

## **Final Report to the Oil Spill Recovery Institute**

Today's date: 5 November 2001

Name of awardee/grantee: Joan F. Braddock

Project title: Biodegradation Potential of Petroleum Hydrocarbons in Beaufort Sea Sediments: Assessment of Long-Range Impacts of Oil Spills (Fellowship)

Dates project began and ended: July 1, 2000 to June 30, 2001

### **Part I- Technical Report**

#### **A. Abstract**

Despite large-scale development on the North Slope of Alaska, no recent studies examining arctic marine sediment microbial communities and their ability to metabolize petroleum compounds have been published. Microbial degradation of spilled petroleum hydrocarbons is a major mechanism for removal of these compounds from the environment. In this study we completed the second year of a study supported by the Coastal Marine Institute of the University of Alaska Fairbanks. Year one of the study was co-supported by the North Slope Borough and focused on sediments collected near Barrow, Alaska. Year two was co-supported, through this fellowship grant, by the Oil Spill Recovery Institute and focused on sediments collected near Prudhoe Bay. In both years we surveyed marine sediment microbial populations in the Arctic Ocean to determine what microorganisms are present and what their metabolic capability is for degradation of various petroleum hydrocarbons. We also examined the effects of sediment on the bioavailability of a polycyclic aromatic hydrocarbon (phenanthrene) to hydrocarbon degrading bacteria. In our survey we found high total numbers of microorganisms in all sediments examined (about  $10^{10}$  cells/g dry wt. sediment). Only about  $10^6$ - $10^8$  of these organisms were culturable in a marine heterotroph medium. Most probable numbers of culturable phenanthrene and hexadecane degraders were fairly high, about  $10^3$ - $10^5$ /g dry wt. sediment each, and populations of both types of organisms were significantly higher offshore Prudhoe Bay than offshore Barrow. In addition culturable crude oil degraders were significantly greater offshore Prudhoe Bay than Barrow. Mineralization potentials were low for both hexadecane and phenanthrene at both geographic locations. Despite the low organic carbon content of these sediments ( $\leq 1.5\%$ ), substantial adsorption to particles occurred; adsorption was rapid. Laboratory bioavailability studies using these same sediments are ongoing. The results of this study will be useful in predicting the fate of spilled petroleum hydrocarbons in the Arctic Ocean.

This fellowship supported the second year of a master's student project for Kathleen Gannon. Ms. Gannon is currently writing her thesis and is expected to defend it about January 2002.

#### **B. Review Objectives**

- a. The primary objective of this study was to understand how sediment microbial communities nearshore and offshore in the Beaufort Sea can respond, and have responded to petroleum hydrocarbon exposure, and to provide that information to

appropriate resource management personnel. The experiments delineated in our proposal to address objective one have all been successfully completed.

- b. A secondary objective of this project was to collect baseline data on marine sediment microbes before further industrial development occurs along the Beaufort Sea coast. The surveys described in our proposal were all successfully completed and the results compiled.
- c. A third objective was to communicate the purpose and results of our work with people residing in the North Slope Borough. We have communicated some of the results from our work through participation in the Minerals Management Information Transfer Meeting held in Anchorage in April 2001. Participants included representatives from the North Slope Borough.

#### C. Problems or Roadblocks

- a. Two roadblocks presented themselves during the past year of work on this project. One was that we were unable to obtain a stable consortium or isolate from Arctic Ocean sediments that degrades the polycyclic aromatic hydrocarbon, phenanthrene. The consortium or isolate was needed to complete bioavailability experiments. We eventually obtained a known marine cold water isolate from the laboratory of Julia Foght at the University of Alberta. The experiments were then completed in fall 2001. The second roadblock was that the master's student supported by this fellowship (K. Gannon) was in a motorcycle accident in April 2001. Her activities were very limited for about a month and somewhat limited for the rest of the summer. However, despite this obstacle we were able to complete all experiments that had been proposed. The data from these experiments, however, is still being reduced and will be included in the master's thesis being written by Ms. Gannon.

#### D. Accomplishments

- a. Surveys of nearshore marine sediment microbial communities from near Barrow locations and from near Prudhoe Bay locations were completed. Total microscopic counts of bacterial populations indicated very high populations, higher than those measured in sediments collected from more temperate regions such as Prince William Sound. Populations of total microorganisms and culturable heterotrophs were not different between Barrow and Prudhoe Bay sediments. However, significant differences were seen in culturable hydrocarbon-degrading microbial populations. Significantly higher populations were seen in some Prudhoe Bay locations for hexadecane degraders, phenanthrene degraders and crude oil emulsifiers. We do not yet understand the reasons for these differences.
- b. Mineralization potentials for hexadecane and phenanthrene were found to be low in all sediments surveyed implying that hydrocarbon-degraders, even if present, are not acclimated to mineralizing these representative hydrocarbon substrates.
- c. Despite the low organic carbon content of sediments collected near Barrow, we found substantial adsorption of the representative polycyclic hydrocarbon, phenanthrene, to particles; adsorption was rapid.

## E. Conclusions

- a. Sediments collected from nearshore sites in the Arctic Ocean had high populations of total bacteria as determined by direct microscopic counts. These populations are high relative to other sediments such as those from Prince William Sound.
- b. Interestingly, locations near Prudhoe Bay had significantly higher populations of culturable hexadecane degraders, phenanthrene degraders and crude oil emulsifiers than did locations near Barrow. These differences may be due to naturally occurring differences in these sediments rather than anthropogenic inputs since hydrocarbon chemistry data collected for sediments in the Prudhoe Bay area in 1999 for the Arctic Nearshore Impact Monitoring in the Development Area (ANIMIDA) project have not shown hydrocarbon contamination.
- c. Low mineralization potentials for hexadecane and phenanthrene imply that the microbial populations in Arctic Ocean sediments are not acclimated to use of these compounds as growth substrates.
- d. Phenanthrene rapidly adsorbed to sediments collected from near Barrow locations. It is anticipated that adsorption will decrease the bioavailability of phenanthrene to phenanthrene degraders. These studies are ongoing.

## F. Appendices

OSRI was acknowledged as a funding source for all talks presented on this research in 2001.

- a. Annual Report to the Coastal Marine Institute (CMI) 2000 (Most data collected prior to support from OSRI).
- b. Annual Report to the Coastal Marine Institute (CMI) 2001.
- c. Abstract to the Alaska Branch of the American Society 17<sup>th</sup> Annual Meeting, Anchorage, April 2001.
- d. Abstract for Minerals Management Information Transfer Meeting, Anchorage, April 2001.

