water in the sediment samples.

The amount of sediment required to produce porewater (PW) concentrations of 41.25 mL pore water/L of elutriate was determined using the following formula:

grams of sediment/L seawater = (41.25 g PW/L seawater)/(g PW/g sediment)

The sediment/seawater mixture was combined in a 1-gallon glass jar and stirred vigorously by a mechanical tumbling device for 30 minutes. The mixture was then allowed to settle for 1 hour. The supernatant was carefully siphoned off, without disturbing the settled material, and immediately used for testing. The elutriate siphoned from the settled material represented 100% of the suspended-particulate porewater test material. Concentrations of 1%, 10%, 50%, and 100% elutriate were made from this material and dilution water prepared from artificial sea salts (bioassay grade Crystal Sea Marine Mix™), resulting in porewater concentrations of 4.125%, 2.0625%, 0.4125%, and 0.04125% pore water. Seawater was used for the negative control.

Sediment toxicity was also evaluated using the 28-day amphipod test using *Leptocheirus plumulosus* following methods in USEPA (2001) and MEC-Weston Protocol MEC-BIO-077. The 28-day *Leptocheirus* test is a static-renewal bedded sediment test that evaluates both survival and growth of neonate amphipods. Test organisms were reared in the laboratory in native sediments. Native sediment was also used for control sediment treatments.

### 2.3.3. Benthic Infauna

Benthic infaunal samples were processed to the lowest practical taxonomic level, which was generally to the species level, by Marine Taxonomic Services (Corvallis, Oregon). Sorting, identification, and quality assurance procedures followed PSEP (1987) protocols.

## 2.4. DATA ANALYSIS

Descriptive statistics for chemical results were performed using Microsoft Excel or SYSTAT (v10) 1999. Statistical analyses, including testing of statistical assumptions (i.e., normality and homogeneity of variance in data), were performed using SYSTAT or Excel. All comparison analyses were conducted using a $p<0.05$ probability level.

Bioassay data were tested for normality and then evaluated using Approximate *t*, Student's *t* or Mann-Whitney tests, as appropriate for the distribution of the data to determine if significant differences occurred between control and test conditions.

Analyses of the replicated benthic species composition data were conducted to determine if differences existed between the paired sites. Descriptive statistics, diversity index values, and major phyla summaries were generated using Microsoft Excel or SYSTAT (v10) 1999. SYSTAT was used to evaluate the data for homogeneity of variances and normality, as well as perform parametric t-tests. All comparison analyses were conducted using the $p<0.05$ probability level.

Stepwise linear regression models were constructed using Statistica 7.1 to identify significant relationships between sediment toxicity and PAHs, sediment grain size, and TOC content, and between benthic responses and these same chemical and physical parameters.

Benthic community classification analysis, using the Bray-Curtis similarity coefficient, was conducted using PRIMER software (Clark and Warwick 2001). Because this software package has a limited data matrix size, only those taxa constituting more than 10% of the total abundance in the study were included (i.e., rare taxa were omitted). Abundances were transformed using log (x+1) to meet the normality of distribution assumption required for multivariate testing.

# 3. RESULTS

## 3.1. PHYSICAL SEDIMENT CHARACTERISTICS

Intertidal sediments at the five pairs of stations were coarse-grained and contained up to 6% fines (silt and clay) (Table 2). Gravel accounted for an average of 61% of the sediment while sand accounted for an average of 37%. Station pairs varied in their respective sand and gravel compositions by 4–28%. The coarse-grained nature of these sediments is typical of Prince William Sound.

## 3.2. SEDIMENT CHEMISTRY

TOC, TPH, and PAHs were analyzed in each of the composite sediment samples collected for synoptic chemical and bioassay testing (Table 2). TOC at all but one of the unoiled stations ranged from 0.156 to 1.39%. TOC at the Herring Bay reference station was anomalously high at 5%, which may have resulted from the inclusion of organisms or detrital material within the sample. The TOC content of sediments from the oiled stations ranged from 0.456 to 5.92% with an average of 3.17%.

Concentrations of TPH were low at the unoiled stations, ranging from less than 10 to 104 mg/kg. In contrast, oiled stations contained 320–3,500 mg/kg TPH. PAHs followed a similar trend with the unoiled stations ranging from 0.005 to 0.016 mg/kg and the oiled stations ranging from 0.954 to 35.5 mg/kg. Variation in TPH and PAH concentrations among oiled stations is likely a function of the amount of oil remaining and its distribution within the sediment column.

The distributions of low molecular weight PAHs (LPAHs) and high molecular weight PAHs (HPAHs) are also shown in Table 2. At the oiled stations, LPAHs were found in consistently higher concentrations than HPAHs, accounting for 67–86% of the total PAH. In contrast, LPAHs at the unoiled stations accounted for a significantly lower percentage ($p$=0.002) of the total PAHs with 33–75% of the total PAH.

TPH was also measured in the top 2 cm of sediment adjacent to each of the eight benthic replicates collected at the oiled and unoiled stations at Disk Island, Northwest Bay, Bay of Isles, and Herring Bay. Values ranged by 1–2 orders of magnitude within each of the oiled stations and up to 1 order of magnitude within the unoiled stations (Table 3). The mean coefficients of variation for the oiled and unoiled stations were 90 and 40, respectively, indicating roughly twice the variability at the oiled stations than at the unoiled stations.

