

## 4.0 Discussion

In this section the results and interpretation of the chemical and biological tests performed on the sediments and tissues from the study are further evaluated in the framework of the null hypotheses presented in Section 1.2. The title and subject of each of the following subsections correspond to a particular null hypothesis, and include an assessment on the validity of the null hypothesis.

### 4.1 Surface Sediments as Contaminant Sinks

Fine-grained, organic-rich sediments are the most common sink in the coastal ocean for organic and trace metal contaminants because of the large, active surface area available for adsorption. Contaminants introduced by municipal discharges to areas surrounding Anchorage and by oil and gas activities to uppermost Cook Inlet will most likely be deposited with such fine-grained sediments. The upper areas of Cook Inlet have been previously shown to contain only minor deposits of fine-grained sediment because the shallow water, large tidal ranges and active hydrography move sediments southward to outermost Cook Inlet and the Shelikof Strait (Sharma and Burrell, 1970; Carlson *et al.*, 1977; Hampton, 1982). Thus, a primary goal of this study was to identify and sample these potential sinks for fine-grained sediments and determine whether they contain contaminants.

Surface sediments collected during this study help identify patterns of deposition for fine-grained silts and clays in outermost Cook Inlet and the Shelikof Strait. A contour map of silt+clay (Figure 4-1) shows a sharp increase in silt+clay from 30 to 40 percent in outermost Cook Inlet to greater than 95 percent in the middle (zone 2) through the lower (zones 3 and 4) portions of the Shelikof Strait (summary data in Table 3-10). Clearly, the trends shown on Figure 4-1 support the initial assumption of the study and identify most of the Shelikof Strait as an important sink for fine-grained sediment. Furthermore, the observed trend supports deposition of more fine-grained sediment toward the southern areas of Shelikof Strait.

The TOC content of the sediment averages about 0.75 percent for the entire study area with a relatively limited number of sites (Homer Harbor and zone 4) containing greater than 1 percent TOC. These concentrations of TOC compare well with values previously reported by Hampton (1983). Concentrations of TOC tend to be highest in samples with greater than 80 percent silt+clay (Figure 4-2), suggesting that organic matter is associated with fine-grained sediments.

A first approximation of the magnitude of sediment deposition in outermost Cook Inlet and the Shelikof Strait is estimated here based on data for riverine discharge of sediment, as well as sediment accumulation rates. Riverine input of sediment from the Susitna-Knik-Matanuska (S-K-M) River system was reported as  $40 \times 10^6$  tons/year (Feely *et al.*, 1982; USGS, 1998). This value is most likely a lower limit for total sediment input from the S-K-M system because the three rivers are estimated to contribute 75 to 90 percent of the total sediment input to upper Cook Inlet. Thus, estimates of total transport of sediment from the S-K-M system range from  $44$  to  $53 \times 10^6$  tons/year. Added to inputs from the S-K-M system is some fraction of the sediment transport of  $107 \times 10^6$  tons/year from the Copper River (Reimnitz, 1966). Based on a sediment composition model described in Section 4.3, sediment derived from the Copper River accounts

for 10 to 20 percent of the total sediment deposited in the study area. Total sediment deposition was determined for each zone (Table 4-1). The average sediment accumulation rate was multiplied by the area of fine-grained sediment in each zone to determine total deposition (Table 4-1). Total sediment deposition based on sediment accumulation is  $\sim 60$  to  $70 \times 10^6$  tons/year for zone 0 to zone 4. Using data for river inputs, the  $44$  to  $53 \times 10^6$  tons/year from the S-K-M system is combined with a 10 to 20 percent contribution from the Copper River (i.e.,  $6$  to  $14 \times 10^6$  tons/year) for a total of  $50$  to  $67 \times 10^6$  tons/year. Thus, the two approaches to determining total sediment deposition in the study area agree relatively well.

Are fine-grained sediments from outermost Cook Inlet and the Shelikof Strait contaminated with trace metals? Identification of metal contamination in sediments requires knowledge of natural levels of metals. All trace metals are present in marine sediments at some natural level that varies as a function of the mineralogy, grain size, and organic matter content of the sample. As previously described, concentrations of trace metals were normalized to Fe to help account for natural variability in sediment composition and concomitant trends in concentrations of trace metals. Metal/Fe ratios can be compared with source sediments from the Susitna and Copper Rivers, surface sediments from pristine sites, or with older, deeper (pre-industrial) sediments from the same site to help identify anthropogenic additions of metals over space and time. If a particular metal/Fe ratio is significantly increased relative to clean, reference material from the area, then that metal can be identified as having some fraction of the total concentration introduced by anthropogenic sources. For example, Figure 4-3 shows a Cr/Fe plot for all surface sediments, along with source material from the Susitna and Copper Rivers. The Cr/Fe plot (Figure 4-3) shows that most points follow a relatively good linear trend that includes source sediment from the Copper River. The Cr/Fe ratios for average continental crust and sediment from the Susitna River (Figure 4-3) are comparable, yet higher than observed for the samples. Thus, with the available data, no samples show a clear deviation from what are believed to be natural concentrations for Cr.

In contrast with the Cr/Fe relationship described above, the Zn/Fe relationship for source material and surface sediment from the study area fit a linear trend with a slope that is about 2 times higher than shown for average continental crust (Figure 4-3). However, this trend of higher Zn levels in Alaskan sediments, relative to Fe, is consistent with data for Alaskan rocks (Table 3-3). Furthermore, Zn concentrations and the Zn/Fe ratio have been constant in sediments deposited in the study area during the past 100 years (e.g., Figures 3-52 through 3-65). Thus, the higher Zn/Fe ratio for Alaskan sediments is a natural reflection of somewhat higher levels of Zn in sediments from the study area relative to average continental crust. Like Cr, concentrations of Be, Ni, Pb, Se, Sn, and Tl (Figures 4-4 and 4-6) follow linear trends versus Fe with a slope less than shown for average continental crust and with no indications of anthropogenic loading. In contrast, concentrations of Ag, As, Ba, Cd, Cu, Hg, Mn, Sb, and V versus Fe are similar to trends described for Zn versus Fe (Figures 4-4 through 4-7). Six points on the Hg/Fe graph (Figure 4-7) show positive deviations from the basic relationship shown for the source samples and other surface sediments. These samples were collected from Z0F1 and Z0F14, both in Kachemak Bay. However, present-day Hg levels in Kachemak Bay (core 98-Z0F1) are comparable with values observed throughout the twentieth century, suggesting that these concentrations are typical for the Kachemak Bay area. Thus, within the present data set, no clearly identifiable anthropogenic inputs of sediment metals are observed between the source rivers and the sediments of outermost Cook Inlet and the Shelikof Strait.

