

*Exxon Valdez* Oil Spill  
Restoration Project Final Report

Microbiology of Subtidal Sediments: Monitoring Microbial Populations

Restoration Project 93047-2  
Final Report

Joan F. Braddock  
Zachary Richter

Institute of Arctic Biology  
P.O. Box 757000  
University of Alaska Fairbanks  
Fairbanks, Alaska 99775-7000

June 1994

## Microbiology of Subtidal Sediments: Monitoring Microbial Populations

### Restoration Project 93047-2 Final Report

**Study History:** This study began as a part of NRDA Air/Water Study Number 2 Petroleum Hydrocarbon-Induced Injury to Subtidal Marine Sediment Resources in 1989. Status reports under this study number were submitted in 1989 and 1990. In 1991 the number of the study was changed to Subtidal Study Number 1. The title remains the same. A status report under the new title was submitted in 1991. The final report for Subtidal Study Number 1 (microbiology) was submitted in June 1992, reviewed, then revised in April 1993. No field work was performed in 1992. In 1993 Restoration Study Number 93047 Exxon Valdez Restoration Project: Subtidal Monitoring was initiated. Our project, Microbiology of Subtidal Sediments: Monitoring Microbial Populations, is part of Restoration Project 93047, Subtidal Monitoring: Recovery of Sediments, and Hydrocarbon-Degrading Microorganisms in the Subtidal Environment. The Restoration project was led by the National Oceanic and Atmospheric Agency with cooperation from the Alaska Department of Environmental Conservation and the University of Alaska Fairbanks. The University of Alaska Fairbanks was responsible for the microbiological portion of the study. Previous findings on microbial numbers and activity in subtidal sediments can be found in two summary reports from Natural Resource Damage Assessment projects: (1) Braddock, J.F., M.R. Brockman, J.E. Lindstrom and E.J. Brown, 1990, Microbial hydrocarbon degradation in sediments impacted by the Exxon Valdez Oil Spill, NOAA Report for contract no. 50-DSNC-8-00141, Washington, DC and (2) Braddock, J.F., B.T. Rasley, T.R. Yeager, J.E. Lindstrom and E.J. Brown, 1993, Hydrocarbon mineralization potentials and microbial populations in marine sediments following the Exxon Valdez oil spill, Exxon Valdez Oil Spill Project Final Report, University of Alaska, Fairbanks, AK. Some of the results from these studies have been published: (1) Brown, E.J. and J.F. Braddock. 1990. Sheen Screen: a miniaturized most probable number technique for oil-degrading microorganisms. *Appl. Environ. Microbiol.* 56:3895-3896 and (2) Braddock, J.F., J.E. Lindstrom and E.J. Brown. 1995. Distribution of hydrocarbon-degrading microorganisms in sediments from Prince William Sound, Alaska following the Exxon Valdez oil spill, *Mar. Pollut. Bull.* 30:125-132. A second manuscript (Braddock, J.F., J.E. Lindstrom, T.R. Yeager, B.T. Rasley and E.J. Brown, Patterns of microbial activity in oiled and unoled sediments in Prince William Sound) has been accepted for publication in the Exxon Valdez Oil Spill Symposium Proceedings.

**Abstract:** An increase in the biodegradation activity of naturally occurring populations of microorganisms can lead to substantial removal of petroleum from the environment. Therefore, measurements of microbial populations are an important component of contaminated site assessment studies. Following the Exxon Valdez oil spill in 1989, we measured numbers of hydrocarbon-degrading microorganisms and hydrocarbon mineralization potentials of microorganisms in oiled and unoled surface sediments from the shore through 100 m depth offshore. We found both temporal and spatial variations in numbers and activity of hydrocarbon-degrading microorganisms with statistically significant higher values at the oiled sites than at reference sites. In the summer of 1993 we returned to ten study sites within Prince William Sound to monitor the changes in the numbers and activities of hydrocarbon-degrading microorganisms at these sites with time. In 1993 the numbers and activities of hydrocarbon-degrading microorganisms were generally very low at all sites although elevated populations and activities were measured in intertidal sub-surface samples at several sites (Northwest Bay, Herring Bay and Sleepy Bay) with observable sub-surface oiling.

## **Table of Contents**

|   |    |
|---|----|
| Executive Summary .....                     | 1  |
| Introduction .....                          | 2  |
| Objective.....                              | 2  |
| Methods .....                               | 3  |
| Sampling .....                              | 3  |
| Microbial Population Estimates .....        | 3  |
| Hydrocarbon Mineralization Potentials ..... | 6  |
| Results .....                               | 7  |
| Discussion .....                            | 32 |
| Conclusions .....                           | 35 |
| Literature Cited .....                      | 36 |
| Appendix .....                              | 39 |

## **List of Tables**

|  |    |
|--|----|
| Table 1. Site names and locations for the <i>F/V Scorpis</i> cruise. ....          | 4  |
| Table 2. Summary of microbial population data. ....                                | 9  |
| Table 3. Summary of hexadecane oxidation rate potentials. ....                     | 16 |
| Table 4. Summary of phenanthrene oxidation rate potentials, 2 day incubation. .... | 23 |
| Table 5. Summary of phenanthrene oxidation rate potentials, 4 day incubation. .... | 25 |

## **Executive Summary**

Assessment of microbial populations is an essential component of oil spill monitoring since a major fate of spilled petroleum depends on the ability of microorganisms to use hydrocarbons as a source of carbon and energy (Leahy and Colwell, 1990). Microbial measurements also can be used to provide evidence for the presence of in situ biodegradation (Madsen et al. 1991). In addition, monitoring microbial populations is a tool for assessing the extent and persistence of oil contamination following a spill.

We have monitored the total numbers and activity of hydrocarbon-degrading microorganisms since 1989 in sediments (intertidal and subtidal) impacted by the *Exxon Valdez* oil spill. We sampled both oiled and unoiled sites in Prince William Sound on six separate cruises spanning 1989-1991. Surface sediment samples were collected from the shoreline (intertidal) through 100 m depth offshore. Both the numbers and activity of microbial populations were assayed in these samples. We used most probable number techniques to estimate the numbers of oil-degrading microorganisms and radiorespirometry to measure the mineralization potentials of the microbial community for various hydrocarbons including benzene, hexadecane, phenanthrene and naphthalene.

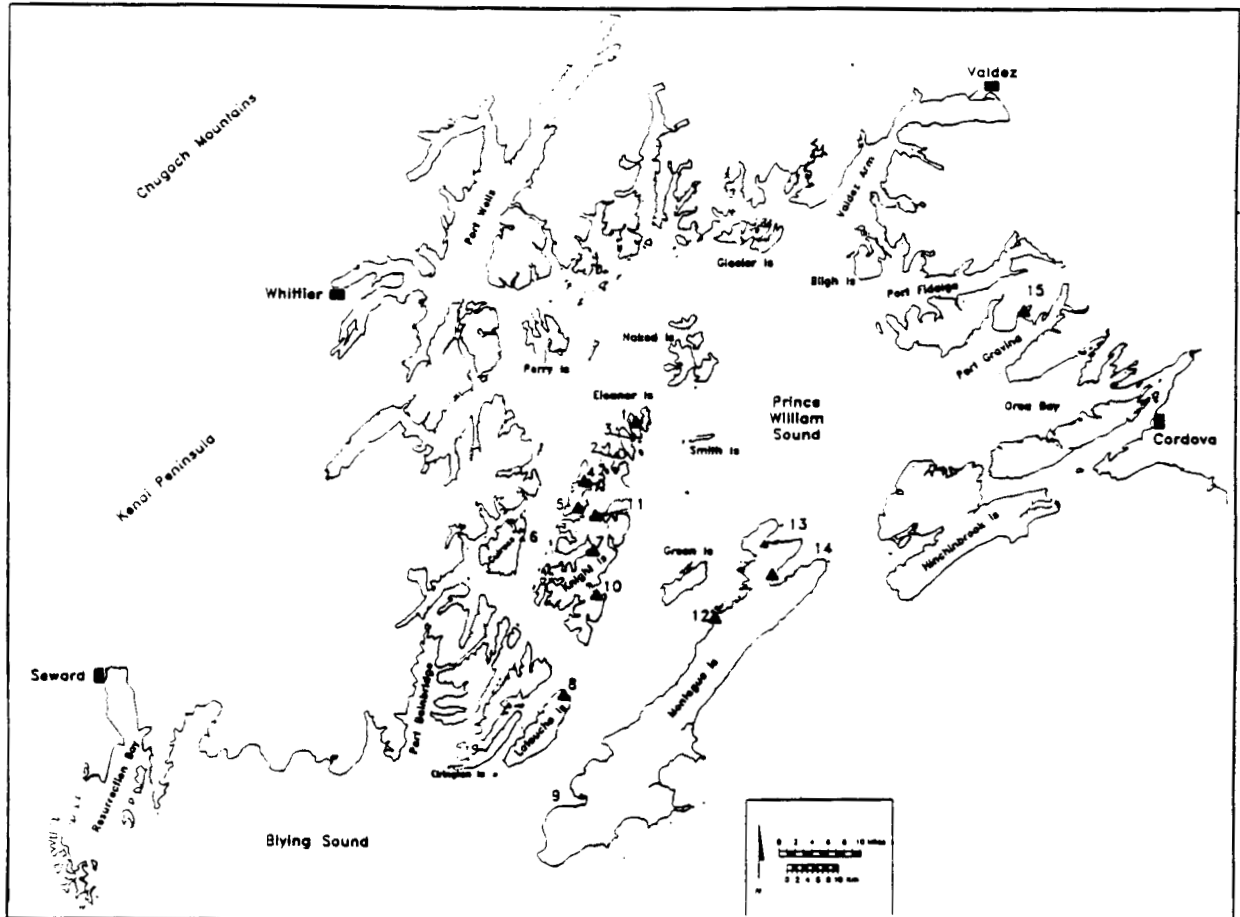
We found that the numbers and activity of hydrocarbon-degrading microorganisms are indicators of the presence of hydrocarbons in sediments from Prince William Sound. We found statistically significant differences in the populations of hydrocarbon degraders in shoreline sediments collected from bays within the path of the oil slick relative to populations at reference sites which were not oiled during the *Exxon Valdez* spill. We also saw differences in populations of hydrocarbon degraders with time following the spill. Populations of hydrocarbon-degrading microorganisms dropped substantially in most surficial intertidal sediments from 1989 to 1991 indicating a reduction in readily biodegradable fractions with time. However, high numbers still existed on several shorelines in 1991 particularly in buried sediments. In these sediments hydrocarbon degrader population numbers and activities were as great as seen in early summer 1989.

We returned to Prince William Sound in the summer of 1993 to ten sites previously sampled for microbial populations and activities. Our objective in this study was to continue to monitor the numbers of hydrocarbon-degrading microorganisms to determine if active populations remained at heavily contaminated sites within Prince William Sound and to validate microbial techniques as relatively cheap and quick assays for monitoring the extent and persistence of hydrocarbon contaminants following an oil spill. In 1993 the numbers and activities of hydrocarbon-degrading microorganisms were generally very low at all sites although elevated populations and activities were measured in intertidal sub-surface samples at several sites (Northwest Bay, Herring Bay and Sleepy Bay) with observable sub-surface oiling. We found that microbial population estimates were good indicators of exposure of sediments in Prince William Sound to oil after the spill.

