

## **2.0 Methods**

In this section, the methods used in field sampling, field measurements, and laboratory analyses are described.

### **2.1 Field Methods and Study Design**

#### **2.1.1 Study Design**

The program study area was identified as outermost Cook Inlet and Shelikof Strait of Alaska. The term “outermost Cook Inlet” has been used to avoid confusion with the lower Cook Inlet salmon district north of the study area. The study encompassed two separate field surveys, the first undertaken in 1997 and the second in 1998. For purposes of the scientific program, five regions or zones within the study area were defined as indicated in Figure 2-1. The first zone (zone 0) was outermost Cook Inlet, including the region from Anchor Point across to approximately Mt. Chinitna, and from the lower tip of Kenai Peninsula to the Barren Islands and across to Cape Douglas. Zone 1 was defined as North Shelikof Strait, stretching from the Barren Islands and Cape Douglas down to the mid-section of Afognak Island (Cape Paramanof) and across the Alaska Peninsula. Zone 2 was defined as the mid-Shelikof Strait region, from the mid-section of Afognak Island (Cape Paramanof) to Hallo Bay on the Alaska Peninsula, and down to Uganik Bay on Kodiak Island and Katmai Bay on the Alaska Peninsula. Zone 3 was defined as the south Shelikof Strait region, stretching down from zone 2 to the widening area of the strait as indicated in Figure 2-1. Finally, zone 4 was added during the 1998 survey and extended to the south of zone 3, just southeast of Kodiak Island.

Stations for sediment sampling were composed of random and fixed stations. The locations of the sampled random and fixed stations are provided in Table 2-1 and shown in Figure 2-1. In zones 1, 2, and 3 (Shelikof Strait), 15 random and 2 fixed stations were sampled in 1997, while 6 random and 2 fixed stations were sampled in each of these zones in 1998. In zone 0 (outermost Cook Inlet), 8 fixed stations were sampled during each of the 1997 and 1998 surveys. Only fixed stations were selected from outermost Cook Inlet due to the limited area where potential depositional environments could be identified (i.e. mud or silt/clay bottom). Three fixed stations were sampled in zone 4, exclusive to the 1998 survey.

Random stations were selected in zones 1, 2, and 3 by establishing a 5-km grid within the 50 fathom depth contour of each zone. This grid resulted in more than 100 blocks fully contained in each zone. Each block within a zone was sequentially numbered. Random numbers were then generated to identify the random stations within each zone. The first 15 stations randomly identified in each zone that contained silt/clay sediment, based on historical data, were established as the primary random stations. The station location was positioned in the center of the random block selected. An additional 10 alternate stations in each zone were identified in the same manner. The selection criteria for alternate stations were defined so that the next closest alternate station was selected if sampling at any random or fixed station was unsuccessful (did not contain silt/clay sediment). At each location, a grab sample was collected to determine if the station sediment was acceptable for sampling. A sediment sample was considered acceptable if it contained greater than 50 percent silt/clay (i.e., mud). The percent silt/clay was estimated by visual observation of the sediments. If the sediment sample was not acceptable,

repeat grabs were attempted at the station, but no more than three attempts were performed. If after repeated grab attempts the station was deemed unacceptable, the next closest alternate station was selected from the list in the sampling and logistics plan (Arthur D. Little, 1997a).

Several alternate stations were sampled during the course of the survey due to inappropriate bottom substrate. In Table 2-1, the alternate stations sampled and the rationale for requiring an alternate station selection are included. The two fixed stations in zones 1, 2, and 3 were selected in deep “holes” that contained depositional sediment. The three fixed stations in zone 4 were selected in deep areas where fine-grained depositional sediment was likely to occur.

The eight fixed stations in zone 0 were selected to obtain representative spatial coverage within the zone (e.g., Kachemak Bay, Kamishak Bay, and Kennedy Entrance). The eight fixed stations were selected from areas where historical grain-size data indicated depositional sediments occurred. In addition, four alternate fixed stations were identified in case depositional sediment could not be collected from any of the eight primary fixed stations.

### **2.1.2 Field Sampling**

The 1997 field survey was conducted aboard the R/V *Alpha Helix*, based out of the University of Alaska, Seward Marine Center, Alaska. The cruise was conducted from July 7 to July 17, 1997, and coincided with the most favorable tidal and current conditions in the program study area. The field team arrived in Seward, Alaska on July 6. Mobilization of the field team and the R/V *Alpha Helix* took place on July 7, and the R/V *Alpha Helix* departed Seward on July 8, 1997. Sediment and fish sampling was conducted from July 9 through July 16. The *Alpha Helix* returned to Seward on July 17 for demobilization at the Seward Marine Center. Field sampling personnel from Arthur D. Little, Inc. (ADL), the Florida Institute of Technology (FIT), Applied Marine Sciences (AMS), EVS Environment Consultants (EVS), and MMS participated in the survey. The scientific team and ship’s crew conducted the work on a 24-hour-a-day shift schedule.

The 1998 field survey was also conducted aboard the R/V *Alpha Helix*, from June 27 to July 5, 1998. After arrival of the field team on June 25, the mobilization of the R/V *Alpha Helix* and the field team occurred on June 26, followed by departure from Seward on June 27, 1998. Sediment and fish sampling was conducted from June 27 through July 5. The *Alpha Helix* returned to Seward on July 5 (one day ahead of schedule) for demobilization at the Seward Marine Center. Field sampling personnel from ADL, FIT, AMS, and MMS participated in the survey, with the scientific team and ship’s crew conducting the work on a 24-hour-a-day shift schedule.

The field sampling methods were conducted in accordance with the ADL Team’s Standard Operating Procedures (SOPs). The field sampling and logistics plan (Arthur D. Little, 1997a; Arthur D. Little, 1998a), prepared for the 1997 field survey, provides detailed explanation of the field methods used in sample collection, equipment decontamination, subsampling of fish tissues and sediment cores, and sediment profile imaging (SPI) film development. In this section, we summarize the methods for station selection, field sampling, and source sample collection.

Sediment samples were collected from 14 fixed stations and 45 random stations, fish samples were collected from 3 stations, and 12 source samples were collected from the Shelikof Strait

and outermost Cook Inlet region in 1997. During the 1998 survey, sediments were collected from 19 fixed stations and 18 random stations, fish samples were collected from 3 locations, and an additional 12 source samples were collected. All samples were analyzed for the appropriate chemical and physical parameters. Figure 2-1 shows the field survey station locations where surface sediment, sediment core, and fish samples were taken. The samples collected are also listed in Table 2-1, which summarizes the station locations and number and type of samples collected at each location and the analyses performed. Additional information is presented in the field survey cruise reports (Arthur D. Little, 1997b; Arthur D. Little, 1998b). The sample analysis results are discussed in Section 3.

The sequence of events at each sampling station followed specific procedures, described in detail in the sampling and logistics plan (Arthur D. Little, 1997a; Arthur D. Little, 1998a), including:

- Identify station (latitude and longitude)
- Navigate to station position within 0.2 nautical mile (nm) radius of the grid location
- Review the acoustic bottom profile for likelihood of depositional sediments
- Deploy seabird conductivity, temperature, and depth (CTD) and collect CTD measurements
- Collect Van-Veen grab samples
- Deploy SPI camera to photograph sediments on the ocean floor (1997 cruise only)
- Deploy box core or gravity core, where appropriate, and collect sediment cores

Equipment decontamination procedures were followed as described in the sampling and logistics plan (Arthur D. Little, 1997a). Decontamination typically included a physical scrub, rinses with seawater and distilled water, and a rinse with ethanol or isopropanol.

Replicate samples were collected as part of the field sampling design. At several locations, sediment samples were taken in triplicate, and at other locations as seven replicates (e.g., seven sample jars for one location). Reproducibility and range of results were demonstrated by analysis of replicate samples.

#### **2.1.2.1 Conductivity, Temperature, and Depth Measurements**

At each station, the seabird CTD was deployed to collect data on CTD. These data were downloaded by a data logger to a computer system where they were analyzed, graphically displayed, and stored electronically. The CTD data were recorded in hard copy and digital format on board the *Alpha Helix*.

For most stations, the CTD was deployed to a depth of 2 m above the ocean floor. However, the depth required for the CTD measurements was redefined due to the extended wire time involved for deployments at deep stations in zones 2, 3, and 4. A maximum CTD depth of 200 m was determined to be acceptable at deep stations. As a result, CTD measurements were collected to a depth of 200 m (or bottom, whichever was shallower) during the R/V *Alpha Helix* cruises.

The CTD data collected will be submitted to the National Oceanographic Data Center (NODC) in electronic format. It was not in the scope of work under this program to analyze the collected CTD data, therefore, no discussions nor interpretation of data are included in this report.

#### **2.1.2.2 Sediment Sampling**

Sediment sampling included the collection of surface sediments and sediment cores. During the collection and handling of sediment samples from the grab sampler, box core, and gravity core, extreme care was taken throughout the subsampling process to avoid contact with metals and hydrocarbon sources. Samples were taken away from the metal sides of the box core and no metal spatulas were used for the trace metal samples. The grab sampler, box core, and gravity core were protected from stack smoke, grease drips from winches and wire, and other potential airborne contaminants during the sampling process.

**Surface Sediments.** The modified Van-Veen grab sampler (0.1 m<sup>2</sup>), constructed of stainless steel and Kynar coated, was the primary equipment used for surface sediment sample (0 to 2 cm depth) collection at all stations except where sediment cores were collected. For sediment cores, a box core was used in addition to the Van-Veen grab sampler. The grab sampler was designed to be deployed from a vessel equipped with a power winch and A-frame or boom system and to collect undisturbed surface sediment samples to a maximum depth of approximately 15 cm. The operation of the grab sampler for collection of a bulk sediment sample (SOP ADL-1018) and the collection and handling of subtidal sediment chemistry samples from the Van-Veen grab sampler (SOP ADL-1019) are summarized below.

The grab sampler required some modifications (a shock cord dampener and adjustable stainless-steel feet) to successfully collect samples at deep stations in the heavy seas encountered during the survey. In addition, the order of gear deployment was modified at some stations due to limitations in the crane wire length (i.e., the grab sampler and CTD were deployed consecutively from the hydrowire winch at many stations).

When the grab was returned to the deck of the vessel, the sample was visually inspected to ensure the bucket was closed and the scissors extended upright. The doors were opened and the sample was visually inspected for sediment and overlying water in the bucket. Overlying water indicates that the sediment sample is undisturbed and that surface sediments remain intact (i.e., there was no leakage of water and hence fine sediment from the grab). If the grab was successful, samples were collected; if not, the grab's contents were discarded and the grab was redeployed.

Subsamples were removed from the grab sampler through the hinged doors on the top of the bucket. Overlying water was removed from the grab by siphoning through a precleaned Teflon<sup>®</sup> tube using a siphon bulb, or by carefully cracking the grab jaws to allow the water to flow out without disturbing the sediments. If used, the Teflon<sup>®</sup> tube was decontaminated prior to use and stored in precleaned aluminum foil.

Sediment samples were collected from the top 2 cm of the grab, which represents recent accumulation. Unconsolidated sediment 2 cm deep was removed from the grab with an aluminum, Kynar-coated scoop. The 2 cm-deep scoop facilitated accurate depth collection of the sediment. The top 2 cm were collected by several scoops up to the volume needed for subsamples and placed directly in appropriate sample containers for organics, metals, total organic carbon (TOC), and grain-size analyses. At stations where toxicity samples were needed, 3 to 6 grabs were necessary to obtain enough sediment volume for toxicity subsamples. Toxicity sediments from multiple grabs were composited in a Kynar-coated bowl. When the appropriate volume was reached, the sample was homogenized in the bowl and then transferred into appropriate precleaned containers. All sampling equipment was decontaminated before use as

outlined in the field sampling and logistics plan (Arthur D. Little, 1997a). Specific subsamples were collected from each grab into their individual container and stored in the freezer or the refrigerator (toxicity samples), as appropriate.

Trace metal samples were removed from the grab sampler with a Teflon® spatula, placed into labeled 48 mL plastic vials, and refrigerated. Samples to be used for grain-size analysis were doubled-wrapped in labeled Ziploc® storage bags and refrigerated.

Sediments for acid-volatile sulfide and simultaneously extracted metals (AVS/SEM) were collected, exclusively during the 1997 sampling event, from the top 2 to 3 cm of the grab sampler using a 50 mL plastic syringe with the lower end cut off such that the barrel was completely open. The syringe was carefully pushed laterally into the sediment as the plunger was pulled back. When the syringe was full, the outer plastic of the syringe was carefully wiped clean and the open end was covered with Parafilm®. Syringes were placed in labeled plastic bags and frozen.

Sediments were collected for hydrocarbon degrading microorganisms (1998 field survey only). A top 2 cm sediment subsample from the grab was collected in a pre-cleaned plastic jar and stored refrigerated. A total of 40 samples for hydrocarbon degrading organisms were collected in 1998 and shipped on ice to the University of Alaska, Fairbanks for analysis.

After the desired subsamples were removed, an open basin was placed beneath the grab on the grab stand. The grab jaws were then opened by releasing tension on the lifting wire and collapsing the scissor mechanism. Any remaining sediment that fell into the basin was discarded. The grab was rinsed with clean seawater from the deck hose and decontaminated with distilled water and ethanol/isopropanol rinses prior to deployment at a new station.

***Sediment Cores.*** After grab samples, CTD measurements, and SPI (1997 cruise only) were collected, an MK III box core was used to collect sediment cores at stations where geochronology cores were specified. In addition, two gravity cores were collected from the 1997 cruise (Table 2-1) and archived frozen for possible future analysis. One gravity core was collected from the 1998 cruise and analyzed to obtain a deep sediment profile.

The box coring device was deployed by a remotely operated winch system to the ocean floor. Prior to deployment, the box coring device was decontaminated according to procedures in the field sampling and logistics plan (Arthur D. Little, 1997a).

After retrieval of the box core, the overlying water was siphoned off as quickly as possible without disturbing the surface sediment layer. The inner box containing the sediment was moved into a covered deck area to further reduce contamination. Sediment cores were collected by carefully pushing four premeasured, 40 cm lengths of cellulose-acetate-butyrate (CAB) tubing down into the box core. Then, one person reached into the sediment and, placing one hand over the lower end of the CAB tubing, pulled the core out from the sampler. Both ends of the core were capped and taped. The cores were labeled and stored upright in a refrigerator until subsectioning was carried out within 24 to 48 hours. The CAB tubing had been precleaned with detergent and water, then rinsed with distilled water.

Sediment cores were subsectioned aboard ship. Cores to be used for analysis of trace metals, organic substances, and grain size were subsectioned into 2 cm intervals over the top 10 cm (5 samples) of the core and at 5 selectively spaced 2 cm intervals over the remainder of the core to obtain 10 samples. Subsections were obtained by placing the core upright in a fixed holder and slowly moving a polyvinyl chloride (PVC) piston up from the bottom of the core to extrude the sediment. Using a ruler, a 2 cm section of sediment was carefully extruded. Then the outer layer of sediment (2 to 3 mm) in contact with the CAB tubing was removed to minimize potential contamination from the core liner or from any smearing during sample collection and/or extrusion. Using a stainless-steel spatula, the extruded sediment was transferred to a clean glass jar and homogenized. After homogenization, about 10 g of sediment (wet weight) for analysis of metals and TOC were transferred to a labeled 48 mL plastic vial and stored in a refrigerator. Sediment in the glass jar was stored frozen to preserve organic substances. Sediment samples obtained from the core were set aside for grain-size analysis by placing them in labeled plastic bags and storing them in a refrigerator. During 1997, whole cores for grain-size analysis were returned to the Marine and Environmental Chemistry Laboratories at FIT and transferred to the Marine Geology Laboratory at FIT. In the Marine Geology Laboratory, the cores were subsectioned following the same sampling criteria outlined for obtaining samples for trace metals and organic substances.