The levels and extent of PAH, from petroleum and other sources, in outermost Cook Inlet and Shelikof Strait are shown in a contour map of the total PAH concentrations (Figure 4-8). The total PAH concentrations generally follow the pattern observed for silt+clay (Figure 4-1), with an increase in total PAH from approximately 0.20 to approximately 0.40 ppm in zone 0 (outermost Cook Inlet) to approximately 0.50 to 1.0 ppm in zones 1, 2, 3, and 4 (Shelikof Strait). Exceptions to this pattern were observed at stations Z0F1 and Z0F14 (Kachemak Bay), which showed elevated concentrations of PAH relative to other zone 0 surface sediments. The relative elevation of PAH at these two stations was likely due to enrichment from local coal and petroleum hydrocarbon inputs. Coal outcrops are a well documented feature of the nearby Kachemak Bay shoreline, and anthropogenic petroleum hydrocarbon influences were identified from the adjacent Homer Harbor source samples.

However, the total PAH concentrations in all zones are comparable to values reported for background hydrocarbons in other studies from offshore coastal waters of Alaska (Boehm, *et al.*, 1991; Bence *et al.*, 1996; Page, *et al.* 1996; Boehm, *et al.*, 1998). Therefore there does not appear to be any identifiable enrichment of petroleum contaminants (as represented by PAH) from anthropogenic activities, including oil and gas production in upper Cook Inlet.

In summary, in the context of the null hypothesis, **the surface sediments of outermost Cook Inlet and the Shelikof Strait are potential traps for contaminants from oil and gas production activities in upper Cook Inlet**, by virtue of the fact that Shelikof Strait is a depositional area. Based on evaluations of the organic and inorganic data, no contamination of the study area's surface sediments was detected that may have originated from oil and gas production activities in upper Cook Inlet.

## **4.2 Concentration of Pollutants Over Time**

### **4.2.1 Geochronology of Metals**

A 50- to 100-year chronology of metal levels in sediments from outermost Cook Inlet and the Shelikof Strait was obtained for each zone. Concentrations of Fe, Al (Table 3-19) and/or the Fe/Al ratio were uniform in each core (Figures 3-52 through 3-65) and thus, for convenience, metal concentrations, rather than ratios to Fe, are used in this section.

Profiles for Pb versus time show that concentrations of Pb varied by less than 10 percent (CV) within each core over time periods extending back as far as the late 1800s. For example, at station Z1F1, Pb levels vary by  $\pm 0.7$   $\mu\text{g/g}$  for a mean of 12.3  $\mu\text{g/g}$  (Figure 4-9). A similar trend was observed for station Z3F2 (Figure 4-9) and each of the other sites. The overall constant and low levels of Pb, with a maximum of 15.2  $\mu\text{g/g}$  relative to 14.8  $\mu\text{g/g}$  for average continental crust, show that no detectable anthropogenic Pb has been deposited at the 14 core sites during the past century.

Based on a total of  $\sim 60 \times 10^6$  tons of sediment deposited in the study area per year, about 60 tons of Pb would need to be introduced to the region to observe a 1  $\mu\text{g/g}$  increase in sediment Pb levels. Previous estimates of anthropogenic Pb inputs to the area were about 2 tons of Pb (Boehm *et al.*, 1998) and are consistent with the sedimentary record. Local instances of Pb contamination are still possible; however, none were detected.

In a manner similar to that described for Pb, concentrations of Ag, Ba, Be, Cu, Hg, Ni, Sb, Tl, V and Zn, or their ratios to Fe, generally show less than 10 to 20 percent variation within a core at all sites. Furthermore, many of the observed higher levels (or ratios to Fe) are observed deeper in the cores (i.e., older sediments). Thus, none of these elements show discernible anthropogenic inputs or changes in metal levels during the past 40 to 50 years of industrial activity in the area.

Concentrations of Cr in cores from sites Z1F1 and Z1F2 are 20 to 30 percent higher during the early 1900s relative to the 1940s (Figure 4-10). Uniform profiles of Cr over time are observed for most other sites with the exception of one or two lower values each in cores from Z0F5, Z0F6, Z1R3B, and Z3F2. Chromium was mined on the Kenai Peninsula near Seldovia at varying times from World War I through the late 1950s. The slightly elevated Cr levels in nearby sites Z1F1 and Z1F2 may reflect inputs of small amounts of Cr tailings from this activity. No such evidence of enrichment was found at the other sites.

Concentrations of Cd in the various cores have the greatest variability (Figure 4-10), with an overall range of 0.07 to 0.27  $\mu\text{g/g}$ . At these levels, Cd concentrations are within the bounds of bottom sediments from the Susitna (0.30  $\mu\text{g/g}$ ) and Copper (0.16  $\mu\text{g/g}$ ) Rivers. The variations over time are believed to be a complex function of biological inputs of Cd-rich organic matter, diagenesis in the sediments, and variable inputs of source sediment.

Surface layers (from 1 to 6 cm thick) at stations Z2R16, Z2R2, and Z3F1 have Mn concentrations greater than 1,000  $\mu\text{g/g}$  in what otherwise are generally uniform Mn profiles at the various sites. This surficial enrichment of Mn is most likely related to dissolution of Mn oxides in reducing layers below 6 cm with diffusion of  $\text{Mn}^{2+}$  up to oxic surface sediments where it precipitated as  $\text{MnO}_2$ . Massoth et al.(1979) previously observed such remobilization of Mn in Shelikof Strait. Arsenic follows a similar trend to that observed for Mn, suggesting that As also may follow Mn in this diagenetic process.