## **Methods**

**Sampling:** Surface sediment (top 0 - 3 cm) samples were previously collected and analyzed for microbial populations and activities on six cruises: R/V Fairweather (Summer 1989), M/V Nautilus (Winter 1989), R/V Cobb (Spring 1990), R/V Davidson (Summer 1990), R/V Cobb (Fall 1990) and F/V Big Valley (Summer 1991)(see Braddock et al., 1993). In 1993 ten sites in PWS were visited from 8 to 16 July on the F/V Scorpius (Summer 1993; Table 1 and Fig. 1). The season designators for the cruises used here are meant to reflect the weather in PWS during the time of the cruise even though the dates of the cruise do not strictly follow traditional season definitions. During the three summer cruises surface sediments were collected in the intertidal zone (referred to as shoreline or 0 m) and at 3, 6, 20, 40 and 100 m depths offshore at mean low tide. On the other cruises surface sediment samples were collected only in the intertidal zone and at the shallower water depths offshore (Winter 1989, 0 and 3 m; Spring 1990 and Fall 1990, 0, 3, 6 and 20 m), due either to the restricted capabilities of the support vessel or abbreviated cruise schedules for those sampling trips. Surface sediments at the 40 or 100 m depths were collected using either a Van Veen grab or a Smith-McIntyre grab. Composite samples at each depth from three grabs were obtained by subsampling surface sediment (top 0-3 cm) into sterile bags. Samples at the 3, 6 and 20 m depths were collected by SCUBA divers while shoreline samples were collected by either SCUBA divers or a shore party in the low intertidal zone as close to low tide as was feasible. The intertidal (shoreline), 3, 6 and 20 m samples were composites of eight subsamples collected at random intervals along a 30 m transect parallel to the shoreline. Only one bag of sediment was collected for each site at each depth on the Summer 1989 cruise while three replicate bags were collected at each depth for all subsequent cruises so that statistical procedures could be used to compare the study sites to reference sites. All sediment and water samples were placed in coolers at the time of collection for transport to the support vessel. Processing for microbiological analyses was performed within three hours of collection. We assayed samples from which all rocks greater than 1 cm in diameter were removed due to the prevalence of rocks and coarse-grained sediments at several locations and depths in PWS.

**Microbial Population Estimates:** Heterotrophic and hydrocarbon-degrading microbial populations were estimated using most probable number (MPN) techniques. While no technique to enumerate specific metabolic types of microorganisms in marine systems is absolute, the MPN technique can give consistent results that are appropriate for relative comparisons among stations and depths. The number of hydrocarbon-degrading microorganisms in each sediment sample was estimated using the Sheen Screen MPN (Brown and Braddock, 1990). Hydrocarbon degrading microorganisms were defined as those microbes capable of dispersing a sterile Prudhoe Bay oil sheen layered on Bushnell-Haas marine mineral salts (Difco Laboratories, Detroit, MI) broth. On the Summer 1989 cruise, duplicate sets of cell well plates were prepared for each subtidal depth at each site. For all other cruises one set of cell well plates was prepared from each replicate sediment sample at a given site and depth to yield triplicate values. The Sheen Screen plates were incubated at approximately 15 °C for three weeks before being scored for disruption of the oil sheen. Marine heterotrophs were enumerated in a similar manner, except that the growth medium was marine broth (Difco Laboratories, Detroit, MI) and growth was indicated by turbidity (Lindstrom et al., 1991). The heterotrophs plates were incubated for 1 week after inoculation before being scored for turbidity.



**Figure 1.** Locations of study sites sampled in 1993 (indicated by bold triangle). Site numbers correspond to sites as follows: 1. Northwest Bay; 4. Herring Bay; 5. Lower Herring Bay; 7. Drier Bay; 8. Sleepy Bay; 10. Snug Harbor; 11. Bay of Isles; 12. Mooselips Bay; 14. Zaikof Bay and; 15. Olsen Bay.

## Results

The distribution of the surface slick of oil after the grounding on Bligh Reef was dependent on winds and currents and generally resulted in a spread of the oil to the southwest (Royer et al., 1990; Galt et al., 1991). By about six days after the spill the surface slick had heavily coated many shorelines around Knight Island and other smaller islands in that area (Fig. 2). The study sites sampled followed, in general, the path of the oil slick (see Fig. 1).

Microbial populations (heterotrophs and hydrocarbon degraders) were estimated at each site in 1993 (Table 2, Appendix A). The numbers of hydrocarbon-degrading microorganisms were generally low at all sites visited in 1993 relative to populations measured at many of these sites in 1989 or 1990 (Fig. 3a-e). In 1993 the only samples which contained high populations of hydrocarbon-degrading microorganisms were collected from sub-surface samples collected by digging shallow pits (approximately 20 cm deep). Northwest Bay, Herring Bay and Sleepy Bay all had visible sub-surface oiling in 1993 and all of these same sites had populations of hydrocarbon degraders greater than  $10^4$ /g sediment. No other sediment samples collected in 1993 were recorded as containing visible oil and the populations of hydrocarbon degraders were  $10^3$ /g sediment or less.

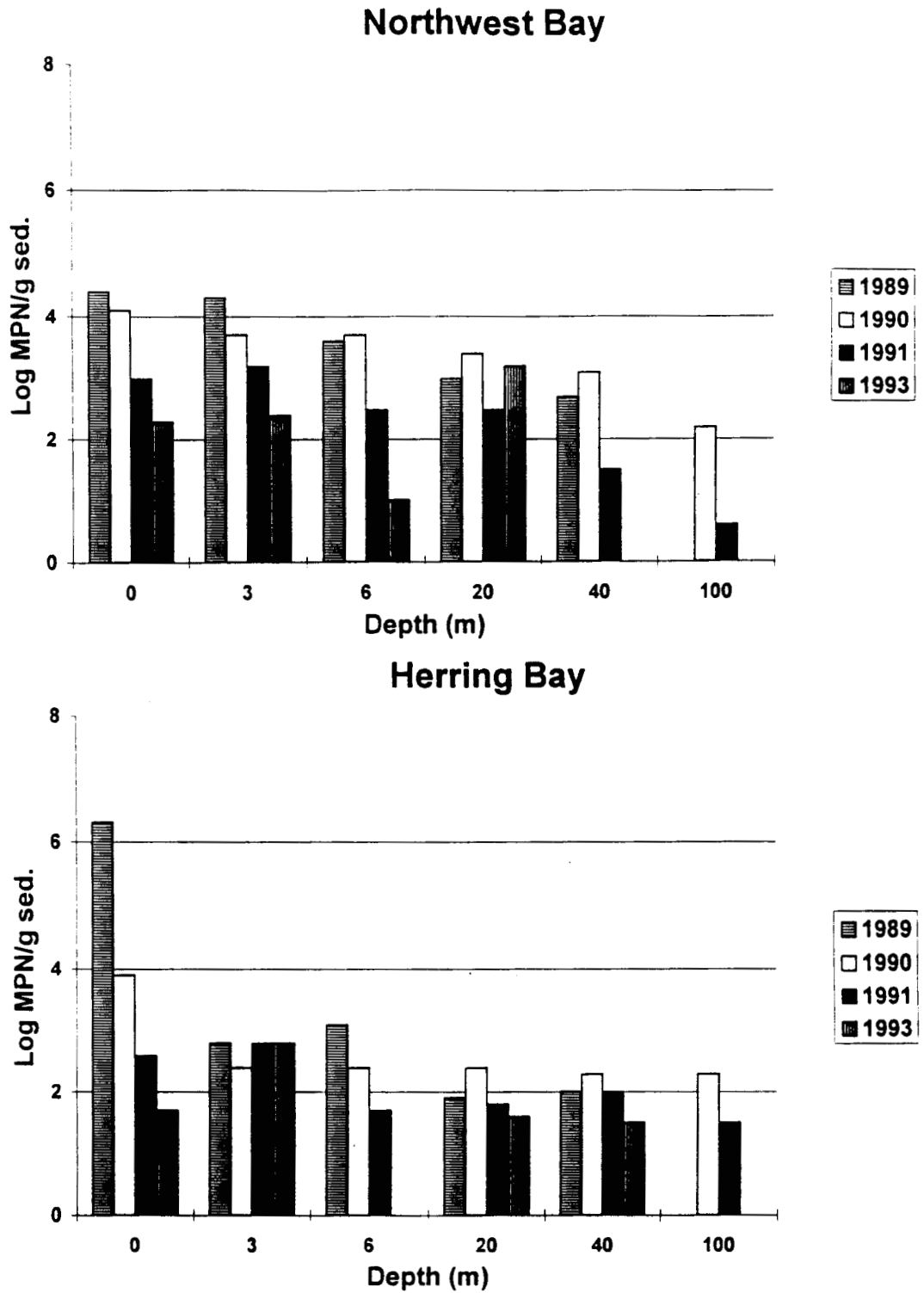
The activity potentials for the hydrocarbon degrader populations were measured by assaying the mineralization of radiolabelled hydrocarbon fractions in laboratory incubations. Hexadecane and phenanthrene were used as representatives of a linear alkane and a polycyclic aromatic hydrocarbon, respectively. Mineralization potentials for hexadecane were low (less than 10% mineralized after a two day incubation; Table 3) except in two sub-surface samples collected at Northwest Bay and Sleepy Bay. Temporal decreases were detected in the mineralization potentials for hexadecane at all sites and depths since 1989 (Fig. 4a-e).

In a similar manner, mineralization potentials for phenanthrene were measured in 1993 sediment slurries after two-day and four-day incubations (Tables 4 and 5, respectively). Phenanthrene mineralized was less than three percent after a two day incubation for all depths and sites except one anomalous value of 7.9% at Drier Bay at 100 m (Table 4). The variability in replicates measured at this site was also high (see Appendix A) and the same trend was not reflected in the hydrocarbon degrader populations measured or the hexadecane or four day phenanthrene mineralization potentials measured. The mineralization potentials measured after four day incubations were less than four percent except in two subsurface samples collected at Northwest and Sleepy Bays. The two day phenanthrene data from 1993 were compared to values measured in samples collected in 1989-1991 (Fig. 5a-e). As with the hexadecane mineralization potential data (Fig. 4a-e) the phenanthrene potentials generally have declined since 1989.

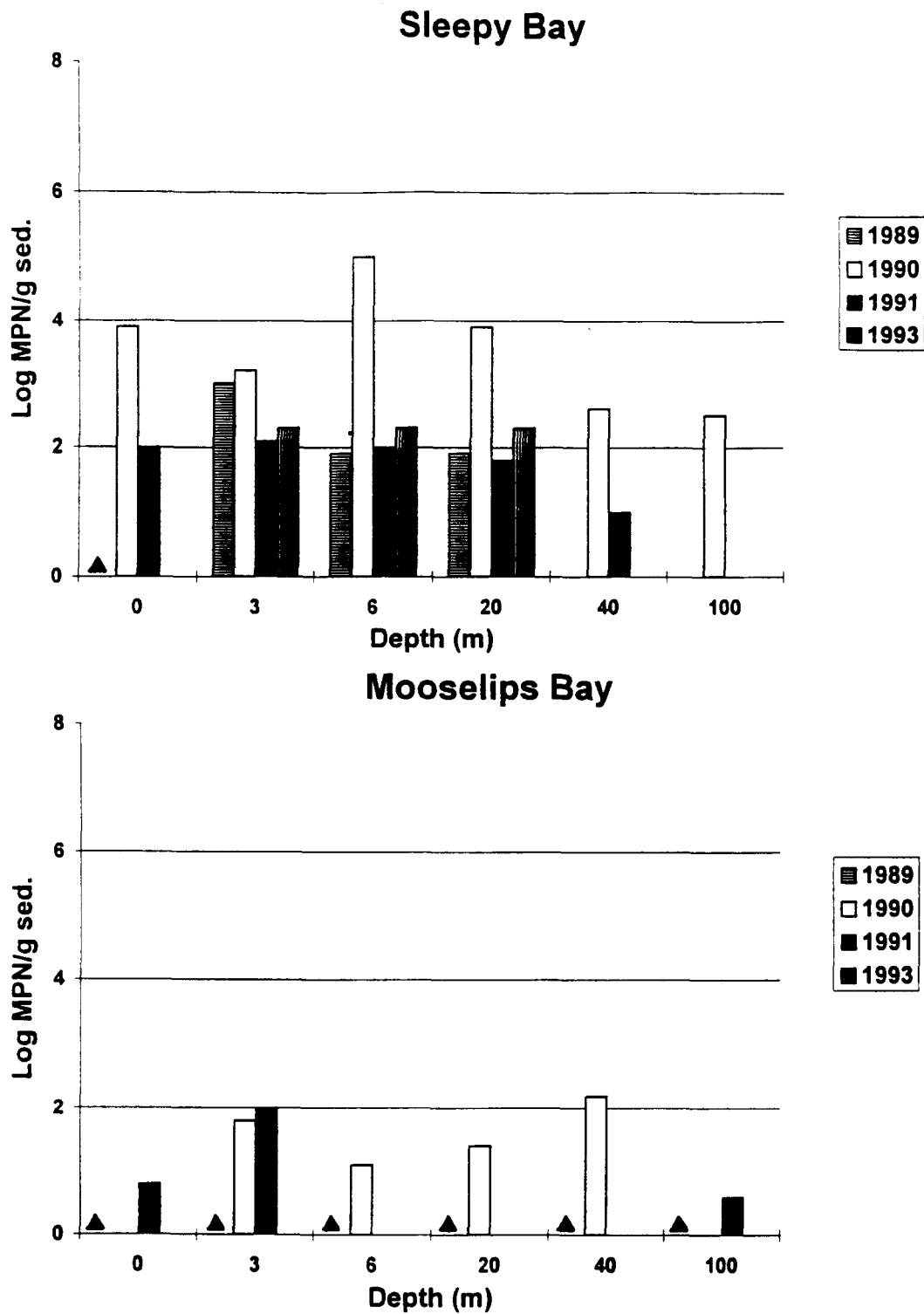


**Table 2.** Summary of microbial population data (heterotrophs and hydrocarbon degraders) from intertidal and subtidal sediments collected in July 1993 (Scorpius cruise).