The third core from each site also was subsectioned aboard ship for determination of sediment accumulation rates. For this core, the top 10 cm were subsectioned into 0.5 cm sections and 1 cm intervals were taken throughout the remainder of the core. Using a ruler, a 0.5 cm or 1 cm layer was carefully extruded and the sediment in contact with the CAB tubing was removed to avoid smearing recent sediment with older sediment during sampling and extrusion of the core. Sampling over 0.5 cm sediment intervals over the top 10 cm was carried out to ensure that the  $^{137}\text{Cs}$  record, dating back only to 1950, was observed. For example, if the sediment accumulation rate was 0.2 cm/y, then the complete  $^{137}\text{Cs}$  record would be found within the top 9.5 cm unless extensive in situ mixing had occurred. Sediment from each interval was placed into a labeled 48 mL plastic vial and refrigerated. The fourth core from each site was archived at Arthur D. Little.

Surface sediments and core samples were shipped to the Arthur D. Little and the Marine and Environmental Chemistry Laboratories at FIT in coolers packed with blue ice and custody sheets. Upon receipt, each sample was logged and the samples were transferred to a refrigerator (trace metals, age dating) or a freezer (organics, AVS/SEM). Samples collected for grain-size analysis were transferred to the Marine Geology Laboratory at FIT.

#### **2.1.2.3 Sediment Toxicity Sample Collection**

The methods used to obtain sediments for toxicity evaluation are described in this section. Details of the location and composition of samples are reported in their respective cruise reports (Arthur D. Little, 1997b; Arthur D. Little, 1998b).

Sediments were collected with the Van-Veen grab and/or the MK III box core. Multiple grabs were often required to obtain sufficient sample quantities for all measured parameters. When this was the case, aliquots of sediments were held in a Kynar-coated stainless-steel bowl. Between grabs, the bowl was covered with clean aluminum foil and held in the dry laboratory. Once sufficient quantities of sediment were obtained, the sample was well mixed and aliquoted into labeled 2 L, wide-mouth (factory-cleaned) polyethylene jars. The samples were held in a

4°C refrigerator while on board the vessel, then shipped to the analytical laboratory (Pacific Eco-Risk Laboratory [PERL]). The sediments for toxicity testing were not frozen when stored and shipped. Chain-of-custody forms accompanied the samples.

#### **2.1.2.4 Sediment Profile Imaging**

The purpose of the SPI survey was to delineate sediment type, provide information on patterns of sediment deposition and erosion, and describe biological community characteristics in the region. During the 1997 cruise on the R/V *Alpha Helix*, 57 stations were sampled by SPI. The SPI survey was not repeated on the 1998 cruise. The sediment profile images were taken by the EVS field crew according to the procedures described below. The complete SPI report was issued by Arthur D. Little as a separate stand-alone document in 1998 (Arthur D. Little, 1998c).

At the beginning of the survey, the time on the SPI camera's internal data logger was synchronized with the internal clock on the computerized navigation system to Greenwich Mean Time (plus 8 hours). Three replicate images were taken at each station; each SPI replicate was identified by the time recorded on the film and on disk, along with vessel position. Even though multiple images were taken at each location, each image was assigned a unique frame number by the data logger and cross-checked with the time stamp in the navigational system's computer data file. Redundant sample logs were kept by the field crew.

Test exposures of the Kodak® Color Separation Guide (Publication No. Q-13) were fired on deck at the beginning and end of each survey day to verify that all internal electronic systems were working to design specifications and to provide a color standard against which final film emulsion could be checked for proper color balance. Charged spare batteries were carried in the field at all times to ensure uninterrupted sample acquisition. After deployment of the camera at each station, the frame counter was checked to make sure that the requisite number of replicates had been taken. In addition, a prism penetration depth indicator on the camera frame was checked to verify that the optical prism had actually penetrated the bottom to a sufficient depth to acquire a profile image. If images were missed (frame counter indicator) or the penetration depth was insufficient (penetration indicator), weights were added or removed and additional replicates taken. Changes in prism weight amounts, the presence or absence of mud doors, and chassis stop positions were noted in the log for each replicate image. All film taken was developed in the field at the end of each survey day to verify successful data acquisition; strict controls were maintained for development temperatures, times, and chemicals to ensure consistent density on the film emulsion. The film was then visually inspected under magnification to determine whether any stations needed resampling.

Following completion of field operations, the color slides were scanned and stored in photo-CD format by ProLab®, Inc., Seattle, Washington. One hundred fifty-six digital images were analyzed from this survey using Image Pro® (Media Cybernetics, Inc.). Calibration information was determined by measuring 1 cm gradations from the Kodak Color Separation Guide. This calibration information was applied to all SPI images analyzed. Linear and area measurements were recorded as number of pixels and converted to scientific units using the calibration information.

Measured parameters were recorded on a Microsoft® Excel™ spreadsheet. These data were subsequently checked by an EVS senior scientist (Dr. J. Germano) as an independent quality

assurance/quality control (QA/QC) review of the measurements before final interpretation was performed.

#### **2.1.2.5 Fish Collection**

The methods used to capture fish for analysis are described in this section. Details of time, location, depth, and species of fish caught were reported in their respective cruise reports (Arthur D. Little, 1997b; Arthur D. Little, 1998b).

The primary fish species targeted by this study were Arrowtooth flounder. However, very few were caught in 1997. The primary target species based on the results of the long-line catches, though five Sablefish (black cod) and five Arrowtooth flounder were caught and analyzed. In 1998, Halibut and Pacific cod were caught in nearly equal numbers in all zones fished, so they were also analyzed. In addition to these two primary species analyzed in 1998, 9 Arrowtooth flounder, 4 Sablefish, 1 Aleutian skate and 1 Pacific halibut were caught and analyzed.

Table F-1 in the appendix lists all of the species caught and analyzed or discarded during both years of the study. Species not analyzed in 1998 out of interest stemming from differences in physiology and life history between elasmobranchs and teleosts (e.g., sharks and fishes).

Fish were captured by long-line. The gear used for this study had approximately 200 circle hooks with a shaft-to-point distance of 2 cm. The hooks were tied to 30 cm leaders of approximately 200 kg breaking strength, which were attached to the main line every 2 m. The main line was approximately 7 mm in diameter, with a breaking strength of approximately 1,000 kg. Each end of the long-line had an anchor/buoy line attached to it, with the long-line attached near the anchor, allowing the hooks to fish on or near the bottom. The buoys located the long-line at the surface. Each hook was baited with a piece of salted herring, and the gear was deployed for 4 to 6 hours and then retrieved (longer fishing times would have precluded returning all unused Halibut alive, as mortality increases with time).

A large tub was filled with seawater prior to retrieving the fishing gear. As the gear was brought on board, fish were removed from the hooks and either placed in the tub or discarded overboard. Fish were discarded if they were not a species of interest, or if they were dead. The species of all fish captured were documented regardless of whether they were kept for analysis. All unused target fish were returned to the ocean alive, after their total length (TL) was measured.

The methods used for tissue sampling of captured fish are described in this section. Weight and length were reported in the first cruise report as reported to the Alaska Department of Fish and Game (Arthur D. Little, 1997b).

Dissections commenced as soon as the fishing gear was retrieved. Dissections were done on several species of fish, but all were accomplished using the following protocol. Dissections were conducted by four scientists: one to record data, one to prepare the sample containers and assist the dissector, the third to weigh and measure, and a fourth to dissect the fish. The fish were removed from the tub, sacrificed with a fatal blow to the head, weighed on deck using a pesola (hanging balance), and then brought into the wet laboratory and placed on a precleaned nylon cutting board. General observations (e.g., parasites, deformations) were recorded. The fish were then measured (TL) and dissected.



All dissection equipment was cleaned with Alconox® detergent and water, and then rinsed with 1 percent hydrochloric acid (HCl) and methanol prior to each dissection. The dissections were performed “in the fish” to minimize the potential for sample contamination. This was accomplished by placing the fish blind side up (for the flatfishes, i.e., the Halibut, flounder, and skates) and making a cut with a cleaned stainless-steel filet knife along the lateral line from the pelvic girdle to the caudal peduncal. Round fish (i.e., Pacific Cod and Sablefish) were laid on their side, but otherwise dissected with the same methodology. The musculature covering the peritoneal cavity was then cut away, and a small portion of the flesh from the caudal end of the fish (without skin) was removed for metals analysis (FIT ancillary samples). General observations (e.g., parasites, deformities, sex of the fish) of the peritoneal cavity were made and then sections of liver for Reporter Gene System (RGS) P450, metals, and organic contaminant analyses were removed and placed in precleaned glass containers. A section of liver was also removed for immunohistochemical analysis of CYP1A (P4501A) and placed in a polyethylene container. Finally, a section of kidney and gill were removed and placed with the liver CYP1A sample. The liver samples for RGS-P450, metals, and organics were composited if sufficient numbers of target species were obtained, or were collected as individual samples where small numbers of individuals were caught; all samples were frozen after collection (Arthur D. Little, 1997b; Arthur D. Little, 1998b). The samples for CYP1A analysis were fixed with 10 percent-neutral-buffered formalin. The muscle samples for metals analysis were placed in plastic bags and frozen. Chain-of-custody forms accompanied the samples to each analytical laboratory.

### **2.1.3 Source Sample Collection**

Source samples were collected in order to compare concentrations and distributions of contaminants in the sediments to potential contaminant sources. Based on the literature review of historical data on the outermost Cook Inlet and Shelikof Strait (Boehm *et al.*, 1998), a number of potential contamination sources for the depositional sediments were identified. These sources include oil and gas activities, oil seeps, coals, municipal discharges, boat harbors, and riverine and coastal inputs. Samples representative of these separate source types were collected as part of the both the 1997 and 1998 sample cruises and are summarized in Table 2-2.

#### **2.1.3.1 Source Oils**

A source sample of Cook Inlet crude oil was collected from the Unocal Trading Bay Production Facility (TBPf) in August 1997 by Northern Testing Labs (NTL). A second oil source sample consisted of Swanson River Field oil, and was collected by UNOCAL in 1998. Finally, oil seep samples were collected from Well Creek, which drains into Oil Bay on the Iniskin Peninsula (Figure 2-1). The samples of seep oil were collected from the surface water of a pond adjacent to the creek. Each oil sample was collected in a precleaned glass jar for organics and metals, then shipped to Arthur D. Little for analysis.

#### **2.1.3.2 Source Coals**

A coal source sample was collected from the bluff area west of Homer Spit by Cook Inlet Regional Citizens' Advisory Council (CIRCAC), in November 1997. Five chunks of beach-washed coal were collected from the beach approximately one mile west of Bishops Beach, in Homer, Alaska (Figure 2-1), placed in a precleaned glass jar, then shipped to Arthur D. Little for analysis. A second coal source sample was collected from scattered pieces along Coal Bay Beach, Homer in 1998 (Figure 2-1) following the R/V *Alpha Helix* cruise.

Additional coal source samples collected in 1998 included those from coal seams in Ninilchik Bluff (Figure 2-1) and at Matanuska (Figure 2-2), one collected from a coal pocket at Coyote Lake (Figure 2-2) and two separate samples from the Beluga Coal Fields provided by CIRCAC.

### **2.1.3.3 Source Sediment**

Several locations were sampled during both surveys to determine the potential influence of resuspended river sediment and coastal bottom sediment to the study area.

The Homer boat harbor sediment source samples were collected during the July 1997 R/V *Alpha Helix* cruise. These sediment source samples were collected just outside the entrance of the boat harbor to the west of the dredged channel in an area where sampling activities did not interfere with boat traffic in the harbor. Two samples were collected from the Van-Veen grab, using a 2 cm-deep, Kynar-coated, stainless-steel scoop, at 0- to 2-cm and 4- to 6-cm intervals.

The Copper River sediment samples were collected in July 1997 by P. Boehm of Arthur D. Little. Figure 2-3 shows the four Copper River sediment sampling locations from Round and Long Islands (CR-2 to CR-4), and upstream near Million Dollar Bridge (CR-1). The samples were collected on the shoreline one meter above water level, from surface sediments (0 to 2 cm) using a stainless-steel spoon and precleaned glass sample jars. Sampling equipment was decontaminated between sample locations by rinsing with distilled water. Additional sediment (CR-5) was collected from the Copper River in 1998 (Figure 2-3). The sediment was placed into glass jars (organics) and plastic vials (metals), and shipped to Arthur D. Little and FIT.

The Susitna River sediment samples were collected in July 1997 by J. Trefry of FIT (Figure 2-3). The two Susitna River samples were taken from the northern bank of the river, 200 yards downstream (west) of the bridge at mile 105 on George Parks Highway (Highway No. 2). Sediment from the Susitna River was collected where there was fine-grained sediment, approximately one meter from the shore. At the time of sampling, the Susitna River was at a relatively high stage and the water was very turbid. The samples were collected using a stainless-steel spoon that was rinsed well with alcohol and Susitna River water. The sediment was placed directly into precleaned glass jars provided by Arthur D. Little. The samples were stored on ice in a cooler and shipped frozen with blue ice to Arthur D. Little.

Other sediment source samples were collected from one location in the influence of the Alaska Coastal Current, up-current of Cook Inlet offshore of the Kenai Peninsula, during the 1998 R/V *Alpha Helix* cruise (Figure 2-1). Additional sediment samples were collected offshore of St. Augustine Island with a Van-Veen grab sampler for grain-size and chemistry analysis (Figure 2-1). A sediment sample was collected near Holgate Glacier (Kenai Peninsula), not for the purpose of source influence, but to be used as a toxicity reference sample representing the fine-grained sediment encountered in the study area (Figure 2-1). This location was selected since it represented fine grained and glacial sediment and was up current from Cook Inlet (would not have Cook Inlet contaminants) and inshore of the influence of the Gulf of Alaska. In May 1998, Matanuska River sediment was collected for both metals and organic analysis (Figure 2-2). Finally, a miscellaneous volcanic ash sample was collected from the beach of St. Augustine Island for metals analysis only (Figure 2-1).

#### **2.1.3.4 Aqueous Sources**

Water samples from the Susitna, Knik, Matanuska, and Copper rivers were collected in 1998 (Figures 2-2 and 2-3) using 1-L, acid-washed polyethylene bottles. Each bottle was submerged, opened, partially filled, and closed. Once closed, the bottle was shaken and the sample discarded as an equipment rinse. Then, the closed bottle was resubmerged, opened, filled, and closed. To minimize sample contamination, all bottles were handled using powder-free polyethylene gloves. The bottles were double-bagged, labeled, placed in a cooler, and shipped cold to FIT for filtration and analysis of the suspended solids.

The municipal discharge produced water source sample was collected by personnel at the Point Woronzof, Anchorage Municipal Wastewater Treatment Facility (WWTF) in August 1997, and shipped to Arthur D. Little for analysis. The sample of final effluent was collected after treatment from the discharge location.

A produced water sample from the Unocal TBPF, representing oil and gas production activities, was sampled by a Unocal contractor. The produced water samples were collected in August 1997 from a final effluent outfall at the facility (TBPF-Outfall). The samples were collected in a precleaned glass jar for organics, and a plastic bottle for metals, and shipped on ice to Arthur D. Little for analysis of the suspended solids.