#### **4.2.2 Geochronology of Organics**

The geochronology of petroleum hydrocarbons was established in 12 sediment cores from the outermost Cook Inlet and Shelikof Strait. A chronology of 20 to 100 years was established, based on the individual core at each station with at least one core with a 50+ year record in each zone. In general, profiles of the concentrations of the totals for the 3 categories of organic compounds (total PAH, TPHC, and total S/T) did not reveal any significant increases over the oil and gas development time period. This finding was consistent between cores over time. Some variation was noted, as the CV for total PAH and total S/T ranged between 8 and 23 percent within each core. The CVs for TPHC were somewhat higher: 14 to 49 percent, showing greater variability for this parameter, but no consistent trend within cores. The one exception was the core from Z3F2, which exhibited a nearly three-fold increase (from 20  $\mu\text{g/g}$  to 53  $\mu\text{g/g}$ ) in the non-specific TPHC parameter between approximately 1987 and present (Figure 3-77). However, no corresponding increase in either total PAH or total S/T was observed during the same time interval, indicating that the TPHC increase was not related to anthropogenic or petroleum hydrocarbon inputs over the last 10 years.

In all cores, the concentrations of PAH analytes are within the same range as the PAH levels in the surface sediments of the corresponding zone. Perylene is an exception. Perylene concentrations generally increased with core depth at each zone, as shown in Figure 4-11. The

increasing concentration of perylene with depth may be related to the well-known process associated with perylene formation during early sediment diagenesis, or may be related to perylene input associated with coal.

#### **4.2.3 Statistical Analysis of Concentrations in Cores**

As discussed in Section 3.3.5, the dated sediment cores were evaluated statistically to determine if there were any significant trends in the cores that could be associated with the onset of petroleum exploration and production activities in upper Cook Inlet. The results of the statistical analysis revealed that there were no significant increases in the concentration of organics and metals in the sediments which could be correlated with the onset of petroleum activities (circa 1963). Several of the trends in specific parameters which were discussed previously (e.g., increasing perylene with core depth, increases in terrigenous hydrocarbon parameters near the surface, and subtle shifts in several petroleum source ratios) were also validated by the statistical analysis.

In summary, in the context of the null hypothesis, **the concentrations of metals and organics in sediments in outermost Cook Inlet and Shelikof Strait have not increased significantly since offshore oil exploration and production began in Cook Inlet (circa 1963).**

### **4.3 Composition of Pollutants Over Time**

#### **4.3.1 Metals**

Average metal values for Ag, As, Ba, Be, Cd, Cr, Cu, Hg, Mn, Ni, Pb, Sb, Se, Sn, Tl, V, and Zn or their ratios to Fe, from each core site are comparable with levels obtained for source sediments from the Susitna and Copper Rivers. This observation is consistent with the concept that no identifiable additions of these metals occur as sediment is being transported from the source rivers to depositional sites in outermost Cook Inlet and the Shelikof Strait. In other words, oil and gas operations and the Point Woronzof outfall have not had a discernible impact on concentrations of these metals at the study sites.

Knowledge of the relative amounts of sediment in the Shelikof Strait that are derived from the S-K-M system versus the Copper River has valuable scientific and management application. Previous work by Hein *et al.* (1979) qualitatively identified the presence of sediments from the Copper River in Shelikof Strait using clay mineralogy. A first approximation of the quantitative proportions of sediment in each zone that are derived from the S-K-M system was calculated during our study using trace metal data.

Five different metal/metal ratios were selected for this calculation. Metal ratios were used to normalize differences in absolute concentrations of metals from various source and sediment samples. For each ratio used, the following conditions were established: (1) the ratio for river bottom sediment was within  $\pm 10$  percent of the value for river suspended solids, (2) the ratio for the combined S-K-M system differed from that for the Copper River by greater than 30 percent. The average values used for the S-K-M system are based on the following fractions of total sediment transport: Susitna (83 percent), Knik (11 percent), and Matanuska (6 percent), using sediment discharge data from the USGS (1999). Based on these criteria, the five ratios used for the model are Ni/Be, Fe/Zn, Sn/Sb, Pb/Tl, and V/Zn (Table 4-2).

For each surface sample and each ratio, the fraction of sediment derived from the S-K-M system was calculated using a simple 2-member mixing model with average ratios from Table 4-2. Results for all 5 ratios were averaged for each site and the grand average for each sample is presented as a mean  $\pm 2$  standard deviation (95 percent confidence interval) in Table 4-3. In zones 0, 1, and 2, the error of the estimate (95 percent confidence interval) is 24 to 30 percent relative to 8 percent for zones 3 and 4. Alaska Coastal Current sediment collected outside the Shelikof Strait has no discernible input from the S-K-M system. Surficial sediment from outermost Cook Inlet and the Shelikof Strait contains an average of  $81 \pm 22$  percent sediment from the S-K-M system. Thus, the dominant source of sediment to the study area at present is from the S-K-M system.

#### 4.3.2 Organics

The sediment core profiles showed that concentrations of the primary hydrocarbon parameters were relatively constant with depth within each core. An evaluation of diagnostic source parameters within the cores revealed that in most cases the source(s) of these hydrocarbons was constant over the time period studied.

As was seen in the surface sediments, double-ratio source plots of C2D/C2P versus C3D/C3P provide a representative fine-tuned summary of the PAH sources in the core samples. Double-ratio source plots for each of the sediment cores are presented in Figures 4-12 through 4-19. Based on an evaluation of the source ratio plots of the cores, several important trends are observed. In the cores from Z0F1 (Kachemak Bay, Figure 4-12) there is greater scatter throughout the cores, likely related to variable local hydrocarbon source inputs (e.g., coal, Homer Harbor sediment, runoff).

The source ratio plot of the sediment core from Z0F5 (Figure 4-13) shows some scatter of the different core sections, with the shallower depth intervals (~1940 to present) clustering between values for “background” sediment and coal. The deeper sections of the core show a trend towards source values corresponding to the Well Creek seep oil value and the Oil Bay seep oil (~1940 and earlier). This result could indicate a possible shift in the source of petroleum hydrocarbons over time. This shift would indicate a greater influence of the Well Creek/Oil Bay seep oil sources to the outermost Cook Inlet sediments in the past (prior to 1940), and an increase in the contribution of the particle-bound “background” hydrocarbons from other petroleum hydrocarbon source(s) to the east (e.g., Katalla, Yakutaga area formations) more recently (post-1940). A mixture of these two “end members” is suggested by the double-ratio plot.

A similar trend is observed in the source ratio plot of the core sample from station Z0F6 (Figure 4-13), where the deeper sections of the core (6 to 25 cm) are more closely associated with the Well Creek seep oil and Oil Bay seep oil sources, and the shallower surface sections (0 to 6 cm) are intermediate to “background” sediment and coal sources. The same follows for the core sample from Z0F8, i.e., the deeper sediments (16 to 20 cm, Figure 4-14) are separated from the cluster of upper sediments and exhibit Well Creek seep oil and Oil Bay seep oil influence. Geochronology for the Z0F6 and Z0F8 cores confirms that each of the deeper sediments within each core is from the 1940s and earlier, establishing a source correlation between the trends observed in these 3 zone 0 core profiles.