**Appendix A**  
**CMI Annual Report**  
**September 2000**

**Petroleum Hydrocarbon Degrading Microbial Communities in Beaufort Sea Sediments**  
by Joan F. Braddock and Kathleen A. Gannon

**Abstract**

Despite large-scale development on the North Slope, no recent studies examining Alaskan arctic marine sediment microbial communities and their ability to metabolize petroleum compounds have been published. Microbial degradation of spilled petroleum hydrocarbons is a major mechanism of removal of these compounds from the environment. In this study we initiated a survey of marine sediment microbial populations in the Arctic Ocean to determine what microorganisms are present and what their metabolic capability is for degradation of various petroleum hydrocarbons. We also began a series of laboratory studies to examine the bioavailability of the polycyclic aromatic hydrocarbon, phenanthrene, to microorganisms in the presence of sediments collected near Barrow, Alaska. In our survey we found high total numbers of microorganisms in the sediments examined (about  $10^9$  cells/g dry wt. sediment). Only about  $10^6$  of these organisms were culturable in a marine heterotroph medium. Most probable numbers of culturable phenanthrene and hexadecane degraders were fairly high, about  $10^4$ /g dry wt. sediment each. Mineralization potentials were low for both hexadecane and phenanthrene. Despite the low organic carbon content of these sediments ( $\leq 1.5\%$ ), substantial adsorption to particles occurred; adsorption was rapid. Laboratory bioavailability studies using these same sediments are ongoing. The results of this study will be useful in predicting the fate of spilled petroleum hydrocarbons in the Arctic Ocean.

**Introduction**

The response to recent lease sales in the Beaufort Sea suggests that Beaufort Sea offshore oil development projects will be increasing in number and frequency over the upcoming decade. Increased offshore oil and gas production can lead to both chronic and acute additions of petroleum hydrocarbons to the Arctic marine environment. One important unanswered question in this context is “what is the long-term fate of petroleum hydrocarbons spilled in the Arctic Ocean?”

Following the *Exxon Valdez* oil spill (EVOS), the most recent high-latitude large oil spill for which published studies are available, the fate of EVOS oil was determined from the most complete and accurate mass balance of any oil spill (Wolfe et al., 1994). The most significant term in the mass balance was approximately 50% of the spilled oil that biodegraded either *in situ* on shorelines or in the water column. It is apparent that the microbial component was the most important of the mechanisms determining the long-term fate of EVOS oil in Prince William Sound (PWS). As noted by Sugai et al. (1997), prediction of hydrocarbon persistence following a spill requires systematic ecosystem-level studies of the abiotic and biotic factors influencing biodegradation. Among these many factors are nutrient availability, oxygen tension, presence of

fine-grained sediments, and presence of an acclimated community of hydrocarbon-metabolizing microbes (Leahy and Colwell, 1990).

In contrast to the many EVOS-generated studies of oil impact and fate, relatively little information is available regarding the potential fate or persistence of oil spilled in the Beaufort Sea. The marine environment of the Beaufort Sea is quite different from that in PWS, so data from the EVOS studies should be extrapolated to the Beaufort Sea environment with caution. Microbial studies conducted in the 1970s and 1980s (Atlas et al., 1978; Haines and Atlas, 1982) found that hydrocarbon-degrading microbes generally increased following oil contamination, but petroleum hydrocarbons were degraded very slowly. In general, following initial abiotic losses from their experimental systems, biodegradation of oil was limited and did not significantly alter the chemical composition of the residual oil. The authors found that oil is degraded in arctic sediments very slowly; biodegradation was only detectable after a full year of exposure of oil to the sediments. They pointed to several factors limiting biodegradation. These included limited populations of hydrocarbon-metabolizing microbes, localized high concentrations of hydrocarbons, low temperatures, unfavorable C:N and C:P ratios, low oxygen tensions and limited circulation of interstitial waters in fine-grained sediments. They also noted that abiotic weathering of the oil also was slow, with “limited loss of low molecular weight aliphatic and aromatic hydrocarbons during 2 years’ exposure.”

Despite large-scale development on the North Slope, no recent studies examining Alaskan arctic marine sediment microbial communities and their ability to metabolize petroleum compounds have been published. In year one we conducted a survey of Arctic Ocean offshore sediments near Barrow, Alaska, and evaluated the sediment microbial communities for their ability to metabolize petroleum hydrocarbons. In addition laboratory acclimation studies using field sediment were used to estimate the adaptability to hydrocarbon metabolism of extant microbial communities. Sediment samples collected were also split and archived cryogenically for potential later analysis of microbial community structure by DNA or phospholipid fatty acid analysis. Samples were also collected for laboratory-based studies on the effects of sediment sorption on bioavailability of petroleum hydrocarbons. These latter studies are designed to assess the effects of Arctic Ocean sediments on biodegradability of petroleum fractions known to be toxic (e.g., phenanthrene or other polynuclear aromatic hydrocarbon).

## **Methods and Materials**

In the first year of this project we focused our efforts on sediments collected near Barrow, Alaska. Sampling occurred 24-25 July 1999. Samples were collected in Elson Lagoon, offshore the former Naval Arctic Research Laboratory (NARL), and offshore the town site of Barrow (see Table 1 for specific locations). At each sampling location five sites were selected and at each site triplicate grabs were collected for a total of 45 sediments collected. From each grab, sediment samples were collected with a disinfected metal spoon. Each replicate sample was placed into sterile containers and kept cold until processing. Additionally a 15 mL cryovial was filled from each grab sample to be frozen and preserved to have available for genetic or fatty acid analysis at a later date. Samples for microbial analysis were chilled until being returned to the laboratory in Fairbanks. Cryovials were frozen (-20 °C) as soon as possible (within about 4 hr. after collection) and stored frozen until returned to Fairbanks at which point they were transferred to an ultracold freezer (-80 °C). The unfrozen sediments were then analyzed for existing hydrocarbon degradation potential as well as hydrocarbon degradation acclimatization potential.

Microbial population and activity assays to determine the existing potential of the sediments were initiated within two days after returning to Fairbanks. Analyses included estimates of total, heterotrophic, crude oil emulsifying, and substrate-specific degrader microbial populations, and assays for metabolic activity. Data derived from these assays was pooled (i.e., six values for each assay from each sampling site) to generate mean and standard error estimates. In addition some samples were analyzed for total carbon content at the UAF Mass Spectrometer Facility and others were analyzed for particle size composition at the University of Washington Analytical Services Laboratory (see Table 2).

Microbial population analyses included most-probable-number assays (MPNs) for crude oil emulsifiers (Brown and Braddock, 1990), marine heterotrophs (Lindstrom et al., 1991), substrate specific assays for phenanthrene and hexadecane (Braddock and McCarthy, 1996; Wrenn and Venosa, 1996; Braddock and Catterall, 1999), and total microscopic direct counts of marine microbes (Braddock et al., 1990). Activities, as well as numbers, of specific types of hydrocarbon degraders were assessed using a technique for extracting kinetic data from the cultures set up for enumeration (Lindstrom et al., 1998). Selected samples were also spiked with <sup>14</sup>C-labeled hydrocarbons in microcosms. To prepare each microcosm, 10 mL of a 1:10 sediment slurry in a mineral salts medium (Bushnell Haas; Atlas 1993) was added to a previously sterilized, 40-ml septum vial (I-Chem Research, Hayward, CA). After the microcosms were constructed, 50 µl of a 2-g/L solution (in acetone) of radiolabeled hydrocarbon was added by syringe to each vial through the septum. The resulting initial concentration of added hydrocarbon was then 100 µg per vial (10 µg/ml culture broth; radioactivity *ca.* 50,000 dpm). Substrates used (Sigma Chemical Co., St. Louis, MO) included the alkane hexadecane (1-<sup>14</sup>C-labeled), and the polynuclear aromatic hydrocarbon (PAH) phenanthrene (9-<sup>14</sup>C-labeled). Each treatment was replicated 5-fold, and killed controls were used to check for abiotic <sup>14</sup>CO<sub>2</sub> evolution. Vials were incubated at 8°C for 96 hours, killed by adding NaOH to stop respiration, and assayed for <sup>14</sup>CO<sub>2</sub> from hydrocarbon mineralization (Brown *et al.*, 1991).

