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Environmental Assessment of the Alaskan Continental Shelf

Annual Reports of Principal Investigators for the year ending March 1980

Volume II: Receptors- Microbiology Ecological Processes

U.S. DEPARTMENT OF COMMERCE
National Oceanic & Atmospheric Administration
Office of Marine Pollution Assessment

U.S. DEPARTMENT OF INTERIOR
Bureau of Land Management

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ACKNOWLEDGEMENT

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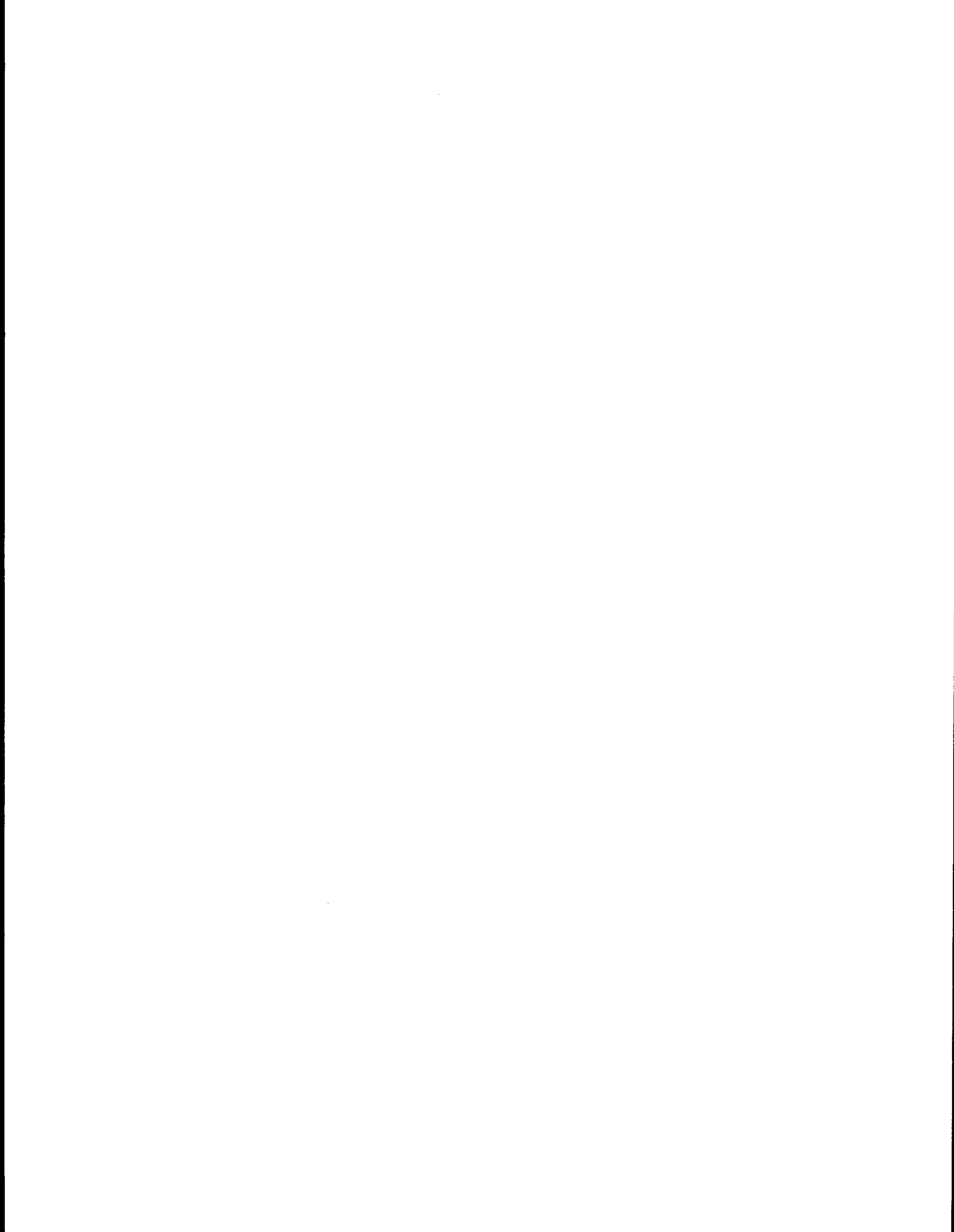

RECEPTORS-MICROBIOLOGY

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ANNUAL REPORT

Assessment of Potential Interactions of Microorganisms and Pollutants Resulting from Petroleum Development on the Outer Continental Shelf of Alaska

RU #29

Contract #03-5-022-85

April 1, 1980

Submitted by:

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Prepared for:

Outer Continental Shelf Energy Assessment Program
National Oceanic and Atmospheric Administration

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Consultants:	Micah Krichevsky Elaine Krichevsky

I. Objectives - Summary

A. In the Beaufort Sea to assess the potential interaction of microorganisms and pollutants produced by petroleum development on the outer continental shelf. Specifically:

1) To examine the biodegradation of petroleum hydrocarbons in Beaufort Sea sediment.
2) To examine the biodegradation and weathering of petroleum in and under sea ice.

B. To determine the distribution of microbiological populations in Cook Inlet and adjacent areas and assess the potential interactions of microorganisms and pollutants produced by petroleum development on the outer continental shelf. Specifically:

1) To determine distribution and densities of microbial populations in sediment and water relative to potential inputs of hydrocarbons from sources in Upper Cook Inlet.
2) To determine the potential rates of biodegradation of petroleum hydrocarbons in water and sediment.
3) To examine denitrification processes in sediment.

C. To determine the distribution of microbiological populations in Norton Sound and to assess the potential interactions of microorganisms and petroleum hydrocarbons. Specifically:

1) To determine if a natural oil seepage in Norton Sound has altered the density of taxonomic distribution of microorganisms.
2) To determine if a natural oil seepage in Norton Sound has altered potential rates of hydrocarbon biodegradation.
3) To examine natural rates of denitrification in the Bering Sea and to determine if a natural oil seepage in Norton Sound has altered potential rates of denitrification.

II. Introduction - Scope of Work

This study is a continuation of an effort to characterize microbial populations and the ability of microorganisms to biodegrade petroleum hydrocarbons in proposed Alaskan OCS oil and gas lease areas. The approach has been to determine the distribution and population levels of several microbiological groups, e.g. hydrocarbon degraders within a geographic area, to extensively characterize selected microorganisms and using numerical taxonomy to determine the diversity of the microbial community and an inventory of the dominant microbial taxa within the geographic area and to examine rates of denitrification. During this year studies were conducted in Cook Inlet and the Bering Sea.

Analyses were performed to follow the chemical changes in crude oil as it undergoes biotic (biodegradation) and abiotic (physical and chemical) weathering in experimentally oiled sediment in the Beaufort Sea.

III. Current State of Knowledge

The state of knowledge concerning microbial populations and hydrocarbon biodegradation in Alaskan OCS areas has been summarized in previous annual reports and in reports of synthesis meetings. New information developed from this project is described below.

IV. Study Area

Studies were conducted in Cook Inlet, Beaufort Sea and Bering Sea (including Norton Sound) regions. The sampling sites used during this study are shown in Figure 1.

V. Methods and Rationale of Data Collection

Enumeration of Microorganisms

Enumerations of bacterial populations were performed using both direct count and viable plate count procedures. For direct counts, samples were preserved with formaldehyde, one part formaldehyde: one part sample. Samples were filtered through 0.2 μm cellulose nitrate black filters and stained with acridine orange according to the procedure of Daley and Hobbie (1975). Samples were viewed with an Olympus epifluorescence microscope with a BG-12 exciter filter and 0-530 barrier filter. Ten fields per filter and two filters per sample were viewed and the counts averaged.

For viable plate counts, surface spread inoculations from serial dilutions were used. For some water samples concentration by filtration through 0.45 μm filters (Millipore Corp.) was used. Marine agar 2216E (Difco) was used to enumerate viable heterotrophic microorganisms. Replicate plates were incubated aerobically at 4° C to enumerate psychrophilic-psychrotrophic populations.

Most Probable Number estimates of hydrocarbon utilizers were performed. Dilutions of samples were added to 30 ml stoppered serum vials containing 5 ml autoclaved Bushnell Haas broth (Difco) with 3% added NaCl, and 50 μl filter sterilized (0.2 μm Millipore filter) Cook Inlet crude oil spiked with 1-^{14}C*n* hexadecane (s.p. act. = 0.9 μCi/ml oil). A 3 tube MPN procedure was used. Following incubation at 5° C for 4 weeks the solutions were rendered alkaline with concentrated KOH to stop microbial activity.

Solutions were then acidified with concentrated HCl and the $^{14}CO_2$ recovered by purging the vials with air and trapping the $^{14}CO_2$ in 1 ml hyamine hydroxide. The hyamine hydroxide was washed from the tubes into scintillation vials with 3 one ml portions of methanol. The counting solution was 10 ml Omnifluor + toluene (New England Nuclear). Counting was with a Beckman liquid scintillation counter. Counts greater than or equal to 2 times control were considered as positive; counts less than 2 times control were considered as negative. The most probable number of hydrocarbon degrading microorganisms were determined from the appropriate MPN Tables and recorded as most probable number per ml for water samples or most probable number per g dry wt. for sediment samples.

Numerical Taxonomic Testing

Approximately 300 phenotypic characteristics were determined for bacterial strains selected at random from enumeration plates. Characterization included morphological, physiological, biochemical, nutritional and antibiotic sensitivity testing. The procedures used in these test procedures have been previously described (e.g. see 1979 annual report). Similarities were estimated with the Jaccard coefficient (S_j) and unweighted average linkage sorting. Testing on all strains isolated to date has been completed, cluster analyses have been completed on all but the data collected on strains from the Bering Sea.

The number of taxonomic groups and the number of individuals in each group, determined by the cluster analyses, were used to calculate the Shannon diversity index, $\bar{H}$. The formula

$$\bar{H} = \frac{c}{N}(N \log_{10} N - \Sigma n_i \log_{10} n_i)$$

will be used, where c = 3.3219, N = total numbers of individuals and n_i = total number of individuals in the ith taxonomic grouping.

Since different population sizes were used in the determination of $\bar{H}$ the equitability (J) was calculated to normalize the data using the formula $J = \frac{\bar{H}}{H'}$, where H' = theoretical maximal diversity for a population of size N.

Denitrification Studies

Surface sediment samples (0-5 cm) were collected in Alaskan Continental Shelf regions (Beaufort Sea, during August-September, 1978; Being Sea-Gulf of Alaska and Cook Inlet regions, during April-May, 1979; Norton Sound-Bering Sea region, during June-July, 1979). Sediment samples were collected with a Smith-MacIntyre or a SutarVan Veen grab sampler in water depths greater than 5 m. Relatively undisturbed surface sediment samples were collected from each of the grab samples. A Kahl mud snapper was used for collection of sediment samples in shallow waters. Each sediment was mixed thoroughly with a small portion of the overlying seawater collected in the grab sampler to produce a slurry to facilitate handling of the sediment.

Salinity determinations were made with either a Beckman RS7A salinity meter or a Plessey Environmental Systems salinity-temperature meter (model 9060). Temperatures were determined with reversing thermometers attached to Nansen water sampling bottles or with the Plessey Environmental Systems instrument.

For nutrient analyses, sediment samples, which had been quick-frozen with dry ice immediately upon collection and maintained in a frozen state at -20° C, were thawed in a warm bath, mixed and then centrifuged for 30 minutes at 0° C at 7700 xG in a Sorvall RC 2B refrigerated centrifuge.

Five to fifteen ml of the supernatant (interstitial water) was recovered and used for nutrient analyses. The interstitial water was diluted and approximately 20 ml of the diluted interstitial water was placed in quartz tubes with 0.3 ml added H_2O_2 and exposed to ultra-violet light for 4 hours. A portion of the diluted interstitial water was used to determine concentrations of nitrate plus nitrite using a Technicon auto-analyser according to the procedures of Callaway, *et al.* (1972). The following modifications to this procedure were made: sample, 0.8 ml min^{-1}; DDW diluture, 1.2 ml min^{-1}; NH_4Cl, 1.0 ml min^{-1}; sulfanilamide, 0.1 ml min^{-1}; N-(1-naphthyl)-ethylenediamine, 0.1 ml min^{-1}. Ammonium concentrations were determined according to the method of Head (1971).

The acetylene blockage of N_2O reduction method (Balderson *et al.*, 1976; Sorensen, 1978a; Yoshinari and Knowles, 1976; Yoshinari *et al.*, 1977) was used for measuring denitrification potentials. For denitrification assays, 5 ml portions of the sediment slurry were used to inoculate replicate 30 ml serum bottles containing either 5 ml Rila Marine mix (40g l^{-1}) (R), 5 ml Rila salts mix plus 0.1% (w/v) proteose peptone #3 (Difco) (RP), 5 ml Rila salts mix plus 0.1% (w/v) KNO_3 (RN), or 5 ml Rila marine salts mix plus nitrate broth (Difco) (RNB). Medium R was used to measure denitrification activity of unamended sediments. Medium RP was used to measure denitrification activity of sediments amended with readily available organic nitrogen and carbon. Medium RN was used to measure denitrification activity of sediments amended with readily available mineral nitrogen. Medium RNB was used to measure denitrification activity of sediments amended with both readily available organic carbon and nitrogen and mineral nitrogen.

After inoculation, the sediment suspension was at a dilution of 1:2 compared with the original sediment. All bottles and solutions were sterilized at 121° C for 15 min. and had been cooled to 5° C prior to inoculation. All sample-media combinations were performed in triplicate. After inoculation the bottles were recapped with their original stoppers and purged with argon for 15 min. Following purging, 5 ml of the headspace gas was withdrawn with a syringe and replaced with 5 ml of acetylene generated from CaC_2. The serum stoppers were then covered with silicone rubber sealant to prevent gas leakage through needle punctures. All bottles were incubated at 5° C. After 48 hours incubation, 0.5 ml saturated KOH solution was injected into replicate bottles containing sediment plus media R, RN or RNB. After injection of the KOH solution to stop biological activity, all bottles were resealed with silicone rubber to prevent leakage from needle punctures. All bottles were maintained at 5° C until the headspace gases could be analysed in the laboratory.

The following controls for this investigation were established. Two sediment samples from the Beaufort Sea were inoculated into serum bottles containing either R, RN, or RNB. Six replicates of each of the media were left active, sterilized by autoclaving or received 0.5 ml 6N HCl. The bottles were recapped after inoculation and purged with argon for 15 min. Replicates of each of the active, sterile, or acidified treatments had 5 ml of headspace gas removed with a syringe and 5 ml of C_2H_2 generated from CaC_2 added. Additional replicates of each media did not receive C_2H_2. All bottle caps were covered with silicone rubber to prevent gas leakage. After 24, 48, 120 and 240 hours of incubation at 5° C, 0.5 ml saturated KOH solution was injected into three replicates each of active,

electron capture detector (ECD) for N_2O determinations. Control experiments also were analysed with the ECD. The instrument was equipped with a 6 m x 0.3 cm Porapak Q column. The conditions for chromatography were: injector, 100° C; oven 40° C; and ECD, 300° C. The carrier for the 5830 GC was agron-methane (19:1) at a flow rate of 25 ml min^{-1}. The injection size was 250 μl. The identities of the compounds detected by gas chromatography were confirmed by comparison of retention times with known compounds and by gas chromatography-mass spectrometry. The gas chromatograph-mass spectrometer used was a Hewlett Packard model 5992, equipped with a 6 m x 0.3 cm Porapak Q column. The carrier gas was He at 30 ml min^{-1}. The conditions for chromatography were: injector, 100° C; and oven, 50° C.

The quantification of N_2O was accomplished by preparing known dilutions of N_2O in argon and determining detector response per unit N_2O. Detector response was plotted against known quantity injections of N_2O to obtain a standard curve. The ECD was found to be linear in response across several orders of magnitude of N_2O concentrations. Experimental results were compared with the standard curve to obtain concentrations of N_2O and the quantity of N_2O produced per gram by weight sediment was calculated. The limit of sensitivity using the thermal conductivity detector was approximately 40 nmoles N_2O (= 1.8 μg N_2O) produced per gram dry weight sediment; with the ECD detector it was possible to detect production of approximately 0.005 nmoles N_2O (= 0.2 ng N_2O) per gram dry weight sediment.

Surface sediments were collected from Elson Lagoon (4 m depth) in the Beaufort Sea near Barrow, Alaska in January, 1978. Portions of the sediment were immediately mixed with 0.5% (w/v) Prudhoe Bay crude oil and other portions were left untreated. Treated and untreated sediments were then placed

separately in 25 cm x 25 cm x 5 cm plexiglas trays and placed *in situ* on the bottom of Elson Lagoon. Similar experiments were begun in Jan. 1979, and Jan. 1980. Sediment samples were recovered from replicate trays after 1 week, 8 month, 1 2/3 year and 2 year periods of incubation.

Oiled and unoiled sediments (5 ml portions) from these experiments were added to R, RP, RN, and RNB solutions. The bottles were then recapped and purged with argon for 15 minutes. After purging, 5 ml of the headspace gas was withdrawn and replaced with C_2H_2 generated from CaC_2. Samples were incubated at 5° C for either 48 or 240 hours. Following incubation duplicate bottles were injected with 0.5 ml KOH solution to stop biological activity. Headspace gases were analysed in the laboratory as described earlier using an electron capture detector.

Recovery and Analysis of Oil from *In Situ* Studies

Residual oil was recovered from ice cores and oil amended sediments by solvent extraction. Ice cores were extracted with diethyl ether using separatory funnels.

Sediments amended with crude oil were recovered from the sample trays and frozen for transport back to the laboratory. The frozen sediments were thawed and extracted as follows. Twenty five grams wet weight of each sediment were weighed into flasks. Each sample was extracted sequentially with diethyl ether, benzene, and methylene chloride by shaking for 2 hours at 200 rpm on a rotary shaker. The ethyl ether and benzene were separated from the samples in separatory funnels. Methylene chloride was separated from the samples by filtration with Whatman #2 paper in a Buchner funnel. After extraction, all extracts were combined and reduced in volume, in flasks with Vigreaux reflux columns. The volume of solvent was reduced

to about 10 ml, and 10 to 20 ml pentane was added and concentrated to about 10 ml. Pentane was added to the samples and reduced in volume three times. After concentration of the samples and replacement of the solvents with pentane, samples were transferred quantitatively to scintillation vials. All samples were then concentrated to 5 ml under a stream of dry nitrogen.

All samples were separated into 3 fractions using silica gel column chromatography. Columns were prepared by suspending previously dried silica gel 100 (E.M. Reagents, Darmstadt, W. Germany) in methylene chloride.

The columns were packed in 25 ml burets with teflon stopcocks. After packing, the methylene chloride was washed from the columns with three volumes of pentane. One half of each sample (2.5 ml) was applied to the surface of the column and drained into the column bed. After standing for 3 to 5 minutes the alkane fraction (f_1) was eluted from the column with 20 ml pentane. Then 5 ml of 20% CH_2Cl_2 in pentane was added to the column and drained into the column bed. The aromatic fraction (f_2) was collected by eluting the column with 45 ml of 40% CH_2Cl_2 in pentane. A third fraction (f_3) was collected by eluting the column with 45 ml of 50% CH_3OH in CH_2Cl_2 and draining the column.

Fractions f_1 and f_2 were concentrated to standard volumes and prepared for gas chromatography and gas chromatography-mass spectrometry. Fraction f_1 was adjusted to 20 ml with pentane after addition of 5 ml redistilled toluene and the internal standard. Fraction f_2 was adjusted to 10 ml after addition of 2.5 ml of toluene and the internal standard and removal of CH_2Cl_2.

Samples of fractions f_1 and f_2 from each sediment sample were then analysed by gc or gc-ms. Conditions for gc were injector, 240° C; temp. program,

70-270° C at 4° C per min; FID, 300° C, and He at 21 cm/sec. The instrument was a Hewlett Packard 5830 reporting gc. After gc analysis of f_2 fractions, several samples were chosen for quantitative mass spectrometry. The conditions for chromatography in the Hewlett Packard 5892 gc-ms were identical to those used in ordinary gc analysis. Both columns were SE54 grade AA from Supelco. Quantification of compounds of interest was performed using selected ion monitoring. Ions of the compounds were selected from National Bureau of Standards reference spectra. The selected ions were monitored for 10 msec per scan. Quantification of all samples, f_1 and f_2 was performed by calculating response factors from data obtained from known injections of known quantities of specific compounds. Injection sizes were corrected for using the internal standard hexamethyl benzene. The quantities of specific compounds were calculated per injection and subsequently related to quantities per gram dry weight sediment.

$$ISF = \frac{\text{area HMB x rf}}{\text{ng/}\mu\text{l HMB}}$$

$$\text{ng/}\mu\text{l} = \frac{\text{area of peak x rf of peak}}{\text{ISF}}$$

$$\mu\text{g/g dry wt} = \text{ng/}\mu\text{l x } \mu\text{l } f_1 \text{ or } (f_2 \text{ x } 2)$$

VI. Results

Denitrification

The sample locations, depths, salinities, and temperatures are shown in Figure 1. The bottom waters of Norton Sound had temperatures between 5.0 and 10.4^{o} C, salinities between 20.0 and 32.1 o/oo, and sample depths between 1.7 and 44 m. The lower salinities in the Norton Sound region occurred due to freshwater input from the Yukon River. In the Beaufort Sea, temperatures varied between -1.0 and 6.0^{o} C, salinities between 10.4 and 29.0 o/oo, and sample depths between 1 and 67 m. The low salinities reflect ice melt and the nearshore trapping of terrestrial runoff by sea ice formations. Bottom temperatures in the Bering Sea varied between -1.8 and 4.7^{o} C, salinities were between 31.5 and 35.0 o/oo, and sample depths were between 1 and 151 m. Temperatures in the northern Bering Sea (adjacent to Norton Sound) were 5-10° C lower during spring than during summer when samples were collected within Norton Sound. Temperatures observed in Upper Cook Inlet were between 4.2 and 7.5^{o} C, salinities were between 30.1 and 33.0 o/oo, and sample depths were between 1 and 297 m.

The concentrations of nitrate plus nitrite and of ammonium ions are shown in Figure 2. Norton Sound nitrate-nitrite concentrations ranged from 0.1 to 31.5 μM; generally lower concentrations of nitrate-nitrite were found within the Sound and higher concentrations occurred near the mouth of the Yukon River and to the west of Norton Sound proper. Nitrate-nitrite concentrations in the Beaufort Sea ranged from 0.3 to 46 μM and in

Cook Inlet 1.6 to 130 μM. The highest concentrations of nitrate-nitrite ions occurred in Kamishak Bay on the western side of Cook Inlet. Ammonium ions concentrations ranged from 6 to 1200 μM in Norton Sound, from 1 to 338 μM in the Beaufort Sea and from 24 to 244 μM in Cook Inlet.

Production of N_2O by microbial denitrification from unamended sediment and sediment amended with an organic carbon-nitrogen source was relatively low during 2 days incubation (Figs. 3, 4); N_2O production was less than 1 μg g^{-1} dry sediment during the 2 day incubation period. Addition of organic carbon produced variable results; in several cases, addition of proteose peptone led to suppression of N_2O evolution by approximately 50%, in others denitrification was either unaffected or slightly stimulated. In the northern Bering Sea and Norton Sound samples, approximately one-third of the unamended samples produced no detectable N_2O (<1 ng N_2O g^{-1} dry sediment); unamended samples from the southern Bering Sea-Gulf of Alaska region all produced measureable N_2O (>34 ng N_2O g^{-1} dry sediment) during the 2 day incubation period.

Analysis for N_2O production from sediment from all four cruises, incubated with R, RN, and RNB solutions for ten day periods yielded much higher rates of N_2O production and showed regional differences. Unamended sediments from the Beaufort Sea produced no detectable N_2O (Fig. 5) using a thermal conductivity detector, but with added NO_3^-, N_2O production was as high as 141 μg g^{-1} dry sediment (Fig. 6); addition of NO_3^- and an organic carbon source resulted in N_2O production up to 713 μg g^{-1} sediment (Fig. 7). In some cases the added carbon stimulated N_2O production (comparison of data in Figs. 6 and 7 at comparable sampling locations).

Unamended sediments from the northern Bering Sea (north of Pribilof Islands) and Norton Sound regions showed N_2O production values between 0 and 500 ng g^{-1} sediment (Fig. 5). Approximately one-third of the northern Bering Sea and Norton Sound samples produced no detectable N_2O from unamended sediments, even during 10 days incubation (Fig. 5). The southern Bering Sea-Gulf of Alaska samples produced between 80 and 750 ng N_2O g^{-1} sediment. All unamended samples in the southern Bering Sea-Gulf of Alaska produced detectable quantities of N_2O. Addition of NO_3^- to the sediment samples stimulated N_2O production from both Bering Sea and Norton Sound sediment samples (Fig. 6). In the upper Bering Sea the stimulation was from two to four orders of magnitude above unamended treatments. Data from the Norton Sound samples also showed that addition of NO_3^- stimulated N_2O production from three to four orders of magnitude compared to unamended samples. In the lower Bering Sea-Gulf of Alaska the degree of stimulation was markedly lower, from one to two orders of magnitude over unamended treatments. Production of N_2O from ice covered areas was generally lower than from the same areas without ice cover when supplemented with NO_3^- (Fig. 6); Norton Sound and the upper Bering Sea were covered by ice during April-May, 1979 and were ice-free in July-August, 1979. Near the mouth of the Yukon River, denitrifying activity was 2-3 times greater with added NO_3^- than from sediments from other areas of Norton Sound. In some cases addition of an organic carbon source stimulated activity by up to 2 times and in other cases added organics apparently suppressed denitrifying activity by about 50%.

Unamended samples collected in Upper Cook Inlet and Shelifkof Strait

(west of Kodiak Island and just south of Cook Inlet) yielded between 30 and 1000 ng N_2O g^{-1} sediment during 10 day incubations (Fig. 5). Addition of NO_3^- to Cook Inlet area samples stimulated N_2O production from 1 to 3 orders of magnitude over unamended sediments (Fig. 6). Addition of an organic carbon source and NO_3^- generally stimulated N_2O production by twice the values observed when NO_3^- was the sole amendment (Fig. 7); in one case though N_2O production was slightly depressed.

The control experiments showed the following. Storage of the vials prior to analysis did not result in significant loss of N_2O from the headspace. Repetitive analysis of vials over an extended period produced repeatable quantification (recovery) of N_2O concentrations. The presence of nitrite at the concentration tested, which was above the concentration of nitrite originally in the samples did not inhibit N_2O production. Essentially identical amounts of N_2O were produced in the presence and absence of 0.1% added nitrite. No significant abiotic N_2O production was found; no detectable N_2O production was observed using sterilized sediments. No significant spontaneous N_2O evolution was found even at low pH; some near background levels of N_2O were detected after 10 day incubation at pH 5.0; measurement of pH for selected active sediment showed none with pH values less than 6.5 even after 10 days incubation with RNB. The presence of acetylene enhanced production of N_2O, i.e., the blockage was effective, lower quantities of N_2O were found in the absence of C_2H_2 than when C_2H_2 was added. Linear rates of N_2O production were found up to, but not in excess of, 48 h in unamended samples and up to 240 h with added NO_3^-. Thus the results from the 2 day unamended incubations can be used to calculate

natural rates; the results from the 10 day nitrate amended experiments can be used to calculate maximal potential rates of denitrification.

Without added nitrate (natural rates) there was a statistically significant difference in the denitrification (N_2O production) between oiled and unoiled sediments (Table 1). This difference was found for both short term (1 week) and long term (up to 2 years) exposures. No N_2O production was observed during the incubation period from any of the oiled sediments. With added nitrate there was not statistically significant difference between oiled and unoiled sediments; i.e., denitrification potentials were not altered by exposure to oil.

Table 1. Effects of crude oil on denitrification.

			Denitrification (natural rate) ng N_2O produced g^{-1} h^{-1}	
Elson Lagoon	Jan. 1980	1 wk.	0.2	0.004
Elson Lagoon	Jan. 1980	2 yr.	0.1	0.004
Elson Lagoon	Aug. 1979	8 mo.	0.005	0.004
Elson Lagoon	Aug. 1979	16 mo.	0.004	0.004

			Denitrification (potential rate + NO_3^-) ng N_2O produced g^{-1} h^{-1}	
Elson Lagoon	Jan. 1980	1 wk.	46.3	48.8
Elson Lagoon	Jan. 1980	2 yr.	56.3	55.2
Elson Lagoon	Aug. 1979	8 mo.	166.3	179.2
Elson Lagoon	Aug. 1979	16 mo.	166.3	190.9

Table 2. Comparison of denitrification and concentrations of fixed forms of organic nitrogen in sediments from different regions of the Alaskan Continental Shelf.

	Denitrification (natural) ng g^{-1} h^{-1}	NH_4^+ μM	$NO_3^- + NO_2^-$ μM
Upper Cook	0.05	-	-
Western Cook	11.25	137	55.2
Shelikof Strait	0.66	83	3.1
Norton Sound	1.91	188	6.4
Beaufort Sea	-	103	12.4

- = not determined

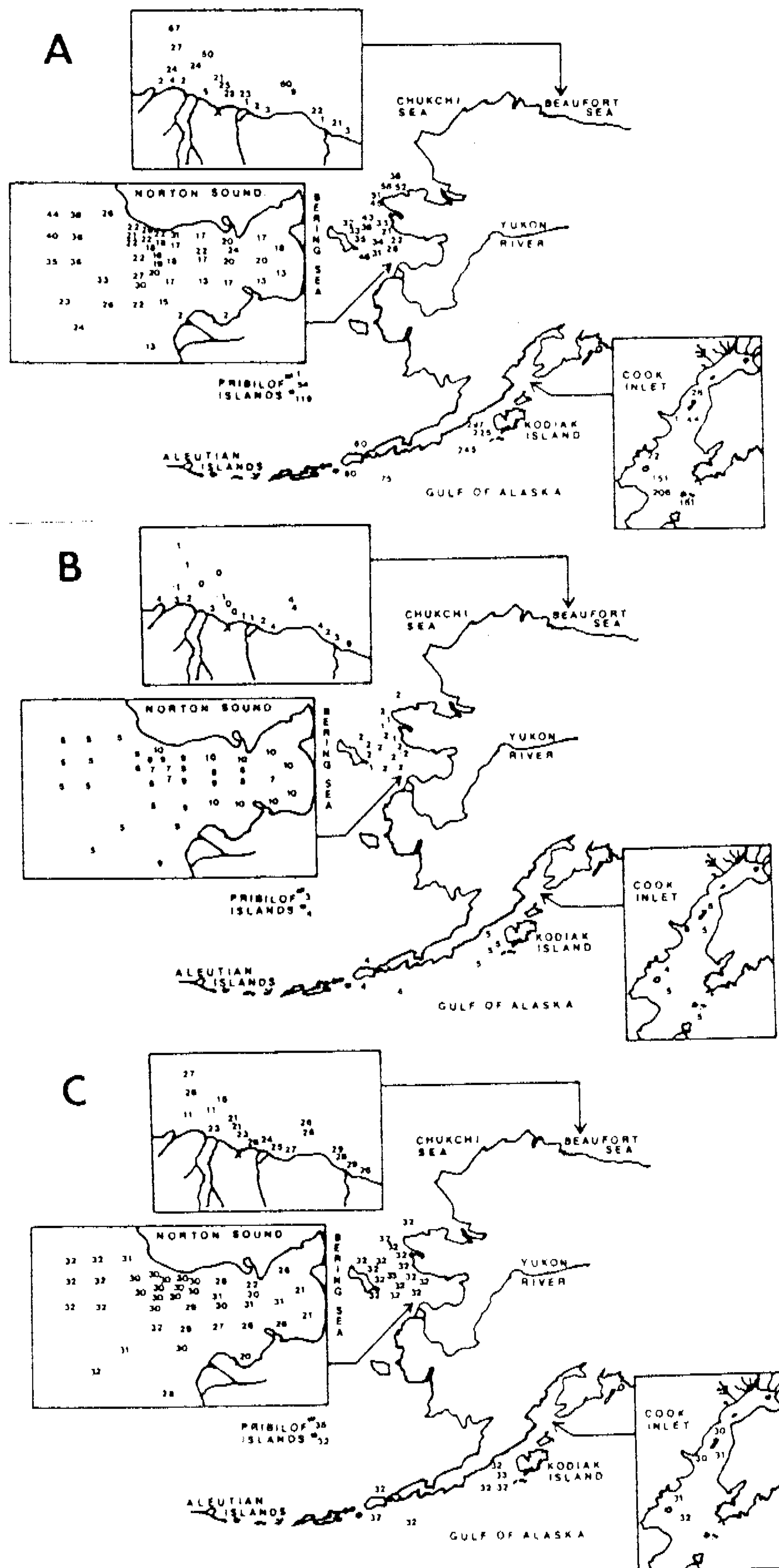

Fig. 1. Chart showing depths (A), bottom salinities (B), and bottom temperatures (C) at times of samples collections.

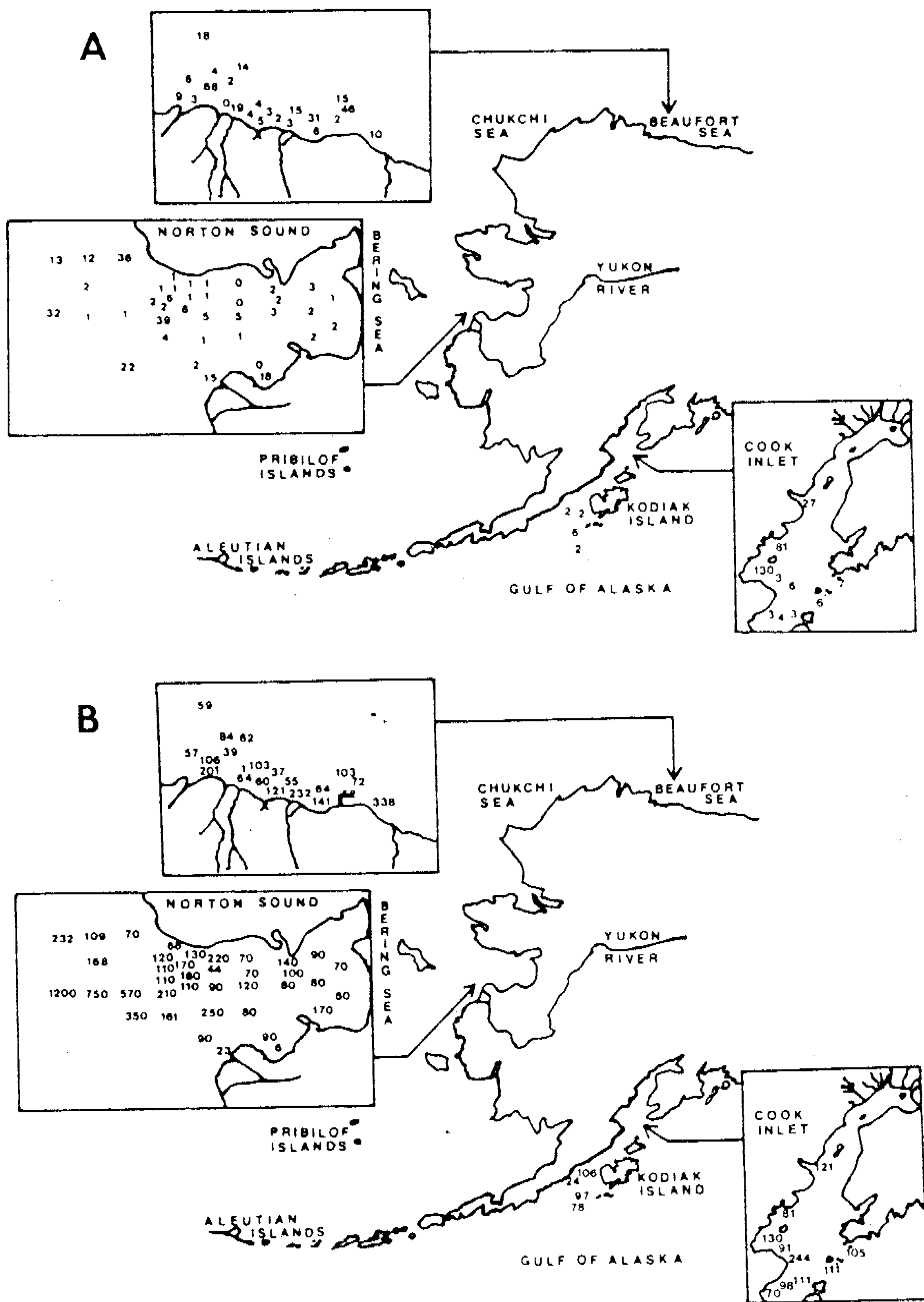

Fig. 2. Concentrations of nitrate-nitrite (μM)(A) and of ammonium (μM) (B) ions in interstitial water.

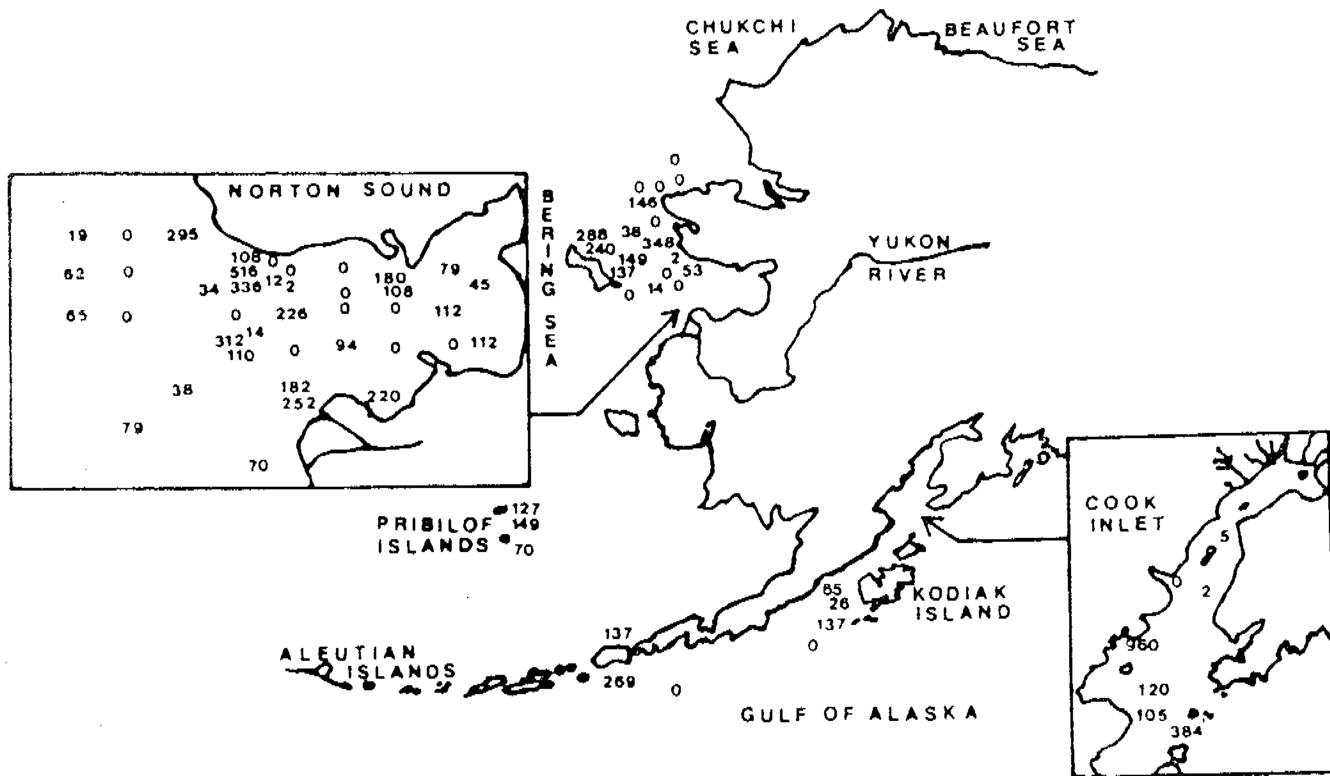

Fig. 3. Natural rates of denitrification - N_2O produced (ng g^{-1}) during 2 day incubation.

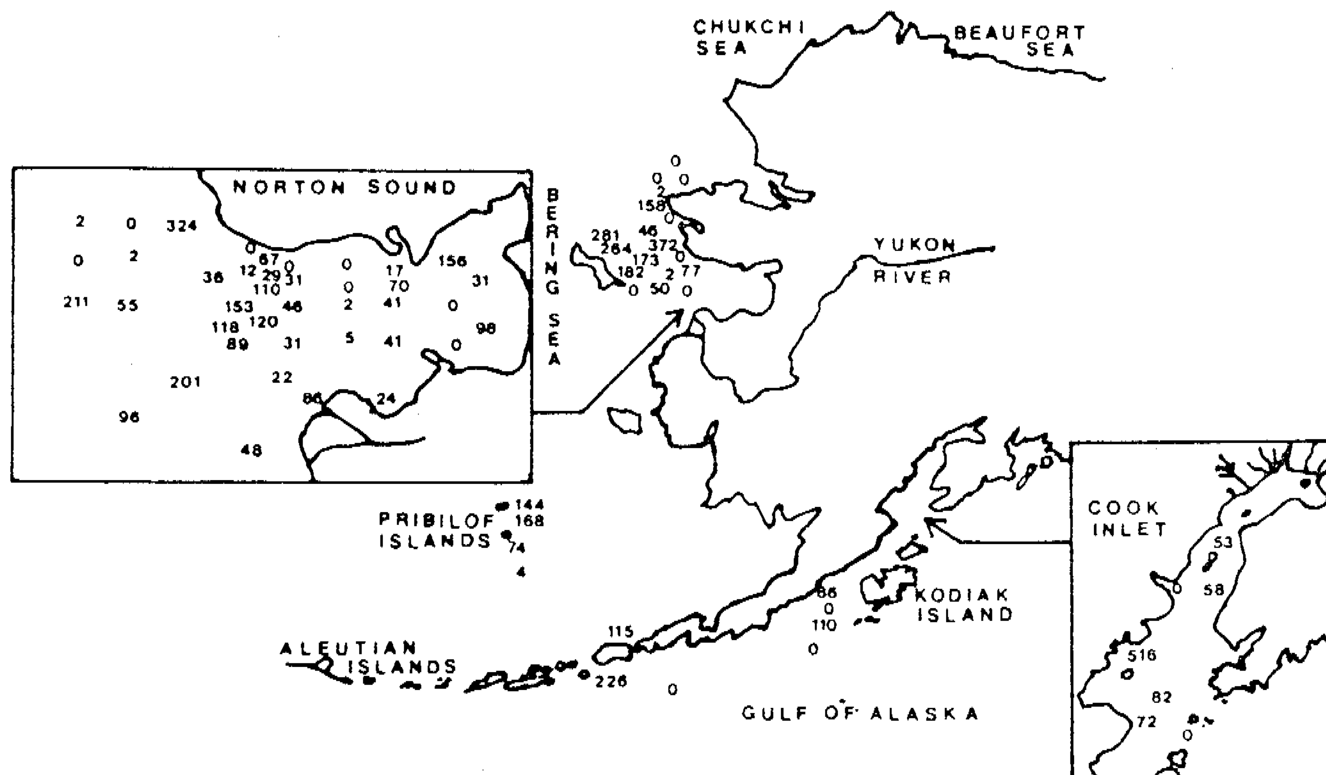

Fig. 4. Effects of organic matter on natural rates of denitrification - N_2O produced (ng g^{-1}) during 2 day incubation with organic C/N supplement.

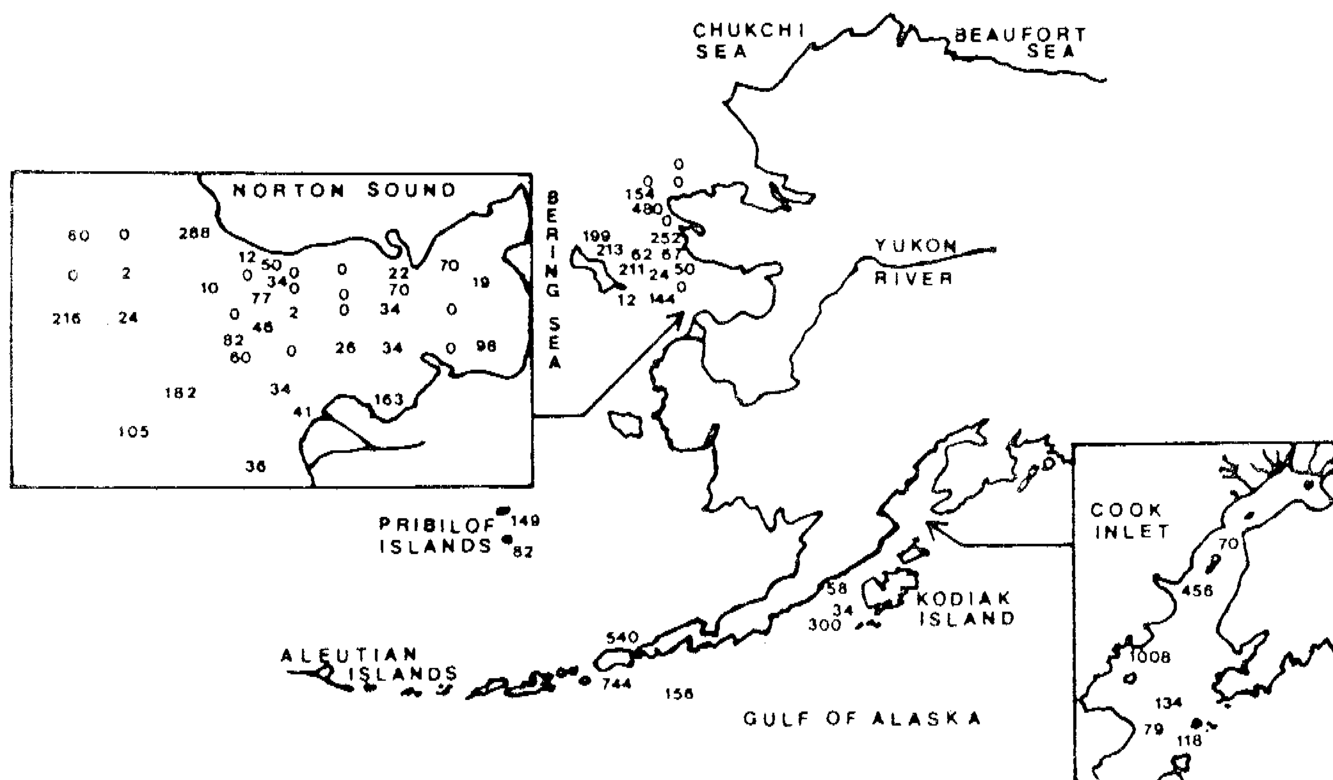

Fig. 5. N_2O produced (ng g^{-1}) during 10 day incubation with no supplements.

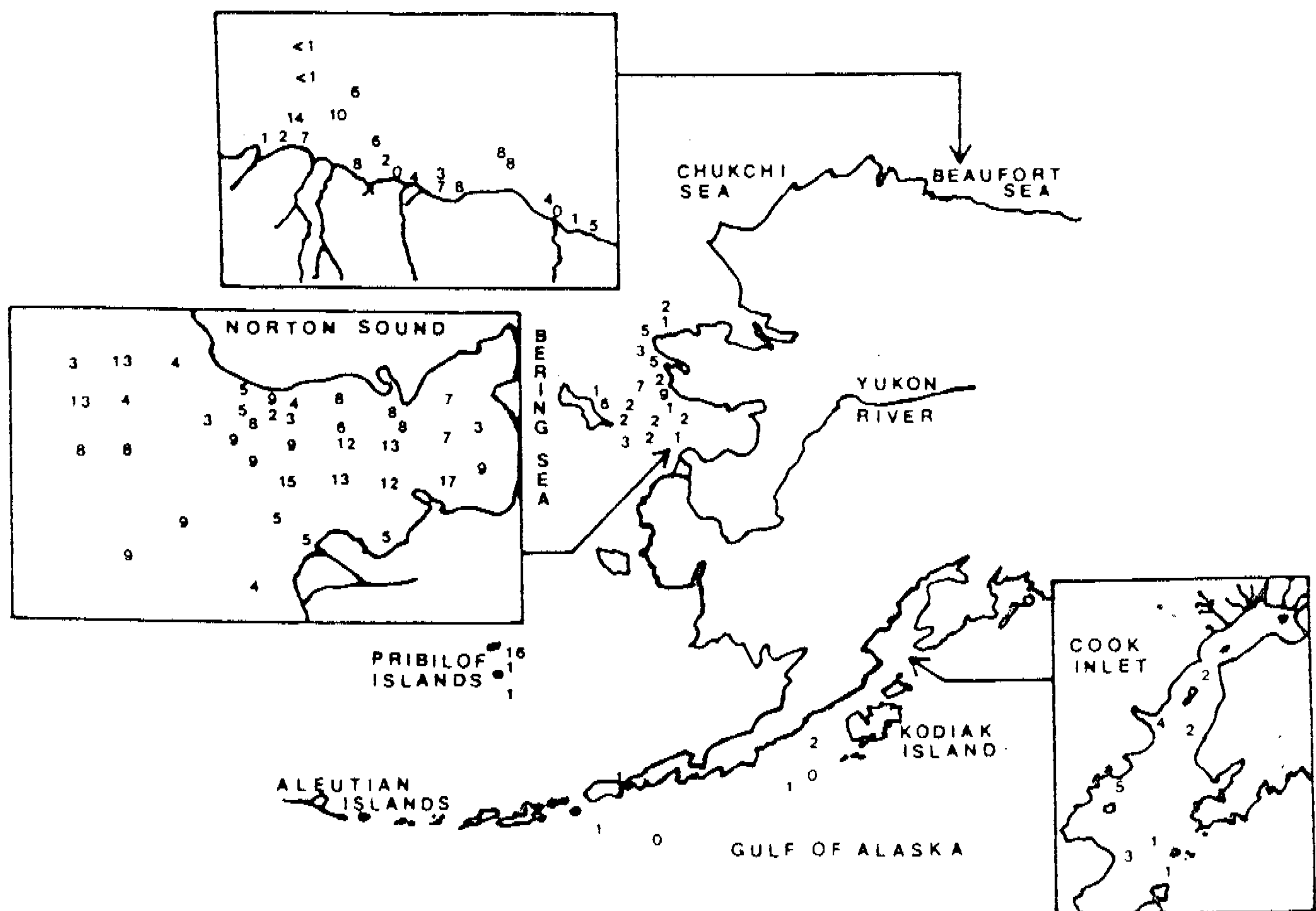

Fig. 6. Potential rates of denitrification - N_2O produced (ng x 10^4 g^{-1}) during 10 day incubation with nitrate added.

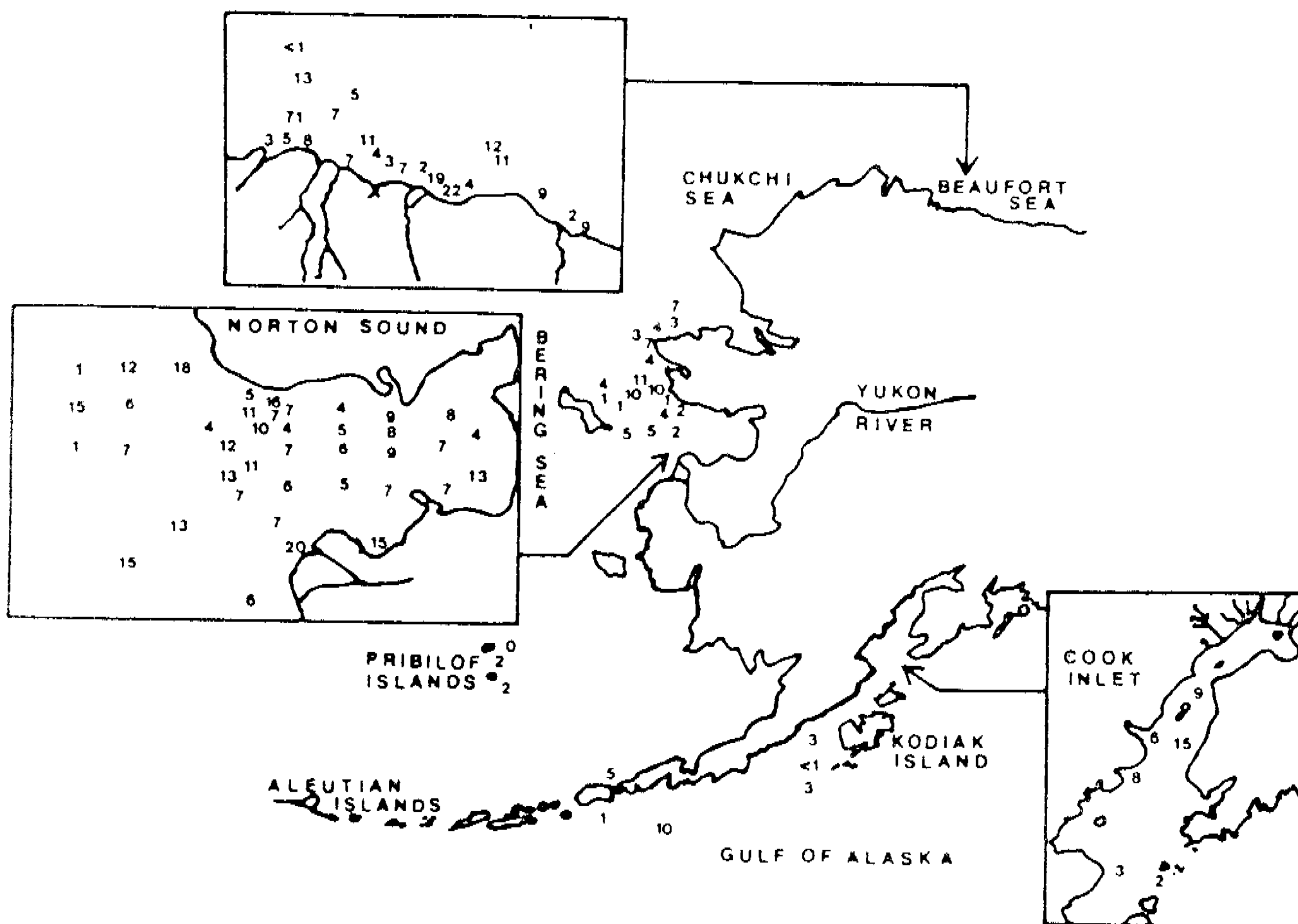

Fig. 7. Effects of organic matter on potential rates of denitrification - N_2O produced (ng x 10^4 g^{-1}) during 10 day incubation with nitrate and organic C/N added.

The total numbers of microorganisms (direct counts) determined from samples are shown in Figures 8-13. A summary of total and viable counts from various cruises is shown in Table 3. As in previous studies there were local spatial variations (generally within one order of magnitude difference) but no similar results were found for the most part for the viable counts (Figs. 14-20). The viable counts in sediment were higher though, in the Bering Sea in ice covered areas than in ice free areas (Fig. 14). There were no major stepwise or gradual increases in the viable counts in water along a northerly transect as had been predicted. Apparently the 1-2 order of magnitude higher viable counts observed in the Bearfort Sea than in the Gulf of Alaska occurs north of the Bering Straits. The input of the Yukon River had only a localized affect on the numbers of microorganisms.

As with the viable counts the numbers of hydrocarbon utilizers were higher in the ice covered regions of the Bering Sea than in ice free regions (Fig. 21). The counts of hydrocarbon utiliers within Norton Sound did not show any elevation in numbers in the vicinity of the gas seepage (Fig. 22). Some higher counts were found at the inner end of the Sound which may reflect inputs of terrestrially derived hydrocarbon.

In contrast to the distribution of hydrocarbon utilizers in Norton Sound there was an interesting distribution pattern of hydrocarbon utilizers within Cook Inlet. Counts of hydrocarbon utilizers were higher in surface waters in Upper Cook Inlet than in Lower Cook Inlet (Fig. 23). This could reflect an enrichment of hydrocarbon utilizers in Upper Cook Inlet due to inputs of petroleum hydrocarbons. In sediment counts were higher in Lower Cook Inlet than in Upper Cook Inlet (Fig. 24). This

pattern would be consistent with sedimentation of hydrocarbons in Lower Cook Inlet but also could be a reflection of differences in grain size distributions in sediments of Upper and Lower Cook Inlet. We have generally found high concentrations of hydrocarbon utilizers in embayments and at the entrances of the Inlet. In general the distribution of hydrocarbon utilizers within Cook Inlet supports a hypothesis that hydrocarbons are transported into the sediments along the west side of the Inlet, at the entrances to the Inlet that the sources of these presumptive hydrocarbon inputs are different.

Talbe 3. COUNTS OF BACTERIA
(number per ml or g)

Area	Time	Water		Sediment	
		Total	Viable	Total	Viable
Norton Sound	July 1979	2.8×10^5	3.0×10^2	2.0×10^8	2.8×10^6
Northern Bering	April 1979	1.1×10^5	1.8×10^2	1.9×10^9	3.2×10^5
Aleutians	April 1979	1.7×10^5	1.8×10^2	8.0×10^8	5.3×10^5
Cook Inlet	April 1979	4.2×10^5	3.7×10^2	2.6×10^9	3.3×10^5
N.W. Gulf	August 1975	3.0×10^5	1.0×10^2	-	6.3×10^5
N.E. Gulf	March 1976	1.4×10^5	1.3×10^2	3.0×10^9	1.0×10^6
Beaufort	August 1975	8.2×10^5	9.6×10^3	6.2×10^8	2.0×10^5
Beaufort	March 1976	1.8×10^5	6.1×10^2	3.7×10^8	2.5×10^5
Beaufort	August 1976	5.2×10^5	5.0×10^4	2.1×10^9	8.3×10^6

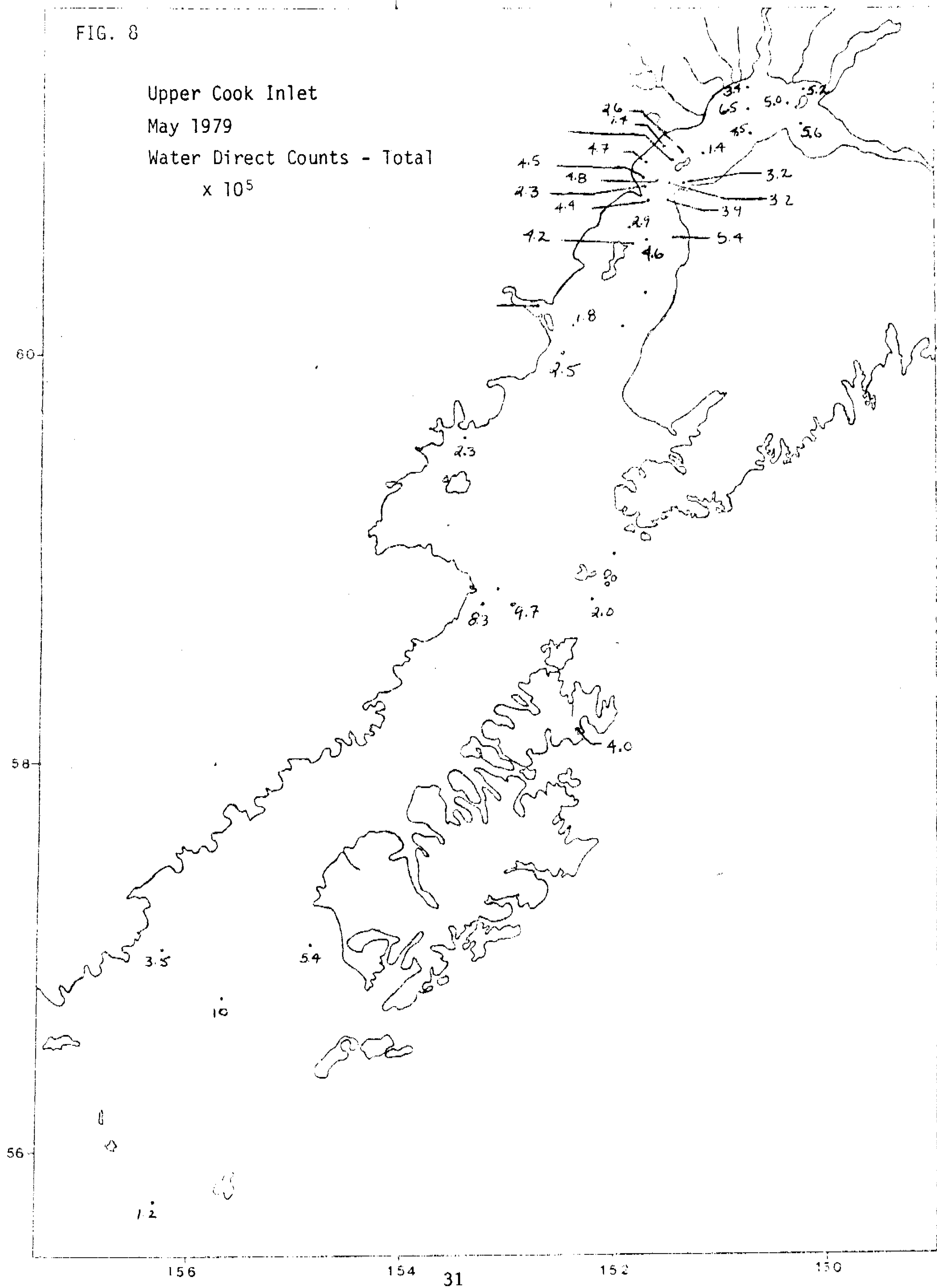
FIG. 8
Upper Cook Inlet
May 1979
Water Direct Counts - Total
x 10^5
60
58
56
156
154
152
150

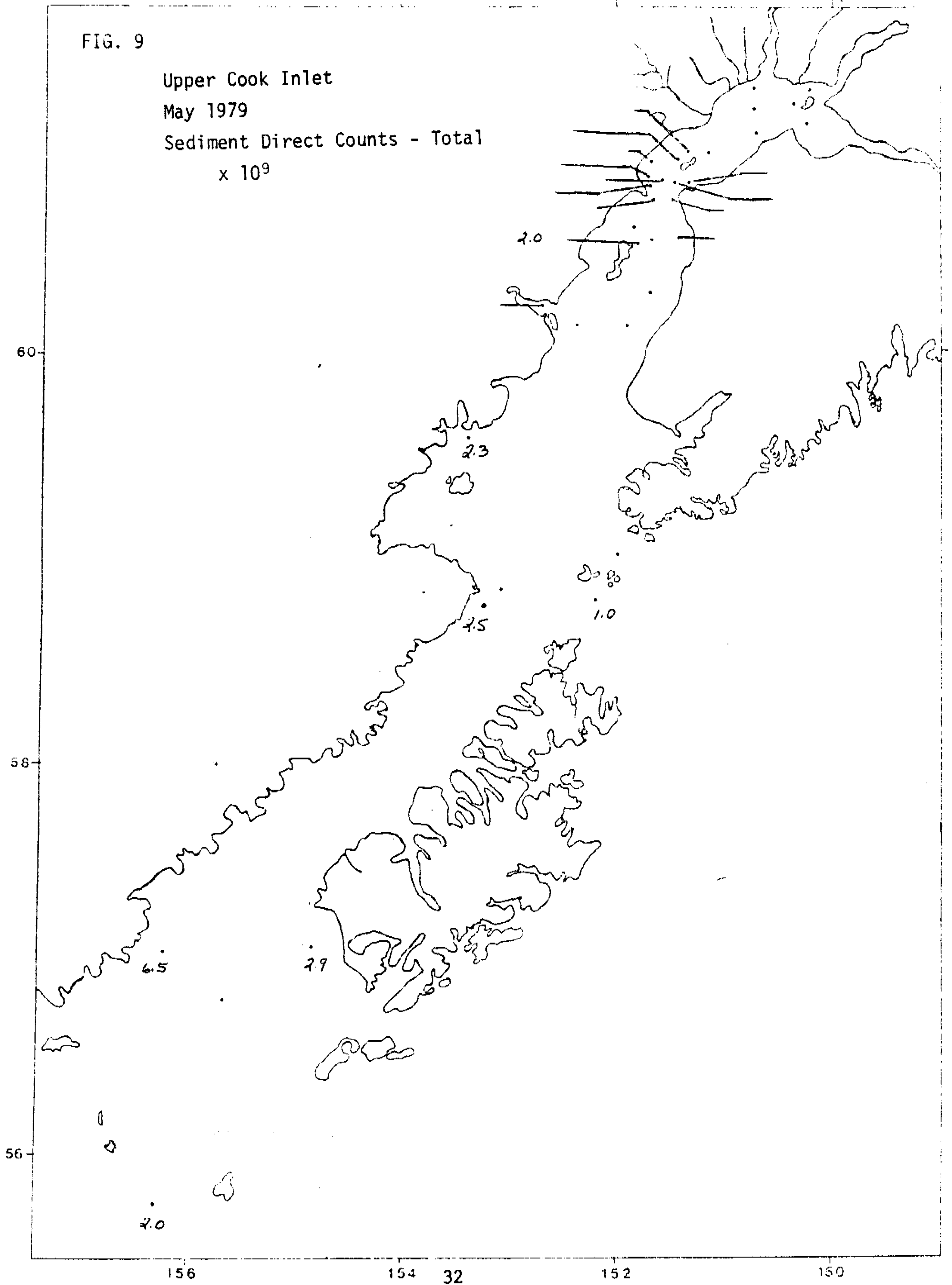
FIG. 9
Upper Cook Inlet
May 1979
Sediment Direct Counts - Total
x 10^9
2.0
2.3
1.5
1.0
6.5
2.9
2.0
60
58
56
156
154
152
150

FIG. 10

Water - Total

x 10^4/ml

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FIG. 11

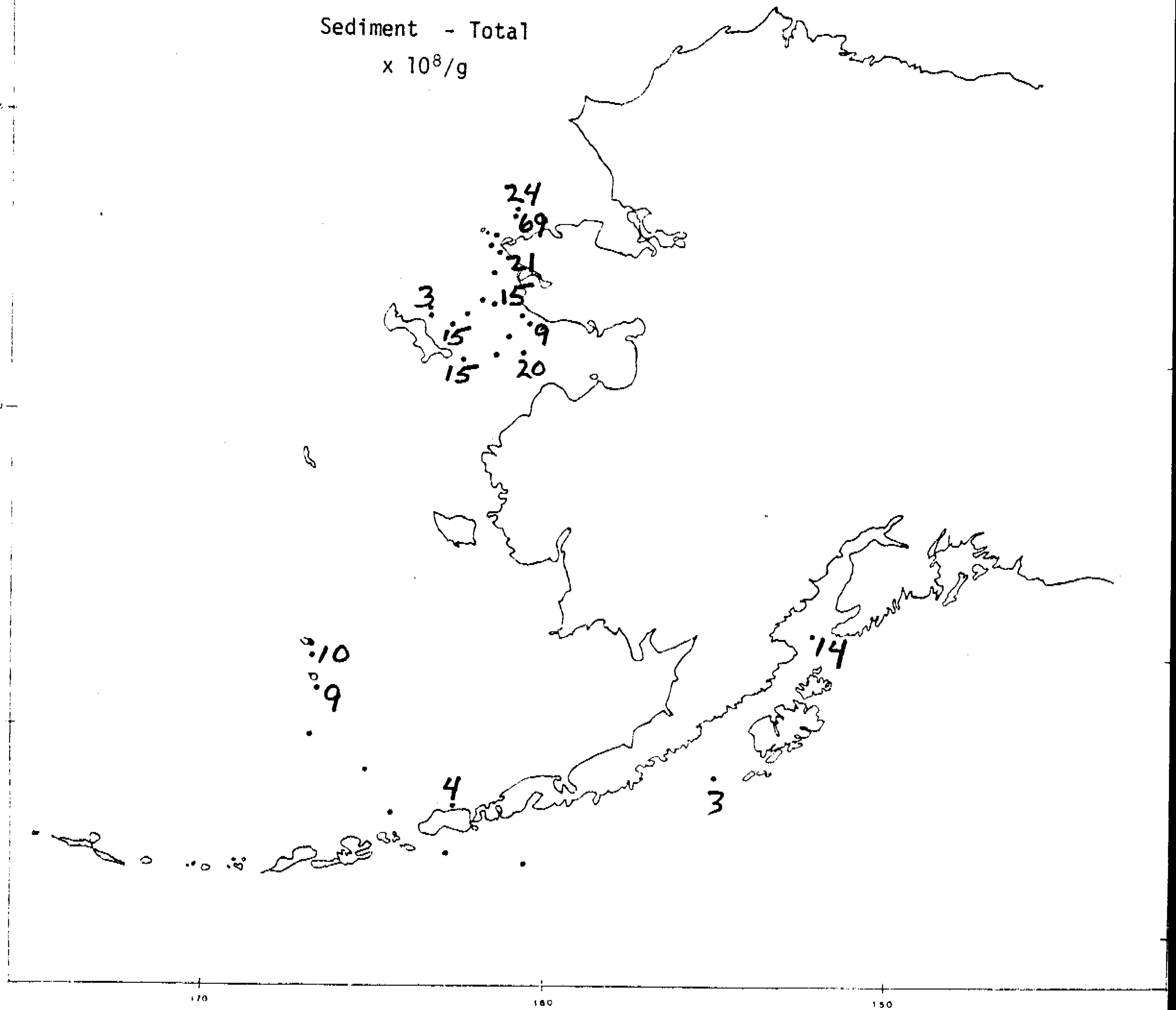


Fig. 12

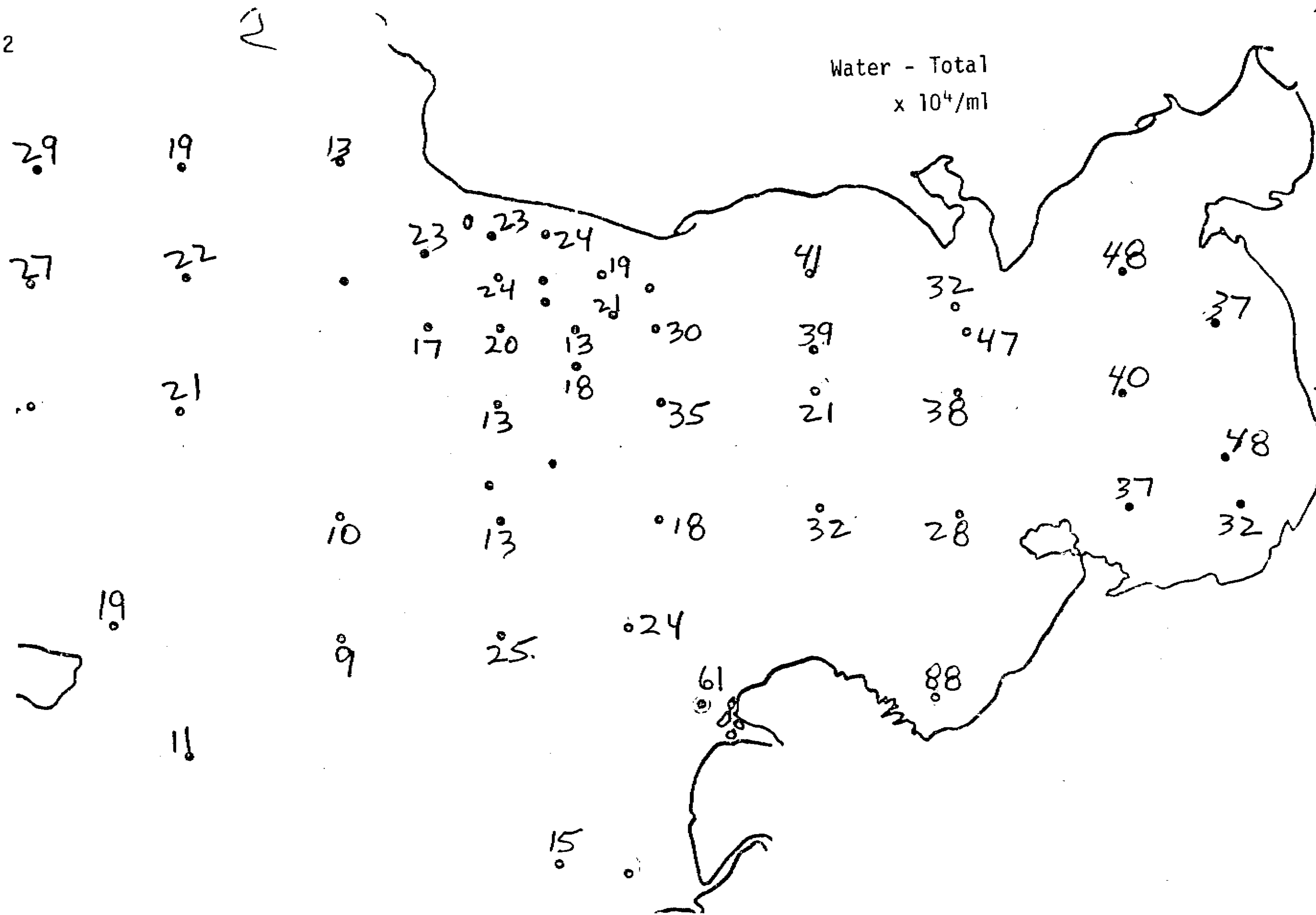
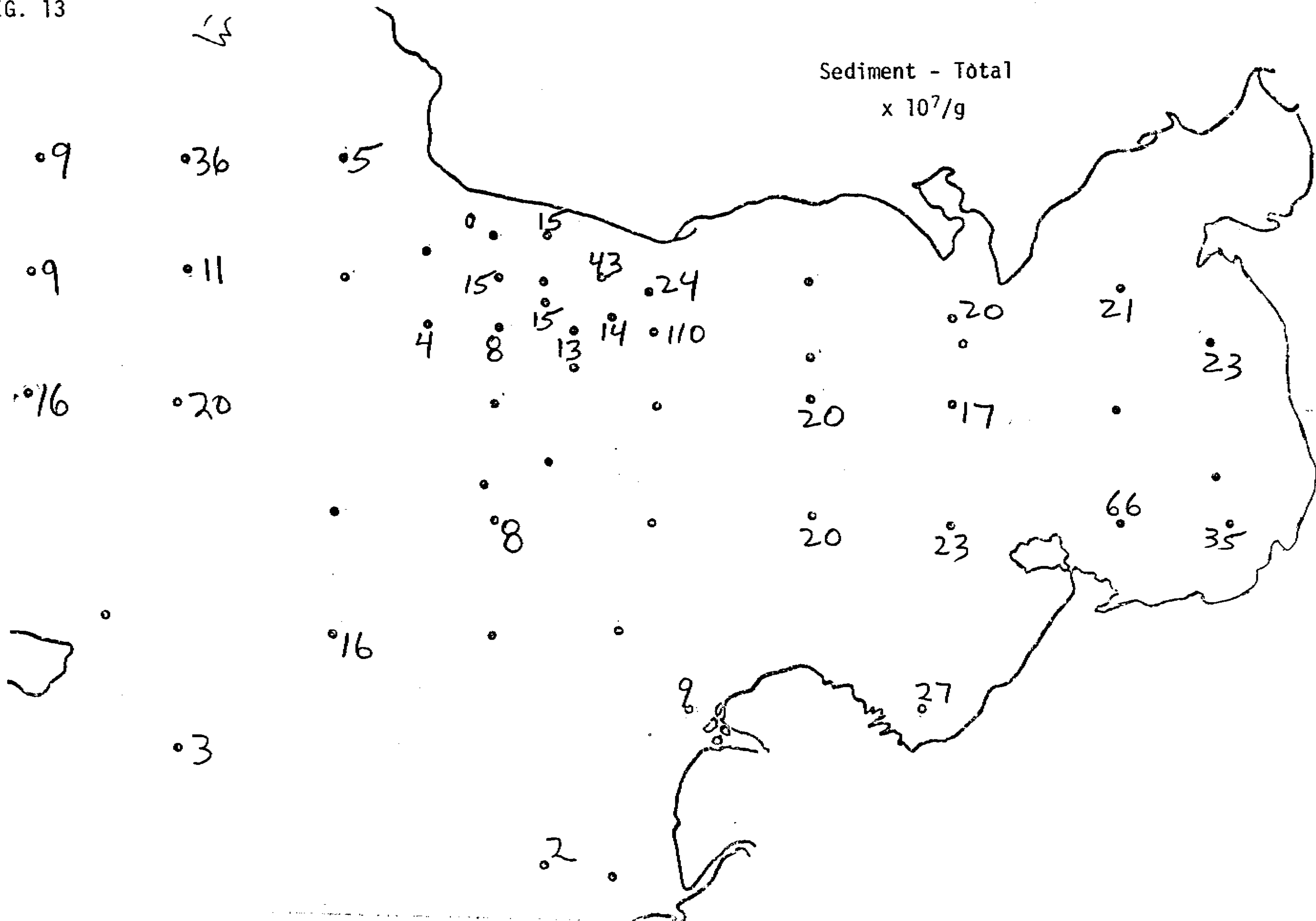


FIG. 13



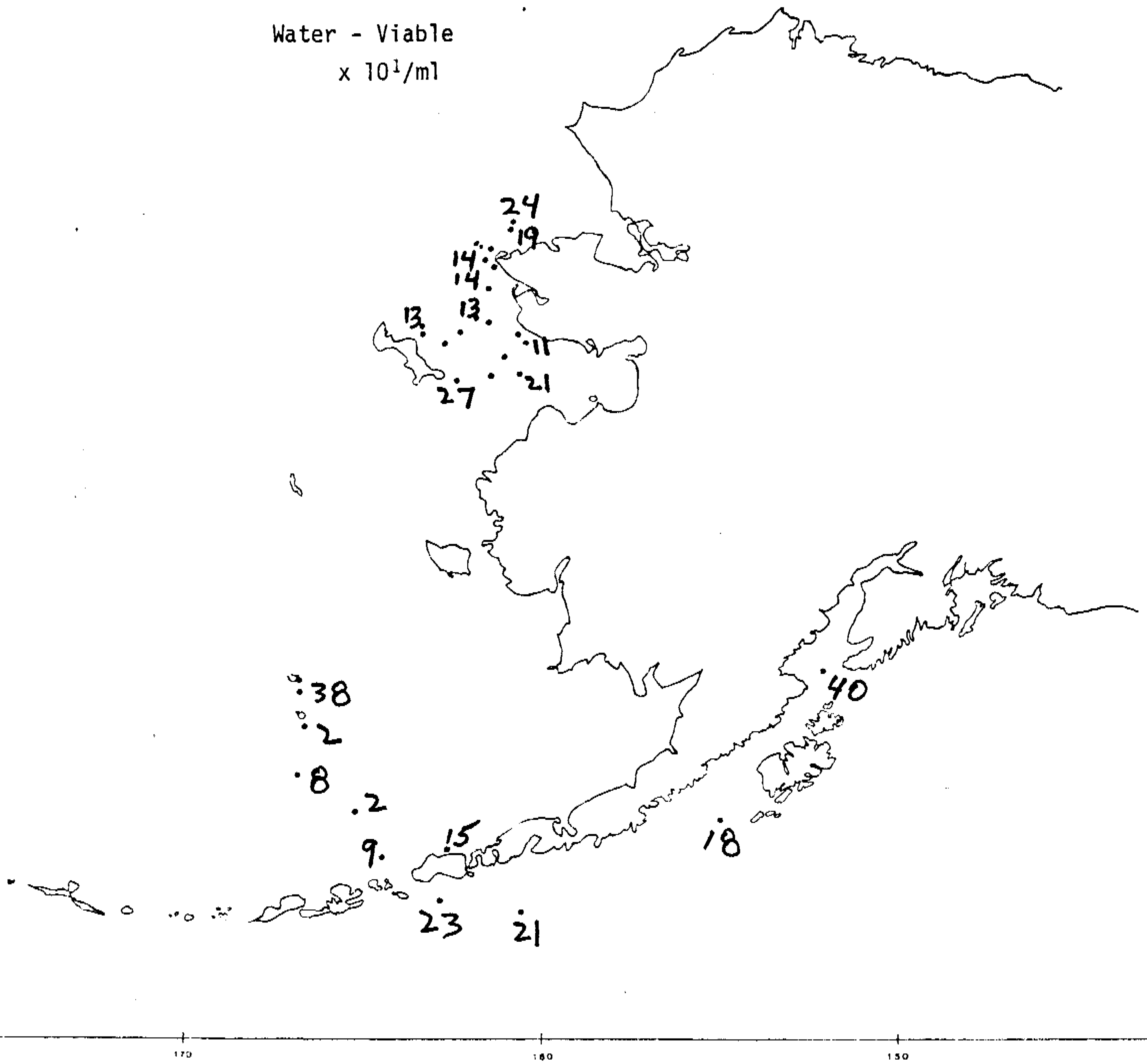


FIG. 14
Water - Viable
x 10^1/ml
170
160
150
24
19
14
14
13
13
11
21
27
38
2
8
2
9
15
23
21
40
18