TPH measurements from the composite chemistry/bioassay sampling locations (Table 2) and from sampling locations adjacent to the benthic samples (Table 3) varied, but generally followed the same trend. Of the oiled stations, Station KN0136A (Bay of Isles) had the highest concentration in each data set while Station EL056C (Northwest Bay) had the lowest

**Table 2. Summary of Chemical Results for Composite Sediment Samples.**

| Site | Station Type | Station | Sediment PAH (mg/kg) | Sediment LPAH (mg/kg) | Sediment HPAH (mg/kg) | Elutriate PAH (µg/L) | Elutriate LPAH (µg/L) | Elutriate HPAH (µg/L) | TPH (mg/kg) | TOC (percent) | Total Solids (percent) | Gravel (percent) | Sand (percent) | Fines (percent) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Disk Island | Oiled | DI067A | 25.7 | 20.0 | 5.7 | 32.6 | 26.6 | 6.0 | 1,540 | 5.92 | 64 | 38 | 58 | 5.9 |
| | Unoiled | DI063A | 0.016 | 0.007 | 0.009 | 0.143 | 0.110 | 0.033 | 104 | 0.938 | 80 | 66 | 31 | 3.4 |
| Northwest Bay | Oiled | EL056C | 1.91 | 1.48 | 0.43 | 1.73 | 1.08 | 0.66 | 620 | 1.56 | 89 | 81 | 18 | 1.6 |
| | Unoiled | EL052B | 0.009 | 0.004 | 0.005 | 0.083 | 0.059 | 0.024 | 69 | 0.698 | 87 | 62 | 37 | 1.7 |
| Bay of Isles | Oiled | KN0136A | 35.5 | 30.4 | 5.1 | 28.3 | 22.5 | 5.9 | 3,500 | 5.78 | 77 | 61 | 35 | 4 |
| | Unoiled | KN0205A | 0.008 | 0.006 | 0.002 | 0.153 | 0.126 | 0.028 | 13 | 1.39 | 78 | 48 | 46 | 6 |
| North Herring Bay | Oiled | KN0109A | 0.954 | 0.640 | 0.314 | 2.35 | 0.764 | 1.585 | 320 | 0.456 | 91 | 71 | 28 | 0.8 |
| | Unoiled | KN0110A | 0.005 | 0.002 | 0.002 | 0.098 | 0.074 | 0.024 | 13 | 0.163 | 96 | 67 | 33 | 0.1 |
| Herring Bay | Oiled | KN0114A | 19.7 | 16.3 | 3.43 | 10.0 | 6.88 | 3.16 | 2,900 | 2.15 | 83 | 60 | 38 | 2 |
| | Unoiled | KN011A | 0.012 | 0.004 | 0.007 | 0.078 | 0.074 | 0.004 | <10 | 5 | 65 | 52 | 43 | 5 |

PAH = Polycyclic aromatic hydrocarbon
LPAH = Low molecular weight ploycyclic aromatic hydrocarbon
HPAH = High molecular weight polycyclic aromatic hydrocarbon
TPH = Total petroleum hydrocarbon
TOC = Total organic carbon

**Table 3. Ranges of TPH Concentrations (mg/kg) at Benthic Stations.**

| Site | Station Type | Station | Minimum | Maximum | Mean | Standard Deviation | Coefficient of Variation |
|---|---|---|---|---|---|---|---|
| Disk Island | Oiled | DI067A | 164 | 9,800 | 3,834 | 3,525 | 92 |
| | Unoiled | DI063A | 26.9 | 152 | 59 | 43 | 73 |
| Northwest Bay | Oiled | EL056C | 68 | 1,050 | 249 | 268 | 108 |
| | Unoiled | EL052B | 26.2 | 91 | 62 | 21 | 34 |
| Bay of Isles | Oiled | KN0136A | 510 | 14,600 | 5,929 | 5,904 | 100 |
| | Unoiled | KN0205A | <10 | 23.2 | 13 | 5 | 38 |
| Herring Bay | Oiled | KN0114A | 240 | 2,900 | 1,230 | 762 | 62 |
| | Unoiled | KN011A | <10 | 14 | 10 | 1.4 | 14 |

concentration in each data set.[2] Station DI067A (Disk Island) and Station KN0114A (Herring Bay) reversed their ranking relative to TPH concentration in the two data sets.

Total PAH, LPAH, and HPAH were compared to effects range low (ERL) and effects range median (ERM) sediment quality guidelines (Long and Morgan 1990) to help understand potential ecological effects in the sediments. Exceedance of these guidelines indicates the potential for adverse effects to biota, with ERM exceedances being more severe than ERL exceedances. Confirmation of effects requires actual biological testing. Each of the oiled stations exceeded either an ERL or an ERM, with ERM exceedances occurring at the Disk Island, Bay of Isles, and Herring Bay oiled stations. ERLs were exceeded at the Northwest Bay and North Herring Bay oiled stations. None of the unoiled stations exceeded the ERL or ERM for LPAH, HPAH, or total PAH.

## 3.3. ELUTRIATE CHEMISTRY

Sediment elutriates were prepared for use in the larval mussel bioassay. Elutriate PAH concentrations are provided in Table 2. The highest concentrations occurred in the Disk Island (32.6 mg/L) and Bay of Isles (28.3 mg/L) oiled sediments. Sediment and elutriate PAH concentrations were significantly correlated ($R^2 = 0.88$, $p<0.001$).

[2] As noted earlier, samples for benthic infauna enumeration were not collected at Station KN0109A in North Herring Bay.

## 3.4. SEDIMENT TOXICITY

### 3.4.1. Larval Mussel Bioassay

Larval mussel survival across the 10 seawater controls averaged 91%, and the proportion of normally developed larvae was 76% due to low numbers of normally developed larvae in two replicates (Table 4). This control test was valid with >90% survival and >70% normal development. When the replicates with low normal development were removed, the mean proportion of normally developed larvae was 94%.

Significant levels of toxicity or abnormal growth to *Mytilus edulis galloprovincialis* were not observed in any of the elutriates tested (Table 4). The proportion of normally developed larvae was essentially the same when normality was based on either the initial stocking density or the number of surviving larvae. No calculable $LC_{50}$ was observed for any of the test treatments, and no significant toxicity was observed in any of the 100% elutriate preparations. Elutriates prepared from oiled and unoiled intertidal sediments were not associated with toxicity in the larval mussel bioassay.