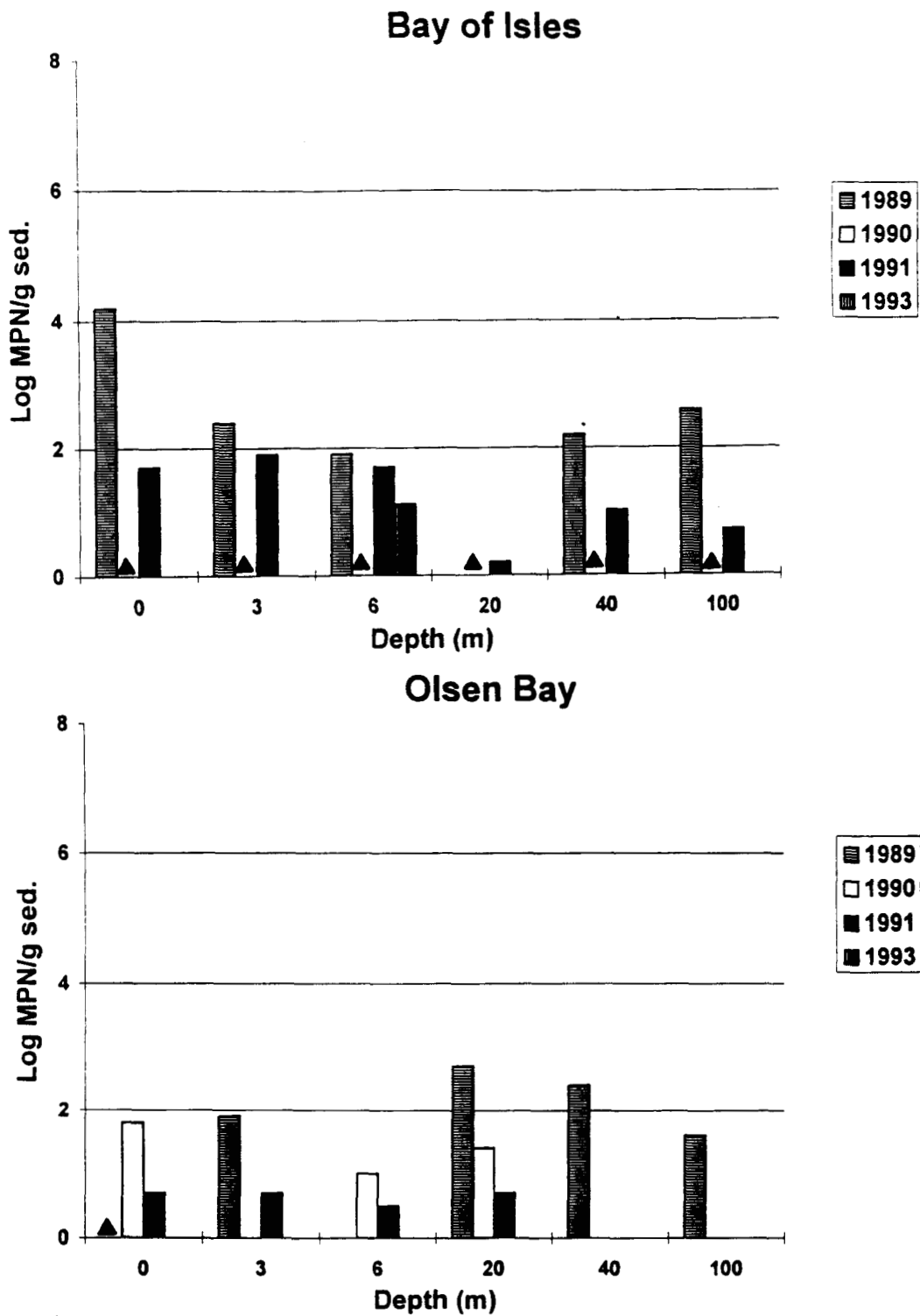
|  | Depth (m) | Heterotrophs (cells/g dry sed.) |           |          | Hydrocarbon Degraders (cells/g dry sed.) |           |          |
|--|-----------|---------------------------------|-----------|----------|--|-----------|----------|
|  |           | Mean                            | Std. Dev. | Log Mean | Mean                                     | Std. Dev. | Log Mean |
| Northwest Bay<br>7/8/93<br>Site #1       | Beach     | 9.0E+05                         | 9.1E+05   | 6.0      | 2.1E+02                                  | 3.3E+01   | 2.3      |
|  | Sub-Surf  | 8.6E+05                         | 8.8E+05   | 5.9      | 1.3E+05                                  | 2.0E+05   | 5.1      |
|  | 3         | 3.0E+05                         | 1.7E+05   | 5.5      | 2.5E+02                                  | 7.2E+01   | 2.4      |
|  | 6         | 7.7E+04                         | 3.4E+04   | 4.9      | 9.7E+00                                  | 1.7E+01   | 1.0      |
|  | 20        | 3.1E+05                         | 3.4E+05   | 5.5      | 1.6E+03                                  | 1.8E+03   | 3.2      |
|  | 40        | 4.7E+04                         | 2.3E+04   | 4.7      | 0.0E+00                                  | 0.0E+00   |          |
|  | 100       | 3.3E+04                         | 1.5E+04   | 4.5      | 0.0E+00                                  | 0.0E+00   |          |
| Herring Bay<br>7/9/93<br>Site #2         | Beach     | 7.4E+05                         | 3.9E+05   | 5.9      | 4.9E+01                                  | 8.1E+01   | 1.7      |
|  | Sub-Surf  | 4.0E+04                         | 3.1E+04   | 4.6      | 3.2E+04                                  | 5.6E+04   | 4.5      |
|  | 3         | 1.1E+05                         | 1.3E+05   | 5.0      | 5.7E+02                                  | 5.7E+02   | 2.8      |
|  | 6         | 6.1E+04                         | 3.2E+04   | 4.8      | 0.0E+00                                  | 0.0E+00   |          |
|  | 20        | 3.2E+04                         | 1.4E+04   | 4.5      | 3.6E+01                                  | 6.2E+01   | 1.6      |
|  | 40        | 2.3E+04                         | 2.8E+04   | 4.4      | 3.0E+01                                  | 2.6E+01   | 1.5      |
|  | 100       | 7.1E+03                         | 7.1E+03   | 3.9      | 0.0E+00                                  | 0.0E+00   |          |
| Lower Herring Bay<br>7/10/93<br>Site #3  | Beach     | 3.5E+05                         | 4.1E+05   | 5.5      | 0.0E+00                                  | 0.0E+00   |          |
|  | Sub-Surf  | 1.2E+04                         | 1.3E+04   | 4.1      | 0.0E+00                                  | 0.0E+00   |          |
|  | 3         | 6.5E+05                         | 9.6E+05   | 5.8      | 0.0E+00                                  | 0.0E+00   |          |
|  | 6         | 2.6E+05                         | 2.5E+05   | 5.4      | 0.0E+00                                  | 0.0E+00   |          |
|  | 20        | 1.9E+04                         | 1.0E+04   | 4.3      | 3.3E+00                                  | 5.7E+00   | 0.5      |
|  | 40        | 9.2E+03                         | 4.7E+03   | 4.0      | 0.0E+00                                  | 0.0E+00   |          |
|  | 100       | 2.5E+04                         | 2.0E+04   | 4.4      | 0.0E+00                                  | 0.0E+00   |          |
| Drier Bay<br>7/11/93<br>Site #4          | Beach     | 1.9E+05                         | 1.3E+05   | 5.3      | 1.3E+02                                  | 8.9E+01   | 2.1      |
|  | Sub-Surf  | 2.4E+04                         | 1.9E+04   | 4.4      | 0.0E+00                                  | 0.0E+00   |          |
|  | 3         | 8.5E+04                         | 3.4E+04   | 4.9      | 0.0E+00                                  | 0.0E+00   |          |
|  | 6         | 1.8E+06                         | 2.1E+06   | 6.2      | 0.0E+00                                  | 0.0E+00   |          |
|  | 20        | 1.1E+05                         | 2.3E+04   | 5.0      | 0.0E+00                                  | 0.0E+00   |          |
|  | 40        | 7.2E+04                         | 8.8E+04   | 4.9      | 0.0E+00                                  | 0.0E+00   |          |
|  | 100       | 3.4E+04                         | 3.7E+04   | 4.5      | 0.0E+00                                  | 0.0E+00   |          |
| Sleepy Bay<br>7/11 to 7/12/93<br>Site #5 | Beach     | 1.2E+05                         | 8.1E+04   | 5.1      | 0.0E+00                                  | 0.0E+00   |          |
|  | Sub-Surf  | 3.6E+08                         | 6.2E+08   | 8.6      | 1.2E+05                                  | 2.1E+05   | 5.1      |
|  | 3         | 4.7E+05                         | 2.5E+05   | 5.7      | 2.0E+02                                  | 1.2E+02   | 2.3      |
|  | 6         | 3.1E+05                         | 1.3E+05   | 5.5      | 1.9E+02                                  | 1.4E+02   | 2.3      |
|  | 20        | 7.5E+04                         | 4.4E+04   | 4.9      | 2.0E+02                                  | 2.4E+02   | 2.3      |
|  | 40        | 9.6E+03                         | 5.0E+03   | 4.0      | 0.0E+00                                  | 0.0E+00   |          |
|  | 100       | 1.5E+04                         | 9.8E+03   | 4.2      | 0.0E+00                                  | 0.0E+00   |          |



**Figure 3a.** Log Most Probable Number of hydrocarbon-degrading microorganisms in surface sediments from the shoreline to 100 m depth offshore collected from Northwest Bay and Herring Bay in the summers of 1989, 1990, 1991 and 1993 (see also Braddock et al., 1993). A bold triangle indicates that data were unavailable for that date or depth. The absence of a bar for a date or depth indicates that the measured value was  $< 1.3$  which reflects the lower sensitivity of the MPN technique used.