## **2.2 Analytical Methods**

### **2.2.1 Physical Parameters**

#### **2.2.1.1 Grain Size**

Surface sediment samples (0 to 2 cm) for grain-size analysis were double-wrapped in Ziploc<sup>®</sup> plastic bags and kept refrigerated while at sea. A separate subcore was collected for grain size at each of the nine coring locations during 1997 and four coring locations during 1998. Water was siphoned from the top of the core and the core was stored in a refrigerator at sea. Surface sediment and core samples were shipped to the Marine Geology Laboratory at FIT in coolers packed with blue ice and a chain-of-custody sheet. Upon receipt, each sample was logged in and prepared for analysis as described below.

Determination of grain-size distribution followed the classic method of Folk (1974). Initially, a dispersant (2 g of sodium hexametaphosphate “Calgon<sup>®</sup>” per 1 L of distilled water) was added to about 20 g of wet sediment to disaggregate and deflocculate the sediment. The subsample was immersed in this solution for 24 hours, prior to wet-sieving the sample through mesh sizes No. 10 (2 mm) and No. 230 (0.063 mm) to separate the gravel, sand, and mud fractions. The gravel and sand fractions were collected in preweighed beakers, dried in an oven at 60°C and reweighed. The mud fraction was collected in an evaporating bowl and transferred to a 1,000 mL cylinder. This fraction was deflocculated with a mechanical mixer for 5 minutes. Dispersant was added to the cylinders to bring the volume to 1,000 mL. The mud subsample was soaked for another 24 hours. Pipette analysis was performed for each cylinder using a 20 mL pipette. The fractions were withdrawn at a depth of 20 cm after 20 seconds (silt) and at a depth of 10 cm after 2 hours (clay). The samples were placed in preweighed beakers and dried. Weights for all size fractions were recorded in a spreadsheet for later calculations. The 2 g/L of deflocculant was subtracted from the final weights during calculations. The final data are presented as percent sand (2 mm to 0.062 mm), silt (0.062 to 0.004 mm), and clay (<0.004 mm). No gravel (particle sizes greater than 2 mm) was found in any samples.

### 2.2.1.2 Total Organic Carbon

Sediment stored in 48 mL plastic vials was initially freeze-dried to a constant mass (approximately 48 hours) after a 2 g portion of wet sediment had been taken for mercury (Hg) analysis. A 0.5 to 1 g portion of the freeze-dried sediment was placed in a 10 mL Pyrex® beaker and treated with 2 mL of concentrated HCl to remove any inorganic carbon present. The sediment was dried at 60°C and reweighed to determine the increase in weight due to the formation of calcium chloride (CaCl<sub>2</sub>) as a result of adding HCl. Then, approximately 5 to 20 mg of pretreated sediment were weighed into tin cups and combusted at 1,020°C in a Carlo-Erba® NA1500 carbon analyzer following the manufacturer's instructions. The TOC content of the sediment samples was determined using a four-point calibration curve with sulfanilamide as the standard. The TOC concentrations were corrected to account for the increase in sediment mass following the addition of HCl. The calibration curve was checked every 10 samples by analyzing standard reference material (SRM) BCSS-1, a marine sediment issued by the National Research Council of Canada (NRC).

### 2.2.2 Organic Parameters

Arthur D. Little provided analytical chemistry services as part of the Sediment Quality in Depositional Areas of the Shelikof Strait and Outermost Cook Inlet study. Arthur D. Little performed selected organic chemical analyses on 302 sediment, 43 tissue, and 27 source samples. This section describes the analytical methods that were used in performing these chemical analyses.

The core target analytes for the sediment and source samples were saturated hydrocarbons (SHC) as reported in Table 2-3, polycyclic aromatic hydrocarbons (PAH) as reported in Table 2-4, and biomarkers (steranes/triterpanes [S/T]) as reported in Table 2-5. Instrumental analysis included gas chromatography/flame ionization detection (GC/FID) for SHC determinations and gas chromatography/mass spectrometry detection (GC/MS) for PAH and biomarker determinations. Samples were grouped together in batches of no more than 20 field samples plus associated quality control (QC) samples. All organic sample analyses were conducted according to Arthur D. Little analytical SOPs.

#### 2.2.2.1 Sample Preparation

**Sediment Extraction.** The sediment samples were extracted based on EPA Method 3550A, Ultrasonic Extraction, which has been modified to include orbital shaking of the sample in extraction solvent for 1 hour following the final sonication. The following is a summary of the procedure used:

A 50 g weight of the homogenized sediment was placed into a Teflon® jar and dried with sodium sulfate. Another 5 g subsample was placed into an aluminum weighing pan for dry weight determination. The sample was serially extracted 3 times with 100 mL of dichloromethane (DCM) and acetone (1:1, volume to volume [V/V]), each time by sonication. The final sonication was followed by orbital shaking for 1 hour. The surrogates were spiked into the sample after the first addition of solvent and before the first extraction. All sediment samples were spiked with low-level surrogates. The surrogates were: naphthalene-d<sub>8</sub>, acenaphthene-d<sub>10</sub>, phenanthrene-d<sub>10</sub>, and benzo[a]pyrene-d<sub>12</sub> for PAH analysis, 5α-androstane and d<sub>50</sub>-tetracosane for SHC analysis, and 5β(H)-cholane and d<sub>66</sub>-dotriacontane for biomarker analysis.

After extraction, samples were concentrated by Kuderna-Danish on a hot water bath and an extract weight was taken, if necessary. Extracts were treated with copper to remove sulfur, and split in half. One-half was archived and the other half processed through a neutral alumina column (optionally, silica gel High-Performance Liquid Chromatography [HPLC] fractionation was performed in addition to the alumina cleanup).

The QC samples processed along with the sediment samples included one procedural blank, one blank spike, and one SRM (sediment SRM 1941a) per batch. The blank spike sample was fortified with PAH matrix spike solution and SHC matrix spike solution.

***Tissue Homogenization.*** Each tissue sample was a composite of the livers of up to five individual fish. Samples were homogenized in a blender prior to extraction. A 30 g aliquot was removed from each homogenized sample, frozen, and sent to Columbia Analytical Services for RGS-P450 analysis. A 10 to 15 g aliquot of frozen liver homogenate was sent to the Marine and Environmental Chemistry Laboratories at FIT for trace metals analysis.

***Tissue Extraction.*** Twenty-five grams of the homogenized wet tissue sample were placed into a Teflon<sup>®</sup> jar and dried with sodium sulfate. Another 5 g subsample was placed into an aluminum weighing pan for dry weight determination. The sample was then serially extracted 3 times with 100 mL of DCM by maceration using a Tissuemizer<sup>®</sup>. The surrogates were spiked into the sample after the first addition of solvent and before the first extraction. The surrogates were: naphthalene-d<sub>8</sub>, acenaphthene-d<sub>10</sub>, phenanthrene-d<sub>10</sub>, and benzo(a)pyrene-d<sub>12</sub> for PAH analysis and 5β(H)-cholane and d<sub>66</sub>-dotriacontane for biomarker analysis. Surrogate compounds were spiked into all tissue samples at the low level.

After extraction and concentration the gravimetric weight (lipid weight) was recorded. Extracts were then processed through an alumina column and a post-alumina gravimetric weight recorded prior to cleanup on the HPLC. Percent solid determinations for the tissue samples are provided in Appendix C.

The QC samples processed with each batch of tissue samples included one procedural blank, one blank spike, one SRM (1974a), and one duplicate analysis. The blank spike sample was fortified with PAH matrix spike solution.

***Source Samples.*** Source samples included crude oils, produced water, sediments, coals, and municipal discharge. These samples were expected to exhibit high concentrations of the targeted analytes. As such, they were segregated from the other samples to avoid the possibility of contaminating low-level samples. Following is a summary of the procedures used for the preparation of source samples.

***Crude Oil:*** A dilution was prepared from each crude oil or seep sample in DCM at an approximate concentration of 5 mg/mL. Each dilution was spiked directly with SHC, PAH, and S/T surrogates at the low level. Extracts were then passed through an alumina column and prepared for instrumental analysis.

***Produced Water and Municipal Discharges:*** Produced water and municipal discharge samples were extracted serially with DCM by the liquid-liquid method. These samples were spiked with

surrogates at the high level. After extraction, the combined extracts were passed through an alumina column.

*Source Sediment Samples:* Source sediment samples from Homer Harbor and the Copper and Susitna rivers were extracted by the sediment extraction procedure described in Section 2.2.2.1.

*Coals:* Coal source samples (approximately 5 g) were first ground to a fine particle size using a mortar and pestle, followed by extraction using the procedure described in section 2.2.2.1. The extracts were then passed through an alumina column and fractionated by silica gel into saturates and aromatics.

#### **2.2.2.2 Organic Extract Cleanup**

**Alumina Column.** Sediment extracts were treated with activated copper to remove sulfur and split in half. One-half was archived, and the other half passed through a neutral alumina column. Silica gel fractionation by HPLC was performed in addition to alumina cleanup to remove additional interferences from the S/T extracts.

Tissue extracts were weighed and passed through a 2 percent deactivated F-20 alumina column, and a post-alumina gravimetric weight was recorded.

**High-Performance Liquid Chromatography.** Tissue sample extracts were cleaned up by HPLC using a preparative gel permeation chromatography (GPC) column. The extracts were split according to total extract weight following the criteria in the SOP. The resulting post-HPLC extracts were analyzed by GC/MS for PAH. The pre-HPLC archive was saved for possible biomarker analysis at a later date.

#### **2.2.2.3 Internal Standard Addition for Instrumental Analysis**

The post-alumina and post-HPLC extracts were spiked with SHC, PAH, and S/T internal standards as appropriate for each extract/fraction. In general, the extracts were concentrated to approximately 250  $\mu$ L before adding the internal standards. The internal standard compounds were: chrysene- $d_{12}$  and fluorene- $d_{10}$  for PAH; chrysene- $d_{12}$  for S/T; and  $d_{62}$ -triacontane for SHC. The amount of SHC internal standard added to the extracts was adjusted to obtain a target concentration of 50  $\mu$ g/mL. The amount of PAH and S/T internal standard added to the extract was adjusted to obtain a target concentration of 1  $\mu$ g/mL. Aliquots (approximately 100  $\mu$ L) were removed from the spiked extracts for GC and GC/MS analyses. Due to the low extract volume all instrumental aliquots were recombined.

#### **2.2.2.4 Instrumental Analysis**

Instrumental analysis included GC/FID and GC/MS analysis of all sediment samples, and GC/MS analysis of tissue samples. A five-point calibration, an Instrumental Reference Material (IRM), a North Slope Crude Reference, and a Cook Inlet Crude Reference were run at the beginning of each instrumental sequence with each batch of samples.

All instruments were calibrated with analytical standards prior to the analysis of sample extracts. Target analyte concentrations were calculated versus the internal standard compound and were corrected for recovery of the surrogate compounds. The recovery of the surrogate compounds was calculated versus the internal standards added to the extracts prior to instrumental analysis.

**Gas Chromatography/Flame Ionization Detection.** Approximately 100 µL of the internal standard containing sample extract were submitted for GC/FID analysis. Sample extracts were injected onto a 30 m long by 0.25 mm inner-diameter (ID) fused-silica capillary column with DB5 bonded phase. This column provides baseline resolution of n-alkanes from n-C<sub>8</sub> to n-C<sub>40</sub> and n-C<sub>17</sub>/pristane and n-C<sub>18</sub>/phytane pairs. The injection port is designed for splitless injection and includes a silanized wide-bore glass liner containing a plug of silanized glass wool to reduce high-molecular-weight mass discrimination.

**Gas Chromatography/Mass Spectrometry Analysis.** The GC/MS analysis of sample extracts for PAH and biomarkers was performed in accordance with EPA Method 8270 modified to include alkyl PAH and selected ion monitoring (SIM). The PAH and S/T analyses were performed on sediments. PAH analyses only were performed on tissues. Approximately 100 µL of extract were submitted for analysis. The sample extract was injected onto a 30 m long by 0.25 mm ID fused-silica capillary column with DB5 bonded phase.

#### **2.2.2.5 Compound Quantification/Identification**

**Saturated Hydrocarbons.** The C<sub>8</sub> through C<sub>40</sub> normal alkanes, pristane, phytane, and selected isoprenoids were determined in the extract per EPA Method 8015 modified (Table 2-3). Two control oil solutions were analyzed with the samples. Quantification of the analytes was based on the internal standard compound (d<sub>62</sub>-triacontane) which was spiked into the sample just prior to analysis. The target analyte concentrations were corrected for surrogate recovery. The SOP includes the acceptability criteria for the calibration, procedural blank, surrogate compound recoveries, and matrix spike recoveries, as well as the corrective action if the criteria are not met, reporting requirements, and method detection limit (MDL) protocols. The data quality objectives (DQO) for this analysis are summarized in Table 2-8.

**Polycyclic Aromatic Hydrocarbons.** The extracts were analyzed by GC/MS in the SIM mode per modified EPA Method 8270, to determine the concentrations of parent and alkylated PAH in the samples (Table 2-4). Two control oils and a 1:10 dilution of SRM 1491 spiked with 250 ng/mL of surrogates and internal standards were also analyzed with the samples.

The concentrations of the individual PAH were calculated relative to one of the two internal standards that were spiked into the sample just prior to instrumental analysis (Table 2-4). The analyte concentrations were corrected for their respective surrogate recoveries (Table 2-4). The target PAH concentrations were quantified using average response factors (RFs) generated from the five-point calibration curve. To quantify the alkyl PAH, homologues were assigned the RF of their respective parent PAH compound.

**Steranes and Triterpanes.** Only sediment extracts were analyzed for steranes and triterpanes by GC/MS in the SIM mode by modified EPA Method 8270 (Table 2-5). An initial four-point calibration was performed before each batch sequence. A control oil (approximately 5 mg/mL) was analyzed to obtain a good biomarker signal and the identification of target compounds was based on the retention times of this oil. There was no analysis of sediment or instrumental SRM, since there was no recognized SRM for these compounds.

The concentrations of all identified S/T were calculated versus the internal standard chrysene-d<sub>12</sub>. All target triterpane concentrations were quantified using the average RF of 17β(H), 21β(H)-hopane (T23) generated from the initial S/T four-point calibration. All target sterane

concentrations were quantified using the average RF of cholestane (S17) in the initial four-point calibration. Analyte concentrations were corrected for surrogate recovery. Surrogate recovery of 5 $\beta$ (H)-cholane was calculated relative to the internal standard. If the determination of 5 $\beta$ (H)-cholane was interfered with, the extract archive was analyzed, using the alternate internal standard triacontane-d<sub>62</sub> and the alternate surrogate dotriacontane-d<sub>66</sub>. Extracted ion chromatograms of m/z 191 for the diterpanes and triterpanes and m/z 217 for the steranes were annotated with peak names and printed out for all samples.

### **2.2.3 Inorganic Parameters**

#### **2.2.3.1 Trace and Major Metals in Sediments**

Initially, each wet sediment sample was homogenized in the original 48 mL plastic vial using a Teflon<sup>®</sup> mixing rod. Then, a portion (approximately 2 g) of each sample was transferred to a preweighed plastic vial to determine water content. Once transferred, the wet sediment and the vial were reweighed. In addition, approximately 2 to 4 g of sample were transferred into a polyallomer centrifuge tube to determine the Hg content of the sediment (element symbols are defined in Table 2-6). Samples intended for water content were frozen, freeze-dried, and reweighed to determine the water content. The dried sediment samples were homogenized again using a Teflon<sup>®</sup> mixing rod.