### 3.4.2 Amphipod Bioassay

Mean percent survival in the controls was 90% for *L. plumulosus*, and mean individual growth was 1.36 mg/individual (Table 5), indicating a valid test. Survival in the test treatments ranged from 0 to 84% survival. Mean percent survival was 0% in all sediments collected from oiled stations and was significantly less ($p$<0.001) than survival at the unoiled stations. Growth for treatments with surviving amphipods (i.e., growth at the unoiled stations) ranged from 0.12 mg/individual in KN0110A to 0.56–0.74 mg/individual in DI036A, EL052B, KN011A, and KN205A. Growth at EL052B, KN011A, and KN0110A was significantly reduced relative to the control ($p$<0.05).

Amphipod response was examined in relation to both the presence of oil as well as sediment grain size and TOC content. Survival was reduced to 0% at each of the oiled stations where TPH concentrations ranged between 320 and 3,500 mg/kg and total PAH ranged from 0.954 to 35.5 mg/kg. LPAH made up a significantly greater proportion of the total PAH at the oiled stations than it did at the unoiled stations ($p$=0.002). The oiled stations had a wide range of TOC (0.456 to 5.92%) and gravel (38 to 81%) contents, while percent fine-grained sediment (i.e., sand and silt) ranged from 0.8 to 5.9%. In contrast, the unoiled stations, where amphipod survival ranged from 37 to 84%, had significantly lower TPH (<10 to 104 mg/kg; $p$=0.003 with log transformed data) and PAH (0.005–0.016 mg/kg; $p$<0.001 with log transformed data) concentrations but similar and not statistically different TOC (0.156 to 5%), gravel (48 to 67%) and percent fines (0.1 to 6%) as the unoiled stations.

**Table 4. Summary of Larval Bioassay Results Using *M. edulis galloprovincialis.* [1]**

| Site | Station Type | Station | Elutriate PAH (μg/L) | Elutriate Concentration (%) | *M. edulis galloprovincialis* Mean Percent Survival | Standard Deviation | Mean Percent Normal Development | Standard Deviation |
|---|---|---|---|---|---|---|---|---|
| Laboratory Control | | | | | 91 | 10 | 94 [2] | 32 [3] |
| Disk Island | Oiled | DI067A | 32.6 | 100 | 97 | 4 | 95 | 5 |
| | | | | 50 | 93 | 4 | 92 | 5 |
| | | | | 10 | 95 | 8 | 94 | 8 |
| | | | | 1 | 96 | 5 | 96 | 5 |
| | Unoiled | DI063A | 0.143 | 100 | 97 | 3 | 96 | 3 |
| | | | | 50 | 98 | 11 | 97 | 11 |
| | | | | 10 | 86 | 6 | 84 | 7 |
| | | | | 1 | 95 | 7 | 94 | 8 |
| Northwest Bay | Oiled | EL056C | 1.73 | 100 | 92 | 7 | 91 | 7 |
| | | | | 50 | 99 | 6 | 98 | 7 |
| | | | | 10 | 94 | 5 | 93 | 6 |
| | | | | 1 | 94 | 6 | 93 | 7 |
| | Unoiled | EL052B | 0.083 | 100 | 87 | 6 | 86 | 6 |
| | | | | 50 | 96 | 2 | 95 | 2 |
| | | | | 10 | 95 | 7 | 94 | 8 |
| | | | | 1 | 94 | 7 | 93 | 6 |
| Bay of Isles | Oiled | KN0136A | 28.3 | 100 | 93 | 11 | 90 | 13 |
| | | | | 50 | 84 | 5 | 73 | 4 |
| | | | | 10 | 96 | 7 | 95 | 7 |
| | | | | 1 | 92 | 12 | 91 | 12 |
| | Unoiled | KN0205A | 0.153 | 100 | 99 | 2 | 99 | 3 |
| | | | | 50 | 99 | 3 | 99 | 3 |
| | | | | 10 | 96 | 7 | 95 | 7 |
| | | | | 1 | 92 | 10 | 91 | 10 |
| North Herring Bay | Oiled | KN0109A | 2.35 | 100 | 94 | 14 | 92 | 15 |
| | | | | 50 | 85 | 15 | 84 | 15 |
| | | | | 10 | 84 | 16 | 83 | 17 |
| | | | | 1 | 95 | 5 | 94 | 6 |
| | Unoiled | KN0110A | 0.098 | 100 | 100 | 0 | 100 | 1 |
| | | | | 50 | 91 | 10 | 89 | 11 |
| | | | | 10 | 90 | 8 | 88 | 8 |
| | | | | 1 | 92 | 8 | 91 | 8 |
| Herring Bay | Oiled | KN0114A | 10.0 | 100 | 96 | 6 | 95 | 6 |
| | | | | 50 | 96 | 5 | 96 | 6 |
| | | | | 10 | 95 | 7 | 94 | 7 |
| | | | | 1 | 90 | 9 | 89 | 8 |
| | Unoiled | KN011A | 0.078 | 100 | 90 | 14 | 89 | 16 |
| | | | | 50 | 95 | 6 | 94 | 7 |
| | | | | 10 | 95 | 4 | 94 | 5 |
| | | | | 1 | 93 | 5 | 92 | 5 |

[1] Bioassay results are mean values of laboratory replicates.
[2] 76% when outliers are included.
[3] 40% when outliers are included.