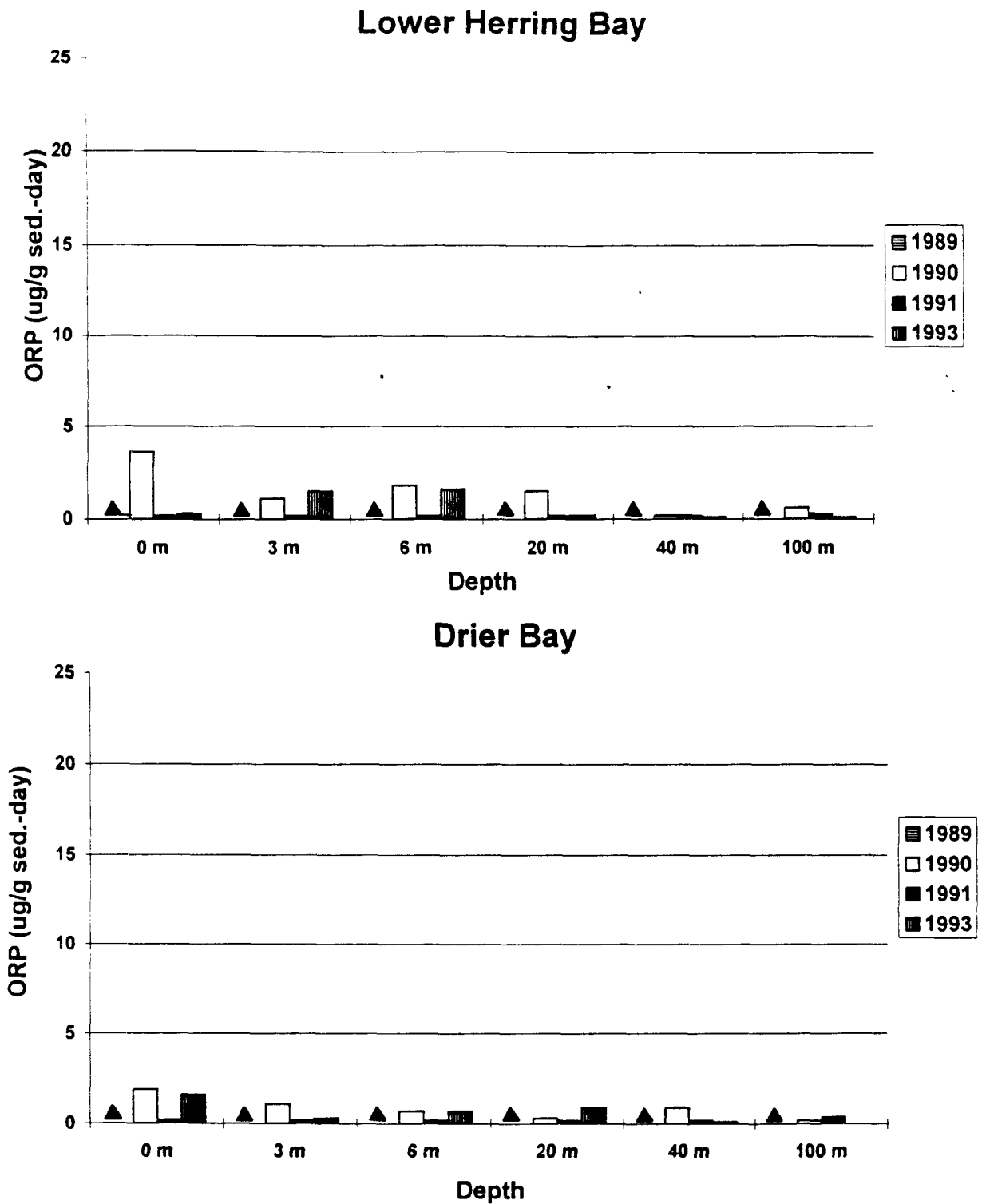
**Figure 3c.** Log Most Probable Number of hydrocarbon-degrading microorganisms in surface sediments from the shoreline to 100 m depth offshore collected from Sleepy Bay and Mooselips Bay in the summers of 1989, 1990, 1991 and 1993 (see also Braddock et al., 1993). A bold triangle indicates that data were unavailable for that date or depth. The absence of a bar for a date or depth indicates that the measured value was  $< 1.3$  which reflects the lower sensitivity of the MPN technique used.



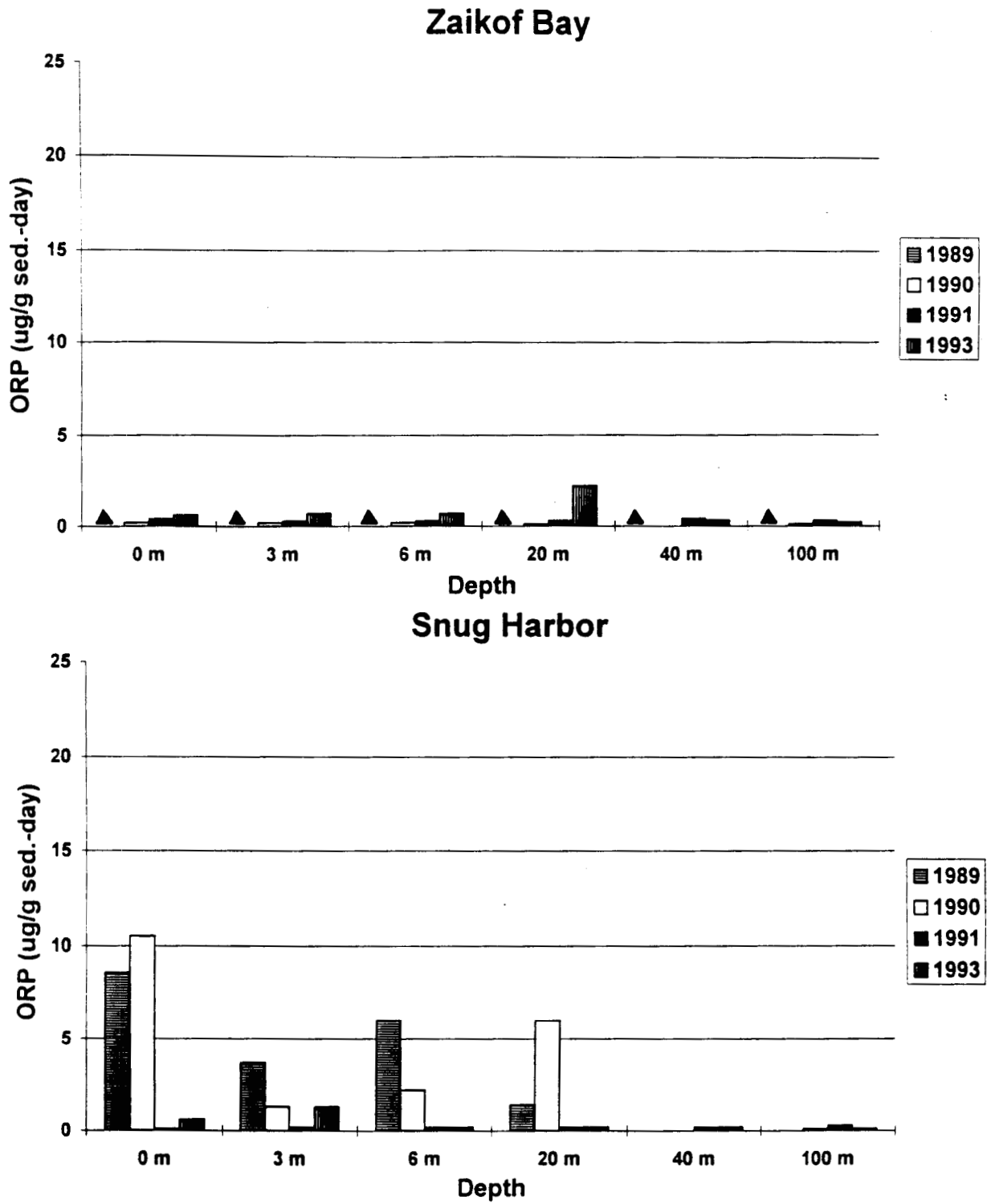
**Figure 3e.** Log Most Probable Number of hydrocarbon-degrading microorganisms in surface sediments from the shoreline to 100 m depth offshore collected from Bay of Isles and Olsen Bay in the summers of 1989, 1990, 1991 and 1993 (see also Braddock et al., 1993). A bold triangle indicates that data were unavailable for that date or depth. The absence of a bar for a date or depth indicates that the measured value was  $< 1.3$  which reflects the lower sensitivity of the MPN technique used.

**Table 3 cont.** Summary of hexadecane oxidation rate potentials (average rate for two day incubation) for sediment slurries from samples collected in July 1993 (Scorpius cruise).

|  | Depth (m) | % Dry Weight | % Mineralized | ORP<br>$\mu\text{g}/(\text{g dry wt.} \cdot \text{day})$<br>(@95% Confid. Level) |   |     |
|--|-----------|--------------|---------------|--|---|-----|
| <b>Mooselips Bay</b><br>7/12 to 7/13/93<br>Site #6 | Beach     | 0.75         | 8.8           | 4.4  | ± | 1.3 |
|  | Sub-Surf  | 0.88         | 0.4           | 0.2  | ± | 0.1 |
|  | 3         | 0.71         | 1.6           | 0.8  | ± | 0.2 |
|  | 6         | 0.70         | 1.0           | 0.5  | ± | 0.1 |
|  | 20        | 0.70         | 1.2           | 0.6  | ± | 0.2 |
|  | 40        | 0.66         | 0.7           | 0.3  | ± | 0.1 |
|  | 100       | 0.71         | 0.4           | 0.2  | ± | 0.1 |
| <b>Zaikof Bay</b><br>7/13/93<br>Site #7            | Beach     | 0.81         | 1.2           | 0.6  | ± | 0.1 |
|  | Sub-Surf  | 0.87         | 0.7           | 0.3  | ± | 0.1 |
|  | 3         | 0.72         | 1.5           | 0.7  | ± | 0.1 |
|  | 6         | 0.61         | 1.4           | 0.7  | ± | 0.1 |
|  | 20        | 0.65         | 4.4           | 2.2  | ± | 1.3 |
|  | 40        | 0.63         | 0.6           | 0.3  | ± | 0.1 |
|  | 100       | 0.74         | 0.5           | 0.2  | ± | 0.1 |
| <b>Snug Harbor</b><br>7/14/93<br>Site #8           | Beach     | 0.75         | 1.2           | 0.6  | ± | 0.1 |
|  | Sub-Surf  | 0.88         | 5.3           | 2.7  | ± | 0.3 |
|  | 3         | 0.76         | 2.6           | 1.3  | ± | 1.1 |
|  | 6         | 0.73         | 0.4           | 0.2  | ± | 0.1 |
|  | 20        | 0.68         | 0.3           | 0.2  | ± | 0.1 |
|  | 40        | 0.57         | 0.3           | 0.2  | ± | 0.1 |
|  | 100       | 0.43         | 0.2           | 0.1  | ± | 0.1 |
| <b>Bay of Isles</b><br>7/15/93<br>Site #9          | Beach     | 0.84         | 7.2           | 3.6  | ± | 2.3 |
|  | Sub-Surf  | 0.85         | 4.0           | 2.0  | ± | 0.5 |
|  | 3         | 0.81         | 0.6           | 0.3  | ± | 0.1 |
|  | 6         | 0.54         | 1.8           | 0.9  | ± | 0.2 |
|  | 20        | 0.23         | 1.0           | 0.5  | ± | 0.2 |
|  | 40        | 0.62         | 0.7           | 0.3  | ± | 0.1 |
|  | 100       | 0.38         | 0.6           | 0.3  | ± | 0.1 |
| <b>Olsen Bay</b><br>7/15 to 7/16/93<br>Site #10    | Beach     | 0.71         | 0.4           | 0.2  | ± | 0.1 |
|  | Sub-Surf  | 0.90         | 0.1           | 0.1  | ± | 0.1 |
|  | 3         | 0.54         | 0.8           | 0.4  | ± | 0.1 |
|  | 6         | 0.53         | 1.1           | 0.5  | ± | 0.1 |
|  | 20        | 0.34         | 1.2           | 0.6  | ± | 0.1 |
|  | 40        | 0.70         | 0.3           | 0.2  | ± | 0.1 |
|  | 100       | 0.44         | 0.3           | 0.1  | ± | 0.1 |



**Figure 4b.** Oxidation rate potentials for hexadecane (average for a two day incubation) in surface sediment slurries from the shoreline to 100 m depth offshore collected from Lower Herring Bay and Drier Bay in the summers of 1989, 1990, 1991 and 1993 (see also Braddock et al., 1993). A bold triangle indicates that data were unavailable for that date or depth. The absence of a bar or triangle indicates a measured value of zero.



**Figure 4d.** Oxidation rate potentials for hexadecane (average for a two day incubation) in surface sediment slurries from the shoreline to 100 m depth offshore collected from Zaikof Bay and Snug Harbor in the summers of 1989, 1990, 1991 and 1993 (see also Braddock et al., 1993). A bold triangle indicates that data were unavailable for that date or depth. The absence of a bar or triangle indicates a measured value of zero.