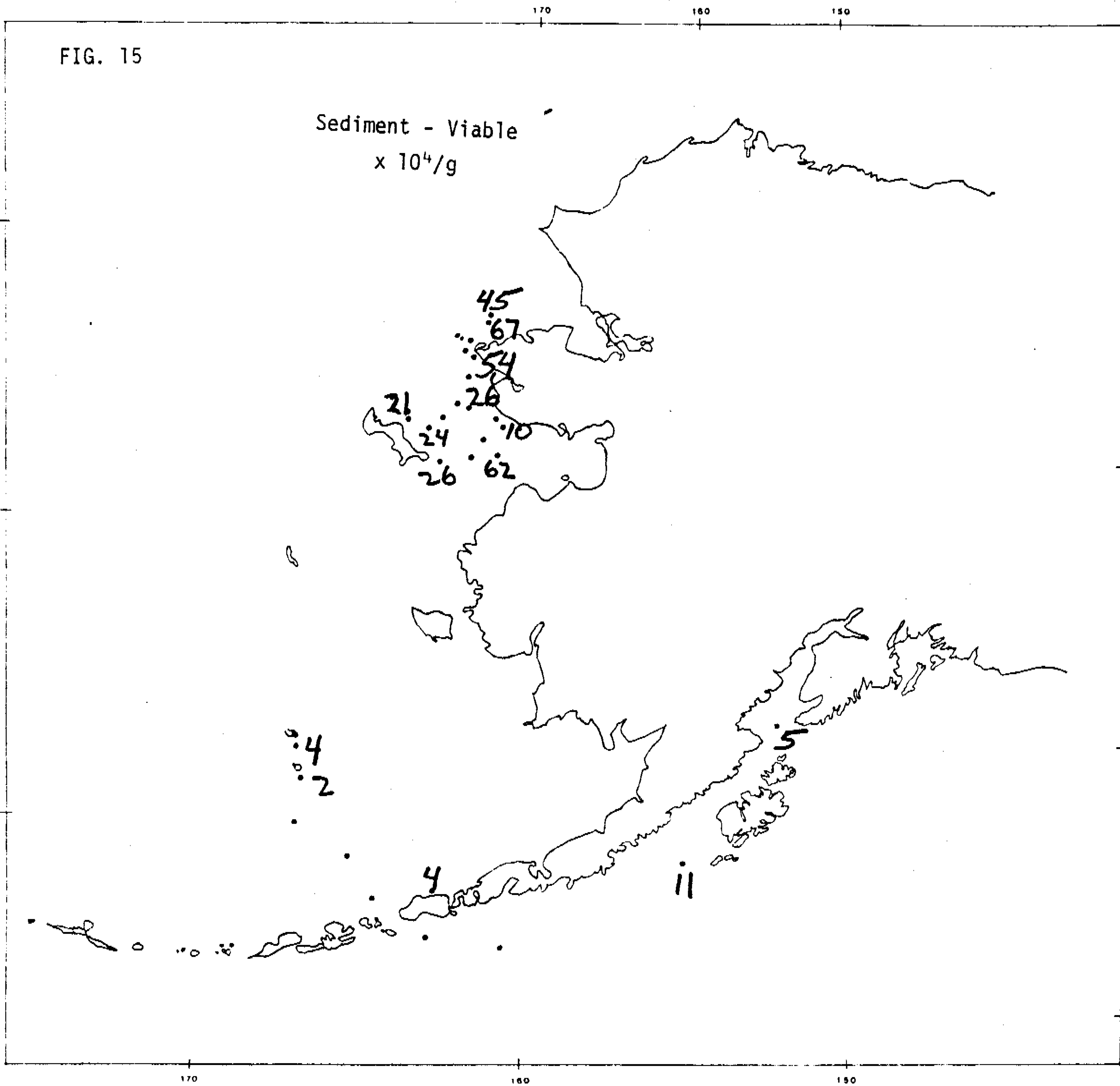


FIG. 15
Sediment - Viable
x 10^4/g
170
160
150
45
67
54
26
21
24
10
26
62
4
2
4
5
11

FIG. 16

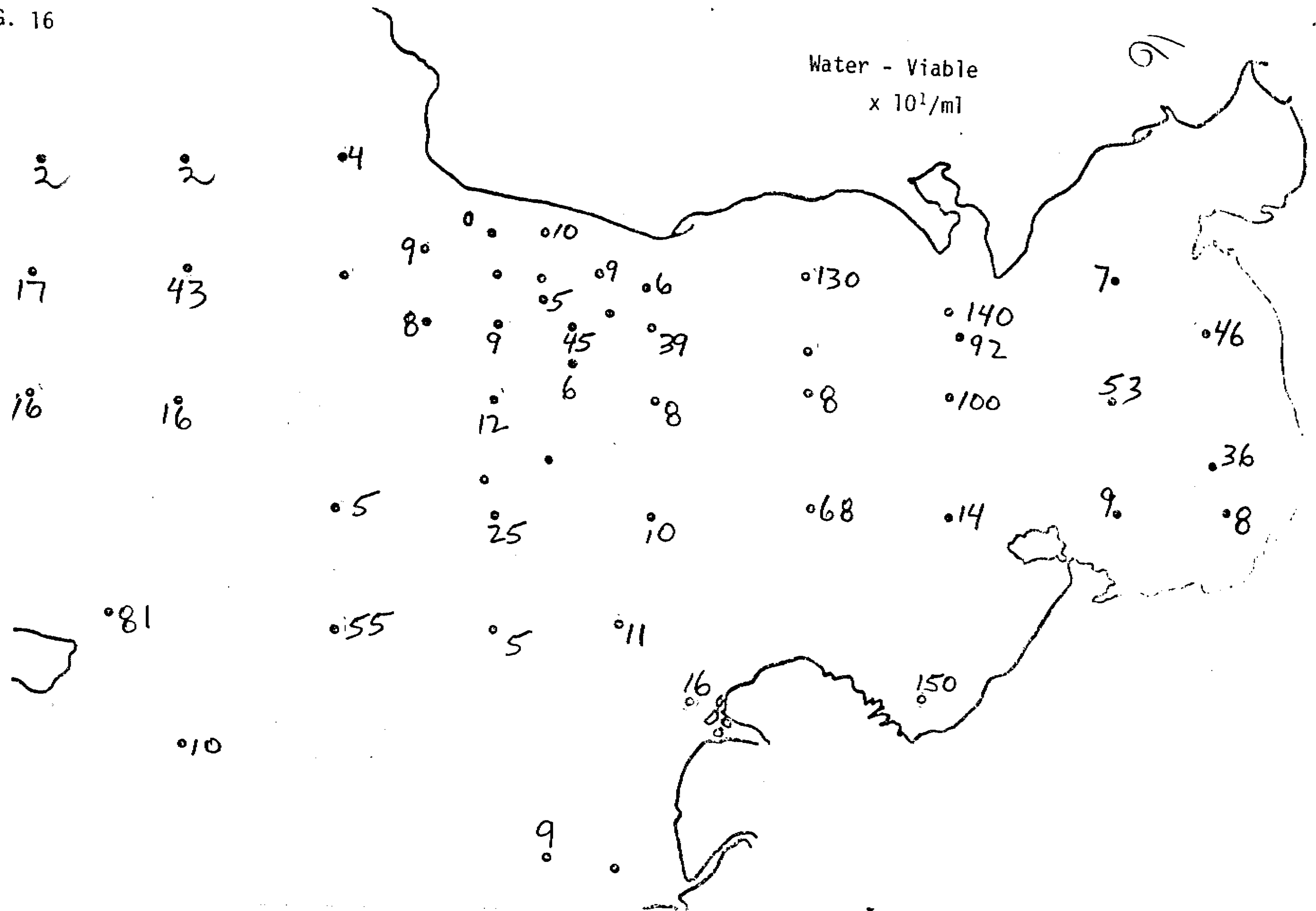
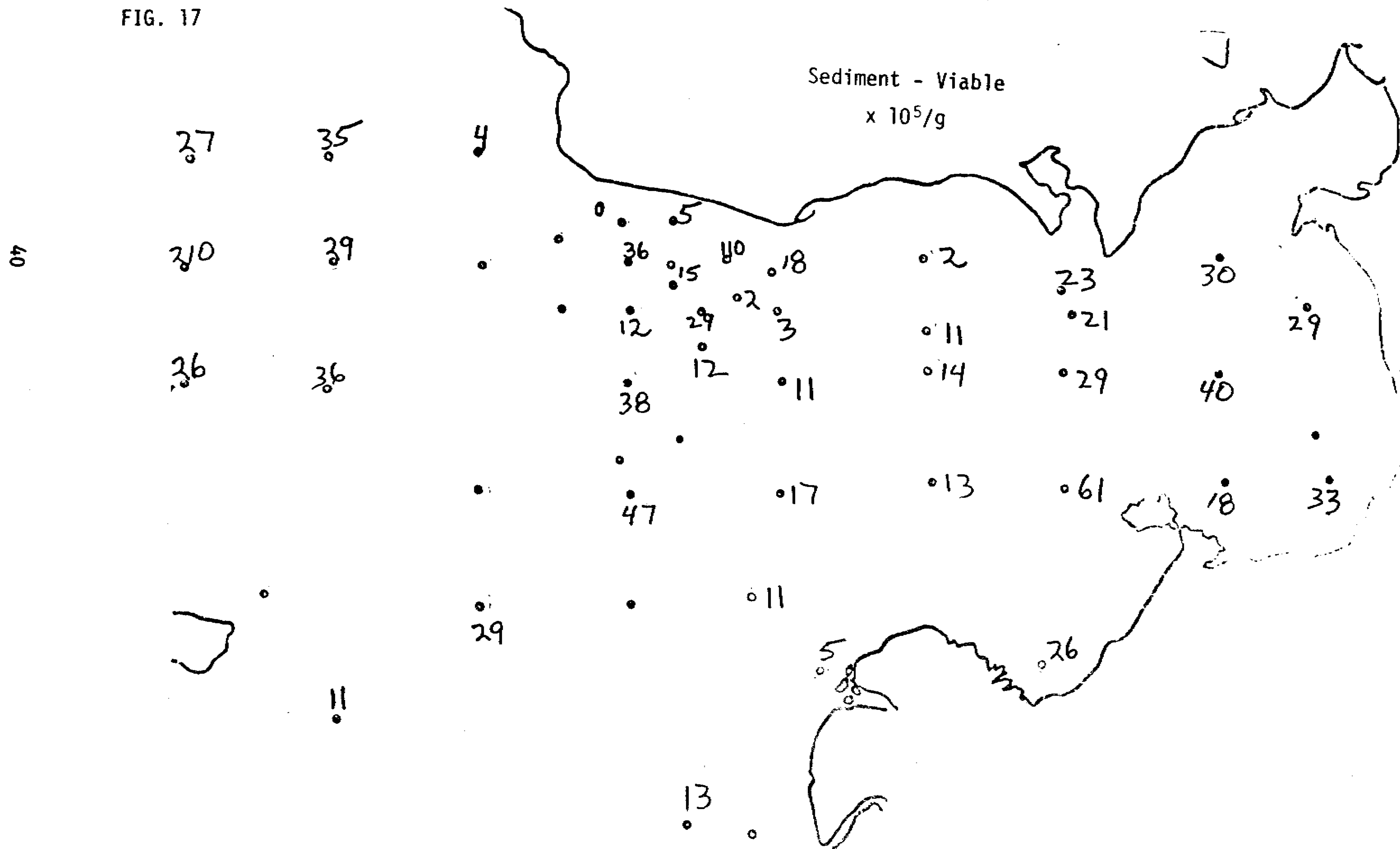


FIG. 17



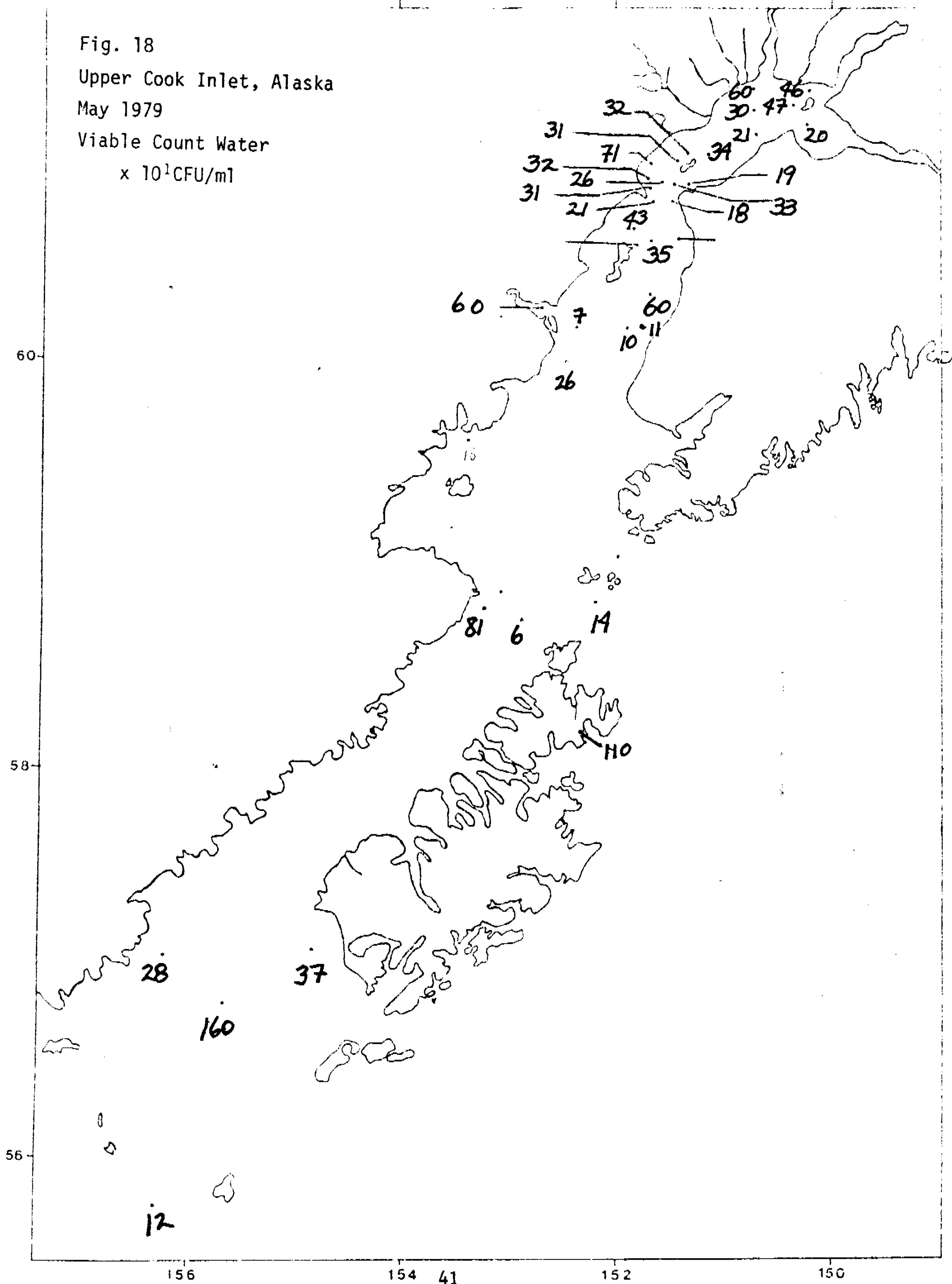


Fig. 18
Upper Cook Inlet, Alaska
May 1979
Viable Count Water
x 10¹CFU/ml
60
46
30
47
32
31
21
20
71
34
32
19
26
31
33
21
18
43
35
60
7
60
10
11
26
81
6
14
110
28
37
160
12
60
58
56
156
154
152
150

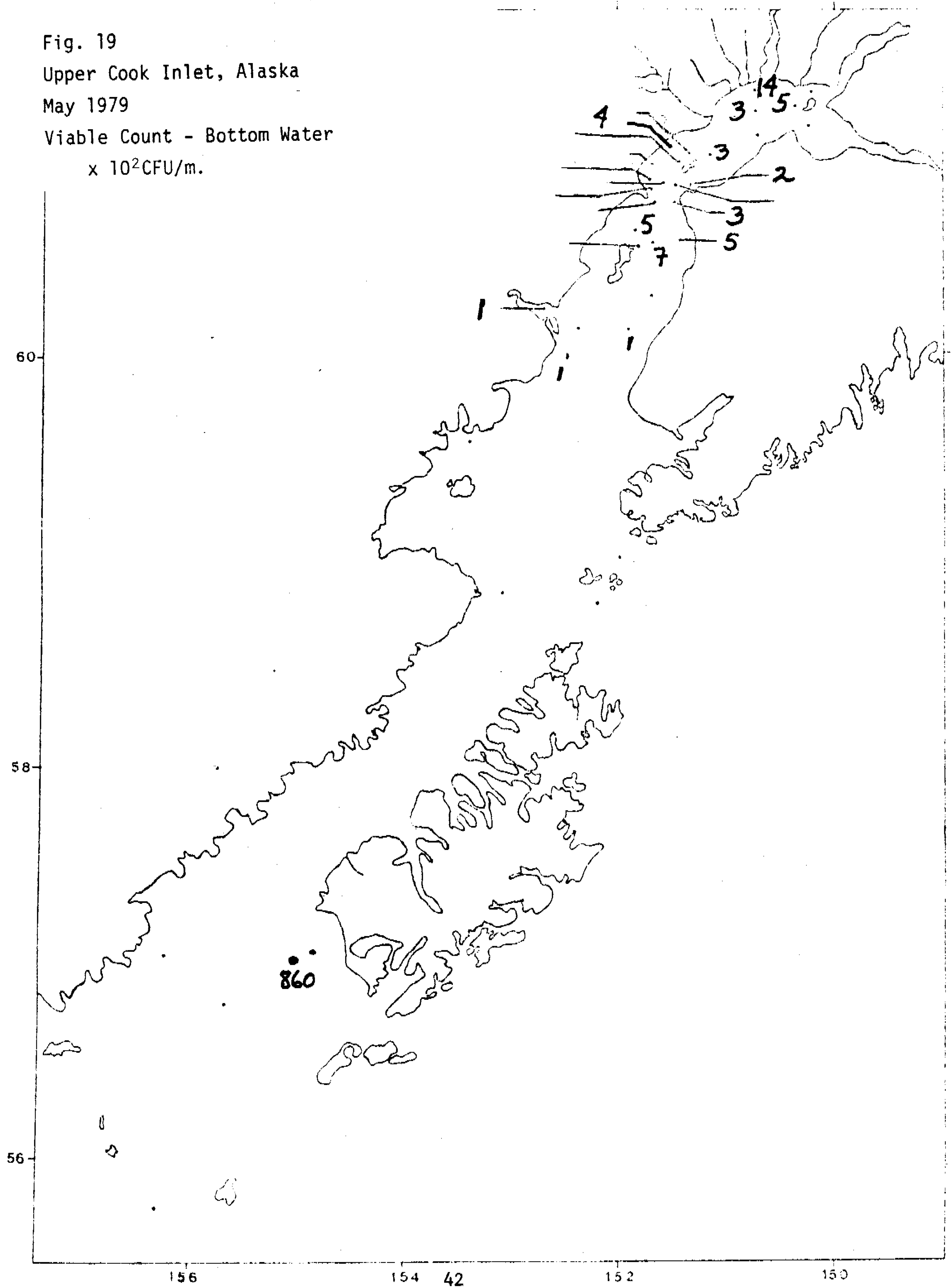


Fig. 19
Upper Cook Inlet, Alaska
May 1979
Viable Count - Bottom Water
x 10^2CFU/m.

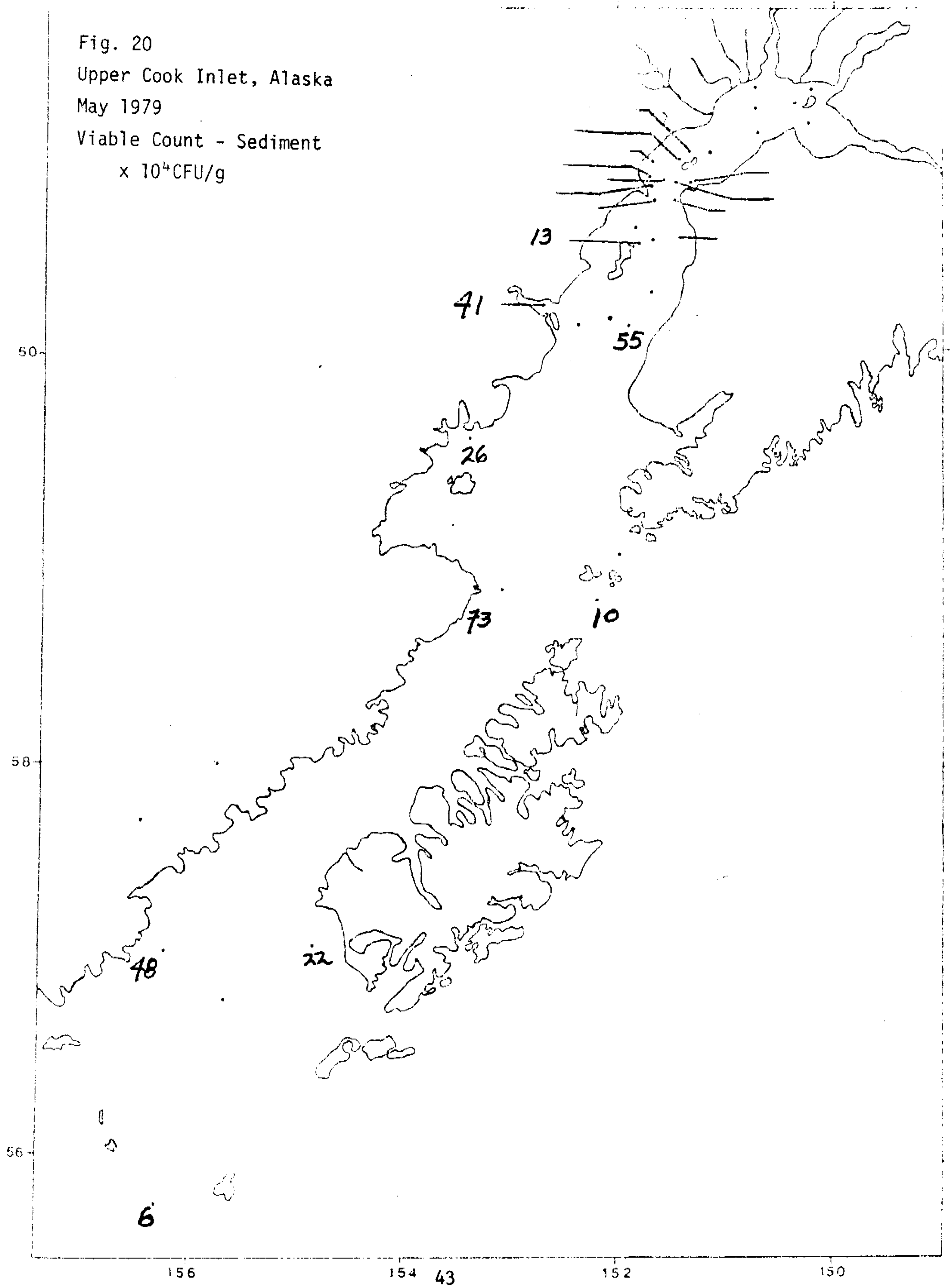
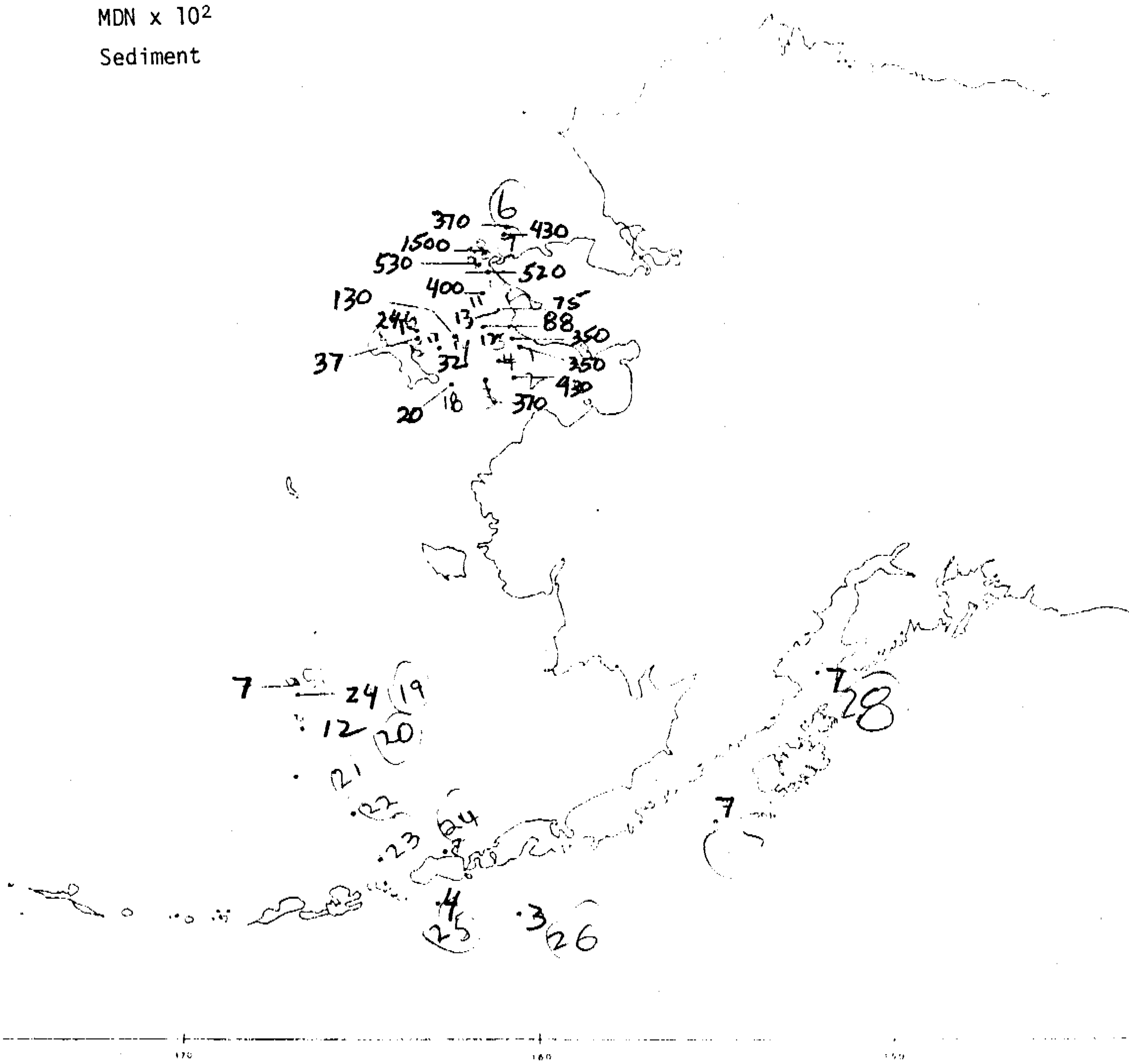


Fig. 20
Upper Cook Inlet, Alaska
May 1979
Viable Count - Sediment
x 10⁴CFU/g
13
41
55
26
73
10
22
48
6
60
58
56
156
154
152
150

Fig. 21 Spring 1979

Bering Sea
4/79 - 5/79
MDN x 10^2
Sediment



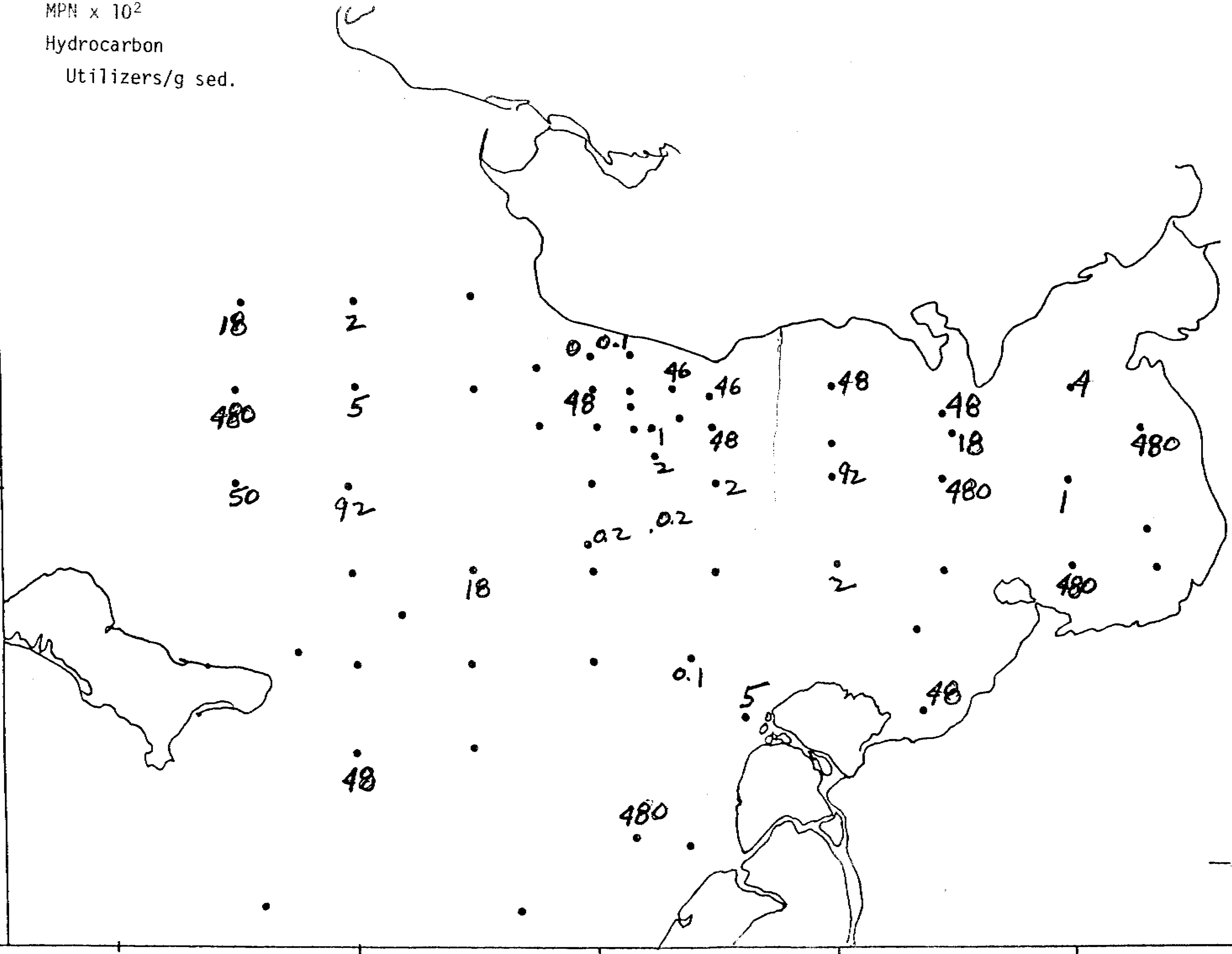


FIG. 22

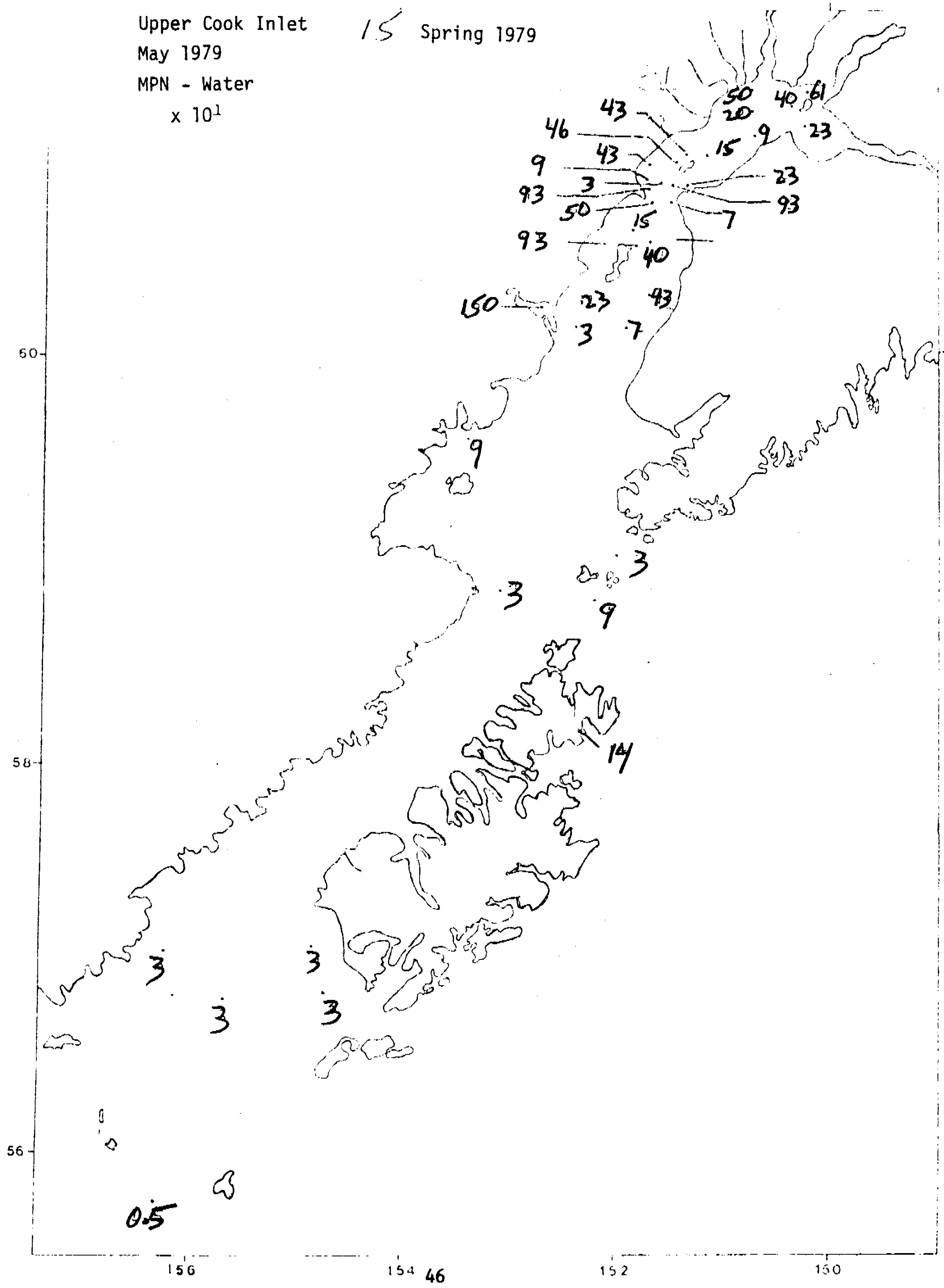
FIG. 23

Upper Cook Inlet

May 1979

MPN - Water

x 10^1



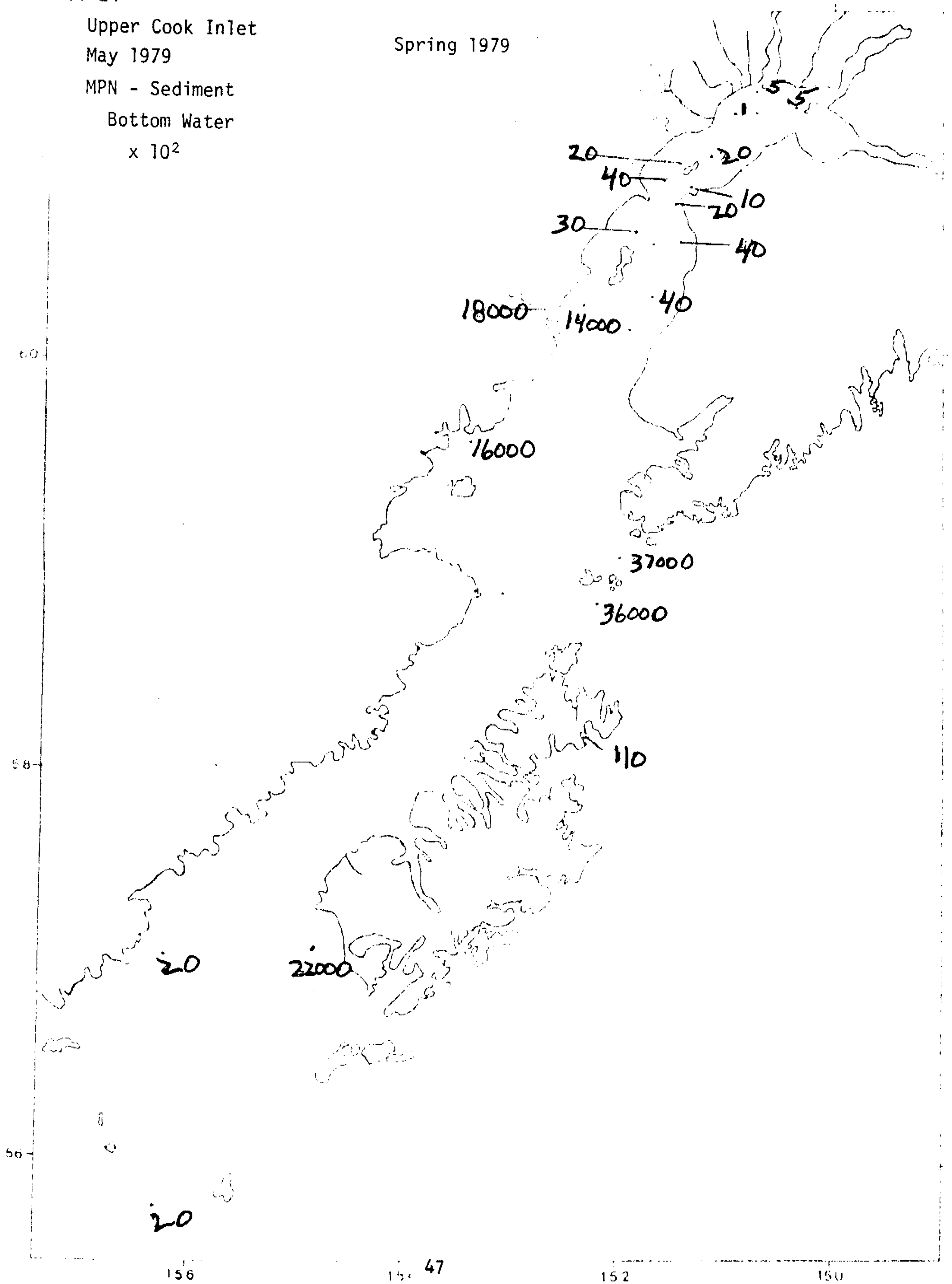


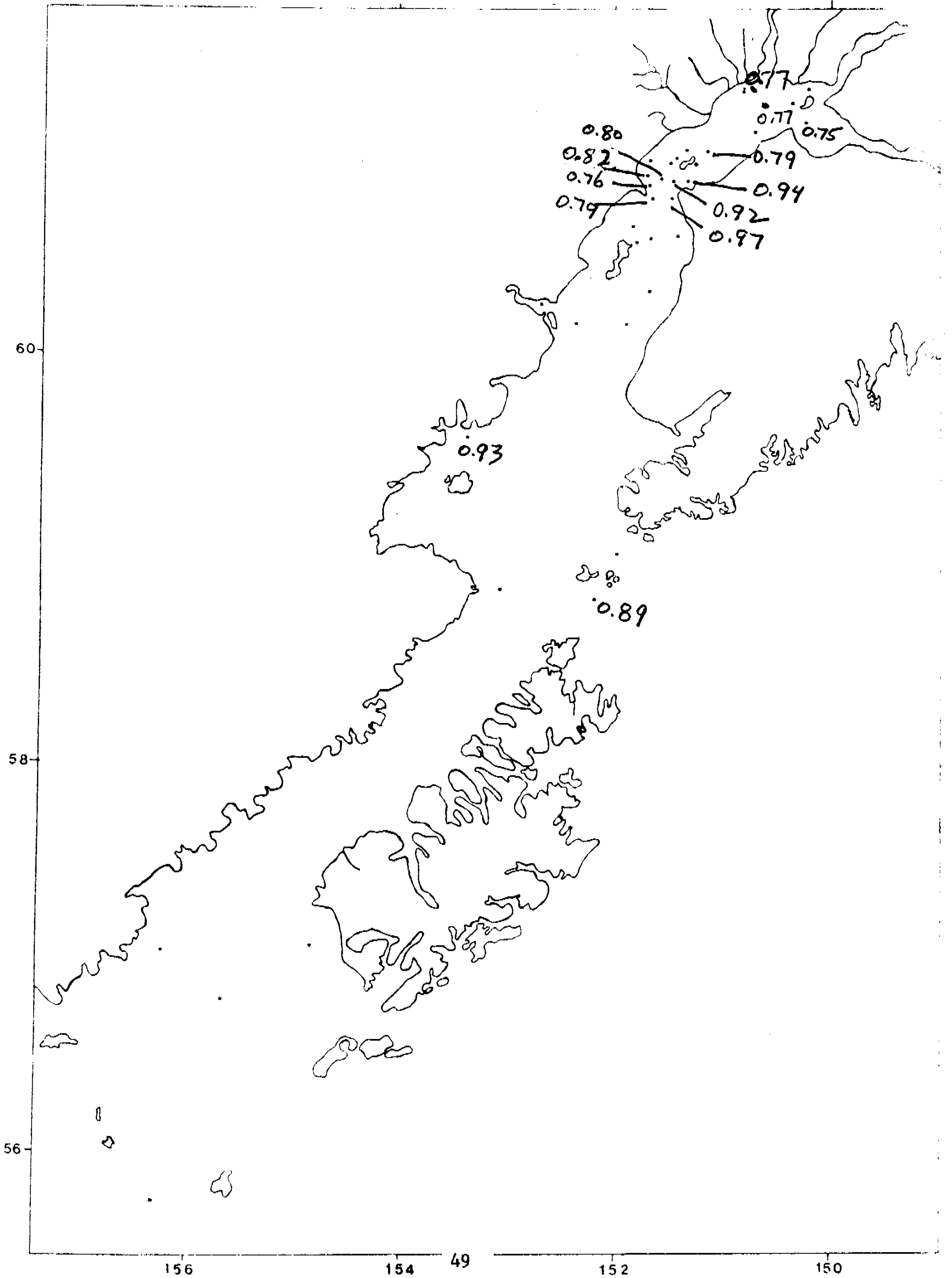
FIG. 24
Upper Cook Inlet
May 1979
MPN - Sediment
Bottom Water
x 10^2
Spring 1979
5
5
.1
20
20
40
10
20
30
40
18000
14000
40
60
16000
37000
36000
58
110
20
22000
56
20
156
152
150

Numerical Taxonomic Testing

A series of scientific manuscripts describing the results of numerical taxonomic testing results are being dispatched separately to the Juneau project office. To avoid duplication and adding to the length of this report those manuscripts are not included in this years report.

Data is shown in Fig. 25 on the equitability of species in Cook Inlet water calculated from the numerical taxonomic analyses. This data is of particular interest this year as it was generated as part of an interdisciplinary cruise effort in Upper Cook Inlet. The taxonomic diversity was somewhat lower in uppermost part of Cook Inlet sampled and along the western side of Upper Cook Inlet than in several stations along the eastern side and in Lower Cook Inlet.

FIG. 25 J - Water



Hydrocarbon Analyses

The analysis of the long term sediment tray experiments yielded several interesting observations. Samples were collected at several times over a 2 year period. The sample collection times were 0, 1/2 hour, 24 hour, 48 hour, 72 hour, 7 days, 14 days, 21 days, 28 days, 3 months, 4 months, 8 months, 1 year, 1 1/4 years, 1 1/2 years, and 2 years (unfortunately due to problems with the experimental field exposure, i.e. the running aground of a NOAA operated vessel amid the trays, these sampling times do not represent a single time course experiment, but a composite of several experiments begun at different times).

Analysis of fractioned samples was performed after preliminary screening of all samples by glass capillary gas chromatography (GC). Alkanes were analyzed by gc and aromatics by gc-ms. After analysis the quantities of specific hydrocarbons were calculated and pristane/heptadecane ratios were calculated. Tables showing the quantities of hydrocarbons and the tracings of the chromatographs are included in the appendix. Table 1 shows the results of the pristane/C_{17} rations observed over 2 years of _in situ_ exposure of oiled sediment to seawater. Prudhoe Bay crude oil had a pristane/C_{17} ratio of 0.86. The lowest ratio observed was 0.73 after 3 months of exposure of oiled sediment. With one exception the pristane/C_{17} ratios observed after 1 year exposure were greater than 1.0. At 1, 1 1/2 years the ratio was 1.19 and at 2 years exposure the pristane/C_{17} ratio was 1.75. The increase of the ratio over time indicates active biodegradation of crude oil. The increase of the ratio over time indicates active biodegradation of crude oil. The increase of the ratio indicates that microorganisms preferentially utilize heptadecane over pristane.

Analysis of alkanes (fraction f_1) demonstrated that crude oil has a tendency to accumulate in pockets in sediments soon after mixing of sediments and oil. "Replicate" samples taken at 0 time had concentrations of specific alkanes between approximately 10 to 60 μg/g dry sediment for C_{12}-C_{22} alkanes. After 0.5 hours the observable range of concentrations for specific alkanes was about 15-20 μg/g dry sediment. At 24 hr. exposure the alkanes were present in the range 15-60 μg/g dry wt. for selected compounds. Samples analyzed from 48 hr to 8 months exposure exhibited relatively stable concentrations of alkanes.

Results

The range of concentrations observed across the exposure times from 48 hr to 8 months was between 3-40 μg/g dry sediment for alkanes from C_{12} to C_{24}. Samples with lower concentrations were found in sediments with longer exposure time. The development of pockets of oil is evident from the range of concentrations of alkanes within any particular exposure time. At 8 months the concentration of undecane began to decline relative to higher molecular weight alkanes. After 1 1/2 years undecane was about 50% of dodecane. After 1 year of exposure the concentrations of alkanes was observed to be less than 10 μg/g dry sediment for specific alkanes. After 1 1/4 years some alkanes were observed in the range of 10-40 μg/g dry sediment presumably due to development of pockets of oil in the sediment. The concentration of alkanes observed after 1 1/2 years exposure was generally less than 15 μg/g dry sediment for selected compounds. The concentrations of alkanes observed after 2 years exposure were in the range of 2 to 10 μg/g dry sediment. In at least one 2 year

sample the lighter alkanes (C_{11}-C_{16}) were not present in detectable quantities.

After screening of aromatic (f_2) samples by GC, samples from each exposure time were chosen for quantitative analysis by mass spectrometry. The compounds found in highest concentration in Prudhoe Bay crude oil were naphthalene, methyl naphthalenes, dimethyl (C_2) naphthalenes, trimethyl (C_3) naphthalenes, phenanthrene, and methyl phenanthrene. The recovery of these compounds from zero time exposures was with one exception close to the quantities of compounds found in analysis of 0.2 ml of Prudhoe Bay crude oil.

After 0.5 hour exposure concentrations of the above compounds had declined to about one half or less of the zero time values. From the 48 hour to 21 day exposure times the concentrations of the selected aromatic compounds were relatively constant at about one half or less of the zero time values.

Results

Recovery of selected aromatics from the 28 day and 3 month exposure time samples demonstrated that the concentrations of the aromatic compounds declined further to less than one fourth of the zero time values. Samples collected from 4 month exposure contained slightly more of the selected aromatics than 3 month samples. After one year of exposure the concentration of the aromatic compounds was reduced further, in some cases to about 10% of the zero time samples. After 1 1/2 years exposure the concentrations of the aromatic components were all less than 10 μg/g dry wt. After 2 years exposure the quantities of aromatic compounds observed was less than 3 μg/g dry sediment except for one compound. Other observations were that

naphthalene and methyl naphthalene declined more rapidly than higher molecular weight aromatic compounds. The development of pockets of oil in sediment was evident in the analysis of these samples also, since concentrations of hydrocarbons were found to vary between samples exposed for the same length of time. A major difference between the alkane analysis and the aromatic analysis was that the concentrations of aromatic compounds declined more uniformly and more rapidly than the alkane concentrations. This observation is probably due to the fact that aromatic compounds are soluble in water at low levels. Losses of aromatic compounds are likely due to dissolution and biodegradation.

Analysis of oil exposed to ice in stainless steel cylinders was also performed. Samples collected at zero exposure time had a pristane to heptadecane ratio of 0.84. At 48 hours, the pristane/heptadecane ratio was 0.82 and at 7 days exposure the pristane to heptadecane ratio was 0.81. The concentrations of oil in the ice cylinders changed rapidly over the period of the experiment. After 48 hours exposure the alkanes had declined in concentration by about 60-70%. After 7 days the concentrations of C_{13}-C_{26} alkanes were similar to the 48 hour samples. Undecane and dodecane had declined relative to the higher molecular weight compounds. Analysis of the aromatic components of the ice cylinder oil exposure experiments indicated that the aromatic compounds behaved similarly to the alkane compounds. After 48 hours exposure, the concentration of aromatics was reduced by about 50-70% and after 7 days exposure the concentration of aromatic compounds was similar to those observed at 48 hours exposure.

Table 1. Pristane to heptadecane ratios from *in situ* sediment tray experiments.

Time	Pristane/heptadecane
Prudhoe Crude	0.86
0 from sediment	0.87
0.5 hours	0.88
24 hours	0.79
48 hours	0.86
72 hours	0.84
7 days	0.80
14 days	0.87
21 days	0.88
28 days	0.73
3 months	0.73
4 months	0.84
8 months	0.83
1 year	1.19
1 1/4 year	0.89
1 1/2 year	1.19
2 year	1.75

VII. Discussion

Denitrification

The role of microorganisms in the biogeochemical cycling of nitrogen on a global scale is well recognized (See reviews by Dalton 1974; Delwiche 1970; Focht and Verstraete 1977; Burns and Hardy 1975 and Payne 1973). There have, however, been relatively few studies which have examined denitrification and nitrogen fixation in marine sediments. Koike and Hattori (1978a) stated that little is know about denitrification in marine sediments. Delwiche (1970) estimated denitrification from marine sources at 40 million tons per year while Rittenberg (1963) calculated a denitrification rate of 8.6 million tons per year. The methodological approaches frequently used for measurement of nitrogen cycling activities in marine sediments, e.g. those which employ ^{15}N tracers, generally do not permit efficient examination of large numbers of samples. The relatively recently developed methods for assaying nitrogen fixation by acetylene reduction and denitrification by acetylene blockage of nitrous oxide reduction permit more extensive determinations of the rates of these critical nitrogen cycling processes.

Several recent studies have examined denitrification in coastal marine sediments. Koike and Hattori (1973a, 1978b) examined denitrification and ammonia formation in coastal sediments. They reported rates of nitrogen gas production in sediment surface layers was about 10^{-2} μg atoms N_2 g^{-1} h^{-1} irrespective of location (3 sediments from Japanese Bays were used in these studies). Oren and Blackburn (1979) reported denitrification rates for sediment cores from Danish fjords of 12.5 nmoles of N_2-N ml^{-1} day^{-1} for the top cm and of 2.0 nmoles of N_2-N ml^{-1} day^{-1} for 1-3 cm. Sorensen (1978b), using the acetylene inhibition technique, reported a maximum rate of denitrification in coastal marine sediment of 35 nmoles of nitrogen ml^{-1} of

sediment day^{-1} at 2.5^{0} C with peak denitrification rates occurring at 2-3 cm. Sorensen (1978c) found the greatest rates of denitrification occurred near the surface sediment in the transitional zone from aerobic to anaerobic conditions. Grundamanis and Murray (1977) also found nitrification and denitrification to be important in surface sediments and implicated bioturbation a major factor affecting the rates of these processes.

Our data indicates that there are major seasonal and spatial differences in the rates of these processes along the Alaskan Continental Shelf. In a previous report Koike and Hattori (1978c) measured a rate of denitrification of 1.2 μg atoms nitrogen g^{-1} dry weight of sediment hour^{-1} in surface sediments of the southern Bering Sea. The paper assumed a uniform rate of denitrification over the entire Bering Sea shelf and estimated the loss of combined nitrogen by denitrification to be 5 x 10^{11} g N year^{-1} over the entire shelf region. In the current study the rate of dentrification at sites comparable to those examined by Koike and Hattori (1978c) averaged 0.9 ng atoms nitrogen g^{-1} dry weight sediment hour^{-1}. Our data was within the range reported in the study by Koike and Hattori. However, there were significant spatial and seasonal differences in the rates of denitrification between the sourther Bering Sea sediments and sediments collected in the northern Bering Sea (an area which is ice covered during part of the year). Rates of denitrification were higher during summer than during spring (when the region was ice covered). These seasonal differences could be due to temperature differences in the environment, perhaps reflecting population differences (our assays were performed at the same temperature and the rates, therefore, do not reflect simple enzyme-temperature relationships artificially created in the methodology.

There were major regional differences in the (data from RU190 Griffiths and Morita) and denitrification which are summarized in Table 2. Rates of nitrogen fixation generally did not correlate with concentrations of fixed forms of nitrogen; rates of denitrification, however, showed a significant correlation with concentrations of nitrate-nitrite but not with concentrations of ammonium ions. Several regional differences in relative rates of denitrification and nitrogen fixation are apparent. In Upper Cook Inlet which has very coarse sediments, natural rates of nitrogen fixation and denitrification were balanced but extremely low. In Shelikof Strait just below Cook Inlet rates of both denitrification and nitrogen fixation were higher and of equal magnitude. In contrast in both Norton Sound and western Cook Inlet (Kamishak Bay) rates of denitrification were significantly higher than rates of nitrogen fixation. In these latter regions the sediments represent a sink for fixed forms of nitrogen; in these sediments fixed forms of nitrogen which entered from terrestrial and river runoff would be expected to be lost from the sediments. Consideration of seasonal and spatial variations can greatly alter estimates of annual fluxes of nitrogen.

The data in the present study suggests that the potential for denitrification is as great or greater in northern sediments as in southern Alaskan Continental Shelf regions. This is suggested by the greater increase in rates of denitrification when nitrate is added to northern sediments indicative of a high potential for denitrification and by the higher incidence of cases where denitrification could not be detected without added nitrate in northern sediments indicative of complete depletion of available substrate. Denitrification in these sediments was apparently limited by "available" nitrate. Nitrification which was not measured in the present study undoubtedly

plays a critical link in the nitrogen cycle in these sediments by controlling the rates at which nitrate becomes available for denitrification. Koike and Hattori (1978a) previously reported that the rate of denitrification is proportional to nitrate concentrations in the range of 0-30 μg atoms nitrogen l^{-1}. They also concluded that denitrification in Bering Sea sediments was apparently controlled by the supply of nitrate and nitrite to the sediments (1978c). In the present study there was a significant correlation (α <0.001) between rates of denitrification and nitrate-nitrite concentrations although the correlation coefficient (r) was only 0.6.

Freshwater input (e.g. from the Yukon River) had an apparent effect on rates of denitrification. This was most likely due to inputs of nitrogenous compounds rather than of organic carbon. Indeed addition of organic carbon-nitrogen generally did not stimulate rates of denitrification but rather in many cases resulted in a 50% depression in these rates. Addition of nitrate with or without organic carbon-nitrogen on the other hand greatly stimulated rates of denitrification in all cases.

Hydrocarbon Analyses

The results of the analysis of hydrocarbons from crude oil mixed with sediment and exposed *in situ* to a shallow water environment indicate that biodegradation of crude oil occurs over a relatively long time period. No substantial change in the ratio of pristane to heptadecane occurred until after 8 months of exposure even though the total quantity of hydrocarbons declined over the same period. After 8 months the ratio of pristane to heptadecane began to increase until the 2 year exposure period which had a pristane/heptadecane ratio of 1.75. The increase of the ratio indicates a preferential utilization of straight chain hydrocarbons by the microbiota of the Elson Lagoon sediments. Similarly, light alkanes began to decline

in concentration compared to longer chain alkanes after 8 months exposure. In at least one sample from the 2 year exposure period C_{11}-C_{17} were not detected. Another clear result of the analysis of the sediment tray experiments is that the added oil tended to gather together in pockets or small pools of oil. The development of these pockets of oil would tend to decrease the rate of biodegradation of oil hydrocarbons since the oil exposed to the active microbiota would be at an effectively lower concentration than originally added to the sediment.

The analysis of the aromatic components of the sediment tray experiments yielded results different than the alkane analysis in that the concentration of the aromatics declined more rapidly than the alkanes and reached a moderately stable level that was constant for about 1 year exposure. After which the concentration of the aromatics declined further. As with the alkane analysis, light compounds such as naphthalene and methyl naphthalene declined in concentration more rapidly than more highly substituted naphthalene or phenanthrene. The rapid changes in the aromatic components of crude oil in these experiments are probably due to the dissolution of the aromatic hydrocarbons in seawater.

Oil exposed to ice rapidly decreased in concentration. Since there was no change in pristane to heptadecane ratios over the period of the experiment, the primary mechanism of oil loss would presumably due to dissolution and other transport phenomena rather than biodegradation. There was no major difference in the behavior of aromatic and alkane hydrocarbons in these experiments.

It is possible to conclude from this work that oil impacting arctic sediments is subject to biodegradation after several months exposure. Dissolution and other transport mechanisms play a role in the removal of oil

from impacted sediments. Crude oil impacting on sediments demonstrates a tendency to aggregate in small pools which further decrease the availability of the oil to organisms capable of degrading the oil. It is possible that the slow onset of biodegradation of oil may be partially due to the accumulations of oil in pools. The processes of biodegradation exhibit a preference for straight chain alkanes over branched chain alkanes as evidenced by the change in pristane to heptadecane ratios. Development of an estimate of the rate of biodegradation of oil in arctic marine sediments will require much additional analysis of existing data and future developments in analytical technique.

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APPENDIX

GAS CHROMATOGRAPHY

MASS SPECTROMETRY

DATA

Please Note: Included in this appendix are the tables derived from gas chromatography and mass spectrometry. The actual printouts are of too poor quality to reproduce in this publication; this office will be happy to supply copies of this information upon request.

SEDIMENT

ANALYSES

Hydrocarbon Analysis

Sample number: PC1 Fresh Prudhoe Crude

Compound	area units	rf	ng/ml	µg/g
HMB	4128	0.014		
undecane	10510	0.011	25.1	50.2
dodecane	10510	0.011	25.1	50.2
tridecane	9466	0.012	24.7	49.4
tetradecane	8406	0.012	21.9	43.8
pentadecane	8790	0.013	24.8	49.6
hexadecane	8328	0.012	21.7	43.4
heptadecane	6804	0.014	20.7	41.4 → .86
pristane	4087	0.02	17.8	35.6
octadecane	6474	0.014	19.7	39.4 → .66
phytane	3022	0.02	13.1	26.2
nonadecane	5822	0.014	17.7	35.4
eicosane	5473	0.0135	16.1	32.2
uncosane	5207	0.0135	15.3	30.6
docosane	4507	0.013	12.7	25.4
tricosane	3838	0.0135	11.3	22.6
tetracosane	3209	0.014	9.8	19.6
pentacosane	2544	0.0145	8.1	16.2
hexacosane	1946	0.015	6.3	12.6
heptacosane	1328	0.0155	4.5	9.0
octacosane	820	0.016	2.9	5.8
nonacosane				
triacontane				
untriacontane				
dotriacontane				

Hydrocarbon Analysis

Sample number: PC1 Fresh Prudhoe Crude

Compound	area units	rf	ng/ul	Mg/
HMB	568364	2×10^{-4}		
naphthalene	437540	2×10^{-4}	19.4	
methyl naphthalene	723169	2×10^{-4}	32.1	
	415560		18.5	
dimethyl naphthalene	619153	2.5×10^{-4}	34.4	
	784752		43.6	
	215568		12.0	
trimethyl naphthalene	55523	2×10^{-4}	2.5	
	51628		2.3	
	336603		15.0	
	224378		10.0	
	117110		5.2	
phenanthrene	68545	5×10^{-4}	7.6	
dibenzothiophene	59832	4×10^{-4}	5.3	
methyl phenanthrene	48974	1.1×10^{-3}	12.0	
	48761		11.9	
methyl dibenzothiophene	47342	4×10^{-4}	4.2	
	20975		1.9	
	9682		0.9	
dimethyl phenanthrene	60284	1.1×10^{-3}	3.3	
dimethyl dibenzothiophene	23834	4×10^{-4}	2.1	
	20796		1.8	
	10345		0.9	
	5031		0.4	
trimethyl phenanthrene	8574	1.1×10^{-3}	2.1	
	19268		4.7	
trimethyl dibenzothiophene	4151		1.0	
	4054	4×10^{-4}	0.4	
	6195		0.6	
	5072		0.5	
	3779		0.3	
	433		0.1	
Benzanthracene	1153	9.6×10^{-3}	2.5	
C4 phenanthrene	11298	1.1×10^{-3}	2.8	
	605		0.1	
	708		0.2	

Hydrocarbon Analysis

Sample number: PC2 2.5 ul Fresh Prudhoe Crude

Compound	area units	rf	ng/ul	ug/l
HMB	3982	0.014		
undecane	9958	0.011	24.9	49.8
dodecane	10070	0.011	25.2	50.4
tridecane	9160	0.012	25.0	50.0
tetradecane	7914	0.012	21.6	43.2
pentadecane	8536	0.013	25.2	50.4
hexadecane	8038	0.012	21.9	43.8
heptadecane	6504	0.014	20.7	41.4
pristane	3936	0.02	17.9	35.8
octadecane	6290	0.014	20.0	40.0
phytane	2923	0.02	13.3	26.6
nonadecane	668	0.014	2.1	4.2
eicosane	5366	0.0135	16.5	33.0
uncosane	5261	0.0135	16.1	32.2
docosane	4726	0.013	14.0	28.0
tricosane	4142	0.0135	12.7	25.4
tetracosane	3577	0.014	11.4	22.8
pentacosane	2873	0.0145	9.5	19.0
heptacosane	2330	0.015	7.9	15.8
heptacosane	1708	0.0155	6.0	12.0
octacosane	1069	0.016	3.9	7.8
nonacosane	698	0.016	2.5	5.0
triacontane				
untriacontane				
dotriacontane				

→ .86 (heptadecane/pristane)

→ .67 (octadecane/phytane)

Hydrocarbon Analysis

Sample number: PC2 Fresh Prudhoe Crude

Compound	area units	rf	ng/μl	μg/l
HMB	595647	2×10^{-4}		
naphthalene	474397	2×10^{-4}	20.2	
methyl naphthalene	701058	2×10^{-4}	29.8	
	409252		17.4	
dimethyl naphthalene	647426	2.5×10^{-4}	34.4	
	777268		41.3	
	250786		13.3	
trimethyl naphthalene	35139	2×10^{-4}	1.5	
	59568		2.5	
	331572		14.1	
	208731		8.9	
	90665		3.9	
phenanthrene	58407	5×10^{-4}	6.2	
dibenzothiophene	45415	4×10^{-4}	3.9	
methyl phenanthrene	39507	1.1×10^{-3}	9.2	
	37227		8.7	
methyl dibenzothiophene	37567	4×10^{-4}	3.2	
	15863		1.4	
	4962		0.4	
dimethyl phenanthrene	48740	1.1×10^{-3}	11.6	
dimethyl dibenzothiophene	18499	4×10^{-4}	1.6	
	17869		1.5	
	8976		0.8	
	4254		0.4	
trimethyl phenanthrene	14473	1.1×10^{-3}	3.4	
	668		0.2	
trimethyl dibenzothiophene	4418	4×10^{-4}	0.4	
	6239		0.5	
	4887		0.4	
	3135		0.3	
	1894		0.2	
Benzanthracene	507	9.6×10^{-3}	1.0	
	12341	1.1×10^{-3}	2.9	
Tet M (C4) Phenanthrene	525		0.1	
	447		0.1	

Hydrocarbon Analysis

Sample number: 0 Time oil 4/78

Compound	area units	rf	ng/µl	µg/g dry wt.
HMB	3525	0.014		
undecane	252	0.011	0.7	1.4
dodecane	280	0.011	0.8	1.6
tridecane	380	0.012	1.2	2.4
tetradecane	367	0.012	1.1	2.2
pentadecane	461	0.013	1.5	3.0
hexadecane	410	0.012	1.3	2.6
heptadecane	391	0.014	1.4	2.8
pristane	did not integrate			
octadecane	369	0.014	1.3	2.6
phytane	did not integrate			
nonadecane	362	0.014	1.3	2.6
eicosane	371	0.0135	1.3	2.6
uncosane	393	0.0135	1.4	2.8
docosane	348	0.013	1.2	2.4
tricosane	361	0.0135	1.2	2.4
tetracosane	288	0.014	1.0	2.0
pentacosane	278	0.0145	1.0	2.0
hexacosane	207	0.015	0.8	1.6
heptacosane	did not integrate			
octacosane	did not integrate			
nonacosane				
triacontane				
untriacontane				
dotriacontane				

Hydrocarbon Analysis

Sample number: 0-Time A4 f1 2.5 µl

Compound	area units	rf	ng/µl	µg/g dry wt.
HMB	4400	0.014		
undecane	9256	0.011	20.8	41.6
dodecane	10430	0.011	23.4	46.8
tridecane	10210	0.011	22.9	45.8
tetradecane	9352	0.012	22.9	45.8
pentadecane	10450	0.013	27.7	55.4
hexadecane	9624	0.012	23.6	47.2
heptadecane	7908	0.014	22.6	45.2 →.83
pristane	4589	0.02	18.7	37.4
octadecane	7568	0.014	21.6	43.2 →.68
phytane	3580	0.02	14.6	29.2
nonadecane	6582	0.014	18.8	37.6
eicosane	6534	0.0135	20.0	40.0
uncosane	6264	0.0135	17.3	34.6
docosane	5490	0.013	14.6	29.2
tricosane	4705	0.0135	13.0	26.0
tetracosane	4029	0.014	11.5	23.0
pentacosane	3188	0.0145	9.4	18.8
hexacosane	2577	0.015	7.9	15.8
heptacosane	1805	0.0155	5.7	11.4
octacosane	1128	0.016	3.7	7.4
nonacosane	699	0.016	2.3	4.6
triacontane				
untriacontane				
dotriacontane				

Hydrocarbon Analysis

Sample number: O Time A4

Compound	area units	rf	ng/ul	µg/g dry wt.
HMB	591702	2×10⁻⁴		
naphthalene	409719	2×10⁻⁴	17.4	17.4
methyl naphthalene	710247	2×10⁻⁴	30.2	30.2
	427843		18.2	18.2
dimethyl naphthalene	724771	2.5×10⁻⁴	38.6	38.6
	816508		43.4	43.4
	277805		14.8	14.8
trimethyl naphthalene	58519	2×10⁻⁴	2.5	2.5
	57524		2.4	2.4
	393653		16.8	16.8
	256848		10.9	10.9
	115357		4.9	4.9
phenanthrene	107268	5×10⁻⁴	11.4	11.4
dibenzothiophene	86856	4×10⁻⁴	7.4	7.4
methyl phenanthrene	81141	1.1×10⁻³	19.0	19.0
	76655		17.9	17.9
methyl dibenzothiophene	84860	4×10⁻⁴	7.2	7.2
	35592		3.0	3.0
	6755		0.6	0.6
dimethyl phenanthrene	93702	1.1×10⁻³	21.9	21.9
dimethyl dibenzothiophene	40547	4×10⁻⁴	3.5	3.5
	36069		3.1	3.1
	17602		1.5	1.5
	8558		0.7	0.7
trimethyl phenanthrene	56856	1.1×10⁻³	13.3	13.3
	5282		1.2	1.2
trimethyl dibenzothiophene	8460	4×10⁻³	0.7	0.7
	10929		0.9	0.9
	16130		1.4	1.4
Benzanthracene	639	9.6×10⁻³	1.3	1.3
C4 Phenanthrene	13478	1.1×10⁻³	3.2	3.2
	696		0.2	0.2
	762		0.2	0.2

Hydrocarbon Analysis

Sample number: Oil 0 Time froz. 2.5 ul

Compound	area units	rf	ng/ul	ug/g dry wt.
HMB	5836	0.014		
undecane	3376	0.011	6.6	13.2
dodecane	8560	0.011	14.5	29.0
tridecane	11630	0.012	21.5	43.0
tetradecane	12590	0.012	23.2	46.4
pentadecane	14720	0.013	29.4	58.8
hexadecane	13920	0.012	25.7	51.4
heptadecane	11350	0.014	24.4	48.8 → .71
pristane	5609	0.02	17.3	34.6
octadecane	11060	0.014	23.8	47.6 → .70
phytane	5425	0.02	16.7	33.4
nonadecane	9942	0.014	21.4	42.8
eicosane	10220	0.0135	21.2	42.4
uncosane	8910	0.0135	18.5	37.0
docosane	7772	0.013	15.5	31.0
tricosane	6798	0.0135	14.1	28.2
tetracosane	5969	0.014	12.9	25.8
pentacosane	4998	0.0145	11.1	22.2
hexacosane	4266	0.015	9.8	19.6
heptacosane	3420	0.0155	8.2	16.4
octacosane	2313	0.016	5.7	11.4
nonacosane	1719	0.016	4.2	8.4
triacontane	870	0.0165	2.2	4.4
untriacontane				
dotriacontane				

Hydrocarbon Analysis

Sample number: Oil 0 Time frozen

Compound	area units	rf	ng/ml	μg/g dry wt.
HMB	585734	2×10^{-4}		
naphthalene	327003	2×10^{-4}	14.2	14.2
methyl naphthalene	841739	2×10^{-4}	36.6	36.6
	492283		21.4	21.4
dimethyl naphthalene	883373	2.5×10^{-4}	48.0	48.0
	1139949		62.0	62.0
	320367		17.4	17.4
trimethyl naphthalene	95395	2×10^{-4}	4.1	4.1
	505849		22.0	22.0
	323421		14.1	14.1
	227345		9.9	9.9
phenanthrene	99174	5×10^{-4}	10.8	10.8
dibenzothiophene	76972	4×10^{-4}	6.7	6.7
methyl phenanthrene	67764	1.1×10^{-3}	16.2	16.2
	65033		15.6	15.6
methyl dibenzothiophene	71785	4×10^{-4}	6.2	6.2
	30359		2.6	2.6
	6149		0.5	0.5
dimethyl phenanthrene	88523	1.1×10^{-3}	21.2	21.2
dimethyl dibenzothiophene	34294	4×10^{-4}	3.0	3.0
	30810		2.7	2.7
	16254		1.4	1.4
	7955		0.7	0.7
trimethyl phenanthrene	74595	1.1×10^{-3}	17.8	17.8
trimethyl dibenzothiophene	4295		1.0	1.0
	7608	4×10^{-4}	0.7	0.7
	11807		1.0	1.0
	7700		0.7	0.7
	5455		0.5	0.5
Benzanthracene	1650	9.6×10^{-3}	3.4	3.4
C4 Phenanthrene	27726	1.1×10^{-3}	6.6	6.6
	1705		0.4	0.4

Hydrocarbon Analysis

Sample number: 4/78S

0 time exposure

Compound	area units	rf	ng/ul	μg/g dry wt.
HMB	542010	2×10^{-4}		
naphthalene	185609	2×10^{-4}	8.6	8.6
methyl naphthalene	339323	2×10^{-4}	15.8	15.8
	165744		7.7	7.7
dimethyl naphthalene	278347	3.5×10^{-4}	22.7	22.7
	300044		24.4	24.4
	96487		7.9	7.9
trimethyl naphthalene	12063	3×10^{-4}	0.8	0.8
	24306		1.7	1.7
	116783		8.1	8.1
	76940		5.4	5.4
	30439		2.1	2.1
phenanthrene	21698	5×10^{-4}	2.5	2.5
dibenzothiophene	15404	5×10^{-4}	1.8	1.8
methyl phenanthrene	19823	1.1×10^{-3}	5.1	5.1
	16084		4.1	4.1
methyl dibenzothiophene	12069	5×10^{-4}	1.4	1.4
	6794		0.8	0.8
	3096		0.4	0.4
dimethyl phenanthrene	21226	1.1×10^{-3}	5.4	5.4
dimethyl dibenzothiophene	7963	5×10^{-4}	0.9	0.9
	8063		0.9	0.9
	4208		0.5	0.5
	2079		0.2	0.2
trimethyl phenanthrene	1314	1.1×10^{-3}	0.3	0.3
trimethyl dibenzothiophene	2830		0.7	0.7
	594	5×10^{-4}	0.1	0.1
	3555		0.4	0.4
	3351		0.4	0.4
Benzanthracene	290	1.1×10^{-2}	0.7	0.7
C4 phenanthrene	146	1.1×10^{-3}	0.1	0.1

Hydrocarbon Analysis

Sample number: 4/785

0 Time exposure

Compound	area units	rf	ng/µl	µg/g dry wt.	
HMB	4488	0.014			
undecane	4798	0.011	10.6	21.2	
dodecane	5065	0.011	11.1	22.2	
tridecane	5288	0.012	12.7	25.4	
tetradecane	4811	0.012	11.5	23.0	
pentadecane	4733	0.013	12.3	24.6	
hexadecane	4433	0.012	10.6	21.2	
heptadecane	3967	0.014	11.1	22.2	→ .86
pristane	2369	0.02	9.5	19.0	
octadecane	3519	0.014	9.9	19.8	→ .72
phytane	1783	0.02	7.1	14.2	
nonadecane	3348	0.014	9.4	18.6	
eicosane	3182	0.0135	8.6	17.2	
uncosane	3152	0.0135	8.5	17.0	
docosane	2983	0.013	7.8	15.6	
tricosane	2851	0.0135	7.7	15.4	
tetracosane	2653	0.014	7.4	14.8	
pentacosane	2357	0.0145	6.8	13.6	
hexacosane	2013	0.015	6.0	12.0	
heptacosane	1580	0.0155	4.9	9.8	
octacosane	963	0.016	3.1	6.2	
nonacosane	567	0.016	1.8	3.6	
triacontane					
untriacontane					
dotriacontane					

Hydrocarbon Analysis

Sample number: 4/78 00

Compound	area units	rf	ng/ul	µg/g dry wt.
HMB	5204	0.014		
undecane	6446	0.011	12.2	24.4
dodecane	6746	0.011	12.8	25.6
tridecane	6952	0.012	14.4	28.8
tetradecane	6330	0.012	13.1	26.2
pentadecane	6290	0.013	14.1	28.2
hexadecane	6063	0.012	12.5	25.0
heptadecane	5371	0.014	13.0	26.0 → .83
pristane	3137	0.02	10.8	21.6
octadecane	4907	0.014	11.8	23.6 → .73
phytane	2502	0.02	8.6	17.2
nonadecane	4634	0.014	11.2	22.4
eicosane	4401	0.0135	10.2	20.4
uncosane	4412	0.0135	10.3	20.6
docosane	4267	0.013	9.6	19.2
tricosane	4014	0.0135	9.3	18.6
tetracosane	3702	0.014	8.9	17.8
pentacosane	3263	0.0145	8.2	16.4
hexacosane	2803	0.015	7.2	14.4
heptacosane	2167	0.0155	6.8	13.6
octocosane	1483	0.016	4.1	8.2
nonacosane				
triacontane				
untriacontane				
dotriacontane				

Hydrocarbon Analysis

Sample number: 4/73 00

Compound	area units	rf	ng/ml	µg/g dry wt.
HMB	730973	2×10^{-4}		
naphthalene	265689	1×10^{-4}	4.6	4.6
methyl naphthalene	438561 270760	2×10^{-4}	15.1 9.3	15.1 9.3
dimethyl naphthalene	532073 549987 140295	2.5×10^{-4}	22.9 23.7 6.0	22.9 23.7 6.0
trimethyl naphthalene	24931 46098 225188 164806 83212	2×10^{-4}	0.9 1.6 7.8 5.7 2.9	0.9 1.6 7.8 5.7 2.9
phenanthrene	74185	3×10^{-4}	3.8	3.8
dibenzothiophene	53858	3×10^{-4}	2.8	2.8
methyl phenanthrene	46782 45392	1.3×10^{-3}	10.5 10.2	10.5 10.2
methyl dibenzothiophene	45617 20623 4903	3×10^{-4}	2.4 1.1 0.3	2.4 1.1 0.3
dimethyl phenanthrene	47314 1144	1.3×10^{-3}	10.6 0.3	10.6 0.3
dimethyl dibenzothiophene	23111 21225 9116 4671	3×10^{-4}	1.2 1.1 0.5 0.2	1.2 1.1 0.5 0.2
trimethyl phenanthrene	20245 2113	1.3×10^{-3}	4.5 0.5	4.5 0.5
trimethyl dibenzothiophene	31253 3217 1734	3×10^{-4}	1.6 0.2 0.1	1.6 0.2 0.1

Hydrocarbon Analysis

Sample number: 48-A
0 Time

Compound	area units	rf	ng/µl	µg/g dry wt.
HMB	3699	0.014		
undecane	798	0.011	2.1	4.2
dodecane	1151	0.011	3.1	6.2
tridecane	1323	0.012	3.9	7.8
tetradecane	1236	0.012	3.6	7.2
pentadecane	1260	0.013	4.0	8.0
hexadecane	1080	0.012	3.2	6.4
heptadecane	1038	0.014	3.5	7.0 → 1.03
pristane	735	0.02	3.6	7.2
octadecane	917	0.014	3.1	6.2 → .94
phytane	598	0.02	2.9	5.8
nonadecane	896	0.014	3.1	6.2
eicosane	839	0.0135	2.8	5.6
uncosane	897	0.0135	3.0	6.0
docosane	824	0.013	2.6	5.2
tricosane	798	0.0135	2.6	5.2
tetracosane	780	0.014	2.7	5.4
pentacosane	726	0.0145	2.6	5.2
hexacosane	614	0.015	2.2	4.4
heptacosane	406	0.0155	1.5	3.0
octacosane	did not integrate			
nonacosane				
triacontane				
untriacontane				
dotriacontane				

Hydrocarbon Analysis

Sample number: 48B

0 Time

Compound	area units	rf	µg/µl	µg/g dry wt.
HMB	4135	0.014		
undecane	1010	0.011	2.4	4.8
dodecane	1475	0.011	3.5	7.0
tridecane	1770	0.012	4.6	9.2
tetradecane	1716	0.012	4.5	9.0
pentadecane	1779	0.013	5.0	10.0
hexadecane	1571	0.012	4.1	8.2
heptadecane	1487	0.014	4.5	9.0 → .96
pristane	994	0.02	4.3	8.6
octadecane	1326	0.014	4.0	8.0 → .83
phytane	769	0.02	3.3	6.6
nonadecane	1301	0.014	4.0	8.0
eicosane	1219	0.0135	3.6	7.2
uncosane	1207	0.0135	3.5	7.0
docosane	1166	0.013	3.3	6.6
tricosane	1147	0.0135	3.4	6.8
tetracosane	1068	0.014	3.3	6.6
pentacosane	958	0.0145	2.9	5.8
hexacosane	814	0.015	2.7	5.4
heptacosane	562	0.0155	1.9	3.8
octacosane	did not integrate			
nonacosane				
triacontane				
untriacontane				
dotriacontane				

Hydrocarbon Analysis

Sample number: 48 D

Compound	area units	rf	ng/ul	μg/g dry wt.
HMB	3414	0.014		
undecane	254	0.011	0.8	1.6
dodecane	376	0.011	1.1	2.2
tridecane	449	0.012	1.5	3.0
tetradecane	433	0.012	1.4	2.8
pentadecane	514	0.013	1.8	3.6
hexadecane	422	0.012	1.4	2.8
heptadecane	384	0.014	1.5	3.0
pristane	did not integrate			
octadecane	339	0.014	1.3	2.6
phytane	did not integrate			
nonadecane	369	0.014	1.4	2.8
eicosane	323	0.0135	1.2	2.4
uncosane	335	0.0135	1.2	2.4
docosane	290	0.013	1.0	2.0
tricosane	326	0.0135	1.2	2.4
tetracosane	289	0.014	1.1	2.2
pentacosane	274	0.0145	1.1	2.2
hexacosane	209	0.015	0.8	1.6
heptacosane				
octacosane				
nonacosane				
triacontane				
untriacontane				
dotriacontane				

Hydrocarbon Analysis

Sample number: 48-1

1/2 hour exposure

Compound	area units	rf	ng/µl	µg/g dry wt.
HMB	4162	0.014		
undecane	2757	0.011	6.6	13.2
dodecane	3504	0.011	8.4	16.8
tridecane	3950	0.012	10.3	20.6
tetradecane	3731	0.012	9.7	19.4
pentadecane	3787	0.013	10.7	21.4
hexadecane	3607	0.012	9.4	18.8
heptadecane	3304	0.014	10.1	20.2 → .86
pristane	2006	0.02	8.7	17.4
octadecane	2968	0.014	9.0	18.0 → .76
phytane	1563	0.02	6.8	13.6
nonadecane	2875	0.014	8.8	17.6
eicosane	2725	0.0135	8.0	16.0
uncosane	2732	0.0135	8.0	16.0
docosane	2634	0.013	7.4	14.8
tricosane	2506	0.0135	7.4	14.8
tetracosane	2318	0.014	7.1	14.2
pentacosane	2088	0.0145	6.6	13.2
hexacosane	1761	0.015	5.7	11.4
heptacosane	1394	0.0155	4.7	9.4
octacosane	898	0.016	3.1	6.2
nonacosane	567	0.016	2.0	4.0
triacontane				
untriacontane				
dotriacontane				

Hydrocarbon Analysis

Sample number: 48-1

1/2 hour exposure

Compound	area units	rf	ng/µl	µg/g dry wt.
HMB	579245	2×10^{-4}		
naphthalene	124502	2×10^{-4}	5.4	5.4
methyl naphthalene	213870	2×10^{-4}	9.3	9.3
	124640		5.4	5.4
dimethyl naphthalene	238391	3.5×10^{-4}	18.1	18.1
	246552		18.8	18.8
	55911		4.3	4.3
trimethyl naphthalene	9651	3×10^{-4}	0.6	0.6
	20980		1.4	1.4
	104469		6.8	6.8
	68060		4.4	4.4
	27848		1.8	1.8
phenanthrene	23856	5×10^{-4}	2.6	2.6
dibenzothiophene	15914	5×10^{-4}	1.7	1.7
methyl phenanthrene	20938	1.1×10^{-3}	5.0	5.0
	17897		4.3	4.3
methyl dibenzothiophene	15399	5×10^{-4}	1.7	1.7
	7237		0.8	0.8
	2030		0.2	0.2
dimethyl phenanthrene	499	1.1×10^{-3}	0.1	0.1
dimethyl dibenzothiophene	23877	5×10^{-4}	5.7	5.7
	9409		1.0	1.0
	8894		1.0	1.0
	4267		0.5	0.5
	2236		0.2	0.2
trimethyl phenanthrene	12280	1.1×10^{-3}	2.9	2.9
	1473		0.4	0.4
trimethyl dibenzothiophene	1955	5×10^{-4}	0.2	0.2
	3941		0.4	0.4
	2634		0.3	0.3
	1828		0.2	0.2
Benzanthracene	277	1.1×10^{-2}	0.7	0.7
C4 Phenanthrene	420	1.1×10^{-3}	0.1	0.1