To begin to understand the acclimation potential of the microbial population and to understand the effect of the presence of particles on biodegradation, enrichment flasks were set up as well abiotic microcosms to assess the adsorptive properties of the sediments collected. An additional set of

experiments in the presence of hydrocarbon degrading populations (bioavailability experiments) is ongoing and will not be reported here.

Phenanthrene solutions for adsorption experiments were prepared using [9-<sup>14</sup>C]phenanthrene (10.0 mCi/mole, 98% purity; Sigma) dissolved in spectranalyzed acetone (99.7% pure; Fisher). Fifty  $\mu$ L (ca. 50,000 dpm) of a stock solution was injected into 10 mL of the appropriate sediment slurry to yield final phenanthrene concentrations of about 1.25, 2.50, 3.75, or 5.0 ng phenanthrene/mL slurry (the solubility of phenanthrene is about 1.0  $\mu$ g/mL in pure water at 25 °C; Shaw, 1989). The chemical extractability of sorbed phenanthrene was determined for each sediment slurry concentration (1, 2%). Microbial activity was inhibited by autoclaving. Samples were spiked with 50  $\mu$ L of the appropriate [9-<sup>14</sup>C]phenanthrene stock solution to yield final concentrations as indicated for each experiment. Triplicate vials of each sediment and phenanthrene concentration were incubated as indicated depending on the experiment. No aging (0 day) samples were extracted as soon as practical after spiking the samples. All glassware were washed, combusted and autoclaved before use. To extract the phenanthrene from the samples, the vials were first centrifuged at approximately 3000  $\times$  g for 1 minutes after which 2.5 mL of the supernatant was removed. This fraction is indicated as the aqueous phase phenanthrene concentration.

## Results and Discussion

Particle size analysis indicated variability in samples in the distribution of sand, silt or clay (Table 2). In the three samples analyzed for organic carbon content, all were very low (about 1.5 % or less).

Direct counts of microorganisms in sediments collected at all sampling locations indicate healthy populations (approximately  $10^9$  cells/g dry sediment) of microorganisms present in surface sediment from Elson Lagoon and off shore Barrow and NARL (Figure 1). These numbers are consistent with total direct counts on average of  $2 \times 10^9$  cells/g dry wt. sediment reported by Kaneko et al. (1978) for the Beaufort Sea. The cultivatable marine heterotrophs were lower (on average about 3 orders of magnitude lower) than direct counts. These numbers are also consistent with Kaneko et al. (1978) who found about  $10^5$  heterotrophic microorganisms/g dry wt sediment. Phenanthrene and hexadecane degraders were between  $10^3$  and  $10^4$  cells/g dry sediment. Even after a long incubation period for the sheen screen plates (crude oil degraders) we found little to no activity present for any sediment (data not shown).

While populations of microorganisms are present in these Arctic Ocean sediments, their ability to readily degrade petroleum hydrocarbons appears to be limited. The 96-well plate kinetic data were hampered by very slow growth rates of phenanthrene and hexadecane degraders. In addition, mineralization potentials for phenanthrene and hexadecane were uniformly low (Table 3). These sediments universally showed higher potential for mineralization of the linear alkane, hexadecane, than the polycyclic aromatic hydrocarbon, phenanthrene. A further indicator of low activity is that our efforts to enrich for a consortium that both degraders hexadecane and phenanthrene have thus far had limited success. We are continuing our efforts to find an isolate or consortium appropriate for the bioavailability experiments.

To begin to understand the adsorptive properties of these Arctic Ocean sediments we initiated a series of isotherm assays (Figure 2). NARL sediment most rapidly adsorbed phenanthrene, a result consistent with the somewhat higher organic carbon content measured for this sediment (Table 2). Aging generally led to increased partitioning into the sediment fraction. The adsorption isotherms were used to calculate partition coefficients ( $K_p$ ) for under various experimental conditions (Table 4). The NARL sediment was clearly the most adsorptive of the three sediments examined.

These results indicate that sediment microbial populations have not changed appreciably since 1976. High numbers of microorganisms exist in these sediments, many of which were culturable with either hexadecane or phenanthrene supplied as a sole carbon source. However, mineralization potentials were low relative to other sites (e.g. Prince William Sound Alaska; Braddock et al., 1990) indicating that the population may only slowly acclimate to biodegradation of these hydrocarbon substrates. Adsorption isotherm experiments with the polycyclic aromatic hydrocarbon, phenanthrene, indicate rapid and extensive adsorption. The three sediments examined in these experiments did show differences in their adsorptive properties. This is likely due in part to differences in organic carbon content and to other unidentified differences among the sediments. Bioavailability experiments are ongoing with these sediments. In addition we have recently collected about 30 samples from offshore Prudhoe Bay and will use these sediments to continue our survey to characterize the sediment microbial community in the Arctic Ocean.

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Table 1. Sampling locations for this study were Elson Lagoon (E), offshore the former Naval Arctic Research Laboratory (NARL; N), and offshore Barrow (B). Three grabs were collected at each of five sampling sites at each location. Latitudes and longitudes were recorded from a handheld GPS unit once at the Elson Lagoon sampling sites and each time a grab was taken at the offshore NARL and Barrow sites.