The source ratio plots of these three zone 0 cores (Z0F5, Z0F6, and Z0F8) all suggest a shift in the contribution of petroleum hydrocarbon sources in the western portion of outermost Cook Inlet around 1940, with an apparent decrease in the influence of Iniskin Peninsula petroleum sources after 1940. A preliminary statistical evaluation of the source ratios from these three cores was performed to test this trend in the same manner as described previously for the post-1963 intervention effect, except in this analysis a 1940 intervention effect was tested. The results of this analysis (Woolcott Smith - personal communication) show a significant increase ( $P < 0.05$ ) in the C2D/C2P and C3D/C3P source ratios after 1940 in all three cores. Several causes for this observed shift in petroleum hydrocarbon sources could be suggested, including earthquake activity around 1940, which may have diminished the flow of the local Iniskin Peninsula seeps, or a shift in the sedimentary or circulatory regime of the area. Further study would be required to refine these theories; however, with respect to the objectives of this monitoring program it is important to note that the observed trend is only found in the western area of outermost Cook Inlet, and occurred prior to any offshore oil exploration and production activities in Cook Inlet.

The source ratio plots for the zone 1, 2, 3, and 4 cores (Figures 4-15 through 4-19) generally show a consistent cluster of source ratio values throughout the depth intervals sampled. This indicates that the source of hydrocarbons to these sediments has not changed since the early 1900s, based on the geochronologies established to date. The source ratios generally plot in the same area as the “background” sediment, indicating that particle-bound petroleum hydrocarbons associated with seeps and petroleum formation source(s) to the east of the study area (e.g., Katalla, Yakutaga, east of Prince William Sound) are likely the primary source of hydrocarbons over this period.

Thus, in the context of the null hypothesis, **the composition (source[s]) of metals in the sediments of outermost Cook Inlet and Shelikof Strait do not appear to have changed since offshore oil exploration and production began in Cook Inlet (circa 1963). The composition of hydrocarbons in sediment cores does show subtle changes over the past 25 to 50 years, but these changes do not appear to be correlated with petroleum production activities or spills.**

#### **4.4 Risk Associated with Pollutant Concentrations: Exposure Assessment**

In the context of this study multiple parameters were used to evaluate biological and ecological risk. The following sections provide a discussion on both sediment quality and biological and ecological effects (sediment quality criteria, sediment toxicity, CYP1A [P450] induction in fish tissues, and RGS analyses of sediment and fish tissue).

##### **4.4.1 Comparison to Sediment Quality Criteria: Metals**

Sediment quality criteria have been used extensively worldwide to assess possible adverse biological effects from metals and PAH. The most utilized criteria are the ERL (Effects Range-Low) and ERM (Effects Range-Medium) presented by Long *et al.* (1995). These guidelines are based on field, laboratory, and modeling studies conducted in the United States that coupled concentrations of contaminants in sediments with biological effects (e.g., Long and Morgan, 1990). The ERM is defined as the concentration of a substance in the sediment that results in an adverse biological effect in about 50 percent of the test organisms and the ERL is defined as the

concentration of a substance that affects 10 percent of the test organisms. Thus, the general application of the criteria has been to state that adverse biological effects are “rarely” observed when metal or PAH levels are less than the ERL, “occasionally” observed when contaminants are present at levels between the ERL and ERM, and “frequently” observed when concentrations exceed the ERM.

Nine of the 17 metals investigated during this study have been assigned ERL and ERM concentrations by Long *et al.* (1995) at this time. None of these 9 metals are found at levels above the ERM in any sediment samples from this study (Figures 4-20, 4-21, and 4-22). Furthermore, 5 of the metals (Ag, Cd, Hg, Pb, and Zn) are present in sediments at levels below the ERL at all sites (Figures 4-20, 4-21, and 4-22). Concentrations of Cr exceed the ERL by less than 20 percent at 2 sites in Kachemak Bay (Z0F1 and Z0F14). In contrast, concentrations of As exceed the ERL of 8.2 µg/g at 38 of 56 sites by less than 1 to as much as 8 µg/g (Figure 4-20). However, the highest As concentration of 16 µg/g in sediment from Z0F1 is still well below the ERM value of 70 µg/g. Suspended solids from the Susitna River carry As levels of approximately 36 µg/g, with no 2 sites from zone 0 having higher As concentrations than this source material.

Concentrations of Cu exceeded the ERL of 34 µg/g at 53 of 56 sites by as much as 18 µg/g (52 µg Cu/g at Z0F1); however, the maximum Cu level in sediments from this study is well below the ERM for Cu of 270 µg/g. Once again, source sediment from the Susitna River (31 µg Cu/g) and the Copper River (43 µg Cu/g) along with Alaskan rock data (Table 3-3), show that natural levels of Cu in the area are close to or above the ERL value chosen by Long *et al.* (1995). Finally, all samples from this study had Ni levels that exceed the ERL of 20.9 µg/g. The average crustal abundance of Ni is 56 µg/g (Wedepohl, 1995), a value that exceeds that ERM level of 51.6 µg/g. Similarly, the Ni content of sediment from both source rivers exceeds the ERL. Even for Ni values greater than the ERM, only 16.9 percent mortality was recorded by Long *et al.* (1995) for their database, and thus the ERL and ERM values for Ni may need to be revised in the second iteration of these sediment quality criteria. Overall, As and Cr in 2 or 3 sites in zone 0 (mainly Kachemak Bay) are present at levels that exceed the ERL; however, these values are comparable with results for river suspended solids and Alaskan rocks.

#### **4.4.2 Comparison to Sediment Quality Criteria: Polycyclic Aromatic Hydrocarbons**

The PAH analytes in the sampled sediments were compared to the ERL and ERM values for organic parameters in a manner similar to the metals. The results of this comparison for the organics were slightly different from the metals results in that the ERL for both individual and total PAH parameters were not exceeded in any zone or any site. This comparison for organics data is illustrated in Figure 4-23 and indicates that overall, the PAH concentrations in the study sediments are at levels that would not be expected to result in adverse biological effects.