**Table 5. Summary of Amphipod Bioassay Results Using *L. plumulosus.* [1,2]**

| | | | | *L. plumulosus* | | | |
|---|---|---|---|---|---|---|---|
| Site | Station Type | Station | TPH (mg/kg) | Mean Percent Survival | Standard Deviation | Mean Growth (mg/individual) | Standard Deviation |
| LaboratoryControl | | | | 90 | 7.9 | 1.36 | 0.80 |
| Disk Island | Oiled | DI067A | 1,540 | **0** | -- | ND | -- |
| | Unoiled | DI063A | 104 | 81 | 17 | 0.74 | 0.11 |
| Northwest Bay | Oiled | EL056C | 620 | **0** | -- | ND | -- |
| | Unoiled | EL052B | 69 | **52** | 30 | **0.56** | 0.22 |
| Bay of Isles | Oiled | KN0136A | 3,500 | **0** | -- | ND | -- |
| | Unoiled | KN0205A | 13 | 84 | 15 | 0.69 | 0.30 |
| North Herring Bay | Oiled | KN0109A | 320 | **0** | -- | ND | -- |
| | Unoiled | KN0110A | 13 | **37** | 28 | **0.12** | 0.10 |
| Herring Bay | Oiled | KN0114A | 2,900 | **0** | -- | ND | -- |
| | Unoiled | KN011A | <10 | **62** | 5 | **0.66** | 0.13 |

[1] Bioassay results are mean values of laboratory replicates.
[2] Shaded values indicate response was significantly different ($p$ <0.05) from the control.
ND = no growth data due to 0% survival.

The relationships between *L. plumulosus* survival and PAH, TPH, TOC, percent gravel, and percent fines were assessed using a stepwise linear regression model. PAH, TPH, and percent fines data were log base 10-transformed prior to analysis due to the distribution of the data. The best predictors of amphipod survival were PAHs and percent fines (adjusted $R^2 = 0.91$; $p$<0.001). PAHs were negatively correlated with survival, whereas fines were positively correlated with survival. Other variables were found to be correlated based on partial correlations (controlling for survival). Significant correlations ($p$<0.05) were observed between PAHs and TPH, TOC, and percent fines, with $R^2$ values of 0.74, 0.61, and 0.73, respectively. Percent sand and percent gravel were also significantly correlated ($R^2 = 0.88$). To evaluate the influence of the results from the oiled stations (which had 0% survival) on the model, the model was rerun using only data from the unoiled stations. No statistically significant stepwise linear regression model was identified; however, using linear regression methods, percent fines was found to be the only predictor of amphipod survival ($R^2 = 0.71$; $p$<0.07) with increasing percent fines correlated with increasing survival.

Amphipod growth at the unoiled stations was also assessed using the stepwise linear regression model. That model did not identify significant relationships.

As mentioned earlier, the composition of the oil differed between the oiled and unoiled stations, with a significantly greater ($p$=0.004) proportion of LPAHs at the oiled stations than at the unoiled stations. When the stepwise linear regression analysis was conducted, HPAH and percent fines were the best predictors of survival ($R^2$=0.92; $p$<0.001). LPAHs were highly correlated with HPAHs ($R^2$=0.92; $p$<0.001) but were not identified as the best predictor of survival because of greater variability. Other researchers (e.g., Boehm et al. 2002) have shown that mortality is greater with higher concentrations of LPAHs relative to HPAHs. The stepwise regression analysis did not define relationships between growth and LPAHs or HPAHs, likely because of the small sample size; however, LPAHs were positively correlated with growth at the unoiled stations using linear regression methods ($R^2$=0.72; $p$=0.07).

## 3.5. BENTHIC INFAUNA

Benthic sampling at the four oiled and four unoiled stations resulted in the identification of 1,997 organisms belonging to 48 taxa. The major taxa groups were broadly represented over the entire data set with 14 polychaete taxa, 13 mollusc taxa, and 13 crustacean taxa, plus 8 other taxa. The three most abundant taxa, accounting for 53% of the total abundance, were Oligochaeta sp. indeterminate (indet.), juvenile *Mytilus* sp., and Nematoda sp. indet.

Thirty-eight taxa were identified from the oiled stations. The numerically dominant taxa at these stations were Oligochaeta sp. indet. (21.6%), Nematoda sp. indet. (20.3%) and juvenile *Mytilus* sp. (10.5%). In contrast, 30 taxa were identified from the unoiled stations. The unoiled stations were numerically dominated by Oligochaeta sp. indet. (34.5%), juvenile *Mytilus* sp. (16.7%), *Littorina scutulata* (10.5%), and Cirripedia sp. indet. (10.2%). The five numerically dominant taxa at each station are listed in Table 6 along with the proportion of the total abundance and richness at each station that were attributed to these five taxa.

Species-area curves were developed to evaluate the appropriateness of the analysis of eight replicate samples in the evaluation of benthic community structure, and, in particular, the probability of missing rare taxa. Over the eight replicates processed, 100% of the taxa encountered were collected in between three and seven replicates. These results indicate a low probability of encountering additional rare taxa had additional replicates been processed, and further suggest that data generated by eight replicates have captured the vast majority of species present.

A power analysis was completed to determine the statistical power associated with the benthic data. The benthic data were log base 10 transformed, and Levene's test ($p$<0.05) was used to test whether the variances between the unoiled and oiled groups were equivalent. The test was not significant, so a one-way power calculation for samples with equal variances was conducted. At $p$<0.05, the power was 0.27 for benthic abundance and 0.32 for benthic richness. These results show a low probability of detecting statistically significant differences between oiled and unoiled treatments due to variability within each treatment group.