<b>Sampling Location</b>	<b>Latitude</b>	<b>Longitude</b>	<b>Approximate Depth (m)</b>
E 1	71 <sup>0</sup> 20.035 N	156 <sup>0</sup> 33.584 W	1.8
E 2	71 <sup>0</sup> 19.176 N	156 <sup>0</sup> 32.473 W	2
E 3	71 <sup>0</sup> 17.529 N	156 <sup>0</sup> 24.707 W	3-4
E 4	71 <sup>0</sup> 22.824 N	156 <sup>0</sup> 26.775 W	3
E 5	71 <sup>0</sup> 21.176 N	156 <sup>0</sup> 30.889 W	2-3
N 1-1	71 <sup>0</sup> 20.000 N	156 <sup>0</sup> 42.878 W	10
N 1-2	71 <sup>0</sup> 19.957 N	156 <sup>0</sup> 42.973 W	
N 1-3	71 <sup>0</sup> 19.942 N	156 <sup>0</sup> 42.986 W	
N 2-1	71 <sup>0</sup> 20.025 N	156 <sup>0</sup> 42.356 W	8
N 2-2	71 <sup>0</sup> 19.944 N	156 <sup>0</sup> 42.523 W	
N 2-3	71 <sup>0</sup> 20.006 N	156 <sup>0</sup> 42.689 W	
N 3-1	71 <sup>0</sup> 19.976 N	156 <sup>0</sup> 41.519 W	6
N 3-2	71 <sup>0</sup> 19.986 N	156 <sup>0</sup> 41.572 W	
N 3-3	71 <sup>0</sup> 19.986 N	156 <sup>0</sup> 41.572 W	
N 4-1	71 <sup>0</sup> 19.880 N	156 <sup>0</sup> 40.975 W	5
N 4-2	71 <sup>0</sup> 19.871 N	156 <sup>0</sup> 41.009 W	
N 4-3	71 <sup>0</sup> 19.863 N	156 <sup>0</sup> 41.031 W	
N 5-1	71 <sup>0</sup> 19.811 N	156 <sup>0</sup> 40.606 W	5
N 5-2	71 <sup>0</sup> 19.811 N	156 <sup>0</sup> 40.634 W	
N 5-3	71 <sup>0</sup> 19.808 N	156 <sup>0</sup> 40.634 W	
B 1-1	71 <sup>0</sup> 18.109 N	156 <sup>0</sup> 46.089 W	3
B 1-2	71 <sup>0</sup> 18.110 N	156 <sup>0</sup> 46.084 W	
B 1-3	71 <sup>0</sup> 18.111 N	156 <sup>0</sup> 46.074 W	
B 2-1	71 <sup>0</sup> 18.175 N	156 <sup>0</sup> 46.494 W	4
B 2-2	71 <sup>0</sup> 18.174 N	156 <sup>0</sup> 46.502 W	
B 2-3	71 <sup>0</sup> 18.174 N	156 <sup>0</sup> 46.502 W	
B 3-1	71 <sup>0</sup> 18.305 N	156 <sup>0</sup> 47.042 W	7
B 3-2	71 <sup>0</sup> 18.279 N	156 <sup>0</sup> 47.164 W	
B 3-3	71 <sup>0</sup> 18.274 N	156 <sup>0</sup> 47.162 W	
B 4-1	71 <sup>0</sup> 18.067 N	156 <sup>0</sup> 47.419 W	7
B 4-2	71 <sup>0</sup> 18.067 N	156 <sup>0</sup> 47.419 W	
B 4-3	71 <sup>0</sup> 18.067 N	156 <sup>0</sup> 47.420 W	
B 5-1	71 <sup>0</sup> 18.260 N	156 <sup>0</sup> 47.992 W	14
B 5-2	71 <sup>0</sup> 18.118 N	156 <sup>0</sup> 47.880 W	10
B 5-3	71 <sup>0</sup> 18.102 N	156 <sup>0</sup> 47.904 W	14

Table 2. Particle size analysis and percent carbon content of selected Arctic Ocean sediment samples.

Sampling Location	%C	Size Class (%)			
		rock > 2 mm	sand 2-0.05 mm	silt 0.05-0.002 mm	clay <0.002 mm
Elson Lagoon					
Site 1	0.088, 0.093	X	92.5	1.25	6.25
Site 2		X	55.0	20.75	16.25
Site 3		X	54.0	10.0	36.0
Site 4		X	3.75	43.75	52.5
Site 5		X	46.25	27.5	26.25
Offshore NARL					
Site 1	1.59, 1.50	X	14.0	54.0	32.0
Site 2		X	26.0	52.0	22.0
Site 3		X	74.0	10.0	16.0
Site 4		X	93.75	2.50	3.75
Site 5		X	96.25	2.50	1.25
Offshore Barrow					
Site 1	0.044, 0.044	9.22	79.36	2.27	9.07
Site 2		X	86.2	5.05	8.75
Site 3*		X, X, X	83.75, 70.0, 58.0	3.75, 10.0, 20.0	12.5, 20.0, 22.0
Site 4		22.6	26.3	18.6	32.5
Site 5		X	50.0	24.0	26.0

\*Five samples (grabs) were collected at each site. Only one sample was analyzed for particle size except at Barrow site 3 where three different samples were analyzed to assess variability at a given site.

Table 3. Mineralization potentials of Arctic Ocean sediments collected August 1999. Values shown are the mean ( $\pm$  SE) of five replicate microcosms.

Location Sampled	Mineralization Potential (ng substrate mineralized/g dry wt. sediment)	
	Hexadecane	Phenanthrene
Elson Lagoon		
Site 1	46 $\pm$ 4	12 $\pm$ 1
Site 2	90 $\pm$ 5	21 $\pm$ 2
Site 3	56 $\pm$ 2	15 $\pm$ 2
Site 4	127 $\pm$ 6	30 $\pm$ 4
Offshore NARL		
Site 1	100 $\pm$ 15	15 $\pm$ 2
Site 2	86 $\pm$ 5	15 $\pm$ 3
Site 3	62 $\pm$ 7	13 $\pm$ 1
Site 4	59 $\pm$ 4	19 $\pm$ 1
Offshore Barrow		
Site 1	57 $\pm$ 2	12 $\pm$ 1
Site 2	38 $\pm$ 4	7 $\pm$ 1
Site 3	35 $\pm$ 4	11 $\pm$ 1
Site 4	134 $\pm$ 4	39 $\pm$ 2
Site 5	51 $\pm$ 4	18 $\pm$ 2

Table 4. Partition coefficients (Kp) for phenanthrene in experiments using 1 or 2% sediment slurries.

Location Sampled	Reaction Time	Kp (mL/g)	
		1% Sediment	2% Sediment
Elson Lagoon	1 minute	26.7	11.9
	2 hours	40.1	28.5
	2 days	64.2	50.3
	4 days	ND	74.7
Offshore NARL	15 minutes	170	231
	2 hours	774	825
	2 days	2130	1610
	4 days	2410	4590
Offshore Barrow	1 minute	35.5	16.8
	2 hours	81.4	48.1
	2 days	49.2	45.0
	4 days	ND	58.9

ND = no data as experiment was not performed under those conditions.

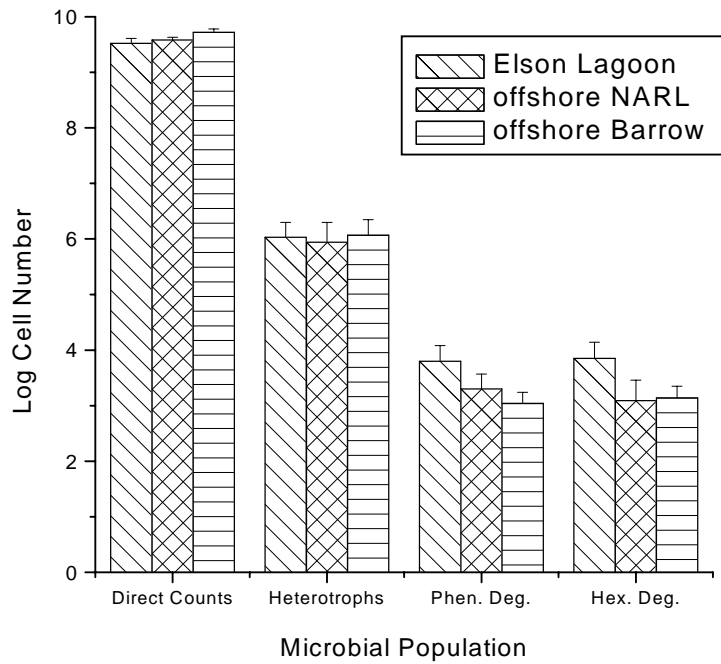


Figure 1. Microscopic direct counts and most probable numbers of heterotrophs, phenanthrene degraders, and hexadecane degraders from sediments collected near Barrow, Alaska in July 1999. Data represent the mean of 15 sediments from each sampling location (five sites per locations and three grabs per site)  $\pm$  one standard error.