#### **4.4.3 Sediment Toxicity Tests**

Sediment bioassays (using amphipods) were conducted in this study to determine if sediments from selected areas of lower Cook Inlet and Shelikof Strait had the potential to cause biological effects. Reduced survival to amphipods may indicate the potential for biological effects to



benthic organisms. However, as the sediments have a large mixture of trace substances, it may be difficult to determine which substance(s), if any, are responsible for the observed reduced survival of amphipods. This portion of the report will attempt to identify possible sources of the observed reduced survival in some of the bioassays conducted during this study.

Statistical analysis (using ToxCalc v5.0 software) of the results from the 1997 sediment bioassays conducted by PERL demonstrate that 15 of the 20 sediment samples resulted in survival significantly less than the Control at  $p < 0.05$ . However, most laboratories conducting sediment bioassays only indicate toxicity if the following two criteria are met:

1. There is a significant difference between the laboratory control and the test using a t- test, as was done here.
2. Mean organism response in the bioassay test was less than 80 percent of the laboratory control value.

Application of the second criterion eliminates the problem of designation of toxicity based only on comparison to controls with low replicate variance.

The 80 percent of control criterion was established by statistical analysis of many amphipod data sets by other investigators (e.g., Thursby and Schlegel, 1993). The two criterion approach is currently being used by the EPA's EMAP Program (Schlimmel *et al.*, 1994), by California's State Bay Protection and Toxic Cleanup Program (BPTCP, 1993) and by the Regional Monitoring Program for Trace Substances in the San Francisco Estuary (SFBRMP, 1995).

Applying these criteria to the bioassay results demonstrates that at 7 of the 20 sites sampled in 1997, amphipod survival was significantly lower than the controls: 2 of 8 from zone 0; 1 of 4 from zone 2; and 4 of 4 from zone 3. None of the 4 sites in zone 1 demonstrated significantly lower survival than controls.

Correlating amphipod survival with the trace contaminants measured in this study is problematic due to two factors:

1. While pristine in comparison with sediments from many areas where amphipods have been used as test organisms to assess sediment toxicity, the tested sediments did contain a large mix of trace substances. In this study, we have used the Effects Range methodology developed by Long and Morgan (1990) to relate contaminant concentrations to survival of test organisms. This methodology defines the concentrations of contaminants which have resulted in deleterious biological effects to test organisms (see Section 4.4.1 for a more detailed discussion of ERL and ERM). It has been demonstrated that contaminant mixtures may have a synergistic or additive effect on test organism survival, and that synergistic or additive effects may not be easily elucidated based on analysis of individual contaminant concentrations (Chapman, 1989; Schwartz, 1988, 1995). Methodologies are being developed that may more completely address additive or synergistic biological effects of the mixture of chemicals widely found in sediment analyses, and may prove useful in future analyses of sediments from this study (Thompson, 1998). However, although four of the sediment metals (As, Cr, Cu, and Ni) concentrations exceeded the ERL, where sediment concentrations are

“occasionally” associated with adverse effects, no concentrations exceeded the ERM, the level at which sediment concentrations are “frequently” associated with adverse effects (Long and Morgan, 1990; Long *et al.*, 1995). Concentrations of organic chemicals in the sampled sediments show a similar result. The ERLs for individual and total PAH were not exceeded in any zone or any site.

2. The amphipod *Eohaustorius estuarius* used in this study in 1997 (and widely used elsewhere) is recognized as tolerating highly variable mixtures of grain size, ranging from 0.6 to 100 percent sand. However, it naturally inhabits sandy sediments and some correlation between survival and grain size has been reported by DeWitt *et al.* (1989), and SAIC (1993a; 1993b), with increased mortality associated with decreasing grain size (higher percent silt) (EPA, 1994).

Statistical analysis of all chemical concentrations and amphipod survival demonstrates that percent survival was significantly correlated to two factors in the 1997 samples: 1) concentrations of Zn in the sediments and, 2) percent silt (fine-grained sediments). The three stations with the greatest percent silt were also the stations where amphipods had the poorest survival. Therefore, sediment particle size composition, as has been found previously for this and other amphipod species, probably affected survival. While further investigations may elucidate other factors affecting test organism survival, all indications from the samples collected in 1997 indicate fine grain size of area sediments as the primary factor contributing to low survival in the 1997 sediment bioassays with *Eohaustorius estuarius*.

Concentrations of AVS and SEM also were determined for the sediments collected in 1997 and used in the *Eohaustorius* toxicity tests. As previously mentioned, the underlying principle for using results from AVS-SEM is that metals bound (SEM) with sulfide (AVS) are not bioavailable. Concentrations of AVS in the 27 sediment samples (0 to 2 cm) from this study were all less than 3.1  $\mu\text{mole/g}$ , except for a value of 18.6  $\mu\text{mole/g}$  at station Z0F14. Only five other sites from this study (Z0F1, Z1R13, Z2R13, Z3R11, and Z3R20) yielded AVS levels greater than 1  $\mu\text{mole/g}$ . An AVS value of less than 1  $\mu\text{mole/g}$  was considered below the limit of applicability of the AVS/SEM technique by DiToro *et al.* (1990). Thus, low AVS values in the sediments from outermost Cook Inlet and the Shelikof Strait restrict use of the AVS/SEM approach. By comparison, results for marine sediments from Long Island Sound and Sapelo Island, Georgia, show values of AVS that ranged from less than 0.1 to about 10  $\mu\text{mole/g}$  in the top 1 cm of the sediment and 8 to 43  $\mu\text{mole/g}$  over the top 10 cm of the sediment column (DiToro *et al.*, 1990).

For the 9 sites in outermost Cook Inlet and the Shelikof Strait where amphipod survival was less than 80 percent, AVS levels averaged  $0.7 \pm 1.0 \mu\text{mole/g}$  relative to  $0.4 \pm 0.7 \mu\text{mole/g}$  at 10 stations where survival was greater than 80 percent (excluding the high AVS site Z0F14). Thus, no significant correlation between AVS levels and amphipod survival was observed for the sediments collected in 1997. Concentrations of SEM also were similar between the two survival groups at  $1.0 \pm 0.2 \mu\text{mole SEM/g}$  for the less than 80 percent survival group and  $0.8 \pm 0.4 \mu\text{mole SEM/g}$  for the greater than 80 percent survival group, again showing no relationship in toxicity results among sites as a function of SEM levels in 1997.