**Table 6. Five Numerically Dominant Taxa at Each Station and Their Contribution to Total Abundance and Richness.**

| Site | Unoiled Stations | | | Oiled Stations | | |
|---|---|---|---|---|---|---|
| Bay of Isles | KN0205A | Abundance (%) | Richness (%) | KN0136A | Abundance (%) | Richness (%) |
| | *Pygospio elegans* (P) | 84 | 31 | Nematoda | 78 | 42 |
| | Cirripedia sp. indet. (C) | | | Nemertinea | | |
| | *Mytilus* spp. (M) | | | Cirripedia sp. indet. (C) | | |
| | *Hemigrapsus oregonensis* (C) | | | *Lottia* spp. (M) | | |
| | *Fabriciola berkeley*i (P) | | | *Mytilus* spp. (M) | | |
| Northwest | EL052B | Abundance (%) | Richness (%) | EL056C | Abundance (%) | Richness (%) |
| | Oligochaeta | 97 | 56 | Oligochaeta | 76 | 23 |
| | *Mytilus* spp. (M) | | | *Accedomoera* spp. (A) | | |
| | *Littorina scutulata* (M) | | | *Littorina scutulata* (M) | | |
| | Nematoda | | | Nematoda sp. indet. | | |
| | Cirripedia sp. indet. (C) | | | *Mytilus* spp. (M) | | |
| Disk Island | DI063A | Abundance (%) | Richness (%) | DI067A | Abundance (%) | Richness (%) |
| | Cirripedia sp. indet. (C) | 72 | 28 | Oligochaeta | 90 | 28 |
| | *Mytilus* spp. (M) | | | Nematoda | | |
| | *Pagarus* spp. (C) | | | *Mytilus* spp. (M) | | |
| | Nemertinea | | | *Lottia* spp. (M) | | |
| | Oligochaeta | | | *Littorina scutulata* (M) | | |
| Herring Ba | KN011A | Abundance (%) | Richness (%) | KN0114A | Abundance (%) | Richness (%) |
| | Oligochaeta | 66 | 26 | Oligochaeta | 89 | 56 |
| | *Pholoe minuta* (P) | | | *Mytilus* spp. (M) | | |
| | *Mytilus* spp. (M) | | | Nematoda | | |
| | *Fabriciola berkeleyi* (P) | | | *Pholoe minuta* (P) | | |
| | *Nematoda* sp. indet. | | | Nemertinea, Hyalidia (A) | | |

A = Amphipoda
C = Other Crustacea
M = Mollusca
P = Polychaeta

## 3.5.1. Comparison of Oiled and Unoiled Stations

Measures of benthic community structure (i.e., abundance, richness, diversity [H'], and evenness [J]) were not significantly different ($p$<0.05) between oiled and unoiled stations in Northwest Bay and Bay of Isles despite significant differences in TPH content (Table 7). At Disk Island, only abundance differed between oiled and unoiled stations with the oiled station (DI067A) having a greater abundance ($p$<0.05) than the unoiled station (DI063A). In Herring Bay, the unoiled station (KN011A) had a significantly greater richness, abundance, and diversity than the oiled station (KN0114A). On average, TPH concentrations at oiled stations were between 4 and 456 times greater than at the unoiled stations.

Graphical representations of the relationship between TPH concentration and benthic abundance and richness are shown in Figure 2. Individual replicates are shown in Figure 2 because the variability in TPH concentrations adjacent to each benthic replicate (Table 3) was large and could have had the potential to affect benthic response. Neither richness nor abundance appeared to be influenced by TPH, and less than 1% of the variability in either abundance or richness was explained by TPH. When the oiled and unoiled replicates were treated as individual groups, $R^2$ values between TPH and the benthic measures were less than 0.12.

A stepwise linear regression model was constructed using physical and chemical parameters to predict benthic abundance and richness. No combination of predictor variables had a statistically significant relationship with either abundance or richness.

## 3.5.2. Multivariate Analyses of Benthic Data

Community classification (i.e., cluster) analyses were performed using Primer software (Clark and Warwick 2001). The replicates for each station were pooled, and a Bray-Curtis dissimilarity coefficient was calculated. All stations were grouped at similarities between ~30 and 60%, indicating a lack of substantially different community structures (Figure 3). This is consistent with data presented earlier that failed to show clear differences in abundance and number of taxa among stations.

Two station groups, Groups A and B, are identified in Figure 3. Characteristics of the groups, which each contain oiled and unoiled stations, are shown in Table 8. Stations in Group A had a significantly greater abundance ($p$=0.049) than those in Group B. Richness, TPH, and grain size did not differ significantly between the groups. The numerically dominant taxa (Table 6) were similar between groups.

**Table 7. Characteristics of Benthic Communities at Oiled and Unoiled Stations.** [1,2,3]

| Site | Station Type | Station | Richness (0.008 $m^2$) | Abundance (0.008 $m^2$) | Shannon-Weiner (H') | Eveness (J') | TPH (mg/kg) | Gravel (percent) | Sand (percent) | Fines (percent) |
|---|---|---|---|---|---|---|---|---|---|---|
| Disk Island | Oiled | DI067A | 6.4 | **35.1** | 1.46 | 0.81 | **3,834** | 50.0 | 47.5 | 2.6 |
| | Unoiled | DI063A | 4.5 | **8.0** | 1.32 | 0.84 | **59** | 65.2 | 32.7 | 2.0 |
| Northwest Bay | Oiled | EL056C | 8.3 | 65.5 | 1.46 | 0.71 | **249** | 81.6 | 16.8 | 1.6 |
| | Unoiled | EL052B | 5.5 | 60.6 | 1.11 | 0.66 | **62** | 62.6 | 35.8 | 1.6 |
| Bay of Isles | Oiled | KN0136A | 7.3 | 31.3 | 1.64 | 0.84 | **5,929** | 61.5 | 35.0 | 3.5 |
| | Unoiled | KN0205A | 6.4 | 27.1 | 1.38 | 0.76 | **13** | 62.5 | 32.5 | 5.1 |
| Herring Bay | Oiled | KN0114A | **3.8** | **5.5** | **1.18** | 0.92 | **1,230** | 66.4 | 32.1 | 1.6 |
| | Unoiled | KN011A | **6.5** | **16.5** | **1.56** | 0.88 | **10** | 53.5 | 42.5 | 3.9 |