Approximately 0.45 g of freeze-dried, homogenized sediment and standard reference sediment (BCSS-1) were totally digested in Teflon<sup>®</sup> beakers using concentrated, high-purity HF-HNO<sub>3</sub>-HClO<sub>4</sub>. Total digestion of the sediments is preferred because then no doubt remains about the absolute amount of metal associated with a sample. In the digestion process, 1 mL HClO<sub>4</sub>, 1 mL HNO<sub>3</sub>, and 3 mL HF were added to the sediment in the Teflon<sup>®</sup> beaker and heated at 50°C with a Teflon<sup>®</sup> watch cover in place until a moist paste formed. The mixture was heated for another 3 hours at 80°C with an additional 2 mL HNO<sub>3</sub> and 3 mL HF before bringing the sample to dryness. Finally, 1 mL HNO<sub>3</sub> and about 30 mL distilled, deionized water (DDW) were added to the sample and heated strongly to dissolve perchlorate salts and reduce the volume. The completely dissolved and clear samples were then diluted to 20 mL with DDW. This technique is 100 percent efficient with no loss of the elements studied and has been used successfully in the FIT laboratory for many years with a variety of sediment types.

Sediment for Hg analysis was digested by heating 2 to 4 g of wet sediment in an acid-washed, polyallomer centrifuge tube with 4 mL HNO<sub>3</sub> and 2 mL H<sub>2</sub>SO<sub>4</sub>. Sample tubes were heated for 1 hour in a 90°C water bath and allowed to cool. Each tube was centrifuged at 2,000 revolutions per minute (rpm) and the supernate was decanted into a 25 mL graduated cylinder. The sediment pellet was rinsed twice with 5 mL DDW, centrifuged, and decanted into the graduated cylinder before diluting to a final volume of 20 mL with DDW.

Labware used in the digestion process was acid-washed with hot, 8N HNO<sub>3</sub> and triple-rinsed with DDW. Two procedural blanks, two duplicate samples, and two SRMs were prepared with each set of 40 samples. SRM BCSS-1 (trace metals except Hg) and MESS-2 (Hg), sediment samples issued by the NRC, and 1646a (Hg), issued by the National Institute of Standards and Technology (NIST), respectively, were used.

Samples, SRM, and procedural and reagent blanks were analyzed by either flame atomic absorption spectrometry (FAAS), Zeeman graphite furnace atomic absorption spectrometry



(ZGFAAS), cold vapor atomic absorption spectrometry (CVAAS), or inductively coupled plasma/mass spectrometry (ICP/MS). The method used for each element and the corresponding MDLs are presented in Table 2-6. All analytical techniques followed manufacturers' specifications, SOPs on file at FIT, and the details provided in the QA/QC section below. These methods are very similar to the EPA methods described for Series 7000 FAAS and ZGFAAS, Series 7470 CVAAS, and Series 6010A ICP/MS as described in EPA 1992. Matrix interferences were carefully monitored for all elements using the method of standard additions.

#### **2.2.3.2 Trace Metals in Tissues**

In preparation for analysis, each tissue sample was homogenized with a Teflon<sup>®</sup> mixing rod (fish liver-composite samples) or stainless-steel instruments (fish flesh). Then, between 0.3 to 1.5 g of tissue were transferred to a preweighed plastic vial and reweighed to determine percent water content (required for Hg analyses). The plastic vial was frozen, freeze-dried, reweighed, and the water content was calculated. In addition, an additional weighed portion of each homogenized wet tissue (approximately 1 g of liver-composite or 2 to 3 g of fish flesh) was transferred to a 50 mL polypropylene centrifuge tube to be digested for total Hg content.

Liver-composite and fish flesh samples for determining concentrations of all metals except Hg were prepared using approximately 4 g of wet sample. The samples were transferred to preweighed, 100 mL glass digestion flasks, reweighed, frozen, freeze-dried, and the percent water content was calculated. These freeze-dried tissues and approximately 0.5 g portions of tissue SRM were totally dissolved by refluxing with concentrated, high-purity HNO<sub>3</sub>, H<sub>2</sub>O<sub>2</sub>, and HCl. Once the tissue samples were completely dissolved, the clear solutions were transferred to graduated cylinders, diluted to 20 mL with DDW rinses of the flasks, and then stored for analysis in 30 mL polyethylene bottles.

The wet tissue samples (1 to 3 g) for Hg analysis, along with 0.2 to 0.4 g portions of tissue SRM, were digested using high-purity HNO<sub>3</sub> and H<sub>2</sub>SO<sub>4</sub>. Each sample was refluxed for 1 hour in a 90°C water bath and allowed to cool. Once cool, the solution was decanted into a graduated cylinder, diluted to a final volume of 20 mL with DDW rinses of the centrifuge tubes, and stored for analysis in a 30 mL polyethylene bottle.

Metal concentrations in the digested tissue samples, tissue SRM, and procedural blanks were determined by FAAS, ZGFAAS, CVAAS, or ICP/MS in a manner compatible with the EPA Series 200.3 techniques (EPA, 1991). The methods used for each element and the corresponding MDL are listed in Table 2-6. In all cases, the manufacturers' specifications were followed and adherence to QA/QC requirements was maintained.

#### **2.2.3.3 Trace Metals in Aqueous and Oil Source Samples**

Initially, the salinity of the aqueous source samples (effluent and produced water) was obtained using a Reichert-Jung<sup>®</sup> Model 10419 refractometer to help determine the appropriate analytical technique or dilution for determining trace metal concentrations. Then, 20 mL of each sample (effluent and produced water) was pipetted into a 30-mL polyethylene bottle. The sample was acidified with 0.2 mL of high-purity HNO<sub>3</sub> and stored for the determination of all metal concentrations. The liquid source samples were not shaken to resuspend particulate matter prior to the transfer.

Subsamples of the crude oils (approximately 1 g) were transferred to glass digestion flasks and digested by refluxing with high-purity  $\text{HNO}_3$ ,  $\text{H}_2\text{O}_2$ , and  $\text{HCl}$  for the determination of all metals except Hg. The digested oil samples were poured into graduated cylinders and diluted to a final volume of 15 mL with DDW rinses of the digestion flask. The Hg concentration of the crude oil was determined using 0.2 g subsamples that were placed in polypropylene copolymer centrifuge tubes and refluxed with high-purity  $\text{HNO}_3$  and  $\text{H}_2\text{SO}_4$  for 1 hour in a 90°C water bath. After the Hg digestion was complete, the sample was transferred into a graduated cylinder, diluted to a final volume of 20 mL with DDW rinses of the centrifuge tube, and stored for analysis in 30 mL polyethylene bottles.

Metal concentrations for the water source samples, crude oil, SRM, and procedural and reagent blanks were determined by FAAS, ZGFAAS, CVAAS, or ICP/MS following methods comparable with EPA Series Method 200 and 200.8 (EPA, 1992). Levels of Ba in produced water and Zn in the crude oil were determined by FAAS using a Perkin-Elmer® Model 4000 instrument. Concentrations of Cd (except produced water), Cr, Cu, Fe, Mn, Ni, and V were determined by ZGFAAS using a Perkin-Elmer® Model 4000 instrument equipped with an HGA-400 graphite furnace, and an AS-40 autosampler. Levels of Ag, As, Be, and Se were determined by ZGFAAS using a Perkin-Elmer® Model 5100 instrument equipped with Zeeman background correction, an HGA-600 graphite furnace, and an AS-60 autosampler. Values for Ba, Pb, Sb, Sn, Tl, and Zn in the low-salinity effluent water and crude oil (except Zn) were determined by ICP/MS using a Perkin-Elmer® ELAN 5000. Dissolved concentrations of Cd, Pb, Sb, Sn, Tl, and Zn in the high-salinity water were determined by ICP/MS using the method of standard additions. All Hg values were determined by CVAAS using a Laboratory Data Control Model 1235 Mercury Monitor. In all cases, the manufacturers' specifications were followed and adherence to QA/QC requirements was maintained.

#### **2.2.3.4 Trace Metals in River Particulate and Coal Source Samples**

River water samples were vacuum filtered through polycarbonate filters (Poretics®, 47-mm diameter, 0.4 µm pore size). Prior to use, the filters had been acid-washed in 5N  $\text{HNO}_3$ , triple-rinsed with DDW, dried, and then weighed to the nearest µg using a Sartorius® Model M3P 6-place electronic balance under cleanroom conditions. Vacuum filtration was carried out in a Class-100 laminar-flow hood in the FIT cleanroom facility using acid-washed glassware. The filters were dried and reweighed prior to digestion for trace metals analysis.

Filters with riverine particulates and milligram quantities of SRM No. 2704, a river sediment issued by the NIST, were digested in stoppered, 15 mL Teflon® test tubes using Ultrex II®  $\text{HNO}_3$ , HF, and  $\text{HCl}$ . The sealed test tubes were placed in an 80°C water bath where refluxing of the acids completely dissolved the particles on the filters. After digestion, the resultant solutions were transferred to acid-washed, labeled 15 mL polyethylene bottles, diluted to approximately 6 mL with DDW rinses of the Teflon® test tubes, and stored in a plastic bag until analysis. No separate digestion was required for Hg.

Metal concentrations for the dissolved particulate samples, SRM, and blanks were determined by FAAS, ZGFAAS, CVAAS, or ICP/MS in a manner compatible with EPA Series 7000, 6010A, and 7470 (EPA, 1992), respectively. Particulate Al, Fe, Mn, and Zn concentrations were measured by FAAS using a Perkin-Elmer® Model 4000 AAS. Silver, As, Be, and Se values were determined by ZGFAAS using a Perkin-Elmer® Model 5100PC AAS equipped with Zeeman background correction, an HGA-600 graphite furnace, and an AS-60 autosampler.

Concentrations of Cr, Cu, Ni, and V were quantified by ZGFAAS using a Perkin-Elmer® Model 4000 AAS equipped with an HGA-400 graphite furnace, and an AS-40 autosampler. Values for Ba, Cd, Pb, Sb, Sn, and Tl were measured by ICP/MS using a Perkin-Elmer® ELAN 5000 spectrometer. Particulate Hg levels were determined by CVAAS using a Laboratory Data Control Model 1235 Mercury Monitor. In all cases, the instrument manufacturers' specifications were followed and adherence to QA/QC requirements was maintained.

Coal samples were digested and analyzed following the methods used for sediments as outlined in Section 2.2.3.1

#### **2.2.3.5 Acid-Volatile Sulfide/Simultaneously Extracted Metals**

Approximately 4 to 9 g of wet sediment were homogenized, weighed, and analyzed for AVS using the cold acid purge-and-trap method (Di Toro *et al.*, 1990). The homogenized sample was placed in a flask containing 45 mL of DDW after the system had been purged with 99.999 percent nitrogen. The sulfide in the sediment was then volatilized by injecting 45 mL of deoxygenated 2N HCl through a septum. The flask was continuously stirred and purged with 99.999 percent nitrogen. The nitrogen was passed through an impinger containing 45 mL of a sulfide anti-oxidant buffer (SAOB) which acted to trap and prevent oxidation of the sulfide. The SAOB buffer consisted of 2M NaOH, 0.1M ascorbic acid, and 0.1M ethylene diamine triacetic acid (EDTA). After a reaction time of 1 hour, the SAOB solution was placed into a 100 mL volumetric flask and brought to a final volume of 100 mL by adding the solution obtained from rinsing the impinger flask with a 1:1 solution of SAOB and DDW. The sulfide concentration of the SAOB solution was determined using a sulfide-specific ion probe (Orion® Model No. 9616BN). The probe was calibrated for each analysis using known concentrations of sodium sulfide/SAOB solution with a five-point curve. The sediment/acid slurry that remained at the end of the reaction was filtered into a 100 mL volumetric flask using Whatman® No. 40 ashless paper filter. The reaction flasks were rinsed with DDW and the rinse was used to bring the filtrate volume to 100 mL. This filtrate was then stored in acid-washed polyethylene bottles until analysis for SEM. Concentrations of Cu, Fe, and Zn were determined by FAAS using a Perkin-Elmer® Model 4000 instrument. Cadmium, Ni, and Pb values were obtained by ZGFAAS using a Perkin-Elmer® Model 4000 instrument equipped with an HGA-400 graphite furnace and an AS-40 autosampler.

#### **2.2.4 Geochronology**

Approximately 8 to 10 g of freeze-dried sediment from each layer (0.5 to 1.0 cm thick) of the sediment cores were ground to a fine powder using a Spex® 8000 mixer mill. Then, each sample was tightly packed in a 2 cm diameter, 5 cm long polycarbonate vial to a depth of  $30 \pm 1$  mm. A rubber stopper was cemented in place with two-part epoxy to seal the vials and prevent leakage of  $^{222}\text{Rn}$  and disruption of secular equilibrium between  $^{226}\text{Ra}$  and  $^{210}\text{Pb}$ . The samples were set aside for at least 20 days to establish secular equilibrium. Activities of the various radionuclides were then determined by counting using a well-type intrinsic germanium detector, "WiGe" (Princeton Gamma Tech® Model IGW11023). The samples were counted for a period of 1 to 2 days or until sufficient counts of the pertinent radionuclides were obtained (greater than 1,000 net counts for  $^{210}\text{Pb}$ ).

The peaks monitored for the purposes of this study were as follows:  $^{210}\text{Pb}$  at 46.5 KeV,  $^{214}\text{Pb}$  at 295.2 KeV and 351.9 KeV,  $^{214}\text{Bi}$  at 609.3 KeV, and  $^{137}\text{Cs}$  at 661.6 KeV. The  $^{226}\text{Ra}$  daughter

isotopes  $^{214}\text{Pb}$  (2 peaks) and  $^{214}\text{Bi}$  were used to determine the activity of  $^{226}\text{Ra}$ . Detector efficiency and counting accuracy were standardized using SRM sediment 4350B ( $^{137}\text{Cs}$ ) issued by the NIST and RGU-1 ( $^{210}\text{Pb}$ ) from the International Atomic Energy Agency.

Sedimentation rates (S) in cm/yr were calculated using the following equations with the reasonable assumption for these samples that sediment mixing was minimal:

For  $^{137}\text{Cs}$ :

$$S = \frac{\text{Depth in cm at which Activity } ^{137}\text{Cs} = \text{maximum}}{(\text{Year} - 1963) \text{ in years}}$$

and/or

$$S = \frac{\text{Depth in cm at which Activity } ^{137}\text{Cs} \text{ not detectable}}{(\text{Year} - 1950) \text{ in years}}$$

For  $^{210}\text{Pb}$ :

$$S = \frac{(-) \text{ decay constant for } ^{210}\text{Pb} (0.0311 \text{ y}^{-1})}{\text{Slope for plot of natural logarithm (ln) excess } ^{210}\text{Pb vs. sediment depth}}$$

The activity of excess  $^{210}\text{Pb}$  was calculated by subtracting the mean of  $A_{(\text{Pb-214, Bi-214})}$  from  $A_{\text{Pb-210}}$ .

## 2.2.5 Biological Parameters

### 2.2.5.1 Sediment Toxicity Tests

The toxicity tests followed guidelines established by the EPA (1994) and were performed on surface sediments collected from both the 1997 and 1998 surveys, and a “reference” sample collected from Aialik Bay near Holgate glacier. This fine-grained sediment was tested to evaluate the test organism’s sensitivity to fine-grained sediment.

Sediment samples from 20 locations in 1997, and 7 locations in 1998, were received at the testing laboratory in Martinez, California. Upon receipt, the sediment samples were stored at 4°C until used to set up the test replicates for the sediment toxicity tests. The test organisms for the 1997 sediments, *Eohaustorius estuarius*, were obtained from a commercial supplier (Northwestern Aquatic Sciences, Newport, Oregon). These organisms were acclimated to the test salinity of 34 ppt (parts per thousand). The test organisms for the 1998 sediments, *Ampelisca abdita*, were obtained from a commercial supplier (John Brezina and Associates, Dillon Beach, California).