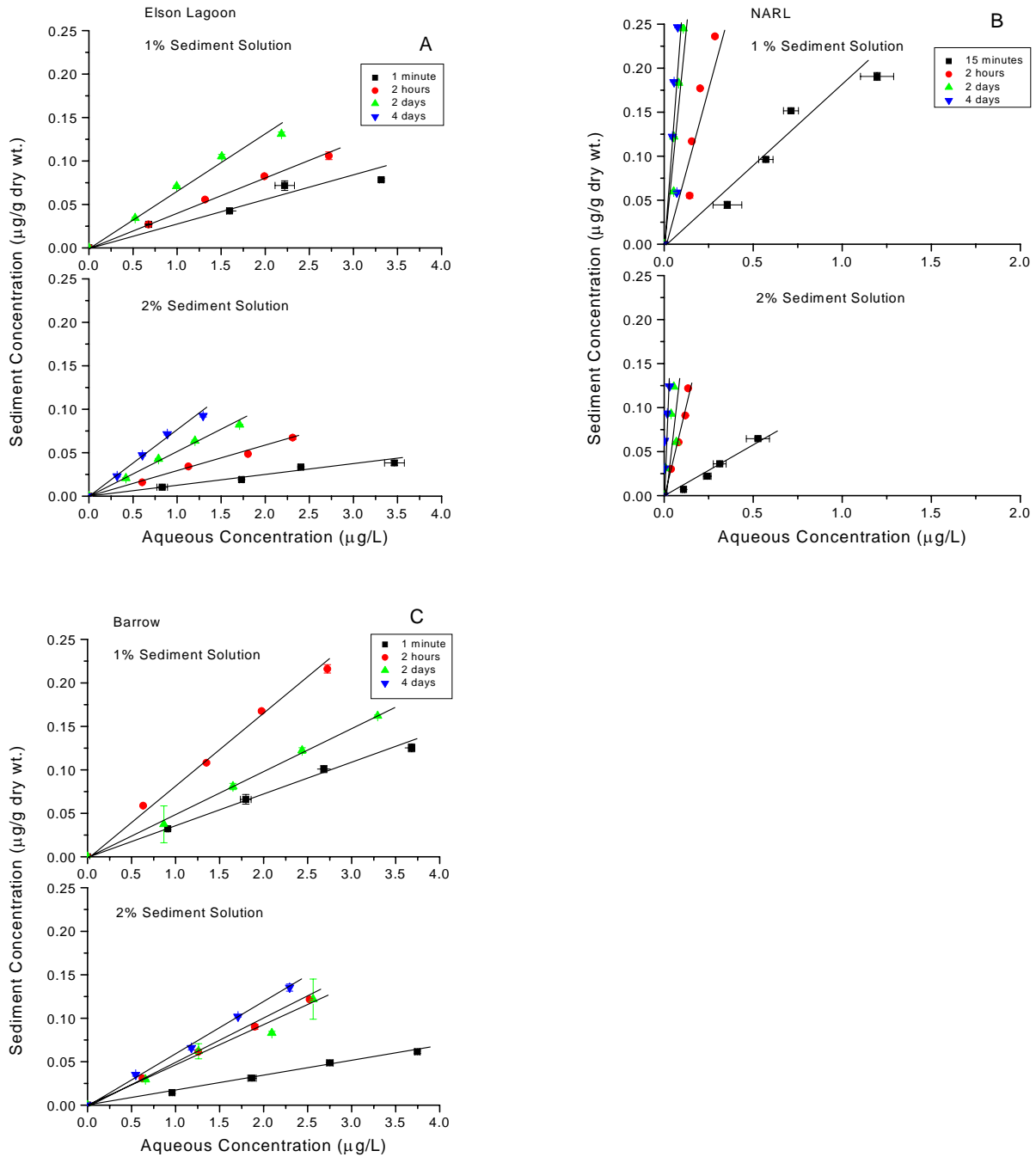


Figure 2. Phenanthrene in solution versus phenanthrene associated with sediment particles in after various reaction times for sediments collected near Barrow, Alaska in July 1999. Experiments were conducted in microcosms with either 1 or 2% sediment slurries. Data points are the mean of triplicates  $\pm$  one standard error.

**Appendix B**  
**CMI Annual Report**  
**September 2001**

**Petroleum Hydrocarbon Degrading Microbial Communities in Beaufort Sea Sediments**

by Joan F. Braddock and Kathleen A. Gannon

**Abstract**

Despite large-scale development on the North Slope, no recent studies examining Alaskan arctic marine sediment microbial communities and their ability to metabolize petroleum compounds have been published. Microbial degradation of spilled petroleum hydrocarbons is a major mechanism of removal of these compounds from the environment. In this study we conducted the second year of a survey (first year near Barrow and second year near Prudhoe Bay) of marine sediment microbial populations in the Arctic Ocean to determine what microorganisms are present and what their metabolic capability is for degradation of various petroleum hydrocarbons. We are also examining the effects of sediment on the bioavailability of a polycyclic aromatic hydrocarbon (phenanthrene) to hydrocarbon degrading bacteria. In our survey we found high total numbers of microorganisms in the all sediments examined (about  $10^{10}$  cells/g dry wt. sediment). Only about  $10^6$ - $10^8$  of these organisms were culturable in a marine heterotroph medium. Most probable numbers of culturable phenanthrene and hexadecane degraders were fairly high, about  $10^3$ - $10^5$ /g dry wt. sediment each, and populations of both types of organisms were significantly higher offshore Prudhoe Bay than offshore Barrow. In addition culturable crude oil degraders were significantly greater offshore Prudhoe Bay than Barrow. It is likely, but not yet determined, that these differences are due to naturally occurring differences in these sediments rather than anthropogenic inputs. Mineralization potentials were low for both hexadecane and phenanthrene at both geographic locations. Despite the low organic carbon content of these sediments ( $\leq 1.5\%$ ), substantial adsorption to particles occurred; adsorption was rapid. Laboratory bioavailability studies using these same sediments are ongoing. The results of this study will be useful in predicting the fate of spilled petroleum hydrocarbons in the Arctic Ocean.

**Introduction**

The response to recent lease sales in the Beaufort Sea suggests that Beaufort Sea offshore oil development projects will be increasing in number and frequency over the upcoming decade. Increased offshore oil and gas production can lead to both chronic and acute additions of petroleum hydrocarbons to the Arctic marine environment. One important unanswered question in this context is “what is the long-term fate of petroleum hydrocarbons spilled in the Arctic Ocean?”