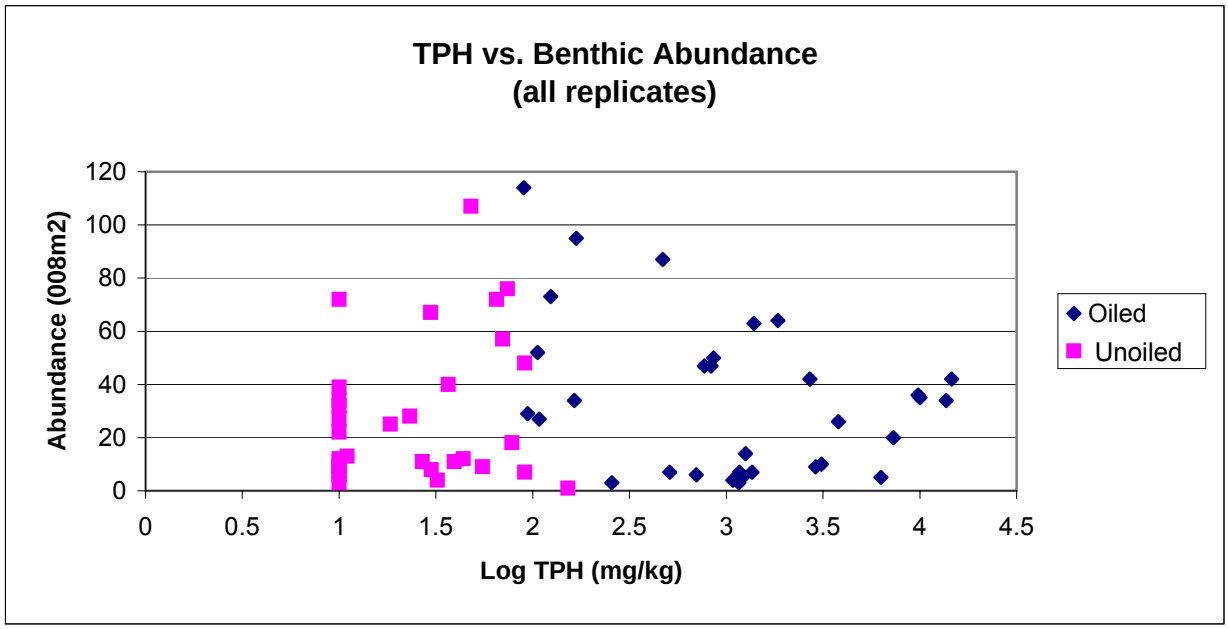
[1] Mean values.
[2] Benthic samples could not be collected at North Herring Bay due to overburden.
[3] Pairs of shaded cells are statistically different at $p$ <0.05.

**Table 8. Characteristics of Benthic Infaunal Cluster Groups.**

| Cluster Group | Stations | Site/Station Type | Mean Abundance (0.008 $m^2$) [1] | Mean Richness (0.008 $m^2$) | TPH (mg/kg) | Gravel (percent) | Sand (percent) | Fines (percent) |
|---|---|---|---|---|---|---|---|---|
| A | EL056C | Northwest Bay, oiled | 65.5 | 8.2 | 249 | 81.6 | 16.9 | 1.5 |
| | KN011A | Herring Bay, unoiled | 16.5 | 6.5 | 10 | 53.6 | 42.5 | 3.9 |
| | KN0136A | Bay of Isles, oiled | 31.2 | 7.2 | 5,929 | 61.5 | 35.0 | 3.5 |
| | DI067A | Disk Island, oiled | 34.9 | 6.4 | 3,834 | 50.0 | 47.4 | 2.6 |
| | EL052B | Northwest Bay, unoiled | 60.6 | 5.5 | 62 | 62.6 | 35.8 | 1.6 |
| | | *Mean* | *41.7* | *6.8* | *2,017* | *61.9* | *35.5* | *2.6* |
| | | *Standard deviation* | *20.7* | *1.0* | *2,719* | *12.2* | *11.6* | *1.1* |
| B | KN0114A | Herring Bay, oiled | 5.5 | 3.7 | 1,230 | 66.4 | 32.1 | 1.5 |
| | DI063A | Disk Island, unoiled | 7.9 | 4.5 | 59 | 65.2 | 32.7 | 2.1 |
| | KN0205A | Bay of Isles, unoiled | 27.1 | 6.4 | 13 | 62.5 | 32.5 | 5.1 |
| | | *Mean* | *13.5* | *4.9* | *434* | *64.7* | *32.4* | *2.9* |
| | | *Standard deviation* | *11.8* | *1.4* | *690* | *2.0* | *0.3* | *1.9* |

[1] Abundance in Group A was significantly different ($p$ <0.049) from Group B. No other significant differences were observed.

There were no consistent differences in benthic community structure between the oiled and unoiled stations, indicating that the subsurface oil was not impacting the resident biota at the community level. The community was primarily dominated by oligochaetes, nematodes, and juvenile barnacles and mussels. Of these, only the oligochaetes and nematodes are sediment deposit feeders. Braddock et al. (1996) and Highsmith et al. (1996) found large increases in oligochaete abundance following the oil spill, which they attributed to be in response to increases in bacterial populations with the capability to consume and break down the oil. Oligochaete densities reached 3,915/$m^2$ in this study at unoiled station EL052B, which was within the range observed by Highsmith et al. (1996) over their reference and oiled stations. Although oligochaetes are characterized as tolerant to organic enrichment (Pearson and Rosenberg 1978), their numerical dominance at stations in Prince William Sound with low TOC may reflect their ability to recruit quickly into areas physically disturbed by waves and ice that contain sufficient organic material for survival.