A more common use of the AVS and SEM data is to subtract the AVS value from the SEM value [i.e., SEM - AVS] and assume that a negative result means that sufficient sulfide is present in the

sediment to bind potentially toxic metals with a high sulfide affinity (Ag, Cd, Cu, Hg, Ni, Pb, and Zn), thereby rendering the sediment less toxic. Overall, excess AVS (a negative result meaning that metals are bound with sulfide) was found at only 4 of the 20 sites sampled in 1997 (2 with greater than 80 percent survival and 2 with less than 80 percent survival), including the site with the lowest percent survival (Z3R11). The SEM-AVS value for the metal-rich site Z0F14 is -17.5  $\mu\text{mole/g}$ , supporting sulfide binding of metals. Once again, no pattern between the results of the amphipod toxicity tests and data for AVS/SEM are observed.

According to the underlying principles of the AVS/SEM test, observed positive numbers for [SEM-AVS] at most sites would correspond with more bioavailable metals. However, in these sediments, a sizeable amount of the SEM is most likely associated with iron oxides that also dissolve with the 1N HCl treatment used. More than 90  $\mu\text{moles Fe/g}$  were released during the SEM treatment, or about 100 times more metal than the total SEM value. Use of the AVS/SEM approach in oxic sediments has been previously shown to be of limited value because of the importance of organic matter and manganese and iron oxides in controlling metal binding (Ankley *et al.*, 1996). The surface few centimeters from all sites were shown by the results of sediment profile imaging to be oxic. Coupled with the observation that most AVS values were less than 1  $\mu\text{mole/g}$ , the lack of relationship between AVS-SEM results and amphipod toxicity is certainly consistent.

In 1998, sediment toxicity tests were conducted on sediments from the seven sites that demonstrated significant toxicity to test organisms in 1997, and in addition, a fine-grained sediment collected from a reference site (figure 2-1) was also tested. This fine-grained reference sediment was tested to assess the test organism's sensitivity to fine-grain size sediments. As discussed in section 3, the test amphipod *Eohaustorius estuarius* (used in 1997) was replaced with the amphipod *Ampelisca abdita*. The site selection and change in test organisms in 1998 was based on the sediment toxicity test results from 1997, which, as discussed above, were not significantly related to measured concentrations of metals or organic compounds. The strongest relationship in the 1997 tests was to grain size, i.e., as grain size decreased, so did survival. *Ampelisca abdita* replaced *Eohaustorius estuarius* in 1998 as an attempt to address the question of the apparent grain-size effect observed with the 1997 sediment toxicity test. *Ampelisca abdita* was selected as an alternative species through conversations with the bioassay laboratory and investigations of the literature. It is thought to be less subject to grain-size effects than *Eohaustorius estuarius*. No significant toxicity (i.e., less than 80 percent) was found in 1998 at the sites with the lowest survival in 1997 or in the fine-grain size "control" site sediments included in 1998. These results indicate that factors affecting survival of test organisms in 1997 were not present in 1998, or that differential survival of test organisms exposed to sediments from the same sites may be explained by differing tolerances between the two amphipod species utilized as test organisms.

#### **4.4.4 P450 Reporter Gene System in Fish and Sediments**

Previous RGS studies have demonstrated that B[a]PEq in excess of 60  $\mu\text{g/g}$  are associated with benthic degradation in sediments. Studies conducted on marine sediments from various parts of the U.S. coastal zone for The National Oceanic and Atmospheric Administration (NOAA) have shown that relatively uncontaminated sediments have produced a response of about 1 to 6  $\mu\text{g/g}$  of B[a]PEq (Anderson *et al.*, 1995; Anderson *et al.*, 1997). The Alaskan sediments analyzed in this study therefore fall within this range of background concentrations. The low levels of

CYP1A induction in most fish tissues analyzed, especially the gills, are also consistent with the low RGS P450 response measured in the sediment extracts and in the fish liver composite samples.

#### **4.4.4.1 Sediments**

Petrogenic PAHs ( $r = 0.689$ ) and Total PAHs ( $r = 0.681$ ) were found to correlate significantly ( $p \leq 0.05$ ) with the P450 RGS responses measured on sediment extracts. Numerous individual hydrocarbons, which were included in the summed petrogenic PAH and Total PAH concentrations, also showed significant correlations with the P450 RGS responses.

Total PAH have been found in numerous NOAA Bioeffects studies to correlate highly with the RGS responses measured in those samples from various coastal regions of the country (Anderson et al. In Press a). These previous studies have demonstrated that RGS responses to sediments less than about 11 mg B[a]PEq/g are not likely to be associated with biological effects. Levels of RGS response to sediments above about 32 mg B[a]PEq/g may be associated with biological effects, and when the measured response reaches 60 mg B[a]PEq/g and greater, there is likely to be a deleterious impact on the organisms associated with the sediments.

The sediments tested in this investigation were all under 11 mg B[a]PEq/g, and most were equivalent to the lowest responses measured previously from very clean portions of the U. S. coastal zones (Northern Puget Sound and Alaska). The range of RGS responses between 0.5 and 5.0 mg B[a]PEq/g, where nearly all of these samples fell is considered to be a very clean environment.

#### **4.4.4.2 Fish Tissue**

The data on P450 RGS responses to fish tissue were expressed on a lipid weight basis, which always produces higher values than normalization on a dry weight basis, because a smaller portion of the tissue is composed of lipid. Very few of the fish tissue samples tested produced fold induction above the solvent control (1.0 fold induction). Those samples that were above 1.0 fold produced B[a]P equivalent values of 0.3 to 1.0 mg B[a]PEq/g lipid, with one arrow tooth flounder sample having a value of 6.5 mg B[a]PEq/g lipid.

In other studies, the highest observed RGS responses to extracts of tissues were from marine mussels deployed in contaminated areas of San Diego Bay (Anderson et al. In Press b). These responses were as high as 295 mg B[a]PEq/g dry weight, and the native clean mussels were at 6 mg B[a]PEq/g dry weight. In a study for the Exxon Valdez trustees the P450 RGS assay was used on 38 samples of whole sand lance collected from various locations in Prince William Sound in 1996, well after the oil spill (1989). These rather lipid-rich fish produced RGS values between 1.5 and 81 mg B[a]PEq/g lipid. The majority of the samples were in the range of 1 to 5, but approximately one-third of the samples were above 10 mg B[a]PEq/g lipid. While it is difficult to compare sand lance directly to the species of fish collected in this investigation, it appears that those in the present study contained much lower levels of inducing compounds.