Biodegradation often is a significant factor in removal of spilled petroleum hydrocarbons from the environment. For example, following the *Exxon Valdez* oil spill (EVOS) it was estimated that, by 1992, approximately 50% of the spilled oil had biodegraded either *in situ* on shorelines or in the water column (Wolfe *et al.*, 1994). In contrast to the many EVOS-generated studies of oil impact and fate, relatively little information is available regarding the potential fate or persistence of oil spilled in the Beaufort Sea. The marine environment of the Beaufort Sea is quite different from

that in Prince William Sound, so data from the EVOS studies should be extrapolated to the Beaufort Sea environment with caution.

Microbial studies conducted in the 1970s and 1980s (Atlas *et al.*, 1978; Haines and Atlas, 1982) found that hydrocarbon-degrading microbes generally increased following oil contamination, but petroleum hydrocarbons were degraded very slowly. In general, following initial abiotic losses from their experimental systems, biodegradation of oil was limited and did not significantly alter the chemical composition of the residual oil. They pointed to several factors limiting biodegradation. These included limited populations of hydrocarbon-metabolizing microbes, localized high concentrations of hydrocarbons, low temperatures, unfavorable C:N and C:P ratios, low oxygen tensions and limited circulation of interstitial waters in fine-grained sediments. They also noted that abiotic weathering of the oil also was slow, with “limited loss of low molecular weight aliphatic and aromatic hydrocarbons during 2 years’ exposure.”

No recent studies examining Alaskan arctic marine sediment microbial communities and their ability to metabolize petroleum compounds have been published. In year one we conducted a survey of Arctic Ocean offshore sediments near Barrow, Alaska, and evaluated the sediment microbial communities for their ability to metabolize petroleum hydrocarbons. In addition laboratory acclimation studies using field sediment were used to estimate the adaptability to hydrocarbon metabolism of extant microbial communities. Sediment samples collected were also split and archived cryogenically for potential later analysis of microbial community structure by DNA or phospholipid fatty acid analysis. Samples were also collected for laboratory-based studies on the effects of sediment sorption on bioavailability of petroleum hydrocarbons. These latter studies are designed to assess the effects of Arctic Ocean sediments on biodegradability of petroleum fractions known to be toxic (e.g., phenanthrene or other polynuclear aromatic hydrocarbon). In year two we continued the study collecting samples in coordination with the Arctic Nearshore Impact Monitoring in the Development Area (ANIMIDA) project at sites near Prudhoe Bay. This report briefly highlights our work to date focusing on the results from year two. The results of this study will be the basis of a master’s degree project in Environmental Chemistry.

## **Methods and Materials**

In the second year of this project we focused our efforts on sediments collected near Prudhoe Bay, Alaska. Sampling was conducted by Arthur D. Little, Inc. (in coordination with the ANIMIDA project) from 19-25 August 2000 (see Table 1 for specific locations). From each grab, sediment samples were collected with a disinfected metal spoon and placed into sterile containers and kept cold until processing. Additionally a 15 mL cryovial was filled from each grab sample to be frozen and preserved to have available for genetic or fatty acid analysis at a later date. Samples for microbial analysis were chilled until being returned to the laboratory in Fairbanks. Cryovials were frozen (-20 °C) as soon as possible (within about 4 hr. after collection) and stored frozen until returned to Fairbanks at which point they were transferred to an ultracold freezer (-80 °C). The unfrozen sediments were then analyzed for existing populations of microorganisms and hydrocarbon degradation potentials.

Microbial population and activity assays to determine the existing potential of the sediments were initiated within two days after returning to Fairbanks. Analyses included estimates of total, heterotrophic, crude oil emulsifying, and substrate-specific degrader microbial populations, and assays for metabolic activity. Data derived from these assays was pooled to generate mean and standard error estimates for each location (see Table 1).

Microbial population analyses included most-probable-number assays (MPNs) for crude oil emulsifiers (Brown and Braddock, 1990), marine heterotrophs (Lindstrom *et al.*, 1991), substrate specific assays for phenanthrene and hexadecane (Braddock and McCarthy, 1996; Wrenn and Venosa, 1996; Braddock and Catterall, 1999), and total microscopic direct counts of marine microbes (Braddock *et al.*, 1990). Mineralization potentials for phenanthrene and hexadecane were also determined using  $^{14}\text{C}$ -labeled hydrocarbons in microcosms. To prepare each microcosm, 10 mL of a 1:10 sediment slurry in a mineral salts medium (Bushnell Haas; Atlas 1993) was added to a previously sterilized, 40-ml septum vial (I-Chem Research, Hayward, CA). After the microcosms were constructed, 50  $\mu\text{l}$  of a 2-g/L solution (in acetone) of radiolabeled hydrocarbon was added by syringe to each vial through the septum. The resulting initial concentration of added hydrocarbon was then 100  $\mu\text{g}$  per vial (10  $\mu\text{g}/\text{ml}$  culture broth; radioactivity *ca.* 50,000 dpm). Substrates used (Sigma Chemical Co., St. Louis, MO) included the alkane hexadecane (1- $^{14}\text{C}$ -labeled), and the polynuclear aromatic hydrocarbon (PAH) phenanthrene (9- $^{14}\text{C}$ -labeled). Each treatment was replicated 3-fold, and killed controls were used to check for abiotic  $^{14}\text{CO}_2$  evolution. Vials were incubated at 8°C for 96 hours, killed by adding NaOH to stop respiration, and assayed for  $^{14}\text{CO}_2$  from hydrocarbon mineralization (Brown *et al.*, 1991).

## Results and Discussion

Direct counts of microorganisms in sediments collected at all sampling locations indicate high populations (approximately  $10^9$ - $10^{10}$  cells/g dry sediment) of microorganisms present in surface sediment from all sample locations (results not shown). There were no significant differences among any of the sites where samples were collected. These numbers are consistent with total direct counts, on average of  $2 \times 10^9$  cells/g dry wt. sediment, reported by Kaneko *et al.* (1978) for the Beaufort Sea. The cultivatable marine heterotrophs were lower (on average about 3 orders of magnitude lower) than direct counts (Figure 1). These numbers are also consistent with Kaneko *et al.* (1978) who found about  $10^5$  heterotrophic microorganisms/g dry wt sediment in samples from the Beaufort Sea. There were no statistically significant differences among locations sampled either near Barrow or near Prudhoe Bay for either total direct counts or culturable heterotrophs.

Phenanthrene and hexadecane degrader populations ranged between  $10^3$  and  $10^4$  cells/g dry sediment. For both carbon substrates, samples collected offshore the townsite of Barrow and offshore the former Naval Arctic Research Laboratory (NARL) had significantly lower populations than samples collected in Elson Lagoon or near Prudhoe Bay (Figure 1). In addition, the culturable population growing on Prudhoe Bay crude oil (oil degraders) was greater at all sites near Prudhoe Bay than at sites near Barrow (including Elson Lagoon)(Figure 1). The reasons for the results are at present unclear. However, it is likely that differences in sediment properties or naturally occurring carbon substrates may account for the results obtained. Since our samples were co-collected with samples, which will be extensively analyzed for physical and chemical properties (ANIMIDA project), we should be able to determine if hydrocarbon chemistry or other

measured properties can account for differences seen among the sites. Based on the data reported in the Draft Final Report for the ANIMIDA project from 1999, we predict that the higher populations of hydrocarbon-degrading microorganisms in samples collected near Prudhoe Bay are more likely due to naturally occurring phenomena rather than anthropogenic hydrocarbon inputs.