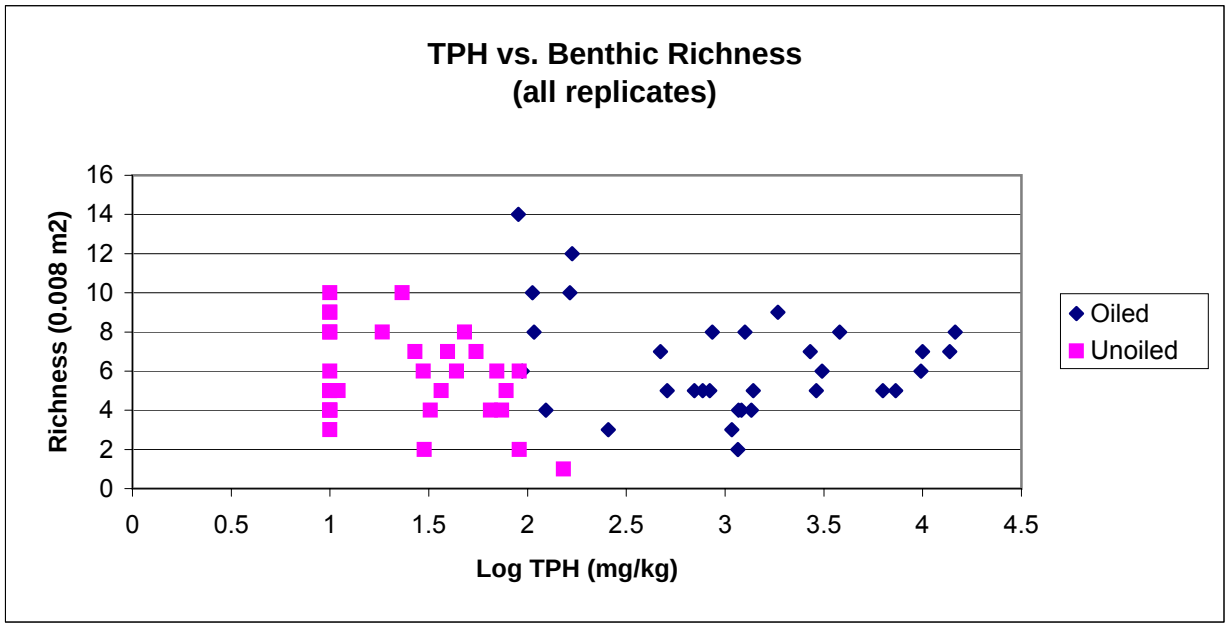




Figure 2. Relationships between TPH and Benthic Abundance and Richness

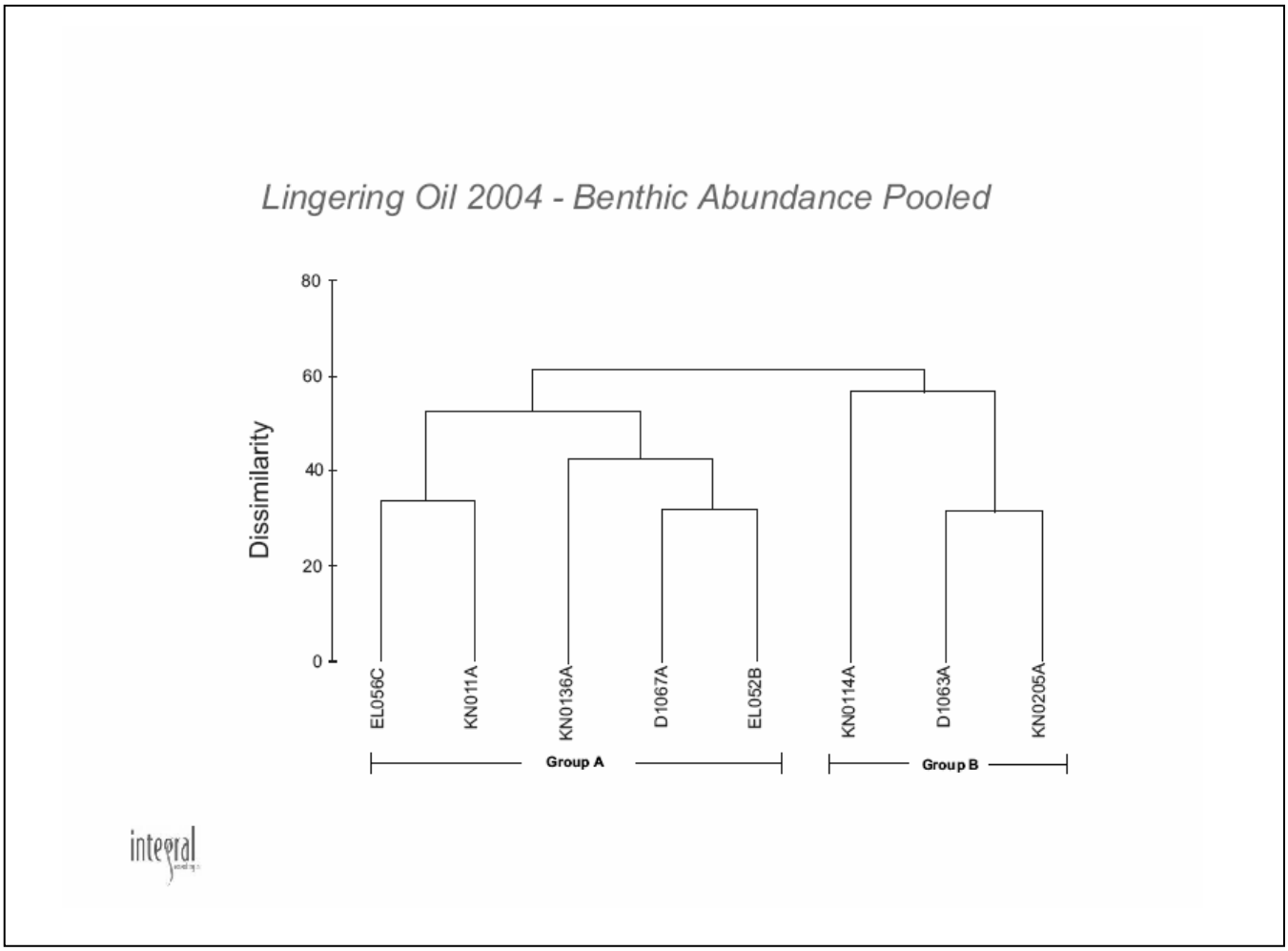



Figure 3. Results of Classification Analysis of Benthic Infauna

# 4. DISCUSSION

The recovery process for intertidal beaches impacted by the 1989 *Exxon Valdez* oil spill can be addressed by assessing both the presence and the ecological effects of lingering oil. As described by Short et al. (2004), beaches containing residual surface and subsurface oil were present in parts of Prince William Sound in 2001. The current study evaluated potential ecological impacts of lingering oil in 2004 by examining sediment quality using chemical analyses and standard bioassay and benthic community structure methods in an approach similar to Chapman's (1990) sediment quality triad. When sediments are found to be toxic, benthic community structure is altered, and chemical concentrations are elevated, there is a weight-of-evidence that ecological impacts are related to elevated chemical concentrations.