The sediment toxicity test replicates were established on 1 day at 4 replicates for each site. Each replicate consisted of a 1 L glass beaker to which approximately 175 ml (approximately 2 cm deep) of sediment was added (each sediment sample was homogenized prior to loading of the test replicate containers). Test replicates were similarly established for a “home” control treatment, which consisted of the same sediment from which the test organisms were originally collected; this sediment was a fine-grained sand mixture. An additional “reference” control, consisting of sediment collected from Aialik Bay, was included in the 1998 sediment toxicity test; this sediment was autoclaved for 30 minutes prior to use. The overlying water consisted of 0.45 µm filtered seawater (collected from the U.C. Bodega Bay Marine Laboratory);

approximately 800 ml of this water was carefully poured into each test replicate so as to minimize disturbance of the sediment. These test replicates were then placed in a temperature-controlled water bath at 15°C (20°C for the *Ampelisca*) under continuous illumination from fluorescent lighting. Each test replicate was gently aerated.

The following day, routine water qualities (temperature, pH, dissolved oxygen [DO], and salinity) were determined for each test replicate. Then, the tests were initiated with the random allocation of 20 randomly selected organisms, into each replicate container (aeration was shut off until the amphipods reburied themselves, approximately 1 hour after their introduction). Each day, for the next 10 days, the temperature, pH, DO, and salinity were analyzed. Also on each day, a sample of the overlying water was collected from each replicate for each sediment treatment containing the *Eohaustorius*, composited, and analyzed to determine the total ammonia at that treatment. For the *Ampelisca*, water was collected on days 2 and 8 for ammonia determination.

After 10 days' exposure, routine water qualities (temperature, pH, DO, and salinity) were determined for each replicate. Then the contents of each replicate beaker were sieved and examined, and surviving amphipods were collected and counted. The resulting percent survival data were statistically analyzed using the ToxCalc statistical software (Tide-Pool Scientific, McKinleyville, California). Comparison of the survival data from each of the sites with the control treatment was made using the Homoscedastic t-Test.

#### **2.2.5.2 CYP1A (P4501A) Determinations**

Preserved sections of liver, gill, heart, and kidney were placed in cassettes in 10 percent neutral buffered formalin, embedded in paraffin, and analyzed immunohistochemically for the presence of CYP1A. Tissue sections (5-µm) mounted on Superfrost Plus slides (Fisher), were deparaffinated and hydrated as before (Smolowitz *et al.*, 1991). Matching serial sections were incubated with 150 µl of 1-12-3p6 monoclonal antibody against scup CYP1A, using modifications of Smolowitz (Smolowitz *et al.*, 1991). Formalin-fixed tissues were embedded in paraffin, and 5-µm sections were mounted on Superfrost Plus slides (Fisher) and analyzed immunohistochemically for the presence of CYP1A as before (Smolowitz *et al.*, 1991). Matching serial sections were incubated using the Shandon™ coverslip system for 2 hours with two 150 µL aliquots of MAbs 1-12-3p6 or with nonspecific purified mouse myeloma protein (UPC-10, IgG2A, Organon Teknika, West Chester, Pennsylvania), each at 1.5 g/ml in 1 percent BSA/TBS added at 0 and 60 minutes. Blocking solutions, secondary antibodies, linker, and color developer were components of the Signet (Medford, Massachusetts) murine immunoperoxidase kit.

Color development was achieved as described before using 2 percent 3-amino-9-ethylcarbazole and 1 percent hydrogen peroxide. Sections were counterstained with Mayer's hematoxylin. Slides were examined with a Zeiss Axioskop microscope and relative staining intensities were determined subjectively by comparing the staining of samples to that of control and highly induced 3,3',4,4' tetrachlorobiphenyl-treated scup liver sections included in each run. Nonspecific staining, if present, was determined by comparison with UPC-10 stained sections. Staining occurrence was scored as 0-no staining (or equal to UPC staining), 1-rare- few cells staining, 2-many cells staining, 3-multifocal and diffuse-all cells staining. The intensity of staining was scored as 0-none (or equal to UPC staining), 1-mild, 2-moderate, 3-medium, 4-strong, 5-very strong. A scaled product of staining occurrence times the staining intensity was

determined for each cell type. Therefore, immunohistochemical (IHC) scores (being the product of 2 numbers, the first from 0 to 3, and the second from 0 to 5) could range from 0 to 15. In this study, scores of 1-5 are considered low, 6-10 are considered moderate, and 11-15 are considered high.

### **2.2.5.3 P450 Reporter Gene System Tests**

The detailed methodology used in this study has been described previously (Anderson et al., 1995; American Public Health Association [APHA], 1996; American Society for Testing and Materials [ASTM], 1997). The P450 Reporter Gene System (P450 RGS) uses human hepatoma cells stably transfected with a luciferase reporter gene downstream of human CYP1A1 promoter. Inducers also trigger a bioluminescent response in affected cells.

Two reference toxicant solutions representing two classes of CYP1A inducers, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and benzo[a]pyrene, (a PAH) and environmental sample extracts are applied at volumes of 2 to 20 mL to replicate wells in 96-well plates of culture media. Both sediment and tissue extracts are applied in the same manner. Both reference toxicants and environmental sample extracts are complex mixtures. Every RGS test uses at least one and normally both reference toxicants, as the performance of the test cells varies from test to test. Use of both reference toxicants allows for consistently accurate comparisons of toxicity of test matrices to benzo[a]pyrene (B[a]PEq) and dioxin (TEQ). Dioxin is an order of magnitude more toxic to the test cells than benzo[a]pyrene. Both were tested in this study. Duplicate plates were dosed such that one plate was incubated for 6h, and the second for 16h. The response due to PAH's is typically measurable after 6 hr. of exposure. Response due to chlorinated hydrocarbons (TEQ, PCB's) is best measured with 16 hr. exposure periods, as it typically requires 16 hours for chlorinated hydrocarbons to induce a response. Due to the unknown nature of contaminants in sediments and fish tissues collected in this study, both exposure periods are used. Response rarely results from a single chemical contaminant, as contaminants most often occur as complex mixtures (sediment, water, or tissues). The RGS values are expressed as both B[a]PEq and Toxic Equivalents (TEQ), since it can not be determined if the response was from exposure to PAH's (B[a]PEq) or from chlorinated hydrocarbons (TEQ). TEQ's also allows comparison of relative toxicity of environmental samples from complex mixtures of contaminants.

After 6h or 16h incubation with the test solutions, the cells were washed with Hank's Balanced Salt Solution (Mediatech, Herndon, Virginia), and lysed with 200  $\mu$ L of buffer containing 1 percent Triton, 25 mM Tricine, pH 7.8, 15 mM MgSO<sub>4</sub>, 4 mM EDTA, and 1 mM dithiothreitol (DTT). Cell lysates were centrifuged at 6,000 rpm for 10s, and 50  $\mu$ L of the supernatant was applied to a 96-well plate, followed by 100  $\mu$ L of 0.1 M potassium phosphate buffer, pH 7.8, containing 5 mM ATP and 10 mM MgCl<sub>2</sub>. Reactions were initiated by injection of 100  $\mu$ L of luciferin, dissolved in 0.1 M potassium phosphate buffer, pH 7.8. Luminescence in relative light units (RLUs) was measured using a ML2250 Luminometer (Dynatech Laboratories, Chantilly, Virginia). Luciferase assay buffers were purchased from PharMingen (San Diego, California).

With each test run, a solvent blank (using a volume of DCM equal to the sample volume being tested) and a reference toxicant (1 ng/mL tetrachlorodibenzo-p-dioxin [TCDD]) were also applied to two separate replicate wells. Mean fold induction of the solvent blank was set equal to 1, and the fold induction of the reference toxicant and each sample were determined by dividing the mean RLUs produced by these samples by the mean RLUs produced by the solvent blank. The coefficient of variation among replicates was acceptable if less than 20 percent.

**Equivalency Calculations.** RGS Toxic Equivalents (TEQ) are a measure of the RGS response if the sample contained only dioxins and furans, and are calculated using the equation below. According to previous concentration-response studies using a standard mixture of dioxins and furans, the RGS response is equivalent to the mixture TEQ in pg/mL (calculated using Toxic

Equivalency Factors established by Safe, 1990). Dividing by 1,000 yields the TEQ in ng/g.

$$\text{RGS TEQ} = (\text{fold induction}/1,000) * ((V_e/V_a)/W_d)$$

Where,

$V_e$  = total extract volume

$V_a$  = volume of extract applied to cells

$W_d$  = dry weight of sample

Similarly, RGS Benzo[a]pyrene Equivalents (B[a]PEq) are a measure of the RGS response if the sample contained only PAH, and are calculated in mg/g using the equation below. Based on RGS concentration-response curves for Benzo[a]pyrene, a fold induction of 60 is produced by 1 mg/mL B[a]P.

$$\text{RGS B[a]PEq} = (\text{fold induction}/60) * ((V_e/V_a)/W_d)$$

Where,

$V_e$  = total extract volume

$V_a$  = volume of extract applied to cells

$W_d$  = dry weight of sample

#### **2.2.5.4 Enumeration of Heterotrophs and Hydrocarbon-Degrading Microorganisms**

Microbial analyses were performed on surface sediments from the 1998 field survey. The analyses were for the enumeration of total heterotrophic microorganisms and hydrocarbon-degrading microorganisms, and was a modification of the most probable number (MPN) technique (Brown and Braddock, 1990; Braddock and McCarthy, 1996). Once the samples arrived in the laboratory they were stored at 4°C and were all processed within a week.

First, each sample was homogenized by mixing the sample in the sample jar with a clean spatula. Then, 10 g ( $\pm$  0.1 g) sediment was diluted into 90 ml of marine Bushnell Heas Broth. These initial dilutions were shaken by hand for one minute before further dilutions for the MPN test. Duplicates (two separate dilution series) were prepared for each sediment. In addition, for heterotrophs, laboratory duplicates were run on each dilution. Finally, approximately 5 to 10 g of sediment was dried overnight at 105°C to determine dry weights for the sediment. Final values reported were all corrected to sediment dry weight.

The cell culture plates for the MPN tests were filled with either Marine Broth (Difco) (96-well plates for heterotrophs) or marine Bushnell Heas Broth (24-well plates for hydrocarbon degraders). Following inoculation, one drop of autoclaved Cook Inlet crude oil was added to each of the wells of the hydrocarbon-degrader plates. The plates were incubated at room temperature. After three weeks and after five weeks, the marine heterotroph plates were scored for growth. Full growth was obtained in the plates at three weeks, confirmed by rescoring the plates at five weeks. After six weeks, the hydrocarbon degrader plates were scored for emulsification of crude oil.

#### **2.2.6 Measuring, Interpreting, and Mapping Sediment Profile Imaging Parameters**

The SPI camera was used to photograph the surface and subsurface layers of sediment on the ocean floor during the 1997 survey. These images can be used to describe benthic community and benthic structure, physical setting (e.g., grain size), biochemical parameters, and depth of the

redox layer.

#### **2.2.6.1 Sediment Type**

The sediment grain-size major mode and range were visually estimated from the color slides by overlaying a grain-size comparator that was at the same scale. This comparator was prepared by photographing a series of Udden-Wentworth size classes (equal to or less than coarse silt up to granule and larger sizes) with the SPI camera. Seven grain-size classes were on this comparator:  $>4 \phi$ ,  $4-3 \phi$ ,  $3-2 \phi$ ,  $2-1 \phi$ ,  $1-0 \phi$ ,  $0-(-)1 \phi$ ,  $<-1 \phi$ . The lower limit of optical resolution of the photographic system was about 62 microns, allowing recognition of grain sizes equal to or greater than coarse silt ( $\geq 4 M$ ). The accuracy of this method has been documented by comparing SPI estimates with grain-size statistics determined from laboratory sieve analyses.

The comparison of the SPI images with Udden-Wentworth sediment standards photographed through the SPI optical system was also used to map near-surface stratigraphy such as sand-over-mud and mud-over-sand. When mapped on a local scale, this stratigraphy can provide information on relative transport magnitude and frequency.

#### **2.2.6.2 Prism Penetration Depth**

The SPI prism penetration depth was measured from the bottom of the image to the sediment-water interface. The average penetration depth was determined by measuring across the entire cross-sectional image. Linear maximum and minimum depths of penetration were also measured. Maximum, minimum, and average penetration depths were recorded in the data file.

Prism penetration is potentially a noteworthy parameter; if the number of weights used in the camera is held constant throughout a survey, the camera functions as a static-load penetrometer. Comparative penetration values from sites of similar grain size give an indication of the relative water content of the sediment. Highly bioturbated sediments and rapidly accumulating sediments tend to have the highest water contents and greatest prism penetration depths.

The depth of the camera's penetration into the bottom also reflects the bearing capacity and shear strength of local sediments. Over-consolidated or relic sediments and shell-bearing sands resist camera penetration. Highly bioturbated, sulfidic, or methanogenic muds are the least consolidated, and deep penetration is typical. Seasonal changes in camera prism penetration are typically observed at the same station and are related to the control of sediment geotechnical properties by bioturbation (Rhoads and Boyer, 1982). The effect of water temperature on bioturbation rates appears to be important in controlling both biogenic surface relief and prism penetration depth (Rhoads and Germano, 1982).

#### **2.2.6.3 Small-Scale Surface Boundary Roughness**

Surface boundary roughness was determined by measuring the vertical distance (parallel to the film border) between the highest and lowest points of the sediment-water interface. The surface boundary roughness (sediment surface relief) measured over a horizontal distance of 15 cm typically ranges from 0.02 to 3.8 cm and may be related to either physical structures (ripples, rip-up structures, mud clasts) or biogenic features (burrow openings, fecal mounds, foraging depressions). Biogenic roughness typically changes seasonally and is related to the interaction of bottom turbulence and bioturbational activities.

The camera must be level to take accurate boundary roughness measurements. In sandy



sediments, boundary roughness can be a measure of sand wave height. On silt-clay bottoms, boundary roughness values often reflect biogenic features such as fecal mounds or surface burrows.

#### **2.2.6.4 Thickness of Depositional Layers**

Because of the camera's unique design, SPI can be used to detect the thickness of depositional and dredged material layers. SPI is effective in measuring layers ranging in thickness from 20 cm (the height of the SPI optical window) to 1 mm. During image analysis, the thickness of the newly deposited sedimentary layers can be determined by measuring the linear distance between the pre- and post-disposal sediment-water interface. Recently deposited material is usually evident because of its unique optical reflectance and/or color relative to the underlying material representing the predisposal surface. Also, in most cases, the point of contact between the two layers is clearly visible as a textural change in sediment composition, facilitating measurement of the thickness of the newly deposited layer.

#### **2.2.6.5 Mud Clasts**

When fine-grained, cohesive sediments are disturbed, either by physical bottom scour or faunal activity (e.g., decapod foraging), intact clumps of sediment are often scattered about the seafloor. These mud clasts can be seen at the sediment-water interface in SPI images. During analysis, the number of clasts was counted, the diameter of a typical clast was measured, and their oxidation state (discussed below) was assessed. The abundance, distribution, oxidation state, and angularity of mud clasts can be used to make inferences about the recent pattern of seafloor disturbance in an area.