Hydrocarbon Analysis

Sample number: 48-2
1/2 hour exposure

Compound	area units	rf	ng/ul	ug/g dry wt.
HMB	3897	0.014		
undecane	2008	0.011	5.1	10.2
dodecane	2700	0.011	6.9	13.8
tridecane	3196	0.012	8.9	17.8
tetradecane	3077	0.012	8.6	17.2
pentadecane	3216	0.013	9.7	19.4
hexadecane	2942	0.012	8.2	16.4
heptadecane	2815	0.014	9.2	19.4 → .85
pristane	1761	0.02	8.2	16.4
octadecane	2511	0.014	8.2	16.4 → .78
phytane	1376	0.02	6.4	12.8
nonadecane	2425	0.014	7.9	15.8
eicosane	2323	0.0135	7.3	14.6
uncosane	2337	0.0135	7.3	14.6
docosane	2246	0.013	6.8	13.6
tricosane	2165	0.0135	6.8	13.6
tetracosane	2000	0.014	6.5	13.0
pentacosane	1784	0.0145	6.0	12.0
hexacosane	1504	0.015	5.2	10.4
heptacosane	1165	0.0155	4.2	8.4
octacosane	669	0.016	2.5	5.0
nonacosane				
triacontane				
untriacontane				
dotriacontane				

Hydrocarbon Analysis

Sample number: 48-3

1/2 hour exposure

Compound	area units	rf	ng/ul	µg/g dry wt.
HMB	3926	0.014		
undecane	2331	0.011	5.8	11.6
dodecane	3063	0.011	7.7	15.4
tridecane	3496	0.012	9.5	19.0
tetradecane	3335	0.012	9.1	18.2
pentadecane	3356	0.013	9.9	19.8
hexadecane	3093	0.012	8.4	16.8
heptadecane	2921	0.014	9.3	18.6 → .87
pristane	1773	0.02	8.1	16.2
octadecane	2600	0.014	8.3	16.6 → .74
phytane	1351	0.02	6.1	12.2
nonadecane	2518	0.014	8.0	16.0
eicosane	2392	0.0135	7.3	14.6
uncosane	2407	0.0135	7.4	14.8
docosane	2291	0.013	6.8	13.6
tricosane	2201	0.0135	6.8	13.6
tetracosane	2057	0.014	6.5	13.0
pentacosane	1863	0.0145	6.1	12.2
hexacosane	1578	0.015	5.4	10.8
heptacosane	1248	0.0155	4.4	8.8
octocosane	735	0.016	2.7	5.4
nonacosane	393	0.016	1.4	2.8
triacontane				
untriacontane				
dotriacontane				

Hydrocarbon Analysis

Sample number: 48-4
1/2 hour exposure

Compound	area units	rf	ng/ul	μg/g dry wt.	
HMB	3914	0.014			
undecane	1677	0.011	4.2	8.4	
dodecane	2335	0.011	5.8	11.6	
tridecane	2959	0.012	8.1	16.2	
tetradecane	3004	0.012	8.2	16.4	
pentadecane	3251	0.013	9.6	19.2	
hexadecane	3050	0.012	8.3	16.6	
heptadecane	2932	0.014	9.3	18.6	→ .92
pristane	1887	0.02	8.6	17.2	
octadecane	2651	0.014	8.4	16.8	→ .77
phytane	1422	0.02	6.5	13.0	
nonadecane	2557	0.014	8.1	16.2	
eicosane	2412	0.0135	7.4	14.8	
uncosane	2438	0.0135	7.5	15.0	
docosane	2273	0.013	6.7	13.4	
tricosane	2187	0.0135	6.7	13.4	
tetracosane	1970	0.014	6.3	12.6	
pentacosane	1708	0.0145	5.6	11.2	
hexacosane	1403	0.015	4.8	9.6	
heptocosane	1083	0.0155	3.8	7.6	
octocosane	591	0.016	2.1	4.2	
nonacosane					
triacontane					
untriacontane					
dotriacontane					

Hydrocarbon Analysis

Sample number: 48-5

1/2 hour exposure

Compound	area units	rf	ng/ul	μg/g dry wt.
HMB	4340	0.014		
undecane	1420	0.011	3.3	6.6
dodecane	2314	0.011	5.3	10.6
tridecane	2844	0.012	7.1	14.2
tetradecane	2803	0.012	7.0	14.0
pentadecane	2953	0.013	8.0	16.0
hexadecane	2735	0.012	6.8	13.6
heptadecane	2633	0.014	7.7	15.4
pristane	1676	0.02	7.0	14.0
octadecane	2355	0.014	6.9	13.8
phytane	1289	0.02	5.4	10.8
nonadecane	2298	0.014	6.7	13.4
eicosane	2227	0.0135	6.3	12.6
uncosane	2268	0.0135	6.4	12.8
docosane	2197	0.013	6.0	12.0
tricosane	2084	0.0135	5.9	11.8
tetracosane	1952	0.014	5.7	11.4
pentacosane	1754	0.0145	5.3	10.6
hexacosane	1449	0.015	4.5	9.0
heptdcosane	1122	0.0155	3.6	7.2
octocosane	703	0.016	2.3	4.6
nonacosane	362	0.016	1.2	2.4
triacontane				
untriacontane				
dotriacontane				

→ .91 (beside heptadecane/pristane)

→ .78 (beside octadecane/phytane)

Hydrocarbon Analysis

Sample number: 48-5

1/2 hour exposure

Compound	area units	rf	ng/ul	μg/g dry wt.
HMB	512471	2×10^{-4}		
naphthalene	55379	2×10^{-4}	2.7	2.7
methyl naphthalene	131824	2×10^{-4}	6.4	6.4
	72706		3.5	3.5
dimethyl naphthalene	149441	3.5×10^{-4}	12.8	12.8
	149869		12.8	12.8
	36322		3.1	3.1
trimethyl naphthalene	10166	3×10^{-4}	0.7	0.7
	10018		0.7	0.7
	59457		4.4	4.4
	43069		3.2	3.2
	20837		1.5	1.5
phenanthrene	16815	5×10^{-4}	2.1	2.1
dibenzothiophene	10563	5×10^{-4}	1.3	1.3
methyl phenanthrene	14616	1.1×10^{-3}	3.9	3.9
	12528		3.4	3.4
methyl dibenzothiophene	11876	5×10^{-4}	1.4	1.4
	4601		0.6	0.6
	1643		0.2	0.2
dimethyl phenanthrene	350	1.1×10^{-3}	0.1	0.1
	17707		4.8	4.8
dimethyl dibenzothiophene	6107	5×10^{-4}	0.7	0.7
	6581		0.8	0.8
	2925		0.4	0.4
	1737		0.2	0.2
trimethyl phenanthrene	6450	1.1×10^{-3}	1.7	1.7
	953		0.3	0.3
trimethyl dibenzothiophene	11737	5×10^{-4}	1.4	1.4
	1406		0.2	0.2
	703		0.1	0.1
C_4 Phenanthrene	393	1.1×10^{-3}	0.1	0.1

Hydrocarbon Analysis

Sample number: 4/78 0-24

24 hr. exposure

Compound	area units	rf	ng/ul	µg/g dry wt.	
HMB	4359	0.014			
undecane	2819	0.011	6.5	13.0	
dodecane	3343	0.011	7.7	15.4	
tridecane	3695	0.012	9.2	18.4	
tetradecane	3438	0.012	8.6	17.2	
pentadecane	3479	0.013	9.4	18.8	
hexadecane	3223	0.012	8.1	16.2	
heptadecane	2976	0.014	8.7	17.4	→ .84
pristane	1751	0.02	7.3	14.6	
octadecane	2667	0.014	7.8	15.6	
phytane	1372	0.02	5.7	11.4	→ .73
nonadecane	2585	0.014	7.5	15.0	
eicosane	2486	0.0135	7.0	14.0	
uncosane	2479	0.0135	7.0	14.0	
docosane	2344	0.013	6.3	12.6	
tricosane	2220	0.0135	6.2	12.4	
tetracosane	2067	0.014	6.0	12.0	
pentacosane	1861	0.0145	5.6	11.2	
hexacosane	1578	0.015	4.9	9.8	
heptacosane	1189	0.0155	3.8	7.6	
octocosane	751	0.016	2.5	5.0	
nonacosane	489	0.016	1.6	3.2	
triacontane					
untriacontane					
dotriacontane					

Hydrocarbon Analysis

Sample number: 4/78 024
24 hour exposure

Compound	area units	rf	ng/µl	µg/g dry wt.
HMB	798526	2×10^{-4}		
naphthalene	132003	1×10^{-4}	2.1	2.1
methyl naphthalene	250367	2×10^{-4}	7.9	7.9
	147903		4.7	4.7
dimethyl naphthalene	306853	2.5×10^{-4}	12.2	12.2
	327769		13.0	13.0
	89395		3.5	3.5
trimethyl naphthalene	23069	2×10^{-4}	0.7	0.7
	158439		5.0	5.0
	103715		3.3	3.3
	46931		1.5	1.5
phenanthrene	53057	3×10^{-4}	2.5	2.5
dibenzothiophene	41111	3×10^{-4}	2.0	2.0
methyl phenanthrene	49820	1.3×10^{-3}	10.3	10.3
	43557		9.0	9.0
methyl dibenzothiophene	45134	3×10^{-4}	2.1	2.1
	18190		0.9	0.9
	5066		0.2	0.2
dimethyl phenanthrene	58206	1.3×10^{-3}	12.0	12.0
dimethyl dibenzothiophene	21716	3×10^{-4}	1.0	1.0
	21269		1.0	1.0
	11241		0.5	0.5
	5200		0.2	0.2
trimethyl phenanthrene	45[illegible]67	1.3×10^{-3}	9.4	9.4
	3064		0.6	0.6
trimethyl dibenzothiophene	4767	3×10^{-4}	0.2	0.2
	7660		0.4	0.4
	4911		0.2	0.2
	3772		0.2	0.2
	1990		0.1	0.1

Hydrocarbon Analysis

Sample number: 710

24 hr. exposure

Compound	area units	rf	ng/µl	µg/g dry wt.
HMB	3463	0.014		
undecane	516	0.011	1.4	2.9
dodecane	800	0.011	2.2	4.5
tridecane	974	0.012	3.0	6.0
tetradecane	1036	0.012	3.2	6.4
pentadecane	1060	0.013	3.5	7.1
hexadecane	976	0.012	3.0	6.0
heptadecane	924	0.014	3.3	6.7 → .77
pristane	503	0.02	2.6	5.2
octadecane	753	0.014	2.7	5.4 → .77
phytane	407	0.02	2.1	4.2
nonadecane	739	0.014	2.6	5.3
eicosane	617	0.0135	2.1	4.3
uncosane	587	0.0135	2.0	4.1
docosane	514	0.013	1.7	3.4
tricosane	507	0.0135	1.7	3.4
tetracosane	467	0.014	1.6	3.3
pentacosane		0.0145		
hexacosane		0.015		
heptacosane		0.0155		
octacosane		0.016		
nonacosane				
triacontane				
untriacontane				
dotriacontane				

Hydrocarbon Analysis

Sample number: 24 hr. oil LB 4/78

Compound	area units	rf	ng/ul	ug/g dry wt.
HMB	3776	0.014		
undecane	did not integrate			
dodecane	332	0.011	0.9	1.8
tridecane	623	0.012	1.8	3.6
tetradecane	693	0.012	2.0	4.0
pentadecane	803	0.013	2.5	5.0
hexadecane	721	0.012	2.1	4.2
heptadecane	682	0.014	2.3	4.6
pristane	297	0.02	1.4	2.8
octadecane	603	0.014	2.0	4.0
phytane	282	0.02	1.3	2.6
nonadecane	595	0.014	2.0	4.0
eicosane	564	0.0135	1.8	3.6
uncosane	610	0.0135	2.0	4.0
docosane	549	0.013	1.7	3.4
tricosane	551	0.0135	1.8	3.6
tetracosane	500	0.014	1.7	3.4
pentacosane	478	0.0145	1.7	3.4
hexacosane	380	0.015	1.4	2.8
heptacosane	285	0.0155	1.1	2.2
octacosane	did not integrate			
nonacosane				
triacontane				
untriacontane				
dotriacontane				

→ .61 (heptadecane/pristane)

→ .65 (octadecane/phytane)

Hydrocarbon Analysis

Sample number: 706 1/5 dilution
24 hr exposure

Compound	area units	rf	ng/ml	μg/g dry wt.
HMB	6458	0.014		
undecane	3863	0.011	5.9	59.2
dodecane	3507	0.011	5.3	53.7
tridecane	3461	0.012	5.7	57.8
tetradecane	2784	0.012	4.6	46.5
pentadecane	2893	0.013	5.2	52.4
hexadecane	2650	0.012	4.4	44.3
heptadecane	2456	0.014	4.7	47.9 → .84
pristane	1455	0.02	4.0	40.5
octadecane	2361	0.014	4.6	46.0 → .70
phytane	1167	0.02	3.2	32.5
nonadecane	2197	0.014	4.2	42.8
eicosane	2157	0.0135	4.0	40.5
uncosane	2188	0.0135	4.1	41.1
docosane	2094	0.013	3.7	37.9
tricosane	1973	0.0135	3.7	37.1
tetracosane	1834	0.014	3.5	35.7
pentacosane	1598	0.0145	3.2	32.2
hexacosane	1394	0.015	2.9	29.1
heptacosane	1051	0.0155	2.2	22.7
octacosane	687	0.016	1.5	15.3
nonacosane				
triacontane				
untriacontane				
dotriacontane				

Hydrocarbon Analysis

Sample number: 706

24 hr exposure

Compound	area units	rf	ng/μl	μg/g dry wt.
HMB	261239	1×10^{-4}		
naphthalene	329460	5×10^{-5}	7.8	15.6
methyl naphthalene	482960	1×10^{-4}	23.0	46.0
	294071		14.0	28.0
dimethyl naphthalene	446835	1×10^{-4}	21.3	42.6
	510021		24.3	48.6
	171258		8.2	16.4
trimethyl naphthalene	27061	1×10^{-4}	1.3	2.6
	206211		9.8	19.6
	128937		6.1	12.2
	53191		2.5	5.0
phenanthrene	33646	2×10^{-4}	3.2	3.2
dibenzothiophene	22707	2×10^{-4}	2.2	4.4
methyl phenanthrene	28078	4×10^{-4}	5.3	10.6
	24211		4.6	9.2
methyl dibenzothiophene	21854	2×10^{-4}	2.1	4.2
	10051		1.0	2.0
	4638		0.4	0.8
dimethyl phenanthrene	32789	4×10^{-4}	6.2	12.4
dimethyl dibenzothiophene	11307	2×10^{-4}	1.1	2.2
	11343		1.1	2.2
	4877		0.5	1.0
	2861		0.3	0.6
trimethyl phenanthrene	13996	4×10^{-4}	2.7	5.4
	2326		0.4	0.8
trimethyl dibenzothiophene	2701	2×10^{-4}	0.3	0.6
	4802		0.5	1.0
	3500		0.3	0.6
	2430		0.2	0.4
	1187		0.1	0.2

Hydrocarbon Analysis

Sample number: 1B
48 hr exposure

Compound	area units	rf	ng/ml	µg/g dry wt.	
HMB	3700	0.014			
undecane	4058	0.011	10.9	21.8	
dodecane	2569	0.011	6.9	13.8	
tridecane	2125	0.012	6.2	12.4	
tetradecane	1973	0.012	5.8	11.6	
pentadecane	2234	0.013	7.1	14.2	
hexadecane	2535	0.012	7.4	14.8	
heptadecane	1739	0.014	5.9	11.8	] 0.92
pristane	1112	0.02	5.4	10.8	
octadecane	2129	0.014	7.3	14.6	] 0.67
phytane	1005	0.02	4.9	9.8	
nonadecane	1650	0.014	5.6	11.2	
eicosane	1754	0.0135	5.8	11.6	
uncosane	1512	0.0135	5.0	10.0	
docosane	1370	0.013	4.3	8.6	
tricosane	1099	0.0135	3.6	7.2	
tetracosane	896	0.014	3.1	6.2	
pentacosane	709	0.0145	2.5	5.0	
hexacosane	525	0.015	1.9	3.8	
heptocosane	247	0.0155	0.9	1.8	
octocosane					
nonacosane					
triacontane					
untriacontane					
dotriacontane					

Hydrocarbon Analysis

Sample number: 2A
48 hr exposure

Compound	area units	rf	ng/ul	µg/g dry wt.
HMB	3022	0.014		
undecane	2722	0.011	8.9	17.8
dodecane	4292	0.011	14.1	28.2
tridecane	4033	0.012	14.4	28.8
tetradecane	3301	0.012	11.8	23.6
pentadecane	3950	0.013	15.3	30.6
hexadecane	3292	0.012	11.8	23.6
heptadecane	2930	0.014	12.2	24.4
pristane	1798	0.020	10.7	21.4
octadecane	1920	0.014	8.0	16.0
phytane	1229	0.020	7.3	14.6
nonadecane	1976	0.014	8.2	16.4
eicosane	1605	0.0135	6.5	13.0
uncosane	1458	0.0135	5.9	11.8
docosane	1189	0.013	4.6	9.2
tricosane	1000	0.0135	4.0	8.0
tetracosane	819	0.014	3.4	6.8
pentacosane	628	0.0145	2.7	5.4
hexacosane	490	0.015	2.2	4.4
heptacosane		0.0155		
octacosane		0.016		
~~nonacosane~~				
triacontane				
untriacontane				
dotriacontane				

→ .88 (heptadecane/pristane)

→ .91 (octadecane/phytane)

Hydrocarbon Analysis

Sample number: 2A
48 hr exposure

Compound	area units	rf	ng/ul	µg/g dry wt.
HMB	265221	4x16^{-4}		
naphthalene	69422	3.x10^{-4}	5.0	5.0
methyl naphthalene	131858	5x10^{-4}	15.7	15.7
dimethyl naphthalene	143080	6x10^{-4}	20.4	20.4
	177992		25.4	25.4
	55188		7.9	7.9
trimethyl naphthalene	7932	5x10^{-4}	0.9	0.9
	14562		1.7	1.7
	76633		9.1	9.1
	50099		6.0	6.0
	22431		2.7	2.7
phenanthrene	13703	5x10^{-4}	1.6	1.6
dibenzothiophene	9177	6x10^{-4}	1.3	1.3
methyl phenanthrene	10825	8x10^{-4}	2.1	2.1
	9844		1.9	1.9
methyl dibenzothiophene	8714	6x10^{-4}	1.2	1.2
	5360		0.8	0.8
	1250		0.2	0.2
dimethyl phenanthrene	14482	8x10^{-4}	2.8	2.8
dimethyl dibenzothiophene	5192	6x10^{-4}	0.7	0.7
	5414		0.8	0.8
	1962		0.3	0.3
trimethyl phenanthrene	6682	8x10^{-4}	1.3	1.3
	862		0.2	0.2
trimethyl dibenzothiophene	1674	6x10^{-4}	0.2	0.2
	1391		0.2	0.2
	1603		0.2	0.2
	1286		0.2	0.2

Hydrocarbon Analysis

Sample number: 2C
48 hr exposure

Compound	area units	rf	ng/ul	µg/g dry wt.
HMB	3776	0.014		
undecane	1525	0.011	4.0	8.0
dodecane	1828	0.011	4.8	9.6
tridecane	3200	0.012	9.2	18.4
tetradecane	3391	0.012	9.7	19.4
pentadecane	3500	0.013	10.8	21.6
hexadecane	2866	0.012	8.2	16.4
heptadecane	2474	0.014	8.3	16.6
pristane	1514	0.020	7.2	14.4
octadecane	1609	0.014	5.4	10.8
phytane	995	0.020	4.7	9.4
nonadecane	1638	0.014	5.5	11.0
eicosane	1455	0.0135	4.7	9.4
uncosane	1342	0.0135	4.3	8.6
docosane	1114	0.013	3.5	7.0
tricosane	934	0.0135	3.0	6.0
tetracosane	730	0.014	2.4	4.8
pentacosane	536	0.0145	1.9	3.8
hexacosane		0.015		
heptacosane		0.0155		
octacosane		0.016		
nonacosane				
triacontane				
untriacontane				
dotriacontane				

.87 (margin notes beside heptadecane/pristane and octadecane/phytane rows: .87, .87)

Hydrocarbon Analysis

Sample number: 3A

48 hr exposure

Compound	area units	rf	ng/ul	μg/g dry wt.
HMB	5620	0.014		
undecane	3660	0.011	6.4	12.8
dodecane	4649	0.011	8.2	16.4
tridecane	5431	0.012	10.4	20.8
tetradecane	5188	0.012	10.0	20.0
pentadecane	5240	0.013	10.9	21.8
hexadecane	4951	0.012	9.5	19.0
heptadecane	4176	0.014	9.4	18.8
pristane	2531	0.020	8.1	16.2
octadecane	3017	0.014	6.8	13.6
phytane	1694	0.020	5.4	10.8
nonadecane	2941	0.014	6.6	13.2
eicosane	2589	0.0135	5.6	11.2
uncosane	2420	0.0135	5.2	10.4
docosane	2119	0.013	4.4	8.8
tricosane	1878	0.0135	4.1	8.2
tetracosane	1634	0.014	3.7	7.4
pentacosane	1393	0.0145	3.2	6.4
hexacosane	1107	0.015	2.7	5.4
heptacosane	825	0.0155	2.0	4.0
octacosane	466	0.016	1.2	2.4
nonacosane				
triacontane				
untriacontane				
dotriacontane				

.86

.7

Hydrocarbon Analysis

Sample number: 3A 48 hr exposure

Compound	area units	rf	ng/μl	μg/g dry wt.
HMB	294766	4×10^{-4}		
naphthalene	70440	3×10^{-4}	4.5	4.5
methyl naphthalene	127114	5×10^{-4}	13.5	13.5
	79746		8.5	8.5
dimethyl naphthalene	132429	6×10^{-4}	16.9	16.9
	150726		19.2	19.2
	49068		6.3	6.3
trimethyl naphthalene	10937	5×10^{-4}	1.2	1.2
	10772		1.1	1.1
	65244		6.9	6.9
	45395		4.8	4.8
	18272		1.9	1.9
phenanthrene	13400	5×10^{-4}	1.4	1.4
dibenzothiophene	9222	6×10^{-4}	1.2	1.2
methyl phenanthrene	9305	8×10^{-4}	1.6	1.6
	10651		1.8	1.8
methyl dibenzothiophene	7530	6×10^{-4}	1.0	1.0
	4192		0.5	0.5
	1261		0.2	0.2
dimethyl phenanthrene	16240	8×10^{-4}	2.8	2.8
dimethyl dibenzothiophene	5072	6×10^{-4}	0.6	0.6
	5324		0.7	0.7
	2770		0.4	0.4
	1270		0.2	0.2
trimethyl phenanthrene				
trimethyl dibenzothiophene				

Hydrocarbon Analysis

Sample number: 3C
48 hr exposure

Compound	area units	rf	ng/ul	µg/g dry wt.
HMB	3373	0.014		
undecane	872	0.011	2.6	5.2
dodecane	1352	0.011	4.0	8.0
tridecane	1753	0.012	5.6	11.2
tetradecane	1860	0.012	6.0	12.0
pentadecane	1996	0.013	6.9	13.8
hexadecane	1737	0.012	5.6	11.2
heptadecane	1522	0.014	5.7	11.4
pristane	843	0.020	4.5	9.0
octadecane	888	0.014	3.3	6.6
phytane	545	0.020	2.9	5.8
nonadecane	1003	0.014	3.7	7.4
eicosane	819	0.0135	3.0	6.0
uncosane	702	0.0135	2.5	5.0
docosane	542	0.013	1.9	3.8
tricosane	402	0.0135	1.4	2.8
tetracosane		0.014		
pentacosane		0.0145		
hexacosane		0.015		
heptacosane		0.0155		
octacosane		0.016		
nonacosane				
triacontane				
untriacontane				
dotriacontane				

.79

.88

Hydrocarbon Analysis

Sample number: 5B
48 hr Control

Compound	area units	rf	ng/µl	µg/g dry wt.
HMB	1967	0.014		
undecane		0.011		
dodecane		0.011		
tridecane		0.012		
tetradecane		0.012		
pentadecane		0.013		
hexadecane		0.012		
heptadecane		0.014		
pristane		0.020		
octadecane	598	0.014	3.8	7.6
phytane		0.020		
nonadecane		0.014		
eicosane		0.0135		
uncosane		0.0135		
docosane		0.013		
tricosane		0.0135		
tetracosane		0.014		
pentacosane		0.0145		
hexacosane		0.015		
heptacosane		0.0155		
octacosane		0.016		
nonacosane				
triacontane				
untriacontane				
dotriacontane				

Hydrocarbon Analysis

Sample number: 5D
48 hr control

Compound	area units	rf	ng/ul	µg/g dry wt.
HMB	2895	0.014		
undecane				
dodecane				
tridecane				
tetradecane				
pentadecane				
hexadecane				
heptadecane				
pristane				
octadecane	581	0.014	2.5	5.0
phytane				
nonadecane				
eicosane				
uncosane				
docosane				
tricosane				
tetracosane				
pentacosane				
hexacosane				
heptacosane				
octacosane				
nonacosane				
triacontane				
untriacontane				
dotriacontane				

Hydrocarbon Analysis

Sample number: oil 72 hr. ② 2.5 μl

Compound	area units	rf	ng/μl	μg/g dry wt.
HMB	4223	0.014		
undecane	8596	0.011	20.1	40.2
dodecane	10010	0.011	23.4	46.8
tridecane	9834	0.012	25.1	50.2
tetradecane	8950	0.012	22.9	45.8
pentadecane	9640	0.013	26.7	53.4
hexadecane	9220	0.012	23.5	47.0
heptadecane	7598	0.014	22.6	45.2
pristane	4459	0.02	19.0	38.0 → .84
octadecane	7280	0.014	21.7	43.4 → .69
phytane	3484	0.02	14.8	29.6
nonadecane	6614	0.014	19.7	39.4
eicosane	6328	0.0135	18.2	36.4
uncosane	6141	0.0135	17.6	35.2
docosane	5355	0.013	14.8	29.6
tricosane	4582	0.0135	13.2	26.4
tetracosane	3923	0.014	11.7	23.4
pentacosane	3191	0.0145	9.8	19.6
hexacosane	2619	0.015	8.4	16.8
heptacosane	1940	0.0155	6.4	12.8
octacosane	1308	0.016	4.5	9.0
nonacosane	773	0.016	2.6	5.2
triacontane				
untriacontane				
dotriacontane				

Hydrocarbon Analysis

Sample number: Oil 72h frozen

Compound	area units	rf	ng/ml	µg/g dry wt.
HMB	3203	0.014		
undecane	4167	0.011	12.8	25.7
dodecane	4646	0.011	14.3	28.6
tridecane	5222	0.012	17.6	35.2
tetradecane	5574	0.012	20.3	40.7
pentadecane	5472	0.013	19.9	39.9
hexadecane	5354	0.012	18.0	36.1
heptadecane	4434	0.014	17.4	34.8 → .9
pristane	2627	0.02	14.7	29.5
octadecane	4466	0.014	17.5	35.1 → .6
phytane	2057	0.02	11.5	23.1
nonadecane	3872	0.014	15.2	30.4
eicosane	3941	0.0135	14.9	29.8
uncosane	3954	0.0135	14.9	29.9
docosane	3615	0.013	13.2	26.4
tricosane	3317	0.0135	12.5	25.1
tetracosane	3019	0.014	11.8	23.7
pentacosane	2578	0.0145	10.5	21.0
hexacosane	2231	0.015	9.4	18.8
heptacosane	1764	0.0135	7.6	15.3
octacosane	1135	0.016	5.1	10.2
nonacosane	777	0.016	3.4	6.9
triacontane				
untriacontane				
dotriacontane				

Hydrocarbon Analysis

Sample number: Oil 72h froz.

Compound	area units	rf	ng/ul	µg/g dry wt.
HMB	710195	2×10^{-4}		
naphthalene	246777	2×10^{-4}	8.8	8.8
methyl naphthalene	458403	2×10^{-4}	16.4	16.4
	253556		9.1	9.1
dimethyl naphthalene	515923	2.5×10^{-4}	23.0	23.0
	537219		24.0	24.0
	113669		5.1	5.1
trimethyl naphthalene	38202	2×10^{-4}	1.4	1.4
	34004		1.2	1.2
	228373		8.2	8.2
	146298		5.2	5.2
	57406		2.1	2.1
phenanthrene	37121	5×10^{-4}	3.3	3.3
dibenzothiophene	29469	4×10^{-4}	2.1	2.1
methyl phenanthrene	29051	1.1×10^{-3}	5.7	5.7
	23256		4.6	4.6
methyl dibenzothiophene	23929	4×10^{-4}	1.7	1.7
	10279		0.7	0.7
dimethyl phenanthrene	30494	1.1×10^{-3}	6.0	6.0
dimethyl dibenzothiophene	11510	4×10^{-4}	0.8	0.8
	11117		0.8	0.8
	5312		0.4	0.4
	2605		0.2	0.2
trimethyl phenanthrene	13093	1.1×10^{-3}	2.6	2.6
	1717		0.3	0.3
trimethyl dibenzothiophene	2949	4×10^{-4}	0.2	0.2
	3298		0.2	0.2
	3044		0.2	0.2
	1990		0.1	0.1
Benzanthracene	337	4.6×10^{-3}	0.6	0.6
C_4 phenanthrene	342	1.1×10^{-3}	0.1	0.1
	402		0.1	0.1

Hydrocarbon Analysis

Sample number: 6A

7 days exposure

Compound	area units	rf	ng/ml	µg/g dry wt.
HMB	4674	0.014		
undecane	2588	0.011	5.4	10.8
dodecane	2537	0.011	5.3	10.6
tridecane	2701	0.012	6.1	12.2
tetradecane	2551	0.012	5.8	11.6
pentadecane	2560	0.013	6.3	12.6
hexadecane	2280	0.012	5.2	10.4
heptadecane	2169	0.014	5.7	11.4
pristane	1156	0.02	4.4	8.8
octadecane	1456	0.014	3.9	7.8
phytane	874	0.02	3.3	6.6
nonadecane	1634	0.014	4.3	8.6
eicosane	1420	0.0135	3.6	7.2
uncosane	1309	0.0135	3.3	6.6
docosane	1118	0.013	2.7	5.4
tricosane	975	0.0135	2.5	5.0
tetracosane	830	0.014	2.2	4.4
pentacosane	617	0.0145	1.7	3.4
hexacosane	452	0.015	1.3	2.6
heptacosane		0.0155		
octacosane		0.016		
nonacosane				
triacontane				
untriacontane				
dotriacontane				

Hydrocarbon Analysis

Sample number: 6A
7 days exposure

Compound	area units	rf	ng/ul	μg/g dry wt.
HMB	306005	4×10^{-4}		
naphthalene	34376	3×10^{-4}	2.1	2.1
methyl naphthalene	65448	5×10^{-4}	6.7	6.7
	40659		4.1	4.1
dimethyl naphthalene	77593	6×10^{-4}	9.5	9.5
	86597		10.6	10.6
	18623		2.3	2.3
trimethyl naphthalene	37540	5×10^{-4}	3.8	3.8
	26031		2.7	2.7
	11396		1.2	1.2
phenanthrene	8798	5×10^{-4}	0.9	0.9
dibenzothiophene	5645	6×10^{-4}	0.7	0.7
methyl phenanthrene	5103	8×10^{-4}	0.8	0.8
	5927		0.9	0.9
methyl dibenzothiophene	4728	6×10^{-4}	0.6	0.6
	2381		0.3	0.3
	617		0.1	0.1
dimethyl phenanthrene	7846	8×10^{-4}	1.3	1.3
dimethyl dibenzothiophene	2824	6×10^{-4}	0.3	0.3
	2961		0.4	0.4
	1364		0.2	0.2
trimethyl phenanthrene	272	8×10^{-4}	0.04	0.04
	1949		0.3	0.3
trimethyl dibenzothiophene	203		0.03	0.03
	383	6×10^{-4}	0.046	0.046
	1042		0.1	0.1
	289		0.04	0.04
	483		0.06	0.06

Hydrocarbon Analysis

Sample number: 6B

7 days exposure

Compound	area units	rf	ng/ml	µg/g dry wt.
HMB	5224	0.014		
undecane	2089	0.011	3.6	7.2
dodecane	2575	0.011	4.9	9.8
tridecane	3222	0.012	6.7	13.4
tetradecane	3350	0.012	6.9	13.8
pentadecane	3579	0.013	8.0	16.0
hexadecane	3364	0.012	7.0	14.0
heptadecane	3285	0.014	7.9	15.8
pristane	1986	0.02	6.8	13.6
octadecane	2520	0.014	6.1	12.2
phytane	1479	0.02	5.1	10.2
nonadecane	2704	0.014	6.5	13.0
eicosane	2419	0.0135	5.6	11.2
uncosane	2333	0.0135	5.4	10.8
docosane	2084	0.013	4.7	9.4
tricosane	1915	0.0135	4.5	9.0
tetracosane	1615	0.014	3.9	7.8
pentacosane	1328	0.0145	3.3	6.6
hexacosane	1029	0.015	2.7	5.4
heptacosane	748	0.0155	2.0	4.0
octacosane		0.016		
nonacosane				
triacontane				
untriacontane				
dotriacontane				

.86

.85

Hydrocarbon Analysis

Sample number: 6C
7 days exposure

Compound	area units	rf	ng/ul	µg/g dry wt.
HMB	4671	0.014		
undecane	3961	0.011	8.4	16.8
dodecane	4044	0.011	8.6	17.2
tridecane	4233	0.012	9.8	19.6
tetradecane	3938	0.012	9.1	18.2
pentadecane	3952	0.013	9.9	19.8
hexadecane	3511	0.012	8.1	16.2
heptadecane	3312	0.014	8.9	17.8
pristane	1898	0.02	7.3	14.6
octadecane	2461	0.014	6.6	13.2
phytane	1392	0.02	5.4	10.8
nonadecane	2513	0.014	6.8	13.6
eicosane	2241	0.0135	5.8	11.6
uncosane	2142	0.0135	5.6	11.2
docosane	1913	0.013	4.8	9.6
tricosane	1722	0.0135	4.5	9.0
tetracosane	1488	0.014	4.0	8.0
pentacosane	1233	0.0145	3.4	6.8
hexacosane	964	0.015	2.3	4.6
heptacosane	687	0.0155	2.1	4.2
octacosane		0.016		
nonacosane				
triacontane				
untriacontane				
dotriacontane				

.82

.82

Hydrocarbon Analysis

Sample number: 6C
7 days exposure

Compound	area units	rf	ng/ul	µg/g dry wt.
HMB	302764	4x10⁻⁴		
naphthalene	70037	3 x10⁻⁴	4.4	4.4
methyl naphthalene	127843	5x10⁻⁴	13.3	13.3
	70089		7.3	7.3
dimethyl naphthalene	97161	6 x10⁻⁴	12.1	12.1
	142910		17.9	17.9
	44497		5.6	5.6
trimethyl naphthalene	12387	5x10⁻⁴	1.3	1.3
	63452		6.6	6.6
	45026		4.7	4.7
	18851		2.0	2.0
phenanthrene	20464	5x10⁻⁴	2.1	2.1
dibenzothiophene	13315	6 x10⁻⁴	1.7	1.7
methyl phenanthrene	15012	8x10⁻⁴	2.5	2.5
	14022		2.3	2.3
methyl dibenzothiophene	13727	6x10⁻⁴	1.7	1.7
	5240		0.7	0.7
	1615		0.2	0.2
dimethyl phenanthrene	17074	8x10⁻⁴	2.8	2.8
dimethyl dibenzothiophene	6609	6 x10⁻⁴	0.8	0.8
	6818		0.9	0.9
	3492		0.4	0.4
	1651		0.2	0.2
trimethyl phenanthrene	549	8x10⁻⁴	0.1	0.1
trimethyl dibenzothiophene	2722		0.5	0.5
	1832		0.3	0.3
	1227		0.2	0.2

Hydrocarbon Analysis

Sample number: 6E
7 days exposure

Compound	area units	rf	ng/ul	µg/g dry wt.
HMB	4328	0.014		
undecane	1157	0.011	2.6	5.2
dodecane	1444	0.011	3.3	6.6
tridecane	1671	0.012	4.2	8.4
tetradecane	1711	0.012	4.3	8.6
pentadecane	1840	0.013	5.0	10.0
hexadecane	1634	0.012	4.1	8.2
heptadecane	1587	0.014	4.6	9.2
pristane	826	0.02	3.4	6.8
octadecane	1065	0.014	3.1	6.2
phytane	682	0.02	2.8	5.6
nonadecane	1255	0.014	3.7	7.4
eicosane	1105	0.0135	3.1	6.2
uncosane	1092	0.0135	3.1	6.2
docosane	963	0.013	2.6	5.2
tricosane	939	0.0135	2.6	5.2
tetracosane	809	0.014	2.4	4.8
pentacosane	734	0.0145	2.2	4.4
hexacosane	572	0.015	1.8	3.6
heptacosane	407	0.0155	1.3	2.6
octacosane		0.016		
nonacosane				
triacontane				
untriacontane				
dotriacontane				

.79
.9

Hydrocarbon Analysis

Sample number: 7A

7 days exposure

Compound	area units	rf	ng/ul	μg/g dry wt.
HMB	4448	0.014		
undecane	593	0.011	1.3	2.6
dodecane	600	0.011	1.3	2.6
tridecane	709	0.012	1.7	3.4
tetradecane	772	0.012	1.9	3.8
pentadecane	854	0.013	2.2	4.4
hexadecane	739	0.012	1.8	3.6
heptadecane	713	0.014	2.0	4.0
pristane	DID NOT INTEG	0.02	—	—
octadecane	444	0.014	1.3	2.6
phytane	—	0.02	—	—
nonadecane	DID NOT INTEG	0.014	—	—
eicosane	519	0.0135	1.4	2.8
uncosane	549	0.0135	1.5	3.0
docosane	461	0.013	1.2	2.4
tricosane	471	0.0135	1.3	2.6
tetracosane		0.014		
pentacosane		0.0145		
hexacosane		0.015		
heptacosane		0.0155		
octacosane		0.016		
nonacosane				
triacontane				
untriacontane				
dotriacontane				

Hydrocarbon Analysis

Sample number: 8B
7 days exposure

Compound	area units	rf	ng/µl	µg/g dry wt.
HMB	1253	0.014		
undecane				
dodecane				
tridecane				
tetradecane				
pentadecane				
hexadecane				
heptadecane				
pristane				
octadecane	633	0.014	6.4	12.8
phytane				
nonadecane				
eicosane				
uncosane				
docosane				
tricosane				
tetracosane				
pentacosane				
hexacosane				
heptacosane				
octacosane				
nonacosane				
triacontane				
untriacontane				
dotriacontane				

Hydrocarbon Analysis

Sample number: 9B

7 days exposure

Compound	area units	rf	ng/ul	μg/g dry wt.
HMB	3897	0.014		
undecane				
dodecane				
tridecane				
tetradecane				
pentadecane	444	0.013	1.3	2.6
hexadecane	406	0.012	1.1	2.2
heptadecane	421	0.014	1.4	2.8
pristane				
octadecane	608	0.014	2.0	4.0
phytane				
nonadecane				
eicosane				
uncosane				
docosane				
tricosane				
tetracosane				
pentacosane				
hexacosane				
heptacosane				
octacosane				
nonacosane				
triacontane				
untriacontane				
dotriacontane				

Hydrocarbon Analysis

Sample number: 9C
7 days exposure

Compound	area units	rf	ng/ul	µg/g dry wt.
HMB	1907	0.014		
undecane		0.011		
dodecane		0.011		
tridecane		0.012		
tetradecane		0.012		
pentadecane		0.013		
hexadecane		0.012		
heptadecane		0.014		
pristane		0.02		
octadecane	479	0.014	3.2	6.4
phytane		0.02		
nonadecane		0.014		
eicosane		0.0135		
uncosane		0.0135		
docosane		0.013		
tricosane		0.0135		
tetracosane		0.014		
pentacosane		0.0145		
hexacosane		0.015		
heptacosane		0.0155		
octacosane		0.016		
nonacosane				
triacontane				
untriacontane				
dotriacontane				

Hydrocarbon Analysis

Sample number: 10D
7 day control

Compound	area units	rf	ng/ml	µg/g dry wt.
HMB	2620	0.014		
undecane				
dodecane				
tridecane				
tetradecane				
pentadecane				
hexadecane				
heptadecane				
pristane				
octadecane	504	0.014	2.4	4.8
phytane				
nonadecane				
eicosane				
uncosane				
docosane				
tricosane				
tetracosane				
pentacosane				
hexacosane				
heptacosane				
octacosane				
nonacosane				
triacontane				
untriacontane				
dotriacontane				

Hydrocarbon Analysis

Sample number: 11A

14 day exposure

Compound	area units	rf	ng/ul	Mg/g dry wt.	
HMB	3328	0.014			
undecane	2232	0.011	6.6	13.2	
dodecane	2559	0.011	7.6	15.2	
tridecane	2733	0.012	8.7	17.4	
tetradecane	2707	0.012	8.8	17.6	
pentadecane	2764	0.013	9.7	19.4	
hexadecane	2436	0.012	7.9	15.8	
heptadecane	2359	0.014	8.9	17.8	.80
pristane	1315	0.02	7.1	14.2	
octadecane	1701	0.014	6.4	12.8	.61
phytane	1026	0.02	3.9	7.8	
nonadecane	1879	0.014	7.1	14.2	
eicosane	1746	0.0135	6.4	12.8	
uncosane	1708	0.0135	6.2	12.4	
docosane	1566	0.013	5.5	11.0	
tricosane	1442	0.0135	5.3	10.6	
tetracosane	1301	0.014	4.9	9.8	
pentacosane	1136	0.0145	4.5	9.0	
hexacosane	929	0.015	3.8	7.6	
heptacosane	608	0.0155	2.5	5.0	
octacosane		0.016			
nonacosane					
triacontane					
untriacontane					
dotriacontane					

Hydrocarbon Analysis

Sample number: 11A
14 day exposure

Compound	area units	rf	ng/ul	μg/g dry wt.
HMB	299753	4×10^{-4}		
naphthalene	70732	3×10^{-4}	4.4	4.4
methyl naphthalene	134872	5×10^{-4}	14.0	14.0
	66996		7.0	7.0
dimethyl naphthalene	114980	6×10^{-4}	14.4	14.4
	125475		15.7	15.7
	25589		3.2	3.2
trimethyl naphthalene	9379	5×10^{-4}	1.0	1.0
	9365		1.0	1.0
	60837		6.3	6.3
	39178		4.1	4.1
	19576		2.0	2.0
phenanthrene	13947	5×10^{-4}	1.5	1.5
dibenzothiophene	10516	6×10^{-4}	1.3	1.3
methyl phenanthrene	10545	8×10^{-4}	1.8	1.8
	10407		1.7	1.7
methyl dibenzothiophene	9274	6×10^{-4}	1.2	1.2
	4036		0.5	0.5
	1366		0.2	0.2
dimethyl phenanthrene	14597	8×10^{-4}	2.4	2.4
dimethyl dibenzothiophene	5493	6×10^{-4}	0.7	0.7
	5727		0.7	0.7
	2049		0.3	0.3
trimethyl phenanthrene	514	8×10^{-4}	0.1	0.1
trimethyl dibenzothiophene	2014	6×10^{-4}	0.3	0.3
	1175		0.1	0.1
	906		0.1	0.1
	1143		0.1	0.1

Hydrocarbon Analysis

Sample number: 12B

14 day exposure

Compound	area units	rf	ng/ul	µg/g dry wt.
HMB	3518	0.014		
undecane	546	0.011	1.5	3.0
dodecane	634	0.011	1.8	3.6
tridecane	769	0.012	2.4	4.8
tetradecane	779	0.012	2.4	4.8
pentadecane	837	0.013	2.8	5.6
hexadecane	759	0.012	2.3	4.6
heptadecane	790	0.014	2.8	5.6
pristane	DID NOT INTEGRATE	0.02		
octadecane	627	0.014	2.2	4.4
phytane		0.02		
nonadecane	593	0.014	2.1	4.2
eicosane	575	0.0135	2.0	4.0
uncosane	583	0.0135	2.0	4.0
docosane	522	0.013	1.7	3.4
tricosane	535	0.0135	1.8	3.6
tetracosane	480	0.014	1.7	3.4
pentacosane		0.0145		
hexacosane		0.015		
heptacosane		0.0155		
octacosane		0.016		
nonacosane				
triacontane				
untriacontane				
dotriacontane				

Hydrocarbon Analysis

Sample number: 12D
14 day exposure

Compound	area units	rf	ng/ul	μg/g dry wt.
HMB	3959	0.014		
undecane	512	0.011	1.3	2.6
dodecane	803	0.011	2.0	4.0
tridecane	1092	0.012	3.0	6.0
tetradecane	1201	0.012	3.3	6.6
pentadecane	1272	0.013	3.8	7.6
hexadecane	1133	0.012	3.1	6.2
heptadecane	1094	0.014	3.5	7.0]-0.69
pristane	528	0.02	2.4	4.8
octadecane	732	0.014	2.3	4.6
phytane		0.02		
nonadecane	785	0.014	2.5	5.0
eicosane	700	0.0135	2.1	4.2
uncosane	696	0.0135	2.1	4.2
docosane	604	0.013	1.8	3.6
tricosane	605	0.0135	1.9	3.8
tetracosane	497	0.014	1.6	3.2
pentacosane	462	0.0145	1.5	3.0
hexacosane		0.015		
heptacosane		0.0155		
octacosane		0.016		
nonacosane				
triacontane				
untriacontane				
dotriacontane				

Hydrocarbon Analysis

Sample number: 12E

14 day exposure

Compound	area units	rf	ng/ul	µg/g dry wt.
HMB	672	0.014		
undecane				
dodecane				
tridecane				
tetradecane				
pentadecane	425	0.013	7.4	14.8
hexadecane				
heptadecane				
pristane				
octadecane	733	0.014	13.7	27.4
phytane				
nonadecane				
eicosane				
uncosane				
docosane				
tricosane				
tetracosane				
pentacosane				
hexacosane				
heptacosane				
octacosane				
nonacosane				
triacontane				
untriacontane				
dotriacontane				

Hydrocarbon Analysis

Sample number: 13B
14 day exposure

Compound	area units	rf	ng/ul	g/g dry wt.
HMB	3137	0.014		
undecane	405	0.011	1.3	2.6
dodecane	465	0.011	1.5	3.0
tridecane	533	0.012	1.8	3.6
tetradecane	550	0.012	1.9	3.8
pentadecane	574	0.013	2.1	4.2
hexadecane	516	0.012	1.8	3.6
heptadecane	546	0.010	2.2	4.4
pristane	DID NOT INTEGRATE	0.02		
octadecane	698	0.014	2.8	5.6
phytane	DID NOT INTEGRATE	0.02		
nonadecane	445	0.014	1.8	3.6
eicosane		0.0135		
uncosane		0.0135		
docosane		0.013		
tricosane		0.0135		
tetracosane		0.014		
pentacosane		0.0145		
hexacosane		0.015		
heptacosane		0.0155		
octacosane		0.016		
nonacosane				
triacontane				
untriacontane				
dotriacontane				

Hydrocarbon Analysis

Sample number: 13D

14 day exposure

Compound	area units	rf	ng/ul	µg/g dry wt.
HMB	3542	0.014		
undecane	1916	0.011	5.3	10.7
dodecane	2122	0.011	5.9	11.8
tridecane	2202	0.012	6.7	13.4
tetradecane	1757	0.012	5.3	10.7
pentadecane	1978	0.013	6.5	13.0
hexadecane	1792	0.012	5.4	10.9
heptadecane	1572	0.014	5.5	11.1 → .83
pristane	924	0.02	4.6	9.3
octadecane	1511	0.014	5.3	10.7 → .72
phytane	770	0.02	3.9	7.8
nonadecane	1472	0.014	5.2	10.4
eicosane	1436	0.0135	4.9	9.8
uncosane	1517	0.0135	5.2	10.4
docosane	1431	0.013	4.7	9.4
tricosane	1301	0.0135	4.4	8.8
tetracosane	1153	0.014	4.1	8.2
pentacosane	1048	0.0145	3.8	7.7
hexacosane	871	0.015	3.3	6.6
heptocosane	666	0.0155	2.6	5.2
octocosane	330	0.016	1.3	2.6
nonacosane				
triacontane				
untriacontane				
dotriacontane				

Hydrocarbon Analysis

Sample number: 13E

14 day exposure

Compound	area units	rf	ng/ul	μg/g dry wt.	
HMB	5721	0.014			
undecane	5218	0.011	9.0	18.0	
dodecane	5497	0.011	9.5	19.0	
tridecane	5626	0.012	10.6	21.2	
tetradecane	4935	0.012	9.3	18.6	
pentadecane	5218	0.013	10.6	21.3	
hexadecane	4700	0.012	8.8	17.7	
heptadecane	4350	0.014	9.5	19.1	→ .85
pristane	2830	0.02	8.2	16.4	
octadecane	4038	0.014	8.8	17.7	
phytane	2128	0.02	6.6	13.2	→ .74
nonadecane	3759	0.014	8.2	16.5	
eicosane	3568	0.0135	7.5	15.1	
uncosane	3547	0.0135	7.5	15.0	
docosane	3203	0.013	6.5	13.1	
tricosane	2863	0.0135	6.0	12.1	
tetracosane	2593	0.014	5.7	11.4	
pentacosane	2178	0.0145	4.9	9.9	
hexacosane	1886	0.015	4.4	8.8	
heptocosane	1545	0.0155	3.7	7.5	
octocosane	973	0.016	2.4	4.8	
nonacosane	819	0.016	2.0	4.0	
triacontane					
untriacontane					
dotriacontane					

Hydrocarbon Analysis

Sample number: 13E
14 day exposure

Compound	area units	rf	ng/ul	μg/g dry wt.
HMB	262877	5×10^{-4}		
naphthalene				
methyl naphthalene	143193	5×10^{-4}	13.8	13.8
	102258		9.8	9.8
dimethyl naphthalene	159836	5.5×10^{-4}	16.9	16.9
	184049		19.5	19.5
	54473		5.8	5.8
	8550		0.9	0.9
trimethyl naphthalene	73508	6×10^{-4}	8.5	8.5
	47534		5.5	5.5
	26318		3.0	3.0
phenanthrene	14889	1×10^{-3}	2.9	2.9
dibenzothiophene	8942	1×10^{-3}	1.7	1.7
methyl phenanthrene	8372	1.8×10^{-3}	2.9	2.9
	10176		3.5	3.5
methyl dibenzothiophene	8281	1×10^{-3}	1.6	1.6
	4073		0.8	0.8
dimethyl phenanthrene	13568	1.8×10^{-3}	4.7	4.7
dimethyl dibenzothiophene				
trimethyl phenanthrene	6236	1.8×10^{-3}	2.2	2.2
trimethyl dibenzothiophene	5347	1×10^{-3}	1.0	1.0
	5401		1.0	1.0
	2439		0.5	0.5
	1207		0.2	0.2

Hydrocarbon Analysis

Sample number: 14A

14 day exposure

Compound	area units	rf	ng/ml	μg/g dry wt.
HMB	4428	0.014		
undecane	2618	0.011	5.8	11.7
dodecane	3103	0.011	6.9	13.8
tridecane	3397	0.012	8.2	16.5
tetradecane	3370	0.012	8.2	16.4
pentadecane	3197	0.013	8.4	16.8
hexadecane	2927	0.012	7.1	14.2
heptadecane	2658	0.014	7.5	15.1 → .89
pristane	1665	0.02	6.7	13.5
octadecane	2545	0.014	7.2	14.4 → .79
phytane	1410	0.02	5.7	11.4
nonadecane	2370	0.014	6.7	13.4
eicosane	2311	0.0135	6.3	12.6
uncosane	2333	0.0135	6.4	12.8
docosane	2175	0.013	5.7	11.4
tricosane	1984	0.0135	5.4	10.8
tetracosane	1828	0.014	5.2	10.4
pentacosane	1590	0.0145	4.6	9.3
hexacosane	1303	0.015	3.9	7.8
heptocosane	986	0.0155	3.1	6.2
octocosane	638	0.016	2.0	4.0
nonacosane				
triacontane				
untriacontane				
dotriacontane				

Hydrocarbon Analysis

Sample number: 14 A
14 day exposure

Compound	area units	rf	ng/ul	µg/g dry wt.
HMB	788770	2×10^{-4}		
naphthalene	172845	1×10^{-4}	2.8	2.8
methyl naphthalene	319152	2×10^{-4}	10.2	10.2
	183982		5.8	5.8
dimethyl naphthalene	323953	3×10^{-4}	15.6	15.6
	351351		16.8	16.8
	101891		4.8	4.8
trimethyl naphthalene	18737	3×10^{-4}	0.8	0.8
	43706		2.0	2.0
	156433		7.6	7.6
	115384		5.6	5.6
	57948		2.8	2.8
phenanthrene	46566	4×10^{-4}	3.0	3.0
dibenzothiophene	35446	4×10^{-4}	2.2	2.2
methyl phenanthrene	41882	5×10^{-4}	3.4	3.4
	34433		2.8	2.8
methyl dibenzothiophene	30541	4×10^{-4}	1.9	1.9
	14606		0.9	0.9
	6397		0.4	0.4
dimethyl phenanthrene	44181	5×10^{-4}	3.5	3.5
dimethyl dibenzothiophene	13643	4×10^{-4}	0.9	0.9
	15645		1.0	1.0
	8436		0.5	0.5
	3905		0.2	0.2
trimethyl phenanthrene	10479	5×10^{-4}	0.8	0.8
	4611		0.4	0.4
	1246		0.1	0.1
trimethyl dibenzothiophene	24252	4×10^{-4}	1.5	1.5
	2866		0.2	0.2

Hydrocarbon Analysis

Sample number: 14C

14 day exposure

Compound	area units	rf	ng/ul	µg/g dry wt.
HMB	4070	0.014		
undecane	2241	0.011	5.4	10.9
dodecane	2641	0.011	6.4	12.8
tridecane	2921	0.012	7.7	15.5
tetradecane	2540	0.012	6.7	13.4
pentadecane	2728	0.013	7.8	15.6
hexadecane	2551	0.012	6.7	13.5
heptadecane	2364	0.014	7.3	14.6 → .91
pristane	1514	0.02	6.6	13.3
octadecane	2264	0.014	7.0	14.0 → .77
phytane	1235	0.02	5.4	10.9
nonadecane	2122	0.014	6.5	13.1
eicosane	2085	0.0135	6.2	12.4
uncosane	2145	0.0135	6.4	12.8
docosane	2011	0.013	5.7	11.5
tricosane	1888	0.0135	5.6	11.2
tetracosane	1727	0.014	5.3	10.6
pentacosane	1545	0.0145	4.9	9.9
hexacosane	1296	0.015	4.2	8.4
heptocosane	1001	0.0155	3.4	6.8
octocosane	590	0.016	2.0	4.0
nonacosane				
triacontane				
untriacontane				
dotriacontane				

Hydrocarbon Analysis

Sample number: 14E
14 day exposure

Compound	area units	rf	ng/ul	µg/g dry wt.
HMB	2937	0.014		
undecane	1057	0.011	3.5	7.1
dodecane	1348	0.011	4.5	9.0
tridecane	1483	0.012	5.4	10.8
tetradecane	1223	0.012	4.4	8.8
pentadecane	1457	0.013	5.8	11.6
hexadecane	1363	0.012	5.0	10.0
heptadecane	1181	0.014	5.0	10.0 → .88
pristane	718	0.02	4.4	8.8
octadecane	1199	0.014	5.1	10.2 → .74
phytane	623	0.02	3.8	7.6
nonadecane	1157	0.014	4.9	9.9
eicosane	1186	0.0135	4.9	9.8
uncosane	1211	0.0135	5.0	10.0
docosane	1136	0.013	4.5	9.0
tricosane	1073	0.0135	4.4	8.8
tetracosane	963	0.014	4.1	8.2
pentacosane	852	0.0145	3.7	7.5
hexacosane	726	0.015	3.3	6.6
heptocosane	523	0.0155	2.4	4.9
octocosane		0.016		
nonacosane				
triacontane				
untriacontane				
dotriacontane				

Hydrocarbon Analysis

Sample number: 15B
14 day control

Compound	area units	rf	ng/µl	µg/g dry wt.
HMB	2892	0.014	40.5	
undecane				
dodecane				
tridecane				
tetradecane				
pentadecane				
hexadecane				
heptadecane				
pristane				
octadecane				
phytane				
nonadecane				
eicosane				
uncosane				
docosane				
tricosane				
tetracosane				
pentacosane				
hexacosane				
heptacosane				
octacosane				
nonacosane				
triacontane				
untriacontane				
dotriacontane				

Hydrocarbon Analysis

Sample number: 16A
21 day exposure

Compound	area units	rf	ng/ul	ug/g dry wt.
HMB	5559	0.014		
undecane	3806	0.011	6.8	13.6
dodecane	4098	0.011	7.3	14.6
tridecane	4407	0.012	8.6	17.2
tetradecane	4132	0.012	8.0	16 0
pentadecane	4199	0.013	8.8	17.6
hexadecane	3935	0.012	7.5	15.0
heptadecane	3629	0.014	8.2	16.5 → .82
pristane	2083	0.02	6.8	13.6
octadecane	3161	0.014	7.2	14.5 → .71
phytane	1558	0.02	5.1	10.2
nonadecane	3047	0.014	7.0	14.0
eicosane	2804	0.0135	6.2	12.4
uncosane	2744	0.0135	6.0	12.0
docosane	2558	0.013	5.4	10.8
tricosane	2440	0.0135	5.3	10.6
tetracosane	2198	0.014	5.0	10.0
pentacosane	1913	0.0145	4.5	9.0
hexacosane	1529	0.015	3.7	7.4
heptacosane	1141	0.0155	2.8	5.6
octacosane	663	0.016	1.7	3.4
nonacosane				
triacontane				
untriacontane				
dotriacontane				

Hydrocarbon Analysis

Sample number: 16B

21 day exposure

Compound	area units	rf	ng/µl	µg/g dry wt.
HMB	5462	0.014		
undecane	1239	0.011	2.2	4.4
dodecane	1509	0.011	2.7	5.4
tridecane	1789	0.012	3.5	7.0
tetradecane	1844	0.012	3.6	7.2
pentadecane	1962	0.013	4.2	8.4
hexadecane	1786	0.012	3.5	7.0
heptadecane	1772	0.014	4.0	8.0
pristane	942	0.02	3.1	6.2 → .77
octadecane	1506	0.014	3.4	6.8 → .67
phytane	711	0.02	2.3	4.6
nonadecane	1503	0.014	3.4	6.8
eicosane	1384	0.0135	3.0	6.0
uncosane	1339	0.0135	3.0	6.0
docosane	1239	0.013	2.6	5.2
tricosane	1205	0.0135	2.6	5.2
tetracosane	1074	0.014	2.4	4.8
pentacosane	968	0.0145	2.3	4.6
hexacosane	811	0.015	2.1	4.2
heptacosane	611	0.0155	1.5	3.0
octacosane		0.016		
nonacosane				
triacontane				
untriacontane				
dotriacontane				

Hydrocarbon Analysis

Sample number: 17A

21 day exposure

Compound	area units	rf	ng/ml	µg/g dry wt.
HMB	3939	0.014		
undecane	4278	0.011	10.7	20.4
dodecane	4361	0.011	10.9	20.8
tridecane	4564	0.012	12.5	25.0
tetradecane	4319	0.012	11.8	23.6
pentadecane	4372	0.013	12.9	24.8
hexadecane	4021	0.012	11.1	22.2
heptadecane	3713	0.014	11.8	23.6 → .82
pristane	2126	0.02	9.7	19.4
octadecane	3147	0.014	10.0	20.0 → .7
phytane	1548	0.02	7.0	14.0
nonadecane	2888	0.014	9.2	18.4
eicosane	2575	0.0135	7.9	15.8
uncosane	2385	0.0135	7.3	14.7
docosane	2245	0.013	6.6	13.4
tricosane	2082	0.0135	6.4	12.8
tetracosane	1902	0.014	6.1	12.2
pentacosane	1673	0.0145	5.5	11.0
hexacosane	1399	0.015	4.7	9.5
heptacosane	1063	0.0155	3.7	7.4
octacosane	675	0.016	2.4	4.8
nonacosane				
triacontane				
untriacontane				
dotriacontane				

Hydrocarbon Analysis

Sample number: 17B

21 day exposure

Compound	area units	rf	ng/µl	µg/g dry wt.
HMB	3966	0.014		
undecane	3000	0.011	7.4	14.9
dodecane	3170	0.011	7.9	15.8
tridecane	3326	0.012	9.0	18.1
tetradecane	3096	0.012	8.4	16.8
pentadecane	3124	0.013	9.2	18.4
hexadecane	2790	0.012	7.5	15.1
heptadecane	2680	0.014	8.5	17.0 → .8
pristane	1505	0.02	6.8	13.6
octadecane	2351	0.014	7.4	14.9 → .6
phytane	1147	0.02	5.2	10.4
nonadecane	2225	0.014	7.0	14.1
eicosane	2064	0.0135	6.3	12.6
uncosane	2032	0.035	6.2	12.4
docosane	1910	0.013	5.6	11.2
tricosane	1813	0.0135	5.5	11.1
tetracosane	1616	0.014	5.1	10.2
pentacosane	1391	0.0145	4.5	9.1
hexacosane	1147	0.015	3.9	7.8
heptacosane	858	0.0155	3.0	6.0
octacosane	488	0.016	1.7	3.5
nonacosane				
triacontane				
untriacontane				
dotriacontane				

Hydrocarbon Analysis

Sample number: 17D

21 day exposure

Compound	area units	rf	ng/ml	μg/g dry wt.
HMB	4861	0.014		
undecane	3881	0.011	7.9	15.8
dodecane	4211	0.011	8.5	17.1
tridecane	4485	0.012	9.9	19.9
tetradecane	4124	0.012	9.1	18.3
pentadecane	4101	0.013	9.8	19.7
hexadecane	3675	0.012	8.1	16.3
heptadecane	3449	0.014	8.9	17.8 → .83
pristane	2008	0.02	7.4	14.8
octadecane	2994	0.014	7.7	15.5 → .71
phytane	1500	0.02	5.5	11.1
nonadecane	2865	0.014	7.4	14.8
eicosane	2651	0.0135	6.6	13.2
uncosane	2570	0.0135	6.4	12.8
docosane	2399	0.013	5.7	11.5
tricosane	2226	0.0135	5.5	11.1
tetracosane	1986	0.014	5.1	10.2
pentacosane	1737	0.0145	4.6	9.3
hexacosane	1451	0.015	4.0	8.0
heptacosane	1115	0.0155	3.1	6.3
octacosane	657	0.016	1.9	3.8
nonacosane				
triacontane				
untriacontane				
dotriacontane				

Hydrocarbon Analysis

Sample number: 17E

21 day exposure

Compound	area units	rf	ng/ul	µg/g dry wt.
HMB	4860	0.014		
undecane	5696	0.011	11.6	23.2
dodecane	5941	0.011	12.1	24.2
tridecane	6260	0.012	13.9	27.8
tetradecane	5731	0.012	12.7	25.4
pentadecane	5719	0.013	13.7	27.5
hexadecane	5468	0.012	12.1	24.3
heptadecane	4925	0.014	12.7	25.5
pristane	2914	0.02	10.7	21.5
octadecane	4349	0.014	11.2	22.5
phytane	2222	0.02	8.2	16.4
nonadecane	4051	0.014	10.5	21.0
eicosane	3761	0.0135	9.4	18.8
uncosane	3658	0.0135	9.1	18.2
docosane	3424	0.013	8.2	16.4
tricosane	3204	0.0135	8.0	16.0
tetracosane	2957	0.014	7.6	15.3
pentacosane	2617	0.0145	7.0	14.0
hexacosane	2295	0.015	6.3	12.7
heptacosane	1802	0.0155	5.1	10.3
octacosane	1161	0.016	3.4	6.8
nonacosane				
triacontane				
untriacontane				
dotriacontane				

→ .8

→ .7

Hydrocarbon Analysis

Sample number: 188

21 day exposure

Compound	area units	rf	µg/ml	Mg/g dry wt.
HMB	4133	0.014		
undecane	1407	0.011	3.3	6.7
dodecane	1629	0.011	3.9	7.8
tridecane	1766	0.012	4.6	9.6
tetradecane	1716	0.012	4.4	8.9
pentadecane	1739	0.013	4.9	9.8
hexadecane	1559	0.012	4.0	8.0
heptadecane	1476	0.014	4.4	8.8
pristane	716	0.02	3.1	6.2
octadecane	1251	0.014	3.8	7.6
phytane	582	0.02	2.5	5.0
nonadecane	1191	0.014	3.6	7.2
eicosane	1096	0.0135	3.2	6.4
uncosane	1086	0.0135	3.1	6.3
docosane	986	0.013	2.7	5.5
tricosane	942	0.0135	2.7	5.5
tetracosane	825	0.014	2.5	5.0
pentacosane	698	0.0145	2.2	4.4
hexacosane	599	0.015	1.9	3.9
heptacosane		0.0155		
octacosane		0.016		
nonacosane				
triacontane				
untriacontane				
dotriacontane				

→ .70 (heptadecane/pristane)

→ .65 (pristane/phytane)

Hydrocarbon Analysis

Sample number: 18B
21 day exposure

Compound	area units	rf	ng/ml	μg/g dry wt.
HMB	691338	2×10^{-4}		
naphthalene	269041	1×10^{-4}	4.9	4.9
methyl naphthalene	501985	2×10^{-4}	18.3	18.3
	288381		10.5	10.5
dimethyl naphthalene	483392	3×10^{-4}	26.4	26.4
	520615		28.4	28.4
	137771		7.5	7.5
trimethyl naphthalene	24604	3×10^{-4}	1.3	1.3
	41661		2.3	2.3
	243862		13.3	13.3
	161162		8.8	8.8
	73282		4.0	4.0
phenanthrene	67406	4×10^{-4}	4.9	4.9
dibenzothiophene	50727	4×10^{-4}	3.7	3.7
methyl phenanthrene	56634	5×10^{-4}	5.1	5.1
	51654		4.7	4.7
methyl dibenzothiophene	45423	4×10^{-4}	3.3	3.3
	20874		1.5	1.5
	10031		0.7	0.7
dimethyl phenanthrene	68864	5×10^{-4}	6.3	6.3
dimethyl dibenzothiophene	24903	4×10^{-4}	1.8	1.8
	24158		1.8	1.8
	12153		0.9	0.9
	5491		0.4	0.4
trimethyl phenanthrene	33815	5×10^{-4}	3.1	3.1
trimethyl dibenzothiophene				

Hydrocarbon Analysis

Sample number: 18C

21 day exposure

Compound	area units	rf	ng/ul	µg/g dry wt.	
HMB	4936	0.014			
undecane	4673	0.011	9.3	18.7	
dodecane	5026	0.011	10.0	20.0	
tridecane	4703	0.012	10.2	20.5	
tetradecane	4509	0.012	9.8	19.7	
pentadecane	4679	0.013	11.0	22.1	
hexadecane	4471	0.012	9.7	19.5	
heptadecane	4002	0.014	10.2	20.4	→ .84
pristane	2382	0.02	8.6	17.3	
octadecane	3535	0.014	9.0	18.0	→ .72
phytane	1783	0.02	6.5	13.0	
nonadecane	3307	0.014	8.4	16.8	
eicosane	3077	0.0135	7.5	15.1	
uncosane	3006	0.0135	7.3	14.7	
docosane	2831	0.013	6.7	13.4	
tricosane	2636	0.0135	6.4	12.9	
tetracosane	2364	0.014	6.0	12.0	
pentacosane	2056	0.0145	5.4	10.8	
hexacosane	1729	0.015	4.7	9.4	
heptacosane	1362	0.0155	3.8	7.6	
octacosane	860	0.016	2.5	5.0	
nonacosane					
triacontane					
untriacontane					
dotriacontane					

Hydrocarbon Analysis

Sample number: 19A

21 day exposure

Compound	area units	rf	ng/ml	μg/g dry wt.	
HMB	5372	0.014			
undecane	5601	0.011	10.3	20.6	
dodecane	5734	0.011	10.5	21.1	
tridecane	6096	0.012	12.2	24.5	
tetradecane	5556	0.012	11.1	22.3	
pentadecane	5476	0.013	11.9	23.8	
hexadecane	5284	0.012	10.6	21.2	
heptadecane	4712	0.014	11.0	22.1	→ .8[illegible]
pristane	2771	0.02	9.2	18.5	
octadecane	4170	0.014	9.7	19.5	→ .72
phytane	2116	0.02	7.0	14.1	
nonadecane	3912	0.014	9.1	18.3	
eicosane	3665	0.0135	8.2	16.5	
uncosane	3556	0.0135	8.0	16.0	
docosane	3315	0.013	7.2	14.4	
tricosane	3084	0.0135	6.9	13.9	
tetracosane	2825	0.014	6.6	13.2	
pentacosane	2499	0.0145	6.0	12.0	
hexacosane	2178	0.015	5.4	10.9	
heptacosane	1688	0.0155	4.3	8.7	
octacosane	1112	0.016	2.9	5.9	
nonacosane					
triacontane					
untriacontane					
dotriacontane					

Hydrocarbon Analysis

Sample number: 19B
21 day exposure

Compound	area units	rf	ng/ul	ug/g dry wt.
HMB	4496	0.014		
undecane	3557	0.011	7.8	15.6
dodecane	3813	0.011	8.3	16.7
tridecane	4087	0.012	9.8	19.6
tetradecane	3755	0.012	9.0	18.0
pentadecane	3752	0.013	9.7	19.5
hexadecane	3362	0.012	8.0	16.0
heptadecane	3183	0.014	8.9	17.8
pristane	1813	0.02	7.2	14.5
octadecane	2762	0.014	7.7	15.4
phytane	1376	0.02	5.5	11.0
nonadecane	2634	0.014	7.3	14.7
eicosane	2449	0.0135	6.6	13.2
uncosane	2375	0.0135	6.4	12.8
docosane	2222	0.013	5.7	11.5
tricosane	2044	0.0135	5.5	11.0
tetracosane	1860	0.014	5.2	10.4
pentacosane	1600	0.0145	4.6	9.2
hexacosane	1407	0.015	4.2	8.4
heptacosane	1071	0.0155	3.3	6.6
octacosane	633	0.016	2.0	4.0
nonacosane				
triacontane				
untriacontane				
dotriacontane				

→ .81 (heptadecane/pristane)
→ .71 (octadecane/phytane)

Hydrocarbon Analysis

Sample number: 19C

21 day exposure

Compound	area units	rf	ng/ul	µg/g dry wt.
HMB	4416	0.014		
undecane	5193	0.011	11.6	23.2
dodecane	5249	0.011	11.7	23.4
tridecane	5454	0.012	13.3	26.6
tetradecane	4970	0.012	12.1	24.2
pentadecane	4911	.013	13.0	26.0
hexadecane	4746	.012	11.6	23.2
heptadecane	4267	.014	12.2	24.4
pristane	2495	.02	10.2	20.4
octadecane	3789	.014	10.8	21.6
phytane	1920	.02	7.8	15.6
nonadecane	3661	0.014	10.4	20.8
eicosane	3473	0.0135	9.6	19.2
uncosane	3459	0.0135	9.5	19.0
docosane	3365	0.013	8.9	17.8
tricosane	3176	0.0135	8.7	17.4
tetracosane	2986	0.014	8.5	17.0
pentacosane	2641	0.0145	7.8	15.6
hexacosane	2279	0.015	7.0	14.0
heptacosane	1818	0.0155	5.7	11.4
octacosane	1127	0.016	3.7	7.4
nonacosane	874	0.016	2.9	5.8
triacontane				
untriacontane				
dotriacontane				

Hydrocarbon Analysis

Sample number: 19C

21 day exposure

Compound	area units	rf	ng/ul	µg/g dry wt.
HMB	773768	2×10^{-4}		
naphthalene	321863	1×10^{-4}	5.3	5.3
methyl naphthalene	498274	2×10^{-4}	16.3	16.3
	285160		9.3	9.3
dimethyl naphthalene	478767	3×10^{-4}	23.5	23.5
	511966		25.2	25.2
	115517		5.7	5.7
	19291		0.9	0.9
trimethyl naphthalene	34470	3×10^{-4}	1.7	1.7
	216122		10.6	10.6
	147469		7.3	7.3
	76680		3.8	3.8
phenanthrene	45963	4×10^{-4}	3.0	3.0
dibenzothiophene	32432	4×10^{-4}	2.1	2.1
methyl phenanthrene	31431	5×10^{-4}	2.6	2.6
	27695		2.3	2.3
methyl dibenzothiophene	26190	4×10^{-4}	1.7	1.7
	12052		0.8	0.8
	3420		0.2	0.2
dimethyl phenanthrene	36749	5×10^{-4}	3.0	3.0
dimethyl dibenzothiophene	13020	4×10^{-4}	0.9	0.9
	6408		0.4	0.4
	3071		0.2	0.2
trimethyl phenanthrene	14612	5×10^{-4}	1.2	1.2
	1768		0.1	0.1
trimethyl dibenzothiophene	3137	4×10^{-4}	0.2	0.2
	4582		0.3	0.3
	2675		0.2	0.2
	2249		0.1	0.1

Hydrocarbon Analysis

Sample number: 19C

21 day exposure

Compound	area units	rf	ng/ul	μg/g dry wt.
HMB	764129	2×10^{-4}		
naphthalene	321276	1×10^{-4}	5.3	5.3
methyl naphthalene	480544	2×10^{-4}	15.8	15.8
	278494		9.1	9.1
dimethyl naphthalene	380370	3×10^{-4}	18.7	18.7
	496964		24.4	24.4
	161714		8.0	8.0
trimethyl naphthalene	34812	3×10^{-4}	1.7	1.7
	211631		10.4	10.4
	145834		7.2	7.2
	78330		3.9	3.9
phenanthrene	55967	4×10^{-4}	3.7	3.7
dibenzothiophene	39101	4×10^{-4}	2.6	2.6
methyl phenanthrene	40740	5×10^{-4}	3.3	3.3
	35272		2.9	2.9
methyl dibenzothiophene	31960	4×10^{-4}	2.1	2.1
	15117		1.0	1.0
	4734		0.3	0.3
dimethyl phenanthrene	48043	5×10^{-4}	3.9	3.9
dimethyl dibenzothiophene	15901	4×10^{-4}	1.0	1.0
	16399		1.1	1.1
	8412		0.6	0.6
	3747		0.2	0.2
trimethyl phenanthrene	3753	5×10^{-4}	0.3	0.3
	6343		0.5	0.5
	4570		0.4	0.4
	3285		0.3	0.3
	1586		0.1	0.1

Hydrocarbon Analysis

Sample number: 19D
21 day exposure

Compound	area units	rf	ng/ul	µg/g dry wt.
HMB	4701	0.014		
undecane	6610	0.011	13.9	27.8
dodecane	6496	0.011	13.6	27.3
tridecane	6576	0.012	15.1	30.2
tetradecane	6035	0.012	13.8	27.7
pentadecane	6000	0.013	14.9	29.8
hexadecane	5754	0.012	13.2	26.4
heptadecane	5126	0.014	13.7	27.4 → .83
pristane	3003	0.02	11.4	22.9
octadecane	4594	0.014	12.3	24.6 → .72
phytane	2338	0.02	8.9	17.9
nonadecane	4363	0.014	11.6	23.3
eicosane	4107	0.0135	10.6	21.2
uncosane	4052	0.0135	10.4	20.9
docosane	3896	0.013	9.6	19.3
tricosane	3699	0.0135	9.5	19.1
tetracosane	3445	0.014	9.2	18.4
pentacosane	3018	0.0145	8.3	16.7
hexacosane	2570	0.015	7.3	14.7
heptacosane	2052	0.0155	6.0	12.1
octacosane	1322	0.016	4.0	8.0
nonacosane	996	0.016	3.0	6.0
triacontane				
untriacontane				
dotriacontane				

Hydrocarbon Analysis

Sample number: 19 D
21 day exposure

Compound	area units	rf	ng/ul	μg/g dry wt.
HMB	726376	2×10^{-4}		
naphthalene	393214	1×10^{-4}	6.8	6.8
methyl naphthalene	584368	2×10^{-4}	20.2	20.2
	347700		12.0	12.0
dimethyl naphthalene	554057	2.5×10^{-4}	23.9	23.9
	626240		27.0	27.0
	159959		6.9	6.9
trimethyl naphthalene	44380	2×10^{-4}	1.5	1.5
	236011		8.1	8.1
	181414		6.3	6.3
	75950		2.6	2.6
phenanthrene	48843	4×10^{-4}	3.4	3.4
dibenzothiophene	35718	4×10^{-4}	2.5	2.5
methyl phenanthrene	39327	8.5×10^{-4}	5.8	5.8
	31516		4.6	4.6
methyl dibenzothiophene	29924	4×10^{-4}	2.1	2.1
	13012		0.9	0.9
	5036		0.4	0.4
dimethyl phenanthrene	1044	8.5×10^{-4}	0.2	0.2
	39534		5.8	5.8
dimethyl dibenzothiophene	15819	4×10^{-4}	1.1	1.1
	15127		1.0	1.0
	7990		0.6	0.6
	3764		0.3	0.3
trimethyl phenanthrene	16331	8.5×10^{-4}	2.4	2.4
	1765		0.3	0.3
trimethyl dibenzothiophene	3653	4×10^{-4}	0.3	0.3
	5499		0.4	0.4
	4076		0.3	0.3
	2492		0.2	0.2
	1454		0.1	0.1
Benzanthracene	266	5.1×10^{-3}	0.2	0.2
C_4 Phenanthrene	562	8.5×10^{-4}	0.1	0.1
	232		0.1	0.1

Hydrocarbon Analysis

Sample number: 20C
21 day control

Compound	area units	rf	ng/ul	µg/g dry wt.
HMB	2794	0.014		
undecane				
dodecane				
tridecane				
tetradecane				
pentadecane				
hexadecane				
heptadecane				
pristane				
octadecane				
phytane				
nonadecane				
eicosane				
uncosane				
docosane				
tricosane				
tetracosane				
pentacosane				
hexacosane				
heptacosane				
octacosane				
nonacosane				
triacontane				
untriacontane				
dotriacontane				

Hydrocarbon Analysis

Sample number: 21A

28 day exposure

Compound	area units	rf	ng/ml	µg/g dry wt.	
HMB	3267	.014			
undecane	868	0.011	2.6	5.2	
dodecane	1158	0.011	3.5	7.0	
tridecane	1130	0.012	3.4	6.8	
tetradecane	886	0.012	2.9	5.8	
pentadecane	1095	0.013	3.9	7.8	
hexadecane	1021	0.012	3.4	6.8	
heptadecane	694	0.014	2.7	5.4	.89
pristane	429	0.02	2.4	4.8	
octadecane	907	0.014	3.5	7.0	.77
phytane	495	0.02	2.7	5.4	
nonadecane	941	0.014	3.6	7.2	
eicosane	970	0.0135	3.6	7.2	
uncosane	1018	0.0135	3.8	7.6	
docosane	980	0.013	3.5	7.0	
tricosane	886	0.0135	3.3	6.6	
tetracosane	766	0.014	3.0	6.0	
pentacosane	644	0.0145	2.6	5.2	
hexacosane	529	0.015	2.2	4.4	
heptocosane	284	0.0155	1.2	2.4	
octocosane					
nonacosane					
triacontane					
untriacontane					
dotriacontane					

Hydrocarbon Analysis

Sample number: 2/A

28 day exposure

Compound	area units	rf	ng/ul	μg/g dry wt.
HMB	686935	2×10^{-4}		
naphthalene	84388	1×10^{-4}	1.5	1.5
methyl naphthalene	151067	2×10^{-4}	5.5	5.5
	79850		2.9	2.9
dimethyl naphthalene	136289	2.5×10^{-4}	5.0	5.0
	144854		6.6	6.6
	46919		2.1	2.1
trimethyl naphthalene	10660	2×10^{-4}	0.4	0.4
	10442		0.4	0.4
	72338		2.6	2.6
	46361		1.7	1.7
	22069		0.8	0.8
phenanthrene	18611	4×10^{-4}	1.4	1.4
dibenzothiophene	13751	4×10^{-4}	1.0	1.0
methyl phenanthrene	16659	8.5×10^{-4}	2.6	2.6
	13978		2.2	2.2
methyl dibenzothiophene	12687	4×10^{-4}	0.9	0.9
	5507		0.4	0.4
	1801		0.1	0.1
dimethyl phenanthrene	15504	8.5×10^{-4}	2.4	2.4
dimethyl dibenzothiophene	6293	4×10^{-4}	0.5	0.5
	6344		0.5	0.5
	2409		0.2	0.2
trimethyl phenanthrene	3804	8.5×10^{-4}	0.6	0.6
trimethyl dibenzothiophene	475	4×10^{-4}	0.1	0.1
	1829		0.1	0.1
	1443		0.1	0.1
	1050		0.1	0.1

Hydrocarbon Analysis

Sample number: 21E

28 day exposure

Compound	area units	rf	ng/ul	ug/g dry wt.
HMB	3039	0.014		
undecane	728	0.011	2.4	4.8
dodecane	857	0.011	2.8	5.6
tridecane	1000	0.012	3.6	7.6
tetradecane	1017	0.012	3.6	7.5
pentadecane	1078	0.013	4.2	8.4
hexadecane	1014	0.012	3.6	7.3
heptadecane	944	0.014	3.9	7.8
pristane	434	0.02	2.6	5.2
octadecane	785	0.014	3.3	6.6
phytane	didn't integrate	0.12		
nonadecane	768	0.014	3.2	6.4
eicosane	711	0.0135	2.8	5.6
uncosane	719	0.0135	2.9	5.8
docosane	636	0.013	2.4	4.8
tricosane	623	0.0135	2.5	5.0
tetracosane	503	0.014	2.1	4.2
pentacosane	434	0.0145	1.9	3.8
hexacosane		0.015		
heptacosane		0.0155		
octacosane		0.016		
nonacosane				
triacontane				
untriacontane				
dotriacontane				

.67

Hydrocarbon Analysis

Sample number: 22B

28 day exposure

Compound	area units	rf	ng/ul	µg/g dry wt.
HMB	2987	0.014		
undecane	640	0.011	2.1	4.2
dodecane	802	0.011	2.7	5.4
tridecane	942	0.012	3.4	6.8
tetradecane	943	0.012	3.4	6.8
pentadecane	1007	0.013	3.9	7.8
hexadecane	897	0.012	3.2	6.4
heptadecane	869	0.014	3.7	7.4
pristane	420	0.02	2.5	5.0
octadecane	726	0.014	3.1	6.2
phytane	did not integrate	0.02	—	—
nonadecane	768	0.014	3.2	6.4
eicosane	711	0.0135	2.9	5.8
uncosane	719	0.0135	2.9	5.8
docosane	636	0.013	2.5	5.0
tricosane	623	0.0135	2.5	5.0
tetracosane	503	0.014	2.1	4.2
pentacosane	439	0.0145	1.9	3.8
hexacosane		0.015		
heptacosane		0.0153		
octacosane		0.016		
nonacosane				
triacontane				
untriacontane				
dotriacontane				