A more recent study conducted for LGL-Alaska under funding by MMS examined the levels of RGS induction produced by fish collected from both Cook Inlet and the Shelikof Straits. The two species that were collected in adequate numbers from each region were the Pacific sand lance and surf smelt. Those samples of the two species collected from Cook Inlet in 1998 were found to be higher in RGS responses than the same species collected from the Shelikof Strait.

The results of these investigations demonstrate that the use of a biomarker screening approach provides a good estimate of the levels of inducing compounds, such as high molecular weight PAHs in both sediment and tissue samples.

The responses (both B[a]PEq and TEQ) of the RGS assay to extracts of fish tissue in 1998 were highly correlated with several analytes. (The RGS values are expressed as both B[a]P and Toxic Equivalents, since without chemical confirmation it can not be determined if the response was from exposure to PAHs [B[a]PEq] or from chlorinated hydrocarbons [TEQ]). It is not surprising that for all fish, RGS responses were correlated with C1-fluorenes, C2-fluorenes, and C1-phenanthrenes, since these compounds would be part of a suite of hydrocarbons that could induce the CYP1A1 gene. However, the correlation of RGS with copper and chromium is merely a matter of co-occurrence in the sediments. The solvent extracts applied to the RGS cells would not likely even contain metals.

For Pacific cod collected in 1998, the observed correlations of RGS with fluorenes, phenanthrenes, and fluoranthrene would be expected as noted above. In this case two other metals (zinc and thallium) show a correlation, but these are again merely the result of co-occurrence in the samples.

In conclusion, the results of the RGS assay are in general agreement with the chemical analyses on the same samples, and both types of measurements demonstrate that the collected sediments and tissues are quite low in the concentrations of PAHs, and chlorinated hydrocarbons which induce the CYP1A1 gene.

#### **4.4.5 CYP1A Response in Fish**

The CYP1A response is considered a very sensitive biological response for assessing exposure to a variety of organic pollution conditions, and is widely considered a useful tool for assessing exposure to specific classes of xenobiotic compounds (e.g., PAHs, coplanar polychlorinated biphenyls [PCBs], polychlorinated dibenzofurans, and dibenzodioxins), CYP1A genes code for the major oxidative enzymes induced in fish and other vertebrates by PAHs and chlorinated hydrocarbons (Stegeman and Hahn, 1994). CYP1A and other measures of induction of the cytochrome P450 enzyme system have become common components of contaminant monitoring and effects studies worldwide (e.g., Bucheli and Fent, 1995, Holdaway et al., 1995, McCain et al., 1996, Wirgin and Waldman, 1998, Collier et al., 1998, and Miller et al., 1999), and in Alaska (e.g., Varanasi et al., 1995 and Jewett et al., 2000). Buchelli and Fent, in their 1995 review, cite 75 field studies, 93% of which showed that CYP1A induction is significantly related to contaminant levels in the environment. When coupled with measures of histopathology, ie., immunohistochemistry (IHC), induction of CYP1A can identify tissue specific-patterns of CYP1A expression to identify target sites and exposure routes for PAH's and other inducing compounds, and can link exposure to xenobiotic compounds to effects at the biochemical, cellular, tissue, and physiological levels. As of yet, however, there are few studies such as this one in which CYP1A has been measured and scored in individual cell types (IHC). This (immunohistochemical ) approach not only relates environmental contaminant concentrations to tissue residues and responses, but may also demonstrate the route of exposure (water, diet, substrate) of biota to environmental contaminants (e.g. Miller et.al., 1989, Spies et al., 1996, Van Veld et. al., 1997).

One study (conducted with a nearly identical methodology) examined cellular response in Embiotocid fish (rainbow surfperch, *Hypsurus caryi*, and rubberlip surfperch, *Rachochilus toxodes*) caught near petroleum seepage in the Santa Barbara Channel, and at control sites. In this study, liver hepatocytes from oiled sites had mean staining indices of 5.6 and 4.2. (rainbow vs rubberlip respectively). These IHC scores have been adjusted based on maximum scores of 15 as used in the present study, as opposed to a maximum score of 25 as used in the surfperch study (the original scores were 9.3 and 7). While at the control sites, liver hepatocyte scores were 2.7 and 2.5 (rubberlip vs rainbow respectively). The original scores being 4.5 and 4.3 (Spies et al., 1996). These values compare to mean values of 2.83, 1.25, and 1.7 (zones 0, 2, and 3 respectively) measured in Pacific halibut (liver hepatocytes) caught in 1997, .25, .09, and 0 (zones 1, 2, and 3 respectively) measured in Pacific halibut in 1998, and 0 to 1.9 measured in all species caught and analyzed in 1998.

There have been several recent studies of hydrocarbon concentrations and effects in Alaska fish stemming from the Exxon Valdez oil spill (EVOS). One study (Jewett et al., 2000) measured the catalytic activity of CYP1A in the form of EROD (ethoxyresorufin *O*-deethylase) as well as using the IHC approach. The measurement of EROD has been the most common means of investigating the CYP1A response to contaminants in both field and laboratory studies up until this point, and the authors are not aware of any other field study of fish from pristine environments in which both approaches (IHC and EROD) were utilized. The fish sampled in the Jewett et al., study (masked greenling, *hexagrammos octogrammus*, and crescent gunnel, *Pholis laeta*) all had mean liver vascular endothelium scores of less than 1, while (with the exception of black cod which had a mean score of 1.2 from Zone 3 in 1997) none of the species from the present study had measurable IHC scores. Vascular endothelium is the first site of contact of inducing compounds in tissues and organs and so should demonstrate early induction and early loss of induction in comparison with other tissues and organs. The total lack of induction (with the exception of black cod) in the fish from the present study indicates either that the fish from Shelikof Strait were less (recently) exposed to inducing compounds than those from the Jewett et al., study, or that there are significant differences between the species in terms of sensitivity to exogenous inducing compounds and or levels of endogenous inducing compounds. Additionally, as exposure through the diet of (metabolized) hydrocarbons lessens with increasing trophic level, it may be expected that higher trophic level fish (halibut and cod) would demonstrate less induction via dietary exposure than lower trophic level fish (gunnells and greenling). The high IHC score for Black cod vascular endothelium is interesting, but difficult to interpret as only 5 fish were sampled, and the mean score of 1.2 came from a single fish with a score of 6.