Depending on their place of origin and the depth of disturbance of the sediment column, mud clasts can be reduced or oxidized. In SPI images, the oxidation state is apparent from the reflectance (MMS, 1998). Also, once at the sediment-water interface, these mud clasts are subject to bottom-water oxygen concentrations and currents. Based on laboratory microcosm observations of reduced sediments placed within an aerobic environment, oxidation of reduced surface layers by diffusion alone is quite rapid, occurring within 6 to 12 hours (Germano, 1983). Consequently, the detection of reduced mud clasts in an obviously aerobic setting suggests a recent origin. The size and shape of the mud clasts are also revealing. Mud clasts may be moved and broken by bottom currents and animals (macro- or meiofauna; Germano, 1983). Over time, large angular clasts become small and rounded.

#### **2.2.6.6 Apparent Redox Potential Discontinuity Depth**

Aerobic near-surface marine sediments typically have higher reflectance relative to underlying hypoxic or anoxic sediments. Surface sands washed free of mud also have higher optical reflectance than underlying muddy sands. These differences in optical reflectance are readily apparent in SPI images; the oxidized surface sediment contains particles coated with ferric hydroxide (an olive or tan color when associated with particles), while reduced and muddy sediments below this oxygenated layer are darker, generally grey to black. The boundary between the colored ferric hydroxide surface sediment and underlying grey to black sediment is called the apparent redox potential discontinuity (RPD).

The depth of the apparent RPD in the sediment column is an important time-integrator of DO conditions within sediment porewaters. In the absence of bioturbating organisms, this high-reflectance layer (in muds) will typically reach a thickness of 2 mm (Rhoads, 1974). This depth

is related to the supply rate of molecular oxygen by diffusion into the bottom and the consumption of that oxygen by the sediment and associated microflora. In sediments that have very high sediment oxygen demand (SOD), the sediment may lack a high reflectance layer even when the overlying water column is aerobic.

In the presence of bioturbating macrofauna, the thickness of the high-reflectance layer may be several centimeters. The relationship between the thickness of this high-reflectance layer and the presence or absence of free molecular oxygen in the associated porewaters must be considered with caution. The actual RPD is the boundary (or horizon) that separates the positive Eh region of the sediment column from the underlying negative Eh region. The exact location of this  $Eh = 0$  potential can be determined accurately only with microelectrodes; hence, the relationship between the change in optical reflectance, as imaged with the SPI camera, and the actual RPD can be determined only by making the appropriate in situ Eh measurements. For this reason, the optical reflectance boundary, as imaged, was described in this study as the “apparent” RPD and it was mapped as a mean value. In general, the depth of the actual  $Eh = 0$  horizon will be either equal to or slightly shallower than the depth of the optical reflectance boundary. This is because bioturbating organisms can mix ferric hydroxide-coated particles downward into the bottom below the  $Eh = 0$  horizon. As a result, the apparent mean RPD depth can be used as an estimate of the depth of porewater exchange, usually through porewater irrigation (bioturbation). Biogenic particle mixing depths can be estimated by measuring the maximum and minimum depths of imaged feeding voids in the sediment column. This parameter represents the particle mixing depths of head-down feeders, mainly polychaetes.

The rate of depression of the apparent RPD within the sediment is relatively slow in organic-rich muds, on the order of 200 to 300 micrometers per day; therefore this parameter has a long time constant (Germano and Rhoads, 1984). The rebound in the apparent RPD is also slow (Germano, 1983). Measurable changes in the apparent RPD depth using the SPI optical technique can be detected over periods of 1 or 2 months. This parameter is used effectively to document changes (or gradients) that develop over a seasonal or yearly cycle, related to water temperature effects on bioturbation rates, seasonal hypoxia, SOD, and infaunal recruitment. Time-series RPD measurements following a disturbance can be a critical diagnostic element in monitoring the degree of recolonization in an area by the ambient benthos (Rhoads and Germano, 1986).

The apparent mean RPD depth also can be affected by local erosion. The peaks of disposal mounds commonly are scoured by divergent flow over the mound. This scouring can wash away fines and shell or gravel lag deposits, and can result in very thin apparent RPD depths. During storm periods, erosion may completely remove any evidence of the apparent RPD (Fredette *et al.*, 1988).

Another important characteristic of the apparent RPD is the contrast in reflectance at this boundary. This contrast is related to the interactions among the degree of organic loading, the bioturbation activity in the sediment, and the concentrations of bottom-water dissolved oxygen in an area. High inputs of labile organic material increase SOD and, subsequently, sulfate reduction rates and the associated abundance of sulfide end products. This results in more highly reduced, lower-reflectance sediments at depth and higher RPD contrasts. In a region of generally low RPD contrasts, images with high RPD contrasts indicate localized sites of

relatively high past inputs of organic-rich material such as phytoplankton or other naturally occurring organic detritus, dredged material, and sewage sludge.

#### **2.2.6.7 Sedimentary Methane**

If organic loading is extremely high, porewater sulfate is depleted and methanogenesis occurs. The process of methanogenesis is indicated by the appearance of methane bubbles in the sediment column, and the number and spatial coverage of all methane pockets is measured. These gas-filled voids are readily discernable in SPI images because of their irregular, generally circular aspect and glassy texture (due to the reflection of the strobe off the gas bubble).

#### **2.2.6.8 Infaunal Successional Stage**

The mapping of infaunal successional stages is readily accomplished with SPI technology. These stages are recognized in SPI images by the presence of dense assemblages of near-surface polychaetes and/or the presence of subsurface feeding voids; both may be present in the same image. Mapping of successional stages is based on the theory that organism-sediment interactions in fine-grained sediments follow a predictable sequence after a major seafloor perturbation. This theory states that primary succession results in “the predictable appearance of macrobenthic invertebrates belonging to specific functional types following a benthic disturbance. These invertebrates interact with sediment in specific ways. Because functional types are the biological units of interest, our definition does not demand a sequential appearance of particular invertebrate species or genera” (Rhoads and Boyer, 1982). This theory is presented in Pearson and Rosenberg (1978) and further developed in Rhoads and Germano (1982) and Rhoads and Boyer (1982).

This continuum of change in animal communities after a disturbance (primary succession) has been divided subjectively into three stages: Stage I is the initial community of tiny, densely populated polychaete assemblages; Stage II is the start of the transition to head-down deposit feeders; and Stage III is the mature, equilibrium community of deep-dwelling, head-down deposit feeders.

After an area of bottom is disturbed by natural or anthropogenic events, the first invertebrate assemblage (Stage I) appears within days after the disturbance. Stage I consists of assemblages of tiny tube-dwelling marine polychaetes that reach population densities of  $10^4$  to  $10^6$  individuals per  $m^2$ . These animals feed at or near the sediment-water interface and physically stabilize or bind the sediment surface by producing a mucous “glue” that they use to build their tubes. Sometimes deposited dredged material layers contain Stage I tubes still attached to mud clasts from their location of origin; these transported individuals are considered as part of the in situ fauna in our assignment of successional stages.

If there are no repeated disturbances to the newly colonized area, then these initial tube-dwelling suspension or surface-deposit feeding taxa are followed by burrowing, head-down deposit-feeders that rework the sediment deeper and deeper over time and mix oxygen from the overlying water into the sediment. The animals in these later-appearing communities (Stage II or III) are larger, have lower overall population densities (10 to 100 individuals per  $m^2$ ), and can rework the sediments to depths of 3 to 20 cm or more. These animals loosen the sedimentary fabric, increase the water content in the sediment, thereby lowering the sediment shear strength, and actively recycle nutrients because of the high exchange rate with the overlying waters resulting from their burrowing and feeding activities.

### 2.2.6.9 Organism-Sediment Index

The Organism-Sediment Index (OSI) is a summary mapping statistic that is calculated on the basis of four independently measured SPI parameters: 1) apparent mean RPD depth, 2) presence of methane gas, 3) low/no dissolved oxygen at the sediment-water interface, and 4) infaunal successional stage. Table 2-7 shows how these parameters are summed to derive the OSI.

The highest possible OSI is +11, which reflects a mature benthic community in relatively undisturbed conditions (generally a good yardstick for high benthic habitat quality). These conditions are characterized by deeply oxidized sediment with a low inventory of anaerobic metabolites and low SOD, and by the presence of a climax (Stage III) benthic community. The lowest possible OSI is -10, which indicates that the sediment has a high inventory of anaerobic metabolites, has a high oxygen demand, and is azoic. Based on Joe Germano's (EVS Environmental Consultants) mapping experience over the past 15 years, he has found that OSI values of 6 or less indicate that the benthic habitat has experienced physical disturbance, eutrophication, or excessive bioavailable contamination in the recent past.

## 2.3 Statistical Methods

### 2.3.1 Coefficient of Variation

The coefficient of variation (CV) was used to describe the variation in several populations of physical and chemical sediment parameters. The CV can be expressed as a percent and is generally defined by:

$$CV = 100 * \sigma / m$$

Where  $\sigma$  is the standard deviation and  $m$  is the population mean (Snedecor and Cochran, 1978). The utility of the measure lies partly in the fact that within many data sets, the mean and standard deviation tend to change in concert. The experimental design associated with the 1997 sediment surface sampling program included three stations possessing seven replicate samples each. Intensive sampling at selected stations was conducted for the purpose of evaluating within station variation.

A modification of the general CV statistic of the equation above was used to describe the within station variation for each of the measured chemical/physical parameters. The following equation was used to generate within station CVs for each measured parameter at the multi-replicate stations:

$$\hat{CV} = 100 * \sqrt{(SS_1 + SS_2 + \dots + SS_i) / (df_1 + df_2 + \dots + df_i) / \bar{X}}$$

Where,

$SS_1, SS_2, \dots, SS_i$  are the parameter-specific sums of squares at each of the stations with seven field replicates;  $df_1, df_2, \dots, df_i$  are the associated degrees of freedom, and  $\bar{X}$  is the grand mean of the parameter of interest from all seven-replicate stations. All data manipulations were aided by the Statistical Analysis System (SAS, ver. 6.12).

Within-zone variation was also explored using the general CV statistic for each zone and chemical/physical parameter. For this analysis, all stations with greater than one replicate per station were included in calculations. Additionally, a parameter-specific “Grand CV” was calculated incorporating all parameter-specific information gathered at stations with greater than one replicate. A CV ratio was then calculated by dividing the zone parameter-specific CV by the Grand CV. Ratios less than one can be used to highlight parameters where within zone variability is less than overall system variability.

### 2.3.2 Analysis of Variance

The General Linear Model (GLM), an application of an analysis of variance (ANOVA), was used to elucidate significant differences between zones. Under the GLM, a continuous response, or dependent, variable (e.g., zinc) is measured under experimental conditions identified by classification, or independent, variables (*i.e.*, zone and year). The variation in the response is explained as being due to effects in the classification, with random error accounting for the remaining variation (Searle, 1971). Prior to running the GLM analyses, mean values were generated for each of the multireplicates. This was deemed appropriate since the variability within stations was much lower than variability between stations, and thus stations can be considered replicates within a zone. Mean station values were subsequently used in all GLM hypothesis testing.

A significant effect identified by the GLM statistic indicates that the classification variables (e.g., Zones) differ for a specific dependent variable (e.g., zinc), but the model does not tell how this difference is manifested (*i.e.*, which Zones are different). A Student-Newman-Keuls (SNK) multiple range test (Steel and Torrie, 1980) was used to identify significant differences ( $p < 0.05$ ) between classification variables when the associated GLM indicated a significant difference. Simply put, the SNK test was used to separate classification variables into significantly different groupings. These tests were performed on the following sets of data:

- Surface sediments collected from 59 stations within zones 0, 1, 2, and 3 in 1997, measured for 23 organic and 26 inorganic parameters
- Surface sediments collected from 35 stations within zones 0, 1, 2, 3, and 4 in 1998, measured for 23 organic, 23 inorganic, and 5 biological parameters

It is well established that certain chemical/physical parameters tend to predict concentrations of other analytes. Because of this, several GLM analyses were performed on the surface sediment data set before and after the transformation (normalization) of metals (dividing metal concentration by percent iron at the station) and organic compounds (dividing by percent total organic carbon at a station).

A GLM analysis was also performed on surface sediment collected from both the 1997 and 1998 sampling periods in order to determine whether significant variance could be attributed to time of collection/analysis. A Bonferroni multiple range test was used to identify significant differences ( $p < 0.05$ ) between classification variables when the associated GLM indicated a significant difference. The Bonferroni multiple range test is conceptually similar to the SNK, however, it is less sensitive to problems associated with an unbalanced experimental design (unequal number of stations, zones, and/or replicates). This test was performed on a subset of

data where station sampling and parameter analysis was repeated during both years and totaled 16 stations (14 stations for the RGS-P450 data).

### **2.3.3 Pearson Product-Moment Correlation**

#### **2.3.3.1 Sediments**

Not all chemical, physical, and biological parameters were measured at all stations. However, subsets of the data were collected to identify significant correlations between measurement parameters and additional differences between zones. These subsets included the following:

- 20 stations in 1997 shared 37 common measurement parameters
- 14 stations where sampling was repeated during both the 1997 and 1998 surveys over 37 measurement parameters

Again, the correlation analyses were performed on both transformed and nontransformed organic/inorganic data. Aquatic toxicity test results were reported as mean percent survival (MPS) and, along with the chemical/physical measurements reported as percents, was transformed by applying an arc sine square root to the fractional data.

Correlative analyses were performed on the separate data sets using the Pearson product-moment which measures the strength of the linear relationship between two variables. If one variable (e.g., length) can be expressed exactly as a linear function of another variable (e.g., weight), then the correlation is 1 if the variables are directly related, or -1 if the variables are inversely related. A correlation of 0 between two variables suggests that each variable has no linear predictive ability for the other. If the values associated with the variables are normally distributed, a correlation of 0 also means the variables are independent of one another. Again, SAS ver. 6.12 was used to perform data manipulations and analysis.

#### **2.3.3.2 Tissues**

Measurements of chemical and biological parameters, similar to those applied to sediments were used in the examination of fish tissues at three stations. Three species of mixed size and sex were acquired. Subsetting of tissues was necessary for microscopic analyses and not all fish were examined for all chemical/biological parameters. Chemical analyses were performed on composite samples of fish tissue (a requirement of chemical analytical methods), while all fish were measured or evaluated for “non-quantity dependent” variables (e.g., length). Data sets included the following:

- In 1997, P450 measurements (Section 2.5.2) were made on 45 individuals and chemical/biological measurements were made on 13 tissue composites.
- In 1998, P450 measurements were made on 116 individuals and chemical/biological measurements were made on 30 tissue composites.

By collecting data in the form of discrete (e.g., length) and composite (e.g., chemical) measurements it was not possible to correlate measures on a one-to-one basis. To evaluate the degree to which two or more variables were related and changed together (correlated), it was necessary to calculate average individual measurements (e.g., length and P450) using groupings defined by the chemical composite groupings. A Pearson’s correlation was applied to the data, after the averaging process, and significant correlations ( $p \leq 0.05$ ) were retained and reported.

#### **2.3.4 Analysis of Variance/Covariance**

GLM and SNK procedures were applied to both the combined chemical/biological and the P450 data sets. In general, fish length positively correlates with fish age, and fish age has been shown to covary with several chemical and biological (P450) parameters. The tendency of one statistical variable to change in relation to another is commonly termed as covariance. Analysis of covariance combines some of the features of a typical regression model with analysis of variance. Typically, a continuous variable (the covariate length in the example above) is introduced into the analysis of variance model in an attempt to lessen the influence of the covariate. GLM processing used length as a covariant for this reason. Additionally, since sex was recorded for each fish sex and is known to correlate with many of the measured parameters sex was examined as a possible covariate during the analytical process.