Hydrocarbon Analysis

Sample number: 22D

Compound	area units	rf	ng/ul	μg/g dry wt.
HMB	3549	0.014		
undecane	1678	0.011	4.7	9.4
dodecane	1825	0.011	5.1	10.2
tridecane	1950	0.012	5.9	11.8
tetradecane	1835	0.012	5.6	11.2
pentadecane	1837	0.013	6.1	12.2
hexadecane	1621	0.012	4.9	9.8
heptadecane	1552	0.014	5.5	11.0
pristane	747	0.02	3.8	7.6
octadecane	1314	0.014	4.7	9.4
phytane	578	0.02	2.9	5.8
nonadecane	1246	0.014	4.4	8.8
eicosane	1129	0.0135	3.9	7.8
uncosane	1115	0.0135	3.8	7.6
docosane	954	0.013	3.1	6.2
tricosane	805	0.0135	2.8	5.6
tetracosane	717	0.014	2.5	5.0
pentacosane	577	0.0145	2.1	4.2
hexacosane		0.015		
heptacosane		0.0155		
octacosane		0.016		
nonacosane				
triacontane				
untriacontane				
dotriacontane				

.69

.62

Hydrocarbon Analysis

Sample number: 22 D

28 day exposure

Compound	area units	rf	ng/ul	µg/g dry wt.
HMB	728589	2×10^{-4}		
naphthalene	117320	1×10^{-4}	2.0	2.0
methyl naphthalene	173765	2×10^{-4}	6.0	6.0
	114440		3.9	3.9
dimethyl naphthalene	204624	2.5×10^{-4}	8.8	8.8
	209108		9.0	9.0
	48007		2.1	2.1
trimethyl naphthalene	8691	2×10^{-4}	0.3	0.3
	17523		0.6	0.6
	90411		3.1	3.1
	63509		2.2	2.2
	34638		1.2	1.2
phenanthrene	26829	4×10^{-4}	1.9	1.9
dibenzothiophene	17835	4×10^{-4}	1.2	1.2
methyl phenanthrene	17606	8.5×10^{-4}	2.6	2.6
	16792		2.5	2.5
methyl dibenzothiophene	12960	4×10^{-4}	0.9	0.9
	6747		0.5	0.5
	2355		0.2	0.2
dimethyl phenanthrene	666	8.5×10^{-4}	0.1	0.1
	23468		3.4	3.4
dimethyl dibenzothiophene	388	4×10^{-4}	0.1	0.1
	7364		0.5	0.5
	7680		0.5	0.5
	4004		0.3	0.3
	1610		0.1	0.1
trimethyl phenanthrene	9277	8.5×10^{-4}	1.4	1.4
	1109		0.2	0.2
trimethyl dibenzothiophene	2130	4×10^{-4}	0.1	0.1
	2439		0.2	0.2
	2210		0.2	0.2
	1507		0.1	0.1

Hydrocarbon Analysis

Sample number: 23B

28 day exposure

Compound	area units	rf	ng/ul	μg/g dry wt.
HMB	3127	0.014		
undecane	416	0.011	1.3	2.6
dodecane	510	0.011	1.6	3.2
tridecane	658	0.012	2.2	4.4
tetradecane	605	0.012	2.4	4.8
pentadecane	741	0.013	2.7	5.5
hexadecane	665	0.012	2.2	4.54
heptadecane	683	0.014	2.7	5.5
pristane		0.02		
octadecane	561	0.014	2.3	4.6
phytane	-	0.02		
nonadecane	572	0.014	2.3	4.6
eicosane	514	0.0135	2.0	4.0
uncosane	518	0.0135	2.0	4.0
docosane	454	0.013	1.7	3.4
tricosane	472	0.0135	1.8	3.6
tetracosane	407	0.014	1.6	3.2
pentacosane		0.0145		
hexacosane		0.015		
heptacosane		0.0155		
octacosane		0.016		
nonacosane				
triacontane				
untriacontane				
dotriacontane				

Hydrocarbon Analysis

Sample number: 24B

28 day exposure

Compound	area units	rf	ng/ml	μg/g dry wt.
HMB	2401	0.014		
undecane	did not integrate	0.011		
dodecane	412	0.011	1.5	3.0
tridecane	537	0.012	1.9	3.9
tetradecane	545	0.012	2.0	4.0
pentadecane	589	0.013	2.3	4.7
hexadecane	512	0.012	1.9	3.9
heptadecane	519	0.011	2.2	4.5
pristane		0.02		
octadecane	401	0.014	1.7	3.4
phytane	413	0.02	2.5	5.1
nonadecane		0.014		
eicosane		0.0135		
uncosane		0.0135		
docosane		0.013		
tricosane		0.0135		
tetracosane		0.014		
pentacosane		0.0145		
hexacosane		0.015		
heptqcosane		0.0155		
octqcosane		0.016		
nonacosane				
triacontane				
untriacontane				
dotriacontane				

Hydrocarbon Analysis

Sample number: 24C

28 day exposure

Compound	area units	rf	ng/ul	µg/g dry wt.
HMB	3884	0.014		
undecane	1490	0.011	3.7	7.5
dodecane	1801	0.011	4.5	9.1
tridecane	2023	0.012	5.6	11.2
tetradecane	1961	0.012	5.4	10.9
pentadecane	1962	0.013	5.9	11.8
hexadecane	1732	0.012	4.8	9.6
heptadecane	1642	0.014	5.3	10.6
pristane	855	0.02	3.9	7.9
octadecane	1403	0.014	4.5	9.1
phytane	634	0.02	2.9	5.8
nonadecane	1327	0.014	4.3	8.6
eicosane	1239	0.0135	4.0	8.0
uncosane	1230	0.0135	3.8	7.6
docosane	1116	0.013	3.3	6.7
tricosane	1044	0.0135	3.2	6.5
tetracosane	916	0.014	2.9	5.9
pentacosane	783	0.0145	2.6	5.2
hexacosane	634	0.015	2.2	4.4
heptacosane	462	0.0155	1.6	3.3
octacosane		0.016		
nonacosane				
triacontane				
untriacontane				
dotriacontane				

→ .74 (heptadecane/pristane)

→ .63 (octadecane/phytane)

Hydrocarbon Analysis

Sample number: 24C

28 day exposure

Compound	area units	rf	ng/ul	μg/g dry wt.
HMB	587929	1 x 10^-4		
naphthalene	79114	5 x 10^-5	1.7	1.7
methyl naphthalene	127450	1 x 10^-4	5.5	5.5
	73485		3.2	3.2
dimethyl naphthalene	144594	1 x 10^-4	6.3	6.3
	149846		6.5	6.5
	38110		1.7	1.7
trimethyl naphthalene	5587	1 x 10^-4	0.2	0.2
	10728		0.5	0.5
	67845		2.9	2.9
	43387		1.9	1.9
	21020		0.9	0.9
phenanthrene	14464	2 x 10^-4	1.3	1.3
dibenzothiophene	9698	2 x 10^-4	0.8	0.8
methyl phenanthrene	9119	4 x 10^-4	1.6	1.6
	9186		1.6	1.6
methyl dibenzothiophene	9798	2 x 10^-4	0.9	0.9
	3799		0.3	0.3
dimethyl phenanthrene	198	4 x 10^-4	<0.1	<0.1
	9841			1.7
dimethyl dibenzothiophene	3841	2 x 10^-4	0.3	0.3
	4408		0.4	0.4
	2007		0.2	0.2
	911		0.1	0.1
trimethyl phenanthrene	468	4 x 10^-4	0.1	0.1
	1118		0.2	0.2
	267		<0.1	<0.1
trimethyl dibenzothiophene	440	2 x 10^-4	<0.1	<0.1
	824		0.1	0.1
	811		0.1	0.1
	400		<0.1	<0.1

Hydrocarbon Analysis

Sample number: 25E

28 day control

Compound	area units	rf	ng/µl	µg/g dry wt.
HMB	2870	0.014		
undecane				
dodecane				
tridecane				
tetradecane				
pentadecane				
hexadecane				
heptadecane				
pristane				
octadecane				
phytane				
nonadecane				
eicosane				
uncosane				
docosane				
tricosane				
tetracosane				
pentacosane				
hexacosane				
heptacosane				
octacosane				
nonacosane				
triacontane				
untriacontane				
dotriacontane				

Hydrocarbon Analysis

Sample number: S1E

3 months exposure

Compound	area units	rf	ng/ul	µg/g dry wt.
HMB	2098	0.014		
undecane	737	0.011	3.4	6.9
dodecane	993	0.011	4.6	9.2
tridecane	1108	0.012	5.7	11.4
tetradecane	1050	0.012	5.4	10.8
pentadecane	1131	0.013	6.3	12.6
hexadecane	1032	0.012	5.3	10.6
heptadecane	994	0.014	5.9	11.9 → .70
pristane	490	0.02	4.2	8.4
octadecane	936	0.014	5.6	11.2 → .64
phytane	421	0.02	3.6	7.2
nonadecane	977	0.014	5.8	11.7
eicosane	1027	0.0135	5.9	11.8
uncosane	1148	0.0135	6.6	13.2
docosane	1136	0.013	6.3	12.6
tricosane	1121	0.0135	6.4	12.9
tetracosane	1078	0.014	6.4	12.8
pentacosane	1028	0.0145	6.3	12.7
hexacosane	864	0.015	5.5	11.1
heptocosane	710	0.0155	4.7	9.4
octocosane	284	0.016	1.9	3.8
nonacosane				
triacontane				
untriacontane				
dotriacontane				

Hydrocarbon Analysis

Sample number: S/E

3 month exposure

Compound	area units	rf	ng/µl	µg/g dry wt.
HMB	406733	2.0×10^{-4}		
naphthalene	37080	1.0×10^{-4}	1.1	1.1
methyl naphthalene	69532	2×10^{-4}	4.3	4.3
	39136		2.4	2.4
dimethyl naphthalene	80278	2×10^{-4}	5.0	5.0
	91241		5.7	5.7
	29276		1.8	1.8
trimethyl naphthalene	3440	2×10^{-4}	0.2	0.2
	6910		0.4	0.4
	44344		2.8	2.8
	30891		1.9	1.9
	12193		0.8	0.8
phenanthrene	13716	5×10^{-4}	2.1	2.1
dibenzothiophene	9375	4×10^{-4}	1.2	1.2
methyl phenanthrene	8871	9×10^{-4}	2.5	2.5
	8144		2.3	2.3
methyl dibenzothiophene	8730	4×10^{-4}	1.1	1.1
	3804		0.5	0.5
	1195		0.1	0.1
dimethyl phenanthrene	8752	9×10^{-4}	2.5	2.5
dimethyl dibenzothiophene	3605	4×10^{-4}	0.5	0.5
	4059		0.5	0.5
	1874		0.2	0.2
	865		0.1	0.1
trimethyl phenanthrene	303	9×10^{-4}	0.1	0.1
	1575		0.4	0.4
trimethyl dibenzothiophene	260	4×10^{-4}	< 0.1	< 0.1
	846		0.1	0.1
	371		< 0.1	< 0.1
	639		< 0.1	< 0.1

Hydrocarbon Analysis

Sample number: S2A

3 month exposure

Compound	area units	rf	ng/ul	µg/g dry wt.
HMB	2666	0.014		
undecane	277	0.011	1.0	2.0
dodecane	630	0.011	2.3	4.6
tridecane	786	0.012	3.1	6.3
tetradecane	647	0.012	2.6	5.2
pentadecane	865	0.013	3.7	7.5
hexadecane	797	0.012	3.2	6.4
heptadecane	615	0.014	2.9	5.8 → .72
pristane	318	0.02	2.1	4.2
octadecane	742	0.014	3.5	7.0 → .68
phytane	362	0.02	2.4	4.8
nonadecane	761	0.014	3.5	7.1
eicosane	772	0.0135	3.5	7.0
uncosane	833	0.0135	3.7	7.5
docosane	801	0.013	3.5	7.0
tricosane	762	0.0135	3.4	6.9
tetracosane	703	0.014	3.3	6.6
pentacosane	601	0.0145	2.9	5.8
hexacosane	522	0.015	2.6	5.2
heptacosane	371	0.0155	1.9	3.8
octacosane		0.016		
nonacosane				
triacontane				
untriacontane				
dotriacontane				

Hydrocarbon Analysis

Sample number: S2B

3 month exposure

Compound	area units	rf	ng/ul	μg/g dry wt.
HMB	2072	0.014		
undecane	391	0.011	1.8	3.7
dodecane	555	0.011	2.6	5.3
tridecane	586	0.012	3.0	6.0
tetradecane	573	0.012	2.9	5.8
pentadecane	578	0.013	3.2	6.5
hexadecane	552	0.012	2.8	5.7
heptadecane	486	0.014	2.9	5.9
pristane		0.02	—	—
octadecane	471	0.014	2.9	5.8
phytane		0.02	—	—
nonadecane	504	0.014	3.1	6.2
eicosane	532	0.0135	3.1	6.2
uncosane	581	0.0135	3.4	6.8
docosane	559	0.013	3.2	6.4
tricosane	563	0.0135	3.3	6.6
tetracosane	540	0.014	3.3	6.6
pentacosane	505	0.0145	3.1	6.2
hexacosane	438	0.015	2.9	5.8
heptacosane	318	0.0155	2.1	4.2
octacosane		0.016		
nonacosane				
triacontane				
untriacontane				
dotriacontane				

Hydrocarbon Analysis

Sample number: S2C

Compound	area units	rf	ng/μl	μg/g dry wt.
HMB	2286	0.014		
undecane	651	0.011	2.8	5.6
dodecane	878	0.011	3.8	7.6
tridecane	1001	0.012	4.7	9.4
tetradecane	915	0.012	4.3	8.6
pentadecane	961	0.013	4.9	9.8
hexadecane	835	0.012	3.9	7.8
heptadecane	757	0.014	4.1	8.3 → .6
pristane	339	0.02	2.6	5.3
octadecane	698	0.014	3.8	7.6 → .5
phytane	285	0.02	2.2	4.4
nonadecane	748	0.014	4.1	8.2
eicosane	731	0.0135	3.8	7.7
uncosane	826	0.0135	4.3	8.7
docosane	759	0.013	3.8	7.7
tricosane	771	0.0135	4.0	8.0
tetracosane	723	0.014	3.9	7.9
pentacosane	686	0.0145	3.9	7.8
hexacosane	509	0.015	3.0	6.0
heptacosane	457	0.0155	2.7	5.5
octacosane		0.016		
nonacosane				
triacontane				
untriacontane				
dotriacontane				

Hydrocarbon Analysis

Sample number: 52D
3 month exposure

Compound	area units	rf	ng/ul	ug/g dry wt.
HMB	4095	0.014		
undecane	1646	0.011	3.9	7.8
dodecane	2104	0.011	5.0	10.0
tridecane	2396	0.012	6.3	12.6
tetradecane	2245	0.012	5.9	11.8
pentadecane	3267	0.013	9.2	18.4
hexadecane	2036	0.012	5.3	10.6
heptadecane	1901	0.014	5.8	11.6 → .76
pristane	1022	0.02	4.4	8.8
octadecane	1670	0.014	5.1	10.2 → .71
phytane	828	0.02	3.6	7.2
nonadecane	1602	0.014	4.9	9.8
eicosane	1544	0.0135	4.5	9.0
uncosane	1556	0.0135	4.6	9.2
docosane	1447	0.013	4.1	8.2
tricosane	1407	0.0135	4.1	8.2
tetracosane	1253	0.014	3.8	7.6
pentacosane	1135	0.145	3.6	7.2
hexacosane	955	0.015	3.1	6.2
heptacosane	718	0.0155	2.4	4.8
octocasane	380	0.016	1.3	2.6
nonacosane				
triacontane				
untriacontane				
dotriacontane				

Hydrocarbon Analysis

Sample number: S2D
3 month exposure

Compound	area units	rf	ng/ul	µg/g dry wt.
HMB	797637	2.0×10^{-4}		
naphthalene	98336	1.0×10^{-4}	1.6	1.6
methyl naphthalene	187051	2.0×10^{-4}	6.0	6.0
	104893		3.3	3.3
dimethyl naphthalene	215324	2.5×10^{-4}	8.5	8.5
	219273		8.7	8.7
	41955		1.7	1.7
trimethyl naphthalene	18369	2.0×10^{-4}	0.6	0.6
	17812		0.6	0.6
	125985		4.0	4.0
	81666		2.6	2.6
	39915		1.3	1.3
phenanthrene	41340	5×10^{-4}	3.3	3.3
dibenzothiophene	24624	4×10^{-4}	1.6	1.6
methyl phenanthrene	33513	1.1×10^{-3}	5.9	5.9
	29070		5.1	5.1
methyl dibenzothiophene	29837	4×10^{-4}	1.9	1.9
	11878		0.8	0.8
	3424		0.2	0.2
dimethyl phenanthrene	710	1.1×10^{-3}	0.1	0.1
	32617		5.7	5.7
dimethyl dibenzothiophene	13942	4×10^{-4}	0.9	0.9
	13782		0.9	0.9
	5484		0.3	0.3
trimethyl phenanthrene	552	1.1×10^{-3}	0.1	0.1
trimethyl dibenzothiophene				

Hydrocarbon Analysis

Sample number: S 2 E

3 month exposure

Compound	area units	rf	ug/ul	ug/g dry wt.
HMB	3721	0.014		
undecane	1331	0.011	3.6	7.2
dodecane	1918	0.011	5.1	10.2
tridecane	2305	0.012	6.7	13.4
tetradecane	2161	0.012	6.3	12.6
pentadecane	2245	0.013	7.1	14.2
hexadecane	2005	0.012	5.9	11.8
heptadecane	1859	0.014	6.3	12.6 → .81
pristane	1041	0.02	5.1	10.2
octadecane	1655	0.014	5.7	11.4 → .68
phytane	807	0.02	3.9	7.8
nonadecane	1582	0.014	5.4	10.8
eicosane	1517	0.0135	5.0	10.0
uncosane	1544	0.0135	5.1	10.2
docosane	1461	0.013	4.6	9.2
tricosane	1413	0.0135	4.7	9.4
tetracosane	1284	0.014	4.4	8.8
pentacosane	1194	0.0145	4.2	8.4
hexacosane	973	0.015	3.6	7.2
heptacosane	725	0.0155	2.7	5.4
octocosane	380	0.016	1.5	3.0
nonacosane				
triacontane				
untriacontane				
dotriacontane				

Hydrocarbon Analysis

Sample number: S2E
3 month exposure

Compound	area units	rf	ng/µl	µg/g dry wt.
HMB	755444	2.0×10^{-4}		
naphthalene	95438	1.0×10^{-4}	1.6	1.6
methyl naphthalene	175472	2.0×10^{-4}	5.8	5.8
	98619		3.3	3.3
dimethyl naphthalene	190856	2.5×10^{-4}	8.0	8.0
	214190		8.9	8.9
	70268		2.9	2.9
	7124		0.3	0.3
trimethyl naphthalene	17509	2.0×10^{-4}	0.6	0.6
	17216		0.6	0.6
	117380		3.9	3.9
	75396		2.5	2.5
	35699		1.2	1.2
phenanthrene	25905	5×10^{-4}	2.2	2.2
dibenzothiophene	18973	4×10^{-4}	1.3	1.3
methyl phenanthrene	20505	1.1×10^{-3}	3.8	3.8
	16367		3.0	3.0
methyl dibenzothiophene	16616	4.0×10^{-4}	1.1	1.1
	7295		0.5	0.5
dimethyl phenanthrene	445	1.1×10^{-3}	0.1	0.1
	15861		2.9	2.9
dimethyl dibenzothiophene	7625	4.0×10^{-4}	0.5	0.5
	7429		0.5	0.5
	2588		0.2	0.2
trimethyl phenanthrene	5874	1.1×10^{-3}	1.1	1.1
	942		0.2	0.2
trimethyl dibenzothiophene	1360	4.0×10^{-4}	0.1	0.1
	2196		0.1	0.1
	1720		0.1	0.1
	1155		0.1	0.1

Hydrocarbon Analysis

Sample number: S 3 A
3 month exposure

Compound	area units	rf	ng/ul	μg/g dry wt.
HMB	2623	0.014		
undecane				
dodecane	252	0.011	1.0	2.0
tridecane				
tetradecane	355	0.012	1.5	3.0
pentadecane	413	0.013	1.8	3.6
hexadecane	343	0.012	1.4	2.8
heptadecane	304	0.014	1.5	3.0
pristane				
octadecane	299	0.014	1.4	2.8
phytane				
nonadecane	325	0.014	1.6	3.2
eicosane	344	0.0135	1.6	3.2
uncosane	410	0.0135	1.9	3.8
docosane	400	0.013	1.8	3.6
tricosane	405	0.0135	1.9	3.8
tetracosane	323	0.014	1.6	3.2
pentacosane	342	0.0145	1.7	3.4
hexacosane	229	0.015	1.2	2.4
heptacosane				
octacosane				
nonacosane				
triacontane				
untriacontane				
dotriacontane				

Hydrocarbon Analysis

Sample number: S3B
3 month exposure

Compound	area units	rf	ng/ml	µg/g dry wt.
HMB	2494	0.014		
undecane	did not integrated	0.011		
dodecane	did not integrated	0.011		
tridecane	202	0.012	1.4	2.9
tetradecane	268	0.012	1.1	2.3
pentadecane	309	0.013	1.4	2.8
hexadecane	219	0.012	0.9	1.8
heptadecane	245	0.014	1.2	2.4 → 1.62
pristane	273	0.02	1.9	3.9
octadecane	323	0.014	2.3	4.6 → .91
phytane	293	0.02	2.1	4.2
nonadecane	365	0.014	1.8	3.6
eicosane	328	0.0135	1.5	3.1
uncosane	350	0.0135	1.7	3.4
docosane	271	0.013	1.2	2,5
tricosane	278	0.0135	1.3	2.7
tetracosane		0.014		
pentacosane		0.0145		
hexacosane		0.015		
heptacosane		0.0155		
octacosane		0.016		
nonacosane				
triacontane				
untriacontane				
dotriacontane				

Hydrocarbon Analysis

Sample number: S3C

3 month exposure

Compound	area units	rf	ng/ul	Mg/g dry wt.
HMB	1345	0.014		
undecane	did not integ.	0.011		
dodecane	did not integ	0.011		
tridecane	—	0.012	—	
tetradecane	—	0.012	—	
pentadecane	—	0.013	—	
hexadecane	—	0.012	—	
heptadecane	—	0.014	—	
pristane	—	0.02	—	
octadecane	260	0.014	2.4	4.8
phytane	291	0.02	3.8	7.7
nonadecane	362	0.014	3.3	6.7
eicosane	367	0.0135	3.3	6.7
uncosane	397	0.0135	3.5	7.1
docosane	335	0.013	2.9	5.8
tricosane	336	0.0135	3.0	6.0
tetracosane	275	0.014	2.5	5.1
pentacosane	242	0.0145	2.3	4.6
hexacosane		0.015		
heptdcosane		0.0155		
octacosane		0.016		
nonacosane				
triacontane				
untriacontane				
dotriacontane				

→ 1.60

Hydrocarbon Analysis

Sample number: S3C 2.9 ml
3 month exposure

Compound	area units	rf	ng/µl	µg/g dry wt.
HMB	2395	0.014		
undecane		0.011		
dodecane		0.011		
tridecane		0.012		
tetradecane		0.012		
pentadecane		0.013		
hexadecane		0.012		
heptadecane		0.014		
pristane		0.02		
octadecane		0.014		
phytane		0.02		
nonadecane		0.014		
eicosane	402	0.0135	2.0	4.0
uncosane	403	0.0135	2.0	4.0
docosane	434	0.013	2.1	4.2
tricosane	434	0.0135	2.2	4.4
tetracosane	391	0.014	2.0	4.0
pentacosane	246	0.0145	1.3	2.6
hexacosane		0.015		
heptocosane		0.0155		
octocosane		0.016		
nonacosane				
triacontane				
untriacontane				
dotriacontane				

Hydrocarbon Analysis

Sample number: S3D
3 month exposure

Compound	area units	rf	ng/ul	µg/g dry wt.
HMB	2904	0.014		
undecane	1060	0.011	3.5	7.0
dodecane	1437	0.011	4.7	9.5
tridecane	1479	0.012	5.3	10.6
tetradecane	1256	0.012	4.5	9.0
pentadecane	1365	0.013	5.3	10.6
hexadecane	1293	0.012	4.6	9.2
heptadecane	938	0.014	3.9	7.8 → .67
pristane	448	0.02	2.6	5.3
octadecane	1141	0.014	4.8	9.6
phytane	559	0.02	3.3	6.7 → .69
nonadecane	1085	0.014	4.5	9.1
eicosane	1078	0.0135	4.3	8.6
uncosane	1107	0.0135	4.4	8.8
docosane	1001	0.013	3.9	7.8
tricosane	800	0.0135	3.2	6.4
tetracosane	666	0.014	2.8	5.6
pentacosane	505	0.0145	2.2	4.4
hexacosane	355	0.015	1.6	3.2
heptacosane		0.0155		
octacosane		0.016		
nonacosane				
triacontane				
untriacontane				
dotriacontane				

Hydrocarbon Analysis

Sample number: S4C
3 month exposure

Compound	area units	rf	ng/ul	µg/g dry wt.
HMB	2103	0.014		
undecane	399	0.011	1.8	3.7
dodecane	631	0.011	2.9	5.9
tridecane	715	0.012	3.6	7.3
tetradecane	705	0.012	3.6	7.2
pentadecane	737	0.013	4.1	8.2
hexadecane	671	0.012	3.4	6.8
heptadecane	630	0.014	3.7	7.5 → .5
pristane	252	0.02	2.1	4.3
octadecane	572	0.014	3.4	6.8 → .6
phytane	251	0.02	2.1	4.2
nonadecane	614	0.014	3.6	7.3
eicosane	623	0.0135	3.5	7.1
uncosane	690	0.0135	3.9	7.9
docosane	674	0.013	3.7	7.4
tricosane	672	0.0135	3.8	7.7
tetracosane	662	0.014	3.9	7.9
pentacosane	615	0.0145	3.8	7.7
hexacosane	498	0.015	3.1	6.3
heptacosane	398	0.0155	2.6	5.2
octacosane		0.016		
nonacosane				
triacontane				
untriacontane				
dotriacontane				

Hydrocarbon Analysis

Sample number: S4D
3 month exposure

Compound	area units	rf	ng/ul	μg/g dry wt.	
HMB	2695	0.014			
undecane	426	0.011	1.5	3.1	
dodecane	653	0.011	2.3	4.7	
tridecane	812	0.012	3.2	6.5	
tetradecane	785	0.012	3.1	6.2	
pentadecane	952	0.013	4.1	8.2	
hexadecane	803	0.012	3.2	6.4	
heptadecane	808	0.014	3.7	7.5	→ .52
pristane	298	0.02	1.9	3.9	
octadecane	731	0.014	3.4	6.8	→ .5
phytane	264	0.02	1.7	3.5	
nonadecane	550	0.014	2.5	5.1	
eicosane	732	0.0135	3.3	6.6	
uncosane	769	0.0135	3.4	6.9	
docosane	741	0.013	3.2	6.4	
tricosane	720	0.0135	3.2	6.4	
tetracosane	680	0.014	3.1	6.3	
pentacosane	607	0.0145	2.9	5.8	
hexacosane	523	0.015	2.6	5.2	
heptacosane	382	0.0155	1.9	3.9	
octacosane		0.016			
nonacosane					
triacontane					
untriacontane					
dotriacontane					

Hydrocarbon Analysis

Sample number: S4E

3 month exposure

Compound	area units	rf	ng/ul	μg/g dry wt.
HMB	2343	0.014		
undecane	561	0.011	2.3	4.6
dodecane	808	0.011	3.4	6.8
tridecane	938	0.012	4.3	8.6
tetradecane	923	0.012	4.2	8.5
pentadecane	997	0.013	4.9	9.9
hexadecane	885	0.012	4.0	8.0
heptadecane	869	0.014	4.6	9.3 → .67
pristane	402	0.02	3.1	6.2
octadecane	795	0.014	4.3	8.6 → .65
phytane	370	0.02	2.8	5.6
nonadecane	818	0.014	4.4	8.8
eicosane	851	0.0135	4.4	8.8
uncosane	937	0.0135	4.9	9.8
docosane	927	0.013	4.6	9.2
tricosane	926	0.0135	4.8	9.6
tetracosane	868	0.014	4.7	9.4
pentacosane	817	0.0145	4.6	9.2
hexacosane	696	0.015	4.0	8.0
heptacosane	572	0.0155	3.4	6.8
octacosane		0.016		
nonacosane				
triacontane				
untriacontane				
dotriacontane				

Hydrocarbon Analysis

Sample number: S4E
3 month exposure

Compound	area units	rf	ng/ml	μg/g dry wt.
HMB	760683	2.0×10^{-4}		
naphthalene	57880	1.0×10^{-4}	1.0	1.0
methyl naphthalene	115087	2.0×10^{-4}	3.8	3.8
	61847		2.1	2.1
dimethyl naphthalene	139060	2.5×10^{-4}	5.8	5.8
	141500		5.9	5.9
	25448		1.1	1.1
trimethyl naphthalene	14069		0.5	0.5
	77045		2.6	2.6
	53513		1.8	1.8
	21269		0.7	0.7
phenanthrene	24399	5×10^{-4}	2.0	2.0
dibenzothiophene	16387	4×10^{-4}	1.1	1.1
methyl phenanthrene	18381	1.1×10^{-3}	3.4	3.4
	18273		3.4	3.4
methyl dibenzothiophene	19788	4×10^{-4}	1.3	1.3
	8008		0.5	0.5
	1966		0.1	0.1
dimethyl phenanthrene	22959	1.1×10^{-3}	4.2	4.2
dimethyl dibenzothiophene	8606	4.0×10^{-4}	0.6	0.6
	8824		0.6	0.6
	4497		0.3	0.3
	2148		0.1	0.1
trimethyl phenanthrene	6212	1.1×10^{-3}	1.1	1.1
trimethyl dibenzothiophene	11487	4.0×10^{-4}	0.8	0.8
	1420		0.1	0.1
Benzanthracene	141	7.9×10^{-3}	0.2	0.2
C_4 Phenanthrene	365	1.1×10^{-3}	0.1	0.1
	204		<.1	<.1

Hydrocarbon Analysis

Sample number: S5B
3 month exposure

Compound	area units	rf	ng/ul	µg/g dry wt.
HMB	4407	0.014		
undecane	565	0.011	1.3	2.6
dodecane	1006	0.011	2.3	4.6
tridecane	1305	0.012	3.2	6.4
tetradecane	1328	0.012	3.3	6.6
pentadecane	1441	0.013	3.8	7.6
hexadecane	1289	0.012	3.2	6.4
heptadecane	1284	0.014	3.7	7.4 → .86
pristane	790	0.02	3.2	6.4
octadecane	1100	0.014	3.1	6.2 → .81
phytane	601	0.02	2.5	5.0
nonadecane	1109	0.014	3.2	6.4
eicosane	1087	0.0135	3.0	6.0
uncosane	1102	0.0135	3.0	6.0
docosane	1032	0.013	2.7	5.4
tricosane	1060	0.0135	2.9	5.8
tetracosane	985	0.014	2.8	5.6
pentacosane	873	0.0145	2.6	5.2
hexacosane	760	0.015	2.3	4.6
heptocosane	591	0.0155	1.9	3.8
octocosane	224	0.016	0.7	1.4
nonacosane				
triacontane				
untriacontane				
dotriacontane				

Hydrocarbon Analysis

Sample number: 55C
3 months exposure

Compound	area units	rf	ng/ul	ug/g dry wt.
HMB	3558	0.014		
undecane	did not integrate			
dodecane	220	0.011	0.6	1.2
tridecane	267	0.012	0.8	1.6
tetradecane	298	0.012	0.9	1.8
pentadecane	387	0.013	1.3	2.6
hexadecane	328	0.012	1.0	2.0
heptadecane	320	0.014	1.1	2.2 → .91
pristane	203	0.02	1.0	2.0
octadecane	292	0.014	1.0	2.0
phytane	did not integrate			
nonadecane	336	0.014	1.2	2.4
eicosane	335	0.0135	1.1	2.2
uncosane	426	0.0135	1.4	2.8
docosane	392	0.013	1.3	2.6
tricosane	447	0.0135	1.5	3.0
tetracosane	449	0.014	1.6	3.2
pentacosane	480	0.0145	1.7	3.4
hexacosane	381	0.015	1.4	2.8
heptocosane	301	0.0155	1.2	2.4
octocosane				
nonacosane				
triacontane				
untriacontane				
dotriacontane				

Hydrocarbon Analysis

Sample number: S5B 3ml
3 month exposure

Compound	area units	rf	ng/ul	ng/g dry wt.
HMB	1847	0.014		
undecane				
dodecane				
tridecane				
tetradecane				
pentadecane				
hexadecane				
heptadecane				
pristane				
octadecane				
phytane				
nonadecane				
eicosane				
uncosane				
docosane				
tricosane				
tetracosane				
pentacosane				
hexacosane				
heptacosane				
octacosane				
nonacosane				
triacontane				
untriacontane				
dotriacontane				

Hydrocarbon Analysis

Sample number: Sed+Oil Zone 4/78
4 month exposure

Compound	area units	rf	ng/µl	µg/g dry wt.	
HMB	3180	0.014			
undecane	678	0.011	2.1	4.2	
dodecane	1061	0.011	3.3	6.6	
tridecane	1163	0.012	3.9	7.8	
tetradecane	884	0.012	3.0	6.0	
pentadecane	1145	0.013	4.2	8.4	
hexadecane	1019	0.012	3.4	6.9	
heptadecane	735	0.014	2.9	5.8	→1.06
pristane	551	0.02	3.1	6.2	
octadecane	1039	0.014	4.1	8.2	→1.21
phytane	893	0.02	5.0	10.0	
nonadecane	880	0.014	3.4	6.8	
eicosane	882	0.0135	3.3	6.6	
uncosane	903	0.0135	3.4	6.8	
docosane	815	0.013	2.9	5.9	
tricosane	712	0.0135	2.7	5.4	
tetracosane	609	0.014	2.4	4.8	
pentacosane	434	0.0145	1.7	3.5	
hexacosane	301	0.015	1.2	2.5	
heptocosane		0.0155			
octocosane		0.016			
nonacosane					
triacontane					
untriacontane					
dotriacontane					

Hydrocarbon Analysis

Sample number: 4/78 Sed + oil

0 Time exposure

Compound	area units	rf	ng/ul	μg/g dry wt.
HMB	723756	2×10^{-4}		
naphthalene	56741	1×10^{-4}	1.0	1.0
methyl naphthalene	122470	2×10^{-4}	4.3	4.3
	67496		2.4	2.4
dimethyl naphthalene	148043	2.5×10^{-4}	6.5	6.5
	153168		6.7	6.7
	42254		1.9	1.9
trimethyl naphthalene	12416	2×10^{-4}	0.4	0.4
	81302		2.9	2.9
	51886		1.8	1.8
	23354		0.8	0.8
phenanthrene	19590	5×10^{-4}	1.7	1.7
dibenzothiophene	13483	4×10^{-4}	0.9	0.9
methyl phenanthrene	14988	1.1×10^{-3}	2.9	2.9
	11652		2.2	2.2
methyl dibenzothiophene	33508	4×10^{-4}	2.4	2.4
	12219		0.9	0.9
	5318		0.4	0.4
dimethyl phenanthrene	209	2.0×10^{-3}	0.1	0.1
dimethyl dibenzothiophene	13729		2.6	2.6
	7272	4×10^{-4}	0.5	0.5
	5863		0.4	0.4
	2740		0.2	0.2
	1498		0.1	0.1
trimethyl phenanthrene	1199	1.1×10^{-3}	0.2	0.2
	4409		0.9	0.9
trimethyl dibenzothiophene	292		0.1	0.1
	432	4×10^{-4}	$<.1$	$<.1$
	1926		0.1	0.1
C_4 Phenanthrene	2584	1.1×10^{-3}	0.2	0.2
	209		$<.1$	$<.1$
	122		$<.1$	$<.1$

Hydrocarbon Analysis

Sample number: AT1 8/78
4 month exposure

Compound	area units	rf	ng/ml	µg/g dry wt.
HMB	4617	0.014		
undecane	3687	0.011	8.0	16.0
dodecane	4147	0.011	8.9	17.8
tridecane	4563	0.012	10.7	21.4
tetradecane	4204	0.012	9.9	19.8
pentadecane	4295	0.013	10.9	21.8
hexadecane	4137	0.012	9.7	19.4
heptadecane	3786	0.014	10.4	20.8 → .86
pristane	2264	0.02	8.9	17.8
octadecane	3451	0.014	9.5	19.0 → .7
phytane	1732	0.02	6.8	13.6
nonadecane	3266	0.014	9.0	18.0
eicosane	3124	0.0135	8.3	16.6
uncosane	3129	0.0135	8.3	16.6
docosane	2948	0.013	7.5	15.0
tricosane	2786	0.0135	7.4	14.8
tetracosane	2557	0.014	7.0	14.0
pentacosane	2263	0.0145	6.4	12.8
hexacosane	1917	0.015	5.6	11.2
heptacosane	1525	0.0155	4.6	9.2
octocosane	963	0.016	3.0	6.0
nonacosane	633	0.016	2.0	4.0
triacontane				
untriacontane				
dotriacontane				

Hydrocarbon Analysis

Sample number: A T 1
4 month exposure

Compound	area units	rf	ng/ul	µg/g dry wt.
HMB	697471			
naphthalene	166450	1×10^{-4}	3.0	3.0
methyl naphthalene	301487	2×10^{-4}	11.0	11.0
	164916		6.0	6.0
dimethyl naphthalene	334017	2.5×10^{-4}	15.2	15.2
	350213		15.9	15.9
	87368		4.0	4.0
trimethyl naphthalene	16061	2×10^{-4}	0.6	0.6
	26179		1.0	1.0
	134197		4.9	4.9
	103540		3.8	3.8
	50550		1.8	1.8
phenanthrene	31177	3×10^{-4}	1.7	1.7
dibenzothiophene	25069	3×10^{-4}	1.4	1.4
methyl phenanthrene	24549	1.3×10^{-3}	5.8	5.8
	19590		4.6	4.6
methyl dibenzothiophene	20755	3×10^{-4}	1.1	1.1
	9445		0.5	0.5
	4157		0.2	0.2
dimethyl phenanthrene	521	1.3×10^{-3}	0.1	0.1
	23335		5.5	5.5
dimethyl dibenzothiophene	10217	3×10^{-4}	0.6	0.6
	9501		0.5	0.5
	5143		0.3	0.3
	2516		0.1	0.1
trimethyl phenanthrene	1[illegible]6	1.3×10^{-3}	2.4	2.4
	[illegible]4		0.3	0.3
trimethyl dibenzothiophene	[illegible]7	3×10^{-4}	0.1	0.1
	3117		0.2	0.2
	2314		0.1	0.1
	1667		0.1	0.1

Hydrocarbon Analysis

Sample number: AT2 8/78
4 month exposure

Compound	area units	rf	ng/µl	µg/g dry wt.
HMB	6225	0.014		
undecane	9526	0.011	15.2	30.4
dodecane	9740	0.011	15.5	31.0
tridecane	10500	0.012	18.3	36.6
tetradecane	9246	0.012	16.1	32.2
pentadecane	9834	0.013	18.5	37.0
hexadecane	8982	0.012	15.6	31.2
heptadecane	7966	0.014	16.2	32.4 → .83
pristane	4625	0.02	13.4	26.8
octadecane	7304	0.014	14.8	29.6 → .66
phytane	3375	0.02	9.8	19.6
nonadecane	599	0.014	1.2	2.4
eicosane	6642	0.0135	13.0	26.0
uncosane	6600	0.0135	13.0	26.0
docosane	6256	0.013	11.8	23.6
tricosane	5883	0.0135	11.5	23.0
tetracosane	5502	0.014	11.2	22.4
pentacosane	4852	0.0145	10.2	20.4
hexacosane	4187	0.015	9.1	18.2
heptacosane	3395	0.0155	7.6	15.2
octocosane	2409	0.016	5.6	11.2
nonacosane	1880	0.016	4.4	8.8
triacontane	959	0.0165	2.3	4.6
untriacontane				
dotriacontane				

Hydrocarbon Analysis

Sample number: AT2

4 month exposure

Compound	area units	rf	ng/ul	µg/g dry wt.
HMB	773758	2×10^{-4}		
naphthalene	325529	1×10^{-4}	5.3	5.3
methyl naphthalene	660942	2×10^{-4}	21.7	21.7
	354291		11.6	11.6
dimethyl naphthalene	698019	2.5×10^{-4}	28.6	28.6
	782227		32.1	32.1
	190529		7.8	7.8
trimethyl naphthalene	45673	2×10^{-4}	1.5	1.5
	50305		1.6	1.6
	347190		11.4	11.4
	226537		7.4	7.4
phenanthrene	89192	3×10^{-4}	4.4	4.4
dibenzothiophene	66021	3×10^{-4}	3.2	3.2
methyl phenanthrene	58237	1.3×10^{-3}	12.4	12.4
	51546		11.0	11.0
methyl dibenzothiophene	56916	3×10^{-4}	2.8	2.8
	24018		1.2	1.2
	7892		0.4	0.4
dimethyl phenanthrene	63361	1.3×10^{-3}	13.5	13.5
dimethyl dibenzothiophene	24857	3×10^{-4}	1.2	1.2
	24185		1.2	1.2
	12012		0.6	0.6
	5897		0.3	0.3
trimethyl phenanthrene	4[illegible]862	1.3×10^{-3}	9.3	9.3
trimethyl dibenzothiophene	[illegible]81		0.7	0.7
	[illegible]04	3×10^{-4}	0.2	0.2
	[illegible]91		0.4	0.4
	5536		0.3	0.3
	4334		0.2	0.2

Hydrocarbon Analysis

Sample number: JT1 9/78 control
8 months exposure

Compound	area units	rf	ng/ul	µg/g dry wt.
HMB	2667	0.014		
undecane				
dodecane				
tridecane				
tetradecane				
pentadecane				
hexadecane				
heptadecane				
pristane				
octadecane				
phytane				
nonadecane				
eicosane				
uncosane				
docosane				
tricosane				
tetracosane				
pentacosane				
hexacosane				
heptacosane				
octocosane				
nonacosane				
triacontane				
untriacontane				
dotriacontane				

Hydrocarbon Analysis

Sample number: JT 2 8/78 control
8 month exposure

Compound	area units	rf	ng/μl	μg/g dry wt.
HMB	2977	0.014		
undecane				
dodecane				
tridecane				
tetradecane				
pentadecane				
hexadecane				
heptadecane				
pristane				
octadecane				
phytane				
nonadecane				
eicosane				
uncosane				
docosane				
tricosane				
tetracosane				
pentacosane				
hexacosane				
heptocosane				
octocosane				
nonacosane				
triacontane				
untriacontane				
dotriacontane				

Hydrocarbon Analysis

Sample number: Con 1 8/78
Control

Compound	area units	rf	ng/ul	µg/g dry wt.
HMB	2714	0.014		
undecane				
dodecane				
tridecane				
tetradecane				
pentadecane				
hexadecane				
heptadecane				
pristane				
octadecane				
phytane				
nonadecane				
eicosane				
uncosane				
docosane				
tricosane				
tetracosane				
pentacosane				
hexacosane				
heptacosane				
octacosane				
nonacosane				
triacontane				
untriacontane				
dotriacontane				

Hydrocarbon Analysis

Sample number: Cow 2 8/78
Control

Compound	area units	rf	ng/ul	µg/g dry wt.
HMB	2537	0.014		
undecane				
dodecane				
tridecane				
tetradecane				
pentadecane				
hexadecane				
heptadecane				
pristane				
octadecane				
phytane				
nonadecane				
eicosane				
uncosane				
docosane				
tricosane				
tetracosane				
pentacosane				
hexacosane				
heptacosane				
octacosane				
nonacosane				
triacontane				
untriacontane				
dotriacontane				

Hydrocarbon Analysis

Sample number: 3/5 Con 1/13
Control

Compound	area units	rf	ng/ul	ug/g dry wt.
HMB	3299	0.014		
undecane				
dodecane				
tridecane				
tetradecane				
pentadecane				
hexadecane				
heptadecane	244	0.014	0.9	1.8
pristane				
octadecane				
phytane				
nonadecane				
eicosane				
uncosane				
docosane				
tricosane				
tetracosane				
pentacosane				
hexacosane				
heptacosane				
octacosane				
nonacosane				
triacontane				
untriacontane				
dotriacontane				

Hydrocarbon Analysis

Sample number: 403
8 month control

Compound	area units	rf	ng/ml	µg/g dry wt.
HMB	2717	0.014		
undecane				
dodecane				
tridecane				
tetradecane				
pentadecane				
hexadecane				
heptadecane				
pristane				
octadecane				
phytane				
nonadecane				
eicosane				
uncosane				
docosane				
tricosane				
tetracosane				
pentacosane				
hexacosane				
heptocosane				
octocosane				
nonacosane				
triacontane				
untriacontane				
dotriacontane				

Hydrocarbon Analysis

Sample number: 404

8 month exposure

Compound	area units	rf	ng/ul	μg/g dry wt.
HMB	4834	0.014		
undecane	5169	0.011	10.5	21.0
dodecane	5573	0.011	11.4	22.0
tridecane	6167	0.012	13.7	27.5
tetradecane	5859	0.012	13.0	26.1
pentadecane	5913	0.013	14.3	28.6
hexadecane	5783	0.012	12.9	25.8
heptadecane	5187	0.014	13.5	27.0
pristane	3110	0.02	11.5	23.1 → .8
octadecane	4598	0.014	11.9	23.9 → .76
phytane	2463	0.02	9.1	18.3
nonadecane	4355	0.014	11.3	22.7
eicosane	4092	0.0135	10.2	20.5
uncosane	3980	0.0135	10.0	20.0
docosane	3708	0.013	~~33~~8.9	17.9
tricosane	3458	0.0135	8.3	16.7
tetracosane	3226	0.014	8.4	16.8
pentacosane	2865	0.0145	7.7	15.4
hexacosane	2461	0.015	6.8	13.7
heptacosane	1972	0.0155	5.6	11.3
octacosane	1439	0.016	4.2	8.5
nonacosane				
triacontane				
untriacontane				
dotriacontane				

Hydrocarbon Analysis

Sample number: 404 old oil
8 month exposure

Compound	area units	rf	ng/ml	µg/g dry wt.
HMB	4287	0.014		
undecane	2699	0.011	6.2	12.4
dodecane	3137	0.011	7.2	14.4
tridecane	3478	0.012	8.8	17.6
tetradecane	3254	0.012	8.2	16.4
pentadecane	3254	0.013	8.9	17.8
hexadecane	2907	0.012	7.3	14.6
heptadecane	2732	0.014	8.0	16.0
pristane	1588	0.02	6.7	13.4
octadecane	2388	0.014	7.0	14.0
phytane	1209	0.02	5.1	10.2
nonadecane	2269	0.014	6.7	13.4
eicosane	2110	0.0135	6.0	12.0
uncosane	2041	0.0135	5.8	11.6
docosane	1952	0.013	5.3	10.6
tricosane	1793	0.0135	5.1	10.2
tetracosane	1666	0.014	4.9	9.8
pentacosane	1465	0.0145	4.5	9.0
hexacosane	1221	0.015	3.8	7.6
heptacosane	940	0.0155	3.1	6.2
octacosane	557	0.016	1.9	3.8
nonacosane				
triacontane				
untriacontane				
dotriacontane				

→ .84 (margin, between heptadecane and pristane rows)

→ .73 (margin, on line between octadecane and phytane rows)

Hydrocarbon Analysis

Sample number: 404 OLD OIL
8 month exposure

Compound	area units	rf	ng/ul	ug/g dry wt.
HMB	655298	2×10^{-4}		
naphthalene	251021	2×10^{-4}	9.7	9.7
methyl naphthalene	467426	2×10^{-4}	18.0	18.0
	242374		9.3	9.3
dimethyl naphthalene	432599	3.5×10^{-4}	29.1	29.1
	467841		31.5	31.5
	117508		7.9	7.9
trimethyl naphthalene	21593	3×10^{-4}	1.2	1.2
	33782		1.9	1.9
	215281		12.4	12.4
	123119		7.1	7.1
	65633		3.8	3.8
phenanthrene	50249	5×10^{-4}	4.8	4.8
dibenzothiophene	35184	5×10^{-4}	3.4	3.4
methyl phenanthrene	36359	1.1×10^{-3}	7.7	7.7
	31617		6.7	6.7
methyl dibenzothiophene	28658	5×10^{-4}	2.8	2.8
	12725		1.2	1.2
	4222		0.4	0.4
dimethyl phenanthrene	814	1.1×10^{-2}	0.2	0.2
	36325		7.7	7.7
dimethyl dibenzothiophene	13734	5×10^{-4}	1.3	1.3
	14381		1.4	1.4
	7498		0.7	0.7
	3463		0.3	0.3
trimethyl phenanthrene	759	1.1×10^{-3}	0.2	0.2
	22431		4.7	4.7
	1975		0.4	0.4
trimethyl dibenzothiophene	3550	5×10^{-4}	0.3	0.3
	5416		0.5	0.5
	3745		0.4	0.4
	2676		0.3	0.3
	1150		0.1	0.1

Hydrocarbon Analysis

Sample number: 406 old oil 4/78
8 months exposure

Compound	area units	rf	ng/ul	ug/g dry wt.	
HMB	4114	0.014			
undecane	2262	0.011	3.9	7.8	
dodecane	2675	0.011	4.6	9.2	
tridecane	3027	0.012	5.7	11.4	
tetradecane	2855	0.012	5.4	10.8	
pentadecane	2851	0.013	5.8	11.6	
hexadecane	2582	0.012	4.8	9.6	
heptadecane	2437	0.014	5.3	10.6	→ .81
pristane	1391	0.02	4.3	8.6	
octadecane	2119	0.014	4.6	9.2	→ .74
phytane	1075	0.02	3.4	6.8	
nonadecane	2045	0.014	4.5	9.0	
eicosane	1937	0.0135	4.2	8.4	
uncosane	1926	0.0135	4.1	8.2	
docosane	1824	0.013	3.7	7.4	
tricosane	1747	0.0135	3.7	7.4	
tetracosane	1652	0.014	3.6	7.2	
pentacosane	1498	0.0145	3.4	6.8	
hexacosane	1298	0.015	3.0	6.0	
heptacosane	1015	0.0155	2.5	5.0	
octacosane	597	0.016	1.5	3.0	
nonacosane					
triacontane					
untriacontane					
dotriacontane					

Hydrocarbon Analysis

Sample number: 406 old oil
8 month exposure

Compound	area units	rf	ng/ul	µg/g dry wt.
HMB	687335	2×10^{-4}		
naphthalene	92393	2×10^{-4}	3.4	3.4
methyl naphthalene	183086	2×10^{-4}	6.7	6.7
	102648		3.7	3.7
dimethyl naphthalene	191745	3.5×10^{-4}	12.2	12.2
	213242		13.6	13.6
	70052		4.5	4.5
	7204		0.5	0.5
trimethyl naphthalene	15214	3×10^{-4}	0.8	0.8
	14806		0.8	0.8
	104303		5.7	5.7
	66560		3.6	3.6
	30622		1.7	1.7
phenanthrene	22783	5×10^{-4}	2.1	2.1
dibenzothiophene	15495	5×10^{-4}	1.4	1.4
methyl phenanthrene	16633	1.1×10^{-3}	3.3	3.3
	13057		2.6	2.6
methyl dibenzothiophene	11147	5×10^{-4}	1.0	1.0
	5459		0.5	0.5
	1797		0.2	0.2
dimethyl phenanthrene	16458	1.1×10^{-3}	3.3	3.3
dimethyl dibenzothiophene	5988	5×10^{-4}	0.5	0.5
	6381		0.6	0.6
	3148		0.3	0.3
	1322		0.1	0.1
trimethyl phenanthrene	5859	1.1×10^{-3}	1.2	1.2
	731		0.1	0.1
trimethyl dibenzothiophene	8165	5×10^{-4}	0.7	0.7
	1072		0.1	0.1
	531		0.1	0.1

Hydrocarbon Analysis

Sample number: 414 old oil
8 month exposure

Compound	area units	rf	ng/ml	µg/g dry wt.
HMB	4179	0.014		
undecane	1332	0.011	3.2	6.4
dodecane	1680	0.011	4.0	8.0
tridecane	2002	0.012	5.2	10.4
tetradecane	1997	0.012	5.2	10.4
pentadecane	2062	0.013	5.8	11.6
hexadecane	1845	0.012	4.8	9.6
heptadecane	1737	0.014	5.3	10.6
pristane	1065	0.02	4.6	9.2
octadecane	1470	0.014	4.5	9.0
phytane	817	0.02	3.6	7.2
nonadecane	1397	0.014	4.3	8.6
eicosane	1272	0.0135	3.7	7.4
uncosane	1204	0.0135	3.5	7.0
docosane	1129	0.013	3.2	6.4
tricosane	1102	0.0135	3.2	6.4
tetracosane	1011	0.014	3.1	6.2
pentacosane	922	0.0145	2.9	5.8
hexacosane	778	0.015	2.5	5.0
heptacosane	594	0.0155	2.0	4.0
octacosane	266	0.016	0.9	1.8
nonacosane				
triacontane				
untriacontane				
dotriacontane				

→ .87 (pristane)

→ .80 (phytane)

Hydrocarbon Analysis

Sample number: Old oil
8 month exposure

Compound	area units	rf	ng/ul	ug/g dry wt.
HMB	4848	0.014		
undecane	3890	0.011	7.9	15.8
dodecane	4765	0.011	9.7	19.4
tridecane	5290	0.012	11.8	23.6
tetradecane	4962	0.012	11.0	22.0
pentadecane	5104	0.013	12.3	24.6
hexadecane	4968	0.012	11.0	22.0
heptadecane	4471	0.014	11.6	23.2 → .89
pristane	2775	0.02	10.3	20.6
octadecane	4027	0.014	10.4	20.8 → .77
phytane	2171	0.02	8.0	16.0
nonadecane	3800	0.014	9.9	19.8
eicosane	3597	0.0135	9.0	18.0
uncosane	3593	0.0135	9.0	18.0
docosane	3423	0.013	8.2	16.4
tricosane	3256	0.0135	8.1	16.2
tetracosane	3008	0.014	7.8	15.6
pentacosane	2653	0.0145	7.1	14.2
hexacosane	2292	0.015	6.4	12.8
heptacosane	1860	0.0155	5.3	10.6
octocosane	1272	0.016	3.8	7.6
nonacosane	823	0.016	2.4	4.8
triacontane				
untriacontane				
dotriacontane				

Hydrocarbon Analysis

Sample number: 701

Compound	area units	rf	μg/ml	μg/g dry wt.	
HMB	408	0.014			
undecane	2866	0.011	7.0	14.0	
dodecane	3297	0.011	8.1	16.2	
tridecane	3611	0.012	9.7	19.4	
tetradecane	3466	0.012	9.3	18.6	
pentadecane	3546	0.013	10.3	20.6	
hexadecane	3169	0.012	8.5	17.0	
heptadecane	3012	0.014	9.4	18.8	→ .80
pristane	1705	0.02	7.6	15.2	
octadecane	2612	0.014	8.1	16.3	→ .73
phytane	1340	0.02	6.0	12.0	
nonadecane	2526	0.014	7.9	15.8	
eicosane	2299	0.0135	6.9	13.9	
uncosane	2293	0.0135	6.9	13.8	
docosane	2097	0.013	6.1	12.2	
tricosane	1920	0.0135	5.8	11.6	
tetracosane	1759	0.014	5.5	11.0	
pentacosane	1537	0.0145	4.9	9.9	
hexacosane	1317	0.015	4.4	8.8	
heptacosane	934	0.0155	3.2	6.4	
octacosane	587	0.016	2.1	4.2	
nonacosane					
triacontane					
untriacontane					
dotriacontane					

Hydrocarbon Analysis

Sample number: 701
8 month exposure

Compound	area units	rf	ng/µl	µg/g dry wt.
HMB	532338	1×10^{-4}		
naphthalene	109651	5×10^{-5}	2.6	2.6
methyl naphthalene	216805	1×10^{-4}	10.3	10.3
	115060		5.5	5.5
dimethyl naphthalene	206127	1×10^{-4}	9.8	9.8
	242979		11.6	11.6
	76958		3.7	3.7
	7763		0.4	0.4
trimethyl naphthalene	16580	1×10^{-4}	0.8	0.8
	15730		0.7	0.7
	106211		5.1	5.1
	66331		3.2	3.2
	29703		1.4	1.4
phenanthrene	21275	2×10^{-4}	2.0	2.0
dibenzothiophene	13099	2×10^{-4}	1.2	1.2
methyl phenanthrene	13008	4×10^{-4}	2.5	2.5
	13651		2.6	2.6
methyl dibenzothiophene	11764	2×10^{-4}	1.1	1.1
	5354		0.5	0.5
	2319		0.2	0.2
dimethyl phenanthrene	285	4×10^{-4}	0.1	0.1
	15198		2.9	2.9
dimethyl dibenzothiophene	5660	2×10^{-4}	0.5	0.5
	6447		0.6	0.6
	2960		0.3	0.3
	1582		0.2	0.2
trimethyl phenanthrene	794	4×10^{-4}	0.1	0.1
	49[illegible]		0.9	0.9
	[illegible]		0.1	0.1
trimethyl dibenzothiophene	1651	2×10^{-4}	0.2	0.2
	2268		0.2	0.2
	2380		0.2	0.2
	1182		0.1	0.1

Hydrocarbon Analysis

Sample number: 702

1 year exposure

Compound	area units	rf	ng/µl	µg/g dry wt.	
HMB	3210	0.014			
undecane	402	0.011	1.2	2.4	
dodecane	618	0.011	1.9	3.8	
tridecane	752	0.012	2.5	5.0	
tetradecane	798	0.012	2.6	5.3	
pentadecane	850	0.013	3.0	6.1	
hexadecane	773	0.013	2.8	5.6	
heptadecane	735	0.014	2.8	5.7	→ 1.19
pristane	608	0.02	3.4	6.8	
octadecane	619	0.014	2.4	4.8	→ 1.18
phytane	511	0.02	2.8	5.7	
nonadecane	548	0.014	3.0	6.1	
eicosane	480	0.0135	1.8	3.6	
uncosane	490	0.0135	1.8	3.7	
docosane	410	0.013	1.4	2.9	
tricosane		0.0135			
tetracosane		0.014			
pentacosane		0.0145			
hexacosane		0.015			
heptacosane		0.0155			
octacosane		0.016			
nonacosane					
triacontane					
untriacontane					
dotriacontane					

Hydrocarbon Analysis

Sample number: 703

1 year control

Compound	area units	rf	ng/ul	µg/g dry wt.
HMB	2916	0.014		
undecane				
dodecane				
tridecane				
tetradecane				
pentadecane				
hexadecane				
heptadecane				
pristane				
octadecane				
phytane				
nonadecane				
eicosane				
uncosane				
docosane				
tricosane				
tetracosane				
pentacosane				
hexacosane				
heptacosane				
octacosane				
nonacosane				
triacontane				
untriacontane				
dotriacontane				

Hydrocarbon Analysis

Sample number: Con 3 DW Control

Compound	area units	rf	ng/ul	ug/g dry wt.
HMB	2824	0.014		
undecane				
dodecane				
tridecane				
tetradecane				
pentadecane				
hexadecane				
heptadecane				
pristane				
octadecane				
phytane				
nonadecane				
eicosane				
uncosane				
docosane				
tricosane				
tetracosane				
pentacosane				
hexacosane				
heptacosane				
octocosane				
nonacosane				
triacontane				
untriacontane				
dotriacontane				

Hydrocarbon Analysis

Sample number: 802 ST
1 1/4 year

Compound	area units	rf	–	µg/g dry wt.
HMB	4244	0.014		
undecane	2314	0.011	5.4	10.8
dodecane	4626	0.011	10.8	21.6
tridecane	6186	0.012	15.8	31.6
tetradecane	5859	0.012	15.0	30.0
pentadecane	6930	0.013	19.2	38.4
hexadecane	6792	0.012	17.3	34.6
heptadecane	5651	0.014	16.8	33.6
pristane	3510	0.02	15.0	30.0
octadecane	5528	0.014	16.5	33.0
phytane	2642	0.02	11.2	22.4
nonadecane	5043	0.014	15.0	30.0
eicosane	4831	0.0135	13.9	27.8
uncosane	4791	0.0135	13.8	27.6
docosane	4548	0.013	12.6	25.2
tricosane	4219	0.0135	12.1	24.2
tetracosane	3909	0.014	11.6	23.2
pentacosane	3375	0.0145	10.4	20.8
hexacosane	2967	0.015	9.5	19.0
heptacosane	2432	0.0155	8.0	16.0
octacosane	1655	0.016	5.6	11.2
nonacosane	1261	0.016	4.3	8.6
triacontane				
untriacontane				
dotriacontane				

→ .89

→ .68

Hydrocarbon Analysis

Sample number: *802 ST*

1 1/4 year exposure

Compound	area units	rf	*ng/ul*	*µ*g/g dry wt.
HMB	547742	2×10^{-4}		
naphthalene	3782	2×10^{-4}	0.2	0.2
methyl naphthalene	10042	2×10^{-4}	0.5	0.5
	6724		0.3	0.3
dimethyl naphthalene	148902	3.5×10^{-4}	12.1	12.1
	26047		2.1	2.1
trimethyl naphthalene	12734	3×10^{-4}	0.9	0.9
	100255		7.0	7.0
	67191		4.7	4.7
	37392		2.6	2.6
phenanthrene	26962	6×10^{-4}	3.8	3.8
dibenzothiophene	22509	6×10^{-4}	3.1	3.1
methyl phenanthrene	36854	1.2×10^{-3}	10.3	10.3
	36360		10.1	10.1
Methyl dibenzothiophene	37328	6×10^{-4}	5.2	5.2
	14984		2.1	2.1
	3528		0.5	0.5
dimethyl phenanthrene	54374	1.2×10^{-3}	15.2	15.2
dimethyl dibenzothiophene	21904	6×10^{-4}	3.1	3.1
	19260		2.7	2.7
	8538		1.2	1.2
	4390		0.6	0.6
trimethyl phenanthrene	21979	1.2×10^{-3}	6.1	6.1
	[illegible]15		0.6	0.6
trimethyl dibenzothiophene	4975	6×10^{-4}	0.7	0.7
	8033		1.1	1.1
	10539		1.5	1.5

Hydrocarbon Analysis

Sample number: 801 Fr_ Th
1 1/2 year exposure

Compound	area units	rf	ng/ul	ug/g dry wt.
HMB	2526	0.014		
undecane	did not integrate			
dodecane	did not integrate			
tridecane	300	0.012	1.3	2.6
tetradecane	230	0.012	1.0	2.0
pentadecane	650	0.013	3.0	6.0
hexadecane	651	0.012	2.8	5.6
heptadecane	394	0.014	2.0	4.0
pristane	314	0.02	2.2	4.4
octadecane	695	0.014	3.5	7.0
phytane	416	0.02	3.0	6.0
nonadecane	719	0.014	3.6	7.2
eicosane	702	0.0135	3.4	6.8
uncosane	778	0.0135	3.8	7.6
docosane	693	0.013	3.2	6.4
tricosane	637	0.0135	3.1	6.2
tetracosane	519	0.014	2.6	5.2
pentacosane	462	0.0145	2.4	4.8
hexacosane	320	0.015	1.7	3.4
heptacosane				
octacosane				
nonacosane				
triacontane				
untriacontane				
dotriacontane				

→ 1.10

→ .86

Hydrocarbon Analysis

Sample number: 801 LT
1 1/2 year exposure

Compound	area units	rf	ng/ul	µg/g dry wt.	
HMB	3038	0.014			
undecane	397	0.011	1.3	2.6	
dodecane	768	0.011	2.5	5.0	
tridecane	1006	0.012	3.6	7.2	
tetradecane	853	0.012	3.0	6.0	
pentadecane	1152	0.013	4.4	8.8	
hexadecane	1133	0.012	4.0	8.0	
heptadecane	782	0.014	3.2	6.4	→ 1.28
pristane	690	0.02	4.1	8.2	
octadecane	1041	0.014	4.3	8.6	
phytane	758	0.02	4.5	9.0	→ 1.05
nonadecane	1013	0.014	4.2	8.4	
eicosane	987	0.0135	3.9	7.8	
uncosane	996	0.0135	4.0	8.0	
docosane	896	0.013	3.4	6.8	
tricosane	782	0.0135	3.1	6.2	
tetracosane	668	0.014	2.8	5.6	
pentacosane	558	0.0145	2.4	4.8	
hexacosane	449	0.015	2.0	4.0	
heptacosane	286	0.0155	1.3	2.6	
octacosane					
nonacosane					
triacontane					
untriacontane					
dotriacontane					

Hydrocarbon Analysis

Sample number: 801 LT *

1 1/2 year exposure

Compound	area units	rf	ng/ul	ug/g dry wt.
HMB	3367	0.014		
undecane	855	0.011	2.5	5.0
dodecane	1456	0.011	4.3	8.6
tridecane	1991	0.012	6.5	13.0
tetradecane	1801	0.012	5.8	11.6
pentadecane	2159	0.013	7.6	15.2
hexadecane	2090	0.012	6.8	13.6
heptadecane	1711	0.014	6.5	13.0 → 1.09
pristane	1319	0.02	7.1	14.2
octadecane	1896	0.014	7.2	14.4 → .92
phytane	1225	0.02	6.6	13.2
nonadecane	1757	0.014	6.6	13.2
eicosane	1749	0.0135	6.4	12.8
uncosane	1800	0.0135	6.6	13.2
docosane	1655	0.013	5.8	11.6
tricosane	1474	0.0135	5.4	10.8
tetracosane	1333	0.014	5.0	10.0
pentacosane	1174	0.0145	4.6	9.2
hexacosane	984	0.015	4.0	8.0
heptacosane	730	0.0155	3.1	6.2
octacosane	359	0.016	1.6	3.2
nonacosane				
triacontane				
untriacontane				
dotriacontane				

Hydrocarbon Analysis

Sample number: 801 LT *

1 1/2 year exposure

Compound	area units	rf	ng/µl	µg/g dry wt.
HMB	529836	2×10^{-4}		
naphthalene	38956	2×10^{-4}	1.9	1.9
methyl naphthalene	90741	2×10^{-4}	4.3	4.3
	50697		2.4	2.4
dimethyl naphthalene	105954	3.5×10^{-4}	8.8	8.8
	124397		10.4	10.4
	39509		3.3	3.3
	4364		0.4	0.4
trimethyl naphthalene	5626	3×10^{-4}	0.4	0.4
	9907		0.7	0.7
	62259		4.4	4.4
	43975		3.1	3.1
	23073		1.6	1.6
phenanthrene	14239	6×10^{-4}	2.0	2.0
dibenzothiophene	10680	6×10^{-4}	1.5	1.5
methyl phenanthrene	11580	1.2×10^{-3}	3.3	3.3
	13490		3.9	3.9
methyl dibenzothiophene	12081	6×10^{-4}	1.7	1.7
	5699		0.8	0.8
	1646		0.2	0.2
dimethyl phenanthrene	20656	1.2×10^{-3}	5.9	5.9
dimethyl dibenzothiophene	7249	6×10^{-4}	1.0	1.0
	7083		1.0	1.0
	2716		0.4	0.4
trimethyl phenanthrene	252	1.2×10^{-3}	0.1	0.1
	10784		3.1	3.1
	1255		0.4	0.4
trimethyl dibenzothiophene	1517	6×10^{-4}	0.2	0.2
	2454		0.4	0.4
	2161		0.3	0.3
	1324		0.2	0.2

Hydrocarbon Analysis

Sample number: Large Tray frozen 2 year exposure

Compound	area units	rf	ng/ul	μg/g dry wt.
HMB	2354	0.014		
undecane	did not integ.	0.011		
dodecane	310	0.011	1.3	2.6
tridecane	321	0.012	2.4	4.8
tetradecane	223	0.012	1.0	2.0
pentadecane	568	0.013	2.8	5.6
hexadecane	478	0.012	2.1	4.3
heptadecane	267	0.014	1.4	2.8 → 1.75
pristane	323	0.02	2.4	4.9
octadecane	684	0.014	3.6	7.3 → 1.39
phytane	672	0.02	5.1	10.2
nonadecane	771	0.014	4.1	8.2
eicosane	766	0.0135	3.9	7.9
uncosane	661	0.0135	3.4	6.8
docosane	574	0.013	2.8	5.7
tricosane	518	0.0135	2.6	5.3
tetracosane	429	0.014	2.2	4.4
pentacosane	263	0.0145	1.4	2.9
hexacosane		0.015		
heptacosane		0.0155		
octacosane		0.016		
nonacosane				
triacontane				
untriacontane				
dotriacontane				

Hydrocarbon Analysis

Sample number: Lg. Troz frozen.
2 year exposure

Compound	area units	rf	ng/ul	µg/g dry wt.
HMB	664349	2x10⁻⁴		
naphthalene	25042	2x10⁻⁴	0.9	0.9
methyl naphthalene	77777	2x10⁻⁴	2.9	2.9
	47216		1.8	1.8
dimethyl naphthalene	212569	2.5x10⁻⁴	10.0	10.0
	35743		1.7	1.7
trimethyl naphthalene	9168	2x10⁻⁴	0.3	0.3
	8305		0.3	0.3
	53387		2.0	2.0
	39756		1.5	1.5
	15637		0.6	0.6
phenanthrene	17117	5x10⁻⁴	1.6	1.6
dibenzothiophene	13153	4x10⁻⁴	1.0	1.0
methyl phenanthrene	13649	1.1x10⁻³	2.8	2.8
	13929		2.4	2.4
methyl dibenzothiophene	11352	4x10⁻⁴	0.9	0.9
	5650		0.4	0.4
dimethyl phenanthrene	372	1.1x10⁻³	0.1	0.1
	14552		3.0	3.0
dimethyl dibenzothiophene	6450	4x10⁻⁴	0.5	0.5
	6553		0.5	0.5
	2977		0.2	0.2
	1468		0.1	0.1
trimethyl phenanthrene	1702	1.1x10⁻³	0.4	0.4
trimethyl dibenzothiophene	569	4x10⁻⁴	0.1	0.1
	1670		0.1	0.1
	1601		0.1	0.1
	1080		0.1	0.1
C4 phenanthrene	225	1.1x10⁻³	0.1	0.1

Hydrocarbon Analysis

Sample number: Large Tray 2
2 year exposure

Compound	area units	rf	ng/µl	µg/g dry wt.
HMB	2207	0.014		
undecane	did not integrate			
dodecane	did not integrate			
tridecane	did not integrate			
tetradecane	did not integrate			
pentadecane	did not integrate			
hexadecane	did not integrate			
heptadecane	did not integrate			
pristane	314	0.02	2.7	5.4
octadecane	435	0.014	2.6	5.2
phytane	258	0.02	2.2	4.4
nonadecane	584	0.014	3.6	7.2
eicosane	577	0.0135	3.4	6.8
uncosane	684	0.0135	4.0	8.0
docosane	698	0.013	3.9	7.8
tricosane	623	0.0135	3.7	6.4
tetracosane	570	0.014	3.5	7.0
pentacosane	494	0.0145	3.1	6.2
hexacosane	437	0.015	2.9	5.8
heptacosane	230	0.0155	2.2	4.4
octacosane				
nonacosane				
triacontane				
untriacontane				
dotriacontane				

→ .85

ICE

ANALYSES

Hydrocarbon Analysis

Sample number: oil ice / 1/10 2.3 oil

Compound	area units	rf	ng/µl	µg/g dry wt.
HMB	7914	0.014		
undecane	3119	0.011	3.9	
dodecane	3077	0.011	3.8	
tridecane	3102	0.012	4.2	
tetradecane	2523	0.012	3.4	
pentadecane	2615	0.013	3.7	
hexadecane	2188	0.012	3.0	
heptadecane	2038	0.014	3.2	
pristane	1170	0.02	2.7	
octadecane	1879	0.014	3.0	
phytane	953	0.02	2.2	
nonadecane	1495	0.014	2.4	
eicosane	1603	0.0135	2.5	
uncosane	1548	0.0135	2.4	
docosane	1467	0.013	2.2	
tricosane	1326	0.0135	2.0	
tetracosane	1215	0.014	1.9	
pentacosane	947	0.0145	1.6	
hexacosane	823	0.015	1.4	
heptacosane	482	0.0155	0.8	
octacosane				
nonacosane				
triacontane				
untriacontane				
dotriacontane				

→ .84 (margin, heptadecane/pristane)

→ .73 (margin, octadecane/phytane)

Hydrocarbon Analysis

Sample number: 2.3 oil Oil ice 2 1/10 dil.

Compound	area units	rf	ng/μl	μg/g dry wt.
HMB	6311	0.014		
undecane	3061	0.011	4.8	
dodecane	2945	0.011	4.6	
tridecane	2884	0.012	4.9	
tetradecane	2396	0.012	4.1	
pentadecane	2311	0.013	4.3	
hexadecane	2102	0.012	3.6	
heptadecane	1935	0.014	3.9	→ .79
pristane	1086	0.02	3.1	
octadecane	1745	0.014	3.5	→ .71
phytane	887	0.02	2.5	
nonadecane	1612	0.014	3.2	
eicosane	1554	0.0135	3.0	
uncosane	1479	0.0135	2.9	
docosane	1359	0.013	2.5	
tricosane	1205	0.0135	2.3	
tetracosane	1047	0.014	2.1	
pentacosane	841	0.0145	1.7	
hexacosane	636	0.015	1.4	
heptacosane	358	0.0155	0.8	1.6
octacosane				
nonacosane				
triacontane				
untriacontane				
dotriacontane				

Hydrocarbon Analysis

Sample number: 2.3 ml oil ice 1/10 dil.

Compound	area units	rf	ng/μl	μg/g dry wt.
HMB	7138	0.014		
undecane	5180	0.011	7.2	
dodecane	4842	0.011	6.7	
tridecane	4970	0.012	7.5	
tetradecane	4169	0.012	6.3	
pentadecane	4214	0.013	6.9	
hexadecane	3683	0.012	5.6	
heptadecane	3383	0.014	6.0	→ .87
pristane	2047	0.02	5.2	
octadecane	3065	0.014	5.4	→ .76
phytane	1605	0.02	4.1	
nonadecane	2795	0.014	5.0	
eicosane	2583	0.0135	4.4	
uncosane	2485	0.0135	4.2	
docosane	2242	0.013	3.7	
tricosane	1955	0.0135	3.3	
tetracosane	1682	0.014	3.0	
pentacosane	1309	0.0145	2.4	
hexacosane	1016	0.015	1.9	
heptacosane	630	0.0155	1.2	
octacosane				
nonacosane				
triacontane				
untriacontane				
dotriacontane				

Hydrocarbon Analysis

Sample number: Oil ice 1/5

Compound	area units	rf	ng/μl	
HMB	621558	2×10^{-4}		
naphthalene	150402	2×10^{-4}	4.9	
methyl naphthalene	294227	2×10^{-4}	12.0	
	146799		6.0	
dimethyl naphthalene	256171	2.5×10^{-4}	13.1	
	311651		15.9	
	102842		5.2	
trimethyl naphthalene	10145	2×10^{-4}	0.4	
	20329		0.8	
	136481		5.6	
	101744		4.2	
	61765		2.5	
phenanthrene	79240	4×10^{-4}	6.5	
dibenzothiophene	48754	4×10^{-4}	4.0	
methyl phenanthrene	63092	1×10^{-3}	12.9	
	56563		11.5	
methyl dibenzothiophene	52216	4×10^{-4}	4.3	
	24615		2.0	
	6339		0.5	
dimethyl phenanthrene	1523	1×10^{-3}	0.3	
dimethyl dibenzothiophene	64139		13.1	
	26217	4×10^{-4}	2.1	
	23876		1.9	
	13118		1.1	
	6355		0.5	
trimethyl phenanthrene	33814	1×10^{-3}	6.9	
	3035		0.6	
trimethyl dibenzothiophene	5579	4×10^{-4}	0.5	
	7490		0.6	
	6479		0.5	
	4136		0.3	
	2238		0.2	

Hydrocarbon Analysis

Sample number: uzl 48 hr 1/78

Compound	area units	rf	ng/µl	µg/g dry wt.	
HMB	3409	0.014			
undecane	4135	0.011	12.0		
dodecane	4698	0.011	13.6		
tridecane	5093	0.012	16.1		
tetradecane	4510	0.012	14.2		
pentadecane	4577	0.013	15.7		
hexadecane	4166	0.012	13.1		
heptadecane	3863	0.014	14.2		
pristane	2223	0.02	11.7		→ .82
octadecane	3510	0.014	12.9		
phytane	1767	0.02	9.3		→ .72
nonadecane	3277	0.014	12.1		
eicosane	3125	0.0135	11.1		
uncosane	3123	0.0135	11.1		
docosane	3020	0.013	9.0		
tricosane	2846	0.0135	8.0		
tetracosane	2727	0.014	8.0		
pentacosane	2360	0.0145	9.0		
hexacosane	2073	0.015	8.2		
heptocosane	1581	0.0155	6.4		
octocosane	1056	0.016	4.4		
nonacosane	650	0.016	2.7		
triacontane					
untriacontane					
dotriacontane					

Hydrocarbon Analysis

Sample number: 48 hr. ayl.

Compound	area units	rf	ng/μl
HMB	727929	2×10^{-4}	
naphthalene	225028	1×10^{-4}	3.9
methyl naphthalene	483454	2×10^{-4}	16.7
	236344		8.1
dimethyl naphthalene	447952	2×10^{-4}	15.4
	490353		16.9
	163184		5.6
trimethyl naphthalene	24050	2×10^{-4}	0.8
	45616		1.6
	194925		6.7
	144981		5.0
	69862		2.4
phenanthrene	39705	5×10^{-4}	3.4
dibenzothiophene	30312	4×10^{-4}	2.1
methyl phenanthrene	30159	9×10^{-4}	4.7
	24185		3.8
methyl dibenzothiophene	25116	4×10^{-4}	1.7
	10388		0.7
	4613		0.3
dimethyl phenanthrene	31869	9×10^{-4}	4.9
dimethyl dibenzothiophene	11918	4×10^{-4}	0.8
	11692		0.8
	5967		0.4
	2895		0.2
trimethyl phenanthrene	591	9×10^{-4}	0.1
	13344		2.1
	1629		0.3
trimethyl dibenzothiophene	2906	4×10^{-4}	0.2
	4840		0.3
	3280		0.2
	2094		0.1
	1197		0.1

Hydrocarbon Analysis

Sample number: 7day wl 1/25/78

Compound	area units	rf	ng/μl	μg/g dry wt.
HMB	4546	0.014		
undecane	1530	0.011	3.3	
dodecane	4502	0.011	9.8	
tridecane	7462	0.012	17.7	
tetradecane	7780	0.012	18.4	
pentadecane	9362	0.013	24.0	
hexadecane	8622	0.012	20.4	
heptadecane	7230	0.014	19.8	
pristane	4133	0.02	16.2	
octadecane	6856	0.014	19.0	
phytane	3297	0.02	13.0	
nonadecane	6226	0.014	17.2	
eicosane	5592	0.0135	14.9	
uncosane	4987	0.0135	13.3	
docosane	4274	0.013	10.9	
tricosane	2979	0.0135	7.9	
tetracosane	2451	0.014	6.7	
pentacosane	1758	0.0145	5.0	
hexacosane	1125	0.015	3.3	
heptacosane	678	0.0155	2.0	
octacosane		0.016		
nonacosane				
triacontane				
untriacontane				
dotriacontane				

0.92

→ .68

Hydrocarbon Analysis

Sample number: 7 day cyl.