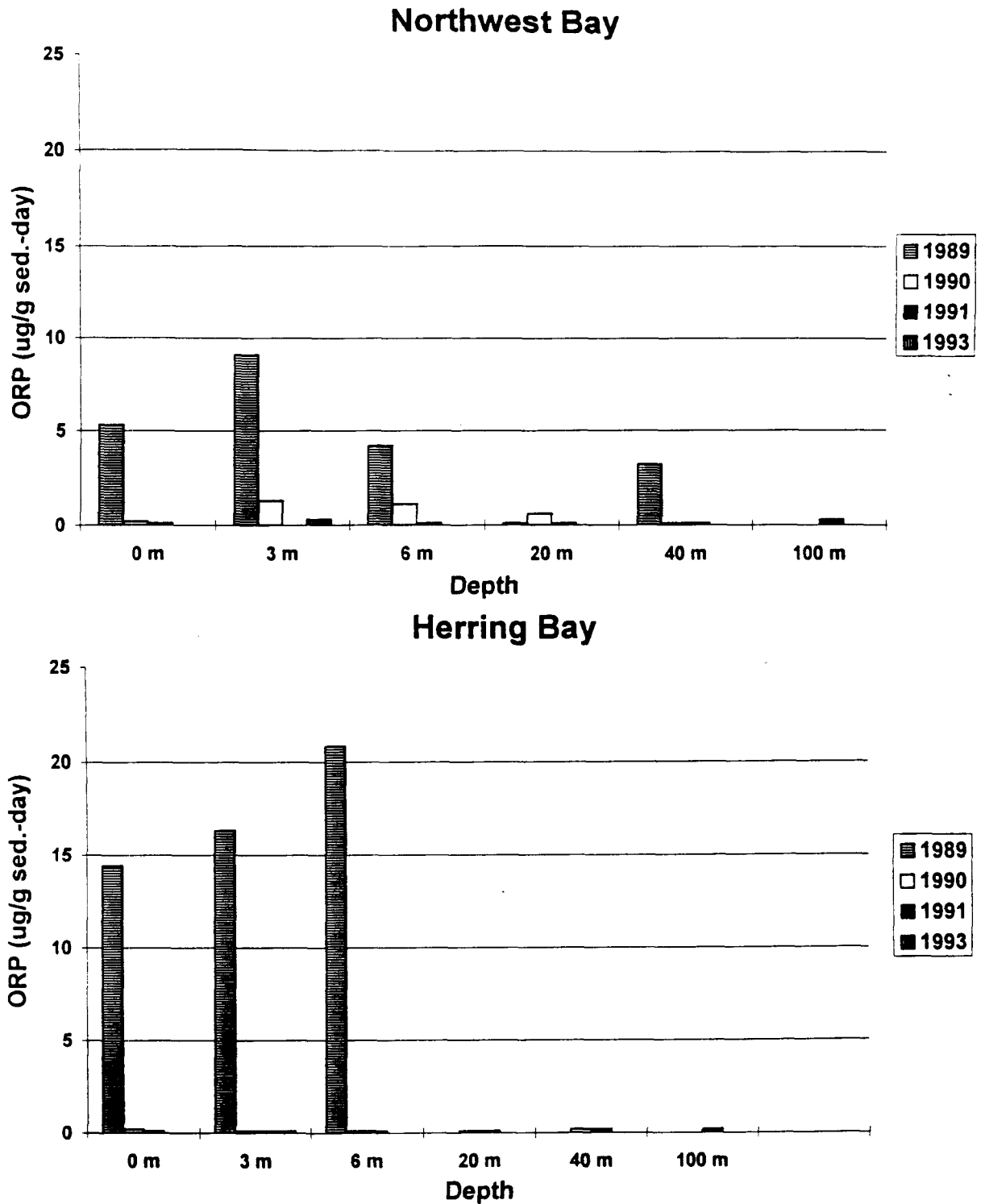
**Table 4.** Summary of phenanthrene oxidation rate potentials (average rate for two day incubation) for sediment slurries from samples collected in July 1993 (Scorpius cruise).

|  | Depth (m) | %Dry Weight | % Mineralized | ORP<br>$\mu\text{g/g}$ dry wt.-day<br>(@95% confid. level) |   |     |
|--|-----------|-------------|---------------|--|---|-----|
| Northwest Bay<br>7/8/93<br>Site #1       | Beach     | 0.88        | 0.0           | 0.0  | ± | 0.0 |
|  | Sub-Surf  | 0.92        | 0.6           | 0.3  | ± | 0.1 |
|  | 3         | 0.80        | 0.0           | 0.0  | ± | 0.0 |
|  | 6         | 0.69        | 0.0           | 0.0  | ± | 0.0 |
|  | 20        | 0.47        | 0.0           | 0.0  | ± | 0.1 |
|  | 40        | 0.70        | 0.0           | 0.0  | ± | 0.0 |
|  | 100       | 0.46        | 0.0           | 0.0  | ± | 0.0 |
| Herring Bay<br>7/9/93<br>Site #2         | Beach     | 0.77        | 0.0           | 0.0  | ± | 0.0 |
|  | Sub-Surf  | 0.72        | 0.2           | 0.1  | ± | 0.1 |
|  | 3         | 0.66        | 0.0           | 0.0  | ± | 0.0 |
|  | 6         | 0.75        | 0.0           | 0.0  | ± | 0.0 |
|  | 20        | 0.74        | 0.0           | 0.0  | ± | 0.0 |
|  | 40        | 0.89        | 0.0           | 0.0  | ± | 0.0 |
|  | 100       | 0.72        | 0.0           | 0.0  | ± | 0.0 |
| Lower Herring Bay<br>7/10/93<br>Site #3  | Beach     | 0.85        | 0.0           | 0.0  | ± | 0.0 |
|  | Sub-Surf  | 0.82        | 0.0           | 0.0  | ± | 0.0 |
|  | 3         | 0.97        | 0.0           | 0.0  | ± | 0.0 |
|  | 6         | 0.91        | 0.0           | 0.0  | ± | 0.0 |
|  | 20        | 0.81        | 0.0           | 0.0  | ± | 0.0 |
|  | 40        | 0.75        | 0.0           | 0.0  | ± | 0.0 |
|  | 100       | 0.37        | 0.0           | 0.0  | ± | 0.1 |
| Drier Bay<br>7/11/93<br>Site #4          | Beach     | 0.97        | 0.8           | 0.4  | ± | 0.3 |
|  | Sub-Surf  | 0.81        | 0.4           | 0.2  | ± | 0.2 |
|  | 3         | 0.90        | 2.3           | 1.1  | ± | 0.9 |
|  | 6         | 0.51        | 0.5           | 0.2  | ± | 0.2 |
|  | 20        | 0.52        | 0.3           | 0.1  | ± | 0.1 |
|  | 40        | 0.63        | 0.4           | 0.2  | ± | 0.1 |
|  | 100       | 0.37        | 7.9           | 4.0  | ± | 2.5 |
| Sleepy Bay<br>7/11 to 7/12/93<br>Site #5 | Beach     | 0.76        | 0.3           | 0.1  | ± | 0.1 |
|  | Sub-Surf  | 0.85        | 1.5           | 0.8  | ± | 0.3 |
|  | 3         | 0.75        | 0.3           | 0.1  | ± | 0.1 |
|  | 6         | 0.75        | 0.2           | 0.1  | ± | 0.0 |
|  | 20        | 0.70        | 0.2           | 0.1  | ± | 0.1 |
|  | 40        | 0.77        | 0.2           | 0.1  | ± | 0.0 |
|  | 100       | 0.66        | 2.7           | 1.4  | ± | 0.7 |

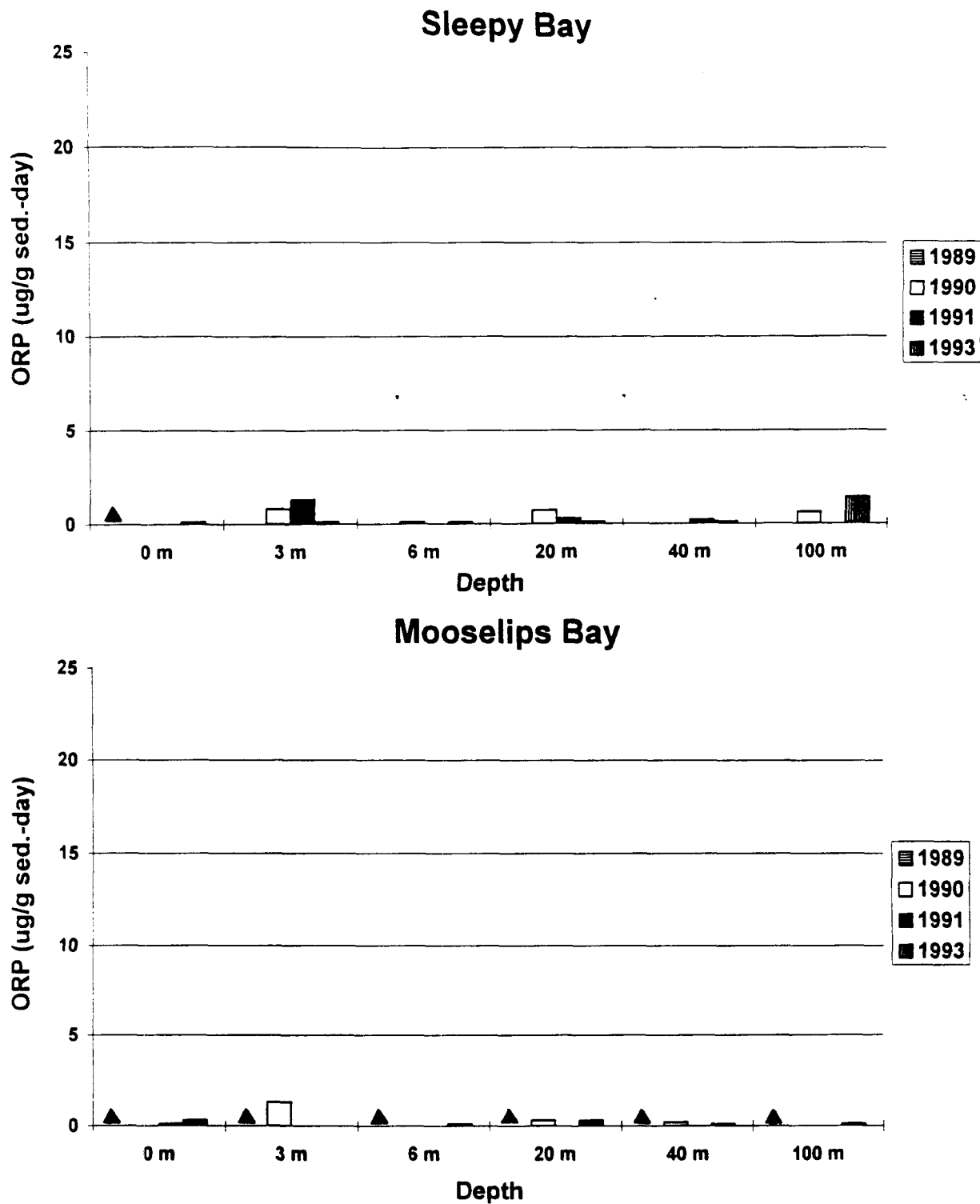


**Table 5.** Summary of phenanthrene oxidation rate potentials (average rate for four day incubation) for sediment slurries from samples collected in July 1993 (Scorpius cruise).