#### **2.3.5 Random Effects Analysis of Variance**

The basic data analysis and statistical questions center around whether there is a consistent and statistically significant pattern associated with the oil and gas development and production operations initiated in 1963. Although concentrations of metals and hydrocarbons associated with post-1963 oil and gas development would be expected to differ by zone and distance from the production sites, as detailed in Section 3.4, indicator ratios of metal and hydrocarbon concentrations might be expected to show consistent patterns even as the absolute concentrations decline with distance from the production site.

Since information from the cores is limited, a parsimonious statistical model was developed to explain post-1963 shifts in metal and organic indices. This statistical analysis models the individual cores as randomized blocks, where each core is considered as a random sample, with a mean effect and an oil and gas development and production effect.

The statistical model for the  $i^{\text{th}}$  core and the  $j^{\text{th}}$  slice is

$$y_{i,j} = \mu + \alpha_i + \beta_i I_{i,j} + \epsilon_{i,j}$$

Where  $\alpha_i$  is the core effect and  $I_{i,j}$  is the indicator variable, which is one if the core is post-1963 and zero otherwise. Within this type of model, there are two modeling approaches. First, we can think of the oil production effect as fixed and constant between cores, thus  $\beta_i = \beta$ , and test the null hypothesis that the effect is zero. The second, and more realistic approach, is to consider the sampled cores as a random sample of all cores, where the oil production effect is consistent, that is, in the same direction, but variable between cores. For the second random effect model, the null hypothesis is that the mean of the random effect differs from zero. Subsequently, the alternate hypothesis is that there is a consistent but variable effect between cores.

Data were analyzed and plotted using the statistical package S-plus. The linear mixed effects models within S-plus were used for the “random cores analysis.” The analysis is based on methods developed in Laird and Ware (1982) using restricted maximum likelihood methods. The distribution theory is approximate for small sample sizes. The P-values reported for the random effects models are only approximated and should be used only to judge the relative strength of the evidence for post-1963 shifts.

## **2.4 Quality Assurance/Quality Control**

As part of our overall QA/QC program, both field and laboratory QA/QC measures were taken. Several types of field QC samples were collected during the survey, including field blanks, equipment blanks, replicate samples, an *Alpha Helix* reference diesel-fuel sample, and a trip blank. These samples were collected to characterize potential influences from equipment (Van-Veen grab, boxcore, core liner, fish dissection tools) and each type of sampling activity (sediment sample collection, fish sampling, and dissection). For the field QA/QC samples, one jar each was collected for metals and organics analyses. Laboratory quality assurance (QA) measures included maintaining detailed laboratory records and comprehensive validation of data packages. In addition, several QC measures were implemented in conjunction with hydrocarbon and metals analyses in order to provide a measure of analytical accuracy, precision, and potential contamination.

### **2.4.1 Field Quality Assurance/Quality Control**

QA/QC samples were collected during the field sampling program to assess overall accuracy and representativeness of the sampling efforts. The number of QC samples collected for this effort is based on the total number of field samples as established in the sampling and logistics plans (Arthur D. Little, 1997a; Arthur D. Little, 1998). Discussion and interpretation of analytical results for these samples are provided in Section 3.6. Quality assurance techniques were used in sampling activities to avoid potential contamination and cross-contamination including use of: precleaned sample containers; clean sampling equipment; decontamination protocol; and good laboratory practices. Standard sampling procedures and protocols were followed. In this section, the field methods used for collecting field QC samples are summarized.



Several types of field QC samples were collected during the field survey, including equipment blanks, field blanks, and trip blanks. For all field QA/QC samples, one jar each was collected for metals and organics analyses.

#### **2.4.1.1 Equipment Blanks**

Three equipment blank samples were collected from rinsate of the grab sampling equipment at locations Z0F1, Z0F8, and Z3R13 in 1997, and three similar rinsates were collected at locations Z1R19, Z2R14A, and Z2R23 in 1998. Both the 1997 and the 1998 surveys included an equipment blank collected from rinsate of fish dissection equipment that was used in the on-board laboratory associated with location Z2R14A. One additional equipment blank sample was collected from rinsate of the box core sampling equipment (and core liner) at location Z0F1. The procedure for collecting the equipment blank samples followed these steps:

- The equipment was decontaminated according to the SOP
- The equipment was rinsed with high-purity, deionized water and the rinsate collected directly into two clean, prelabeled water sample containers
- A precleaned stainless-steel funnel was used to assist in the collection
- The rinsate equipment blank sample was refrigerated at 4°C

#### **2.4.1.2 Field Blanks**

Field blanks were collected during sampling, representing atmospheric or other contamination that the field samples may have been subject to. Three field blank samples were taken during the collection of sediment samples. One field (deck) blank was collected during sediment sampling in 1997 at location Z1R9 when a forest fire smoke smell was noticed in the air. The other two field blank samples, deck blanks, were collected during sediment sampling at location Z2F1 and location Z2R23, in 1997 and 1998, respectively.

To collect field blank samples, a clean, prelabeled sample jar of the same batch used for sample collection was carried into the working area, opened during the collection of one sample, and returned to the laboratory with the field samples. For each field blank, two sample containers were collected for metals and organics analysis, respectively. The field blanks were stored under the same conditions as their associated field samples.

#### **2.4.1.3 Trip Blanks**

A trip blank sample was prepared to accompany the samples from location Z0F1 during the 1997 survey. The trip blank was a sample jar that was never opened. The trip blank was treated similar to other field samples during storage and shipment.

#### **2.4.1.4 Field Source Sample**

A source sample of the R/V *Alpha Helix* diesel fuel was taken during the 1997 field survey. The purpose of this sample was to, if necessary, be able to characterize any potential sample contamination believed to originate from the shipboard diesel fuel (e.g., exhaust and surface sheen). The ship's engineer collected a sample of diesel fuel in a precleaned glass jar. The sample was stored separately from the other samples at room temperature, and shipped packaged in two plastic bags to prevent leaking or cross-contamination to other samples.

## 2.4.2 Organics Analysis Quality Assurance/Quality Control

### 2.4.2.1 Quality Assurance

**Laboratory Records.** All laboratory operations were documented and placed in three-ring binders. Documentation included the following:

- Lot number and vendors for reagents and standards
- Preparation of stock solutions, standards, and spiking solutions
- Sample preparation
- Analytical procedures
- Analytical instrument and conditions
- Dates of analysis of standards
- Dates of analysis of samples
- Problems encountered
- Corrective actions

All entries were initialed and dated by the analyst at the time of entry. Any deviations from SOP were explained, initialed, and dated. All the raw data and chromatograms acquired on the data systems linked with GC/FID and GC/MS instruments were archived in both hardcopy and electronic form.

**Data Validation.** All chemistry data generated by Arthur D. Little's laboratories were assembled in data packages and validated by the designated team member in charge of each analysis to ensure that the data quality objectives (DQOs) for accuracy and precision were met, that the data were generated in accordance with the Laboratory Quality Assurance (QA) Plan, and that data are both traceable and defensible. Data packages were also reviewed by the Project Manager to ensure compliance with procedures and (DQOs) specified in the QA Plan. Data were also reviewed for their consistency with expected petroleum hydrocarbon, PAH, or saturated hydrocarbon distributions.

When data validation was successfully completed by each facility, all data sets were submitted to the QA Officer for a formal audit. This formal audit included a 100 percent review on all hand-entered and calculated data, i.e., preparation documentation, standard amounts, weights, etc. Approximately 20 percent of each data set that was generated by an automated system was checked for accuracy. This involved tracking the final reported concentrations back to the raw data. After any necessary corrections were made, the data were approved by the auditor and forwarded to the Case Leader for review. A formal report documenting the audit findings was generated and maintained in the QA Unit files.

### 2.4.2.2 Quality Control

**Data Quality Requirements.** DQOs are established to ensure that analytical data are of the quality necessary to achieve project objectives. Our DQOs are designed to enhance our ability to identify and accurately quantify source-specific oils. The DQO limits are listed in the specific laboratory and analytical SOP. PAH and SHC DQOs and criteria are summarized in Table 2-8. DQOs for biomarker analysis are summarized in Table 2-9.

Target analyte concentrations, surrogate recoveries, and QC sample results were determined at the respective GC/FID and GC/MS facilities. After careful checking and review by the facility's

manager, these data were arranged in Excel spreadsheet format. Diagnostic graphics were also generated and submitted to the program manager for review. Any subsequent changes to or updates of the data in the spreadsheet were performed by the respective facility. The data packages containing all the information (e.g., chain-of-custody sheets, sample preparation data) required for QA audits were submitted to the QA data auditor.

The auditor prepared a concise audit report for each type of analysis. Comments and action items in the audit report were addressed by the Arthur D. Little laboratory manager and instrument facility supervisors.

## **2.4.3 Metals and Total Organic Carbon Analysis Quality Assurance/Quality Control**

### **2.4.3.1 Quality Assurance**

**Sample Tracking Procedure.** Upon receipt, each sediment, tissue, and source sample received by the Marine and Environmental Chemistry Laboratories at FIT was carefully inspected to ensure that it was intact and that the identification number on the sample container matched that found on the custody sheet. All sediment and source samples were kept refrigerated ( $\sim 1^{\circ}\text{C}$ ) and all tissue samples were kept frozen ( $\sim 18^{\circ}\text{C}$ ) until processed for analysis.

### **2.4.3.2 Quality Control**

For this project, QC measures included balance calibration, instrument calibration (FAAS, ZGFAAS, CVAAS, ICP/MS, and NCS analyzer), matrix spike analysis for each metal, duplicate sample analysis, SRM analysis, procedural blank analysis and standard checks. With each batch of up to 40 samples, 2 procedural blanks, 2 SRM, 2 duplicate samples, and 2 matrix spiked samples also were analyzed. DQOs for these QC measurements are provided in Table 2-10.

**Instrument Calibration.** Electronic balances used for weighing samples and reagents were calibrated prior to each use with certified (NIST traceable) standard weights. All pipets (electronic or manual) were calibrated prior to use. Each of the spectrometers used for metal analysis was initially standardized using a three- to five-point calibration curve with a linear correlation coefficient of  $r \geq 0.999$  required before experimental samples could be analyzed. Analysis of complete three- to five-point calibrations and/or single standard checks alternated every 5 to 10 samples until all the analyses were complete. The relative standard deviation (RSD) between complete calibration and standard check was required to be less than 15 percent or recalibration and reanalysis of the affected samples was performed.

**Matrix Spike Analysis.** Matrix spikes were prepared for a minimum of 5 percent of the samples analyzed and included each metal to be determined. Results from matrix spike analysis using the method of standard additions provided information on the extent of any signal suppression or enhancement due to the sample matrix. If necessary (i.e., spike results outside an 80 to 120 percent limit), all samples were analyzed by the method of standard additions.

**Duplicate Sample Analysis.** Duplicate samples from homogenized field samples (as distinct from field replicates) were prepared in the laboratory for a minimum of 5 percent of the total samples. These laboratory duplicates were included as part of each set of sample digestions and analyses to provide a measure of analytical precision.

**Procedural Blank Analysis.** Two procedural blanks were prepared with each set of 40 samples to monitor potential contamination resulting from laboratory reagents, glassware, and processing

procedures. These blanks were processed using the same analytical scheme, reagents, and handling techniques as used for the experimental samples.

**Standard Reference Material Analysis.** A common method used to evaluate the accuracy of environmental data is to analyze SRM, samples for which consensus or "accepted" analyte concentrations exist. The following SRM were used: Marine Sediments, BCSS-1 and MESS-2 (NRC); Estuarine Sediment 1646a (NIST); Buffalo River Sediment 2704 (NRC); Oyster Tissue 1566a (NIST); Dogfish Muscle DORM-2 (NRC); Lobster Hepatopancreas TORT-2 (NIST), River Water SLRS-3 (NRC) and Trace Elements in Water 1643d (NIST). Metal concentrations obtained for the SRM were required to be within  $\pm 20$  percent of accepted values for greater than 85 percent of other certified analyses. When no certified values exist for a metal, matrix spikes were used to evaluate analytical accuracy.

**Filter Weighing.** All weighing-related manipulation of the filters used for suspended particulate quantification took place under cleanroom conditions, including controlled temperature and relative humidity. Each filter was weighed twice in random order, with a minimum of 5 percent of the filters being weighed in triplicate. Static effects during filter weighing were controlled by placement of two  $^{210}\text{Po}$  antistatic devices near the weighing pan within the balance. The standard deviation for the mass of each filter was required to be less than 2  $\mu\text{g}$  for the value to be accepted.

#### **2.4.4 Biology Quality Assurance/Quality Control**

##### **2.4.4.1 Sediment Toxicity Tests**

The methods used in conducting these tests followed the guidelines established by the EPA manual *Methods for measuring the toxicity of sediment-associated contaminants with estuarine and marine amphipods* (EPA, 1994). The following methodological QA/QC criteria were met at test initiation and validate the results obtained:

- Adult organisms, 3 to 5 mm and in good condition, were used at test initiation; all organisms were from the same source
- Tests were started within 2 days of sediment sample receipt, well within acceptable holding time limits
- Test chambers were identical and contained the same amount of sediment and overlying water
- All instruments used for routine measurements of chemical and physical characteristics were calibrated each day according to the instrument manufacturer's instructions

##### **2.4.4.2 CYP1A (P4501A) Determinations**

- Internal standards were included in each staining run to ensure the consistency and quality of a run, and to determine maximum (occurrence 3 times intensity 5=15) and minimum (0) staining
- All tissues were stained with UPC 10 to determine if nonspecific staining was present
- As part of the standard Signet protocol, slides were presoaked in 3 percent  $\text{H}_2\text{O}_2$  to eliminate endogenous peroxidase activity
- Any slides with questionable staining were rerun

##### **2.4.4.3 P450 Reporter Gene System Determinations**

Measures of QA/QC were taken during testing of each batch of environmental samples. The reference inducer (TCDD) at a concentration of 1 ng/mL, and a solvent blank, typically DCM, are each applied to replicate wells. The fold induction response, that is, the mean RLU of the TCDD divided by the mean RLU of the DCM blank, is compared to a long-term QC chart. The response to 1 ng/mL TCDD must be within 2 standard deviations of the running mean (approximately  $100 \pm 30$ ). Calibration of the luminometer is performed monthly, using a luciferase control kit purchased from Pharmingen.

Environmental extracts are applied to 2 (for both 6h and 16h time periods) or 3 (for only the 16h time period) replicate exposure wells, and the CV is evaluated for each sample. A CV that is in excess of 20 percent is unacceptable, and that extract must be re-tested. In addition, any extract that produces a fold induction response greater than 100 percent must be diluted and retested. Typically, an extract is diluted 1:10 in DCM, and applied to 3 replicate wells.