Compound	area units	rf	ng/μl	
HMB	591369	2×10^{-4}		
naphthalene	118170	2×10^{-4}	5.0	
methyl naphthalene	397583	2×10^{-4}	16.9	
	245078		10.4	
dimethyl naphthalene	587281	2.5×10^{-4}	31.2	
	610534		32.5	
	133910		7.1	
trimethyl naphthalene	45266	2×10^{-4}	1.9	
	42852		1.8	
	290065		12.3	
	180943		7.7	
	76284		3.2	
phenanthrene	47973	4×10^{-4}	4.1	
dibenzothiophene	39141	4×10^{-4}	3.3	
methyl phenanthrene	36327	1×10^{-3}	7.7	
	28896		6.1	
methyl dibenzothiophene	29919	4×10^{-4}	2.5	
	13172		1.1	
	4014		0.3	
dimethyl phenanthrene	782	1×10^{-3}	0.2	
	31804		6.8	
dimethyl dibenzothiophene	15326	4×10^{-4}	1.3	
	13875		1.2	
	6484		0.6	
	3230		0.3	
trimethyl phenanthrene	18754	1×10^{-3}	4.0	
	1469		0.3	
trimethyl dibenzothiophene	3826	4×10^{-4}	0.3	
	3535		0.4	
	2083		0.3	
	4162		0.2	

Hydrocarbon Analysis

Sample number: 7 day oil ugl 1/25/78

Compound	area units	rf	ng/µl	g/g dry wt.
HMB	4122	0.014		
undecane	1469	0.011	3.5	
dodecane	3897	0.011	9.3	
tridecane	6428	0.012	16.8	
tetradecane	6888	0.012	18.0	
pentadecane	7428	0.013	21.0	
hexadecane	7170	0.012	18.7	
heptadecane	6412	0.014	19.6	
pristane	3677	0.02	16.0	
octadecane	5928	0.014	18.1	
phytane	2964	0.02	12.9	
nonadecane	5579	0.014	17.0	
eicosane	5394	0.0135	15.8	
uncosane	5384	0.0135	15.8	
docosane	5122	0.013	14.5	
tricosane	4819	0.0135	14.2	
tetracosane	4490	0.014	13.7	
pentacosane	3888	0.0145	12.3	
hexacosane	3416	0.015	11.1	
heptacosane	2689	0.0155	9.1	
octacosane	1886	0.016	6.5	
nonacosane	1389	0.016	4.8	
triacontane	683	0.016	2.3	
untriacontane		0.016		
dotriacontane				

Hydrocarbon Analysis

Sample number: 7 day oyl

Compound	area units	rf	ng/µl	
HMB	776353	2×10^{-4}		
naphthalene	117311	1×10^{-4}	1.9	
methyl naphthalene	434144	2×10^{-4}	14.0	
	258164		8.3	
dimethyl naphthalene	593391	2×10^{-4}	19.1	
	720655		23.2	
	243320		7.8	
trimethyl naphthalene	65825	2×10^{-4}	2.1	
	359484		11.6	
	236747		7.6	
	91770		3.0	
phenanthrene	104911	5×10^{-4}	8.5	
dibenzothiophene	84237	4×10^{-4}	5.4	
methyl phenanthrene	78025	9×10^{-4}	11.3	
	72415		10.5	
methyl dibenzothiophene	83648	4×10^{-4}	5.4	
	32289		2.1	
	5120		0.3	
dimethyl phenanthrene	89411	9×10^{-4}	13.0	
dimethyl dibenzothiophene	34528	4×10^{-4}	2.2	
	34112		2.2	
	16673		1.1	
	7795		0.5	
trimethyl phenanthrene	1122	9×10^{-4}	0.2	
	40167		5.8	
	3661		0.5	
trimethyl dibenzothiophene	6463	4×10^{-4}	0.4	
	12532		0.8	
	13748		0.9	

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Study of Microbial Activity and Crude Oil-Microbial Interactions in the Waters and Sediments of Cook Inlet and the Beaufort Sea

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SUMMARY STATEMENT

A. Overview

This annual report was designed to provide a summary and synthesis of all of the studies that we have conducted for OCSEAP since RU #190 was established in the spring of 1975. This report includes four major sections under the headings of "Cook Inlet", "Beaufort Sea", "Norton Sound", and "Effects". These sections are tied together by the appropriate cross-references but they were each designed to be complete statements of our work in each area. We have organized this report in this way so that those interested in just one area will be able to find all of the relevant information for that area within the appropriate section.

We understand that people with greatly varying scientific backgrounds may have use for the information contained in this report. With this in mind, we have attempted to explain our results and conclusions using nontechnicial terms whenever possible. In order to aid in the understanding of the text, we have included a glossary of terms at the end of this report which we hope will be of help to those unfamiliar with some of the terms used.

We strongly urge all of those using this report to read parts B.1. and B.2. of the "Discussion" in Section IV. In this section, we have summarized the role of bacteria in the marine environment and the importance of microbial function in the overall productivity of marine ecosystems. Later in the same section, we describe the effects of crude oil on microbial function as it effects productivity in the marine environment.

B. General objectives of our studies

In each geographic area that we have studied, we have measured various microbial functions related to the overall productivity of the respective ecosystems. The types of variables that we have studied are outlined in detail in the main body of this report and will not be discussed in this section. In addition to collecting these baseline data, we have studied both long and short-term effects of crude oil and the dispersant Corexit 9527 on these and other functions. The results of these studies are presented in Section IV.

C. Relevance of this study to management decisions

Whenever possible, we have tried to relate our findings to management decisions concerning the production and transport of crude oil. Most of the statements are presented in part I of each major section. We have also outlined the potential adverse effects of crude oil on the overall productivity of Alaskan marine ecosystems in part 7 of the "Discussion" in Section IV.

D. Summary of major findings

We have found that crude oil alters microbial function in marine sediments. This altered function will have three major impacts on normal biological activity. (1) It will reduce overall productivity by interfering with the normal flow of food through the detrital food chain. Recent estimates show that 50-80% of food available to all animals present is ultimately derived from this source. (2) Crude oil will interfere with the process that converts the nitrogen and phosphorous that is tied up in organic material into inorganic nitrogen and phosphorous that is required for plant growth. Without these inorganic nutrients, plants can not produce the new organic material required to feed the

animals present. (3) Crude oil changes microbial activity in the sediments so that the chemical environment of the sediment surface is changed. It seems quite likely that these changes will remain long after the initial crude oil toxicity has abated. This will greatly alter the normal recruitment of animals back into the impacted area.

Of the areas that we have studied, the inshore regions of the Beaufort Sea (especially near major rivers) and the major bays of Cook Inlet would be the areas where crude oil perturbation would have the most significant effect. Our observations also suggest that the duration of impact would be much longer in the Beaufort Sea than in other Alaskan waters that we have studied.

Our studies also indicate that greatest impact would be found in areas where crude oil became incorporated into fine grained marine sediments. With this in mind, we have recommended that any procedures be avoided that would allow the incorporation of crude oil into these sediments. This would be of particular importance in inshore sediments where the microbial activity is unusually high or in areas where most of the inorganic nutrients required for algae growth is generated locally by bacterial activity.

E. <u>Recommendation for future study</u>

In the Bering Sea, we recommended that more studies of microbial function be conducted during cruises in this region. This area of particular importance because of the transition from Arctic to sub-Arctic environments found in this body of water. From the small amount of microbiological data that is currently available from this area, it is evident that different processes may be taking place in these two environments.

The Bering Sea studies should also include measurements of methane oxidation and methane production so that transport mechanisms in areas such as the North Aleutian Shelf and St. George's Basin can be more clearly defined.

We also feel that multidisciplinary studies of crude oil effects be continued both at the Kasitsna Bay laboratory and in the Beaufort Sea. Emphasis should be placed on food web interactions and inorganic nutrient cycling. Crude oil effects studies should also be conducted on Bering Sea sediments that are incubated *in situ* but are analyzed at the Kasitsna Bay facility.

A more detailed description of proposed studies are listed under part VI of each section.

F. Acknowledgements

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Section I

I. Summary of objectives, conclusions, and implications with respect to OCS oil and gas development.

A. Objectives

The first three cruises (October, 1976, April, 1977 and November 1977) were designed to produce baseline data on the rates at which microorganisms utilize and mineralize organic nutrients and the rates at which they fix atmospheric nitrogen. During the November, 1977; April, 1978 and April, 1979 cruises, we were to make the above mentioned observations in addition to measuring the short-term (acute) effects of Cook Inlet crude oil on these processes. These observations were to be made in coordination with hydrocarbon chemists to determine if microbial populations adjusted to the presence of crude oil (April, 1978 and 1979). These investigations were coordinated with the studies conducted by another group of microbiologists (Atlas RU #29).

In addition to these measurements, we were to collect data on the abiotic variables of salinity, temperature and the biotic variables of inorganic nutrient concentrations of NH_4, NO_3, and PO_4. These latter variables are mainly microbially controlled.

Our studies were designed to determine when and where the impact of crude oil would be the greatest in Cook Inlet. Our conclusions based on the observations made during these five cruises and other information collected during the existence of RU #190 are listed below.

B. Conclusions and Implications

During the course of our studies, we have determined that crude oil and the dispersant Corexit 9527 have adverse short and long-term effects on microbial processes in both Arctic and sub-Arctic waters and sediments of the Alaskan Continental Shelf. The net result of this impact is the reduced overall productivity in the perturbed region. These results are presented and analyzed in Section IV of this report. What we will present in this section is a summary of our recommendations relative to gas and oil development in Cook Inlet using all of the information available to us from all of our OCSEAP studies to date. In this discussion, we have also included potential impacts to Shelikof Strait which is essentially an extension of Cook Inlet.

1. Oil spilled in the Upper Cook Inlet would become associated with the large concentration of suspended matter in these waters. The crude oil would then be transported into the sediments of Kamishak Bay and the Shelikof Strait.

2. Oil spilled in the Lower Cook Inlet would most probably drift into Kamishak Bay where it could become incorporated with the sediments of that region. If crude oil became dispersed throughout the water column, it could migrate into the sediments of Shelikof Strait.

3. Oil spilled in Kachemak Bay would have a significant impact on the overall productivity of that region; i.e., a significant reduction in commercially valuable species along with all other organisms. Of all areas within and near Cook Inlet, this is probably the area that is the most susceptible to crude oil perturbation.

4. Our studies have shown that microorganisms within the water column are initially stressed by the presence of either crude oil or the dispersant Corexit 9527. This perturbation would not have a significant impact on the overall productivity of the perturbed area except under certain conditions, i.e., where crude oil remains in contact with the water column for an extended period of time or where crude oil becomes associated with suspended matter in the water column.

5. The major impact would undoubtedly occur in cases where the oil became incorporated into the sediments. We strongly recommend any procedures which would prevent allowing crude oil to become incorporated into the sediments. Conversely, any procedures used in crude oil production and transport or in crude oil spill control which increases the chances of crude oil becoming incorporated into the sediments should be avoided.

6. The presence of crude oil in Alaskan marine sediments will adversely affect the overall productivity of the impacted region. This occurs in several ways. We have shown that crude oil reduces the rate at which bacteria mineralize organic nutrients by over 50% under certain conditions. This reduces the rate at which inorganic nutrients (NH_4, NO_4, NO_3, and PO_4) are released from organic matter. This could, in turn, reduce primary productivity rates. It also greatly reduces the rate at which atmospheric nitrogen is fixed in a form that can be used by all organisms. In addition, crude oil

appears to interfere with the transfer of organic nutrients from bacteria to the balance of the detrital food chain.

7. In our judgement, the areas where crude oil would have the greatest impact would be in Kachemak Bay. Impacts on Kamishak Bay and Shelikof Strait would be of lesser but still significant importance.

8. Since the impact of crude oil in marine sediments probably remains for several years, the season during which a spill occurs is probably of little direct importance to the severity of the impact. There could be secondary effects such as the frequency of storms which would tend to drive crude oil into the sediments.

II. Study areas

We had one cruise in the North East Gulf of Alaska (NEGOA). The stations at which samples were collected are shown in Fig. 1. During the course of our Cook Inlet studies, we have participated in five major cruises; (October, 1976, April, 1977, November, 1977, April, 1978 and April, 1979). The stations at which samples were collected are illustrated in Figures 2-8. During the first cruise, 37 water and 12 sediment samples were collected; during the second, 44 water and 12 sediment samples were collected; during the third, 60 water and 20 sediment samples were collected; during the fourth cruise, 83 water and 30 sediment samples were collected; and during the last cruise, 49 water and 14 sediments were collected.

III. Methods

A. Sampling procedures

The water samples were taken in sterile Niskin plastic water sample bags fitted on Niskin "butterfly" water samplers. In most cases, water samples were taken within one meter of the surface. Once the water sample was taken, it was placed in an ice chest for storage and transported back to the laboratory for analysis. The analyses of microbial activity were initiated within two hours after sampling was terminated. During transport and storage, the samples were kept at or below the *in situ* temperature.

The majority of the sediment samples in the earlier cruises were taken with a Sutar-Van Veen bottom grab with some samples taken with a Shipek grab in coarse sediments. During the last two cruises, most of the sediments were taken from a one m^2 box corer. When practical, the top 2 cm were collected for use. The sediments that were collected from a small boat near shore, were sampled using a Kahl mud snapper. Water associated with the sample was used to make a sediment slurry for use in the subsequent analysis.

B. Relative microbial activity and percent repiration (mineralization) determinations.

The procedure used in these studies involved adding a U-^{14}C compound to replicate subsamples which were contained in 50 ml serum bottles.

After addition of subsamples, the 50 ml serum bottles that were used for reaction vessels were sealed with rubber serum bottle

caps fitted with plastic rod and cup assemblies (Kontes Glass Co., Vineland, M. J: K-882320) containing 25 x 50 mm strips of fluted Whatman #1 chromatography paper. The samples were incubated in the dark within 0.5 C of the *in situ* temperature. After the incubation period, the bottles were injected through the septum with 0.2 ml of 5N H_2SO_4 in order to stop the reaction and release the $^{14}CO_2$. After the addition of the acid, 0.15 ml of the CO_2 adsorbent, β-phenethylamine, was injected onto the filter paper. The serum bottles were then shaken on a rotary shaker at 200 rpm for at least 45 minutes at room temperature to facilitate the adsorption of CO_2. The filter papers containing the $^{14}CO_2$ were removed from the cup assemblies and added to scintillation vials containing 10 ml of toluene based scintillation fluor (Omifluor, New England Nuclear).

The subsamples were filtered through a 0.45 μm membrane filter (Millipore). The trapped cells on the filter were washed with three 10 ml portions of seawater at 0-3 C. The filters were dried and then added to scintillation vials containing 10 ml of the above mentioned fluor. The vials were counted in a Beckman model LS-100 liquid scintillation counter located in our laboratory at Oregon State University.

In the sediment samples, a 10.0 ml subsample was diluted 1,000 times (v/v) with a 32 o/oo (w/v) solution of sterile artificial seawater. Ten ml subsamples of the sediment slurry were dried and weighed to determine the dry weights. These dry weights were used to calculate the observed uptake rates in terms of grams dry weight of sediment.

During these studies, C^{14} labeled glucose with specific activity of approximately 300 mCi/mM and a final concentration of about 4 μg/liter

was used in sediment samples and glutamic acid with a specific activity of about 230 mCi/mM and a final concentration of about 5.5 μg/liter was used in water samples.

Triplicate subsamples were analyzed for each sample. The channels ratio method for determining counting efficiencies was used. The observed CPM was converted to DPM before the mean value was calculated. The percent respiration (mineralization) was calculated by dividing the amount of labeled carbon taken up by the cells (both cell and CO_2 radioactivity) and multiplying this ratio by 100. All samples were incubated in the dark at a temperature within 0.5 C of the *in situ* temperature.

C. Heterotrophic potential studies

The technique used in these studies were basically those of Hobbie and Crawford (1969) as further modified by Harrison, Wright, and Morita (1971). This procedure involves the addition of different concentrations of U-^{14}C labeled substrate to identical subsamples. The procedures used to process the samples was identical to that described above. The only difference in these two methods is in the number of substrate concentrations used. In the first method, only one substrate concentration is used, in this method, four concentrations were used for each sample. Comparison of these two techniques were made by us (Griffiths et al., 1977) and it was concluded that both could be used to measure relative microbial activity.

The following equation was used to calculate the kinetic parameters:

$$\frac{Cut}{c} = \frac{K_t + S_n}{V_{max}} + \frac{A}{V_{max}}$$

where c = radioactivity assimulated plus that respired as $^{14}CO_2$ by the heterotrophic population in disintegrations/min; S_n = the natural substrate heterotrophic population in disintegrations/min; S_n = the natural substrate concentration in μg/liter; A = the added substrate in μg/liter; C = 2.2 x 10^6 μCi of ^{14}C; u = amount of ^{14}C labeled substrate added/sample bottle in μCi; t = incubation time in hours; V_{max} = the maximum velocity of uptake in μg x liter $^{-1}$ x h^{-1}; and K_t = the transport constant in mg/liter. From this equation can also be calculated the time (T_t) in hours required by the natural microbial population to utilize the natural substrate in seawater sample. For the derivation of this equation and the assumptions on which it is based, see Wright and Hobbie (1966). Saturation curves were converted to the best fitting straight line using least squares and modified Lineweaver-Burk equation.

D. Direct Cell Counts

Ten ml of seawater was fixed in the field laboratory by adding it to 1.0 ml of membrane (0.45 μm) filtered formaldehyde (37%). The vials containing the fixed water samples were sealed and stored until they could be counted in our laboratory at Oregon State University. In the sediment studies, the final dilution of the sediments in the heterotrophic potential studies was used and treated the same as the seawater samples.

From 5 to 17 ml of sample were filtered through a 0.2 μm Nuclepore filter. When a relatively high number of organisms was present, the samples were diluted with membrane filtered artificial seawater. The number of organisms per field was kept within acceptable limits and the volume filtered was kept above 5 ml. Controls were

run using filtered artificial seawater with all of the reagents used in the staining and mounting procedure. These counts were not more than 5% of the those found in the samples and were considered insignificant.

The staining procedure used was that of Zimmermann and Meyer-Reil (1974). This procedure involves staining the cells trapped on the membrane filter with acridine orange and then destaining with iso-propyl alcohol. The membranes were dried and mounts on microscopic slides with a mounting medium of cinnamaldehyde and eugenol (2:1).

The bacterial cells were counted using a Zeiss IV Fl epi-fluorescence condenser microscope with filters KP 500, KP 490, FT 150, and LB 520. The eyepiece was used KpT W 12.5 x and the objective was plan 100 x. Approximately 50 restriction fields were counted per sample. Representative fields were counted from the center of the membrane filter to the outside edge of the filtration circle.

Only bodies with distinct fluorescence (either orange or green), clear outline and recognizable bacterial shape were counted as being bacterial cells.

E. Nitrogen fixation in sediments

Nitrogen fixation in the sediments was determined in the field by using the acetylene reduction method (Stewart et al, 1967). Ten ml subsamples of sediment were added to respective 50 ml serum bottles: one control and two duplicate samples were used for each analysis. After the bottles were sealed with a rubber stopper, the

samples were gassed for one min with helium at a flow rate of 10 cc/sec. Ten ml of acetylene was then added to each bottle and the bottles were allowed to incubate for 24 hr before incubation was terminated with one ml of saturated $HgCl_2$ solution. The controls were treated in the same way before incubation and were used to determine the amount of ethylene that was released abiotically. After the incubation was terminated, the tops of the rubber stoppers were sealed with silicone cement. The bottles were kept at or below 4 C until they could be assayed for ethylene in our laboratory at Oregon State University. The analysis for ethylene was made on a Hewlett Packard model 5830A gas chromatograph. The column used was 1.9 meter of 1/8" stainless steel tubing packed with Porapak R (80-100 mesh) and the column temperature was 40 C. The carrier gas was nitrogen flowing at a rate of 29 cc/min. The resulting levels of ethylene were normalized using incubation times and gram dry weight conversions. All rates were calculated in terms of nmoles nitrogen fixed per gram dry weight of sediment per hour. A factor of 0.33 was used to convert the amount of ethylene produced to the theoretical amount of nitrogen fixed.

F. Nutrient analysis

1. Water sample nutrients

a. Frozen samples were thawed in a warm water bath and then aspirated into a four channel Technicon Autoanalyzer system. The samples were subdivided with a stream divider into four sample flows

which were used to analyze ammonia, phosphate, nitrate and nitrite concentrations.

b. The total concentrations of nitrate and nitrite were determined following the procedures of Callaway et al. (1972). The following modifications were made to this procedure: sample, 0.8 cc/min; DDW diluture, 1.2 cc/min; ammonium chloride, 1.0 cc/min; sulfanilamide, 0.1 cc/min; N-1-naphylethylene 0.1 cc/min. The debubbler before the cadmium column is pumped out of the system (1.0 cc/min) with the remaining water forced through the cadmium column.

c. The nitrite concentration was determined using the same chemistry as the above analysis except there is no ammonium chloride, cadmium column, DDW diluter, or first air bubble. A 2.3 cm cc/min sample tube was used.

d. The phosphate concentration determinations were made using the method of Calloway et al. (1972) without modification.

e. The ammonium ion concentration was made using the technique of Head (1971).

2. Sediment samples

a. Sediment samples were thawed in a warm water bath, mixed and then centrifuged for 30 min at 0°C and 8000 x g.

b. Five to fifteen ml of the supernatant was removed and used in the nutrient analysis. Approximately 20 ml of the diluted sediment water was placed into quartz tubes with 0.3 ml of H_2O_2. These samples were then treated with UV light for 4 hours.

c. Soluble oxidizable nitrogen was determined as nitrate on the Auto-analyzer. The remainder of the diluted sediment water was diluted further for the ammonia determination.

d. When H_2S was present, approximately 0.15 ml of a 2% $CuSO_4$ solution was added to remove sulfide ions from solution which would interfere with the nutrient assays.

e. The total carbon content of the sediment was determined by the following procedure. A subsample of the sediment was treated with HCl to remove all traces of inorganic carbon. The sediment was centrifuged and the supernatant removed. The sediment was dried and combusted using the technique of Pella and Columbo (1973).

G. Statistical analysis

The correlation coefficients used in this report were computed using the following equation:

$$r = \frac{N\Sigma XY - (\Sigma X)\ (\Sigma Y)}{[N\Sigma X^2 - (\Sigma X)^2] - [(N\Sigma Y^2 - (Y)^2]}$$

The significance of differences between mean values were made using Student's "t" test. A critical value of 0.05 was used in these determinations. Whenever it is stated that there was a "significant" difference between two mean values, the difference fulfills the above conditions.

IV. Results

A. Gulf of Alaska (NEGOA)

In March, 1976, we participated in an oceanographic cruise on board the NOAA ship Discoverer. Twenty-seven water and twenty sediment samples were taken at the stations shown in Figure 1. The kinetics of glucose and glutamic acid uptake were measured along with percent respiration and bacterial concentrations in both water and sediment samples (Table 1). From the kinetic data we were able to calculate the maximum potential rate of substrate uptake (V_{max}) which, in turn, can be used as an index of relative microbial activity. In the water samples, the mean V_{max} value was 1.4 ng x l^{-1} x h^{-1} which was approximately 1/2 of the mean value observed one month later in Beaufort Sea waters (Table 1) and much lower than that observed in Beaufort summer water samples. The mean V_{max} value observed in the sediments however was much higher than any V_{max} means that were observed in Beaufort Sea or in the Cook Inlet. This unusually high activity was not however reflected in an unusually high bacterial concentration. In fact, the mean bacterial concentration in NEGOA was only 50% higher than that observed in the winter Beaufort Sea sediments even though the relative microbial activity was almost 100 times as high.

There were no significant geographical patterns observed for relative microbial activity in the NEGOA water samples; however, there was a pattern of relative microbial activity in the sediments that corresponded to areas of high concentrations of detritus feeding benthic organisms (H. Feder, personal communication).

B. Cook Inlet

Between October, 1976 and April, 1979, we participated in five major cruises in the Cook Inlet. The first three taken in October, 1976, April, 1977 and November, 1977 were designed primarily as a means of collecting baseline information on microbial function in this area. The remaining two cruises in April, 1978 and 1979 were designed as coordinated study to be conducted with chemical oceanographers.

1. Relative microbial activity and respiration percentages in water.

a. Relative microbial activity was on the average lower in the waters collected during the November, 1977 cruise and higher in the April, 1979 waters than samples collected during any of the other cruises (Table 2). Both glucose and glutamic acid uptake rates (relative microbial activity) were significantly higher in water samples collected in April, 1979 than at any other time ($p<0.05$). The differences between the mean relative microbial activity values observed during the November, 1977 cruise and the April, 1978 and 1979 cruises were statistically significant but the differences between the mean observed during the November, 1977 cruise and the other two cruises were not statistically significant. The ratio of glucose to glutamic acid uptake was highest in the April, 1978 and 1979 water samples. The differences seen in the mean percent respiration ratios were not statistically significant.

b. A series of experiments were conducted which were designed to determine if the waters near the beach (in the surf zone) showed higher relative microbial activities than those observed further

off-shore (10 meters off-shore from the surf zone). In every case where this was measured in Cook Inlet and in the Gulf of Alaska, the relative microbial activity was highest in the surf zone.

c. With the exception of the April, 1979 cruise, patterns of relative microbial activity were similar in all Cook Inlet cruises. These patterns reflect differences in the microbial communities associated with the major water masses in this region. These water masses are roughly defined by surface water salinities. These patterns are best illustrated in the data collected during the April and November, 1977 cruises (Fig. 9). There are essentially two major water masses in Cook Inlet. One is open ocean water which is characterized by the higher salinities found in the south-eastern portion of the Inlet. The other water mass originating from the Upper Cook Inlet, is characterized by realtively low salinities.

As shown in Figure 10, the relative microbial activities in the northern waters are highest observed in this region, the lowest values were observed to the south and east of the inlet and in open ocean waters. Intermediate values were observed in samples collected along the western side of the inlet.

Consistent respiration percentage patterns were also seen when the results of the data collected were compared from various cruises (Fig. 11). The values in the area near Kalgin Island and Tuxedni Bay are very low ranging from 31 to 40%. Contours of increasing values run in lines which run diagonally from the north-

east to the southwest. Intermediate values are found along these contours in the center of the inlet and the highest values are found in the southeastern portion of the inlet and in the open seawater.

During the April, 1979 cruise, the levels of relative microbial activity were unusually high (Table 2). These high activity levels seemed to mask the patterns of microbial activity that we had seen in the past cruises. We interpret these high values as being a response by the microbial population to a spring phytoplankton bloom. This phenomenon altered patterns of relative microbial activity more than it did respiration percentages.

2. Relative microbial activity and respiration percentages in sediments.

Both the relative microbial activity and respiration percentages observed in the sediments collected during all five cruises showed little variation regardless of the season in which they were sampled (Table 3). In general, the highest rates of microbial activity were observed in the major bays within the Inlet. These values were consistnntly higher than those observed in the Shelikof Strait. These geographical patterns were best illustrated during the April and November, 1977 cruises when the most comprehensive sediment sampling took place (Figs. 12 and 13).

3. Nitrogen fixation rates in sediments.

With the exception of the October, 1976 cruise, measurable nitrogen fixation rates were observed in sediments collected during

all of the Cook Inlet cruises. There were differences observed in the mean rates; but are compared on a station to station bases, there was no statistical significance in these differences (Table 4). During three of these cruises (11/77, 4/78, 4/79), there were enough samples taken in the Shelikof Strait so that a valid comparison could be made between sediment nitrogen fixation rates observed there and rates observed in the Cook Inlet (Table 4). In all three cases, the mean values observed in the Shelikof Strait were higher than those observed in the Cook Inlet. In two of these studies, the difference was significant at the $p<0.05$ level. The differences observed in Shelikof Strait nitrogen fixation rates between cruises were not statistically significant. The mean values for nitrogen fixation observed during both the Beaufort Sea cruises and the Norton Sound cruise were significantly lower than those observed in the Shelikof Strait (Table 4). In addition to the higher values observed in the Shelikof Strait, there were consistently high values observed in Kachemak Bay within Cook Inlet. These geographical trends are best illustrated in the November, 1977 data shown in Figure 14.

C. Data storage

All of the cruise data is currently being stored at NIH under the direction of Dr, Krichevsky, RU #s 391 and 371. This file contains information on station location, salinity, temperature, cell concentrations, uptake rates with and without crude oil, respiration percentages with and without crude oil, nitrogen fixation rates with and without oil, and all inorganic nutrient data for

water and sediment samples. The data collected for each cruise are located in a different file. The file numbers for the Cook Inlet cruises are as follows:

	Sample # series	File #
October, 1976	GW/B 300	237
April, 1977	GW/B 400	254
November, 1977	GW/B 500	259
April, 1978	GW/B 600	306
April, 1979	GW/B 800	260

V. Discussion

A. Relative microbial activity and percent respiration (mineralization).

1. Comparison between activities in sediments and the overlying water columns.

a. Our studies have shown that in regions where there are fine-grained sediments and the water column is relatively shallow, a vast majority of the microbial activity for the whole system resides in the sediments. As a result of our summer, 1975 Beaufort Sea study, we concluded that in the shallow waters behind the barrier islands, where the average water depth at the sample locations was 3 meters, the relative microbial activity in the sediments was 400 times that found in the overlying column (see Section II). During the Beaufort, 1975 study, the ratio of the uptake rates in water/sediments was 14. This was computed by comparing uptake per liter of seawater compared to uptake per g dry wt of sediment. When the mean values for water and sediment uptake in the Cook Inlet are compared in the same way, the ratio is 16. Thus the relative importance of microbial activity in sediments compared to the water column is about the same in the Cook Inlet as that observed in the Beaufort Sea. Of course the mean water depth in Cook Inlet is greater than 3 meters. A more realistic figure would be 30 meters. Even at that depth, the microbial activity in the sediments should be at least 40 times greater than that in the overlying water column in areas where there are fine grained sediments.

b. Another difference that was observed between pelagic and benthic microbial function was the percent respiration values.

In all Cook inlet field studies, the mean values for both glucose and glutamic acid uptake in sediments were equal to or less than that observed in the water samples. The same phenomenon has been observed during our Beaufort Sea studies (see Section II). It is felt that this reflects a qualitative difference in the nutrients available to the microorganisms in these two environments. When the percent respiration is low, a greater proportion of the nutrients taken into the cells are being converted into new microbial biomass. This usually occurs when most of the essentialy growth factors are available to the organisms. This concept will be explained in greater detail later in this report.

c. The Cook Inlet studies have also shown that the level of suspended matter in the water column is also an important feature of the pelagic microbial environment. The comparison between relative microbial activity in offshore (10 meters from the surf zone) and surf zone waters have shown greater activity in the later. This is undoubtedly due to the increased suspended matter load in the surf zone sediments. A more direct connection between suspended particulates and relative microbial activity was observed in the waters of the Upper Cook Inlet. During two cruises, a comparison was made between relative microbial activity and water turbidity. We found that these two variables were highly correlated (correlation coefficients of 0.87 and 0.89). It is quite possible that in high energy environments like those present in the Upper Cook Inlet, the most important locus of microbial activity may be in the water column instead of the sediments. This is opposite of

what we have observed in low energy regions where fine grained sediments are found. The implications of these conclusions will be discussed below.

2. Seasonal differences

a. Unfortunately, all of the five Cook Inlet cruises in which we participated took place either in the spring during the month of April or in the late fall (October and November). Thus, the data generated from these cruises represent only two seasons making interpretation of seasonal variations very difficult (data was not available for the seasons of greatest extremes). In February, 1979, we initiated a series of studies at a field station in Cook Inlet (Kasitsna Bay). Although our main purpose was to study the effects of crude oil on microbial processes, we did collect data on a large number of sediment and water samples at various times of the year. These data are reported in greater detail in Section IV. In summary, we found that microbial activity in the water column was highest in April after (or in association with) the spring diatom bloom and it was lowest in February. The microbial activity in the sediments showed much greater seasonal stability. The highest values were observed in August and the lowest in October and April.

b. Another interesting aspect of these studies was the fact that the ratio of glucose uptake to glutamic acid uptake changed seasonally. Glucose uptake was proportionately greater in pelagic populations during the time when there was greatest phytoplankton activity. This suggests that the microbial population was adjusting to the

presence of organic nutrients released by the phytoplankton during periods of high primary productivity. Similar relationships have been suggested by Gillespie et al. (1976) and by Albright (1977).

c. With the data available from the Kasitsna Bay studies, it is possible to put the values of microbial activity which were observed during the Cook Inlet cruises in perspective. The glucose uptake rates and the ratios of glucose to glutamic acid uptake observed in the October, November and April, 1977 cruises were taken under conditions that were closer to that expected under "winter" conditions. The April, 1978 cruise was conducted at a time when the spring phytoplankton bloom was just beginning and the April, 1979 cruise was conducted when the spring phytoplankton bloom was probably near its peak (at Kasitsna Bay, the diatom bloom had begun at least 4 weeks before the cruise began). During this cruise, we observed microbial activity patterns which did not fit those that had been observed during any other cruise. There were spikes of very high activity throughout the Cook Inlet and Shelikof Strait. This indicated that there was a great deal of patchiness in the occurrence of these blooms. This is consistant with the data reported by Dr. Larrance in his final report for RU #425 (Annual Reports of Principal Investigators for the Year ending March, 1977. Vol 10:1-136).

3. Geographical differences

a. As was mentioned in the Results section, consistent trends in surface water relative microbial activity and percent respiration patterns were observed. The resulting patterns, when interpreted

in light of what is known about the hydrography and chemistry of the region, produce an overall picture of the dynamics of the system which will assist those in government and industry in making a more accurate assessment of the potential problems related to crude oil production in Cook Inlet.

A discussion of the conclusions drawn from these data as well as the facts and assumptions on which these were based have already been mentioned in section I.B of Section I. An analysis of these data have also been submitted for publication (Griffiths et al., 1980d). The following is an amplification of the data presented in that section.

There are two distinct water masses present in Cook Inlet; one to the north that is very turbid and of relatively low salinity and one to the south and southeast which is more typical of open ocean water. We have found that both of these water masses have characteristic patterns of microbial activity and respiration. Glutamic acid uptake studies in surface waters have shown that the relative microbial activity is very high and the respiration percentages are very low in the northern water mass. The reverse pattern is seen in the water mass to the south. Intermediate values were observed in regions where these two water masses meet in the area to the north and east of Augustine Island. This is the same region in which a gyre has been observed by other investigators. In general, the patterns of surface water microbial activity and respiration reflect the net surface circulation patterns reported by Miller and Allen (1976).

As far as we know, this is the first study made in which patterns of microbial activity in marine waters have been used to characterize more than one distinct water mass and to indicate regions of interaction between those water masses. The two water masses in question are clearly shown by the surface water salinity data illustrated in Fig. 9. This is a similar pattern to that observed by Kinney at al. (1969). This is also the same type of pattern that one would expect from the current data presented by Miller and Allen (1976). Since these observations were taken at various times, it would appear that this is a relatively consistent feature in Cook Inlet. These same patterns are clearly shown in the relative levels of microbial activity and respiration percentages observed in the same region (Figs. 10 and 11) during all three Cook Inlet cruises.

At this point it is important to reflect on what these observations mean in terms of what is occurring in these water masses. The water mass to the southeast is coastal water which is being pushed into the inlet by inshore currents moving to the west. These waters probably contain very low levels of available organic nutrients. As a result, the level of microbial activity is low and the percent respiration is high. It has already been established by a number of investigators (Wright and Hobbie, 1966; Vaccaro and Jannasch, 1966; Crawford et al., 1974; and Carney and Colwell, 1975) that the uptake rate of simple labeled amino acids and sugars by natural microbial populations usually reflect the levels of nutrients present in the surrounding water. The significance of the percent

respiration data is less clear. A relatively high percent respiration value indicates that the population is using proportionately more of the nutrient as an energy source and less of it to produce cellular material. There are at least two conditions in which this might occur. If the cells are starved, the cells will utilize most of added nutrient for energy requirements before biosynthesis is initiated. A more likely explaination is that growth factors (lack of available nitrogen or phosphorus) are not present in sufficient concentration to allow biosynthesis to occur even though nutrients are available to the cells during the course of the experiment.

The high levels of relative microbial activity and low respiration rates found in the northern waters indicate that these waters contain nutrients that are qualitatively and/or quantitatively different than those found in the southern waters. The regions where these two water masses mix show intermediate values between these two extremes. These intermediate values could be caused by at least two factors. As the northern water mass moves south along the western edge of the inlet, the nutrients present are being consumed by the microorganisms present. At the same time, low nutrient waters from the south are being mixed with other water thus diluting the nutrients.

Drs. Cline and Feely (1977) have shown in their studies that the water masses in Cook Inlet are usually well mixed vertically. We conducted relative microbial activity at three locations and at various depths during the November cruise. We found no significant microbial activity stratification with depth. It would thus appear that the observations made in the surface waters should hold true for the entire water column in most locations.

We have also observed that the relative levels of microbial activity are directly related to the levels of suspended matter in the surface waters. When relative microbial activity as measured using glutamic acid is compared with turbidity in the same samples, correlation coefficients of 0.87 and 0.89 were observed for all water samples collected during the April and November 1977 cruises respectively. The correlation is also substantiated by the suspended matter patterns reported by Feely and Cline (1977). There is a striking similarity between these patterns and the patterns of microbial activity and respiration percentages reported here. During our determinations of bacterial concentrations using epifluorescent microscopy, we have observed that 70-80% of the bacteria present in water samples are associated with the particulate matter.

Feely and Cline (1977) also reported that much of the suspended matter found in the northern waters probably makes its way into the sediments of the Shelikof Strait. Our studies of relative microbial activity in the Cook Inlet tend to support this hypothesis. During all cruises, relatively high rates of microbial activity were observed in the sediments of Tuxedni Bay, Kachemak Bay and the southern portion of Kamishak Bay. The high rates of activity seen in Kachemak Bay are probably due to the trapping of nutrients within the bay. It has been observed by other investigators that the net flow of water through this bay is very low.

The high levels of microbial activity observed in Tuxedni Bay sediments are probably due to the sedimentation of the microbiologically active suspended matter in the water column in this area. Assuming

that the bacterial populations associated with these particles remain active even after they have settled into the sediments, then measurements of microbial activity in the sediments can be used as a tracer to determine the sedimentation patterns of the suspended matter found in the northern water mass. If we make this assumption, it would appear at least some of this matter settles into the sediments in the southern Kamishak Bay area.

b. The consistantly high rates of microbial activity found in Kachemak Bay may have a direct bearing on the high fisheries productivity in the same region. Dr. Feder (RU #281) has conducted studies of the food webb in Cook Inlet. He has reported that both littoral and offshore benthic organisms are very much dependent on detrital food sources (Lower Cook Inlet, Alaska - A Preliminary Environmental Synthesis). He has also reported that immature forms of crab and adult shrimp ingest sediment directly to extract the nutrients contained in the sediments. From what is now known about detrital food chains, it can be assumed that these organisms are obtaining most of their nutrients from the bacteria that are associated with the detrital particles within the sediment. D. C. Lees (RU #417) has also reported (Final Report - Reconnaissance of the Intertidal and Shallow Subtidal Biotic - Lower Cook Inlet, 1977) that the productivity of macrophytes is higher in Kachemak Bay than in any other region in Cook Inlet. The growth rates found here were as high as any reported in the current literature. Recent studies of macrophyte decomposition and utilization of macrophyte biomass by other organisms indicate that ca. 80% of this biomass is routed through bacteria before it can be used as a usable food source by

the higher trophic levels (Fenchel and Jorgensen, 1977). There is therefore, a g reat deal of presumptive evidence that benthic bacteria play a vital role in the overall productivity of this region.

B. Nitrogen fixation

1. Seasonal differences

a. The availability of fixed nitrogen is one of the limiting nutrients in the ocean and therefore productivity of any marine ecosystem relies on the availability of nitrogenous compounds. The only mechanism that can fix dissolved N_2 in the marine environment is through the agency of microbes (including the cyanobacteria). As was the case in interpreting the relative microbial activity on a seasonal bases, it is very difficult to make statements about seasonal nitrogen fixation based on the results of the cruises. Fortunately, during the Kasitsna Bay study, we conducted measurements of nitrogen fixation on a seasonal basis (see section IV). These results show that the highest rates of nitrogen fixation occurred in the months of February, April and October and that the lowest rates occurred in July. On a seasonal basis, April nitrogen fixation rates in the Beaufort Sea were the highest observed in that region. Although the differences observed in the mean values for nitrogen fixation during the Cook Inlet cruises were not statistically significant, it is interesting that the November, 1977 rates were the highest recorded in both Cook Inlet and Shelikof Strait.

The reason for these seasonal variations probably relate to both the quality and quantity of the nutrients coming into the

sediments. At Kasitsna Bay, we conducted a series of experiments where sediments were augmented with chitin, starch, and dried grass (Cerophyl). The sediments to which starch had been added showed nitrogen fixation rates that were approximately 10 times greater than the controls. Sediments to which the other two substrates were added showed nitrogen fixation rates well below the controls. When we were adding the starch, only organic carbon was being added to the system; however, the other two substrates contained both organic carbon and nitrogen. The stimulation of nitrogen fixation by the addition of soluble organic carbon has been well documented in the literature (Knowles and Wishart, 1977; Fay, 1976; Keirut Brezonik, 1971; and Herbert, 1975).

The low nitrogen fixation rates observed in the July sediments probably reflected the input of nutrients with a low carbon:nitrogen ratio; i.e., phytoplankton, and zooplankton. Later in the season (November), much of the available nitrogen was converted to bacterial biomass which was cropped from the sediments to be utilized by higher trophic levels. This resulted a nutrient source in the sediment with a high carbon:nitrogen ratio that was still readily available for utilization by bacteria. These are the conditions which are most favorable for nitrogen fixation. Further on in the year (February and April), the available carbon was in a more recalcitrant form requiring the bacteria to hydrolyze the compounds with extracellular enzymes before they could be utilized. In this case, the availability of carbon becomes limiting to nitrogen fixation, a reaction that requires a great deal of energy. We have

also measured the activity of several hydrolases on a seasonal basis which supports the above hypothesis. Both the seasonal nitrogen fixation and relative microbial activity measurements made in the sediments near Kasitsna Bay suggest that even though the phytoplankton bloom may take place in April, the input of these nutrients into the sediment are not reflected in our measurements until the summer.

2. Geographical differences

During the cruises, we consistantly found the highest rates of nitrogen fixation in Kachemak Bay and Shelikof Strait. Occasionally, high nitrogen fixation rates were also observed in Kamishak Bay as well. These patterns can be interpreted in light of data collected at Kasitsna Bay, data collected on the currents in Cook Inlet and the denitrification data collected by Dr. Atlas and his associates. The current patterns described by Miller and Allen (1976) suggest that the net flow of water out of the Inlet is south along the west side of the inlet and Shelikof Strait. This pattern is consistant with the results of studies conducted by Feely and Cline (1977) and ourselves. It can thus be assumed that detrital particles that are produced in Cook Inlet, would move to the west side of the Inlet and then south through Shelikof Strait.

It seems likely that as the particles migrate south, the ratio of carbon:nitrogen (C:N) increases as the bacteria use up the available nitrogen and are cropped (a similar mechanism that was suggested for the seasonal changes observed in the Kasitsna Bay study). Particles that settle out in Kamishak Bay would have lower

C:N ratios than those found in Shelikof Strait which is further to the south. Qualitatively, the organic nutrients found these sediments would then be similar to those found in the "winter" sediments of Kasitsna Bay. This would explain why the relative microbial activity is low and the nitrogen fixation rates are high in the Shelikof Strait and the reverse pattern is found in Kamishak Bay.

Additional support for this hypothesis comes from a comparison of nitrogen fixation and denitrification rates in these two areas (Table 5). These data are also presented by Haines et al. (1980). In the western Cook Inlet (Kamishak Bay), the natural rates of denitrification (the conversion of fixed nitrogen to atmospheric nitrogen) were much greater than natural rates of nitrogen fixation (the opposite process). These rates are probably out of balance because of an exogenous input of fixed nitrogen into this area; i.e. detrital particles with low C:N ratios. This is the same situation that was observed in Norton Sound where the exogenous source of fixed nitrogen was terrestrial detritus from the Yukon River. In the Shelikof Strait, the situation was different. In this system, the amount of nitrogen being fixed was approximately equal to the amount of fixed nitrogen that was being transformed back to atmospheric nitrogen. This would suggest that there was very little exogenous nitrogen being supplied to the system; i.e. Shelikof Strait.

3. Significance of nitrogen fixation in overall productivity

The nitrogen fixation rates observed in Shelikof Strait and Kachemak Bay were significantly higher than those rates observed in either the Beaufort Sea or Norton Sound. This indicates that this

process is probably of greater significance in and near Cook Inlet than in Arctic waters. How then do these rates compare with those reported by other investigators in different areas? Of the studies reported, the one that most closely approximates ours was that of Herbert (1975). This was an *in situ* study of nitrogen fixation in sediment cores taken at a location on the northeast coast of Scotland. Herbert observed a maximum nitrogen fixation rate of 1.84 ng nitrogen fixed per g dry wt per h. This rate is the *average* rate of nitrogen fixation observed by us in all sediment samples analyzed during the November 1977 Cook Inlet cruise. The maximum rate that we observed was 6.3 ngN x^{-1} x h^{-1}. In another study, Brooks et al. (1971) reported a range of nitrogen fixation in 8 sediments taken from a Florida estuary of from 0.64 to 6.0 ngN x g^{-1} x h^{-1}. The highest value that they observed was very close to the highest value that we observed in November Homer boat basin). Marsho et al. (1975) reported an average annual nitrogen fixation rate of 2.9 ngN x g^{-1} x h^{-1} in sediments taken from 7 stations in the Rhode River close to Chesapeake Bay. These data suggest that the rates of nitrogen fixation that we observed in Cook Inlet and the Shelikof Strait are close to that observed in other marine sediments and relatively high when compard to sediments that were most similar (the Herbert study).

In the Kasitsna Bay study, we found that the yearly mean value for nitrogen fixation rates in sediments analyzed near there as being approximately 1 ng x g dry wt^{-1} x h^{-1}. This rate was sufficient to replace all of the fixed nitrogen (NH_4, NO_3, and NO_2) in the interstitial water every 24 hours. It is also a rate that is

sufficient to account for an annual production of bacterial biomass in Kachemak Bay of 400 tons.

Under most conditions, nitrogen is usually not limiting to bacterial growth in seawater; however, the same may not be true in sediments. Inshore sediments often contain detritus particles which have very high carbon to nitrogen ratios. Studies of detrital food chains have shown that nitrogen fixation in sediments may be a very important factor in the effective utilization of detritus food particles by higher trophic levels (Mann, 1972; Fenchel and Jørgensen, 1977). This is particularly important when one realizes that the majority of organic nutrients available to support all of the animal population in the inlet probably come from detritus particles. In order for this to become available as a food source for animals from the level of the protozoa on up, the detritus particles must become colonized by bacteria. In order for bacteria to grow, they need fixed nitrogen.

VI. Conclusions

1. In general, the sediments showed very high microbial activity when compared to the water samples studied. This fact suggests that the sediments might be important loci of crude oil degradation. It is not known what rates of biodegradation could be expected in Arctic marine sediments; however, there is evidence that suggests that certain components of crude oil may persist in sediments for long periods of time.

2. Water samples taken along the shoreline consistently show higher levels of microbial heterotrophic activity than those taken offshore. This indicates that initial rates of crude oil biodegradation may be higher in waters next to the beach than in offshore waters.

3. The waters taken from the northern section of Cook Inlet showed much higher levels of activity than those taken near the mouth of the inlet.

4. Measurements of relative microbial activity and respiration percentages can be used to characterize specific water masses and give some information about the organic nutrients found in these waters. The above measurements should be made in both the water column and sediments in new lease areas where this information is not available. These data would provide information about potential transport mechanisms as well as data about biological productivity potential.

5. Nitrogen fixation in the sediments of Cook Inlet and the Shelikof Strait may be an important contributing factor to the overall productivity of the detritus based food chain in that area.

6. Our studies on the effects of crude oil on nitrogen fixation in natural sediment samples showed that the presence of crude oil had little or no short term adverse effect on this process. As indicated in Section IV, long-term exposure of marine sediments did cause a dramatic decrease in nitrogen fixation rates.

7. Crude oil did have an inhibitory effect on glucose respiration in natural marine microbial populations. This effect was noted when either crude oil, crude oil aqueous extract or weathered crude oil was used. This effect probably reflects stress which could cause a reduction in species diversity such as that already observed in Arctic marine waters exposed to crude oil over extended periods.

8. Evidence is accumulating which suggests that crude oil which is spilled in the turbid waters of the Upper Cook Inlet may become associated with the suspended matter found in these waters. If this occurs, then

crude oil components would become associated with the sediment when these particles settle out of the water column. Our studies and the studies of Drs. Feely and Cline suggest that these particles probably settle out into the sediments of the southern Kamishak Bay and/or the sediments of the Shelikof Strait.

9. The regions which would most probably be impacted the most by a crude oil spill would be Kachemak and Kamishak Bays and Shelikof Strait. This is assuming that the crude oil becomes associated with the sediments. Of these three areas, the one which would be most severely impacted would be Kachemak Bay. If significant portions of the sediment in this Bay was perturbed with crude oil, it is quite likely that the fisheries in this region would be adversely effected for an extended period of time.

10. Our effects studies at Kasitsna Bay have shown that virually every benthic microbial process that we have studied show significant changes when marine sediments are exposed to crude oil. These results are reported in Section IV of this report.

VII. Needs for further study

We have collected all of the field data in Cook Inlet and Shelikof Strait required to have a general understanding of relative microbial activity and nitrogen fixation rates in these areas. Future cruise work should be conducted in the Bering Sea where thre is very little information available about microbial processes. We do, however, strongly recommend that the effects studies that we have initiated at Kasitsna Bay be continued.

Table 1. Data summary of the average values measured during 1975-1976 field studies. (*) Average values calculated with one example excluded; a value which we consider more typical.

Factor	Units	Beaufort Sea Summer 1975		Beaufort Sea Winter 1976		Beaufort Sea Summer 1976		Lower Cook Inlet Fall 1976		Gulf of Alaska Winter 1976	
		Ave.	Range	Ave.	Range	Ave.	Range	Ave.	Range	Ave.	Range
V_{max} (Offshore water)	ng/liter/hr	40	4 to 118	3.1	0.2 to 14	21	0.4 to 85	28 (9.3)*	0.2 to 405	1.4	0.3 to 3.4
V_{max} (beach water)	ng/liter/hr	-	- -	-	- -	-	- -	84	0.7 to 404	63.0	9.7 to 113
$V_{max} \times 10^{-1}$ (sediments)	μg/g dry wt/h	5.2	0.2 to 17	0.5	0.04 to 1.8	6.9	0.2 to 64	7.1 (2.0)*	0.2 to 63	45	2 to 103
Percent Respiration (water)	%	59	44 to 76	85	52 to 100	46	20 to 59	58	40 to 78	72	53 to 93
Percent Respiration (sediments)	%	43	32 to 71	45	35 to 87	28	14 to 35	46	38 to 53	44	30 to 72
Number of bacteria x 10^5 (seawater)	cells/ml	4.5	0.1 to 11.9	1.5	0.8 to 2.7	3.7	1.6 to 2.7	4.2	0.2 to 16.5	1.9	1.2 to 2.7
Number of bacteria x 10^8 (sediments)	cells/g dry wt	6.3	0.1 to 41.1	10	0,5 to 19	106	24 to 267	41	4 to 130	15	0.1 to 31
Sample temperature (surface water)	°C	1.2	-0.8 to 3.2	-1.9	-2.0 to -1.5	0.3	-1.3 to 1.8	8.3	-1.5 to 12	3.8	2.0 to 5.0
Sample salinity (surface water)	o/oo	20.5	9.0 to 26.5	24	17 to 29	12.3	5.1 to 20.5	23.8	20.5 to 27.5	31.9	30.7 to 35.5

Table 2. Relative microbial activity of Cook Inlet waters, excluding Homer Boat Basin and Shelikof Strait.

Activity	Units	Oct. 1976		Apr. 1977		Nov. 1977		Apr. 1978		Apr. 1979	
		$\bar{x}$	range	$\bar{x}$	range	$\bar{x}$	range	$\bar{x}$	range	$\bar{x}$	range
Glutamate uptake (T)	ng/l/hr	8.0	0.2-53	9.7	0.5-665	5.3	0.4-61	7.3	0.1-75	18.9	0.8-58
Glucose uptake (S)	ng/l/hr	3.0	0.2-33	1.6	0.2-12	0.8	0.2-6.6	4.5	0.1-20	11.9	0.7-72
Ratio S/T	-	0.38		0.16		0.15		0.62		0.63	
Glutamate Respiration	%	58	40-78	48	27-70	55	32-70	50	32-71	54	35-70
Glucose Respiration	%	28	10-84	41	5-45	31	14-89	29	14-53	35	13-48

Table 3. Relative microbial activity of Cook Inlet sediments, excluding Shelikof Strait.

Activity	Units	Oct. 1976		Apr. 1977		Nov. 1977		Apr. 1978		Apr. 1979	
		$\bar{Y}$	range	$\bar{Y}$	range	$\bar{Y}$	range	$\bar{Y}$	range	$\bar{Y}$	range
Glut. uptake	ng/g/hr	131	30-360	240	80-370	103	20-252	131	10-600	208	72-550
Glucose uptake	ng/g/hr	3.3	0.9-7.1	8.3	2-18.4	6.1	0.7-23.3	6.5	0.3-38.4	5.2	0.9-17.8
Glut. Resp.	%	46	38-53	39	40-49	49	43-57	46	31-61	46	39-51
Glucose Resp.	%	28	20-43	23	16-36	22	13-40	26	10-33	22	14-28

Table 4. Nitrogen fixation rates[1] in field station sediments 1977-1979.

		Apr. 1977	Nov. 1977	Apr. 1978	Apr. 1979	Sep. 1977	Aug. 1978	Jul. 1979
Cook Inlet[2]	$\bar{X}$	0.23	0.46	0.31	0.06			
	range	0-0.52	0.05-1.70	0.10-0.90	0-0.14			
Shelikof Strait	$\bar{X}$		1.3	0.51	0.68			
	range		0.3-4.4	0.30-1.10	0.45-0.95			
Beaufort Sea	$\bar{X}$					0.10	0.20	
	range					0.02-0.21	0-1.40	
Norton Sound	$\bar{X}$							0.22
	range							0-0.88

[1]rates expressed as ng N fixed/g dry weight sediment/hr.

[2]excluding Shelikof Strait and Homer boat basin.

Table 5. Comparison of mean rates of nitrogen fixation, denitrification and concentrations of fixed forms of organic nitrogen in sediments from different regions of the Alaskan Continental Shelf.

	N_2 fixation ng g^{-1} h^{-1}	Denitrification (natural) ng g^{-1} h^{-1}	NH_4^+ µM	NO_3^- + NO_2^- µM
Upper Cook	0.08	0.05	-	-
Western Cook	0.28	11.25	137	55.2
Shelikof Strait	0.68	0.66	83	3.1
Norton Sound	0.22	1.91	188	6.4
Beaufort Sea	0.20	-	103	12.4

Table 6. Variations seen in V_{max} values observed in samples taken at the same geographical location at different times.

Station No. summer 1975	Water samples	
	Date	V_{max} *
1	9/5	51
1	9/11	18
1	9/17	47
2	8/21	83
2	9/5	10
2	9/11	13
2	9/17	13
3	8/21	90
3	9/5	15
3	9/11	4
3	9/17	11
4	8/21	91
4	9/5	9
4	9/11	9
53	9/8	77
53	9/12	60
55	9/8	113
55	9/12	69
70	9/12	19
70	9/14	12
71	9/12	20
71	9/14	5
Winter 1976		
3	4/5	5.4
3	4/9	4.3
3	4/10	0.7
14a	4/5	12
14a	4/18	< 0.2

Table 6 (continued).

Station No. summer 1976	Water samples	
	Date	V_{max}*
72	8/23	85
72	8/24	41

	Sediment Samples	
Summer 1975	Date	V_{max}*
1	9/5	1.51
1	9/17	0.61
2	9/5	0.13
2	9/11	0.13
2	9/17	0.77
3	9/5	0.07
3	9/11	0.02
3	9/17	<0.02
51	9/8	0.78
51	9/13	0.80

*V_{max} values in water samples are reported as ng glutamic per liter per hour and V_{max} values in sediments are reported as μg glutamic acid per gram dry weight per hour.

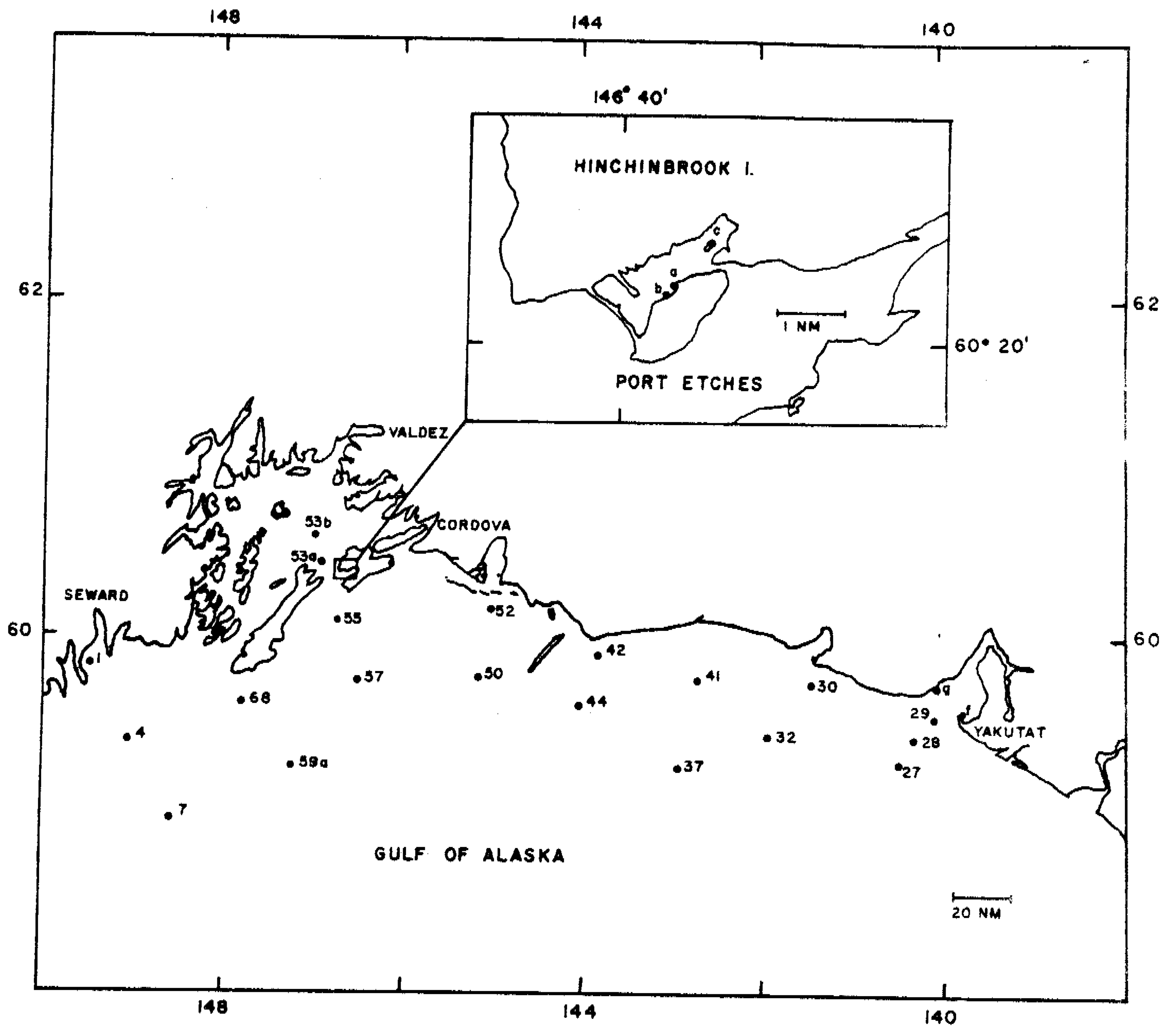


Figure 1. Stations sampled in the Gulf of Alaska during the March, 1976 cruise.

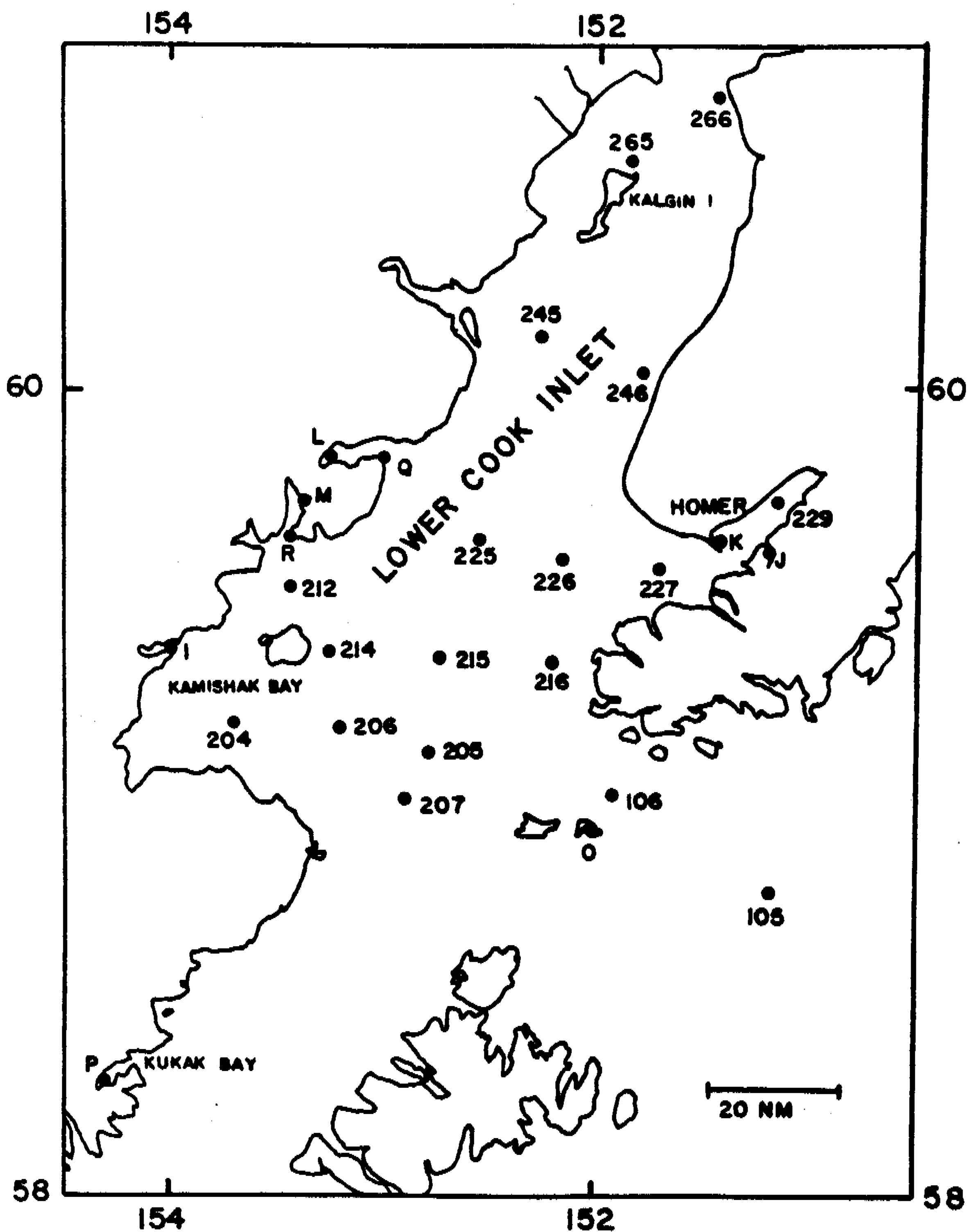


Figure 2. Stations sampled in Cook Inlet during the October, 1976 cruise.

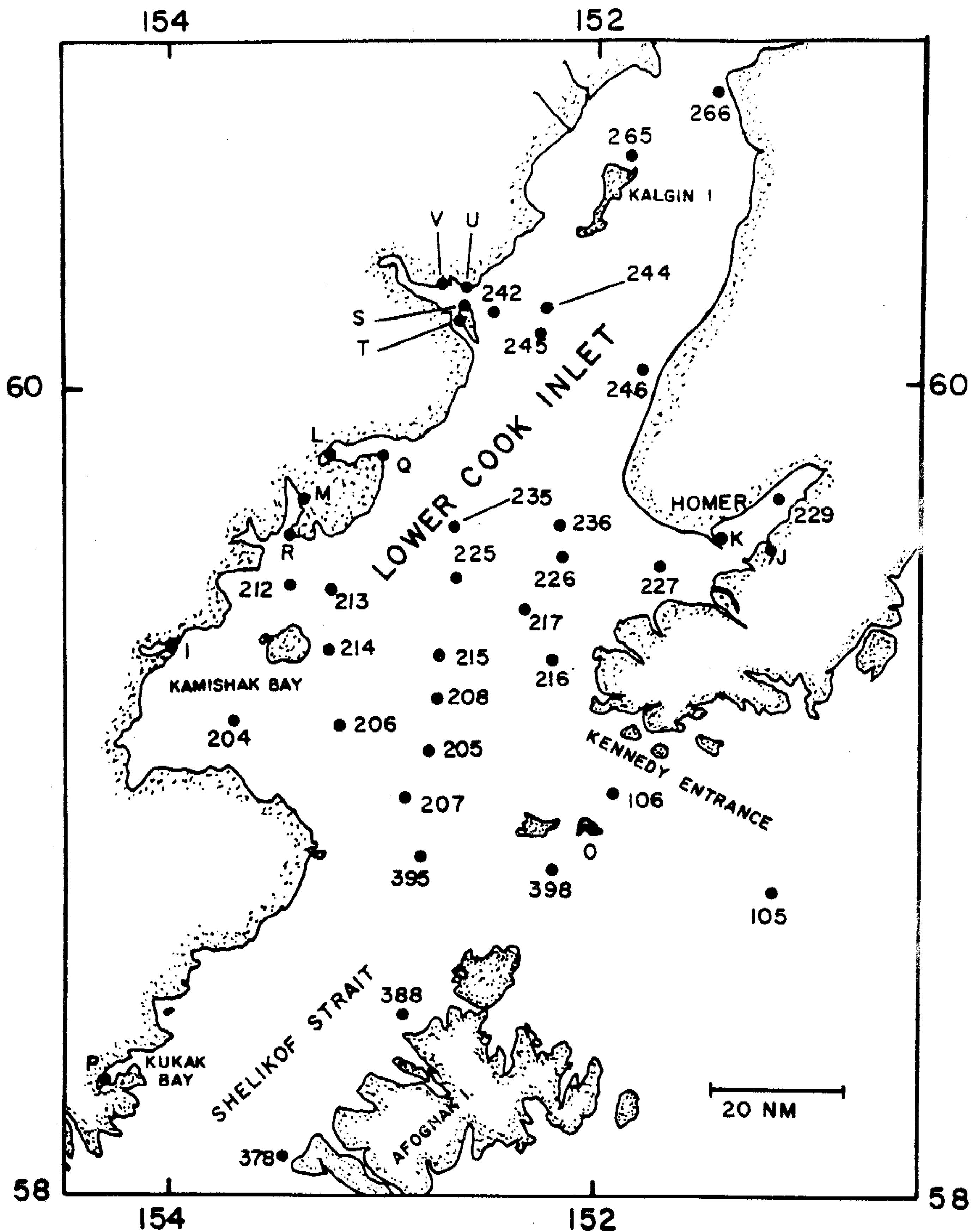


Figure 3. Stations sampled in Cook Inlet during the April, 1977 cruise.

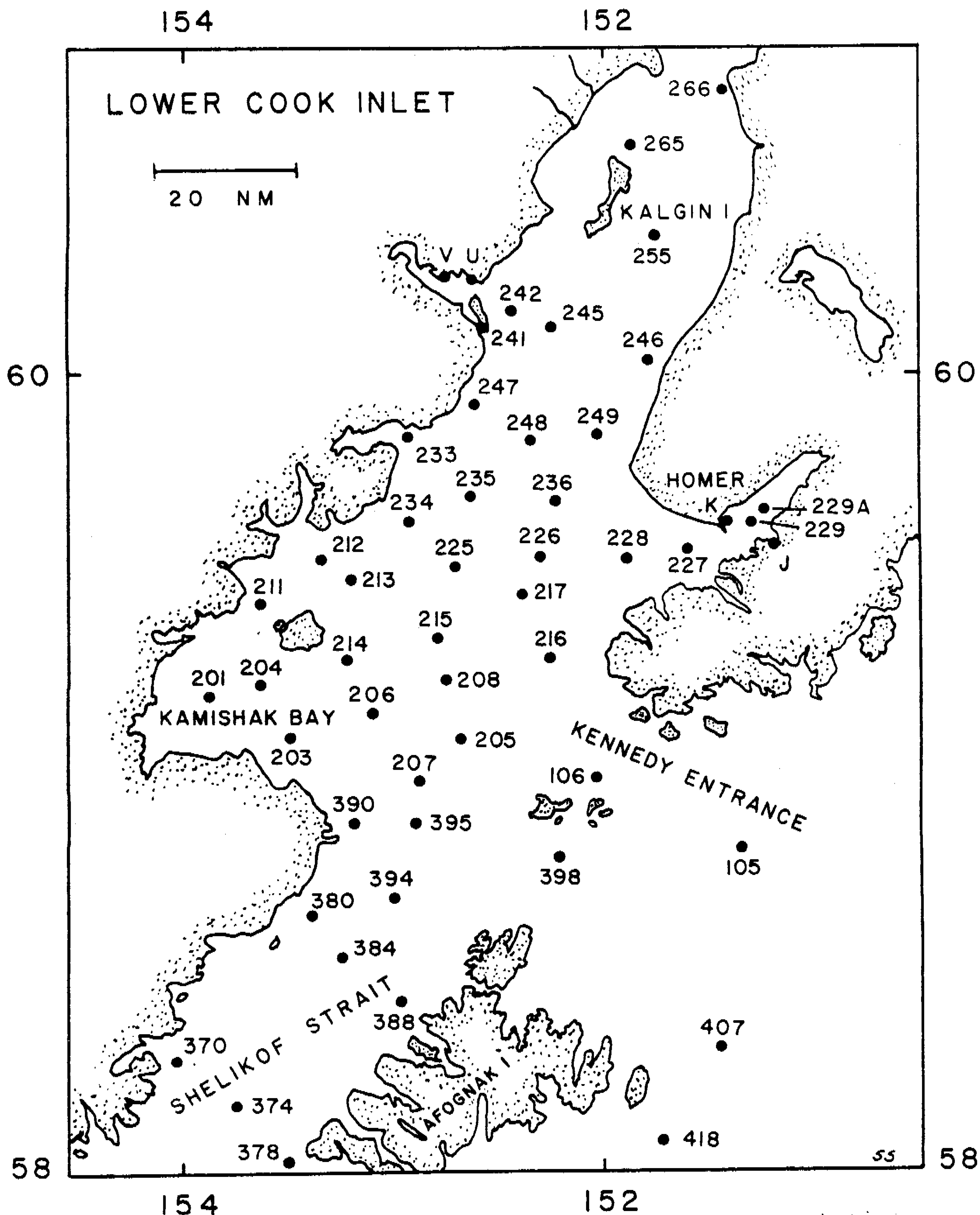


Figure 4. Stations sampled in Cook Inlet during the November, 1977 cruise.

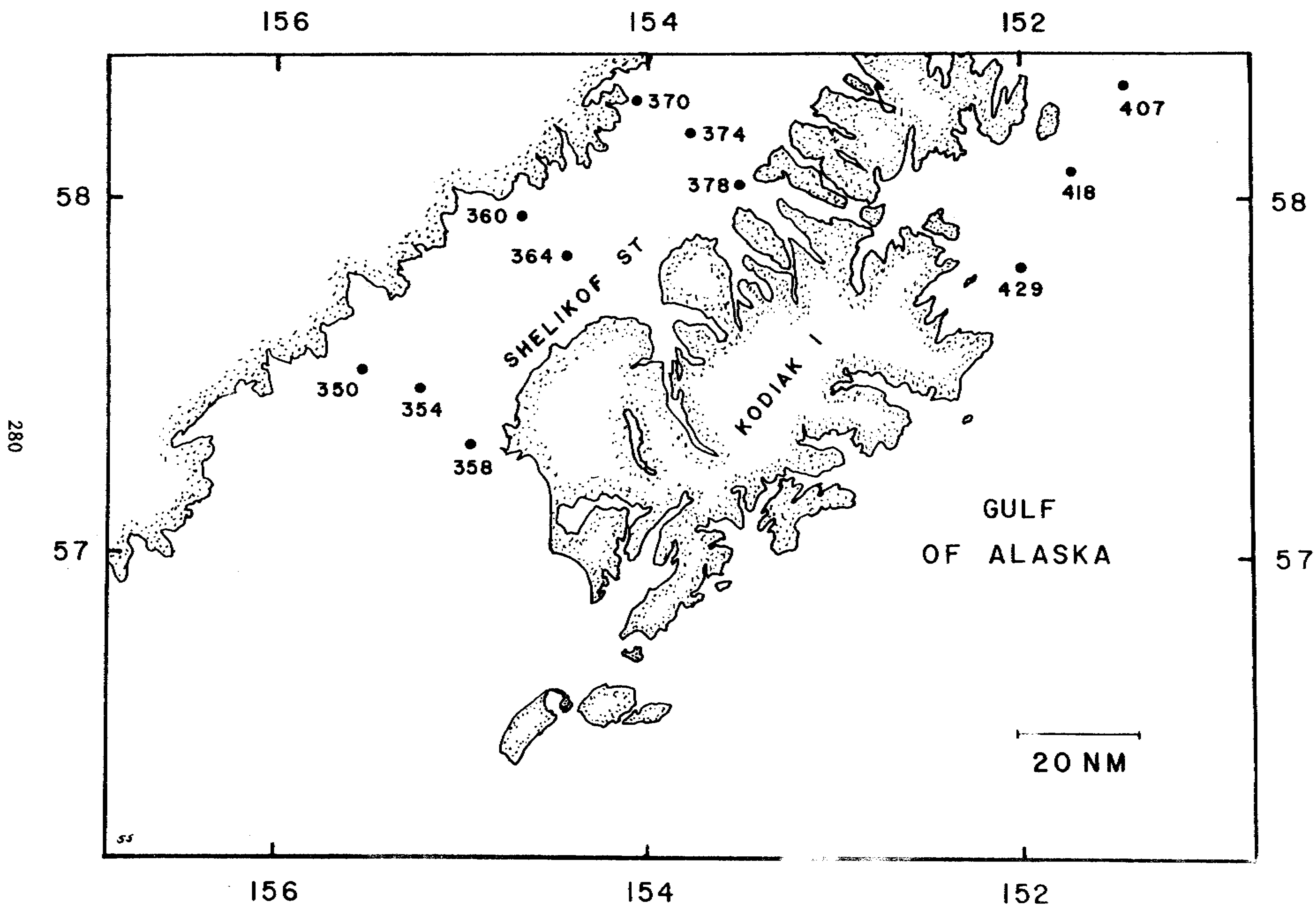


Figure 5. Stations sampled near Kodiak I. during the Novembe , 1977 cruise.

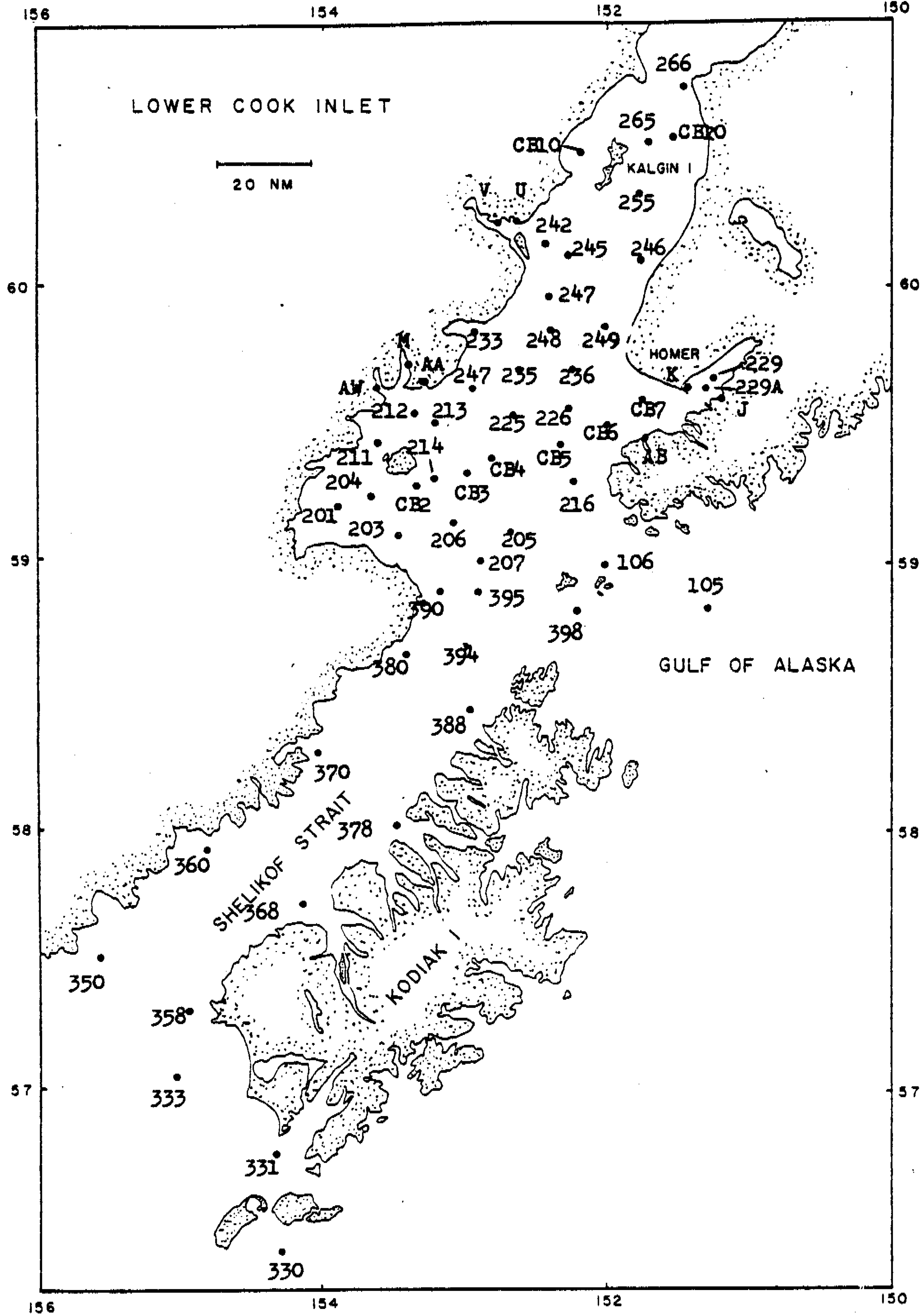


Figure 6. Location of stations sampled during the April, 1978 Cook Inlet cruise.

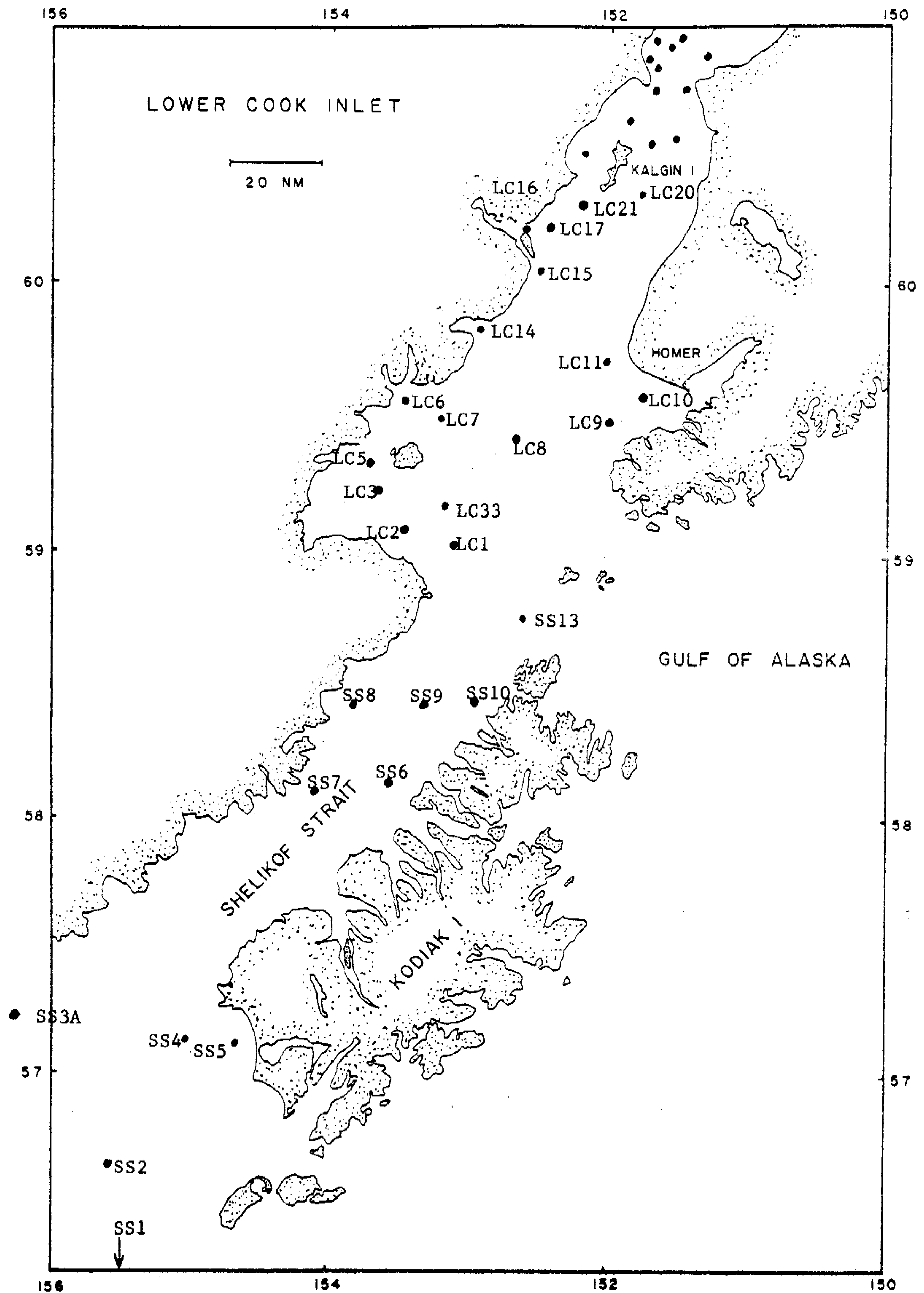


Figure 7. Stations sampled in Cook Inlet during the April, 1979 cruise.