Each of the oiled stations exceeded either ERL or ERM sediment quality guidelines for total PAH, LPAH, or HPAH, whereas the unoiled stations did not. These exceedances, especially the ERM exceedances at Disk Island, Bay of Isles, and Herring Bay, indicate the possibility of ecological impacts. Confirmation of impacts requires biological testing as was also performed in this study.

A broad weight of evidence for ecological impacts was not found in the current study. The power associated with the benthic data was low, making it difficult to identify potential statistical differences in benthic indices. However, the community classification analysis also showed a lack of distinctly different assemblages (Figure 3), and there was substantial overlap in the numerically dominant species at oiled and unoiled stations. Of the two population-level bioassay tests conducted, the larval mussel test, which is generally considered to be a sensitive test, showed no toxicity associated with the elutriate concentrations tested, indicating the lack of a population-level effect. The lack of amphipod survival at the oiled stations indicates acute toxicity; however, the stepwise linear regression found that while oil was negatively correlated with survival, percent fines was positively correlated with survival. Along with the known grain-size requirements of *L. plumulosus*, these results point to toxicity associated with oil as well as a negative effect on survival due to coarse sediment grain size.

The amphipod *L. plumulosus* was chosen as a test organism because it is known to be tolerant of a wide range of sediment grain sizes and sediments in Prince William Sound were known to be coarse. USEPA (2001) states that *L. plumulosus* can be used for toxicity testing when the percent fines content of the sediment is >5%. The fines content in this study ranged from 0.1 to 6.0%, which was outside of the recommended grain size range at most stations. However, other amphipod test species routinely used in sediment bioassays are even less tolerant to coarse sediments. In natural habitats, *L. plumulosus* constructs a tube from fine sediment particles and resides within the tube. The lack of fine-grained sediment may prevent the construction of a suitable tube. Emory et al. (1997) found that *L. plumulosus* growth increased when the sand content increased to about 75%, but growth, survival, and reproduction were significantly reduced when exposed to pure sand. The substrate in Prince William Sound is exceptionally

coarse due to wave exposure, uplifting associated with the 1964 Good Friday Earthquake, and a lack of nearby sediment sources. The lack of fine-grained material in the sediments tested may have negatively affected the organisms used in the test and added to the physiological stress caused by the presence of oil.

The potential grain size effect in the *L. plumulosus* test does not explain the 100% mortality observed in the oiled sediments. However, the test organisms exposed to oiled sediments were likely already under stress due to the lack of fine-grained sediment for tube-building. This stress may have made the test organisms more susceptible to physiological impacts when they were exposed to elevated concentrations of oil in the test sediments.

The low power associated with the benthic community data, resulting from variability in species abundance, substantially reduced the ability to statistically detect differences among stations. Factors that may have influenced this variability include periodic physical disturbance in the intertidal zone and toxicity associated with the lingering oil. Intertidal beaches in Prince William Sound are coarse due to wave action and a lack of sediment sources. Beaches are also physically disturbed by ice. Physical disturbance may keep the benthic community in a perpetual state of recolonization, as evidenced by the presence of early colonizers, such as oligochaetes and nematodes, at most stations. The presence of lingering oil within the sediment column, and the potential for phototoxicity near the sediment surface, also may have led to patchiness that lessened the ability to statistically detect differences between oiled and unoiled stations.

The experimental methods used in this project were limited to those that might be employed for routine long-term monitoring of intertidal sediment quality. Additional studies on PAH bioaccumulation at these same locations were simultaneously conducted by the National Marine Fisheries Service. Together these studies provide information on PAH exposure and associated ecological effects due to lingering oil from the *Exxon Valdez* oil spill. A better understanding of the transport pathways for the oil to reach the sediment surface, and the likely future residence time of oil within the sediments, would be valuable additions to our overall knowledge base of oil-related benthic effects on Prince William Sound beaches.

In the event that future investigations or monitoring of the ecological impacts of lingering oil on benthic communities are conducted in Prince William Sound using methods similar to those used in this survey, future investigators will need to review the bioassay tests available to them in light of these results. Although the larval mussel test is recognized as a sensitive test with broad regulatory acceptance, other larval tests may be more sensitive to oil. The lack of response in this test, in association with the abundance of juvenile *Mytilus* sp. collected in the benthic samples, indicates a lack of toxicity associated with the oiled sediment for this taxonomic group.

Amphipods are also sensitive indicators of sediment quality, hence their routine use as sediment toxicity test organisms. Future investigators should consider use of a different

amphipod species, with better tolerance for very coarse sediment, for future testing of Prince William Sound sediments.

Assessment of the benthic community is the most direct measurement of actual conditions on intertidal beaches with lingering oil; however, the statistical power associated with the benthic data collected for this study was low and likely affected the ability to statistically detect potential differences between oiled and unoiled stations. In the future, a reconnaissance survey to evaluate the effectiveness of different sampled areas, screen sizes, and numbers of replicates on reducing replicate variability and improving the associated statistical power of the data is recommended. Although the results of this investigation indicate that lingering oil does not appear to be impacting the benthos at the community level, additional survey work that builds upon this study may improve the statistical power and yield more conclusive results.

# 5. ACKNOWLEDGMENTS

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