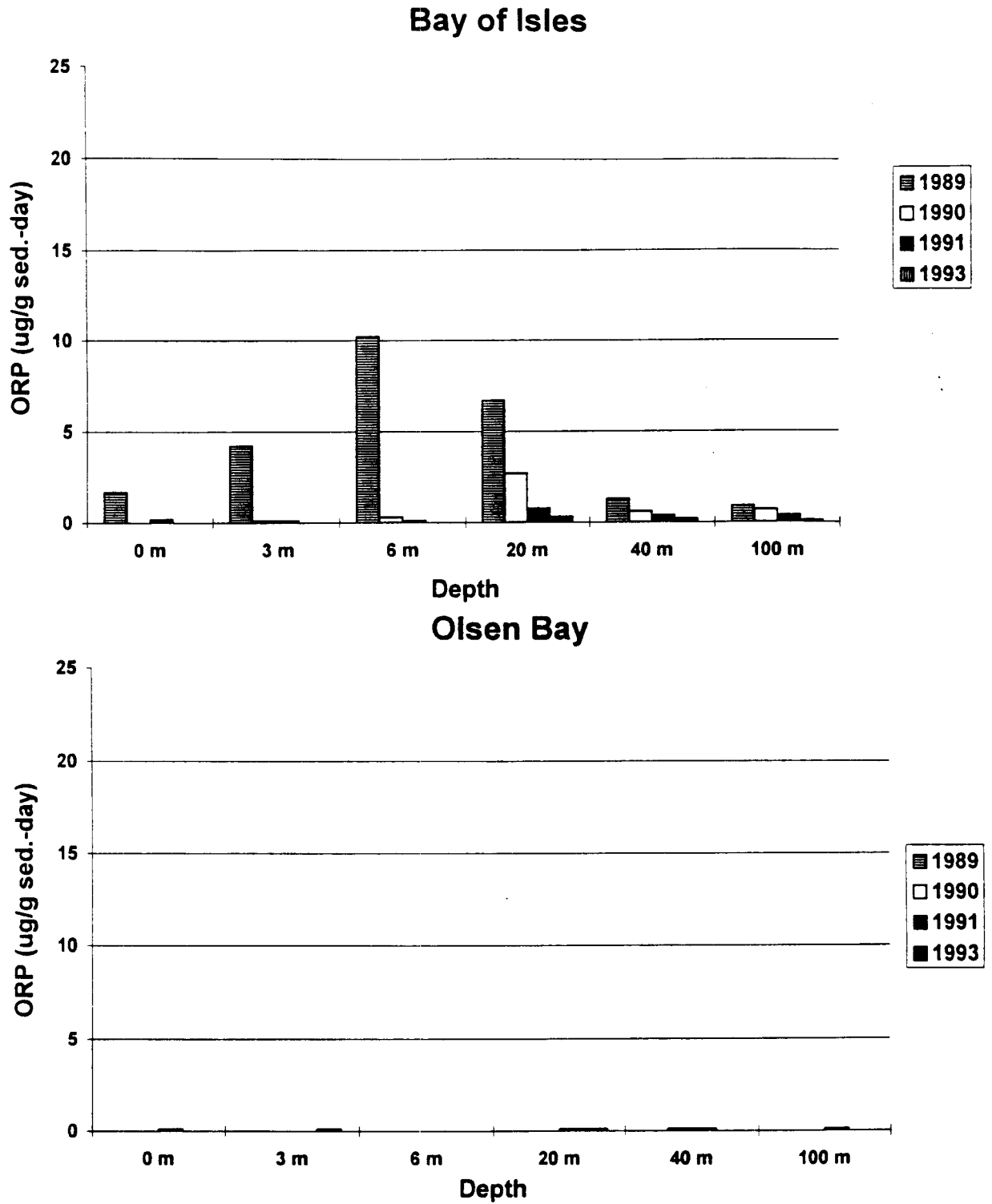
|   | Depth (m) | % Dry Weight | % Mineralized | ORP<br>μg/g dry wt.-day<br>(@95% confid. level) |   |     |
|---|-----------|--------------|---------------|---|---|-----|
| <b>Northwest Bay<br/>7/8/93<br/>Site #1</b>       | Beach     | 0.88         | 0.0           | 0.0   | ± | 0.0 |
|   | Sub-Surf  | 0.92         | 10.1          | 2.5   | ± | 0.9 |
|   | 3         | 0.80         | 0.0           | 0.0   | ± | 0.0 |
|   | 6         | 0.69         | 0.0           | 0.0   | ± | 0.0 |
|   | 20        | 0.47         | 0.0           | 0.0   | ± | 0.1 |
|   | 40        | 0.70         | 0.0           | 0.0   | ± | 0.0 |
|   | 100       | 0.46         | 0.0           | 0.0   | ± | 0.0 |
| <b>Herring Bay<br/>7/9/93<br/>Site #2</b>         | Beach     | 0.77         | 0.0           | 0.0   | ± | 0.0 |
|   | Sub-Surf  | 0.72         | 0.6           | 0.2   | ± | 0.2 |
|   | 3         | 0.66         | 0.0           | 0.0   | ± | 0.0 |
|   | 6         | 0.75         | 0.0           | 0.0   | ± | 0.0 |
|   | 20        | 0.74         | 0.0           | 0.0   | ± | 0.0 |
|   | 40        | 0.89         | 0.0           | 0.0   | ± | 0.0 |
|   | 100       | 0.72         | 0.0           | 0.0   | ± | 0.0 |
| <b>Lower Herring Bay<br/>7/10/93<br/>Site #3</b>  | Beach     | 0.85         | 0.0           | 0.0   | ± | 0.0 |
|   | Sub-Surf  | 0.82         | 0.0           | 0.0   | ± | 0.0 |
|   | 3         | 0.97         | 0.0           | 0.0   | ± | 0.0 |
|   | 6         | 0.91         | 0.0           | 0.0   | ± | 0.0 |
|   | 20        | 0.81         | 0.0           | 0.0   | ± | 0.0 |
|   | 40        | 0.75         | 0.0           | 0.0   | ± | 0.0 |
|   | 100       | 0.37         | 0.1           | 0.0   | ± | 0.1 |
| <b>Drier Bay<br/>7/11/93<br/>Site #4</b>          | Beach     | 0.97         | 0.2           | 0.0   | ± | 0.0 |
|   | Sub-Surf  | 0.81         | 0.1           | 0.0   | ± | 0.0 |
|   | 3         | 0.90         | 0.5           | 0.1   | ± | 0.1 |
|   | 6         | 0.51         | 0.7           | 0.2   | ± | 0.2 |
|   | 20        | 0.52         | 0.4           | 0.1   | ± | 0.2 |
|   | 40        | 0.63         | 3.9           | 1.0   | ± | 0.9 |
|   | 100       | 0.37         | 0.7           | 0.2   | ± | 0.1 |
| <b>Sleepy Bay<br/>7/11 to 7/12/93<br/>Site #5</b> | Beach     | 0.76         | 0.1           | 0.0   | ± | 0.0 |
|   | Sub-Surf  | 0.85         | 4.4           | 1.1   | ± | 1.0 |
|   | 3         | 0.75         | 0.2           | 0.1   | ± | 0.0 |
|   | 6         | 0.75         | 0.2           | 0.0   | ± | 0.0 |
|   | 20        | 0.70         | 0.3           | 0.1   | ± | 0.0 |
|   | 40        | 0.77         | 0.2           | 0.0   | ± | 0.0 |
|   | 100       | 0.66         | 1.5           | 0.4   | ± | 0.3 |



**Figure 5a.** Oxidation rate potentials (average for a two day incubation) for naphthalene (1989) and phenanthrene (1990, 1991 and 1993) in surface sediment slurries from the shoreline to 100 m depth offshore collected from Northwest Bay and Herring Bay in the summers of 1989, 1990, 1991 and 1993 (see also Braddock et al., 1993). A bold triangle indicates that data were unavailable for that date or depth. The absence of a bar or triangle indicates a measured value of zero.



**Figure 5c.** Oxidation rate potentials (average for a two day incubation) for naphthalene (1989) and phenanthrene (1990,1991 and 1993) in surface sediment slurries from the shoreline to 100 m depth offshore collected from Sleepy Bay and Mooselips Bay in the summers of 1989, 1990, 1991 and 1993 (see also Braddock et al., 1993). A bold triangle indicates that data were unavailable for that date or depth. The absence of a bar or triangle indicates a measured value of zero.



**Figure 5e.** Oxidation rate potentials (average for a two day incubation) for naphthalene (1989) and phenanthrene (1990, 1991 and 1993) in surface sediment slurries from the shoreline to 100 m depth offshore collected from Bay of Isles and Olsen Bay in the summers of 1989, 1990, 1991 and 1993 (see also Braddock et al., 1993). A bold triangle indicates that data were unavailable for that date or depth. The absence of a bar or triangle indicates a measured value of zero.

*Exxon Valdez* oil by very expensive and time-consuming advanced chemical hydrocarbon fingerprinting techniques. These additional sources of oil in PWS confound the use of standard chemical analyses alone in the collection of data for damage assessment purposes. If this older weathered oil is present (in surface sediments) at our study sites then our data indicate that the older oil has not led to increased populations of hydrocarbon degraders. The response of microbial populations may then be a useful complement to hydrocarbon chemistry data in defining the extent of recent pollution occurring from the spill.

There was no statistically significant correlation between the total numbers of hydrocarbon-degrading bacteria and mineralization potentials for hexadecane. However, when high hexadecane mineralization potentials were measured the numbers of hydrocarbon oxidizers were generally also high. Increased populations of Sheen Screen positive organisms represent an increased potential for biodegradation of hydrocarbons in oil (Bartha and Atlas, 1987). But other factors such as salinity, temperature, mineral nutrient availability, oxygen availability, hydrocarbon concentration, and acclimation of the microbial population to a particular hydrocarbon component of the oil can affect the mineralization potential as well (Bartha and Atlas, 1987; Leahy and Colwell, 1990). Experiments in this study were designed to minimize as many of these factors as possible (including hydrocarbon availability) except the in situ microbial biomass and its potential to degrade the hydrocarbons added experimentally (see Brown et al., 1991). Using this "optimal" procedure, rates of hexadecane, naphthalene and phenanthrene mineralization among sites and sampling stations could be compared. For example, mineralization potential samples were run in a mineral salts medium with nutrients such as nitrogen and phosphorus, were well oxygenated and were incubated at a constant temperature (15 °C). In addition, a relatively large amount of hydrocarbon substrate was added to each sample to be assayed so that the final rate was dependent on added substrate rather than the hydrocarbon substrate in the original sample. The concentrations of the specific hydrocarbon we added greatly exceeded ambient levels in heavily oiled samples collected in 1989 (see Brown et al., 1991). The reported potentials thus reflect the potential of the in situ microbial populations (Bartha and Atlas, 1987; Aelion and Bradley, 1991) to transform hydrocarbons when incubated in conditions that are standardized and essentially optimal.

Bartha and Atlas (1987) summarized the results of a number of published studies on biodegradation rates (potentials) of samples from marine systems. They found a range of 5-2,500  $\mu\text{g/g-day}$  for seawater communities under partially optimized conditions. These rates for nutrient enriched samples were found to be as much as 300-fold higher than for non-nutrient enriched samples. Values from our study range from 0 to approximately 40  $\mu\text{g/g sed-day}$ . Therefore, our values fall at the low end of those reported by Bartha and Atlas for samples incubated under partially optimized conditions. A more recent study (Karl, 1992) conducted about six weeks after the grounding of the *Bahia Paraiso* in Antarctica in 1989 found extremely low rates for hexadecane mineralization potential (0.13-1.21  $\text{pmol/g sed-day}$ ) in samples run without nutrient amendments and incubated at 1 °C (sub-optimal conditions).

We found apparent differences in microbial preference for hexadecane, naphthalene or phenanthrene in Prince William Sound sediments temporally and with depth. Hexadecane potentials nearly always exceeded those for PAH except for sediments collected in the spring of

hydrocarbon levels should be measured periodically for several years following other major oil spill events to monitor transport of petroleum compounds in the environment.

### **Conclusions**

1. Populations of hydrocarbon-degrading microorganisms were good indicators of the distribution of *Exxon Valdez* oil following the spill.
2. Populations of hydrocarbon-degrading microorganism generally declined with time at most sites probably as a result of a decline in readily biodegradable hydrocarbon fractions. By 1993 few oiled sites sampled had populations of hydrocarbon degraders that were significantly higher than those measured at reference sites. High populations were only measured in samples collected from the intertidal 10-20 cm below the surface.
3. The numbers of hydrocarbon degraders were highest in sediments offshore heavily oiled beaches in summer 1990. These populations declined to levels observed at reference sites by 1991 and continued to be low in 1993.
4. Mineralization potentials for hexadecane and for phenanthrene were not statistically correlated to the populations of hydrocarbon-degrading microorganisms measured in sediment samples. Hexadecane potentials were generally higher than those for phenanthrene (or naphthalene).