While direct comparisons of data from IHC versus EROD based studies are somewhat tenuous, the lack of other IHC based studies on Alaska fish initiated the need to make comparisons from studies utilizing other methodologies. EROD is generally considered to be a more sensitive measure of CYP1A induction than IHC. Jewett et al., 2000, utilized EROD due to the low IHC scores in fish from un-oiled sites. Unfortunately, IHC and EROD were not measured in the same fish, making direct comparisons impossible. However, the EROD scores of fish from un-oiled sites were generally lower than those from oiled sites. Another study of Alaska fish stemming from EVOS (Varanasi et al., 1995) utilized aryl hydrocarbon hydroxylase (AHH) to measure P-4501A (CYP1A) induction in fish from over 50 sites in Prince William Sound, Lower Cook Inlet, and embayments along the Kenai Peninsula and the Alaska Peninsula. AHH is a very

similar measure to EROD. The findings in the (Varanasi et al., 1995) study were similar to the Jewett et al., 2000 study in that AHH levels decreased with time and distance from EVOS.

In the tissue assays conducted on fish collected in 1997, the only significant differences observed between zones were in the CYP1A concentrations in the halibut kidney tubules, although a similar progression between zones was seen for all three cell types. In addition, when all three CYP1A response variables were regressed against all of the tissue contaminant concentrations, kidney tubule response did not correlate positively with any tissue measures. Similar results were obtained for the tissues assayed in 1998. In 1997, Halibut were the only fish species caught in significant numbers in all sampled zones, while in 1998, both Halibut and Pacific Cod were caught in sufficient quantities in all sampled zones to allow investigation of differences between CYP1A scores for both species. The results, however, are similar to 1997 in that there was no significant correlation between or within species, scores, and zones. The exception to this is cytoplasmic vacuolation in hepatocytes, which is a non-specific response of these cells that may in some circumstances be linked to contamination.

There is the possibility that the CYP1A responses seen in some cells, i.e, kidney tubules and kidney vascular endothelium, could be due to unmeasured factors. Relatively high levels of kidney cell induction have been noted in other species in circumstances where contaminants are apparently not a factor (Stegeman, personal communication). These tissues could be responding to natural inducers, either endogenous or exogenous.

The low to moderate levels of induction observed in the fish collected in this study could also be a response to contaminants not measured in this study, e.g., chlorinated hydrocarbons such as the PCB. Chlorinated hydrocarbons, especially the coplanar forms of PCB, dibenzofurans, and dioxins, are known inducers of CYP1A in fish (Stegeman *et al.*, 1992). Halibut are piscivorous, and therefore relatively high on the marine food chain, and likely to accumulate some of these globally ubiquitous and slowly metabolized contaminants. In addition, it is well understood that polar, and possibly sub-polar environments, are apparently sinks for chlorinated hydrocarbons that are transported long distances in the atmosphere from lower latitudes. Recent measurement of chlorinated hydrocarbons in the tissues of harbor seals (Small *et al.*, 1998) and killer whales (Matkin *et al.*, unpublished) have confirmed high tissue concentrations of PCBs (high parts per million) that confirm that the Gulf of Alaska receives significant inputs of these biologically active molecules. It is therefore almost certain that fish in the northern Gulf of Alaska ecosystem are also contaminated with such compounds, but probably at 10 to 100 times lower concentrations. One recent study (Ewald et al., 1997) of biotransport of organic pollutants (PCBs and DDTs) by Alaskan Sockeye salmon to their inland spawning lakes in the copper river drainage, found muscle lipid concentrations of PCBs of 670 ng/g and DDTs of 221 ng/g in fish caught in the Gulf of Alaska just prior to upriver migrations. These concentrations are far below levels of concern for human health from consumption, and are 10 times lower than reported in Atlantic salmon from the Baltic Sea (Larsson et al., 1996), and 20 times lower than those reported for salmonids in Lake Ontario (Oliver and Niimi, 1988).

The low induction of CYP1A in gill tissues of all three fish species would be consistent with waterborne sources of contamination being extremely low for these bottom-dwelling fish in lower Cook Inlet and Shelikof Strait. This finding is also consistent with the low concentrations of PAH and the very low P450 RGS responses seen in sediment extracts in this study. On the other hand, the responses in the liver hepatocytes and the two cell types in the kidney are

consistent with some level of inducing compounds in the diet. With the currently available data it is not possible to assign this low to moderate level of CYP1A induction to any particular group of compounds. While serving as a useful benchmark for measures of CYP1A induction in fish from Shelikof Strait and other relatively pristine areas of the world, future studies of fish from this region should attempt to collect greater numbers of fish from which to base statistical analyses and should also collect non-migratory species from lower trophic levels, due to the ability of fish to metabolize PAH. Additionally, other studies of fish from Alaska and other regions intent on determining exposure to xenobiotics have included measurement of bile hydrocarbons (Varanasi et al., 1995, Jewett et al., 2000). These measurements have proven useful in determining recent exposures to PAH. The lack of tissue residue analysis for other CYP1A inducing compounds in the present study hindered the authors in attempting to relate CYP1A induction to PAH alone.

#### **4.4.6 Assessment of Ecological Risk**

Sediment and fish tissue sampling in 1997 and 1998 has provided a picture of contaminants and potentially toxic trace substances in the environment at very low concentrations with an attendant low biological risk. The concentrations of trace metals are consistently below the risk levels identified by Long and Morgan (1990), except for Ni, which has a crustal abundance higher than the designated ERL and ERM concentrations. The concentrations of PAH detected in sediments are also below the ERL identified by Long and Morgan (1990). The P450 RGS results also indicated low to negligible biological risk associated with extractable organic compounds, namely PAH, in the sediments. Sediment bioassays with amphipods produced some low survival rates, but these appear to be related to testing sediments with a high silt content rather than any trace chemicals in the sediments, be they natural or anthropogenic in origin. The levels and patterns of induction of CYP1A in cells of bottom-dwelling fish are consistent with some mild induction by contaminants, but with weak induction in the gills they appear not to be waterborne, but rather from the diet. None of the measured contaminants in the fish tissues correlated with CYP1A induction, but chlorinated hydrocarbons were not measured.

In summary, in the context of the null hypothesis, **our results indicate that the concentrations of organic and metal parameters in the sediments of outermost Cook Inlet and Shelikof Strait do not appear to pose any immediate ecological risk to the marine organisms in the study area.**