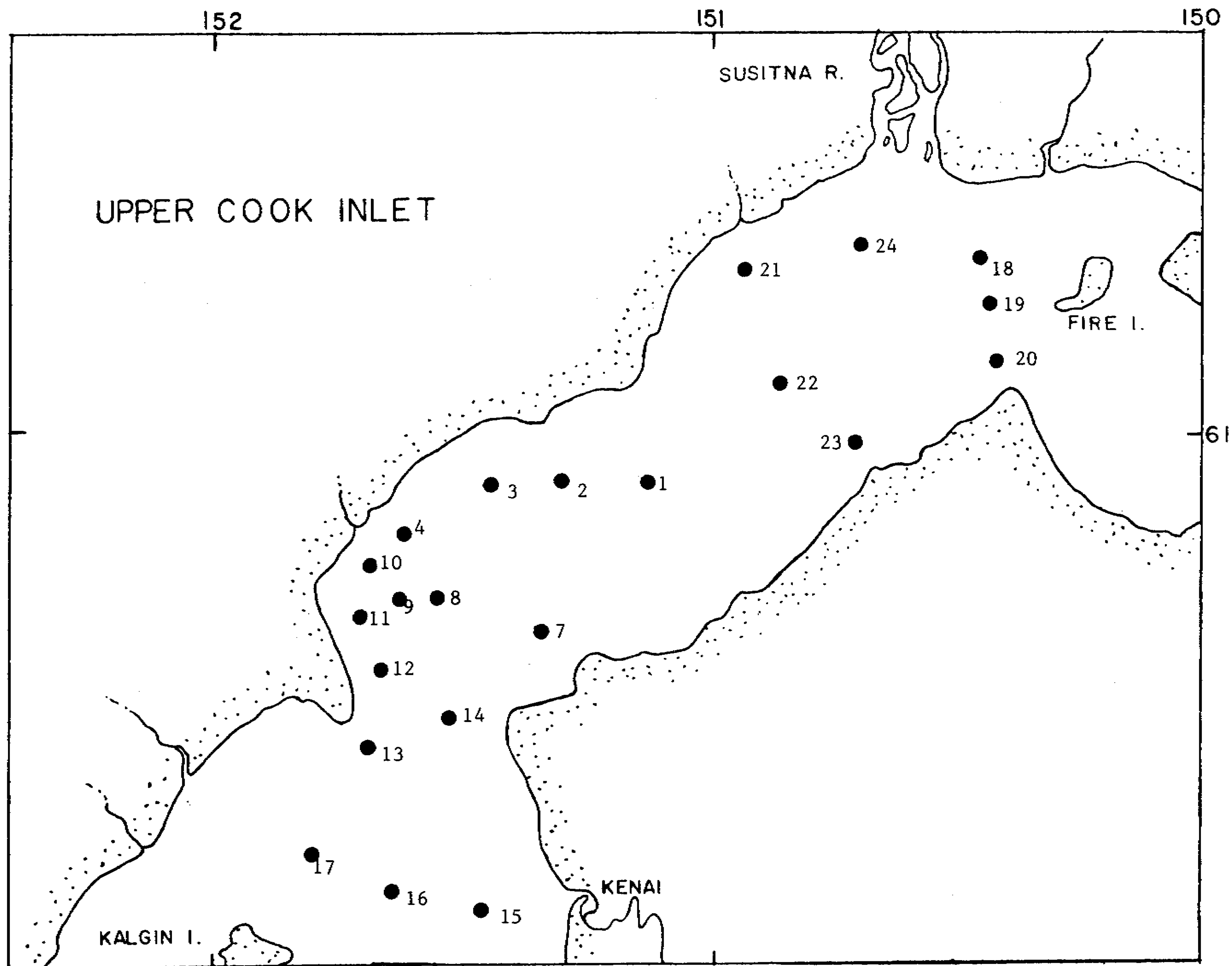


Figure 8. Stations sampled in the Upper Cook Inlet during the April, 1979 cruise.

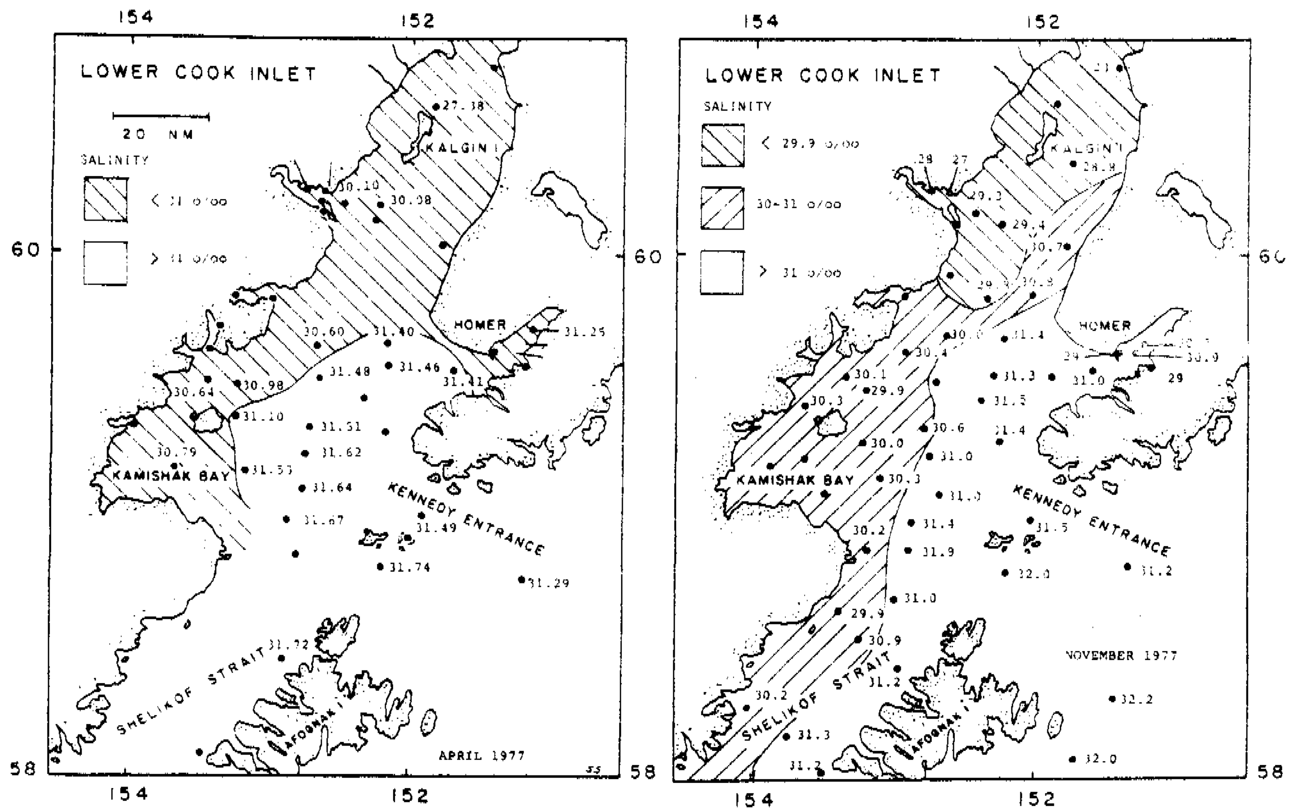
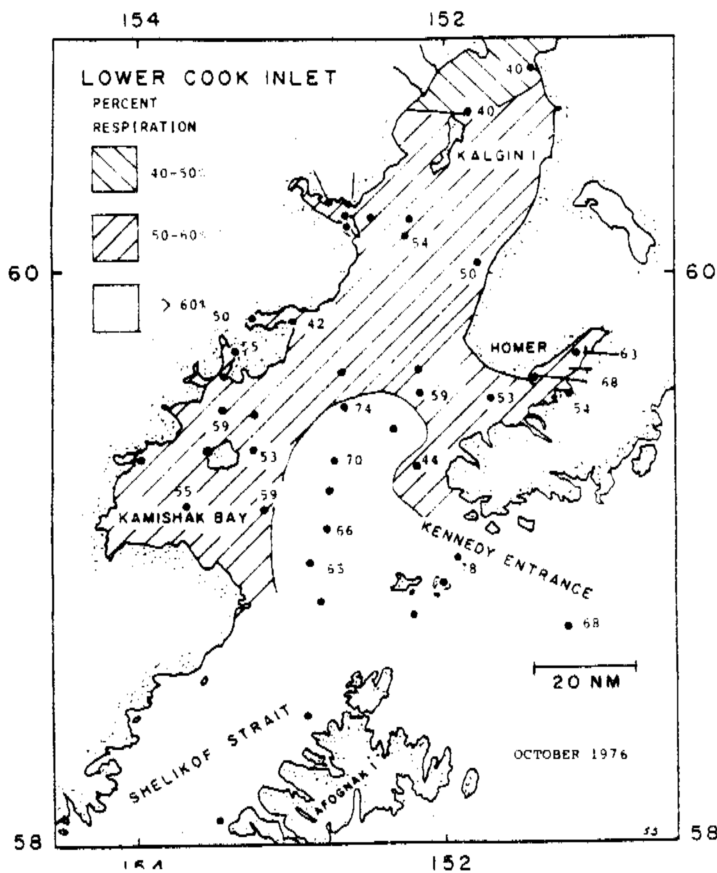
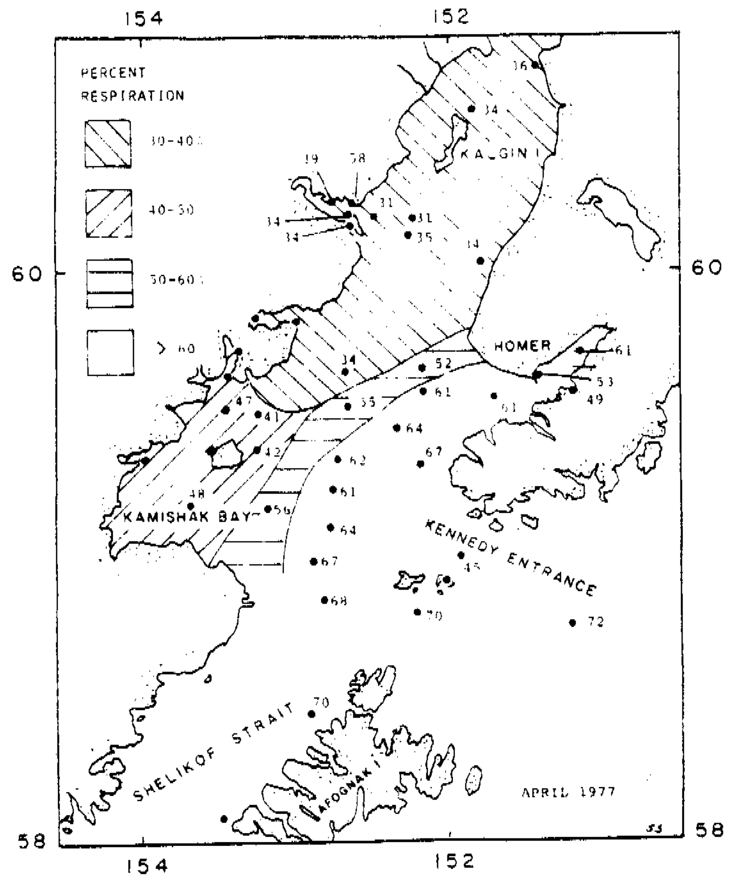


Figure 9. Surface salinities in Cook Inlet during the April and November, 1977 cruises.

Figure 10. Water masses in Cook Inlet characterized by relative activities during three sampling periods.





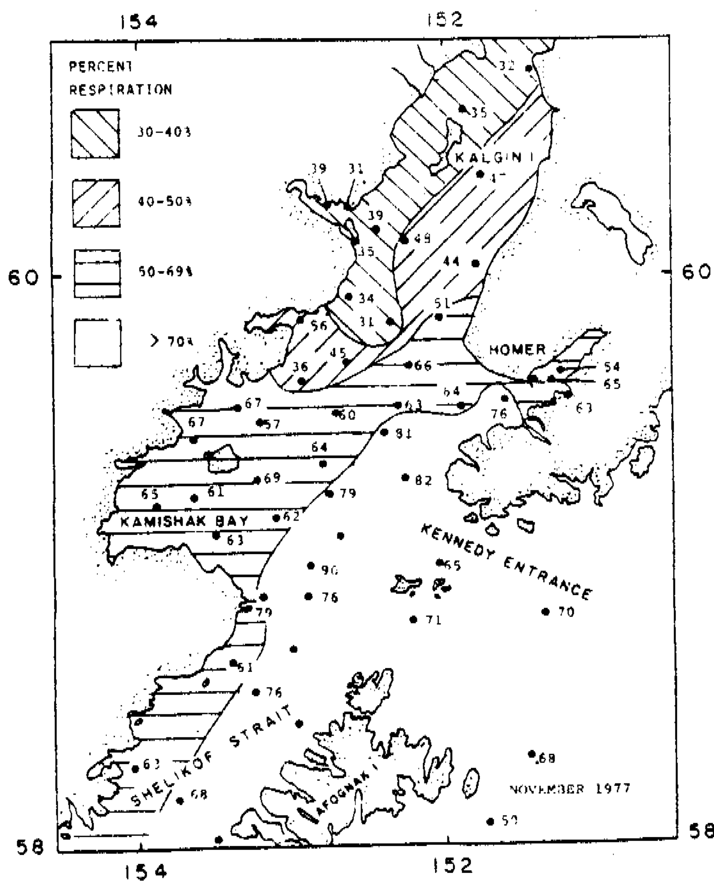


Figure 11. Water masses in Cook Inlet characterized by percent respiration during three sampling periods.

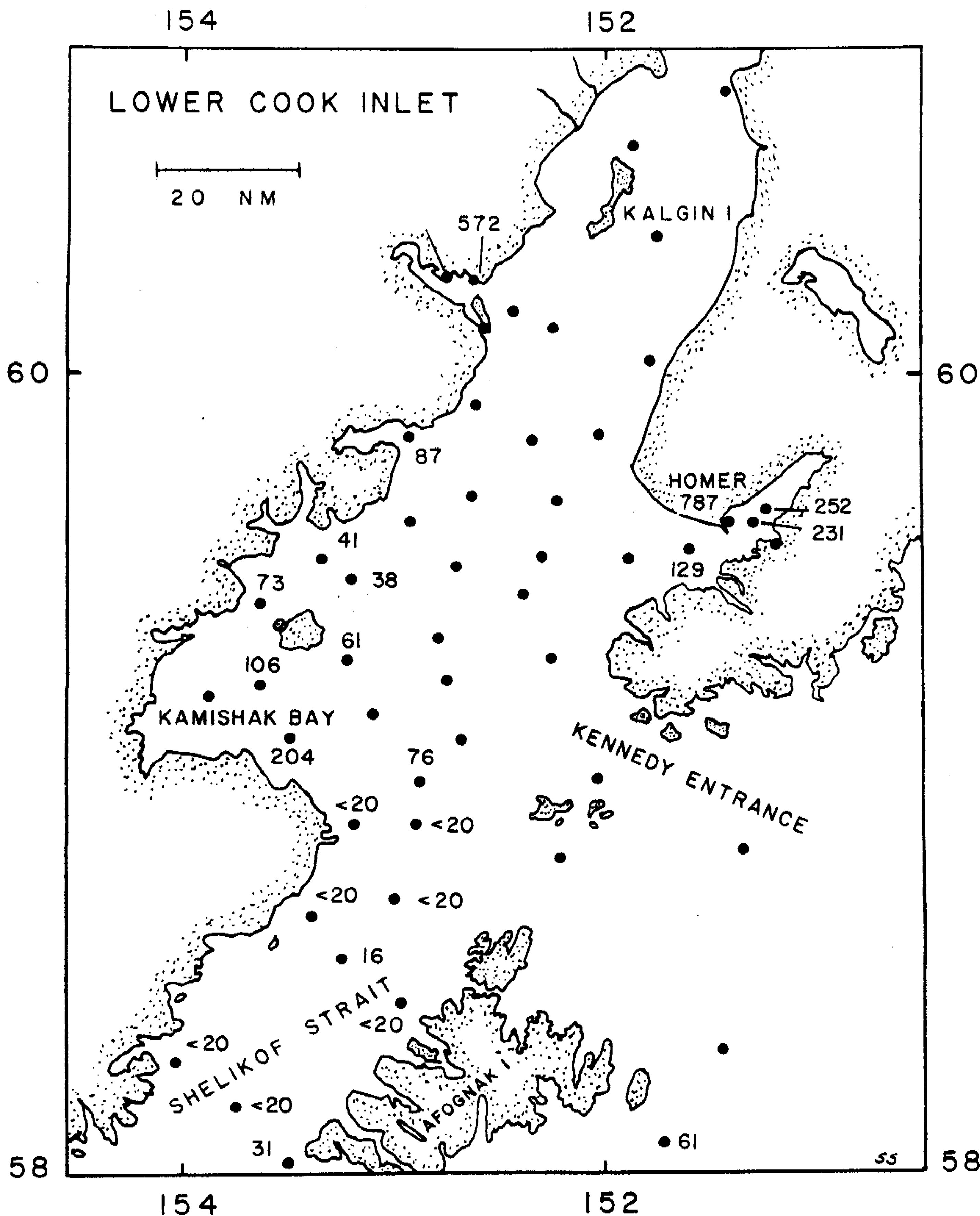


Figure 12. Relative microbial activity in sediment samples measured using glutamic acid during the November, 1977 cruise. Units are ng/ g dry wt./ h.

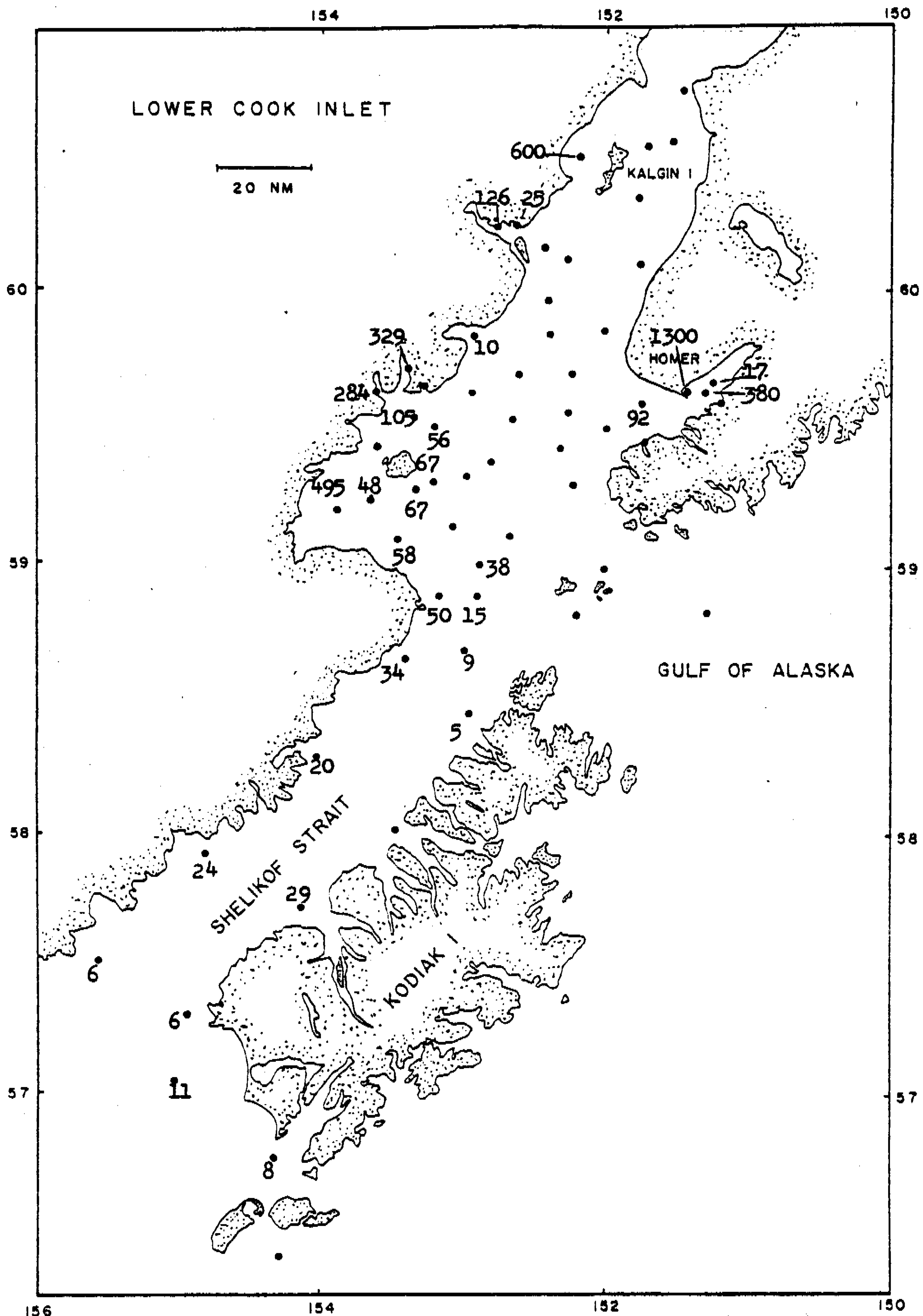


Figure 13. Relative microbial activity in the marine sediments collected during the April, 1978 Cook Inlet cruise. Units used are ng glutamate/g/h.

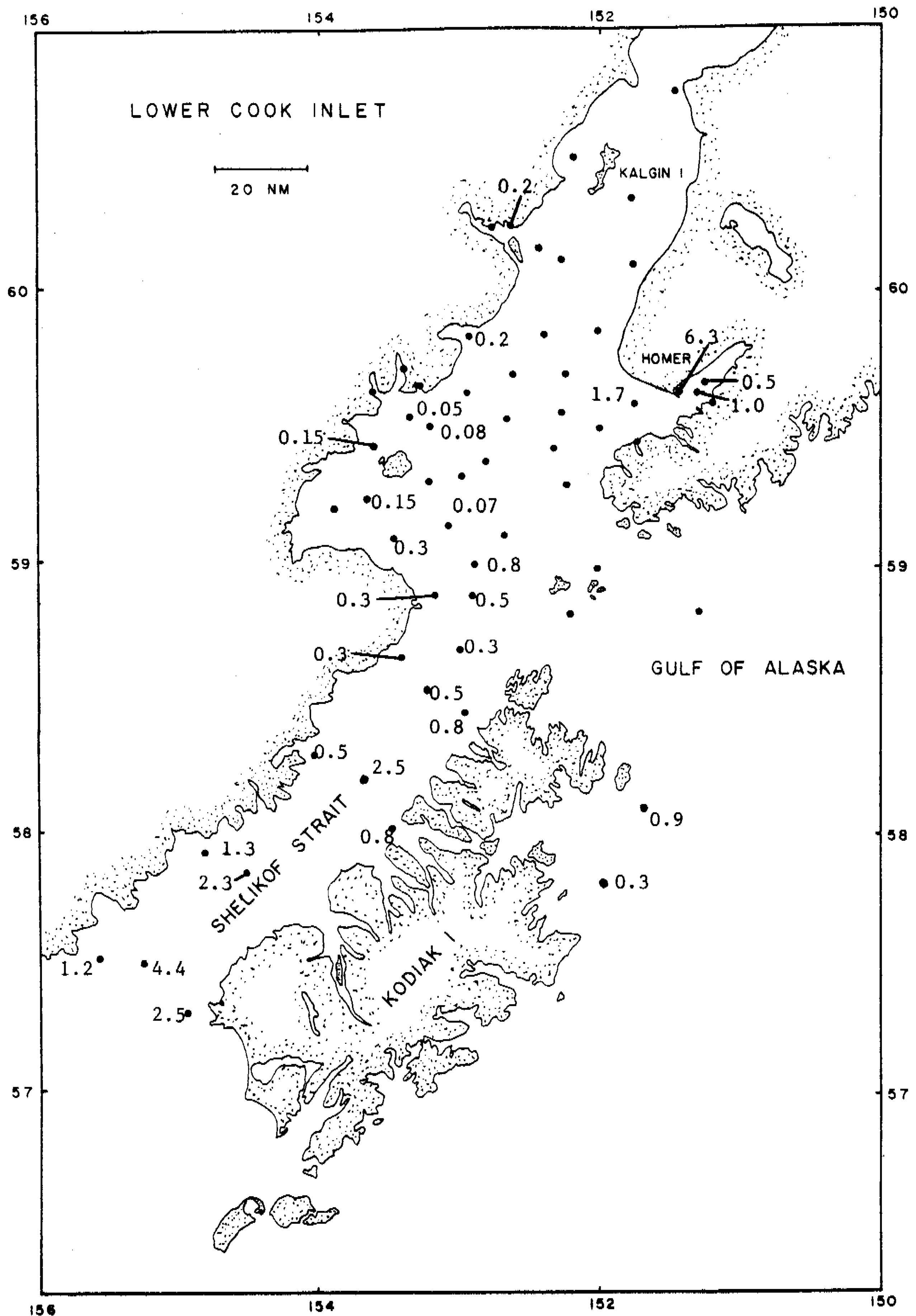


Figure 14. Nitrogen fixation rates in sediment samples collected during the November, 1977 cruise. The units are ng N_2 fixed/g dry wt. /h.

BEAUFORT SEA

Section II

I. <u>Summary of objective, conclusions and implications with respect to OCS oil and gas development.</u>

A. Objectives.

During the early studies, our main objectives were to obtain baseline data on microbial activity and bacterial cell concentrations in the waters and sediments of the Beaufort Sea. These data were to be augmented with salinity, temperature and inorganic nutrient data. These studies were to be designed to define when and where the microbial populations were the most active. As the study progressed, we were to start measuring nitrogen fixation rates in the sediments and the short-term effects of crude oil on these processes. In July, 1977, a study was initiated in Elson Lagoon which was designed to show what effects if any, crude oil had on benthic microbial populations. Those studies have been conducted from that time to the present. During all of these studies, we were to work in close cooperation with Dr. Atlas (RU #29).

B. <u>Conclusions and implications with respect to OCS oil and gas development.</u>

1. Our studies to date have shown that crude oil has significant long and short-effects on microbial function in Arctic and sub-Arctic marine environments. This has some important implications concerning an overall productivity of an impacted area. Some of the long-term effects of crude oil in sediments include reduced rates of organic nutrient mineralization. This and reduced transformation rates of key nitrogen cycle components suggest that crude

oil could reduce the rate at which inorganic nutrients such as nitrate and phosphate are made available to phytoplankton to be used in primary productivity.

2. There is also evidence to suggest that secondary productivity, through the detrital food chain, could also be adversely affected by the presence of crude oil in sediments. Such a reduction in secondary productivity would have a profound effect on the food available to support life at all trophic levels. This reduction would most probably be of greatest importance in the shallow inshore communities; especially those areas near the major rivers where terrestrial carbon is washed in seasonally. Recent studies on the dynamics of detrital food chains suggest that over 50% of phytoplankton carbon and 80-90% of macrophytic and terrestrial carbon passes through bacterial biomass before being utilized by higher trophic levels. We have typically observed reductions in microbial activity of 50% or more in waters and sediments exposed to crude oil. By inference, crude oil should have a profound effect on overall biological productivity in an impacted area. The aquaria studies at Kasitsna Bay have shown that there is a large accumulation of detrital material on the surface of oiled sediments. This did not occur in the non-oiled control sediments (see Section IV for details of this study). Under these conditions, the blockage of the detrital food chain by the presence of crude oil was dramatically illustrated.

3. Beaufort Sea inshore sediments were shown to have approximately 400 times the microbial activity as that found in the overlying water column. This illustrates the relative importance of the

sediments in the system. As mentioned above, it is known that crude oil has a profound effect on microbial activity in these sediments. We therefore recommend that during the planning and execution of oil production, those procedures which could cause crude oil to become accidentally incorporated into the sediments should be avoided. Likewise, procedures which would tend to drive crude oil into the sediments during cleanup operations after a spill should be avoided.

4. The onset of measurable long-term crude oil effects took considerably longer in Beaufort Sea sediments than they did in Cook Inlet sediments (up to 10 times longer). It seems quite likely that the crude oil effects that we are observing in the Elson Lagoon samples will persist for a long time. We have seen many of these effects persisting in Cook Inlet sediments one year after exposure. From these observations and what we know about what takes place in Beaufort Sea sediments, we would predict that crude oil would affect benthic microbial function for several years.

5. In a recent study conducted by Dr. Schell (RU #537), it was concluded that the Beaufort Sea amphipod Gammarus steosus feeds directly on peat. This shows a direct link between cellulose utilization and transfer of this carbon to higher trophic levels. Actually, the amphipods are probably digesting off the bacteria which colonize the peat. It has also been shown by Busdosh and Atlas (1977) that Beaufort Sea amphipods avoid feeding in areas contaminated with crude oil. In our Kasitsna Bay study, we also observed a reduction in the utilization of detritus because the detritivores were either killed or avoided feeding in crude oil

contaminated sediments. This leaves us with the question of what happens to the detritus if it is not utilized by the detrital food chain? Our work at Kasitsna Bay shows that the detritus accumulates on the surface of the sediments and the sediments become anaerobic. These anaerobic conditions would undoubtedly effect the long-term distribution of the normal benthic organisms since most of the sediments we have sampled in the Beaufort Sea appear to be well oxidized.

II. Study areas.

During the summer of 1975, we conducted a two month study of microbial activity in marine waters and sediments near Point Barrow and Prudhoe Bay. The locations that were sampled are illustrated in Figs. 15 and 16. At this time, 50 water samples (including 5 ice samples) and 24 sediment samples were collected and analyzed. During the 1976 field studies, the locations shown on Fig. 17 were sampled. During April, 1976, 26 water samples (including 3 ice samples) and 14 sediment samples were analyzed. During the August, 1976 Glacier cruise, 18 water and 13 were collected. The sediment samples that were returned to us from the September, 1977 cruise were sampled at the locations given in Fig. 18. During the August, 1978 cruise, we collected and analyzed 42 water and 38 sediment samples from the locations given in Fig. 19. The location for the Elson Lagoon oiled tray experiment was approximately 1/2 way between stations 2 and 3 in Fig. 16.

III. Methods.

The methods used for most routine field operations are presented in Section I. The methods used in the crude oil and Corexit effects studies are presented in Section IV of this report. The only method that is not covered in the other methods sections in this report was the one used to measure ATP, ADP, and AMP.

A. Analysis of adenylate concentrations.

The procedure used was a modification of that described by Bullard (1978). One ml of sediment contained in a 50 ml beaker was added to 8 ml of extraction buffer (0.04 M Na_2HPO_4 adjusted to pH 7.70 with 0.02 M citric acid) and placed in a boiling water bath for 2 minutes. With the exception of the time when the samples were boiled they were kept cold by placing the beakers in crushed ice. Evaporation was minimized by covering the beakers with a watch glass. One tenth ml of 10^{-6}M ATP was added to both one sediment sample and the control containing no sediment. This was used to determine the extraction efficiency. Duplicate subsamples of each sediment were extracted. Following extraction, samples were removed to an ice bath, then added to centrifuge tubes, allowing a small volume distilled H_2O rinse to completely recover the extracted sample. After centrifuging at 8000 x g at 0 C for 10 min, the clear supernatant was removed to a screw cap vial, its volume brought up to 10.0 ml with distilled H_2O, and frozed at -20 C until the time of analysis.

charge was calculated using the following relationship:

$$\text{Energy Charge} = \frac{\text{ATP} + 1/2\ \text{ADP}}{\text{ATP} = \text{ADP} + \text{AMP}}.$$

IV. Results.

Our first study in the Beaufort Sea was conducted during the months of July, August and September, 1975. This study resulted in an extensive survey of relative microbial activity in the waters and sediments near Barrow, Prudhoe Bay and landward of the Barrier Islands between these two locations (Figs. 15 and 16). These first studies were published in 1978 (Griffiths et al.). We also participated in two cruises in the Beaufort Sea. During the first cruise (August, 1976 Glacier cruise), we studied relative microbial activity at offshore locations between Point Barrow and Prudhoe Bay (Fig. 17). During our second cruise conducted in August, 1978, we studied relative microbial activity and nitrogen fixation rates in samples collected from the Colville River to the US-Canadian border (Fig. 19). We did not participate in the September, 1977 Glacier cruise, but Dr. A. G. Carey (OSU) brought back sediment samples to us. We measured nitrogen fixation rates and hydrocarbon biodegradation potentials in these samples (Fig. 18).

We have also participated in another study in collaboration with Dr. Atlas (RU #129). This study was designed to assess the effects of crude oil on microbial function in Elson Lagoon sediments. The results of these studies are presented and analyzed in Section IV of this report.

A. Relative microbial activity and respiration percentages.

In the studies mentioned below, the relative microbial activities are expressed in V_{max} values. This is equivalent to the substrate uptake rates used in the previous section.

1. Local variations.

Variations in the maximum potential uptake rates (V_{max} values) of water samples taken at the same location within a few weeks of

one another are shown in Table 6. In some cases, the variation seen in the water samples was greater than 10-fold at the same station. The variation was somewhat less in the sediment samples. These variations are much greater than one would expect from experimental error alone since the variation between identical subsamples is generally near 10% for the technique used. The variations seen in the water samples probably reflect differences in water masses present at each location. This supposition is supported by the fact that stations sampled on the same date usually showed the same relative pattern of microbial activity even though the average level of activity may change dramatically from one week to the next. All of these samples were taken near or within the Barrier Islands in very shallow seas. These waters are greatly influenced by wind-driven currents and the effects of freshwater runoff. The variations in the sediments probably reflect inaccuracies in station location and the patchiness of the sediment due to ice gouging.

2. Geographical differences.

During the first three field-study periods, bacterial concentrations, relative microbial activity (V_{max}), temperature, salinity, and pH were measured in water and/or sediment samples. During the summer 1975 study period, these measurements were compared in samples that were taken in the Barrow and Prudhoe Bay areas (Table 7). In the water samples, there was a significant difference seen between the mean percentage of respiration and the mean salinity values observed in these two regions. Both values were greater in the Barrow area.

A somewhat different result was obtained from similar measurements made on sediment samples taken in these two regions. The only factors that showed a significant difference in the mean values were the pH and the relative microbial activity (V_{max}). Both the mean values for relative microbial activity and pH were higher in the Prudhoe Bay sediments. The mean bacterial concentration was about twice as high in the Prudhoe Bay area but the variations were high and a statistical analysis of the data revealed that the difference was not significant. The difference seen in the bacterial concentrations did, however, reflect the same difference seen in the V_{max} values when these two regions were compared. In general, the values presented in Table 7 were probably representative of values normally found in the bay waters of the Beaufort Sea in late summer months.

In most cases, the nearshore water samples taken farthest from shore showed the lowest V_{max} values. This is best illustrated in the data collected in the Prudhoe Bay area (Table 8). A comparision was made between the distance of the sampling site from shore and the V_{max} value observed in the water sampled at that site. On the three sampling dates for which there are sufficient data to make a valid comparison, there was a negative correlation between the distance from shore and the V_{max} values observed at that distance. This same trend, however, was not seen in samples taken along the offshore transects sampled in the winter and summer of 1976 (Fig. 17). The sediment samples collected during the September, 1977

cruise showed higher relative microbial activities in the area near Point Barrow than the areas to the east (Fig. 20). During the August, 1978 cruise, we were able to sample a more comprehensive sampling grid (Fig. 19). These data show that the relative microbial activity was highest in the areas near the major rivers along the North Slope. This phenomenon was observed in both water and sediment samples (Figs. 21 and 22). In addition, we observed reduced respiration percentages in the waters adjacent to these rivers (Fig. 23). The patterns of uptake and respiration in the waters were essentially the same as that observed in the Cook Inlet (described in Section I) and in the Norton Sound (described in Section III).

3. Comparison of relative microbial activity in Beaufort Sea and other regions.

Using V_{max} values, we have shown that the relative microbial activity in Beaufort waters was roughly equivalent to that observed during the 1976 Cook Inlet cruise and during the NEGOA cruise the same year (Table 1, Section I). With the exception of the unusually high activity observed in NEGOA sediments, the same can be said for relative microbial activity in the sediments as well.

Comparisons of relative microbial activity can also be made in the studies in which glutamate uptake at one concentration was used (Table 9). If uptake rates in both water and sediments in the Beaufort Sea are compared with data collected in both Cook Inlet (including Shelikof Strait) and Norton Sound, the rates are very similar. With the exception of the two water samples collected in Elson Lagoon in April, 1978, all of the mean values are within the

same order of magnitude. With the exception of the January Beaufort Sea sediment samples, the same can be said for the relative microbial activities in the sediments as well.

Unfortunately, there is very little data in the current literature with which we can compare the Beaufort Sea data. The data that is available from other geographical regions (Table 10), indicate that the rates observed in the Beaufort Sea during the summer, 1975 study was roughly equivalent to that reported by others.

4. The influence of freshwater input on cell concentrations and microbial activity.

One of the many unique characteristics of the Beaufort Sea is the wide range of salinities encountered. During our studies, the widest salinity range observed was seen during the 1976 summer cruise where surface and near-surface samples showed salinities ranging from 5 to 21%. This salinity variation in the area of study was the result of freshwater coming from melting of the pack ice. As a result, the surface-water slinity was much lower than that found at 5 m and below. Seawater samples that were diluted 50% with sterile fresh water showed no significant change in the ability of the natural microflora to utilize glutamic acid even though marked changes in salinity resulted in this treatment. Studies were also made on the relative microbial activity seen in melted ice in contrast to the same measurement made in the water adjacent to the ice (Table 11). Ice when melted showed a high degree of variability which was undoubtedly due to its diverse origin.

The microbial activities and bacterial concentrations in the surface water and melted ice were similar. When seawater and melted ice were mixed 1:1 there was no adverse effect on glutamic acid utilization. These observations were further supported by relative microbial activity measurements made at the surface at 15 m (Table 12). The input of fresh water from melted icepack at the surface did not reduce the concentration of bacteria present nor did it adversely affect the uptake of glutamic acid when these values were compared with samples taken from more saline waters at 15 m.

5. Seasonal variations.

The two 1976 Beaufort Sea studies were designed to compare relative microbial activities and bacterial cell concentrations in water and sediment samples taken in the summer and winter at the same stations. Additional data were obtained from other stations during the 1975 summer. The average V_{max} values observed in both water and sediment samples during the winter study were about one order of magnitude lower than that seen in either summer study (Table 13). The data presented in Table 14 illustrate this trend in water samples taken at the same stations during these two seasons. The bacterial concentrations also decreased in the winter but the decrease was not as dramatic as that seen in the V_{max} data. However, there was very little difference seen between the cell concentrations observed in the 1975 summer sediments taken from the inshore stations and the values observed in the 1976 winter sediments taken from the offshore stations even though a 10-fold difference was seen in the V_{max} values in the same samples.

A statistical analysis of these data shows that there was a significant difference in the V_{max}, percentage of respiration, cell concentration, and salinity between the water samples taken in the winter and those taken during both summers. In the sediment samples, there was a significant difference seen between the V_{max} values during the same sampling seasons. The cell concentrations in the sediments were significantly lower in the winter as compared to the summer 1976 measurements but they were not significantly different from those observed in the summer of 1975.

The differences seen in the microbial activity in the sediment and surface-water samples during the summer and winter studies probably reflect variations in nutrient availability during these two seasons rather than changes in water temperature. The widest difference in the average surface-water temperature observed between winter and summer conditions was about 3°C (Table 13). A study was conducted to determine the effects of incubation temperature on four water samples taken in the Barrow area during the 1975 summer study (Fig. 24). These data indicate that a drop in temperature of 3°C will decrease the V_{max} value by about 50%.

In addition to these data, we also collected seasonal data during the Elson Lagoon study (Table 15). In these studies, we compared the relative microbial activity in water and sediment samples using single concentrations of glucose and glutamic acid. The microbial activity in the water samples was low during the January and April sampling periods but much higher during August.

In the sediments, the activity was lowest in January and highest in April and August.

6. Comparison of microbial activity in sediments and in the water column.

The sediments of shallow waters have long been recognized as an important area of microbial activity in marine ecosystems. It has also been known that microbial activity in sediment is much higher than that found in water. This trend was also observed in this study (Table 13). In the summer 1975 study, the average value for the potential rate of glutamic acid uptake and mineralization was 0.52 μg glutamic acid per gram dry weight of sediment per hour. There is no way to compare directly this figure to potential activity in a comparable volume of water but a reasonable approximation can be made. A relative comparison between microbial activity in sediments and seawater can be made by contrasting the activity in a given volume of seawater with an equal volume of sediment-seawater slurry. In the summer 1975 study, the average V_{max} in the undiluted sediment slurries was 2.5×10^{2} μg per liter per hour. This is roughly four orders of magnitude higher than the average figure of 3.8×10^{-2} μg observed in the water samples.

Another comparison of potential microbial activity in sediments and seawater can be made by comparing the total activity in an average water column with activity in the underlaying sediment. To make such a comparison, at least two assumptions must be made. The first is that most of the microbial activity is taking place in the first 2 cm of the sediment. This assumption is based on the findings of ZoBell (1942) and others who have shown that the

vast majority of bacteria in sediments are found in the top 2 cm. The other assumption is that diluting the sediment sample with sterile artificial seawater does not significantly affect the resulting observed activity.

Keeping these assumptions in mind, the potential rate of glutamic acid utilization in the average water column (1 m^2 x 3 m deep) can be compared with the same potential in the sediment below that square meter of water. The maximum potential for glutamic acid utilization in an average water column in the test area was 0.1 mg glutamic acid in the water column per hour. If our assumptions are correct, the sediments in the water columns studied in the inshore stations (summer 1975) had, on the average, 400 times greater activity than the entire overlaying water column. This figure should be considered an underestimation of what is probably the true value because: (1) we have assumed that there is no microbial activity in the sediments below 2 cm, (2) the value was calculated in terms of a slurry which contained roughly 50% seawater, (3) the actual sediment samples studied contained material to a depth of 4 cm.

7. Adjustment of microbial populations to hydrocarbons in the environment.

During the course of our studies, we have collected two sets of data which shows that benthic microbial populations may be changing in response to the inputs of hydrocarbons into the Beaufort Sea. An analysis of biodegradation potentials in sediments collected

during the September, 1977 cruise indicates that the benthic microbial populations in sediments to the east of Prudhoe Bay have the highest potential for degrading crude oil (Fig. 25). Sediments collected during the August, 1978 cruise shows that the benthic microorganisms in this same general area are affected the least by the addition of crude oil on a short-term basis (Fig. 26). In these studies, the lack of a reduction in the amount of glucose taken up by the microorganisms in the presence of crude oil can be interpreted as an indication of prior exposure.

B. Rates of nitrogen fixation in Beaufort Sea sediments.

1. Geographical distrubution.

We measured rates of nitrogen fixation in two sets of sediments during two cruises in the Beaufort Sea (Figs. 27 and 28). The set of data that was collected in September, 1977 covered the largest geographical area. In this study, the highest rates were observed in sediment samples collected near Point Barrow. The nitrogen fixation rates to the east were generally lower. During the second cruise which included the area between the Colville River and the Canadian-US border, there are no significant geographical trends noted. If one compares these data with similar data collected in Cook Inlet, Shelikof Strait and Norton Sound, there were differences noted in the mean values (Table 4, Section I). The mean nitrogen fixation rate observed in Norton Sound was not significantly different from that observed in the Beaufort. Generally the rates observed in Cook Inlet were higher but the differences were not statistically

significant. The mean values for nitrogen fixation in Shelikof Strait were significantly higher than those observed in the Beaufort Sea.

2. Seasonal differences.

There has been only one study in which we have collected nitrogen fixation data on a seasonal basis in the Beaufort Sea. We measured nitrogen fixation rates in sediments located near the oiled tray experiment in Elson Lagoon (Table 15). Three sets of observations were made; January, 1978, April, 1978 and August, 1978. The lowest rates were observed in January, 1978; intermediate values in August and the highest value in April. The August figure was approximately 1/3 of the mean observed in sediments analyzed during the NorthWind cruise later that same summer. Unfortunately, there was one non-oiled sediment analyzed in April, 1978. In all, there were five sediment samples analyzed (one control and four oiled). The mean value for all five was 1.3 ng x g^{-1} x h^{-1}. This value is exactly one order of magnitude higher than the rate observed in January, 1978.

C. Total adenylates in Elson Lagoon sediments.

The combined concentrations of ATP, ADP, and AMP was measured in the sediments of Elson Lagoon in August, 1978 and January, 1979. The total concentration of these three chemical species (total adenylates) was higher in the summer than in the winter. From this information, we were also able to calculate energy charge. The higher the energy charge, the more metabolically active the population is. The mean energy charge in summer sediments was 0.97 and in the winter it was 0.25 (Table 41, Section IV).

D. Crude oil toxicity studies.

During the August, 1978 Beaufort Sea cruise, we measured the acute toxic effects of Prudhoe Bay crude oil on nitrogen fixation rates and relative microbial activity in both water and sediment samples. During the Elson Lagoon oiled tray experiment, we measured these variables and the long-term effects of crude oil on these variables. The results of these studies are reported and analyzed in Section IV of this report.

E. Data storage.

A complete data set from the Beaufort Sea is located in the following NIH data files:

	#
Summer, 1975	
August, 1976 Glacier cruise	236
September, 1977 " "	307
September, 1978 Northwind	309

V. Discussion

A. Geographical differences.

When one compares the relative microbial activity observed in the Beaufort Sea with similar observations made by us and others in different regions, the rates are very close to rates measured elsewhere. This is true even in regions where the mean water temperature is much higher. This suggests to us that the microbial populations in the Beaufort Sea are very well adapted to functioning in this extreme environment. This supposition is supported by data collected by Dr. Atlas (RU #29). He consistently finds more bacterial strains growing on agar plates incubated at 4 C than those incubated at 15 C when he innoculates his plate with seawater or sediments collected in the Beaufort Sea. Thus it cannot be assumed that metabolic processes are inherently slow in the Beaufort Sea because the temperatures are lower than those found in other regions.

Within the Beaufort Sea we did see regional differences in microbial function. The offshore area near Barrow showed higher levels of both nitrogen fixation and microbial activity than the areas to the east. The same pattern was seen when we compared relative microbial activities in the waters and sediments of inshore samples collected in Barrow and Prudhoe Bay during the summer of 1975. The one exception to this is the area near Barter Island. It is quite likely that these variations are related to the sedimentation rates in these areas.

During the Prudhoe Bay study which was conducted landward of the Barrier Islands, we found that the relative microbial activity was highest nearshore. In other studies where samples were collected

seaward of the Barrier Islands along tracks normal to the coast, there was no consistent pattern of microbial activity along those lines. These data suggest to us that nutrients become trapped behind the Barrier Islands. Most of these nutrients entering the system are probably from the major rivers as indicated by the data collected during the August, 1978 cruise (Figs. 21, 22, and 23). The patterns of relative microbial activity and respiration percentages observed near the rivers is very similar to those observed in the Cook Inlet and Norton Sound. The region in which this phenomenon is best documented is in the Cook Inlet (see Section 1). In all three areas where there is large plume of freshwater coming into the environment, there is an region where the relative microbial activity is very high and the respiration percentages are very low. We feel that this indicates a significant nutrient input from terrestrial sources. In these regions, the quality and quantity of the nutrients available are such that the microorganisms are growing very rapidly; this is reflected in high metabolic activity and high production of bacterial biomass. This biomass, in turn, represents a major nutrient source for organisms at higher trophic levels.

In total, these geographical trends suggest to us that the areas landward of the barrier islands, particularly near the major rivers, would be the place where crude oil would most profoundly affect microbial function. As we will explain in detail in Section IV, a crude oil spill in these areas could greatly effect the overall productivity since we have shown that long-term exposure

of sediments to crude oil can alter overall productivity in several ways.

The other regional patterns that we have observed concern the adaptation of natural microbial populations to the presence of hydrocarbons. We have used two methods to determine if a population of microorganisms had been exposed for an extended period of time to hydrocarbons. The association between prior exposure and the variables that we measure is based on the assumption that a microbial population adjusts to the presence of crude oil. A more detailed explanation is given in Section IV of this report.

If a natural microbial population is exposed to hydrocarbons, then a greater portion of that population is capable of degrading hydrocarbons. The more hydrocarbon degrading bacteria present, the higher the crude oil biodegradation potential. This type of association has been established in the Cook Inlet studies reported by Roubal and Atlas (1978). Our study of Beaufort Sea sediments showed that the biodegradation potentials were highest in the sediments collected to the east of Prudhoe Bay (Fig. 25).

The other variable that we have studied is the acute effect of crude oil on glucose uptake in Beaufort Sea sediments. We have consistently observed that the microbial populations in both waters and sediments that have not had a prior exposure to hydrocarbons, show reduced rates of glucose uptake in the presence of crude oil (Griffiths et al., 1980b). In samples where there is little or no acute crude oil effect on glucose uptake, it can be assumed that

this population has had prior exposure to hydrocarbons. In Cook Inlet, we have observed essentially the same patterns using this technique as those reported by Roubal and Atlas (1978). In the Beaufort Sea, the area where we measured little or no acute effects of crude oil on glucose uptake was to the east of Prudhoe Bay. Since essentially the same results were obtained by two different techniques, we must assume that the benthic microbial populations in this region have been exposed to hydrocarbons. The most likely source of hydrocarbons in this area is crude oil.

B. Seasonal differences.

Whenever we have compared relative microbial activity in Beaufort Sea waters seasonally, we have observed significant differences summer and winter (January and April) rates. In the winter, the activities are typically ten times lower than in the summer. We have observed about the same difference in our seasonal studies in Kasitsna Bay (Section IV). It is also of the same magnitude as the seasonal changes reported by Carney and Colwell (1976) who attributed this seasonal fluctuation to temperature differences. Our study on the effects of incubation temperature on apparent microbial activity (Fig. 24) shows that the seasonal temperature changes could not account for the seasonal differences that we have observed in the Beaufort Sea. If temperature was the only factor involved, we would anticipate a change by a factor of 2 instead of a factor of 10. An alternative explaination, is that nutrients are limiting in the winter. Under winter conditions, there would be very little if any terrestrial input and very little from phytoplankton until

the first under-ice phytoplankton bloom which often occurs in April (Dr. Horner, personal communication).

If the observed seasonal differences were due to temperature changes, we would expect to find essentially the same magnitude of change in the microbial activity in sediments. This was not observed. Typically, there was a 2 to 3 fold seasonal change in benthic microbial activity. There is, of course, a larger reservoir of nutrients in the sediments than is the overlaying water column. There are undoubtedly enough nutrients in the sediments during the winter months to keep the microorganisms active during this time. The mineralization of nutrients during the winter months would result in microbial biomass which could be used by benthic organisms as a food source at a time when other food sources are minimal. Busdosh and Atlas (1977) have observed that amphipods are very active during the winter months in Elson Lagoon. It is possible that these and other organisms obtain food (either directly or indirectly from microbial biomass during these months. Another byproduct of nutrient mineralization by microorganisms is inorganic nitrogen and phosphorous. These inorganic nutrients, which are required by phytoplankton for growth, may accumulate during the winter months as a result of detrital mineralization by bacteria. The build-up of these nutrients in the winter may supply much of the nitrogen and phosphorous required for the spring phytoplankton blooms. We have observed that crude oil adversely effects this mineralization process. It is therefore very likely that if crude oil became incorporated into marine sediments, that the availability of inorganic nutrients for phytoplankton growth may be significantly reduced (see Section IV for details).

A seasonal variation in the percentage of gluctamic acid respired was noted in seawater samples. In the winter, the average percentage respiration in the water was higher than that found in the summer. This shift in the percentage respiration could reflect the quality and the quantity of nutrients available to the organisms during these two seasons. During the winter months, the nutrient concentrations are presumably low and there may be deficiencies in growth factors required for biosynthesis. As a result, a larger percentage of the utilized glutamic acid is used for the energy requirements of the cells under nutrient-limited conditions. The glutamic acid would thus be respired to CO_2 rather than incorporated into cell material.

This concept is supported by the percentage respiration data collected in sediments. As shown in Table 6, the average percent respiration was lower in the sediments than in the water samples during each of the three field-study periods. Nutrients are known to be concentrated in the sediments. As a result, one would expect to find a greater percentage of glutamic acid being incorporated into cell material in bacteria growing in sediments.

Seasonal variations were also observed in nitrogen fixation rates (Elson Lagoon oiled tray experiment). The lowest rates were observed in the month of January. The highest rates observed were in April and intermediate rates were observed in the summer. This is a different seasonal pattern than that observed during the Kasitsna Bay study (Section IV). In this study, the highest rates were observed in November and the lowest rates in July. These differences suggest that there may be basic qualitative and quantitative

and quantitative differences in the types and relative utilization of sediments of these two regions. In other systems that we have studied, an increase in nitrogen fixation rates usually indicates the availability of a readily utilizable nutrient source with a high carbon:nitrogen ratio. It is possible that, in the Beaufort Sea sediments, the conditions that are favorable for nitrogen fixation do not occur until April, whereas in the sediments of Cook Inlet, they take place in the late fall.

One curious aspect of the seasonal data collected during the Elson Lagoon experiment was the unusually high relative microbial activity associated with the control sediment during the April, 1978 field study. Unfortunately, we analyzed only one control during that field trip. If the mean uptake rates are calculated for all five samples (including the oiled samples which usually have rates lower than the control) the values are 6.2 and 234 ng x g^{-1} x h^{-1} for glucose and glutamic acid respectively. When compared to the other seasonal glucose values observed in Elson Lagoon, these are approximately the same as those observed in August, 1978 and it is 2 times greater than that observed in January, 1978 and 1979 (Table 15). The mean value for glutamic acid uptake was greater in April, 1978 than at any other time. This increased benthic microbial activity comes at the same time as the highest nitrogen fixation rates. We know from the water data that there are no organic nutrients coming into the sediments via the water column at that time. These data suggest that the detrital food chain is being activated in the spring. This is very different from what we have observed in Kasitsna Bay. The net effect of this

activity would be to provide a food source for the benthic community prior to or in conjunction with the spring under-ice phytoplankton bloom. It would also provide some of the inorganic nutrients required for that phytoplankton bloom by mineralization of organic nitrogen and phosphorous.

Another set of variables that show distinct seasonal trends is the total concentration of adenylates and the energy charge that is calculated from the ratios of specific adenylate species (Table 41, Section IV). Since all living things contain adenylates, this assay is not specific for microorganisms. The total concentration of adenylates (ATP + ADP + AMP) can be used as an index of total biomass in the sample analyzed. In our experiments, no organisms larger than 2 mm were included. In August, 1978, the mean adenylate concentration in the controls was 164 nM x g dry wt^{-1}. In January, 1979 the mean was 15 nM x g dry wt^{-1} which was approximately 10 times less than that observed in August. This is a much greater seasonal variation than that observed in Kasitsna Bay (Section IV).

From the adenylate data one can also calculate an energy charge value which should reflect the metabolic state of the population (Wiebe and Bancroft, 1975). In general terms, higher the ratio, the more metabolically active the population as reflected by proportionally higher concentrations of ATP. The summer ratio was approximately 4 times greater than that observed in the winter. The seasonal difference in these ratios was also much greater than that observed in Kasitsna Bay. These data lead us to conclude that the overall metabolism of the benthic community slows down much more during the winter in the Beaufort Sea than it does in Cook Inlet.

C. The role of microorganisms in sea ice.

The sea ice plays an important role in the function of the ecosystem in the Beaufort Sea but almost nothing is known about the effects of freezing and thawing seawater on the natural microflora. It was felt that these processes may have a profound effect on nutrient recycling in these waters. The relative microbial activity in the melted ice was compared with microbial activity in the surrounding seawater. It was found that 6 of the 8 ice samples tested showed activities as high or higher than that observed in the associated seawater (Table 11). There are several possible explanations for this observation: (1) the organisms that do survive freezing are the ones that are most actively utilizing glutamic acid, (2) there is a significant number of marine bacteria that survive freezing and are able to grow so that there is not net loss in activity, (3) many bacteria are not actually frozen but are concentrated into highly saline pockets of water within the ice. Of these possibilities, the latter seems the most likely.

It is currently thought that when seawater is frozen, small pockets of highly saline seawater remain throughout the ice. It is quite likely that nutrients and bacteria are also concentrated in these saline pockets. One study has been made of the number of colony-forming units (CFU) found in ice cores taken in Beaufort Sea ice (R. M. Atlas, personal communication). It was found that the bacterial populations showed a high degree of patchiness throughout the core. These data tend to support the above concept. If the bacteria are concentrated in highly saline pockets, the high salinity itself may afford some degree of freeze-injury protection.

Salinity profiles of waters in the Beaufort Sea (taken when there was a significant icepack melting) showed a shallow lens of brackish water at the surface of the water column. These waters showed relative microbial activities and bacterial cell concentrations that were as high or higher than that found in the much more saline waters at 15 m. This along with the seawater dilution studies shown in Table 12 suggest that the freshwater input from melting sea ice does not significantly alter the parameters studied.

D. Effects of crude oil and the dispersant Corexit 9527 on microbial function.

The results of both short-term and long-term exposure experiments conducted in the Beaufort Sea are reported and analyzed in Section IV of this report. There were, however, several basic differences observed in long-term effects of crude oil between the Elson Lagoon and the Kasitsna Bay studies that need to be stressed. The long-term effects of crude oil took much longer to be expressed in Beaufort Sea sediments than in Kasitsna Bay sediments. Reduced microbial activity was observed in sediments exposed to crude oil for only 5 weeks in Kasitsna Bay. It took up to a year before Elson Lagoon sediments showed the same shift. The same was true with percent respiration changes. From this, it seems quite likely that the effects of crude oil should last longer in Beaufort Sea sediments than in sediments from Cook Inlet.

In the Kasitsna Bay study, we found that the rate of nitrogen fixation can be depressed by 50-95% in oiled sediments. This change required only a few days exposure before measurable changes

could be found. We have not observed a consistent reduction in nitrogen fixation rates in oiled Elson Lagoon sediments. Haines et al. (1980) have reported that denitrification rates are depressed in oiled Elson Lagoon sediments. It appears from their data, that the step affected involves the oxidation of ammonium ion to nitrite. This observation has serious implications relative to the overall impact of crude oil in Beaufort Sea sediments since nitrification is required to convert fix nitrogen in the form of ammonia to nitrate which is the form preferred by phytoplankton. Thus, if this transformation is blocked by the presence of crude oil, the overall productivity of the area could be reduced.

VI. Needs for further study

We feel that most of the cruise data (offshore) that is required for crude oil impact assessment has been collected. This is not true of the inshore areas, especially inshore environments near the major rivers and landward of the Barrier Islands. We feel that there is a very definite need for intensive study of microbial processes in the inshore sediments; especially those functions that relate to primary and secondary productivity. This would include both seasonal field studies and long-term effects studies. We have collected some of these data during the Elson Lagoon study; however, this study has not been comprehensive enough in terms of numbers of samples and geographical representation. We feel that a study of areas adjacent to one or two major rivers along the North Slope would be essential in defining the dynamics of microbial function and the potential effects of crude oil on those essential processes.

There are also some preliminary studies which could be conducted in areas that have already been impacted by crude oil; i.e. the oiled plots used by Dr. Dave Mason (RU #356) during the study "Environmental assessment of selected habitats in the Beaufort and Chukchi Sea littoral systems". Such a study would increase our knowledge of long-term crude oil effects in at least two diverse locations. These data would provide a good basis of comparison with what we have observed in Elson Lagoon and Kasitsna Bay.

One issue that has not been addressed at all in the Beaufort Sea is the effects of drilling muds on benthic microbial function. There are a number of components of drilling mud which could adversely affect a wide range of microbial functions and one component (paraformaldehyde) which would stop all microbial activities; that is why it is placed in drilling muds. A study of drilling mud effects could be conducted using the same methodologies that we are using to assess crude oil and dispersant effects.

Table 7. Data summary of measurements made in samples collected in the Barrow and Prudhoe Bay areas.

Measurement	Units	Barrow			Prudhoe Bay		
		n	$\bar{Y}$	s	n	$\bar{Y}$	s
		Water					
V_{max}*	ng/l/h	37	37	34	13	44	28
Percent respiration*	%	37	62	7	13	54	5
Temperature (*in situ*)	°C	37	1.2	1.5	13	1.0	1.1
Salinity	o/oo	37	22.7	3.1	13	16.7	4.4
pH		37	7.9	0.1	13	7.9	0.1
Bacterial concentration+	10^5 cells/ml	37	4.4	2.9	13	4.5	2.7
		Sediment					
V_{max}*	µg/g dry wt/h	15	0.40	0.37	18	0.83	0.40
Percent respiration*	%	14	41	11	18	32	7
pH		3	7.2	0.1	11	7.5	0.1
Bacterial concentration+	10^8 cells/g dry wt	15	4.4	5.3	18	9.2	12.0

Note: n - number of observation; $\bar{Y}$ = mean value; s = standard deviation.

*These measurements were made using ^{14}C labeled glutamic acid.

+Bacterial concentrations estimated using direct observations with epifluorescent microscopy.

Table 8. V_{max} measurements in water samples taken at stations in Prudhoe Bay.

Date	Station No.	V_{max}*	Distance from shore km	Correlation coefficient
9/8	53	77	0.4	-0.39
"	55	113+	2.1	
"	55	65	2.1	
"	51	38	2.0	
"	54	78	0.8	
9/12	53	60	0.4	-0.85
"	55	69	2.1	
"	56	28	4.0	
"	70	19	5.4	
"	71	20	7.8	
9/13	50	48	0.7	
"	52	32	2.4	
9/14	57	29	4.2	
"	70	12	5.4	
"	71	5	7.8	-0.95
"	72	20	4.3	
"	73	40	2.6	

*V_{max} values reported as ng glutamic acid taken up per litre per hour.

+Sample taken at a depth of 2 m, all others taken at the surface.

Table 9. Comparison of glutamate uptake activity in field station waters and sediment samples.

Area		Water (ng/l/hr)	Sediment (ng/g/hr)
Beaufort Sea	Aug 1976	8	80
	Aug 1978	14	96
Beaufort Sea, Elson Lagoon			
	Jan 1978	4	14
	Apr 1978	0.5	480
	Aug 1978	20	85
	Jan 1979	2	8
Cook Inlet and Shelikof Strait			
	Apr 1977	16	217
	Nov 1977	5	64
	Apr 1978	13	86
Norton Sound	July 1979	19	154

Table 10. Comparison of reports of relative microbial activity in marine water and sediment samples taken in various geographical areas. All measurements were made using ^{14}C labeled glutamic acid. Except as noted, the same methods were used for all studies.

Ocean	V_{max}*	No. of samples	Investigation
	Water samples		
Antarctic	10.9	23	Morita *et al.* (1977)
Antarctic	11.2	8	Gillespie *et al.* (1976)
Tasman Bay New Zealand	40.0	1	Gillespie (unpublished)
Eastern Tropical Pacific+	15.2	10	Hamilton and Preslan (1970)

*V_{max} values are reported as ng glutamic acid taken up per unit of sample per hour. The unit of sample in sediments is 1 g dry weight.

+No CO_2 data included.

Table 11. Relative microbial activity (V_{max}) and cell concentrations in samples of melted ice, associated seawater, and 50/50% mixtures of the two.

Sample No.	Sample type	V_{max}	10^5 cells/ml	Salinity o/oo
		Summer 1975		
82*	Ice	1	-	1.2
83*	Ice	22	-	0.5
84*	Ice	9	-	2.0
81	Water	24	-	25.0
81 + 84	Mixture	22	-	13.5
†		16		
93*	Ice	34	-	9.0
94*	Ice	68	-	6.0
95	Water	28	-	26.0
93 + 95	Mixture	57	-	17.5
†		31		
		Winter 1976		
BI 120	Ice	4.1	1.3	-
BW 120	Water	2.1	1.2	25.5
BI 122	Ice	2.0	1.6	-
BI 122	Water	2.1	2.7	29.0
BW 123	Ice	0.7	2.2	-
BW 123	Water	4.7	1.3	28.0

*Float ice collected at one location.

†Theoretical result of mixing V_{max} values reported as ng glutamic acid per litre per hour.

Table 12. The V_{max} values and cell concentrations in surface waters and water samples taken at 15 m at four stations in the Beaufort Sea.

Station No.	Surface water		Water at 15 m	
	V_{max}*	10^5 cells/ml	V_{max}*	10^5 cells/ml
24	6	3.2	6	2.3
23	27	4.6	5	4.5
22	6	3.0	0.4	1.9
15a	7	3.7	2	5.4

*V_{max} values are reported as ng glutamic acid per litre per hour.

Table 13. Data summary for observations made on all samples taken in the Beaufort Sea.

Measurement	Units	Summer 1975			Winter 1976			Summer 1976		
		n	$\bar{Y}$	s	n	$\bar{Y}$	s	n	$\bar{Y}$	s
V_{max}* (water)	ng/1/h	50	44	30	21	3.1	3.7	16	21	24
V_{max}* (sediments)	μg/g dry wt/h	33	0.61	0.47	14	0.06	0.04	11	0.83	1.20
Percent respiration (water)*	%	50	59	7	23	85	14	16	46	11
Percent respiration (sediment)*	%	33	37	4	14	39	13	11	23	6
Bacterial concentration (water)+	10^5 cells/ml	50	4.5	2.7	23	1.5	0.5	18	3.7	1.3
Bacterial concentration (sediment)+	10^8 cells/g dry wt	33	6.6	8.9	13	10	6.7	11	106	7.9
Salinity	o/oo	50	20.5	5.0	23	24	24	18	15.3	4.7
Temperature	°C	50	1.2	1.0	23	-1.9	-1.9	18	-0.1	0.3

Note: n = number of observations; $\bar{Y}$ = mean value; s = standard deviation.

*These measurements were made using ^{14}C labeled glutamic acid.

+Bacterial concentrations estimated using direct observations with epifluorescent microscopy.

Table 14. A seasonal comparison of V_{max} values observed at several stations.

Station No.	V_{max}*		
	Summer 1975	Winter 1976	Summer 1976
2	30†	1.1‡	–
3	30†	3.5‡	–
15a	–	4.2	7
16	–	2.1	56
21	–	<0.2	4
22	–	0.2	6
23	–	0.2	27
24	–	0.4	6
74	–	2.8	85
80	–	0.7	34

*V_{max} values are expressed a ng glutamic acid taken up per litre per hour.

†Average of four measurements.

‡Average of three measurements.

Table 15. Seasonal comparison of microbial uptake and nitrogen fixation rates in water and sediment samples from Elson Lagoon.

	WATER		SEDIMENTS		
Jan 1978	Glucose[1]	Glutamate[1]	Glucose[2]	Glutamate[2]	N_2fix[3]
401	0.4	4.8	-	-	-
401 ice	0.5	4.5	-	-	-
402	0.3	3.8	3.4	22.6	0.15
403	0.3	3.4	7.8	27.6	0.15
407	-	-	0.2	2.8	0.10
408	0.3	2.4	1.2	6.0	0.11
Mean	0.4	3.8	3.1	15	0.13
Apr 1978					
501	0.3	0.6	-	-	
502	0.3	0.4	-	-	
503	0.1	-	12.2	479	
Mean	0.2	0.5	[6.2][4]	[234][4]	[1.4][4]
Aug 1978					
601	15.6	34.2	2.7	19	1.1
604	2.3	4.6	-	-	-
606	433.9	21.6	-	-	-
607	-	-	13.2	161	0.3
Mean	21	20	8	90	0.7
Jan 1979					
701	0.2	0.6	-	-	-
702	0.2	2.7	-	-	-
703	-	-	2.4	7.0	-
704	-	-	3.7	7.9	
Mean	0.2	1.7	3.1	7.5	-

1 - ng/l/hr
2 - ng/g dry wt/hr
3 - ngN fixed/g dry wt/hr
4 - mean of 5 control and oiled trays

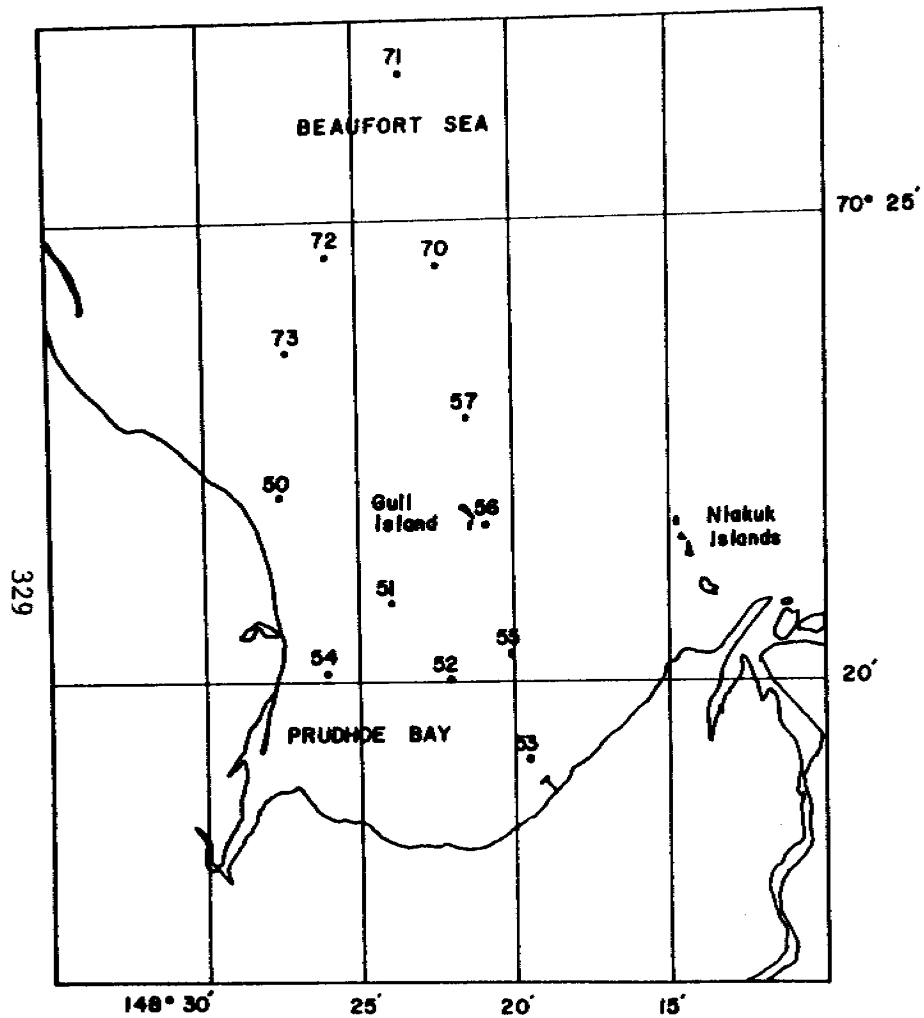


Figure 15. Stations sampled in the Prudhoe Bay area during April, 1976.

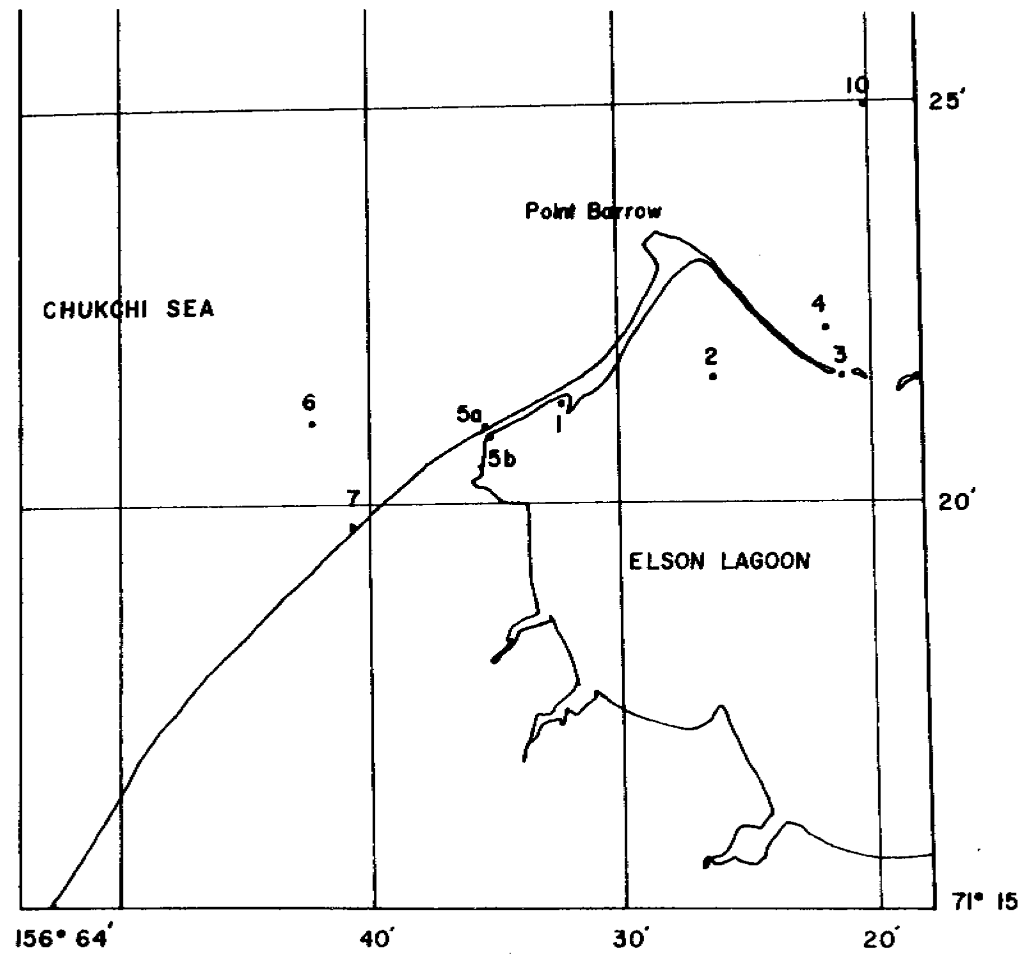


Figure 16. Stations sampled in the Barrow area during April, 1976.

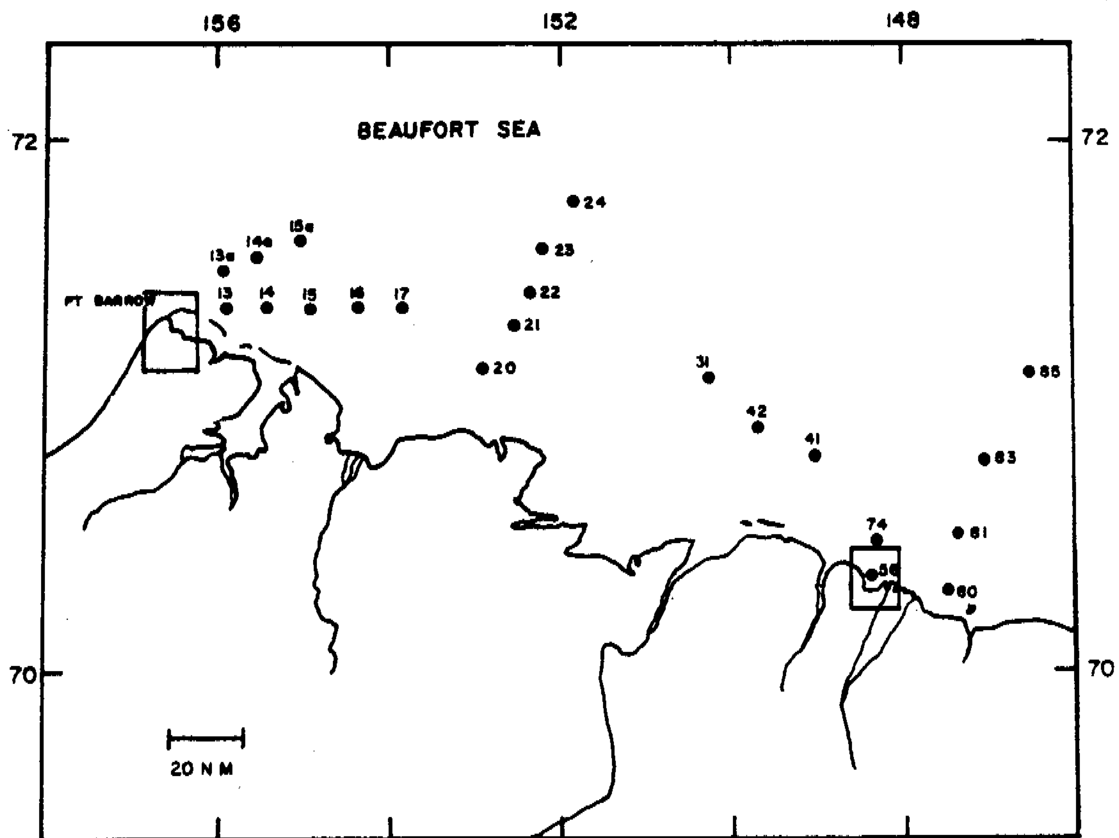


Figure 17. Stations sampled in the Beaufort Sea during April and August, 1976.

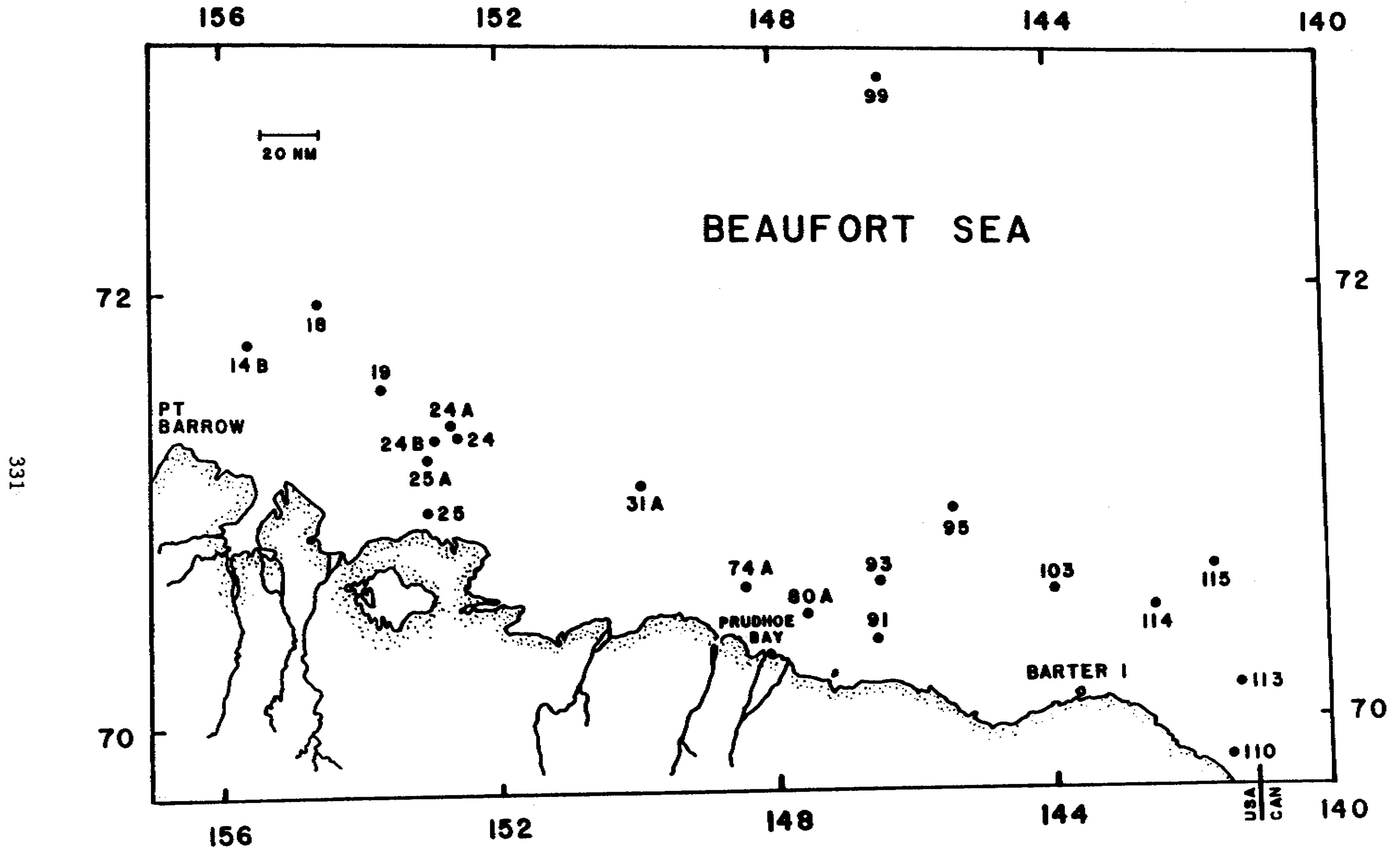


Figure 18. Stations sampled in the Beaufort Sea during the September, 1977 cruise.

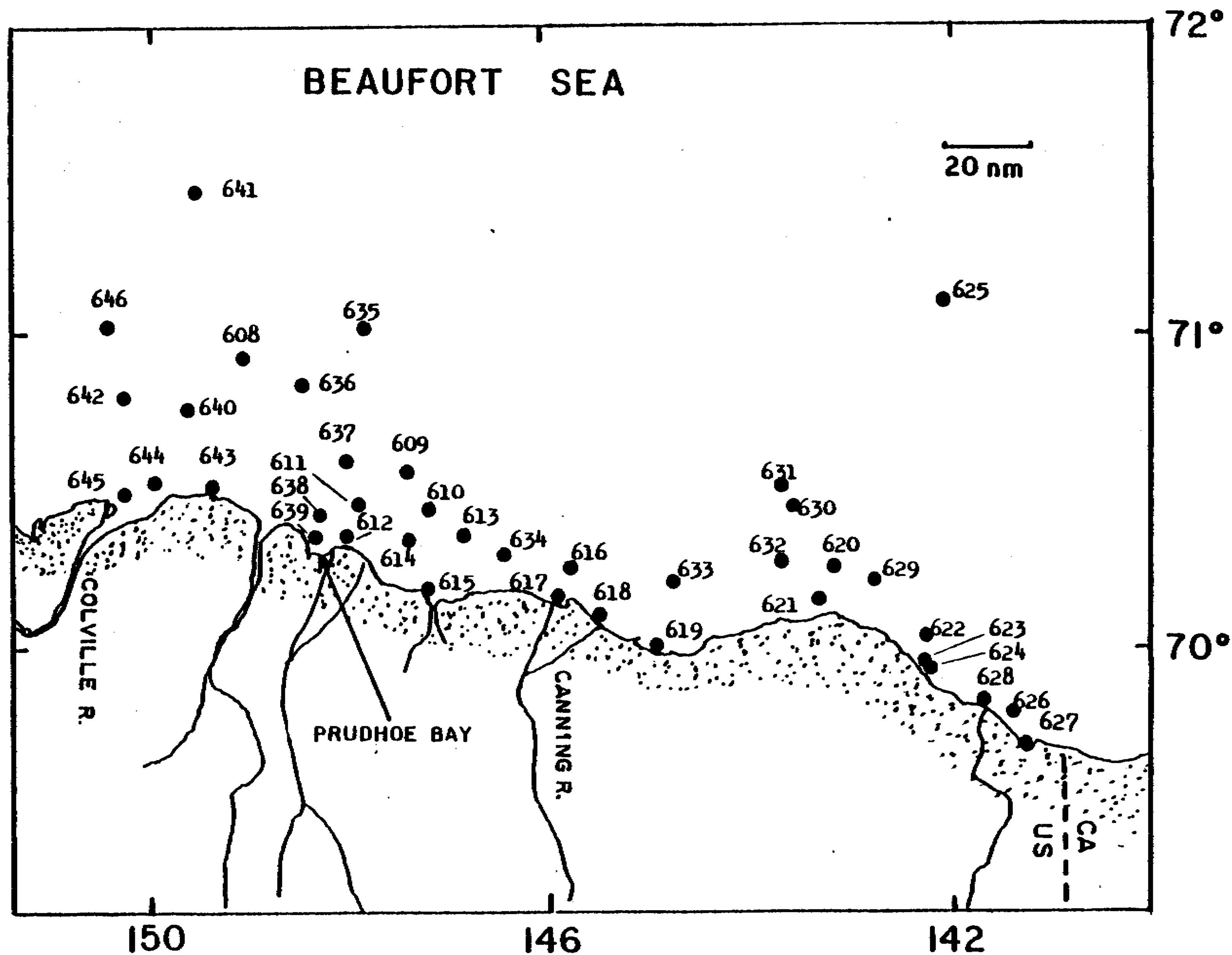


Figure 19. Stations sampled in the Beaufort Sea during the August, 1978 cruise.