While populations of microorganisms are present in these Arctic Ocean sediments, their ability to readily degrade petroleum hydrocarbons appears to be limited. As was seen for samples collected near Barrow in year one of this project (see Braddock and Gannon, 2000), mineralization potentials for phenanthrene and hexadecane were uniformly low in sediments collected near Prudhoe Bay (Table 2). These sediments universally showed higher potentials for mineralization of the linear alkane, hexadecane, than the polycyclic aromatic hydrocarbon, phenanthrene. A further indicator of low activity is that our efforts to enrich for a consortium that both degrades hexadecane and phenanthrene have been difficult. However, we have recently acquired both a consortium and isolates that have high levels of activity for phenanthrene and hexadecane.

The results of this study indicate that sediment microbial populations have not changed appreciable since 1976. High numbers of microorganisms exist in these sediments, many of which were culturable with either hexadecane or phenanthrene supplied as a sole carbon source. But, interestingly, there were significant differences in populations among sites with Prudhoe Bay sites generally having higher populations than those seen for sites near Barrow. However, mineralization potentials were low at all sites sampled relative to other sites (e.g. Prince William Sound Alaska; Braddock *et al.*, 1990) indicating that the populations may only slowly acclimate to biodegradation of these hydrocarbon substrates. Adsorption isotherm experiments with the polycyclic aromatic hydrocarbon, phenanthrene, indicate rapid and extensive adsorption (see Braddock and Gannon, 2000). The sediments examined in these experiments did show differences in their adsorptive properties. This is likely due in part to differences in organic carbon content and to other unidentified differences among the sediments. Bioavailability experiments are ongoing with these sediments. Finally, as the results of the ANIMIDA survey from summer 2000 become available, we will hopefully be able to determine what factors might lead to the elevated populations of hydrocarbon degrading microorganisms we saw in samples collected near Prudhoe Bay. The results of this study will be useful in predicting the fate of spilled hydrocarbons in the Arctic Ocean. This study has supported the master's thesis work of K. Gannon who anticipates defending her thesis for a degree in Environmental Chemistry from the University of Alaska Fairbanks in winter 2000.

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Table 1. Locations and depths of samples collected in Aug. 2000 near Prudhoe Bay.

<b>Sampling Sites Grouped by Location</b>	<b>Latitude</b>	<b>Longitude</b>	<b>Approximate Depth (m)</b>
<b>Liberty</b>			
L04	70° 17.032	147° 39.897	5
L06	70° 16.881	147° 33.978	7
L07	70° 16.789	147° 31.966	7
L08	70° 16.701	147° 30.298	6
L09	70° 16.568	147° 27.130	7
<b>Northstar</b>			
N 12	70° 27.321	148° 42.078	6
N 13	70° 27.004	148° 43.552	5
N 14	70° 25.978	148° 40.459	4
N 15	70° 26.710	148° 44.570	2
N 18	70° 29.082	148° 42.151	11
N 21	70° 26.819	148° 40.587	5
N 22	70° 29.340	148° 41.868	9
N 23	70° 29.340	148° 41.868	11
5 F	70° 26.486	148° 49.550	2
<b>East of Liberty</b>			
3 A	70° 16.988	147° 05.470	7
3 B	70° 17.917	147° 02.549	5
<b>Boulder Patch</b>			
4 A	70° 18.460	147° 40.289	5
4 B	70° 21.034	147° 40.007	7
L01	70° 18.930	147° 27.130	7
<b>Between Liberty &amp; Northstar</b>			
4 C	70° 26.144	147° 42.957	9
5 D	70° 24.488	148° 33.605	2
5 H	70° 22.210	147° 47.744	7
5 (0)	70° 22.210	148° 47.744	5
5 (1)	70° 25.024	148° 03.569	6
5 (5)	70° 26.106	148° 18.127	7
5 (10)	70° 27.323	148° 29.980	8
<b>Colville River</b>			
COL-01	70° 15.96	150° 49.29	
COL-02	70° 11.36	150° 52.12	
<b>Kuparuk River</b>			
KUP-01	70° 17.70	148° 59.37	
KUP-02	70° 17.70	148° 59.37	
<b>Sag. River</b>			
SAG-01	70° 01.68	148° 33.77	

Table 2. Mineralization potentials, 96 hour assays, for hexadecane and phenanthrene for samples collected Aug. 2000.

Sampling Location	Mineralization Potential (ng substrate mineralized /g dry wt sediment)	
	Hexadecane	Phenanthrene
<b>Liberty</b>		
L04	53 ± 8	20 ± 5
L06	84 ± 11	30 ± 2
L07	61 ± 8	27 ± 2
L08	42 ± 19	38 ± 8
L09	62 ± 6	19 ± 3
<b>Northstar</b>		
N 12	65 ± 19	35 ± 5
N 13	103 ± 19	38 ± 4
N 14	122 ± 7	48 ± 4
N 15	45 ± 4	14 ± 1
N 18	71 ± 13	27 ± 1
N 21	157 ± 9	53 ± 3
N 22	44 ± 4	13 ± 0
N 23	81 ± 11	33 ± 1
5 F	97 ± 9	32 ± 3
<b>East of Liberty</b>		
3 A	81 ± 14	34 ± 2
3 B	67 ± 5	28 ± 2
<b>Boulder Patch</b>		
4 A	64 ± 7	27 ± 2
4 B	99 ± 6	52 ± 5
L01	63 ± 4	40 ± 4
<b>Between Liberty &amp; Northstar</b>		
4 C	55 ± 10	31 ± 2
5 D	81 ± 13	36 ± 3
5 H	50 ± 8	26 ± 2
5 (0)	72 ± 8	30 ± 2
5 (1)	73 ± 8	31 ± 2
5 (5)	59 ± 7	30 ± 2
5 (10)	27 ± 8	21 ± 2
<b>Colville River</b>		
COL-01	35 ± 0	15 ± 1
COL-02	46 ± 4	19 ± 3
<b>Kuparuk River</b>		
KUP-01	58 ± 10	19 ± 1
KUP-02	133 ± 10	21 ± 2
<b>Sag River</b>		
SAG-01	50 ± 4	19 ± 1