**Table 2-2: 1997-1998 Source Samples from the Outermost Cook Inlet/Shelikof Strait Region**

Station ID (plot ID)	Station Location	Matrix	Date	Time	Comments
Copper River-1 (CR-1)	Million Dollar Bridge	Sediment	07/15/97	13:38	30 m upstream of Sonar facility @ 1 m above water level.
Copper River-2 (CR-2)	Northwest end of bridge, Long Island (N)	Sediment	07/15/97	14:22	In "catch basin" west side of hwy (milepost 35-36).
Copper River-3 (CR-3)	South end of Long Island	Sediment	07/15/97	14:53	NW end of bridge separating Round and Long Islands.
Copper River-4 (CR-4)	South end of Round Island	Sediment	07/15/97	15:08	Mainland and Round Island.
Homer 0-2 cm (H-2) and 4-6 cm (H-4)	Homer Harbor	Sediment	07/16/97	06:56	Just outside boat harbor entrance, west of dredged channel (59°36.37, 151°19.94) (39 m depth).
Susitna-01 (SR-1)	Susitna River	Sediment	07/20/97	13:30	Northern bank, west of bridge at mile 105 on George Parks Hwy.
Susitna-02 (SR-2)	Susitna River	Sediment	07/20/97	16:30	Northern bank, west of bridge at mile 105 on George Parks Hwy.
Crude TBPF	Cook Inlet crude	Crude Oil	08/14/97	02:55	UNOCAL TBPF/NTL.
Swanson River Field Oil (SW-O)	Swanson River crude	Oil	1998	NA	None.
TBPF-Outfall (TB-W)	UNOCAL TBPF	Produced Water	08/26/97	03:00	UNOCAL TBPF/NTL.
Coal-1 (H-C1)	Homer Spit, Homer, AK	Coal	11/09/97	15:30	Beach-washed coal (5 chunks), approximately 1 mile W. of Bishops Beach/ CIRCAC.
WWTF Final Effluent	Point Woronzof, Anchorage	Water	08/12/97	11:00	Municipal WWTF Final Effluent.
AC1 (AK-C-S)	Offshore Kenai Peninsula	Sediment	06/27/98	15:59- 17:21	Offshore Alaska Coastal Current station. Shake-down gear, sampling with 2 shifts.
St. Augustine Ash	St. Augustine Island	Ash	07/04/98	07:51- 08:48	Beach sample collected onshore St. Augustine Island for volcanic ash.
St. Augustine Sediment (AI-S)	Offshore St. Augustine Island	Sediment	07/04/98	09:20	Offshore Island sediment grab for evidence of ash layer.
Toxicity Reference	Holgate Glacier	Sediment	07/05/98	15:42	Sediment Collected as a toxicity reference sediment sample, near the Holgate Glacier
Homer Coal (H-C2)	Coal Bay Beach, Homer	Coal	07/07/98	15:00	Coal source sample collected from Coal Bay Beach, Homer.
Ninilchik Coal (N-C)	Ninilchik Bluff	Coal	07/07/98	13:00	Coal source sample collected from coal seam in Ninilchik bluff.
Well Creek Oil (WC-O)	Well Creek "beaver ponds"	Oil Seep	07/07/98	12:55- 14:09	Oil seep sample collected at Well Creek "beaver ponds". Transported by helicopter.
Matanuska Coal (M-C)	Northwest of Sutton, AK	Coal	05/16/98	PM	Coal collected from exposed coal seam.
CLC (CL-C)	Coyote Lake Sutton, AK	Coal	05/16/98	PM	Coal collected from coal pocket.
Susitna-03 (SR-W1)	Deshka Landing, Susitna River	Surface Water	05/13/98	AM	Approximately mile 82.5 on George Parks Hwy
Matanuska-01 (MR-W1)	Matanuska River	Surface Water	05/13/98	AM	Collected at intersection of Old Glenn Hwy and Matanuska River
Matanuska-02 (MR-S)	Matanuska River	Sediment	05/13/98	AM	Collected at intersection of Old Glenn Hwy and Matanuska River.
Knik-01 (KR-W1)	Knik River	Surface Water	05/13/98	AM	Collected at intersection of Old Glenn Hwy and Knik River.

**Table 2-2: 1997-1998 Source Samples from the Outermost Cook Inlet/Shelikof Strait Region**

Station ID (plot ID)	Station Location	Matrix	Date	Time	Comments
Copper River- W	Copper River	Surface Water	05/16/98	06:30- 07:30	Sample "C1" from Copper River Hwy mile 25, and "C2" from mile 28.
Copper River-5 (CR-5)	Copper River	Sediment	05/16/98	07:30	Copper River Highway, mile 25.
Susitna-04	Susitna River	Surface Water	06/25/98	PM	Collected at intersection of Susitna River and Parks Hwy, @ approximately mile 101.
Matanuska- 03	Matanuska River	Surface Water	06/25/98	AM	Collected at intersection of Old Glenn Hwy and Matanuska River.
Knik-02	Knik River	Surface Water	06/25/98	AM	Collected at intersection of Old Glenn Hwy and Knik River.
SSCoal 4 (SS-C4)	Beluga Coal Fields	Coal	06/22/98 06/23/98	NA	None.
SSCoal 5 (SS-C5)	Beluga Coal Fields	Coal	06/22/98 06/23/98	NA	None.

**Table 2-3: Saturated Hydrocarbons Target Analyte List**

Compound	Internal Standard/ Surrogate Reference	Compound	Internal Standard/ Surrogate Reference
nC8	A/1	nC28	A/1
nC9	A/1	nC29	A/1
nC10	A/1	nC30	A/1
nC11	A/1	nC31	A/1
nC12	A/1	nC32	A/1
nC13	A/1	nC33	A/1
1380	A/1	nC34	A/1
nC14	A/1	nC35	A/1
1450	A/1	nC36	A/1
nC15	A/1	nC37	A/1
1650	A/1	nC38	A/1
nC16	A/1	nC39	A/1
nC17	A/1	nC40	A/1
Pristane	A/1		
nC18	A/1		
Phytane	A/1		
nC19	A/1		
nC20	A/1		
nC21	A/1	<b>Internal Standard</b>	
nC22	A/1	Triacontane-d <sub>62</sub>	A
nC23	A/1		
nC24	A/1	<b>Surrogate</b>	
nC25	A/1	Tetracosane-d <sub>50</sub>	1/A
nC26	A/1	5-a-Androstane	2/A
nC27	A/1		



**Table 2-4: Polycyclic Aromatic Hydrocarbons Target Analyte List**

Compound	Surrogate/ Internal Standard Reference	Compound	Surrogate / Internal Standard Reference
Naphthalene (C0N)	2, A	Benzo[a]anthracene	3, B
C1-Naphthalenes (C1N)	2, A		
C2-Naphthalenes (C2N)	2, A	Chrysene (C0C)	3, B
C3-Naphthalenes (C3N)	2, A	C1-Chrysenes (C1C)	3, B
C4-Naphthalenes (C4N)	2, A	C2-Chrysenes (C2C)	3, B
		C3-Chrysenes (C3C)	3, B
Acenaphthene (ACE)	2, A	C4-Chrysenes (C4C)	3, B
Acenaphthylene (ACEY)	2, A		
Biphenyl (BIP)	2, A	Benzo[b]fluoranthene	3, B
		Benzo[k]fluoranthene	3, B
Fluorene (C0F)	2, A	Benzo[a]pyrene (BAP)	4, B
C1-Fluorenes (C1F)	2, A	Benzo[e]pyrene (BEP)	4, B
C2-Fluorenes (C2F)	2, A	Perylene (PER)	4, B
C3-Fluorenes (C3F)	2, A	Indeno[1,2,3-c,d]pyrene	4, B
		Dibenzo[a,h]anthracene	4, B
Dibenzothiophene (C0D)	3, A	Benzo[g,h,i]perylene	4, B
C1-Dibenzothiophenes (C1D)	3, A		
C2-Dibenzothiophenes (C2D)	3, A		
C3-Dibenzothiophenes (C3D)	3, A		
Phenanthrene (C0P)	3, A	<b>Surrogate Compounds</b>	
Anthracene (C0A)	3, A	Naphthalene-d8 (D8N)	1, A
C1-Phenanthrenes/Anthracenes	3, A	Acenaphthene-d10	2, A
C2-Phenanthrenes/Anthracenes	3, A	Phenanthrene-d10	3, A
C3-Phenanthrenes/Anthracenes	3, A	Benzo[a]pyrene-d12	4, B
C4-Phenanthrenes/Anthracenes	3, A		
Fluoranthene (FLANT)	3, A		
Pyrene (PYR)	3, A	<b>Recovery Standards</b>	
C1-Fluoranthenes/Pyrenes (C1F/P)	3, A	Fluorene-d10 (D10FL)	A
C2-Fluoranthenes/Pyrenes (C2F/P)	3, A	Chrysene-d12 (D12C)	B
C3-Fluoranthenes/Pyrenes (C3F/P)	3, A		

**Table 2-5: Steranes/Triterpanes Target Analyte List**

<b>Steranes/Triterpanes</b>	<b>Peak Number</b>
Diacholestane	S4
Diacholestane	S5
Methylcholestane	S24
Ethylcholestane	S25
Ethylcholestane	S28
Diterpane	T4
Tricyclitriterpane	T9
Tricyclitriterpane	T10
Trisnorhopane (TS)	T11
Trisnorhopane (TM)	T12
Norhopane	T15
Oleanane	T18
Hopane	T19
Homohopane	T21
Homohopane	T22
<b>Internal Standards</b>	
Chrysene-d12	
Triacontane-d62 (Alternate)	
<b>Surrogates</b>	
5B(H)-Cholane	
Dotriacontane-d66 (Alternate)	

**Table 2-6: Summary of Instrumental Methods and Method Detection Limits (MDL) for Metal Analysis of Sediment and Fish**

Metal	Sediments		Fish	
	Method	MDL (µg metal/g dry sediment)	Method	MDL (µg metal/g tissue dry wt.)
Ag - Silver	ZGFAAS	0.01	ZGFAAS	0.004
Al - Aluminum	FAAS	6	FAAS	2.3
As - Arsenic	ZGFAAS	0.1	ZGFAAS	0.03
Ba - Barium	ICP/MS	0.1	ICP/MS	0.006
Be - Beryllium	ICP/MS	0.1	ZGFAAS	0.002
Ca - Calcium	FAAS	5	Not analyzed	--
Cd - Cadmium	ICP/MS	0.02	FAAS	0.3
Cr - Chromium	FAAS	1	GFAAS	0.003
Cu - Copper	FAAS	2	FAAS	0.7
Fe - Iron	FAAS	5	FAAS	2.5
Hg - Mercury	CVAAS	0.001	CVAAS	0.001
K - Potassium	FAAS	5	Not analyzed	--
Mg - Mannesium	FAAS	1	Not analyzed	--
Mn - Manganese	FAAS	2	FAAS	1.1
Ni - Nickel	ICP/MS	0.2	Not analyzed (Year 1)	—
Pb - Lead	ICP/MS	0.1	ICP/MS	0.001
Sb - Antimony	ICP/MS	0.2	ICP/MS	0.001
Se - Selenium	ZGFAAS	0.05	ZGFAAS	0.03
Sn - Tin	ICP/MS	0.1	ICP/MS	0.001
Tl - Thallium	ICP/MS	0.1	ICP/MS	0.0001
V - Vanadium	FAAS	10	GFAAS	0.007
Zn - Zinc	FAAS	1	FAAS	0.4
<b>Other Parameters</b>				
Grain-Size	Sieve and Pipet	--		
TOC	Carlo Erba NCS System	0.1		

Notes:

FAAS = Flame Atomic Absorption Spectrometry

GFAAS = Graphite Furnace Atomic Absorption Spectrometry

ZGFAAS = Zeeman Graphite Furnace Atomic Absorption Spectrometry

CVAAS = Cold Vapor Atomic Absorption Spectrometry

ICP/MS = Inductively Coupled Plasma-Mass Spectrometry

NCS = Nitrogen - Carbon - Sulfur Analyzer

TOC = Total Organic Carbon

**Table 2-7: Calculation of the Sediment Profile Imaging Organism-Sediment Index**

Parameter	Range/Type	Index Value
<b>A. Mean RPD Depth</b> (choose one)		
	0.00 cm	0
	> 0 - 0.75 cm	1
	0.76 - 1.50 cm	2
	1.51 - 2.25 cm	3
	2.26 - 3.00 cm	4
	3.01 - 3.75 cm	5
	> 3.75 cm	6
<b>B. Successional Stage</b> (choose one)		
	Azoic	-4
	Stage I	1
	Stage I II	2
	Stage II	3
	Stage II III	4
	Stage III	5
	Stage I on III	5
	Stage II on III	5
<b>C. Chemical Parameters</b> (choose one or both if appropriate)		
	Methane Present	-2
	No/Low Dissolved Oxygen <sup>a</sup>	-4

Notes:

Organism-sediment Index = Total of above subset indices (A+B+C); Range: -10 to +11.

<sup>a</sup>This is not based on a Winkler or polarographic electrode measurement, but on the imaged evidence of reduced, low reflectance (i.e., high-oxygen-demand) sediment at the sediment-water interface.

**Table 2-8: Data Quality Objectives for Saturated Hydrocarbons and Polycyclic Aromatic Hydrocarbons Analyses**

Element or Sample Type	Minimum Frequency	Data Quality Objective/Acceptance Criteria
Initial Calibration	Prior to every instrument sequence for GC/MS analysis and as needed for GC/FID analysis	5-point curve, percent RSD < 35 percent for all CC target analytes, 90 percent must be < 25 percent
Continuing Calibration (CC)	After every 12 samples or 16 hours, whichever is more frequent, and at end of instrument sequence	Percent RSD < 35 percent for all CC target analytes; 90 percent must be < 25 percent
Oil Reference Standard	Two with each instrument sequence (One North slope Crude and one Cook Inlet Crude)	North Slope Crude < 35 percent D from laboratory mean for target compounds (use surrogate corrected values) except for compounds below the reporting limit
Procedural Blank	One per batch	No analyte to exceed 5X the MDL unless sample amount is > 10X blank amount
Blank Spike	One per batch	Recovery between 35 and 125 percent
Instrument SRM (1491)	One per instrument sequence Not applicable to SHC analysis	Values must be <15 percent difference of true value for all certified analytes
Sediment SRM (1941a) Tissue SRM (1974a)	One per batch as appropriate	Values must be within 30 percent of the true value on average for all analytes, not to exceed 35 percent of true value for more than 2 analytes
Duplicate Analysis	One per 40 field samples	RPD < 30 percent for all analytes >10 times the MDL; Mean RPD<30 percent
Surrogate Standards	Every sample	Recovery between 45 and 125 percent

**Table 2-9: Quality Control Summary for Sterane and Triterpane Analyses**

<b>Element or Sample Type</b>	<b>Minimum Frequency</b>	<b>Data Quality Objective/Acceptance Criteria</b>
Initial Calibration	Prior to every instrument sequence	4-point curve, percent RSD < 25 percent for all target analytes
Continuing Calibration	After every 12 samples or 16 hours, whichever is more frequent, and at end of each sequence	Percent RSD < 25 percent for all analytes
Oil Reference Standard (North Slope Crude)	One with each instrument sequence	< 35 percent Dif. from laboratory mean for target compounds (use surrogate corrected values) except for compounds below the reporting limit
Procedural Blank	One per batch	No analyte to exceed 5X the MDL unless sample amount is > 10X blank amount
Surrogate Standards	Every sample	Recovery between 45 and 125 percent

**Table 2-10: Data Quality Objectives and Criteria for Metals and Total Organic Carbon**

Element or Sample Type	Minimum Frequency	Data Quality Objective/Acceptance Criteria
Initial Calibration	Prior to every batch of samples	3- to 5-point curve depending on the element and a blank. Standard curve correlation coefficient $r \geq 0.999$ for all analytes
Continuing Calibration	Must end every analytical sequence; for flame, repeat all standards every five samples; for graphite furnace and ICP/MS, recheck standard after every 8 to 10 samples	Percent RSD < 15 percent for all analytes
Standard Reference Materials	One per batch of 20 samples	Values must be within $\pm 20$ percent of accepted values for > 85 percent of the certified analytes and within $\pm 25$ percent for Hg
Method Blank	One per batch of 20 samples	No more than 2 analytes to exceed 5X MDL unless analyte not detected in associated sample
Matrix Spike and Spike Method Blank	One per batch of 20 samples	Analyte recoveries between 80 to 120 percent
Laboratory Duplicate	One per batch of 20 samples	RSD < 25 percent for 65 percent of the analytes