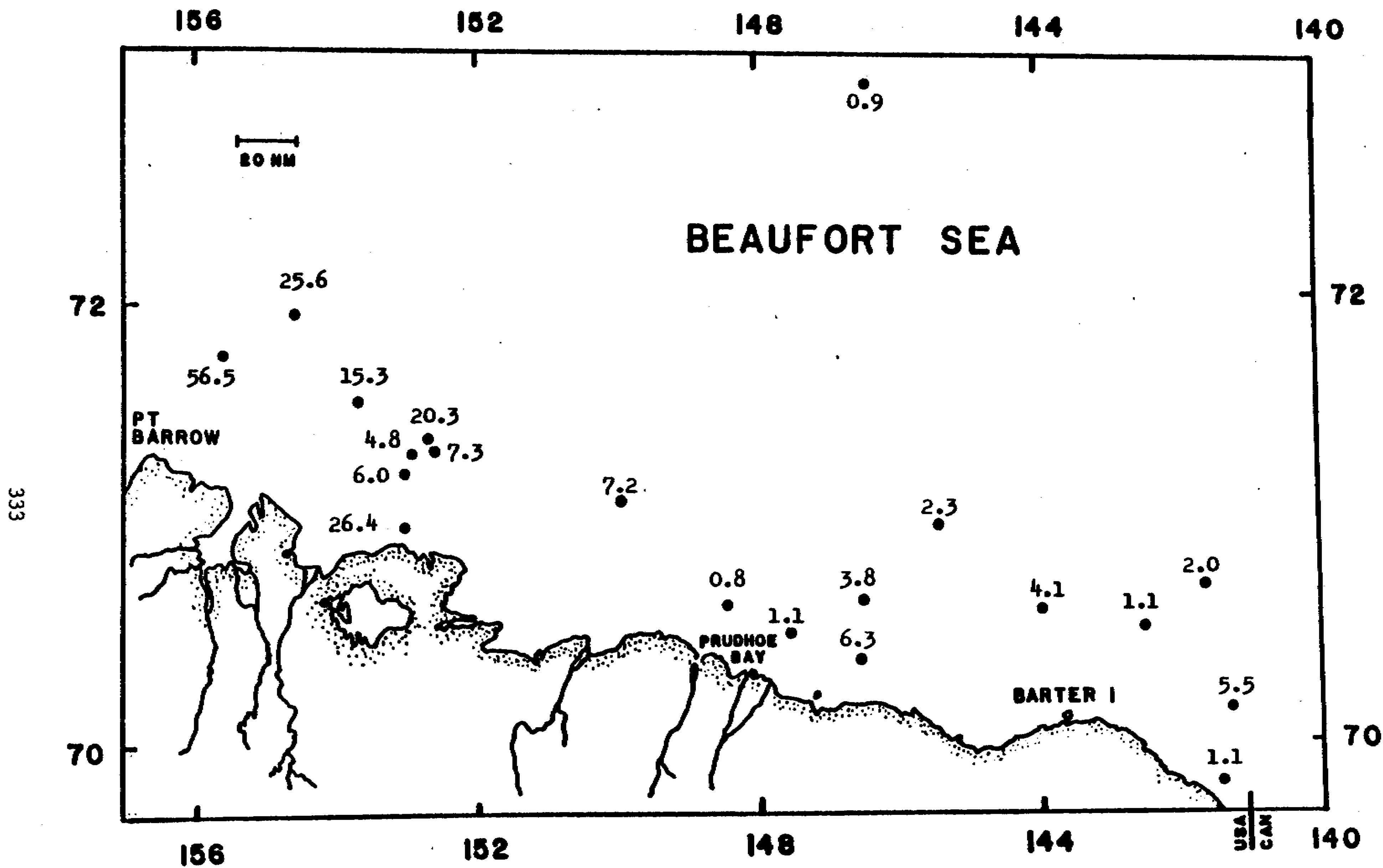


Figure 20. Relative microbial activity in sediments during the September, 1977 cruise. The units are ng glucose/g dry wt./h.

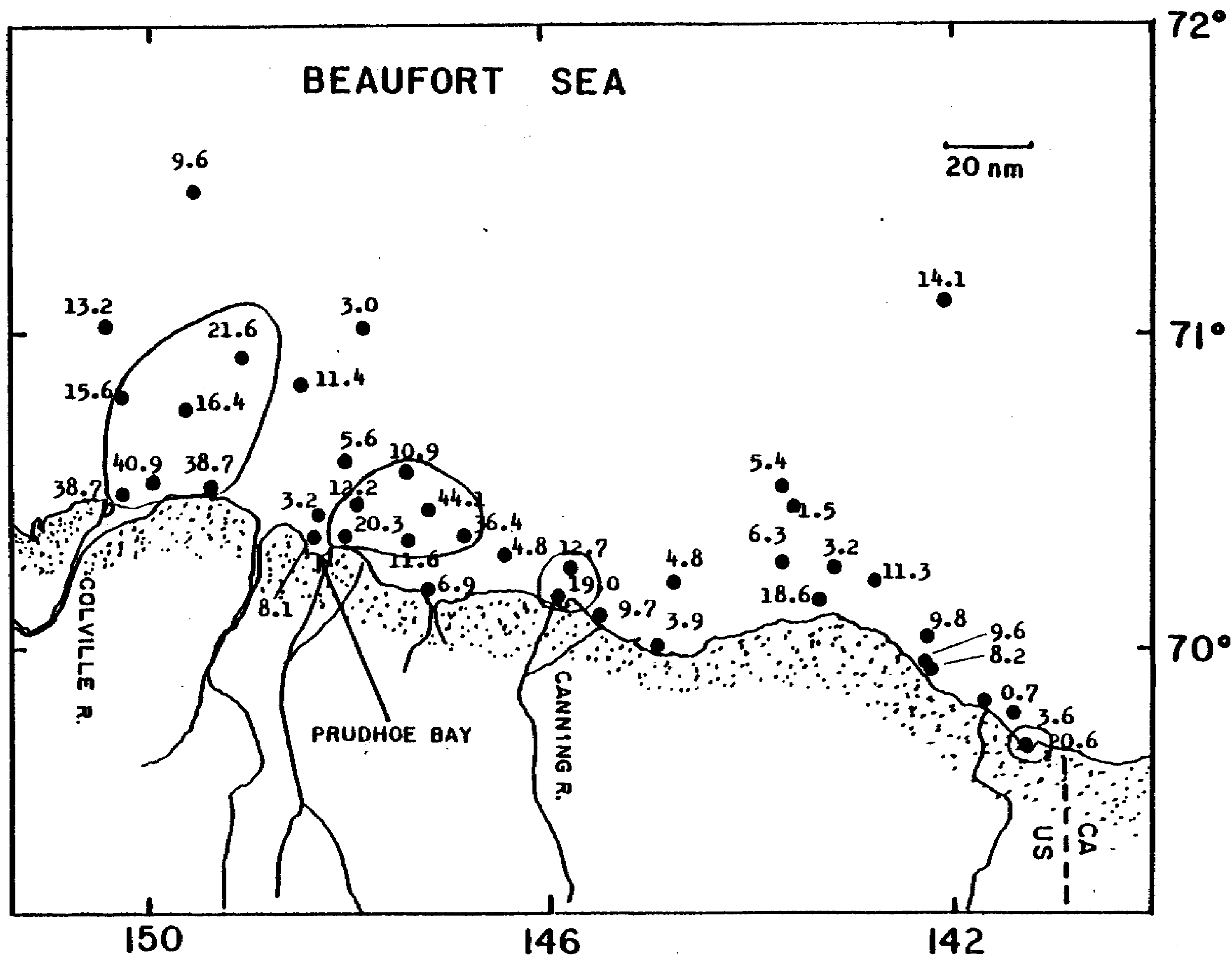


Figure 21. The rate of glutamic acid uptake in water samples collected in August, 1978. The units are µg/litre/h.

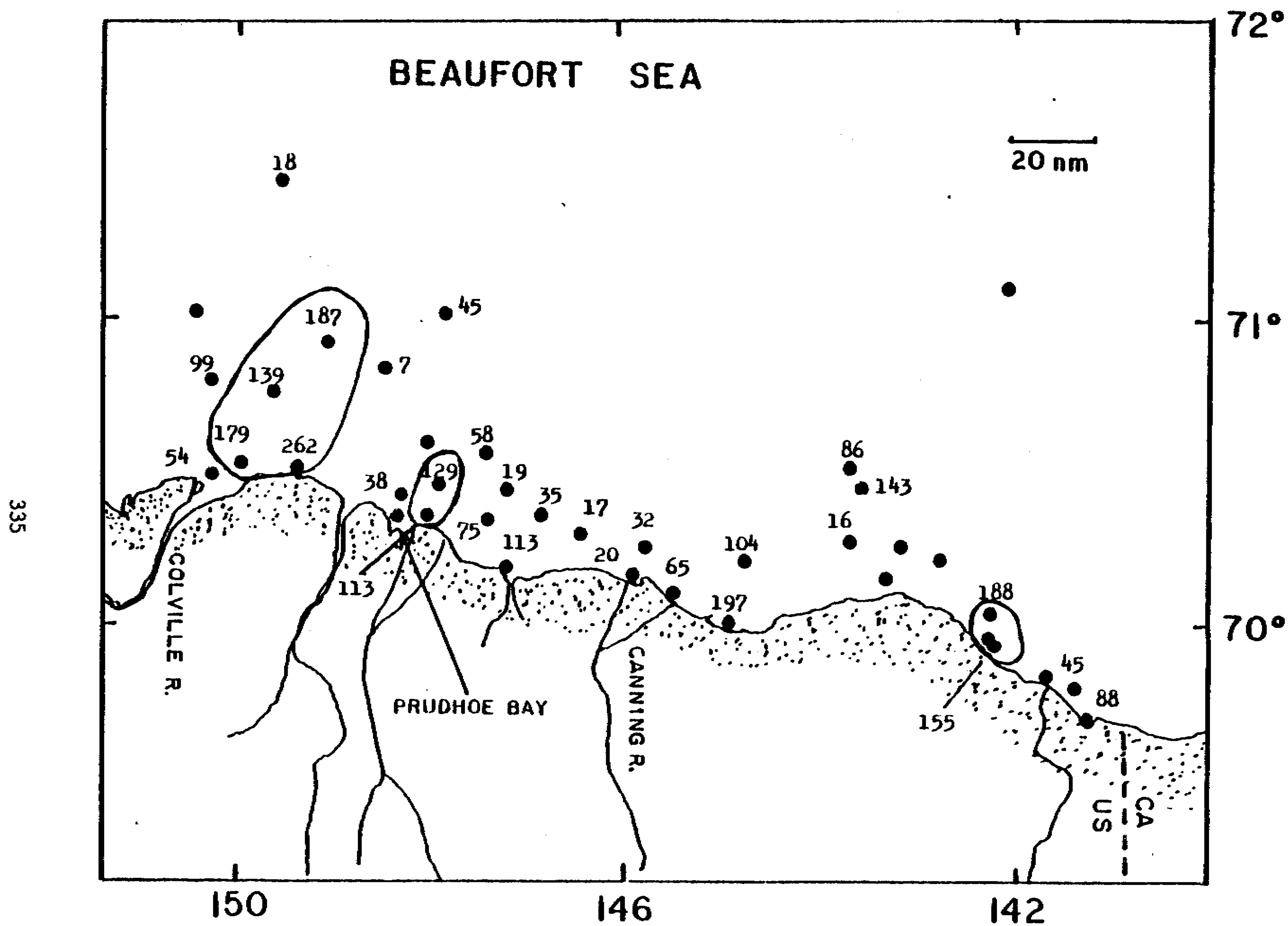


Figure 22. Glutamic acid uptake in sediments collected in August, 1978, expressed as ng/g dry wt./h.

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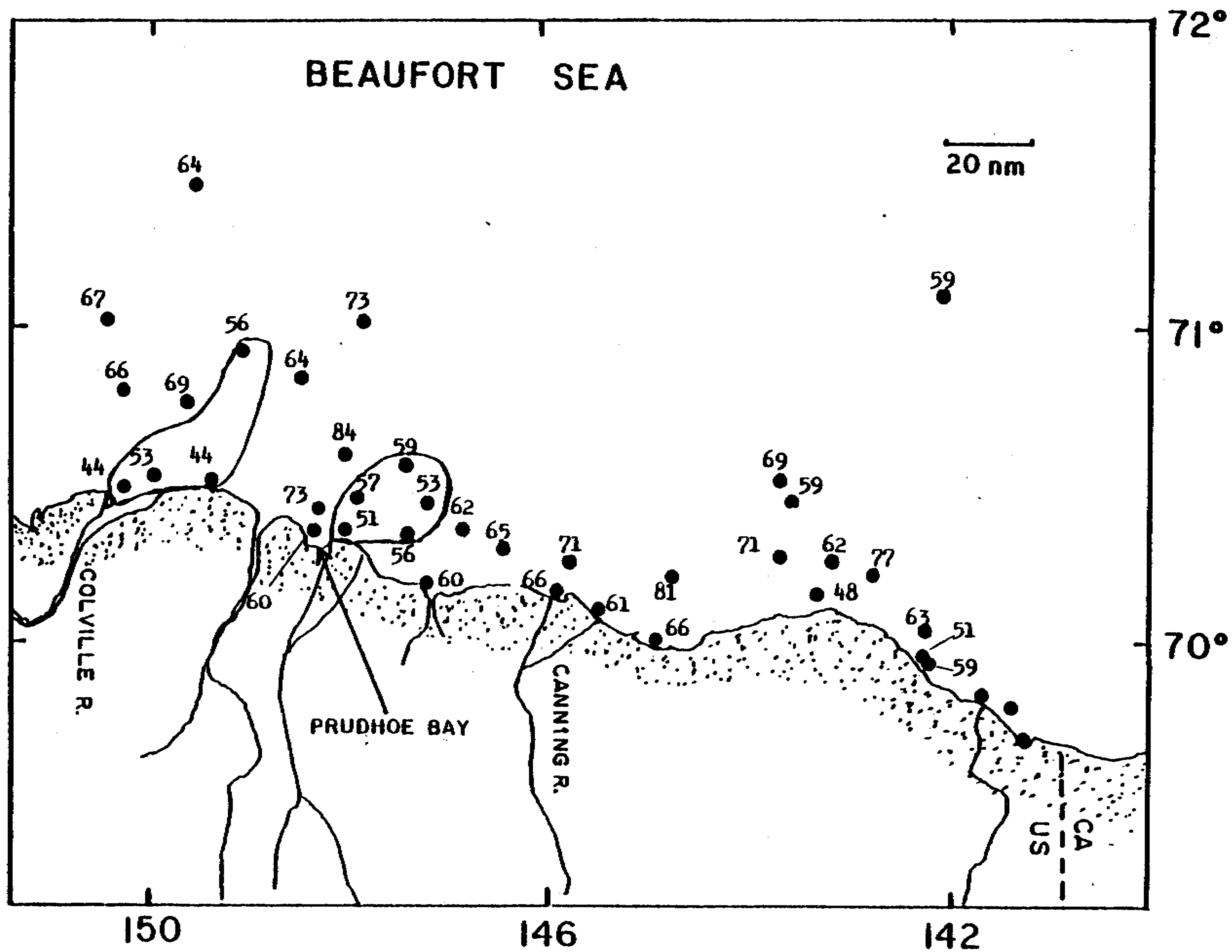

Figure 23. Glutamic acid percent respiration in water samples collected in August, 1978.

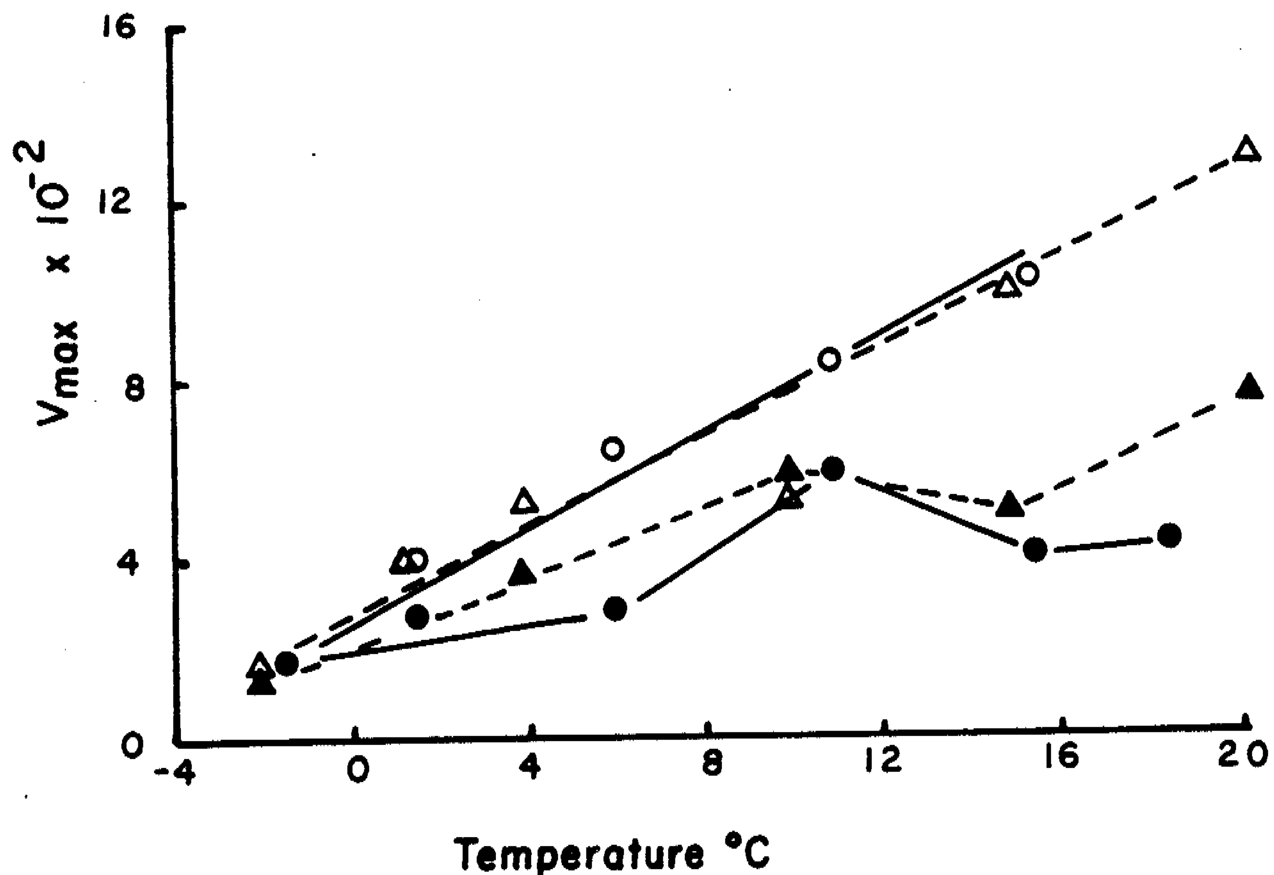

Figure 24. The effect of incubation temperature on the maximum velocity of glutamic acid uptake (V_{max}) in natural microbial populations of water samples taken at stations 5a and 5b. (△)(○), measurements made on samples taken at station 5a. (▲)(●), measurements made on samples taken at station 5b. V_{max} values reported as ng glutamic acid taken up per litre per hour, in units of 10.

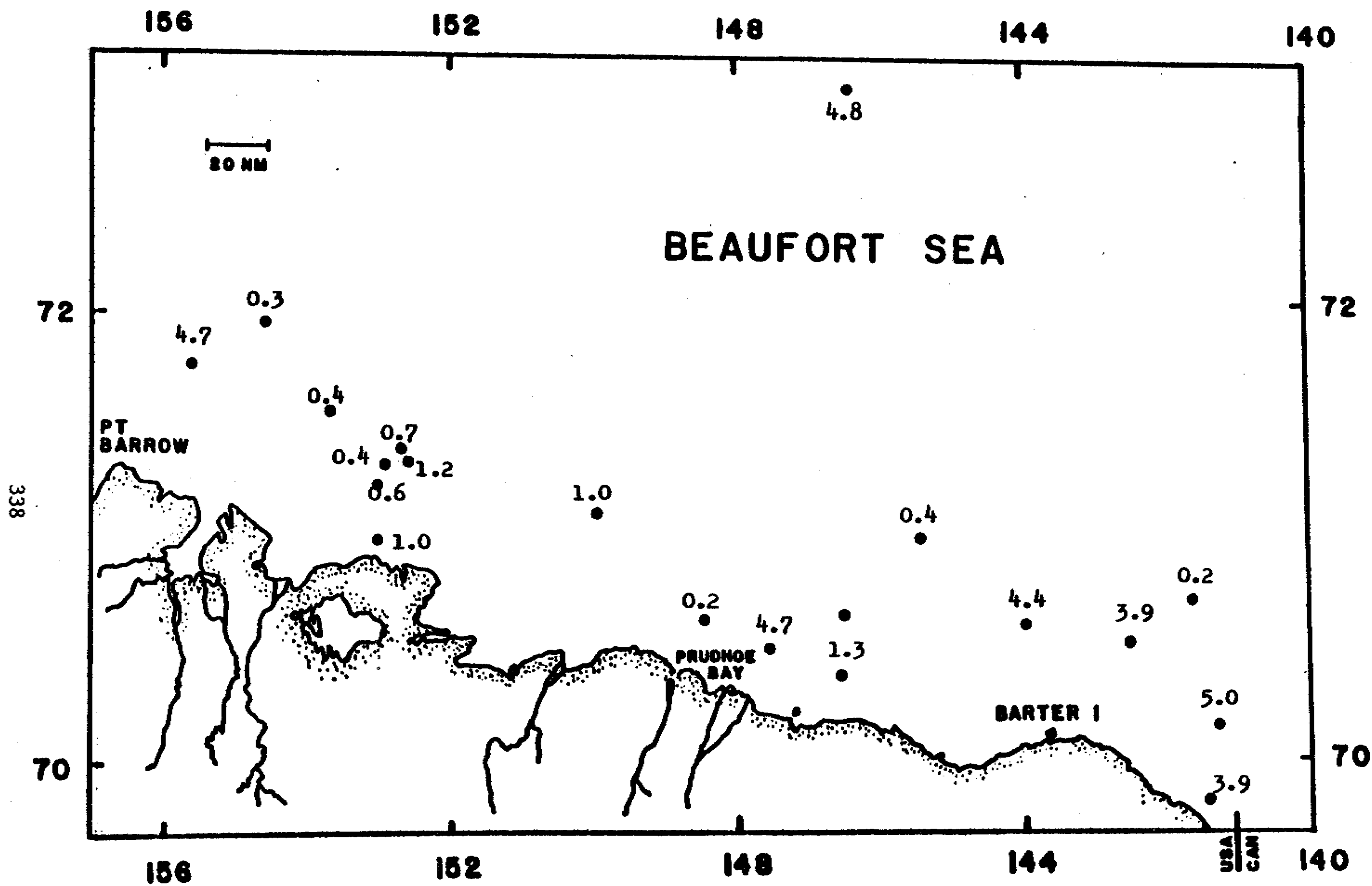

Figure 25. Crude Oil biodegration potentials in sediments collected during September, 1977, expressed as thousand DPM/g dry wt.

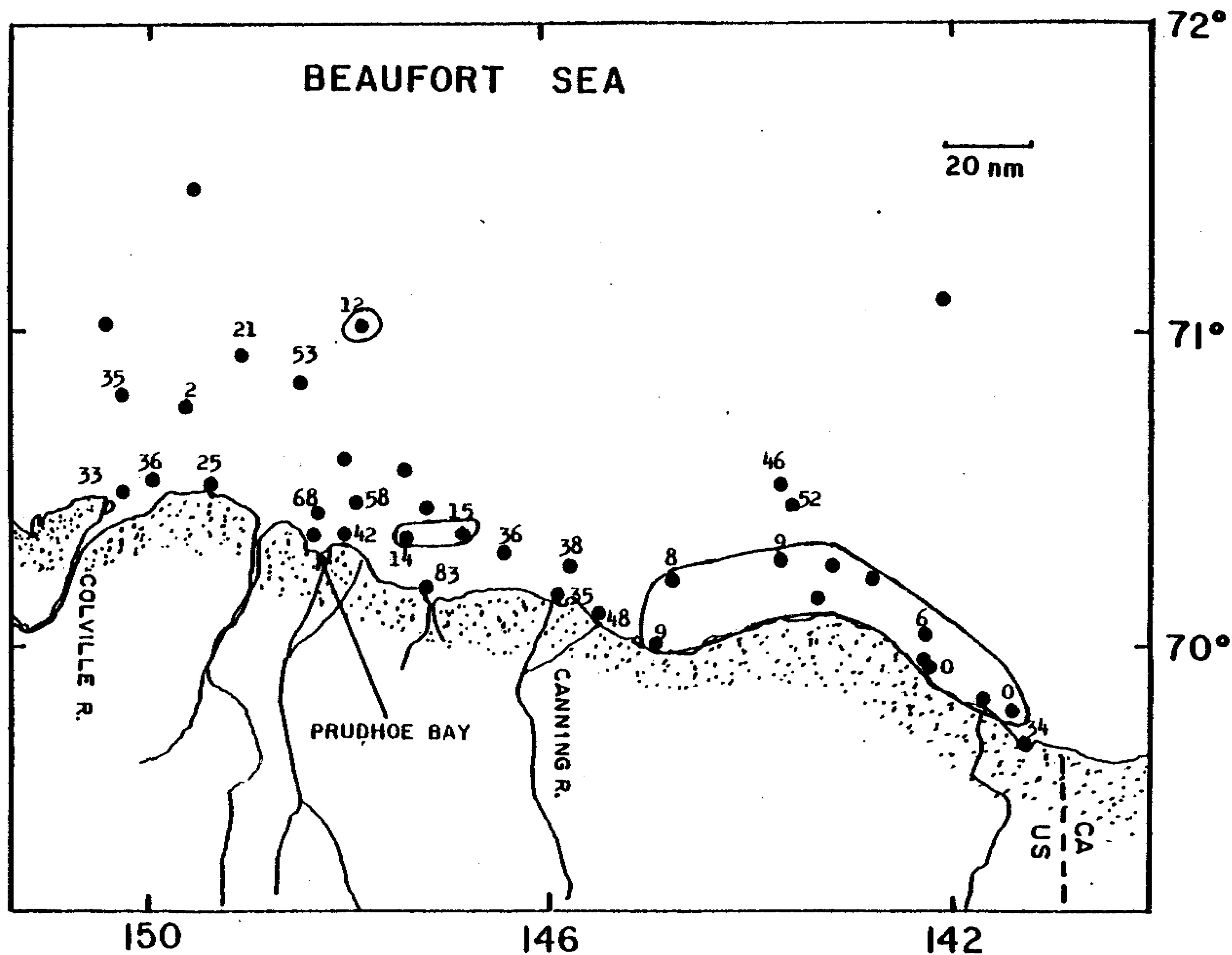

Figure 26. Percent reduction in the rate of glucose uptake in sediment samples exposed to Prudhoe crude oil when compared to nonoiled samples. Sediments were collected during the August, 1978 cruise.

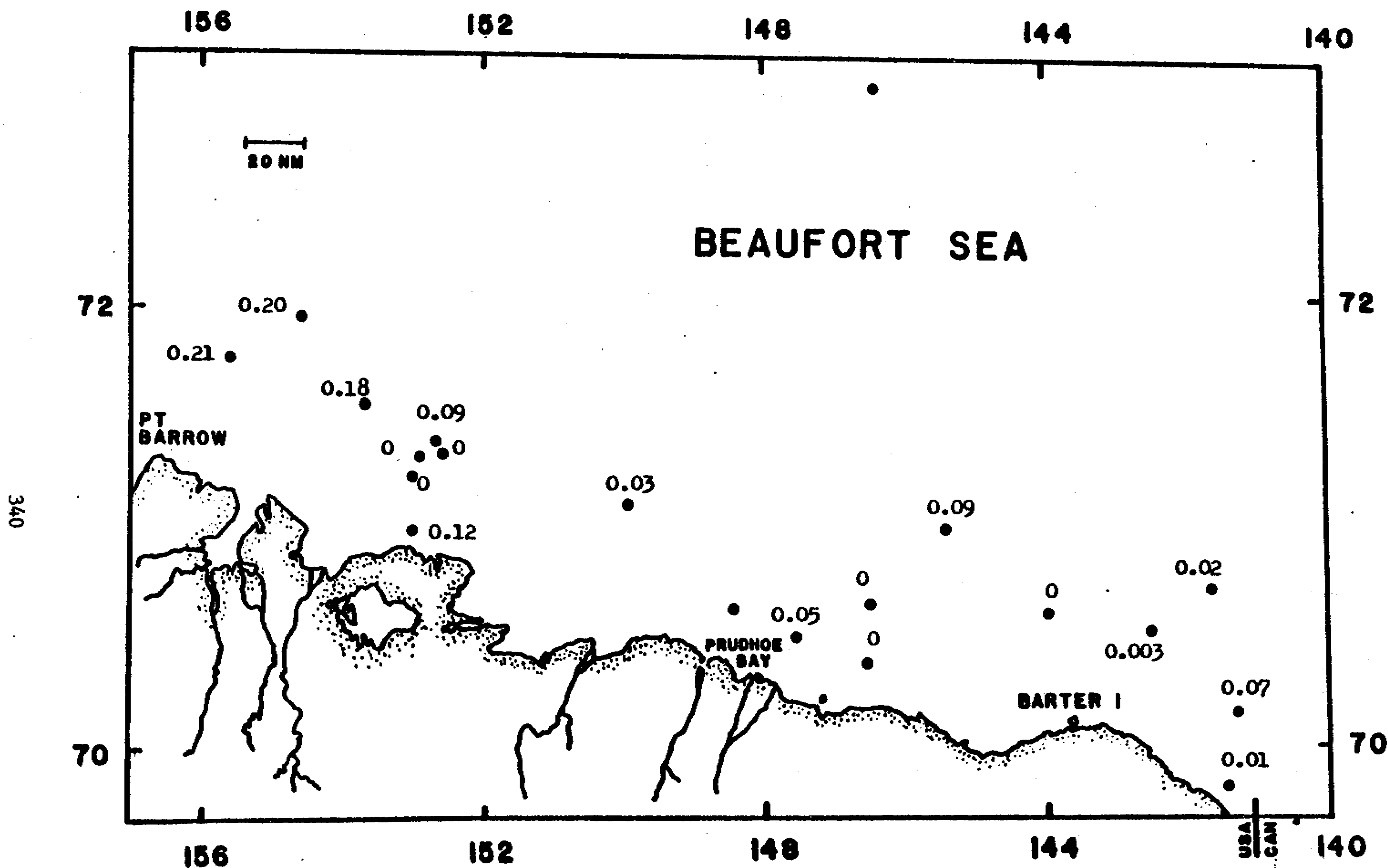

Figure 27. Nitrogen fixation rates in sediments collected during the September, 1977 cruise, expressed as ng N_2 fixed/g dry wt./h.

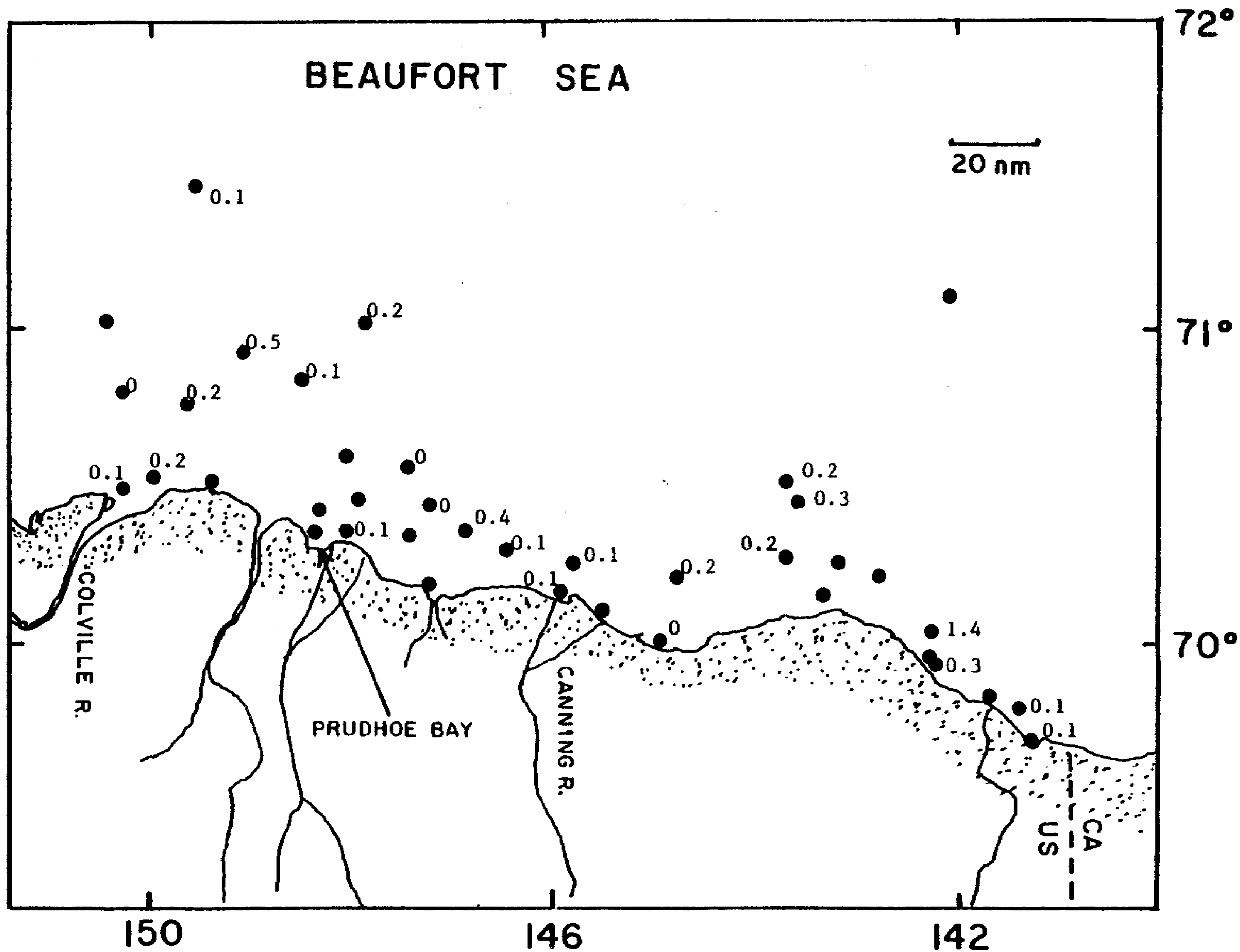

Figure 28. Nitrogen fixation rates in sediments collected during the August, 1978 cruise, expressed as ng N_2 fixed/g dry wt./h.

NORTON SOUND

Section III

I. Summary of objectives, conclusions, and implications with respect to OCS oil and gas development.

A. Objectives.

Our main objective during the July, 1979 Norton Sound cruise was to determine if the microorganisms in areas near the gas vents in Norton Sound showed altered activity which would indicate the presence of petroleum hydrocarbons. There had been a study conducted in this area by Cline and Holmes (1977) which indicated there might be a natural seep of petroleum hydrocarbons in the Norton Sound to the south of Nome, AK. We were to coordinate our research efforts with those of Drs. Cline, Kaplan, and Feeley and with Dr. Atlas. In addition, we were to conduct measurements of relative microbial activity in both water and sediment samples and measure rates of nitrogen fixation in this region. There had not been any previous studies of microbial function in Norton Sound, thus we were to provide information to help fill a significant data gap that had existed in this region. It was important to obtain these data since it was assumed that a significant terrestrial nutrient input was coming into this area from the Yukon River.

B. Conclusions and implications.

1. Data collected by Dr. Atlas, Dr. Cline, Dr. Kaplan, and ourselves indicate that the gas seep south of Nome that had previously been reported by Cline and Holmes (1977) did not contain significant levels of petroleum hydrocarbons. In fact, Kaplan, who was studying heavy hydrocarbons in the sediments of Norton Sound, did not find any evidence for the presence of petroleum hydrocarbons in this

whole area. Our data did not show any consistent patterns of reduced acute crude oil effects in either the waters or the sediments of this area (reduced acute effects in this application would indicate prior exposure to petroleum hydrocarbons).

2. Patterns of relative microbial activity and percent respiration show that there is a significant input of terrestrial carbon coming from rivers flowing into Norton Sound. The same patterns were observed here as we have seen in both Cook Inlet and Beaufort Sea where there is a significant freshwater input from a large landmass drainage. Similar patterns were also seen in the relative microbial activities in the sediments.

3. With the significant terrestrial carbon input into Norton Sound, there is a good likelihood that incorporation of crude oil into the sediments of Norton Sound could significantly reduce the overall productivity of this area.

4. The sediments along the north coast of Norton Sound showed the highest nitrogen fixation rates. If crude oil became incorporated into sediments in this area, the most significant rates of nitrogen fixation in Norton Sound would be affected.

II. Study area

During this cruise, we collected 62 water and 35 sediment samples at the locations illustrated in Fig. 29.

III. Methods

The methods used during this cruise were essentially the same as those described in Section I of this report.

IV. Results

A. Relative microbial activity

1. The mean values for relative microbial activity using both glucose and glutamic acid in the waters and sediments of Norton Sound were generally higher than that observed in the Beaufort Sea during two cruises (Table 16). These differences are not considered significant, however, because most of the locations sampled in the Norton Sound were in the unusually active region associated with Yukon River terrestrial carbon input. If we compare the relative microbial activity in Norton Sound with similar areas in the Beaufort Sea where there is terrestrial carbon input from major rivers, there is very little difference. The same general statement can be made when comparing Norton Sound data with that collected in the Cook Inlet (see Table 2, Section I).

2. Geographical distribution.

In the Norton Sound, we have seen the same patterns of relative microbial activity and respiration percentages that we observed in areas of major terrestrial carbon input in both Cook Inlet (Section I) and in the Beaufort Sea (Section II). The patterns of surface water salinity (Fig. 30) show that there are two major water masses in the area. One of these water masses has characteristics similar to open ocean water that we have studied in the past. This water mass was found in a region which was within a 75 mile radius from the eastern tip of St. Lawrence Island. These waters showed high salinity, low relative levels of microbial activity and high respiration percentages in the microbial populations (Figs. 31 and 32). The waters analyzed at the other locations showed the reverse pattern.

The lower salinities and higher relative levels of microbial activity show the impact of the freshwater input from the Yukon River (a major feature of the Norton Sound). A statistical analysis of the relationship between salinity and relative microbial activity indicates that there is an inverse relationship that is significant at the $p<0.0005$ level. The highest levels of microbial activity were observed in freshwater from the Yukon River.

If the water samples that are associated with the high salinity to the west (group A) and the water associated with the low salinity (group B) are compared, the differences become apparent. The mean rate of glucose uptake in group A was 1/2 that of group B but the significance of this difference with 45 degrees of freedom was only $p = 0.068$. If the same comparison is made with the respiration percentages measured at the same time, the mean value for group A was 38% and the mean value for group B was 24%. With 45 degrees of freedom, the significance of this difference was very high with $p = 0.000004$.

In this region, there are also two water masses which form during the summer months in Norton Sound. One is located a few meters from the bottom which is colder and more saline and one on top of this that extends to the surface. We found the mean rate of glucose uptake (relative microbial activity) to be twice as high in the bottom waters as that observed in the overlaying waters. This comparison was made at 14 locations and the significance of this difference was at the $p = 0.02$ level.

There were also geographical trends found in glucose uptake rates in the sediments of Norton Sound (Fig. 33). With the exception

of the sample collected at station 41, the highest rates were observed in sediments collected in the fine-grained sediments in eastern Norton Sound.

B. Nitrogen fixation in Norton Sound sediments.

The mean nitrogen fixation rate observed during this cruise was approximately equal to that observed in the Beaufort Sea, but it was significantly lower (roughly 1/2 the rate) than that observed in the Shelikof Strait (see Table 4 in Section I). The highest rates of nitrogen fixation were observed along the north coast (Fig. 34).

C. Crude oil effects studies.

During this cruise, we measured the acute effect of crude oil on the uptake of glucose in both water and sediment samples (Figs. 35 and 36). The percent reduction in glucose uptake was determined by comparing the uptake rates of control samples with those exposed to crude oil. No significant geographical trends were noted.

V. Discussion

The patterns of relative microbial activity, respiration percentages, and nitrogen fixation rates all suggest that there is a significant input of terrestrial carbon from the Yukon River and other runoff from the areas around Norton Sound. The relatively low salinity and high microbial activity found in Norton Sound surface waters are an indication of this carbon input. The low respiration percentages in these waters suggest that the microbial population is actively growing because proportionately more of the carbon that is being utilized by the microorganisms is being incorporated into biomass than is respired as CO_2

If the natural rates of nitrogen fixation are compared with denitrification rates (Table 5, Section I), it can be seen that the denitrification rates are much higher. It is felt that this condition reflects the input of exogenous organic carbon into the system. In the Norton Sound, the most likely source of the organic carbon would be the Yukon River. The high rates of microbial activity in the center and eastern end of Norton Sound also reflects the input of exogenous carbon.

The high microbial activity in the bottom waters of Norton Sound probably reflect the release of organic carbon from the sediments or a suspension of particles from the sediments. This is another case in which a distinct water mass can be characterized by measuring microbial function. In every major region that we have now studied, we have been able to characterize large water masses by studying microbial activity and/or respiration percentages of these same populations.

One of the basic assumptions on which this cruise was based was that there was a petroleum seep in the Norton Sound. The methods that we used to determine chronic exposure of microbial populations to petroleum

hydrocarbons showed no consistent patterns which would indicate the chronic input of petroleum hydrocarbons. Using a different methodology, Dr. Atlas also came to the conclusion that there were no chronic inputs of petroleum hydrocarbons. The recent studies conducted by the hydrocarbon chemists have also come to the same conclusion.

VI. Needs for future research.

The data collected during this cruise should be considered as a small part of a much larger study that has to be conducted in the Bering Sea. At the present time, there is very little known about microbial processes in the Bering Sea. This is a very important region for a number of reasons. There is a large number of oil-lease tracts which are to be leased in the Bering Sea. Several of these have high potential for production of significant amounts of petroleum hydrocarbons. At this time, we do not know what the impact the production of petroleum will have on microbial processes in this region. The preliminary work that we have done in Norton Sound indicates that this system may work in essentially the same way as that found in the Beaufort Sea. We also know that crude oil affects microbial processes in different ways when we compare long-term effects in Beaufort Sea and Cook Inlet sediments. Since the Bering Sea represents a transition between these two extremes, it would be a very important region to study. We therefore recommend that both comprehensive cruise data and long-term crude oil effects data be collected in the Bering Sea.

Table 16. Comparison of microbial uptake rates for Norton Sound and Beaufort Sea summer water and sediment samples.

Location		Water				Sediment			
		Glucose[1]		Glutamate[1]		Glucose[2]		Glutamate[2]	
		mean	range	mean	range	mean	range	mean	range
Norton Sound	Jul 1979	12	1.1-110	19	1.5-182	28	0.1-154	127	3-1063
Beaufort Sea	Aug 1976	5	1-13	8	0.5- 24	4	1-15	80	20-180
Beaufort Sea	Aug 1978	7	1-44	14	1-14	9	1-24	96	7-262

1 - ng/l/hr

2 - ng/g dry wt/hr

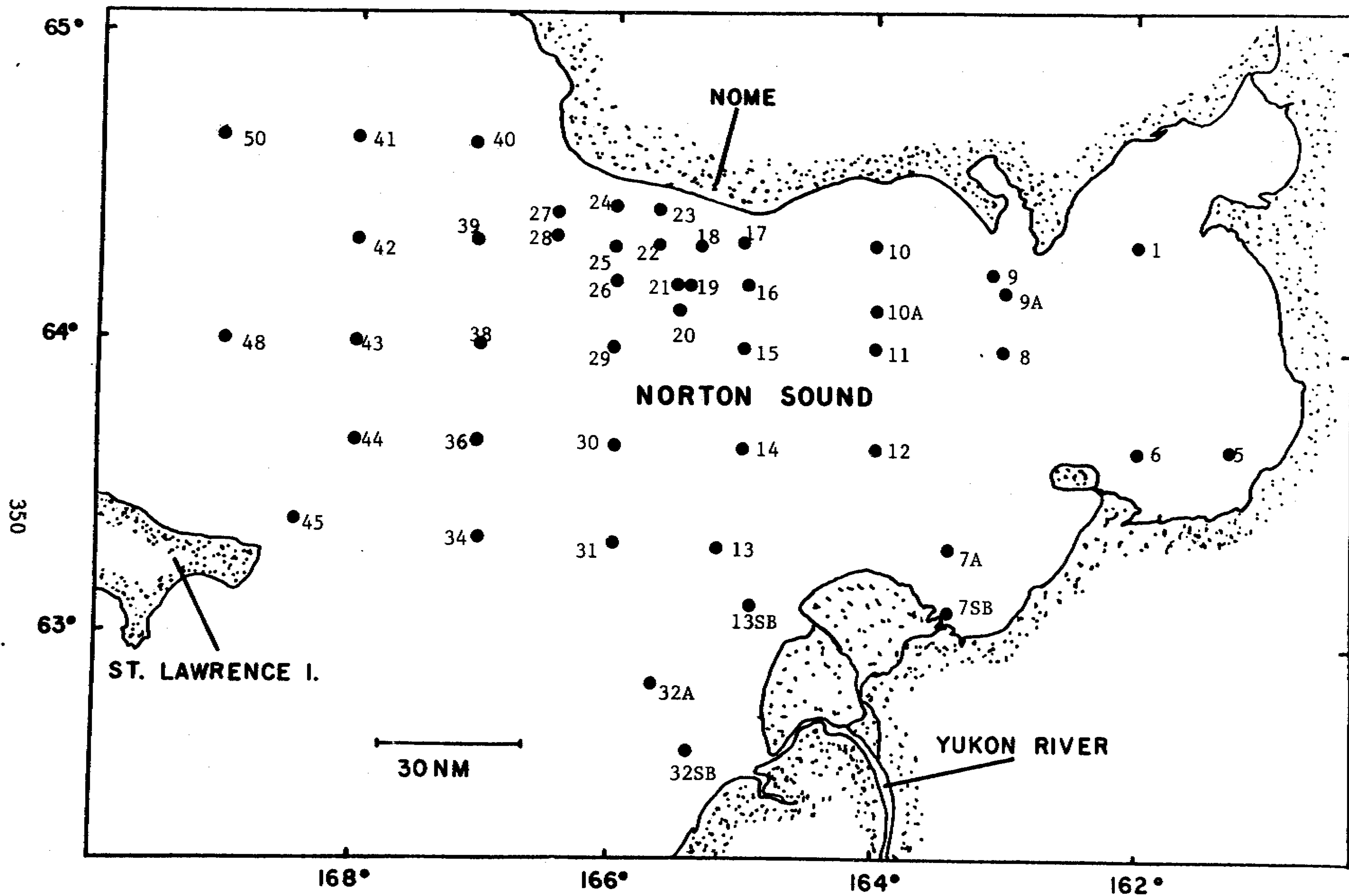

Figure 29. Stations sampled during the Norton Sound cruise in July, 1979.

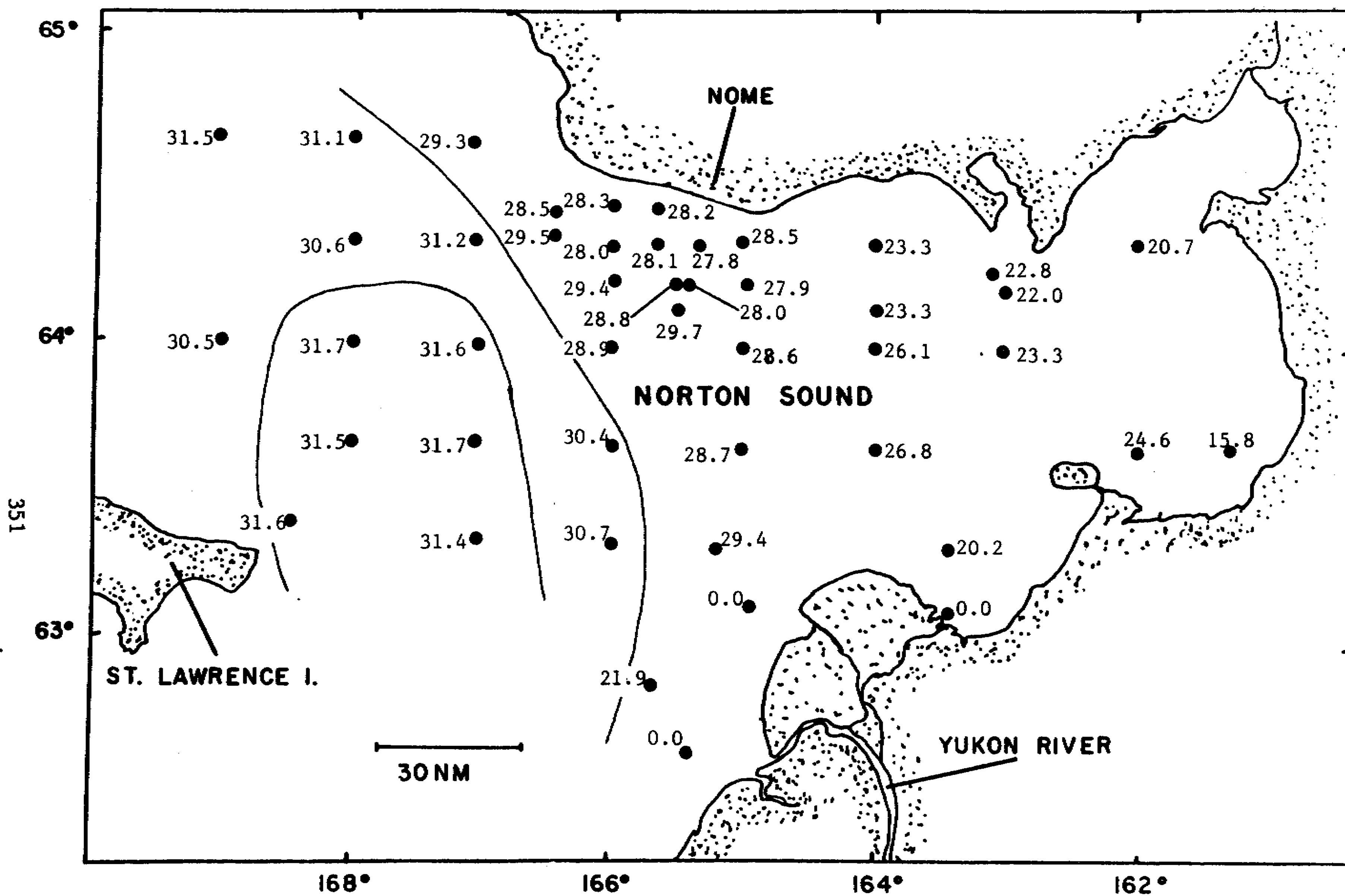

Figure 30. Surface water salinity as parts per thousand during the July, 1979 cruise.

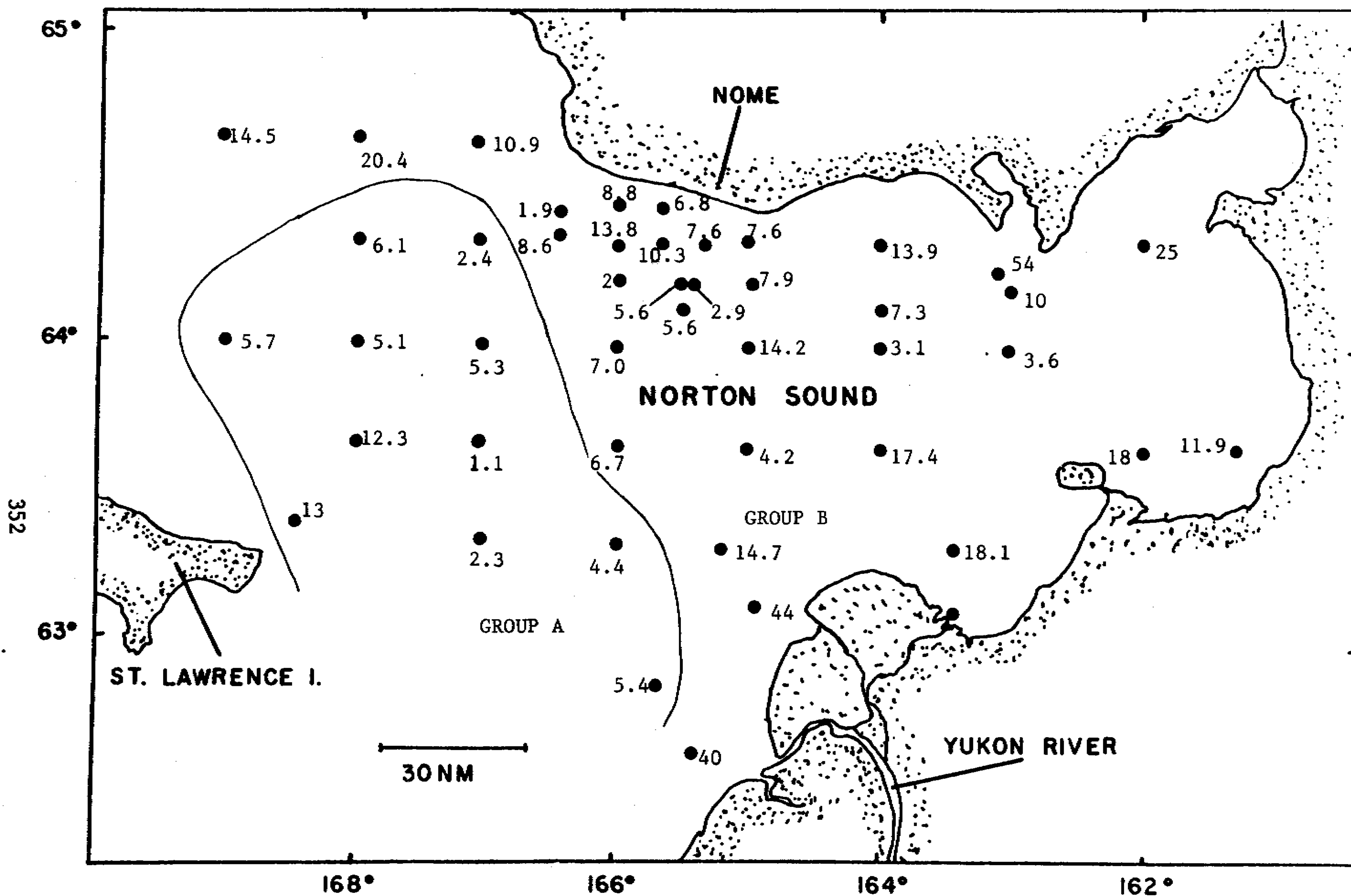

Figure 31. Glucose uptake in ng/l/h, in surface water samples during the July, 1979 cruise.

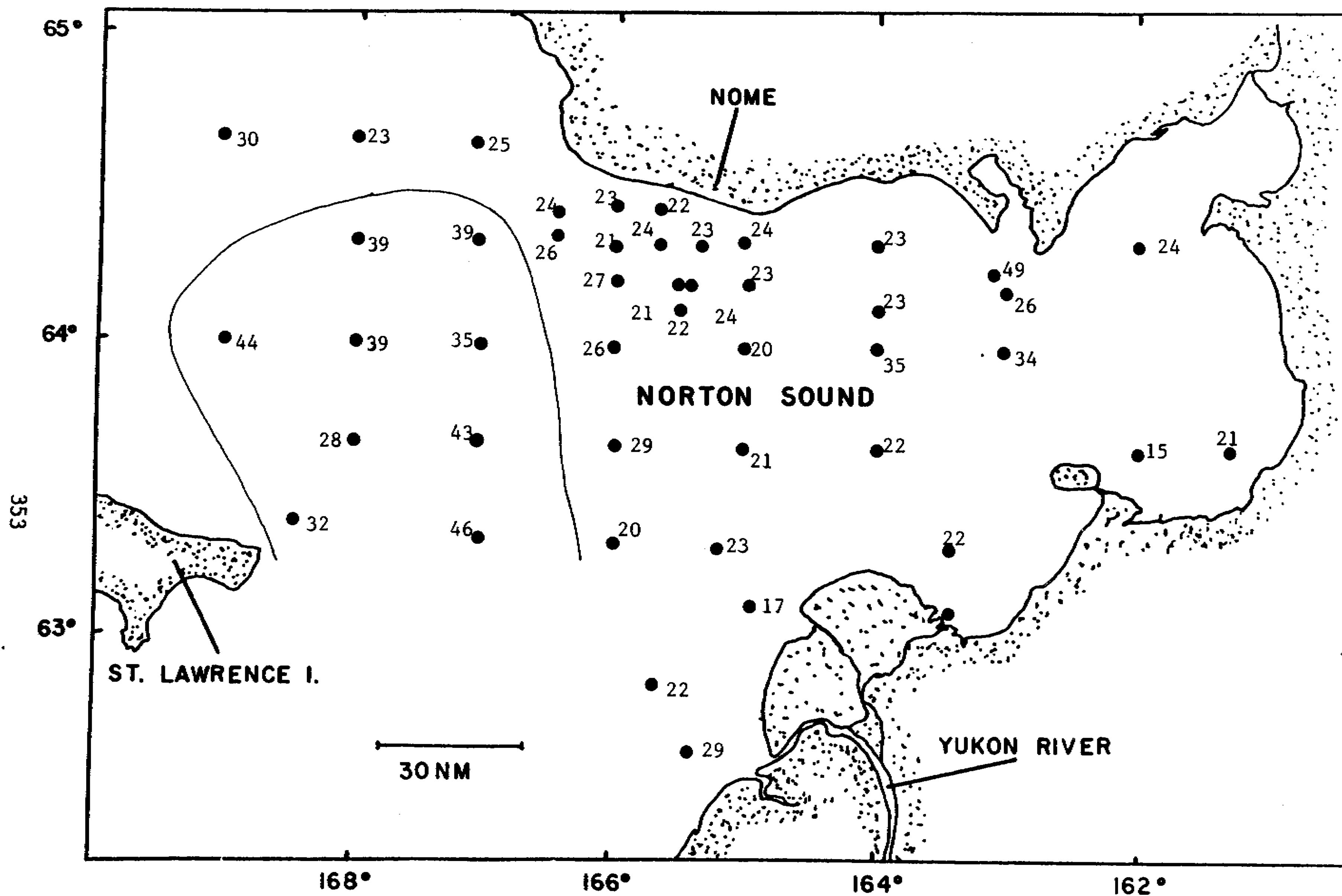

Figure 32. Glucose percent respiration in surface waters during the July, 1979 cruise.

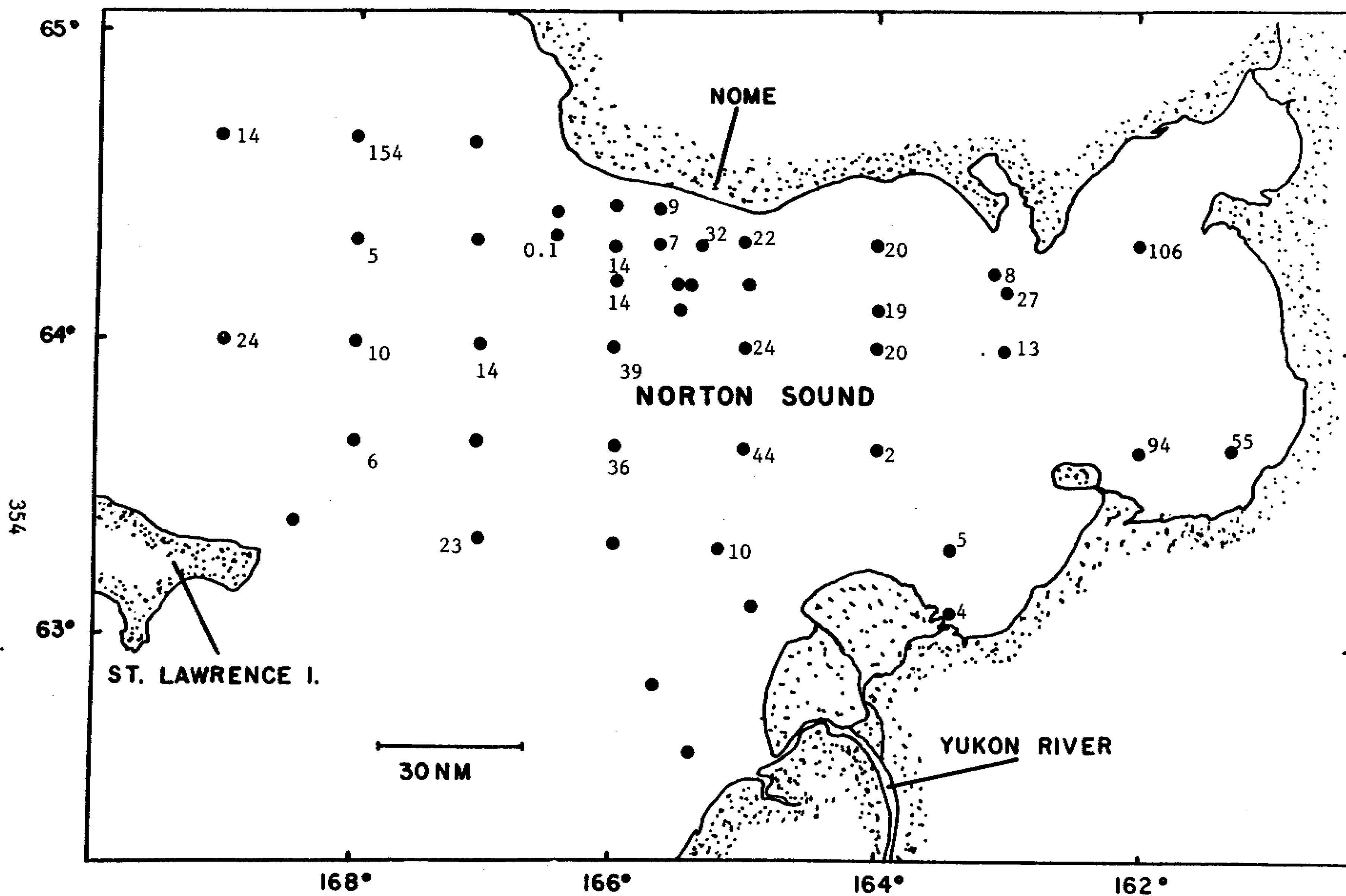

Figure 33. Glucose uptake in sediments, expressed as ng/g dry wt./h., during the July, 1979 cruise.

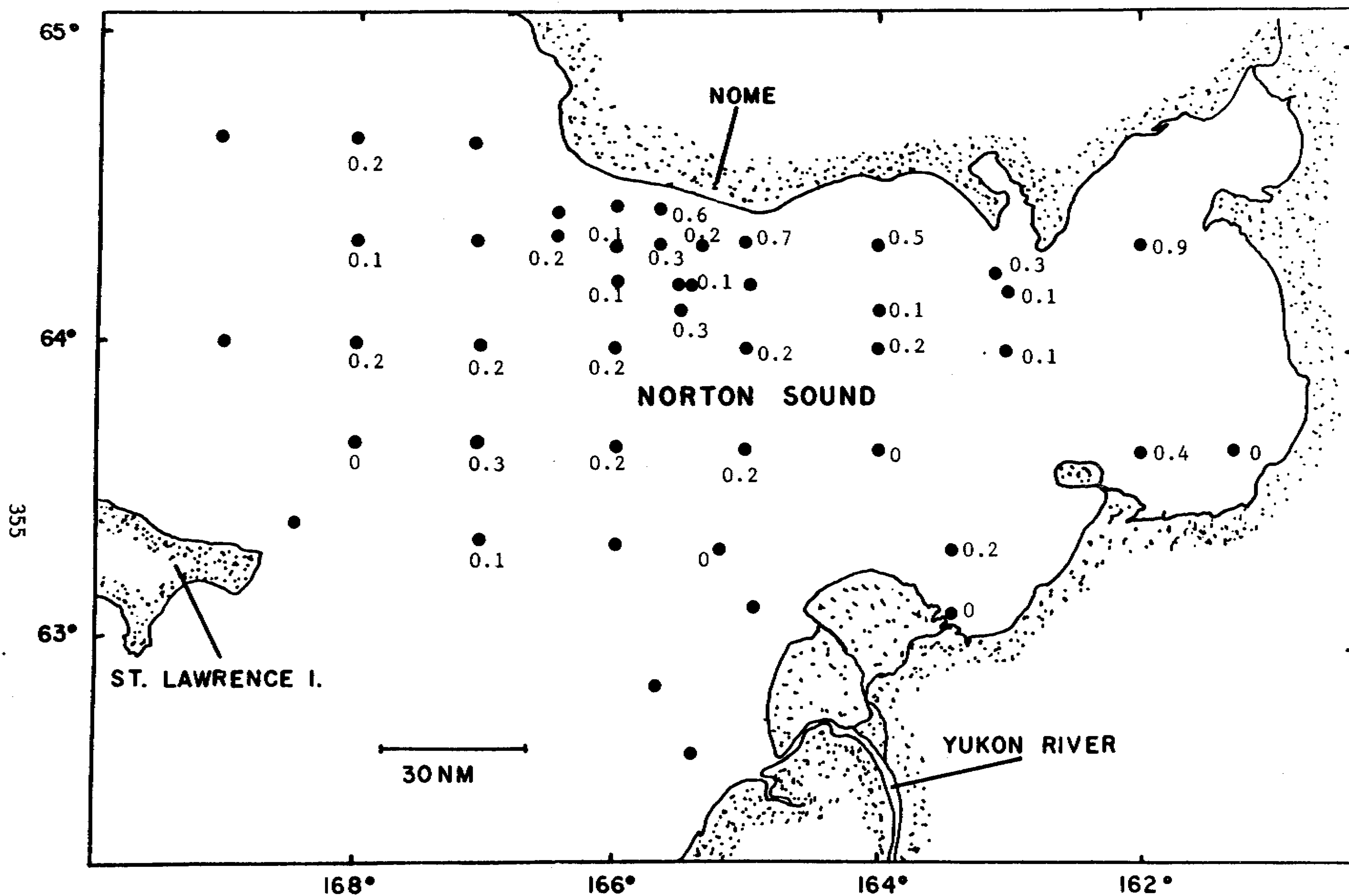

Figure 34. Nitrogen fixation rates in sediments during the July, 1979 cruise. The units are ng N_2 fixed/g dry wt./h.

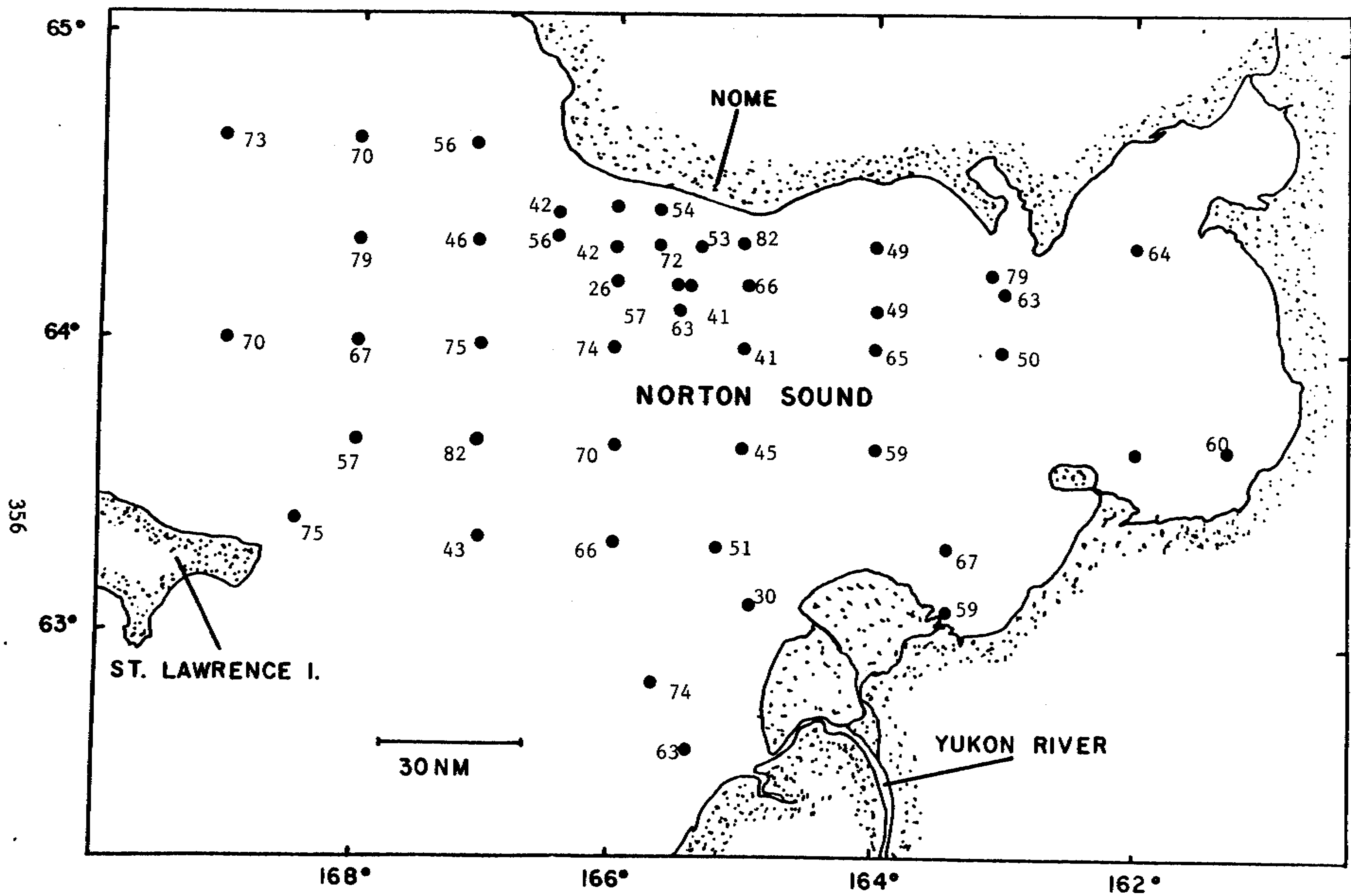

Figure 35. Percent reduction in glucose uptake in surface water samples exposed to crude oil. July, 1979 cruise.

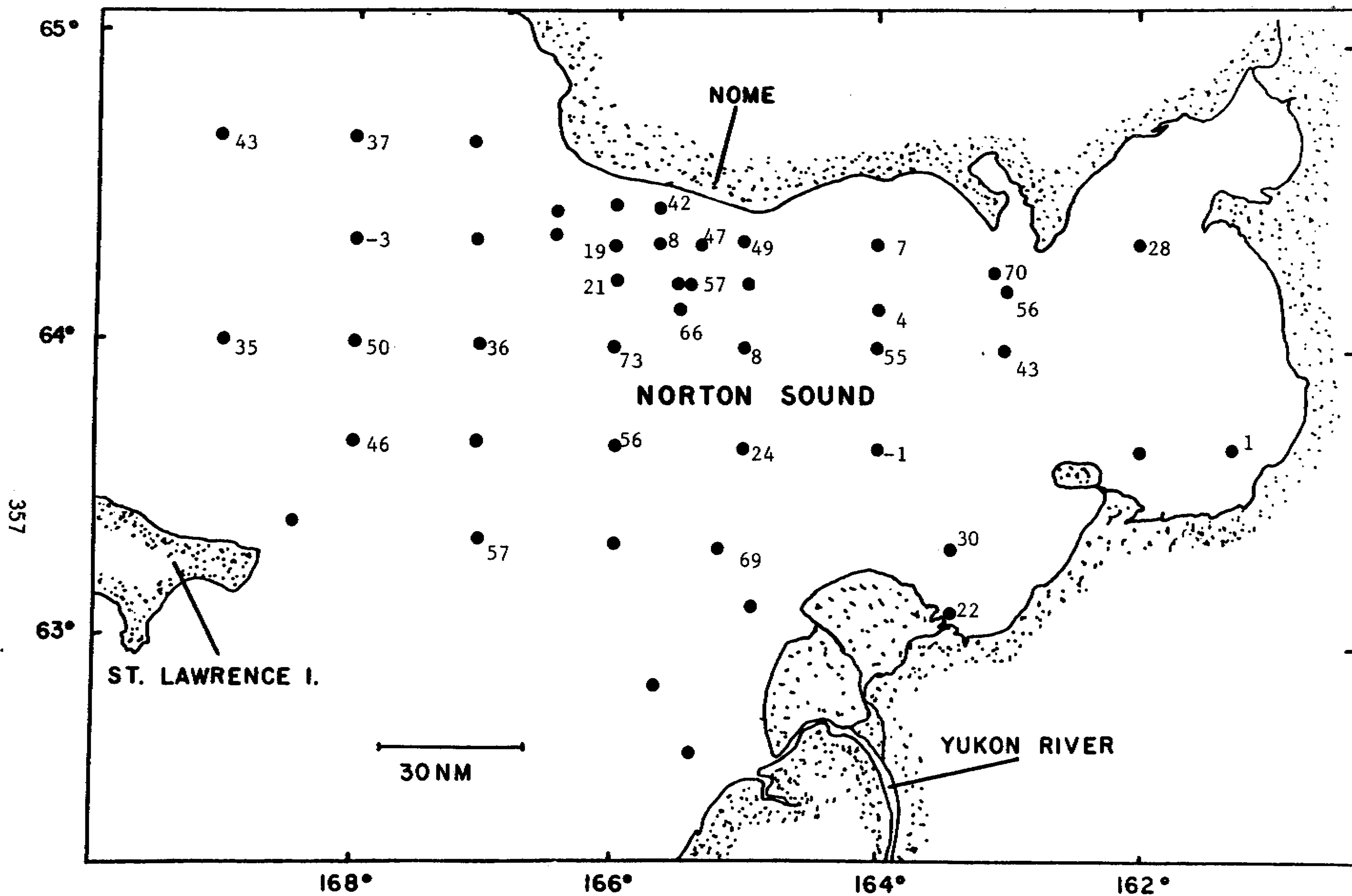

Figure 36. Percent reduction in glucose uptake in sediments exposed to crude oil. July, 1979 cruise.

EFFECTS STUDIES

Section IV

I. <u>Summary of objectives, conclusions, and implications with respect to OCS oil and gas development</u>.

A. Objectives.

Our main objectives in these studies were to determine what effects crude oil and the crude oil dispersant Corexit 9527 have on major microbial functions in the waters and sediments of the Beaufort Sea and Cook Inlet. Due to time and technical constraints, we had to restrict our studies to short-term effects during the cruises in these areas. During these cruises, however, we were able to collect baseline data which would enable us to evaluate the potential impact of crude oil perturbations in various regions.

Two major studies were initiated to define the long-term effects of crude oil on microbial activities. One of these studies was initiated in Elson Lagoon (Beaufort Sea) in July, 1977 and the other was initiated in February, 1979 at Kasitsna Bay (Cook Inlet). These studies were designed to evaluate the long-term effects of crude oil and Corexit on relative microbial activity, nitrogen fixation rates, denitrification rates, respiration percentages, and the concentration of inorganic nutrients.

B. Conclusion and implications.

1. We have found a broad range of both short-term (less than 24 h) and long-term (up to 2 years) effects of crude oil on major microbial functions.

2. Both crude oil and Corexit had an adverse (short-term) effect on relative microbial activity in water and sediments. When both of these were added in combination, the effects were even more

dramatic. The net affect of these perturbations was to place the microorganisms under stress which would undoubtedly reduce the species diversity of the organisms present over a period of time. Since long-term experiments were not conducted in water samples because of the technical difficulties involved, we do not know how this stress effects pelagic microbial function over long-term exposure.

3. Longterm exposure of marine sediments to crude oil altered a number of important functions. These included reduced microbial activity, increased respiration percentages, decreased bacterial biomass, decreased total biomass, reduced metabolic activity by benthic organisms, decreased nitrogen fixation rates, decreased denitrification rates, increased production of CO_2 and methane, increased sediment surface acidity and decreased surface redox potentials (reduced O_2 levels), decreased infaunal borrowing activities and increased accumulation of detrital particles on the sediment surface.

4. The implications of these changes are described in detail under "Discussion" in this section. The major implications are that exposure of sediment to crude oil can act to reduce both primary and secondary productivity of the whole system over an extended period of time (years). In addition, the chemical composition of the sediments surface is altered so that normal recruitment of benthic organisms into the impacted area will also be altered for an extended period of time. This condition could well extend past the time when the direct toxic effects of the crude oil are no longer a factor in killing, injuring or repelling the organisms.

5. Of the functions that we have studied, those associated with nitrogen cycle transformations appear to be impacted to the greatest extent. As such, these reactions may be the most important ones to monitor during assessments of environmental damage associated with a major oil spill. Such studies should also include measurements of relative microbial activity and respiration percentages. In addition, concentration determinations should be made for compounds which are associated with anaerobic fermentation; i.e. H_2S, methane, and ammonia. Measurements of oxygen concentrations in interstitial and interface waters would also be helpful.

6. We feel that the altered functions that we have observed would occur under actual spill conditions. The concentrations of crude oil that we used in our studies are high but even higher concentrations have been reported in oiled sediments after an actual spill. Unfortunately, the types of measurements that we are conducting in our studies have rarely been conducted in studies of actual oil spills; however, where these observations have been made, they correlate well with our findings.

7. We have concluded that every effort should be made during the planning of crude oil production and transport to reduce the risk of incorporating crude oil into marine sediments. This would also apply to procedures used to clean up an oil spill as well. This could possibly lead to some difficult choices during the control of a crude oil spill. It can no longer be assumed that one of the main objectives of control is to remove the slick from the surface of the water. If this is done by driving the oil into the water column and then into the sediments, the risk of killing birds and

mammals will have to be weighed against the possible reduction in the food supply for all organisms present for many years.

8. Of the areas that we have studied, the inshore waters of the Beaufort Sea and Kachemak Bay in Cook Inlet would appear to be the most vulnerable. We feel that the Beaufort Sea is particularly vulnerable since it appears that the effects will last longer there than in other areas we have studied; i.e. Cook Inlet. Also, this area may be more dependent than other areas on bacterial regeneration of the inorganic nutrients required for the spring phytoplankton bloom. In Kachemak Bay, the major carbon input is thought to come from macrophytes and land plants. Most of this material must be cycled through the detrital food chain and is thus susceptable to crude oil perturbation.

II. Study areas.

Samples for the short-term effects studies conducted during cruises were collected at the locations shown in previous sections. The long-term crude oil effects study in Elson Lagoon was conducted at a location approximately half way between stations 2 and 3 shown in Fig. 16 of Section II. The sample used in the Kasitsna Bay study was collected at the location in Kasitsna Bay shown in Fig. 42.

III. Methods

Except for the procedures described below, the techniques used in these studies were the same as those described in Section I and II of this report.

A. Sample collection and manipulation.

Sediment was collected from the bottom of Kasitsna Bay using a pipe dredge. All of these sediments were combined into 120 liter plastic containers and thoroughly mixed. Subsamples of the resulting sediment mixture were treated with 400 ml of fresh Cook Inlet crude oil for every 8 liters of sediment or they were left untreated to act as controls. These sediments were then dispensed in two ways. One set was placed into plexiglass trays measuring 30.5 x 30.5 x 10.2 cm. Once the trays were filled with 8 liters of sediment, they were lowered to the bottom of the bay in 20 meters of water by SCUBA divers. The trays were removed in the same way after the desired incubation period. The trays were covered with plexiglass lids to reduce sediment washout while they were being collected. Since the analysis of the trays took approximately two days, the trays were kept in a trough through which fresh seawater was being circulated at a rate of ca. 8 liters per min. This kept the sediments

at *in situ* temperature and insured the replenishment of dissolved gases. Subsamples were removed from the tray using a 50 ml plastic syringe with the end of the barrel removed. Five of the resulting cores were removed and combined in a plastic 500 ml jar. These samples were then homogenized by vigorous shaking and analyzed. Redox potentials were measured with a platinum electrode (Orion) in non-disturbed sediments.

The other set was placed into 115 liter glass aquaria which were fitted with a plexiglass divider 10 cm high which was placed across the bottom of the aquarium. This separated the sediments in each aquarium into sections which could be sampled separately. Fresh seawater was supplied at one end of each aquarium and removed at the other end by a constant level siphon system which replaced the volume 4 to 6 times each hour. The seawater flow system at the laboratory consisted of a stainless steel pump located 125 meters offshore pumping seawater through a PVC plastic plumbing system. The only materials that the seawater came into contact with were PVC plastic, natural latex rubber tubing, nylon and stainless steel. Each aquarium contained 32 liters of sediment.

B. Procedures for assaying the acute effects of crude oil and Corexit on relative microbial activity.

The assay was conducted essentially the same way as that described in Section I except 10 μl of crude oil was added to the reaction mixture. The cell activity was assayed by withdrawing 5 ml. of sediment dilution from underneath the surface slick with a syringe fitted with a stainless steel needle. The cells were washed and trapped as usual and the resulting counts were doubled

to account for the reduced volume used. In the Corexit experiments, 10 μl of a Corexit solution was added so that the final concentration was either 50 or 15 ppm. In the assays where the cells were exposed crude oil and Corexit, the Corexit was added after the crude oil.