- Karl, D.M. 1992. The grounding of the *Bahia Paraiso*: microbial ecology of the 1989 antarctic spill. *Microb. Ecol.* 24:77-89.
- Kvenvolden, K.A., F.D. Hostettler, J.B. Rapp and P.R. Carlson. 1993. Hydrocarbons in oil residues on beaches of islands of Prince William Sound, Alaska. *Mar. Pollut. Bull.* 26: 24-29.
- Leahy, J.G. and R.R. Colwell. 1990. Microbial degradation of hydrocarbons in the environment. *Microbiol. Rev.* 54:305-315.
- Lindstrom, J.E., R.C. Prince, J.C. Clark, M.J. Grossman, T.R. Yeager, J.F. Braddock and E.J. Brown. 1991. Microbial populations and hydrocarbon biodegradation potentials in fertilized shoreline sediments affected by the T/V *Exxon Valdez* oil spill. *Appl. Environ. Microbiol.* 57:2514-2522.
- Lizarrago-Partida, M.L., F.B. Izquierdo-Vicuna and I. Wong-Chang. 1991. Marine bacteria on the Campeche Bank oil field. *Mar. Pollut. Bull.* 22:401-405.
- Madsen, E.L., J.L. Sinclair and W.C. Ghiorse. 1991. In situ biodegradation: microbiological patterns in a contaminated aquifer. *Science* 252:830-834.
- Page, D.S., P.D. Boehm, G.S. Douglas and A.E. Bence 1993. The natural petroleum hydrocarbon background in subtidal sediments of Prince William Sound, Alaska. O89, p.37 Abst 14th Annu. Meet. Soc. Environ. Toxicol. Chem. 1993.
- Prince, R.C., J.R. Clark and J.E. Lindstrom. 1990. Bioremediation monitoring program. U.S. Coast Guard, Report, Alaska Department of Environmental Conservation, Anchorage, AK.
- Prince, R.C., J.R. Clark, J.E. Lindstrom, E.L. Butler, E.J. Brown, G. Winter, M.J. Grossman, P.R. Parrish, R.E. Bare, J.F. Braddock, W.G. Steinhauer, G.S. Douglas, J.M. Kennedy, P.J. Barter, J.R. Bragg, E.J. Harner and R.M. Atlas. 1994. Bioremediation of the *Exxon Valdez* oil spill; monitoring safety and efficacy. Proceedings of the International Symposium on In Situ and On Site Bioremediation, April 1993.
- Roubal, G. and R. M. Atlas. 1978. Distribution of hydrocarbon-utilizing microorganisms and hydrocarbon biodegradation potentials in Alaska continental shelf areas. *Appl. Environ. Microbiol.* 35:897-905.
- Royer, T.C., J.A. Vermersch, T.J. Weingartner, H.J. Niebauer and R.D. Muench 1990. Ocean circulation influencing the *Exxon Valdez* oil spill. *Oceanography* 3: 3-10.
- Shiaris, M.P. 1989. Seasonal biotransformation of naphthalene, phenanthrene, and benzo[a]pyrene in surficial estuarine sediments. *Appl. Environ. Microbiol.* 55:1391-1399.

## APPENDIX A

Raw data and calculations for microbial  
populations and activities in sediments  
collected in 1993, F/V Scorpius cruise.





HEXADECANE - 2 Day Incubations

|  |          | Depth (m) | % Dry Weight | Day 0 (DPM) | Sediment A (DPM) |       | Sediment B (DPM) |       | Sediment C (DPM) |       | mean dpm | std. dev. | corr mean dpm | % Mineral. | ORP<br>µg/(g dry wt. · day)<br>(95% Confid. Level) |     |  |
|--|----------|-----------|--------------|-------------|------------------|-------|------------------|-------|------------------|-------|----------|-----------|---------------|------------|--|-----|--|
|  |          |           |              |             | rep 1            | rep 2 | rep 1            | rep 2 | rep 1            | rep 2 |          |           |               |            |  |     |  |
| Mooslips Bay<br>7/12 to 7/13/93<br>Site #6 | Beach    | 0.75      | 121          | 290         | 815              | 3032  | 3347             | 4474  | 4006             | 2661  | 1717     | 2546      | 8.8           | 4.4        | ±  | 1.3 |  |
|  | Sub-Surf | 0.88      | 363          | 123         | 141              | 403   | 454              | 134   | 179              | 239   | 149      | 124       | 0.4           | 0.2        | ±  | 0.1 |  |
|  | 3        | 0.71      | 161          | 736         | 428              | 564   | 922              | 355   | 341              | 558   | 232      | 443       | 1.0           | 0.8        | ±  | 0.2 |  |
|  | 6        | 0.70      | 70           | 472         | 466              | 371   | 309              | 312   | 344              | 379   | 73       | 264       | 1.0           | 0.5        | ±  | 0.1 |  |
|  | 20       | 0.70      | 120          | 294         | 313              | 785   | 609              | 246   | 313              | 427   | 218      | 312       | 1.2           | 0.6        | ±  | 0.2 |  |
|  | 40       | 0.66      | 112          | 229         | 305              | 231   | 374              | 303   | 300              | 290   | 54       | 175       | 0.7           | 0.3        | ±  | 0.1 |  |
|  | 100      | 0.71      | 57           | 240         | 307              | 151   | 171              | 268   | 210              | 225   | 59       | 110       | 0.4           | 0.2        | ±  | 0.1 |  |
| Zaikof Bay<br>7/13/93<br>Site #7           | Beach    | 0.81      | 258          | 578         | 499              | 463   | 536              | 450   | 458              | 497   | 51       | 382       | 1.2           | 0.6        | ±  | 0.1 |  |
|  | Sub-Surf | 0.87      | 146          | 340         | 335              | 278   | 275              | 385   | 410              | 337   | 55       | 222       | 0.7           | 0.3        | ±  | 0.1 |  |
|  | 3        | 0.72      | 223          | 475         | 287              | 607   | 621              | 719   | 466              | 529   | 152      | 414       | 1.5           | 0.7        | ±  | 0.1 |  |
|  | 6        | 0.61      | 98           | 531         | 529              | 434   | 419              | 393   | 348              | 442   | 74       | 327       | 1.4           | 0.7        | ±  | 0.1 |  |
|  | 20       | 0.65      | 112          | 250         | 269              | 259   | 262              | 2942  | 3256             | 1206  | 1469     | 1091      | 4.4           | 2.2        | ±  | 1.3 |  |
|  | 40       | 0.63      | 120          | 274         | 264              | 293   | 281              | 247   | 251              | 268   | 18       | 153       | 0.6           | 0.3        | ±  | 0.1 |  |
|  | 100      | 0.74      | 132          | 242         | 264              | 242   | 233              | 268   | 258              | 251   | 14       | 136       | 0.5           | 0.2        | ±  | 0.1 |  |
| 43 Snug Harbor<br>7/14/93<br>Site #8       | Beach    | 0.75      | 65           | 550         | 343              | 434   | 522              | 616   | 356              | 470   | 110      | 355       | 1.2           | 0.6        | ±  | 0.1 |  |
|  | Sub-Surf | 0.88      | 50           | 2522        | 1710             | 1284  | 1516             | 2206  | 2287             | 1918  | 493      | 1803      | 5.3           | 2.7        | ±  | 0.3 |  |
|  | 3        | 0.76      | 160          | 346         | 306              | 233   | 360              | 230   | 3704             | 863   | 1393     | 748       | 2.6           | 1.3        | ±  | 1.1 |  |
|  | 6        | 0.73      | 46           | 290         | 167              | 149   | 344              | 295   | 139              | 231   | 89       | 116       | 0.4           | 0.2        | ±  | 0.1 |  |
|  | 20       | 0.68      | 45           | 194         | 191              | 229   | 196              | 232   | 128              | 195   | 38       | 80        | 0.3           | 0.2        | ±  | 0.1 |  |
|  | 40       | 0.57      | 50           | 174         | 168              | 208   | 216              | 181   | 183              | 188   | 19       | 73        | 0.3           | 0.2        | ±  | 0.1 |  |
|  | 100      | 0.43      | 53           | 184         | 190              | 151   | 111              | 126   | 122              | 147   | 33       | 32        | 0.2           | 0.1        | ±  | 0.1 |  |
| Bay of Isles<br>7/15/93<br>Site #9         | Beach    | 0.84      | 197          | 2079        | 717              | 685   | 350              | 1852  | 8874             | 2426  | 3234     | 2311      | 7.2           | 3.6        | ±  | 2.3 |  |
|  | Sub-Surf | 0.85      | 113          | 964         | 1590             | 2073  | 481              | 1219  | 2208             | 1423  | 664      | 1308      | 4.0           | 2.0        | ±  | 0.5 |  |
|  | 3        | 0.81      | 104          | 293         | 298              | 407   | 305              | 294   | 255              | 309   | 51       | 194       | 0.6           | 0.3        | ±  | 0.1 |  |
|  | 6        | 0.54      | 147          | 505         | 765              | 385   | 484              | 371   | 390              | 483   | 149      | 368       | 1.8           | 0.9        | ±  | 0.2 |  |
|  | 20       | 0.23      | 139          | 191         | 216              | 176   | 197              | 212   | 209              | 200   | 15       | 85        | 1.0           | 0.5        | ±  | 0.2 |  |
|  | 40       | 0.62      | 147          | 232         | 292              | 319   | 274              | 236   | 301              | 276   | 35       | 161       | 0.7           | 0.3        | ±  | 0.1 |  |
|  | 100      | 0.38      | 137          | 212         | 233              | 188   | 155              | 236   | 209              | 206   | 30       | 91        | 0.6           | 0.3        | ±  | 0.1 |  |
| Olsen Bay<br>7/15 to 7/16/93<br>Site #10   | Beach    | 0.71      | 46           | 248         | 212              | 236   | 242              | 236   | 218              | 232   | 14       | 117       | 0.4           | 0.2        | ±  | 0.1 |  |
|  | Sub-Surf | 0.90      | 48           | 177         | 206              | 187   | 188              | 64    | 154              | 163   | 51       | 48        | 0.1           | 0.1        | ±  | 0.1 |  |
|  | 3        | 0.54      | 39           | 277         | 267              | 298   | 286              | 294   | 226              | 275   | 26       | 160       | 0.8           | 0.4        | ±  | 0.1 |  |
|  | 6        | 0.53      | 51           | 346         | 330              | 348   | 291              | 328   | 341              | 331   | 21       | 216       | 1.1           | 0.5        | ±  | 0.1 |  |
|  | 20       | 0.34      | 41           | 271         | 241              | 319   | 260              | 282   | 270              | 274   | 26       | 159       | 1.2           | 0.6        | ±  | 0.1 |  |
|  | 40       | 0.70      | 47           | 235         | 192              | 213   | 175              | 209   | 213              | 206   | 21       | 91        | 0.3           | 0.2        | ±  | 0.1 |  |
|  | 100      | 0.44      | 47           | 156         | 176              | 158   | 177              | 155   | 155              | 163   | 11       | 48        | 0.3           | 0.1        | ±  | 0.1 |  |