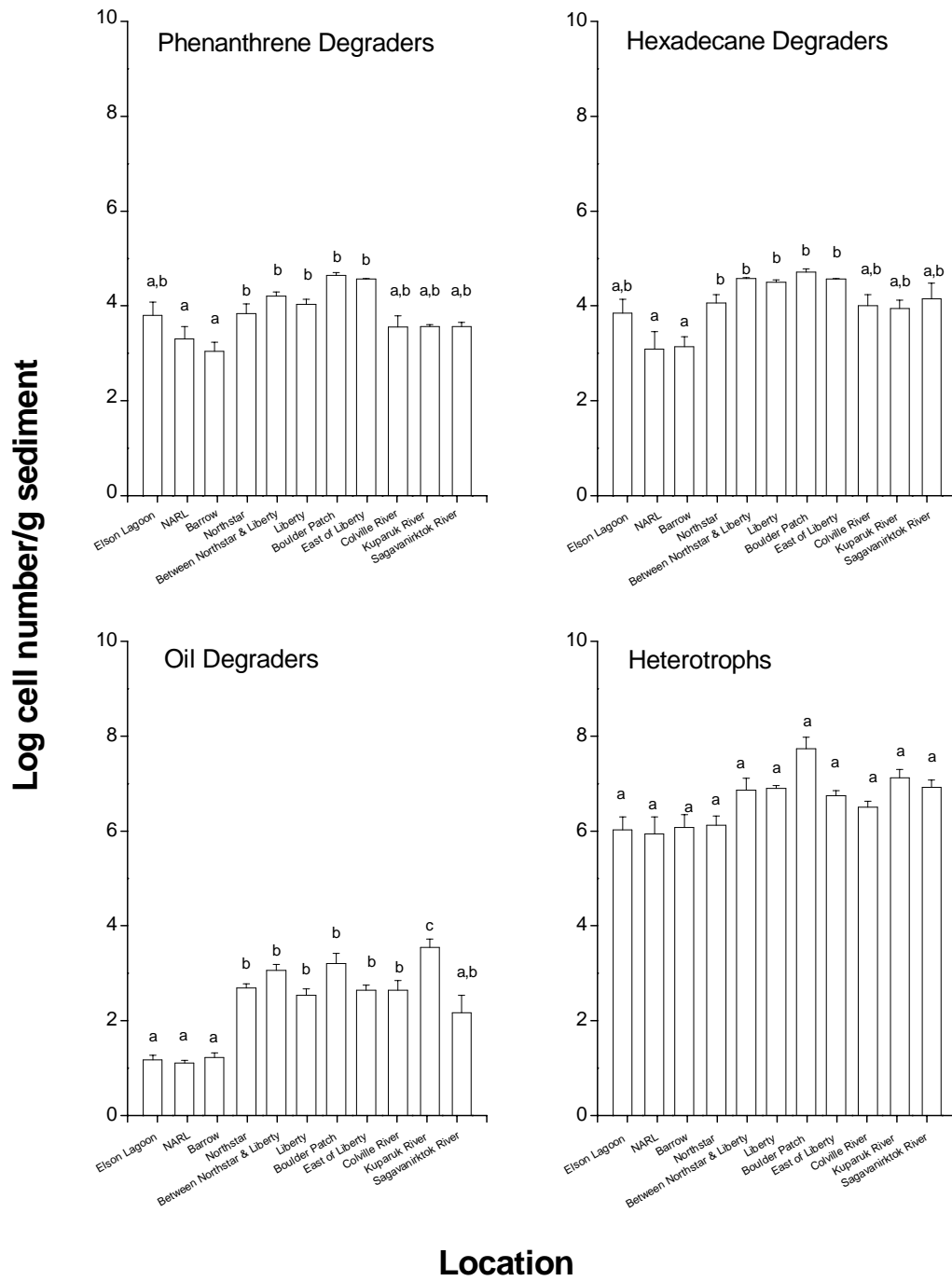


Figure 1. Populations of culturable microorganisms in sediments collected from sites near Barrow and near Prudhoe Bay, AK. Different letters appearing above the bars indicate significant differences among sites. Values represent the mean  $\pm$  SE.

## **Appendix C**

Abstract of paper presented at the 17<sup>th</sup> Annual Meeting of the American Society for Microbiology, Anchorage, April 2001.

Petroleum Hydrocarbon Degrading Microbial Communities in Beaufort Seas Sediments  
Kathleen Gannon and Joan Braddock

There have been no published studies on microbial populations in Beaufort Sea sediment since the 1970's when oil reserves were first developed. However, a number of factors can change the size and composition of the microbial community with time, including potentially the oil development that has occurred in the past 25 years. Enumerations of the microbial communities of Chukchi and Beaufort Sea sediment were conducted for total bacteria, viable heterotrophs, hexadecane degraders, phenanthrene degraders, and crude oil degraders. In addition, the mineralization potentials for phenanthrene and hexadecane were determined for the different communities by radiorespirometry. We also examined the partition coefficients,  $K_p$ , for sediment with varied composition, from three Barrow locations, using the polycyclic aromatic hydrocarbon (PAH), phenanthrene. We found that even though the total bacterial counts were relatively similar between the Chukchi and Beaufort Seas, all of the other populations were significantly higher in the Beaufort Sea around Prudhoe Bay than they were in the Chukchi Sea around Barrow. Despite the location, the mineralization potentials for both hexadecane and phenanthrene were low with hexadecane exhibiting a slightly higher potential. We found that the partition coefficients of phenanthrene decreased as the organic carbon content of the sediment decreased, but for the organic poor sediments, the percent of clay mineral appeared to govern the  $K_p$  pattern with higher content increasing the affinity of phenanthrene for the sediment. These data provide a useful monitor of the populations of microorganisms occurring in these sediments as oil development has progressed since the 1970's. The study also provides information that will help assess the fate and effects of petroleum hydrocarbons in this environment if a spill were to occur.

## Appendix D

Abstract from paper presented at the Minerals Management Information Transfer Meeting, Anchorage, April 2001.

### PETROLEUM HYDROCARBON DEGRADING MICROBIAL COMMUNITIES IN BEAUFORT SEA SEDIMENTS

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There have been no published studies on hydrocarbon degrading populations and activities of microorganisms in Beaufort Sea sediment since the 1970's when oil reserves were first developed. In samples collected in 1999 and 2000, we enumerated populations of total bacteria, viable heterotrophs, and hexadecane (a linear alkane) and phenanthrene (a polycyclic aromatic hydrocarbon) degraders in Chukchi and Beaufort Sea sediments. In addition, we used radiorespirometry to measure mineralization potentials for phenanthrene and hexadecane, and examined the partition coefficients,  $K_p$ , for phenanthrene in sediments with different physical and chemical properties. We found that even though the total bacterial counts were relatively similar between the Chukchi and Beaufort Seas, all of the other microbial populations measured were significantly higher in the Beaufort Sea around Prudhoe Bay than they were in the Chukchi Sea around Barrow. Despite the location, mineralization potentials for both hexadecane and phenanthrene were low with hexadecane exhibiting slightly higher potentials at most locations. These results indicate that the populations present are not acclimated to use of these hydrocarbons. As expected, we found the partition coefficients for phenanthrene increased as the organic carbon content of the sediment increased, but for the organic poor sediments, other properties of the sediment also affected adsorption. These data provide a useful monitor of the populations of microorganisms occurring in these sediments as oil development has progressed since the 1970's. The study also provides information that will help assess the fate and effects of petroleum hydrocarbons in this environment if a spill were to occur.