Mean killed control = 115 dpm

PHENANTHRENE-- 2 Day Incubations

|   |          | Depth (m) | %Dry Weight | Day 0 (DPM) | Sediment A (DPM) |       | Sediment B (DPM) |       | Sediment C (DPM) |       | Mean DPM | Std. Dev. | Corr. Mean DPM | % Mineral. | ORP<br>μg/g dry wt.-day<br>(@95% confid. level) |     |     |
|---|----------|-----------|-------------|-------------|------------------|-------|------------------|-------|------------------|-------|----------|-----------|----------------|------------|---|-----|-----|
|   |          |           |             |             | rep 1            | rep 2 | rep 1            | rep 2 | rep 1            | rep 2 |          |           |                |            |   |     |     |
| Mooselips Bay<br>7/12 to 7/13/93<br>Site #6 | Beach    | 0.75      | 226         | 444         | 440              |       | 510              | 248   | 259              | 380   | 119      | 260       | 0.6            | 0.3        | ±   | 0.1 |     |
|   | Sub-Surf | 0.88      | 322         | 338         | 287              |       | 360              | 315   | 320              | 378   | 333      | 33        | 213            | 0.4        | 0.2   | ±   | 0.0 |
|   | 3        | 0.71      | 116         | 161         | 96               |       | 189              | 225   | 111              | 121   | 151      | 50        | 31             | 0.1        | 0.0   | ±   | 0.0 |
|   | 6        | 0.70      | 173         | 257         | 247              |       | 178              | 102   | 211              | 378   | 229      | 92        | 109            | 0.3        | 0.1   | ±   | 0.1 |
|   | 20       | 0.70      | 111         | 184         | 170              |       | 506              | 773   | 349              | 424   | 401      | 225       | 281            | 0.6        | 0.3   | ±   | 0.1 |
|   | 40       | 0.66      | 204         | 118         | 112              |       | 356              | 501   | 167              | 162   | 236      | 158       | 116            | 0.3        | 0.1   | ±   | 0.1 |
|   | 100      | 0.71      | 143         | 205         | 186              |       | 171              | 268   | 150              | 146   | 188      | 45        | 68             | 0.2        | 0.1   | ±   | 0.0 |
| Zaikof Bay<br>7/13/93<br>Site #7            | Beach    | 0.81      | 234         | 180         | 202              |       | 182              | 281   | 173              | 189   | 201      | 40        | 81             | 0.2        | 0.1   | ±   | 0.0 |
|   | Sub-Surf | 0.87      | 122         | 174         | 196              |       | 183              | 183   | 176              | 276   | 198      | 39        | 78             | 0.1        | 0.1   | ±   | 0.0 |
|   | 3        | 0.72      | 204         | 183         | 197              |       | 202              | 257   | 155              | 183   | 196      | 34        | 76             | 0.2        | 0.1   | ±   | 0.0 |
|   | 6        | 0.81      | 171         | 185         | 191              |       | 178              | 218   | 158              | 184   | 186      | 20        | 66             | 0.2        | 0.1   | ±   | 0.0 |
|   | 20       | 0.65      | 138         | 126         | 189              |       | 161              | 220   | 206              | 194   | 183      | 34        | 63             | 0.2        | 0.1   | ±   | 0.0 |
|   | 40       | 0.63      | 118         | 170         | 107              |       | 185              | 205   | 176              | 111   | 159      | 41        | 39             | 0.1        | 0.1   | ±   | 0.0 |
|   | 100      | 0.74      | 166         | 206         | 212              |       | 268              | 134   | 342              | 375   | 256      | 91        | 136            | 0.3        | 0.1   | ±   | 0.1 |
| 45<br>Snug Harbor<br>7/14/93<br>Site #8     | Beach    | 0.75      | 189         | 72          | 118              |       | 187              | 453   | 79               | 80    | 165      | 148       | 45             | 0.1        | 0.0   | ±   | 0.1 |
|   | Sub-Surf | 0.88      | 234         | 128         | 436              |       | 166              | 254   | 287              | 360   | 272      | 116       | 152            | 0.3        | 0.1   | ±   | 0.1 |
|   | 3        | 0.76      | 49          | 84          | 192              |       | 119              | 88    | 82               | 90    | 109      | 43        | 0              | 0.0        | 0.0   | ±   | 0.0 |
|   | 6        | 0.73      | 67          | 81          | 90               |       | 156              | 103   | 85               | 69    | 97       | 31        | 0              | 0.0        | 0.0   | ±   | 0.0 |
|   | 20       | 0.68      | 62          | 100         | 109              |       | 103              | 91    | 92               | 98    | 99       | 7         | 0              | 0.0        | 0.0   | ±   | 0.0 |
|   | 40       | 0.57      | 62          | 102         | 100              |       | 128              | 120   | 116              | 118   | 114      | 11        | 0              | 0.0        | 0.0   | ±   | 0.0 |
|   | 100      | 0.43      | 62          | 127         | 138              |       | 159              | 140   | 121              | 122   | 135      | 14        | 15             | 0.1        | 0.0   | ±   | 0.1 |
| Bay of Isles<br>7/15/93<br>Site #9          | Beach    | 0.84      | 126         | 125         | 54               |       | 121              | 115   | 106              | 108   | 105      | 26        | 0              | 0.0        | 0.0   | ±   | 0.0 |
|   | Sub-Surf | 0.85      | 121         | 200         | 212              |       | 170              | 169   | 236              | 165   | 192      | 29        | 72             | 0.1        | 0.1   | ±   | 0.0 |
|   | 3        | 0.81      | 139         | 152         | 122              |       | 117              | 122   | 97               | 106   | 119      | 19        | 0              | 0.0        | 0.0   | ±   | 0.0 |
|   | 6        | 0.54      | 149         | 128         | 135              |       | 202              | 111   | 190              | 121   | 148      | 38        | 28             | 0.1        | 0.0   | ±   | 0.1 |
|   | 20       | 0.23      | 120         | 117         | 95               |       | 213              | 307   | 250              | 172   | 192      | 81        | 72             | 0.5        | 0.3   | ±   | 0.2 |
|   | 40       | 0.62      | 179         | 402         | 338              |       | 523              | 255   | 140              | 138   | 299      | 152       | 179            | 0.5        | 0.2   | ±   | 0.1 |
|   | 100      | 0.38      | 129         | 285         | 286              |       | 140              | 120   | 135              | 122   | 181      | 81        | 61             | 0.3        | 0.1   | ±   | 0.1 |
| Olsen Bay<br>7/15 to 7/16/93<br>Site #10    | Beach    | 0.71      | 57          | 127         | 141              |       | 101              | 128   | 132              | 113   | 124      | 14        | 4              | 0.0        | 0.0   | ±   | 0.0 |
|   | Sub-Surf | 0.90      | 56          | 103         | 108              |       | 109              | 97    | 168              | 105   | 115      | 26        | 0              | 0.0        | 0.0   | ±   | 0.0 |
|   | 3        | 0.54      | 53          | 145         | 500              |       | 137              | 122   | 109              | 133   | 191      | 152       | 71             | 0.2        | 0.1   | ±   | 0.1 |
|   | 6        | 0.53      | 62          | 101         | 124              |       | 127              | 167   | 115              | 139   | 129      | 23        | 9              | 0.0        | 0.0   | ±   | 0.0 |
|   | 20       | 0.34      | 80          | 147         | 137              |       | 120              | 109   | 99               | 125   | 123      | 18        | 3              | 0.0        | 0.0   | ±   | 0.1 |
|   | 40       | 0.70      | 54          | 129         | 112              |       | 96               | 75    | 112              | 100   | 104      | 18        | 0              | 0.0        | 0.0   | ±   | 0.0 |
|   | 100      | 0.44      | 58          | 109         | 113              |       | 119              | 82    | 109              | 119   | 109      | 14        | 0              | 0.0        | 0.0   | ±   | 0.0 |

PHENANTHRENE-- 4 Day Incubations

|  | Depth (m) | % Dry Weight | Sediment A (DPM) |       | Sediment B (DPM) |       | Sediment C (DPM) |       | Mean DPM | Std. Dev. | Corr. Mean DPM | % Mineral. | ORP<br>μg/g dry wt.-day<br>(@95% confid. level) |   |     |
|--|-----------|--------------|------------------|-------|------------------|-------|------------------|-------|----------|-----------|----------------|------------|---|---|-----|
|  |           |              | rep 1            | rep 2 | rep 1            | rep 2 | rep 1            | rep 2 |          |           |                |            | ±   | ± | ±   |
| <b>Mooselips Bay</b><br>7/12 to 7/13/93<br>Site #6 | Beach     | 0.75         | 1311             | 395   | 249              | 255   | 193              | 282   | 448      | 428       | 328            | 0.7        | 0.2   | ± | 0.2 |
|  | Sub-Surf  | 0.88         | 366              | 391   | 336              | 303   | 106              | 163   | 278      | 116       | 158            | 0.3        | 0.1   | ± | 0.1 |
|  | 3         | 0.71         | 143              | 140   | 248              | 144   | 335              | 406   | 236      | 114       | 116            | 0.3        | 0.1   | ± | 0.1 |
|  | 6         | 0.70         | 959              | 1108  | 381              | 368   | 1157             | 687   | 776      | 352       | 656            | 1.5        | 0.4   | ± | 0.2 |
|  | 20        | 0.70         | 124              | 135   | 290              | 323   | 164              | 227   | 211      | 83        | 91             | 0.2        | 0.1   | ± | 0.1 |
|  | 40        | 0.66         | 323              | 426   | 370              | 344   | 176              | 231   | 312      | 92        | 192            | 0.5        | 0.1   | ± | 0.1 |
|  | 100       | 0.71         | 188              | 204   | 209              | 182   | 177              | 233   | 199      | 21        | 79             | 0.2        | 0.0   | ± | 0.0 |
| <b>Zaikof Bay</b><br>7/13/93<br>Site #7            | Beach     | 0.81         | 206              | 220   | 175              | 194   | 209              | 330   | 222      | 55        | 102            | 0.2        | 0.1   | ± | 0.0 |
|  | Sub-Surf  | 0.87         | 227              | 197   | 206              | 228   | 215              | 298   | 228      | 36        | 108            | 0.2        | 0.1   | ± | 0.0 |
|  | 3         | 0.72         | 213              | 205   | 203              | 222   | 174              | 264   | 214      | 30        | 94             | 0.2        | 0.1   | ± | 0.0 |
|  | 6         | 0.61         | 204              | 176   | 200              | 189   | 202              | 268   | 207      | 32        | 87             | 0.2        | 0.1   | ± | 0.0 |
|  | 20        | 0.65         | 177              | 178   | 196              | 198   | 206              | 218   | 195      | 16        | 75             | 0.2        | 0.0   | ± | 0.0 |
|  | 40        | 0.63         | 134              | 185   | 130              | 185   | 155              | 254   | 174      | 46        | 54             | 0.1        | 0.0   | ± | 0.0 |
|  | 100       | 0.74         | 188              | 223   | 130              | 339   | 210              | 184   | 212      | 70        | 92             | 0.2        | 0.1   | ± | 0.0 |
| <b>Swig Harbor</b><br>7/14/93<br>Site #8           | Beach     | 0.75         | 98               | 121   | 91               | 101   | 101              | 102   | 102      | 10        | 0              | 0.0        | 0.0   | ± | 0.0 |
|  | Sub-Surf  | 0.88         | 97               | 217   | 112              | 190   | 180              | 212   | 168      | 51        | 48             | 0.1        | 0.0   | ± | 0.0 |
|  | 3         | 0.76         | 95               | 106   | 90               | 113   | 125              | 108   | 106      | 13        | 0              | 0.0        | 0.0   | ± | 0.0 |
|  | 6         | 0.73         | 89               | 101   | 91               | 93    | 161              | 89    | 104      | 28        | 0              | 0.0        | 0.0   | ± | 0.0 |
|  | 20        | 0.68         | 105              | 102   | 104              | 101   | 101              | 111   | 104      | 4         | 0              | 0.0        | 0.0   | ± | 0.0 |
|  | 40        | 0.57         | 130              | 122   | 107              | 140   | 146              | 133   | 130      | 14        | 10             | 0.0        | 0.0   | ± | 0.0 |
|  | 100       | 0.43         | 121              | 135   | 112              | 126   | 154              | 153   | 134      | 17        | 14             | 0.1        | 0.0   | ± | 0.1 |
| <b>Bay of Isles</b><br>7/15/93<br>Site #9          | Beach     | 0.84         | 127              | 136   | 86               | 200   | 158              | 123   | 138      | 38        | 18             | 0.0        | 0.0   | ± | 0.0 |
|  | Sub-Surf  | 0.85         | 200              | 219   | 177              | 181   | 193              | 153   | 187      | 22        | 67             | 0.1        | 0.0   | ± | 0.0 |
|  | 3         | 0.81         | 122              | 129   | 128              | 121   | 132              | 128   | 127      | 4         | 7              | 0.0        | 0.0   | ± | 0.0 |
|  | 6         | 0.54         | 46               | 296   | 126              | 118   | 283              | 353   | 204      | 123       | 84             | 0.3        | 0.1   | ± | 0.1 |
|  | 20        | 0.23         | 166              | 166   | 179              | 222   | 282              | 188   | 201      | 45        | 81             | 0.8        | 0.1   | ± | 0.1 |
|  | 40        | 0.62         | 353              | 231   | 302              | 322   | 291              | 296   | 299      | 40        | 179            | 0.5        | 0.1   | ± | 0.0 |
|  | 100       | 0.38         | 252              | 264   | 321              | 96    | 218              | 222   | 229      | 75        | 109            | 0.5        | 0.1   | ± | 0.1 |
| <b>Olsen Bay</b><br>7/15 to 7/16/93<br>Site #10    | Beach     | 0.71         | 124              | 133   | 137              | 132   | 115              | 108   | 125      | 11        | 5              | 0.0        | 0.0   | ± | 0.0 |
|  | Sub-Surf  | 0.90         | 135              | 108   | 114              | 119   | 78               | 115   | 112      | 19        | 0              | 0.0        | 0.0   | ± | 0.0 |
|  | 3         | 0.54         | 130              | 127   | 119              | 128   | 123              | 133   | 127      | 5         | 7              | 0.0        | 0.0   | ± | 0.0 |
|  | 6         | 0.53         | 152              | 162   | 111              | 141   | 124              | 120   | 135      | 20        | 15             | 0.0        | 0.0   | ± | 0.0 |
|  | 20        | 0.34         | 142              | 150   | 149              | 142   | 95               | 86    | 127      | 29        | 7              | 0.0        | 0.0   | ± | 0.1 |
|  | 40        | 0.70         | 133              | 116   | 84               | 80    | 111              | 90    | 102      | 21        | 0              | 0.0        | 0.0   | ± | 0.0 |
|  | 100       | 0.44         | 112              | 120   | 128              | 125   | 125              | 86    | 116      | 16        | 0              | 0.0        | 0.0   | ± | 0.0 |

47