C. Enzyme assays

The assays for phosphatase and arylsulfatase were conducted using modifications of the techniques originally described by Tabatabai and Bremner (1969 and 1970). These assays are based on the release of p-nitrophenol for the appropriate chromogenic substrate. One ml of 30 o/oo Rila Marine Mix at pH 7.5 was added to one ml of sediment slurry. To this was added one ml of either 0.005 M p-nitrophenyl phosphate or 0.005 M p-nitrophenyl sulfate. The reaction mixture was incubated at the original *in situ* temperature for 1 hr. The reaction was then terminated with 2 ml of 0.5 M NaOH and 0.5 ml of 0.05 M $CaCl_2$. The sample was centrifuged and the resulting clear supernatant decanted for measurement of optical density at 410 nm to determine the concentration of p-nitrophenol released during the course of the reaction.

The assay for polysaccharide hydrolases (amylase and cellulase) was conducted using the technique described below. This assay is based on the release of glucose from the polysaccharide by enzyme activity. Toluene is used to limit microbial metabolism during the reaction. The concentration of glucose is determined by a reaction with dinitrosalicylic acid. Three ml of the substrate solution (1% of soluble starch or carboxymethyl cellulose in 30 o/oo Rila Marine Mix at pH 7.5) was added to three ml of sediment slurry. To this reaction mixture was added 0.3 ml of toluene. The reaction mixture

was then incubated at the original *in situ* temperature for 24 hr. At the end of the incubation period, the sample was centrifuged and 2 ml of the clear supernatant was removed. The supernatant was then mixed with 2 ml of a DSNA solution. The DSNA solution was prepared by dissolving 1 g of dinitrosalicylic acid in 20 ml of 2N NaOH. To this was added 30 ml of distilled water and 30 g of sodium potassium tartrate. The solution was then brought up to a volume of 100 ml using distilled water.

After the DSNA solution was added to the reaction mixture, it was boiled for 10 minutes and cooled. The reaction mixture was centrifuged and the optical density was determined at 540 nm to determine the glucose content. The optical density reading was then converted to a glucose concentration by the use of a standard curve. The results of the enzyme assays were normalized to nM x g dry wt^{-1} x h^{-1}.

D. Studies of sediments augmented with organic nutrients.

These experiments were established in the same manner as the oiled tray experiments described in part A above. The size of the trays were the same but they were divided into four equal sections with plexiglass dividers. Each quadrant was treated differently. One quadrant contained sediment that had no nutrient added; one contained Cerophyl (dehydrated cereal grass leaves from Cerophyl Laboratories, Kansas City, MO, USA), one contained starch (unmodified wheat starch, Sigma) and the fourth contained chitin (Sigma). The concentration used was 5% w/v. The oiled trays were first treated with Cook Inlet crude oil at a concentration of 50 ppt before the nutrients were added.

IV. Results

A. Short-term effects of crude oil and/or Corexit 9527 (24 hrs or less).

1. Crude oil.

a. Effects on relative microbial activity.

We have studied the effects of Alaskan crude oil on 215 water and 162 sediment samples collected from three very different regions along the Alaskan coast. These are all areas which could potentially be impacted by crude oil as the result of crude oil production and transportation. One of the goals of this study was to determine if the presence of crude oil would alter microbial function in these diverse marine environments. It was found that in the 7 field studies where the effect of crude oil on pelagic microorganisms was analyzed, there was a statistically significant difference between the treated and non-treated samples (Table 17). The range in the mean percent reduction values observed for glucose uptake rates was from 37 to 58%. The statistical significance of these differences ranged from $p<0.035$ to $P<0.00001$. In seven out of eight of the field studies where sediment samples were analyzed, there was also a statistically significant difference between glucose uptake rates in treated and non-treated sediments (Table 17). The mean percent reduction values observed in the sediment samples ranged from 14 to 36%. The differences in the mean values observed (when comparing different regions) are probably not significant since the range in values observed at one location (Kasitsna Bay) were comparable. Even though both pelagic and benthic microorganisms were affected by the presence of crude oil, the benthic microorganisms were affected to a lesser degree. The significance of that difference

is not known; perhaps the benthic microorganisms are more consistently exposed to biogenic hydrocarbon.

All of the above studies were conducted using labelled glucose. We wanted to determine if a similar crude oil effect might be observed if an amino acid was used as a substrate in the heterotrophic potential method. During the 1978 Cook Inlet cruise, the same experiment was conducted on 35 water and 7 sediment samples using labeled glutamic acid. The mean percent reduction observed was 33 and 18 respectively. The difference in uptake rates between treated and nontreated water samples was significant at the $p<0.0003$ level but the difference in the sediment samples was not statistically significant. It can thus be said that, at least in pelagic microbial communities, the effect of crude oil on heterotrophic rates is not limited to glucose uptake and respiration.

Since the single concentration method (4) was used to measure changes in uptake and respiration rates in populations exposed to crude oil, it could be argued that the differences observed might have been caused by some component or components of the crude oil which are competing for the same transport mechanisms that are being used to take up both glucose and glutamic acid. In order to determine if this is the case, we elected to use the Wright and Hobbie (1966) technique for measuring uptake kinetics. By using this technique the maximum potential uptake rate (V_{max}), the turnover time required to utilize all of the naturally occurring substrate by the microbial population (T_t) and transport constant plus the natural substrate concentration (K_t+S_n) can be calculated. If some component of crude oil is being transported into the cells via the

same mechanism as glucose, the V_{max} value should not change but the T_t and the K_tS_n values should increase. A study on the effects of crude oil on the kinetics of glucose uptake was conducted on 6 water samples collected in the Beaufort Sea (Table 16). The mean V_{max} value decreased from 14.6 in the non-treated samples to 3.7 μg per liter per hr in the treated samples. The mean T_t value increased from 177 to 492 hr and the K_t+S_n value remained unchanged. The differences observed in both the V_{max} and the T_t values were statistically significant. It thus appears that crude oil is acting as a metabolic inhibitor.

During the course of our studies we also measured the percent respiration. We did observe differences in the percent respiration between treated and non-treated samples; however, these differences were not consistent or statistically significant in most cases. The mean values for treated samples were usually slightly higher than those observed in non-treated samples. These data suggest that the microbial function most affected is substrate transport. If either biosynthetic or respiratory functions were constantly affected, there would be a significant change in the percent respiration values in treated samples.

In one of the studies, we measured the effects of both fresh and "weathered" crude oil and an aqueous extract of fresh crude oil on respiration rates in 20 sediment samples. The average percent reduction was 20, 21, and 23% respectively. It would thus appear that the effects of these various treatments were essentially the same.

Even though the glucose uptake rates were generally reduced in the presence of crude oil, a wide range of effects was observed and in some cases, the uptake rate was actually higher in the treated sample (Table 17). The degree to which water samples were affected by the presence of crude oil was analyzed in terms of sample location. During the 1978 Cook Inlet cruise, the patterns of effect in water samples relative to geographical location suggest that populations exposed to chronic petroleum perturbation may not be as greatly affected by crude oil as those that have not been exposed (Fig. 37). A series of consecutive water samples was collected on a transect starting near Augustine Island and ending near Homer, Alaska. The water samples collected in the center of the Inlet showed less effect than those collected at either end of the transect. The center samples were located in a region of little net water flow which is also in the center of the shipping lane. One sample tested in this region showed higher uptake and respiration rates in the subsample exposed to crude oil than that observed in the control. It is assumed that petroleum products are constantly being introduced into these waters as the result of shipping. Water samples were also taken at two locations just north of Augustine Island. The one to the east was taken in Oil Bay which is so named because of a natural oil seep in that region. The one to the west was taken in a similar bay in which no seep has been reported. The reduction in glucose uptake in the water sample taken from Oil Bay was only 12% as contrasted to a reduction of 67% observed at the other location (the reduction in the glutamic acid uptake in the same samples was 0% and 61% respectively). A series of observations was also made

in water samples collected at three sample locations, one near Homer and two to the north near Kalgin Island. The range of values observed at these locations are illustrated in Figure 37. The percent reduction values observed in water samples collected at the northern stations are significantly lower than those observed in the station located near Homer. The water mass at the sample site near Homer consists primarily of open ocean water which should have had little prior exposure to petroleum products. The northern sample sites are located near oil wells in the Inlet and also near an oil refinery with associated shipping facilities. Roubal and Atlas (1978) reported high concentrations of hydrocarbon-utilizing bacteria in the water samples collected at the two northern sample sites. There have been numerous reports in the literature which link elevated concentrations of hydrocarbon-utilizing bacteria with petroleum contamination in the marine environment (Horowitz and Atlas, 1977 and Mulkins-Phillips and Stewart, 1974). It would thus appear that there is a chronic input of petroleum hydrocarbons at the northern sampling sites.

b. Acute effects of crude oil on nitrogen fixation rates.

We conducted a series of experiments in which we added crude oil to sediments during the 24 hour incubation period used to measure nitrogen fixation. In some of the studies, we added sucrose to the reaction vessel to stimulate nitrogen fixation rates. The studies were conducted on samples collected from various locations within three regions (Table 19). A total of 75 sediment samples were analyzed during the course of this study. In none of the studies was there a statistical difference between the mean values observed.

than in the sediments. For Corexit alone, the statistical significance of this difference was at the $p<.001$ level and it was at the $p<0.04$ level for the Corexit with crude oil treatments.

The above mentioned analyses were conducted using the same concentration of labeled glucose. It is possible that some component of the Corexit produced the same effect as adding non-labeled glucose to the reaction mixtures, thus reducing apparent uptake rates. One method that could be used to determine if this is taking place is to observe the effect of Corexit on the kinetics of glucose uptake using several concentrations and using the equations of Wright and Hobbie (1966). If Corexit is not acting as a metabolic inhibitor, the turnover time (T_t) and the transport constant plus the natural substrate concentration (T_t+S_n) values may change but the maximum potential velocity (V_{max}) of glucose uptake should not change. When this experiment was conducted in three water samples, the V_{max} values decreased in the presence of Corexit suggesting that it is acting as a metabolic inhibitor (Table 22). It should be noted that the incubation times of from 1 to 4 hours were used in these experiments. This indicates that Corexit probably affects glucose transport soon after exposure.

The effect of Corexit on percent respiration in both water and sediment samples was also observed (Tables 23 and 24). If the transport of glucose into the cells was the only function effected by Corexit, one would expect to see no changes in the percent respiration. There was a significant difference between the respiration percentages observed in treated and non-treated samples. For both water and sediment samples, 4 out of 6 studies showed significant

differences in respiration percentages. Of those studies showing a significant difference in the sediment samples, all mean values increased in samples exposed to Corexit (Table 23). In the water samples, two showed increases (Table 24). In the Norton Sound study, there was a slight increase in the mean percent respiration in the treated samples but the difference was not statistically significant. If, however, the water samples were analyzed as two groups, one group showed an increase in the mean and the other showed the reverse trend. The differences in both groups are very significant ($p<0.006$). The two sets of samples taken from two different locations in and near the Norton Sound are shown in Figure 31. The water samples in group A were collected to the west. Surface salinity and relative microbial activity measurements taken during the same cruise suggest that there are two water masses present in the area which are roughly defined by the line in Figure 31. The one to the west is more saline and shows lower levels of microbial activity than does the one to the east. These data suggest that different microbial populations may be affected in different ways by the presence of Corexit.

The relationship between Corexit concentration and its effect on glucose uptake in 9 Kasitsna Bay water samples was studied using three concentration ranges: 0-100, 0-50, and 0-20 ppm (Table 25). Figure 39 graphically illustrates the results of one of the three studies. Both this study and the one conducted over a range of from 0-100 ppm indicate that most of the effect takes place at concentrations below 20 ppm with little further change observed at 50 and 100 ppm. In the 0-20 ppm study, the samples showed decreases in

2. Short-term effects of the dispersant Corexit 9527.

a. Effects on relative microbial activity and respiration percentages.

During 6 different sampling periods, a total of 149 water samples were studied to determine the effects of the crude oil dispersant Corexit 9527 and Corexit with crude oil on glucose uptake during the initial 8 hours exposure (Table 20). With the exception of the Beaufort Sea study, all water samples were exposed to 50 ppm Corexit. The mean value for the percent reduction in glucose uptake rates ranged from 58 to 95% in samples exposed to Corexit alone. When the microbial populations were exposed to Corexit and crude oil, the mean reduction ranged from 75 to 95%. In 3 of the 5 studies where the effects of both treatments were observed, the mean percent reduction in glucose uptake rates was higher in the samples treated with both corexit and crude oil. These differences were very significant ($p<0.001$) in the Upper Cook Inlet and the July Kasitsna Bay studies.

The effect of Corexit and Corexit with crude oil on glucose uptake was also measured in sediment samples collected during the same sampling periods (Table 21). The mean percent reduction in the glucose uptake rates ranged from 15 to 60% in the presence of Corexit and from 40 to 79% in the presence of Corexit and crude oil. In four out of the five studies, the latter treatment showed a greater effect. These differences were statistically significant ($p<0.02$) in all cases. The effects of Corexit and Corexit with crude oil on glucose uptake rates were greater in water samples

uptake rates of 10 and 12% when exposed to Corexit at 1 ppm. Using the best fitting power curve to describe the data, the mean concentration at which 50% of the original uptake rate was lost was calculated to be 12 ppm. These data suggest that the alterations in microbial activity that were observed in the 50 ppm studies would represent the maximum effect possible.

b. Corexit and crude oil effects on bacteriovorous activity by a marine ciliate.

The ingestion of bacterial cells by protozoa in the presence of crude oil and/or Corexit 9527, is expressed as percentage of the control treatment (Table 26). Crude oil, at a concentration sufficient to produce a surface slick, caused slight but significant ($p<0.01$) decrease. This concurs with the observations of Andrews and Floodgate (1974) that protozoa are essentially unharmed by the presence of crude oil and may even take up droplets concurrent with feeding activities. When 15 ppm Corexit 9527 was added to the crude oil the ingestion of bacteria was decreased approximately 20-fold. Corexit (15 ppm) alone produced almost a 10-fold reduction in ingestion. Venezia and Fossato (1977) point out that many of the initial reports of toxicity of crude oil and dispersants are probably due to increased contact with the oil rather than any significant effect of the dispersant itself. Our results indicate that in addition to accentuating the effects of crude oil, Corexit 9527 alone has a detrimental effect on the feeding of the marine ciliate tested. The combined action of crude oil and Corexit appears to be synergistic since the observed ingestion of bacterial cells is about half the value expected if the effects of crude oil and Corexit were independent (4.9 vs 10.1%).

When concentrations of Corexit 9527 were increased from 1 to 100 ppm, there were significant decreases in the uptake of bacterial cells. For Corexit treatments in the 1 to 50 ppm range the effects of each concentration was also significantly decreased ($p<0.01$) from the preceeding concentration (i.e., % uptake control>1 ppm>5>10>15>50). The depression in ingestion rates observed at 50 ppm was essentially the same as that observed at 100 ppm.

It should be noted that the levels of Corexit 9527 used in this study were high relative to levels anticipated under actual field application in open waters (Lindblom, 1978). In one study where concentrations of a related Corexit compound were monitored under these conditions, the highest reported concentration was 1 ppm (McAuliffe et al., 1975). This does not however, rule out the possibility that higher concentrations may be encountered when this dispersant is applied in the near-shore environment; especially in areas where there is little vertical mixing or current activity. Under these conditions it is conceivable that much higher concentrations may be found which would result in reduced cropping rates of bacteria by protozoa. This, in turn, would result in reduced rates of carbon and energy transfer through the detrital food chain.

B. Long-term effects of crude oil on microbial activities in marine sediments.

We have participated in two long-term effects studies, one which was initiated in July, 1977 in Elson Lagoon (near Point Barrow, AK) and the other which was initiated in February, 1979 at Kasitsna Bay (near Homer, AK in Cook Inlet). The initial observations from the Kasitsna Bay study have been reported in an article that has been recently submitted for publication (Griffiths, et al., 1980c).

1. Effects on relative microbial activity as measured by glucose, glutamic acid and acetate uptake.

a. The Kasitsna Bay study.

During the Kasitsna Bay study, we have participated in 4 field study periods after the study was initiated: April, 1979, July-August, 1979, October-November, 1979 and January, 1980. Data summaries from these studies are presented in Tables 27-30. In all 4 studies, the mean relative microbial activity was shown to be decreased in the presence of crude oil when glucose or glutamic uptake rates were compared with non-oiled sediment samples. The mean percent reduction for all 4 studies was 45% and 53% for glucose and glutamic acid respectively. When the same measurement was made using labeled acetate during the January, 1980 field trip, a 74% reduction was observed. With the exception of the differences observed during November study, all of the differences observed between activities in oiled and non-oiled samples were statistically significant. When the November results were first analyzed, it was felt that the reduced effects observed at that time indicated that

after 8 months exposure, the population was starting to recover. A subsequent study conducted in January, 1980, on the same trays and aquaria showed that this was not the case. The differences observed in January (after the sediments had been exposed for 11 months) showed differences that were as great as those observed after 6 weeks and 5 months of exposure.

During one of the study periods, we also compared tray and aquaria sediments that had been treated with both crude oil and the dispersant Corexit 9527 to non-oiled sediments (Tables 31 & 32). In most cases, sediments that were treated with both crude oil and Corexit showed larger differences when compared with the controls than did sediments treated with crude oil alone. Most of these differences; however, were not statistically significant.

The changes in relative microbial activity described above were measured using the one substrate concentration method (Tables 27-30). We also measured relative microbial activity using the multi-substrate concentration technique of Wright and Hobbie (1966). This procedure is described in detail in Section I of this report. In three of the four studies, we used this technique to measure the effects of crude oil on relative microbial activity (V_{max}) and on the time required for the natural microbial population to utilize the naturally occurring substrate available (T_t). In all cases, the relative microbial activity decreased and the T_t values increased; all of these differences were statistically significant. The transport constant plus the natural substrate concentration (K_t + S_n) value was also measured but this value was not consistantly affected. Detailed results on the observations made on trays and aquaria during the July, 1979 field season are presented in Table 32.

During these studies, we also conducted experiments to determine what the acute effects of crude oil, crude oil and Corexit or Corexit would have on sediment samples that had been exposed to crude oil or crude oil and Corexit for extended periods (Table 33). The effects that were observed in the non-treated controls were essentially the same as those reported for acute effects in part "A" of this Section. The effects of crude oil on relative microbial activity was about the same as Corexit at a concentration of 50 ppm. Crude oil and Corexit had an additive effect which was greater than either one alone. The sediments that were previously treated with crude oil or crude oil and Corexit showed very little difference whether they were exposed to Corexit and crude oil or Corexit alone in the actual challenge experiments. In the April and July studies, sediments that had been pretreated with crude oil or crude oil and Corexit, did not show a significant decrease in relative microbial activity when they were exposed to crude oil in an acute challenge experiment. In the November and January experiments, this was not the case. The crude oil treated sediments exposed again during the acute challenge experiments with fresh crude oil showed a decrease in microbial activity relative to the controls.

2. Effects of crude oil on respiration percentages by benthic microorganisms.

During our studies, we measured the effects of crude oil on respiration percentages. A summary of these results are reported in Tables 27-30. In all cases, the mean percent respiration was higher in sediments exposed to crude oil than those that were not regardless of the substrate used to make that determination. The

overall increase in the mean values for all studies were 54%, 18%, and 65% for glucose, glutamic acid, and acetate respectively. The effect of crude oil exposure on respiration percentages were consistently greater when glucose was used as the test substrate.

3. The Elson Lagoon study.

We also saw similar effects of crude oil on relative microbial activity in Elson Lagoon sediments to those effects we found in the Kasitsna Bay sediments. The main difference was that instead of taking as little as 5 weeks to observe an effect, as it did in Kasitsna Bay, it took 9-12 months of exposure before measurable differences were observed (Table 34). Once the effects of crude oil on microbial activity were initiated, they were as great as those found in the Kasitsna Bay sediments. Reduced microbial activity was observed in sediments that had been exposed to crude oil for up to 2 years (the latest observation). We also observed increases in the respiration percentages in all oiled sediments analyzed. These changes were much lower than those observed in the Kasitsna Bay sediments. Even though we observed an increase in respiration percentages in each case, these differences were not statistically significant.

4. The Glacier Bay study.

In June, 1979, a cruise ship spilled fuel oil into Glacier Bay near Juneau, AK. In October, 1979, we collected three oiled and three non-oiled sediments for analysis at the Kasitsna Bay laboratory. Care was taken to collect non-oiled sediments which approximated the physical characteristics of the oiled samples. We conducted a series of uptake experiments on these sediments using glucose to

determine the effects of the oil on relative microbial activity and respiration percentages (Table 36). We then compared the resulting measurements on a sample by sample basis; comparing oiled and non-oiled sediments with the same physical characteristics. If the microbial populations in the exposed sediments were affected by the presence of the crude oil, we would expect to find reduced relative microbial activity, increased respiration percentages and reduced effects on relative microbial activity in acute fresh crude oil challenge experiments. In 2 out of 3 samples, the relative microbial activity was reduced and the percent respiration was higher. In all three samples, there was a lower reduction in microbial activity in the oiled samples when the samples were exposed to fresh crude oil.

5. Long-term crude oil effects on nitrogen fixation rates.

The long-term effects of crude oil on nitrogen fixation rates were also measured during all 4 Kasitsna Bay field studies (Tables 27-30). In this case, the nitrogen fixation rates are expressed as nM x g dry wt^{-1} x h^{-1} ethylene produced. The reduction in nitrogen fixation rates were very significant in every case with the lowest mean percent reduction being 85%. The average percent reduction was 93%. The observed reductions were as high after 11 months exposure as they were after 5 months. This same effect; however, was not observed in Elson Lagoon sediments that had been exposed to crude oil for up to 8 months (Table 37).

We conducted a study to determine how long an exposure was required before a measurable reduction was found. This study was conducted in July, 1979 at Kasitsna Bay. In this sediment, there

was a measurable reduction with the first 24 hours of incubation (Fig. 40). Within 10.5 days, the nitrogen fixation rate has decreased to only 18% of the original rate.

During the November, 1979 field study, we observed rates of nitrogen fixation in sediments that had been augmented with organic nutrients. These nutrients included Cerophyl (dried grass leaves), chitin, and starch. The substrates were added to nontreated sediments and sediments that were treated with crude oil (Table 38). Regardless of the treatment, crude oil had a significant effect on nitrogen fixation rates. In the sediments augmented with starch, the nitrogen fixation rates were increased to a level 25 times higher than the control. Even under the conditions that were ideal for nitrogen fixation, crude oil depressed activity by 90%. The rates of nitrogen fixation in the non-oiled sediments reflected the amount of organic nitrogen present in the sediment in a negative way. Sediments that were augmented with Cerophyl and chitin both showed nitrogen fixation rates below the controls; both of these substrates contain organic nitrogen whereas starch does not.

6. Long-term effects of crude oil on denitrification rates.

We conducted studies on the long-term effects of crude oil on denitrification rates during the November, 1979 and January, 1980 field periods (Table 39). During the first study, the sediments were augmented with nitrate to produce a potential denitrification rate. At this point the sediments had been exposed to crude oil for 8 months. The average rate of reduction in oiled sediments was 57%. The significance in the difference between the means was at the $p<0.009$ level. Sediments exposed to crude oil for 2 weeks did

not show a reduction in denitrification rates. In the sediments that had been augmented with organic carbon, also showed decreased denitrification rates when oiled and non-oiled sediments were compared.

During the January study, both natural and potential denitrification rates were measured. Natural denitrification rates in oiled sediments were reduced below the level of detection (approximately 0.1 nM N_2O). Thus the reduction was greater than 97%. When the potential denitrification rates were measured, they were found to be approximately 1,000 times the natural rate. Crude oil exposure reduced denitrification rates by 74% under those conditions. The significance of the differences between the means was $p<0.004$.

7. Long-term crude oil effects on CO_2 evolution rates.

During the time course experiment mentioned above (nitrogen fixation section) we also measured CO_2 evolution rates with time after a sediment had been exposed to crude oil (Fig. 41). Within the first two days, the CO_2 production rates were lower than the control. After 5 1/2 days exposure, the CO_2 rate was about twice as great as the control. At the end of 10 1/2 days, the rate was still higher.

CO_2 evolution rates were observed in oiled and non-oiled sediments during the November and January field periods (Table 40). After 8 months exposure, the CO_2 evolution rates from oiled sediments were 50% higher than the controls. In the January study, 3 months later, the rates were 58% higher than the controls. In both cases, the differences of the means were statistically significant.

During the January study, we also analyzed CO_2 evolution rates from

sediment collected in Elson Lagoon. These sediments included two non-oiled sediments, one that had been exposed to crude oil for 2 years and one that had been exposed for only a few hours. The evolution rate in the sample exposed for 2 years showed a rate very close to the non-oiled sediment and the "0 time" sediment showed a reduced rate.

During the November study, we measured CO_2 evolution rates from the sediments that had been augmented with various organic nutrients (Table 40). When unaugmented, oiled sediments were compared with those that were augmented with Cerophyl or chitin, the augmented sediments showed increased CO_2 evolution rates. The opposite was true in the oiled sediment augmented with starch. In this study, we are assuming that the rate of CO_2 evolution in the oiled sample - the rate observed in the nonoiled samples = the rate of CO_2 that is generated from the oxidation of crude oil. This experiment illustrates the need for fixed nitrogen in crude oil degradation. In the samples where an increase in CO_2 evolution rates were observed in the oiled samples, there was sufficient organic nitrogen available for the degradation of the crude oil. In the starch augmented sample, there was very little fixed nitrogen available and thus the crude oil degradation was reduced or eliminated.

8. Long-term crude oil effects on methane production.

During the January, 1980 field period, we measured rates of methane production in oiled and nonoiled sediments from both Elson Lagoon and Kasitsna Bay (Table 40). In the Kasitsna Bay samples, the rate of methane production was higher in the oiled sediments; a

difference that was statistically significant. There was no statistical difference between the means of the oiled and nonoiled sediments from Elson Lagoon.

9. Long-term crude oil effects on total adenylates and energy charge.

During two field periods in the Elson Lagoon study, we measured the concentrations of ATP, ADP, and AMP in oiled and nonoiled sediments (Table 41). The concentration of total adenylates is calculated by adding the individual concentrations of all three adenylate species. In all sediments that had been exposed to crude oil for 4 months or more, the concentration of total adenylates was significantly lower in the oiled samples. The same measurements were made in Kasitsna Bay sediments during two field periods (Tables 27 and 28). The same phenomenon was observed in these sediments as well.

Although there was a great seasonal change in the energy charge calculated from the adenylate concentrations in the Elson Lagoon samples, there was no significant difference between the energy charge observed in oiled and nonoiled sediments. In both Kasitsna Bay studies, the mean energy charge values were lower in the oiled samples. This difference was statistically significant in only one of the two studies.

10. Long-term crude oil effects on bacterial concentrations.

During the April, July, and November, 1979 Kasitsna Bay field periods, we measured the concentrations of bacteria in oiled and nonoiled sediments using a direct counting technique (epifluorescent microscopy). In both studies we observed mean value differences in

oiled and nonoiled sediments (Table 42). There was a 33% and a 45% reduction in bacterial cell concentrations in the oiled sediments for the April and July studies respectively. These differences were statistically significant. During the November study, there was no significant differences seen between the mean values.

11. Long-term crude oil effects on sediment surface pH and Eh.

The effects of crude oil on sediment surface pH and Eh was measured during the Kasitsna Bay study. During each field period when these were measured, the surface hydrogen ion concentration (pH) was higher and the Eh (redox potential) was lower in the oiled sediments (Tables 27-29). The differences in the means for both of these variables were statistically significant during the first two studies but not in the third (November, 1979).

12. Long-term crude oil effects on sediment enzymes.

The effects of crude oil on phosphatase, arylsulfatase, amylase and cellulase (P, S, A, C) activity was measured in both Elson Lagoon and Kasitsna Bay sediments (Table 43). In the August, 1979 Elson Lagoon study, we observed reductions in phosphatase, arylsufatase, amylase and cellulase activities and an increase in cellulase activity. The differences of the means were statistically significant only in the arylfulfatase and amylase activities. In the samples collected during January, 1980, there was an increase in P and C, a decrease in S and no change in A. None of these differences were statistically significant. Sediments collected in January, 1979 were also analyzed for cellulase activity. There was an increase of 157% in mean activities in the oiled sediments.

These enzyme activities were also measured in the Kasitsna Bay sediments. In most cases, there was a decrease in P and A activities in sediments that had been exposed to crude oil for 5 months or more. In these same sediments, C activity was increased in the oiled sediments.

C. Crude oil weathering rates in Kasitsna Bay sediments.

At the present time, the only evidence we have of crude oil degradation rates is the difference in CO_2 evolution rates between oiled and nonoiled sediments. We have however, frozen subsamples of all sediment samples that have been analyzed from the beginning of the Kasitsna Bay study. Many of these samples are to be analyzed by hydrocarbon chemists who will be contracted by OCSEAP. Once these analyses have been completed, a great deal of information will be obtained concerning the <u>in situ</u> rates of crude oil degradation in the sediments of Cook Inlet. This information will be of particular importance because of all the other data that have been collected on the same samples.

D. Seasonal observations of microbial function in the sediments and waters near Kasitsna Bay.

1. Relative microbial activity.

During our Kasitsna Bay studies, we measured relative microbial activity at the locations shown in Figure 45. The seasonal trends in relative microbial activity were essentially the same when either glucose or glutamic acid uptake rates were used in the determinations (Table 44). In the water samples, the lowest rates were observed in February, 1979 and the highest rates were observed in April only 6 weeks later. The rates then decreased each season from April through the following January.

The relative microbial activity in the sediments showed a different trend. The levels of activity were relatively constant throughout the year with the exception of August. In August, the mean uptake rates were significantly higher than that observed at any other time of the year.

2. Nitrogen fixation (nitrogenase) rates and adenylate analyses.

The mean nitrogen fixation rate was lowest in July and highest in November, 1979. The difference of the mean values observed at these two times was statistically significant (Table 45).

The concentration of total adenylates increased from a low of 4.4 nM/g dry wt in February, 1979 to a high of 7.7 nM/g dry wt in July, 1979 (Table 45). The calculated energy charge values decreased from a high of 0.31 in February to a low of 0.26 in July. These differences were not statistically significant.

V. Discussion

A. Short-term effects of crude oil and/or Corexit 9527.

1. Crude oil effects

a. There have been a number of studies (Atlas et al., 1976; Calder and Lader, 1976; Griffin and Calder, 1977; Hodson et al., 1977; and Walker and Colwell, 1975) that have addressed the question: What effects do crude oil, refined petroleum products, or pure hydrocarbons have on marine microbial populations? Several of these studies were concerned with the effects of crude oil on microbial growth rates. For the most part, these studies have shown that crude oil has very little effect on growth; however, reduced growth rates have been shown in populations exposed to refined petroleum products (Walker and Colwell, 1977) or aromatic hydrocarbons (Calder and Lader, 1976; Griffin and Calder, 1977). In a study of the effects of crude oil on cell numbers in seawater taken from Yaquina Bay, Oregon, no significant changes in plate counts were observed in water samples exposed to Prudhoe Bay crude oil for periods of up to 40 days (Griffiths, unpublished data). Atlas et al. (1976) reported that Prudhoe Bay water exposed to crude oil showed an increase in cell number relative to the control after 42 days. Although no significant change in species diversity was observed in this experiment, a more recent study by Atlas (personal communication) using numerical taxonomical techniques has shown shifts in the composition of bacterial populations in Arctic marine waters exposed to Prudhoe crude oil for several months. These data suggest that although populations of marine heterotropic bacteria may not decrease when exposed to crude oil, the crude oil

may act as an environmental stress which could affect species composition and thus microbial function.

Hodson et al. (1977) reported that crude oil-aqueous solutions inhibited the uptake and mineralization of ^{14}C labeled glucose by pelagic microorganisms. These observations were made in seawater samples contained in large plastic bags during the course of the CEPEX project (Menzel and Case, 1977). In our study, a large number of pelagic and benthic microbial populations collected from a wide variety of sources were exposed to crude oil. The uptake and mineralization of labeled glucose was monitored to determine if altered function could be detected.

b. Both this study and that reported by Hodson et al. (1977) indicate that there is a significant reduction in the heterotrophic uptake and mineralization rates in pelagic microbial populations when they are first exposed to crude oil and/or refined oils. This effect was observed when either glucose or glutamic acid was used as the test substrate indicating that this phenomenon is not restricted to glucose (the substrate used by Hodson). The phenomenon is caused by a number of crude oil types and crude oil products and affects a large cross section of natural marine microbial populations (although pelagic populations appear to be affected to a greater extent than benthic populations. There was a wide variation found in the percent reduction observed from one location to another. The geographic location of the samples showing these variations indicate that the degree to which a population is affected may be related to the degree of prior exposure to crude oil or petroleum products.

c. Our studies have also shown that crude oil does not affect nitrogen fixation under short-term exposure. This is essentially the same conclusion reached by Knowles and Wishart (1977) in a similar study made on samples collected in and near the Beaufort Sea. The results of the short-term exposure experiments are in marked contrast to our findings of long-term crude oil effects on nitrogen fixation rates. The results of these studies are presented later in this section.

2. Corexit effects.

a. There has been growing evidence that dispersants may have adverse effects on a wide range of aquatic organisms. Hagstrom and Lonning (1977) showed that the dispersant (Corexit 9527) interfered with fertilization and development of a sea urchin with some adverse effects observed at concentrations as low as 0.0003 ppm. Hsiao et al. (1978) showed that crude oil-Corexit mixtures were inhibitory to primary production in Arctic marine phytoplankton. There has been very little information published concerning the impact of dispersants on microbial function. Gunkel (1968) states that 90 percent of marine bacteria were killed in the presence of 10 ppm "emulsifier". Mulkins-Phillips and Stewart (1974) showed that two of four dispersants studied slowed initial growth rates. This increased lag phase was more pronounced at an incubation temperature of 10 C than at 25 C.

b. Our Corexit acute effects studies have shown that this dispersant reduces the level of microbial activity in both water and sediment samples. This effect is probably that of an induced stress on the microbial population. In the Mulkins-Philips and Stewart study

(1974), it was shown that even though some dispersants caused an increase in the lag phase of growth, growth was not inhibited. When natural populations were exposed to dispersants and crude oil, however, there were qualitative shifts in the microbial population. In one case, the resulting population consisted of 100% pseudomonads. One of the experiments conducted during the CEPEX project described by Menzel and Case (1977) consisted of observing the results of adding crude oil and Corexit 9527 to one of the seawater enclosure systems. At the end of the experiment, the microbial population was essentially a pure culture (G. G. Gessey, personal communication). It is quite possible that what we are observing in this study is the initial phase of a selection process which is acting to reduce the diversity of the population.

3. Effects of exposing natural microbial populations to both crude oil and Corexit 9527.

We have consistently observed that the addition of Corexit to crude oil in the acute exposure experiments increased the effect to a level greater than would be expected from either one alone. We feel that this indicates an additive stress condition.

4. Short-term effects and management decisions.

Most of the effects of Corexit 9527 that we have observed are probably relatively unimportant in pelagic microorganisms associated with a well-mixed water mass. With the possible exception of the bacteriovorous activity of protozoa, most of the stress effects associated with addition of Corexit to the system would be greatly reduced by the introduction of new species from other waters. In

water massess where there is little mixing, the reduction in the diversity of the population could cause long-term changes in microbial function.

Essentially the same could be said for crude oil effects in the water column. If the crude oil becomes incorporated into the sediments, however, we know that many microbial functions will be altered for a significant period of time. For these reasons, efforts should be made to reduce the risk of allowing crude oil in water masses in which there is little net mixing or transport. In the case of an oil spill, the use of the dispersant Corexit 9527 should be restricted in waters with little mixing or net transport.

B. Long-term effects of crude oil on microbial function.

1. Role of bacteria in marine systems - an overview.

Before the effects of crude oil on marine microorganisms can be fully evaluated, one must understand the roles of these organisms in the marine system and the relative importance of these roles in the overall productivity of the system. The main functions or roles of marine bacteria are the following:

a. Bacteria form the major link between primary and secondary production. This works in two ways: bacteria package carbon produced by the primary producers in a form that eventually feeds essentially all of the consumers at all trophic levels, and, bacteria convert organic nitrogen and phosphate to the inorganic forms required by algae for new plant material.

b. Bacteria control most of the biogeochemical processes which directly effect the productivity of the system. These processes

control the concentrations of NH_4, NO_3, NO_2, PO_4, H_2S, H_2, O_2, CO_2, methane, and many other important chemical species. The rate at which these compounds are converted from one form to another under a given set of circumstances will often determine the chemical environment at a given point. Because of the reduced diffusion and mixing rates found in marine sediments relative to the water column, the control of the environment by bacterial processes is of particular importance in the sediments.

c. Bacteria can produce endocrine compounds that may be required for growth of other organisms. These "growth factor" requirements may be particularly important to phytoplankton productivity under certain conditions.

d. Bacteria and blue-green algae (now also classified as bacteria) are the only organisms that are capable of fixing atmospheric nitrogen into a fixed form that is required by all living organisms. On a global scale, bacterial transformations are also the most important means of converting fixed nitrogen to atmospheric nitrogen.

2. The role and relative importance of bacteria in the detrital food chain.

Before proceeding, we would like to define the "detrital food chain" or web. This is the movement of all organic carbon (organic nutrients) from one trophic level to the next which originated from sources <u>other than</u> the direct ingestion and direct utilization of plant material. Thus the nutrients that form the basis for the detrital food chain includes <u>all</u> organics which are not associated with living plant material that is eventually utilized directly by some consumer. Up to a few years ago, the bacterial processes were

relegated to a dead-end box at the bottom of flow diagrams which suggested that all of the organic material that could not be used directly by higher trophic levels was mineralized by the bacteria and thus lost from the system. With renewed interest in this problem and the availability of new techniques, much more has been learned about the mechanisms involved in the detrital food chain. As we will show below, the bacteria do indeed mineralize organic compounds; however, they also fill a very important role in the overall productivity of the system that has not become fully appreciated until recently.

In an extensive study of the role of the detrital food chain in juvenile salmon production Naiman and Sibert (1979) stated the following: "The results of our study support the concept of Mann (1972) and Pomery (1974) that the ocean's food web is detrital". Pomery suggested that the main route for the food web in the marine environment was plants - microorganisms (primarily bacteria)-consumers. This is in contrast with the classical view of algae-herbivores-larger consumers, where the bacteria act only as mineralizers.

Mann (1972) concluded that microorganisms provided the main link in the detrital food web while studying the chemical composition of plant material during decomposition. The same conclusion was reached by Thayer et al. (1977) during a similar study of eelgrass decomposition. In a 1975 study, Harrison and Mann showed that the cropping of bacteria by protozoa increased the decomposition rate of eelgrass leaves. Thus, the efficient degradation of this plant material required not only microbial colonization but the cropping of that population by higher trophic level consumers as well.

In the classical scheme, the majority of organic carbon produced by phytoplankton was consumed directly by zooplankton. Recent studies (Smith and Wiebe, 1976; and Larrson and Hagstrom, 1979) have shown that a significant portion of this carbon may be released as soluble carbon and that this in turn, is converted to particulate carbon by bacteria. Paerl (1978) has shown that bacteria colonize the surface of actively growing phytoplankton (over 50% showed colonization). This suggests that the bacteria are utilizing the nutrients being released by actively growing phytoplankton. Using radioactive tracer techniques, Cole and Likens (1979), concluded that bacteria are the major agents of phytoplankton decomposition.

As has been shown above, bacteria are important to many aspects of the detrital food chain. They are the most efficient organisms known in taking up and utilizing soluble organic compounds. It has been estimated that if there were no bacteria in the water column, that the level of soluble organic compounds would be at least 100 times greater than that currently found in marine waters. This means that these soluble organics are converted to particulate organic carbon in the form of bacterial biomass which can then be used as a food source for higher organisms. Bacteria also produce enzymes which hydrolyze such recalcitrant compounds as cellulose, chitin and lignin. The resulting soluble compounds are taken up by bacteria and converted to bacterial biomass. The detrital material is colonized by bacteria which is then ingested by higher organisms. The bacteria are digested off the detrital material and detrital particles are then released back into the environment as fecal pellets. These pellets are recolonized by bacteria and reingested.

The end result of this process is a food source which has low C:N:P ratios and thus is an excellent balanced organic nutrient. The enrichment of both nitrogen and phosphorous is made possible by the extreme efficiency with which bacteria are able to take up these elements.

In a 1977 review article, Fenchel and Jorgensen presented evidence that quantified the relative importance of the detrital food chain in nutrient transfer through the system. They showed that only 10% of macrophytic and seagrass biomass was grazed on directly by herbivores. Considering the composition of most terrestrial carbon, we assume this would hold true for this potential nutrient source as well. If the estimates that over 50% of the phytoplankton produced carbon is also routed through the detrital food chain are correct, the total nutrient flow via this route is substantial. Fenchel and Jorgensen (1977) estimated that on the average 50% of all primary productivity is utilized via this route in the worlds oceans. The figure for nearshore environments runs near 80%.

3. The role and importance of bacteria in the mineralization process.

During the mineralization process, the carbon, nitrogen, phosphorous, sulfur and trace elements that are incorporated into organic molecules are converted into inorganic forms that are then reincorporated into organic molecules by biological activity (primarily through the actions of plants). The high metabolic activity found in bacteria, make them the most important factor in this process. In systems in which fixed nitrogen is limiting for phytoplankton growth, the rates at which bacteria are capable of

mineralizing organic material may directly effect the rate of primary productivity by phytoplankton. During a seasonal study of relative microbial activity in waters near Vancouver Island, B.C., a pulse of high microbial activity was observed both before and after the major spring phytoplankton bloom (Albright, personal communication). It was felt that the first pulse in microbial activity might have released the inorganic nutrients required for the spring bloom. Schell (1974) reported that there was strong evidence that microbial mineralization processes were the most important factor in the accumulation of inorganic nutrients during the winter months in the Beaufort Sea. It was suggested that this was the source of inorganic nitrogen required for the spring phytoplankton bloom. We have observed an increase in inorganic nutrient concentrations in the waters near Kasitsna Bay in the winter months. These concentrations decreased 10 times during the spring diatom bloom in April, 1979. There was also a temporary buildup of ammonia in the waters near Kasitsna Bay in July which was greatly reduced in August after a dinoflaggelate bloom in August. The buildup of these nutrients was probably the result of microbial mineralization processes.

4. The role and importance of microbial nitrogen fixation in the marine environment.

In recent years, a new, sensitive, fast and relatively inexpensive assay for measuring *in situ* rates of nitrogen fixation has been developed (Stewart, 1967). This has greatly facilitated the study of this process in both terrestrial and marine systems. At this point there have been a number of studies which have reported rates

of nitrogen fixation in marine sediments. Rates that have been reported in colder marine environments compare well with those that we have observed in our studies. There is, however, a great deal of difficulty in determining what significance this has to the total nitrogen budget of a given body of water. In the last two years, there have been reports of studies where the relative importance of nitrogen fixation to the nitrogen requirements of specific marine plant species have been estimated (Capone et al., 1979; Teal et al., 1979; and Zuberer and Silver, 1978). In all three cases, nitrogen fixation was thought to account for about 30% of the nitrogen requirements for the plants studied.

Although we do not have the information required to report a complete nitrogen budget for the areas we have studied, we do have indirect evidence that nitrogen fixation may be important to the function of the detrital food chain in marine sediments. It is generally accepted that nitrogen is limiting for carbon utilization in many marine sediments. This would be particularly true in sediments where the primary organic carbon imput was low in organic nitrogen; i.e., macrophytic and terrestrial carbon. During cruises in Cook Inlet, Shelikof Strait, and Norton Sound, we measured nitrogen fixation rates while Dr. Atlas and his associates measured natural rates of denitrification. Thus, the net fixed nitrogen generated in the sediments was compared to the removal of fixed nitrogen to atmospheric nitrogen by denitrification. In Kamishak Bay, the mean rate of denitrification was much greater than the mean rate of nitrogen fixation. This indicated to us that detrital particles generated in the Lower Cook Inlet during a period of phytoplankton

blooms were settling out of the water column and into the sediments. This would bring in exogenous organic nitrogen which could be mineralized (ammonification). The end result of that processes would make more nitrate available for denitrification. In addition, the presence of organic nitrogen would inhibit nitrogen fixation.

We know that currents on the west side of Cook Inlet travel south into the Shelikof Strait. As detritus that is generated in Cook Inlet is carried south by this current, we would anticipate that the nitrogen content would be decreased as the available organic nitrogen is used up. The bacteria associated with the detritus should preferentially utilize organic N-containing compounds (mostly protein). The bacteria would then be cropped, thus removing the nitrogen from the particles. The end result of these processes would be the deposition of detritus material with high carbon to nitrogen ratios, i.e. low concentrations of organic nitrogen. If this were the case, one would predict that the rates of nitrogen fixation would be relatively high and that the rate of nitrogen fixation would approximately equal the rate of denitrification. This is exactly what was observed in Shelikof Strait. The mean value for nitrogen fixation was higher in the Shelikof Strait than in any other region that we have studied (see Section I for details). These data suggest to us that nitrogen fixation is an important and constant feature of the detrital food chain in the Shelikof Strait.

A similar pattern was observed on a seasonal basis in our Kasitsna Bay studies. In the sediments near Kasitsna Bay, there was a major input of carbon into the sediments during the summer which was reflected in much higher benthic relative microbial

activity than at any other time of the year. It was during this same season that we observed the lowest mean value for nitrogen fixation. The highest mean value was observed in November. We feel that the detritus that settles out of the water column during the late spring and summer contains a relatively low carbon:nitrogen ratio; thus the low nitrogen fixation rates. As the bacteria preferentially utilize the compounds containing relatively high concentrations of nitrogen and this nitrogen is removed as the bacteria are cropped, the carbon:nitrogen ratio in the detritus increases; i.e., there is a net loss of fixed nitrogen. This results in high rates of nitrogen fixation in the late fall when there are still relatively large amounts of easily utilizable carbon available but very little fixed nitrogen. As the season progresses, the nitrogen fixation rates decrease because carbon in a readily available form becomes depleted.

The basic assumptions on which the above hypothesis is based, is substantiated by the studies in which we add organic carbon to sediments (Table 38). In the sediments to which nutrients containing organic nitrogen were added, the nitrogen fixation rates were reduced by 75%. In the sediment to which starch was added (containing chemical energy but no nitrogen), the nitrogen fixation rate increased 25 times.

One other observation leads us to conclude that nitrogen fixation might be important to the detrital food chain. Using the mean yearly rate of nitrogen fixation in the sediments near Kasitsna Bay, we calculated that the concentration of all forms of fixed nitrogen in the interstial waters of the same sediments could be replaced by nitrogen fixation every 24 hours.

5. Long-term crude oil effects on microbial function.

a. Overview

1. Changes related to the detrital food chain perturbation.

   a. Decreased bacterial cell biomass.

   b. Decreased microbial activity, increased respiration percentages.

   c. Decreased total adenylates (decreased total biomass).

   d. Decreased nitrogen fixation rates.

   e. Increase in detritus accumulation on sediment surface.

   f. Decreased infaunal activity such as borrowing.

2. Changes related to mineralization.

   a. Decreased alkaline phosphatase activity.

   b. Decreased microbial activity.

   c. Decreased ammonia oxidation and nitrogen fixation.

   d. Decreased oxygenated zone and extension of this zone via infaunal borrowing.

3. Changes related to altered chemical environment.

   a. Increase in hydrogen ion concentrations in surface sediments.

   b. Decreased redox potential in surface sediments (anoxic conditions prevail).

   c. Presumed increase in hydrogen sulfide concentrations.

b. Crude oil effects on the detrital food chain.

There now exists in the literature, extensive documentation of the effects of crude oil on benthic organisms (Carr and Reish, 1977; Giere, 1979; Swenmark, 1973; and Taylor and Karinen, 1977). If these organisms are killed or driven out by the presence of

crude oil, several important changes will take place in the system. The borrowing activity is reduced which is now known to have an important function in the overall metabolism of the sediment by increasing the total oxidized surface area. Borrowing activity is also responsible for the turning over of the sediments (more will be said about this in the next section). Of greater importance to the detrital food chain is the interruption in the flow of bacterial biomass which is utilized by higher trophic levels. If there are no organisms present that are capable of cropping the bacteria, then the sediments become in effect, carbon sinks. The organic carbon that would normally be used as the basis for most secondary productivity will remain in the sediments and will not be used as a food source for the rest of the food chain.

Our observation of oiled sediments indicates that this is what is taking place. The non-oiled sediments showed very little accumulation of detrital material even at the height of the spring phytoplankton bloom. They also contained the normal 2-3 cm of oxidized sediment on the surface and extensive evidence of infaunal borrowing. In contrast to this, the oiled sediments had extensive detrital deposits on the surface and there was essentially no oxidized layer on the surface of the sediments. In addition, there was no evidence of borrowing activity.

Our measurements of total adenylates indicate that there was reduced total biomass in the oiled sediments. This reduction was almost twice that which would be expected from the known reduction in bacterial biomass as determined by direct counts (epifluorescent microscopy). We therefore conclude that the balance of the reduction

was due to reduced infauna which are potentially bacteriovorus. We also have other indirect evidence for the reduction in the number of detritivores. In most oiled sediments, the level of cellulase activity was increased over the controls. This indicates to us that more cellulose is becoming incorporated into the oiled sediments. The reason for this, we believe, is that the organisms that would normally ingest the cellulose are absent. Under normal conditions, the bacteria that hydrolyze cellulose would reside in the guts of these organisms rather than in the sediments.

There have been a number of studies in which it has been shown that microbial activity is actually stimulated when bacteria are being actively cropped (Harrison and Mann, 1975 and Fenchel and Jorgensen, 1977). We have observed both a reduction in microbial activity and a reduction in bacterial biomass in oiled sediments. This could, in part, be due to the absence of bacteriovores in the oiled sediments. It is more likely that this reduction is in response to the toxic effects of the crude oil itself. We have shown that microbial activity is reduced in the presence of crude oil under short-term (8 hour) exposures. In addition, we have seen that the respiration percentages are increased in presence of crude oil. This means that proportionately more of the nutrients that are utilized by the bacteria are being respired as CO_2 and less is being incorporated into bacterial biomass. Even if the bacteria were being cropped, this would mean a greatly reduced efficiency in converting detrital carbon into usable carbon for secondary productivity.

Another indication of stress induced by the toxic properties of crude oil is the decreased energy charge ratios observed in oiled sediments. These ratios, which are generated by comparing relative levels of ATP, ADP, and AMP, are an indication of the metabolic activity of microorganisms (Wiebe and Bancroft, 1975). The higher the ratio, the more active the population; conversely, the lower the ratio, the less metabolically active the population.

c. Crude oil effects on mineralization and nitrogen fixation.

During our studies, we have observed changes due to the presence of crude oil that strongly suggest that the rate of mineralization is reduced. These effects could have a direct bearing on primary productivity rates of marine algae. We have direct evidence that mineralization rates for simple soluble organics are reduced by over 50% (the glucose, glutamic acid and acetate uptake rates shown in Tables 27-30). These reduced rates may, in part, be related to the reduced oxygenated zone observed in the oiled sediments. Most normal marine sediments contain two zones; a top oxidized zone in which most of the mineralization of organic material by microorganisms takes place and an anaerobic zone beneath it (Vanderborght et al., 1977). In a recent review of mineral cycling by Fenchel and Blackburn (1979), it was shown that the presence of the top oxygenated zone is essential for normal mineralization of organics. Without this zone, the products of anoxic metabolism can escape from the sediments. Many of these products are toxic to most higher (eucaryotic) organisms. Under most conditions, more than 90% of all mineralization takes place in the oxygenated zone near the sediment surface. Our studies show that this zone is essentially eliminated in oiled sediments.

Classical studies of marine sediments often treat sediments as a two dimensional layered system. Recent sutdies have shown that this is a gross oversimplification of what takes place in marine sediments (Rhodes and Young, 1970 and Gerlach, 1978). For example, the borrowing activity of infauna can greatly increase the extent of the oxidized layer mentioned above, it can act to physically turn over the sediments bringing to the surface nutrients that would be left at depth and the pumping action by some organisms can bring oxygen and oxygenated compounds into the deeper sediments. Dennis Lees (personal communication) has calculated that the borrowing activities of one species of clam would increase the surface area in Western Cook Inlet sediments by roughly 2.5 times. This is only one of many species present which could provide this function. If crude oil eliminates key infaunal species, the rates of mineralization would certainly be reduced even if there were no other detrimental effects.

In addition to the general reduction in mineralization rates, we also have conducted observations that suggest that specific reactions which are involved in mineralization are affected by the presence of crude oil. These effects would be additive to those described above. We have observed that the natural rates of denitrification are reduced below detectable levels in sediments exposed to crude oil for periods of up to one year (Table 39); the longest exposure time studied by us in Kasitsna Bay. This would represent at least a 97% reduction in activity. When the same experiments were conducted in sediments to which nitrate was added, the crude oil treated sediments showed a 57% reduction in denitrification

rates. The denitrification reaction converts nitrite to nitrous oxide and then to atmospheric nitrogen. If an excess of nitrate is added to the system, this is converted to nitrite which is then utilized in the above reaction. In the non-augmented samples, the activity in oiled sediments was reduced to less than 3% of the control. This is in contrast to a rate that was 33% of the control when nitrate was added. It thus appears that the reaction that converts ammonium ion to nitrite may be impaired in the oiled sediments. This transformation (ammonium oxidation) is an important step in the conversion of organic nitrogen to nitrate which is the nitrogen form preferred by phytoplankton. Recent measurements of denitrification in the Elson Lagoon oiled sediments by Dr. Atlas have shown similar results. In his study, there was no difference between oiled sediments and the controls in experiments where nitrate was added but there was a significant reduction in natural rates. In the near future we plan to assay for crude oil effects on ammonia oxidation.

Another function of microorganisms is the conversion of organic phosphate to inorganic phosphate. The enzyme that is responsible for this reaction is phosphatase. In both the oiled sediments from Elson Lagoon and Kasitsna Bay, we have observed lower phosphatase activity in these sediments relative to the controls.

Another microbial process that adds nitrogen to the system is nitrogen fixation. In the Kasitsna Bay sediments, we have consistently observed rates of nitrogen fixation in oiled sediments which are less than 5% of the controls. Although this reduction was not observed in sediments exposed for 24 h or less, longer exposures of

a few days started to show an effect. In one experiment, we observed an 87% reduction at the end of 10 1/2 days incubation.

d. Crude oil effects on nutrient chemistry.

We have observed several phenomena which suggest that the chemical properties of sediments are altered by the presence of crude oil. These changes undoubtedly reflect changes in microbial activities caused by the crude oil. There is a marked reduction in the redox potential in the surface of the sediments. This is probably due to the reduction in the available O_2 present. These anaerobic conditions are also reflected in the increased rates of methane production that we have observed in oiled sediments. Although we have not measured it directly, there is strong presumptive evidence that another product of anaerobic metabolism, hydrogen sulfide (H_2S) is also being produced. The human nose is very sensitive to hydrogen sulfide (rotten egg smell). Our noses have consistently detected H_2S in oiled sediments. In addition, we have observed the growth of bacteria known to utilize H_2S on the surface of oiled sediments.

We have also observed changes in the sediment surface hydrogen ion concentration (pH) during the first 5 months of exposure. The hydrogen ion concentration increases (pH decreases) in the oiled sediments. Under these acidic conditions, there should be a significant shift in the chemical balance of a number of potentially important chemical species (Fenchel and Blackburn, 1979).

These data suggest that crude oil causes significant changes in the sediment surface which will undoubtedly effect recruitment

of benthic organisms into the impacted area. These effects may be in affect long after the initial toxicity of the crude oil has dissipated.

6. Long-term effects of the dispersant Corexit 9527 on microbial activities.

During our Kasitsna Bay studies we have observed the long-term effects of Corexit on a number of microbial functions. At a concentration of 500 ppm, the only effect observed was that expected from the addition of an organic nutrient to the sediment. These observations did not; however, include measurements of bacterial species diversity. We did, however, observe increased effects when Corexit was added to crude oil. The Corexit appeared to enhance the toxic effect of the crude oil. This same phenomenon has also been reported by others who have studied other marine organisms (Swedmark et al., 1973).

7. The importance of long-term crude oil effects relative to overall productivity.

From the estimates that have been made by other investigators we can assume that at least 50% of the net primary productivity in the offshore areas is routed through the detrital food chain to provide the nutrients for all organisms present. If the disruption of this food chain was 100% effective, we would expect to find a 50% reduction in all species if a large enough areas was impacted. In the highly productive Kachemak Bay where the majority of the carbon imput is thought to come from macrophytic and terrestrial sources (Lees, personel communication), the reduction would be closer to 80%. This is an impact that would have serious consequences in the commercial fishing industry.

In addition to the direct impact on the detrital food chain, we would expect a significant reduction in primary productivity due to reduced mineralization and nitrogen fixation rates. At this time we do not have enough information to make an educated guess as to what this would mean in terms of overall productivity. The effect would undoubtedly be of greatest importance in inshore environments.

The actual impact of a major spill to the overall productivity of the impacted area would depend on a great number of variables. Some of these include the type, degree of weathering and concentration of oil incorporated into the sediments. It would also depend on how thoroughly the oil was mixed with the sediments and the extent of the impacted area. In addition, it would also depend on the relative importance of the area to the juvenile stages of key species. It is obvious that much more work remains to be done before a realistic estimate of potential environmental impact can be made for a given set of circumstances.

The duration of the impact is another factor that must be kept in mind. In the introductory statement made by Vandermeulen (1978) during a symposium on "Recovery Potential of Oiled Marine Northern Environments", he suggested that it might take 5-15 years before the oil impacted region would return to normal. We have seen nothing that would dispute that estimate. In our Kasitsna Bay studies, we have observed no real difference between the effects found after 5 weeks exposure and those observed after almost one year exposure. In the Elson Lagoon study, measurable changes in many variables did not occur until the sediments had been exposed

for 9 months. Once these changes were initiated, they persisted for at least 2 years (the length of the study thus far). The results of our studies suggest that the effects of crude oil in the sediments of the Beaufort Sea would be in affect for a much greater period of time than that observed in the more temperate climate of Cook Inlet. Judging by the delayed onset of measurable change, the impact could last up to 10 times longer in the Beaufort Sea. This is probably due to the length and degree of metabolic depression during the winter months in the Beaufort Sea inshore sediments.

8. The relevence of our observations to actual spill conditions.

We designed our experimental conditions so that the changes that we observed would be similar to those observed under conditions of maximum impact; e.g., we thoroughly mixed fresh crude oil directly into the sediments at relatively high concentrations. We did this in order to make sure that we would be able to measure changes that might take place. As it turned out, the observed changes were so dramatic that this measure may not have been needed. Although the concentration used in these experiments seemed high at the time we initiated this experiment, a recent report by Vandermeulen et al (1979) showed crude oil concentrations twice this magnitude in sediments along the coast of France following the Amoco Cadiz spill (110 ppt). It would thus appear that the concentrations that we used were high but yet possible under actual spill conditions.

During the same oil spill, chemists studying the sediments observed increased rates of methane production and decreased redox potentials in oiled sediments (D. Ward, personnel communication).

These are the same observations that we have made in the oiled Kasitsna Bay sediments.

During the study of crude oil effects associated with the Tsesis oil spill, it was reported that a number of benthic organisms that were normally present in the region were absent from the oiled sediments. In addition, reduced O_2 levels were observed in waters near the sediment interface. Both of these observations correlate well with our findings. In addition, a rapid transport of oil into the surface of the sediments was observed which was due to the sedimentation of particulate material. This suggests a mechanism by which oil might become incorporated into the sediments.

Our study of impacted Glacier Bay sediments indicate that changes in microbial activity and respiration percentages can also occur under actual spill conditions.

Unfortunately, the types of observations that we have been making during the course of our studies have not been routinely conducted during studies of past major spills. Hopefully, after the results of our studies have been published, this situation will change.

9. Recommendations regarding the types of measurements that should be made during a major spill to assess environmental impact.

During past environmental impact studies of major oil spills, the primary emphasis has been in assessing the direct impact of crude oil on higher organisms and the type and concentration of petroleum hydrocarbons associated with the impacted area. More recently, assessments of crude oil degradation rates and direct effects on marine algae have also been made. The results of our

studies strongly suggest that an additional set of variables should also be measured. These would assess the impact on primary and secondary productivity over an extended period of time. The functions that appear to be the most susceptible to crude oil perturbation are the transformations involved in nitrogen cycling. We would therefore recommend the rates of nitrogen fixation, denitrification and possibly ammonia oxidation be measured. These are very sensitive reactions that could act as an indicator of general impact on microbial processes. Relative microbial activity and percent respiration should also be considered since this will provide an indicator of altered mineralization rates which could ultimately effect primary porductivity. Since crude oil tends to reduce O_2 levels and the concentrations of gases and ions that are associated with anaerobic processes should also be monitored. This would pinpoint areas where the sediment surface chemistry is altered by the presence of crude oil.

Our pilot study of oiled sediments from Glacier Bay suggests that even without baseline data available for comparison, one can show the impact of petroleum hydrocarbons by measuring microbial function. This study did show us however, that "control" (non-oiled) sediments should be collected and analyzed along with the oiled sediments. Care should be taken in selecting "control" sediments with physical characteristics similar to the oiled sediments and the number of non-oiled sediments should approximately equal the number of oiled sediments analyzed.

VI. Needs for further study.

A. The recent Bering Sea synthesis meetings conducted by NOAA have shown that very little information is available concerning the microbiology of the Bering Sea. Since several areas in the Bering Sea will be on the BLM lease schedule over the next few years, it is imperative that microbial studies be conducted in this region. Although a great deal can be learned about general microbial function during cruises on NOAA ships, virtually nothing can be learned about the long-term effects of crude oil on microbial metabolism. Through our work at Kasitsna Bay, we have proven the validity of measuring these effects in trays and aquaria located in the field. We have also shown that a well-equipped field laboratory is required to properly analyze these samples.

During our oiled sediments experiments in both the Beaufort Sea (Elson Lagoon) and the Cook Inlet (Kasitsna Bay), we have shown that there are both similarities and differences in the way in which benthic microorganisms react to crude oil perturbation. In both Beaufort Sea and Cook Inlet exposed sediments, the relative microbial activity and the concentration of adenylates are depressed while the percent respiration (mineralization) and CO_2 evoluation are increased. However, we have also seen basic differences between benthic microorganisms from these two regions. Different enzymes appear to react differently to crude oil perturbation indicating different effects on the mineralization of specific organic compounds. Differences were also noted in the crude oil effects on nitrogen cycling.

These results suggest that even though there are many similarities in the way in which benthic microorganisms from various areas react to crude oil perturbation, there are also some basic differences. For this reason, we are recommending that two types of studies be conducted at the NOAA Kasitsna Bay laboratory. One approach would be to set out a minimum of 4 sets of sediments trays in representative areas of the Bering Sea. Each set would consist of 4-5 treated and the same number of non-treated sediments collected from the nearshore environment (the area most likely to be impacted by crude oil). After being left on location for approximately 6 months, subsamples would be collected for analysis at the Kasitsna Bay laboratory. Subsamples would also be collected and analyzed on a periodic basis after that time. This would give information about the basic differences and similarities observed between locations in the Bering Sea and between the Bering Sea and the Cook Inlet.

The second approach would be to continue our studies of long-term crude oil perturbation in Kachemak Bay sediments as outlined below. Since we have observed similarities between the effect of crude oil on both Beaufort Sea and Cook Inlet sediments, we can assume that the same will hold for the Bering Sea. We feel that this is justification to continue our in-depth study of crude oil effects in the sediments near Kasitsna Bay. Such a study would not be feasible at this time in the Bering Sea.

B. The long-term exposure experiments should be continued past FY 80. This will give us a much better estimate of projected crude oil biodegradation rates. This would include monitoring existing trays and establishing new ones so that a better statistical analysis

of the results can be made. In addition to providing information about potential biodegradation rates, the duration of perturbed microbial function can also more accurately be estimated. These extended observations would be particularly important in assessing long-term effects of low crude oil concentrations and long-term effects in crude oil overlays.

C. At the end of the current study, we will have accumulated a great deal of information about changes in sediments that have been exposed to a "worst case" situation; high concentrations of fresh crude oil thoroughly mixed into the sediment. This represents the theoretical maximum effect. The next step should be to study conditions that more closely approximate an actual spill situation including manipulations which might be used to control the oil; i.e., adding dispersants. This would provide information about the low concentration limits of crude oil effects and information about the effects of sediment surface contamination on microbial function in the rest of the sediment. These data would provide a better information base from which to predict crude oil effects under actual spill conditions.

During the Petroleum Effects Workshop held in San Diego in Nov., 1978, recommendations were made concerning crude oil effect studies in Cook Inlet. One of their recommendations (Group B) was to conduct a series of studies of sediments contained in tanks fitted with a seawater flow system, temperature control, and wave generator (pages 38 and 39). They also recommended a multidisciplinary research effort so that a wide range of variables could be monitored.

What we are recommending is a scaled down version of their proposed experimental design. We agree with their statement that an experimental design be adapted which provides the greatest control. From our experience with both aquaria and trays, we feel that both systems are valid and should be continued. We recommend that the concept of the aquarium be expanded to larger containers and that a temperature control feature be incorporated into the system. These tanks or aquaria could be used to simulate actual spill conditions by adding oil directly onto the surface of the water rather than homogenating it into the sediments. By removing and replacing the water, tidal action could be simulated. Using this same approach, the effects of Corexit could be monitored when it is added to crude oil under simulated "normal" application instead of mixed directly into the sediments as we have done in our studies to date. By using the small tanks, chemical and biological changes in both the water column and the sediments could be tested. The conditions of the experiment could be controlled and monitored as best they can under "field" conditions. To gain maximum benefit from these studies, investigators specializing in hydrocarbon chemistry, benthic ecology, planktology, and microbiology should be involved in this study.

For the purposes of determining chemical and microbiological changes as well as changes in the smaller benthic infauna under "field" conditions, the trays would produce essentially all of the information that we would need. Consideration might be made of oiling small (<100 m^3) plots in the intertidal zone to obtain effects information about the larger infaunal and epifaunal groups.

D. One of the more important questions that will not be addressed in much detail by the end of this FY is the rate at which organic carbon has been transferred through the detrital food chain. This is a very difficult problem to study. We have made only modest gains in understanding the magnitude of this carbon input. At the beginning of this project we assumed that we would be measuring a large number of variables to screen for a few that might show change in crude oil perturbed systems. As the study was to progress, we planned to document these few changes and use the balance of our efforts in solving the organic carbon transfer problem. As things turned out, virtually every variable that we measured showed an effect. Documenting these changes has taken all of our research resources to date. A continuation of the project into FY 81 would give us additional time to devote to this important problem.

E. Our most recent studies on the effects of crude oil on microbial function have shown that microbial transformations in the nitrogen cycle are very sensitive to crude oil toxicity. Not only is nitrogen fixation greatly effected but denitrification and ammonia oxidation may also be severely effected. If, on further study, ammonia oxidation is shown to be adversely affected by the presence of crude oil, this would have important implications concerning microbial regeneration of inorganic nutrients required for primary productivity. Future research should be intensified in the whole area of inorganic regeneration and the processes that control this function.

F. We have information that suggests that crude oil and Corexit reduces the diversity of microbial populations. The direct measurement of diversity indexes should be made in the future to document these changes.

Table 17. Percent reduction of glucose uptake rates in microbial populations exposed to crude oil.

Sample	$\bar{Y}$	SD	n	range	$^{*}p<$
Water					
Beaufort Sea, September, 1978	52	20	40	-8 - 88	0.0001
Cook Inlet, April, 1978	45	30	32	-43 - 86	0.01
Cook Inlet, May, 1979	58	20	47	-22 - 92	0.0005
Norton Sound, July, 1979	57	15	60	18 - 82	0.0001
Kasitsna Bay, February, 1979	37	13	7	22 - 83	0.012
Kasitsna Bay, April, 1979	58	16	6	36 - 72	0.035
Kasitsna Bay, July, 1979	56	11	23	0 - 72	0.0001
Sediment					
Beaufort Sea, September, 1977	°35	18	20	0 - 65	0.002
Beaufort Sea, September, 1978	32	22	34	0 - 68	0.001
Cook Inlet, April, 1978	14	32	14	-73 - 35	NS
Cook Inlet, May, 1979	29	42	14	-52 - 78	0.048
Norton Sound, July, 1979	36	22	34	1 - 73	0.0001
Kasitsna Bay, February, 1979	25	14	12	0 - 49	0.01
Kasitsna Bay, April, 1979	16	13	11	-2 - 42	0.01
Kastisna Bay, July, 1979	30	12	23	0 - 72	0.0001

° = CO_2 data only.
* = level of statistical significance between treated and non-treated samples.
NS = not statistically different at the $p<0.05$ level.

Table 18. The effects of crude oil, Corexit 9527, and a combination of the two on the kinetics of glucose uptake in water samples collected during the summer, 1978 Beaufort Sea field studies.

Sample Number		No Oil	Crude Oil	Corexit	Corexit + Oil
(1) Maximum Potential Uptake Rate (V_{max})					
BW611		12.4	4.3	3.8	2.5
BW614		10.8	3.7	3.1	1.2
BW616		5.7	2.1	1.2	0.9
BW619		8.1	2.7		
BW632		8.6	1.5		
BW644		38.7	6.6		
BW645		17.8	5.0		
	$\bar{x}$	14.6	3.7	2.7	1.5
(2) Turnover Time (T_t)					
BW611		306	597	1046	1337
BW614		286	480	1693	2000
BW616		429	689	697	1650
BW619		246	384		
BW644		58	35		
BW645		42	767		
	$\bar{x}$	177	492	1145	1662
(3) Transport Constant + Natural Substrate Concentration ($K_t + S_n$)					
BW611		3.8	2.6	4.0	3.4
BW614		3.1	1.8	5.2	2.5
BW616		2.5	1.5	0.9	1.5
BW619		2.0	1.1		
BW644		2.2	0.2		
BW645		0.8	3.9		
	$\bar{x}$	1.9	1.9	3.4	2.5

(1) = Values in ng/liter/h.
(2) = Values in hours.
(3) = Values in μg/liter.

Table 19. Acute effects of crude oil on nitrogen fixation rates.[1]

	n	Field Samples		Sucrose Added	
		Control	Oil	Control	Oil
Yaquina Bay	5	0.7	0.4	8.7	7.1
Cook Inlet, Apr. 1977	5	0.3	0.3	3.7	3.2
Cook Inlet, Nov. 1977	12	0.3	0.3	0.5	0.6
Cook Inlet, Apr. 1978	17	0.6	0.5	0.7	0.5
Beaufort Sea, Sept. 1977	9	0.1	0.1	0.5	0.4
Beaufort Sea, Jan. 1978	9	0.1	0.1	0.3	0.2
Beaufort Sea, Apr. 1978	5	1.3	1.2	-	-
Beaufort Sea, Aug. 1978	13	0.15	0.16	-	-

[1]mean rates for n samples expressed as ng N-fixed/g dry wt/hr.

TABLE 20

PERCENT REDUCTION IN GLUCOSE UPTAKE RATES IN WATER SAMPLES

Sampling Location	Treatment							
	Corexit				Corexit 9527 + Crude Oil			
	$\bar{y}$	SD	n	range	$\bar{y}$	SD	n	range
*Beaufort Sea	58	22	14	38-79	76	20	14	54-91
Cook Inlet	88	9	37	59-98	94	5	37	78-100
Norton Sound	85	11	60	58-98	-	-	-	-
Kasitsna Bay, 2/79	68	11	7	53-83	59	20	7	29-83
Kasitsna Bay, 4/79	95	3	6	92-98	95	5	6	85-99
Kasitsna Bay, 7/79	82	19	25	38-98	91	10	24	50-99

*These samples treated with 15 ppm Corexit, all other samples treated with 50 ppm Corexit.

TABLE 21

PERCENT REDUCTION IN GLUCOSE UPTAKE RATES IN SEDIMENT SAMPLES

Sampling Location	Treatment: Corexit 9527				Treatment: Corexit 9527 + Crude Oil			
	$\bar{y}$	SD	n	range	$\bar{y}$	SD	n	range
*Beaufort Sea	15	11	12	0-38	40	17	12	0-62
Cook Inlet	57	17	6	39-81	79	13	6	54-93
Norton Sound	54	13	34	30-84	-	-	-	-
Kasitsna Bay, 2/79	38	14	12	20-61	52	14	12	38-74
Kasitsna Bay, 4/79	44	24	11	17-75	44	24	11	6-76
Kasitsna Bay, 7/79	60	9	20	45-80	74	6	20	65-85

*These samples treated with 15 ppm Corexit, all other samples treated with 50 ppm Corexit.

TABLE 22

EFFECTS OF COREXIT ON THE KINETICS OF GLUCOSE UPTAKE IN WATER SAMPLES

Sample Number	Control				Corexit			
	Percent Respiration	V_{max}	K_t+S_n	T_t	Percent Respiration	V_{max}	K_t+S_n	T_t
313	22	0.30	5	19	16	0.90	8	84
314	29	0.016	0.2	13	15	0.0015	0.03	19
315	32	0.11	8	77	22	0.011	3	257

V_{max} = the maximum potential rate of glucose uptake reported as μg/liter/h.

K_t+S_n = the transport constant plus the natural glucose concentration reported as μg/liter.

T_t = the time in hours required for the microbial population to utilize the naturally occurring glucose.

TABLE 23

PERCENT RESPIRATION OBSERVED IN SEDIMENT SAMPLES TREATED WITH COREXIT

	Control				Corexit 9527				
Sampling Location	$\bar{y}$	SD	n	range	$\bar{y}$	SD	n	range	*p =
Beaufort Sea	31	9	18	22-57	34	9	18	23-60	0.022
Cook Inlet	44	26	11	14-78	32	13	11	21-60	NS
Norton Sound	28	8	38	15-66	35	6	38	22-53	0.00003
Kasitsna Bay, 2/79	20	5	15	13-30	24	6	15	9-33	NS
Kasitsna Bay, 4/79	17	8	12	6-34	18	5	12	9-25	0.007
Kasitsna Bay, 7/79	18	3	20	12-23	23	5	20	16-34	0.000008

*Level of statistical significance between treated and non-treated samples.

TABLE 24

PERCENT RESPIRATION OBSERVED IN WATER SAMPLES TREATED WITH COREXIT

	Control				Corexit 9527				
Sampling Location	$\bar{y}$	SD	n	range	$\bar{y}$	SD	n	range	*p =
Beaufort Sea	40	14	13	18-58	28	11	13	15-51	0.0005
Cook Inlet	28	15	39	11-70	32	13	39	14-67	0.013
Norton Sound	26	7	62	15-49	28	7	62	11-44	NS
Kasitsna Bay, 2/79	32	3	6	28-38	35	7	6	29-46	0.040
Kasitsna Bay, 4/79	37	7	7	28-46	47	10	7	34-62	NS
Kasitsna Bay, 7/79	34	9	24	21-50	26	10	24	13-53	0.007
Norton Sound Group A	24	6	48	11-49	28	5	48	18-42	0.00001
Norton Sound Group B	34	8	14	20-46	25	10	14	11-44	0.0006

*Level of statistical significance between treated and non-treated samples.

Table 25. The concentration of Corexit at which the glucose uptake rate was reduced to one half of the control.

Sample Number	Goodness of Fit to Power Curve	Corexit Concentration ppm	Range of Concentrations Tested - ppm
271	0.94	15	0-100
273	0.96	18	0-100
275	0.92	11	0-100
285A	0.99	7	0-20
285B	0.99	7	0-20
242	0.98	10	0-50
247	0.82	10	0-50
250	0.98	10	0-50
255	0.98	10	0-50

mean = 12 ppm (to reduce uptake 50%)

Table 26. Ingestion of *Vibrio alginolyticus* cells by a marine ciliate when exposed to crude oil and/or Corexit.

TREATMENT		PERCENT INJESTION OF CELLS[a]	p-VALUE[b]
Control[c]		100.0 ± 2.9	
Oil (10 ppm)		82.7 ± 5.2	0.01
Oil + Corexit (15 ppm)		4.9 ± 1.0	0.001
Corexit 9527	1 ppm[d]	75.3 ± 5.4	0.01
	5 ppm[d]	51.1 ± 2.5	0.001
	10 ppm[d]	27.2 ± 3.1	0.001
	15 ppm[d]	12.2 ± 2.5	0.001
	50 ppm[d]	2.3 ± 0.9	0.001
	100 ppm[d]	1.7 ± 0.3	0.001

[a]percent ingestion compared to control with mean ± standard deviation.

[b]significance level (p) of treatment compared with control.

[c]control arbitrarily set at 100%.

[d]power curve equation for Corexit concentrations 1 to 100 ppm:

$$Y \text{ (percent ingestion)} = 134 \times (\text{ppm})^{-0.923}$$

Table 27. Changes observed in sediment samples exposed to crude oil for 6 weeks.

	Trays				Aquaria				
	Control	Oil	Dif.	%	Control	Oil	Dif.	%	p<
Variable	$\bar{Y}$	$\bar{Y}$	Δ	Δ	$\bar{Y}$	$\bar{Y}$	Δ	Δ	
Glucose uptake	22	11	11	-50	20	13	7	-35	0.005
Glutamate uptake	335	92	243	-73	257	116	141	-55	0.0001
Glutamate Vmax	430	160	270	-63	300	176	124	-41	0.002
Glutamate Kt+Sn	20	14	6	-30	11	28	17	+155	NS
Glutamate Tt	85	135	50	+59	54	227	173	+320	0.03
Glucose % Resp.	20	38	18	+90	23	45	22	+110	0.0001
Glutamate % Resp.	44	64	20	+45	48	65	17	+26	0.00001
Ethylene Production	0.2	0	0.2	-100	0.75	0.06	0.69	-91	0.004
Total Adenylates	4.5	2.2	2.3	-51	3.8	1.4	2.4	-63	0.0001
Energy Charge	0.33	0.26	0.07	-21	0.34	0.24	0.10	-29	0.011
Hydrogen Ion Conc.	1.4	3.5	2.1	+150	0.5	3.1	2.6	+520	0.01
Redox Potential (Surface)	90	20	70	-78	18	-229	247	-1372	0.03

Under each heading, the mean value measured in the control (non-treated) and the oiled sediments is reported along with the difference between the means and the percent of that difference relative to the control mean. The "p" value is the level of statistical significance between the mean values of all control and treated sediments analyzed.

	Units
Glucose uptake	ng x g. dry wt.$^{-1}$ x h^{-1}
Glutamate uptake	" " "
Glutamate Vmax	" " "
Glutamate Kt+Sn	μg x liter^{-1}
Glutamate Tt	h.

	Units
Glucose % Resp.	%
Glutamate % Resp.	%
Ethylene Production	nM x g. dry wt.$^{-1}$ x h^{-1}
Total Adenylates	(μM x g. dry wt.$^{-1}$
Energy charge	ratio - see methods
Hydrogen ion conc.	x E-8 M
Redox potential	mV

Table 28. Changes observed in sediment samples exposed to crude oil for 5 months.

Variable	Trays				Aquaria				
	Control $\bar{Y}$	Oil $\bar{Y}$	Dif. Δ	% Δ	Control $\bar{Y}$	Oil $\bar{Y}$	Dif. Δ	% Δ	p<
Glucose uptake	77	31	46	-60	140	44	96	-69	0.0001
Glutamate uptake	605	178	427	-71	815	221	594	-73	0.0001
Glutamate Vmax	1040	232	808	-78	1060	300	760	-72	0.001
Glutamate Kt+Sn	44	18	26	-60	18	17	1	-6	0.01
Glutamate Tt	60	130	70	+117	26	84	58	+223	0.003
Glucose % Resp.	14	51	37	+264	21	49	28	+133	0.00001
Glutamate % Resp.	46	50	4	+9	49	42	7	+14	0.007
Ethylene Production	1.1	0.2	0.9	-85	0.64	0.04	0.60	-93	0.00002
Total Adenylates	5.6	1.5	4.1	-74	1.7	0.6	1.1	-65	0.005
Energy Charge	0.34	0.23	0.09	-26	0.39	0.32	0.07	-18	NS
Hydrogen Ion Conc.	1.5	4.3	2.8	+187	2.9	4.2	1.3	+45	0.0005
Redox Potential	85	-238	323	-380	61	-290	351	-576	0.000001

Under each heading, the mean value measured in the control (non-treated and the oiled sediments is reported along with the difference between the means and the percent of that difference relative to the control mean. The "p" value is the level of statistical significance between the mean values of all control and treated sediments analyzed.

	Units
Glucose uptake	ng x g. dry wt.$^{-1}$ x h^{-1}
Glutamate uptake	" " "
Glutamate Vmax	" " "
Glutamate Kt+Sn	μg x liter^{-1}
Glutamate Tt	h.

	Units
Glucose % Resp.	%
Glutamate % Resp.	%
Ethylene Production	nM x g. dry wt.$^{-1}$ x h^{-1}
Total Adenylates	(μM x g. dry wt.$^{-1}$
Energy charge	ratio - see methods
Hydrogen ion conc.	x E-8 M
Redox potential	mV

Table 29. Changes observed in sediment samples exposed to crude oil for 8 months.

	Trays and Aquaria				
	Control	Oil	Dif.	%	p<
Variable	$\bar{Y}$	$\bar{Y}$	Δ	Δ	
Glucose uptake	55	34	-11	-38	NS (0.27)
Glutamate uptake	448	353	-95	-21	NS
Glucose % Resp.	16	35	19	+119	0.003
Glutamate % Resp.	46	49	3	+7	NS
Ethylene Production	0.37	0.02	-0.35	-95	0.003
Denitrification	3.3	1.6	-1.7	-52	0.009
CO_2 Production	1.3	2.6	1.3	+50	0.002
Hydrogen Ion Conc.	2.7	5.4	2.7	+50	NS
Redox Potential	+33	-71	104	-315	NS (0.07)

Under each heading, the mean value measured in the control (non-treated) and the oiled sediments is reported along with the difference between the means and the percent of that difference relative to the control mean. The "p" value is the level of statistical significance between the mean values of all control and treated sediments analyzed.

	Units		Units
Glucose uptake	ng x g. dry wt.$^{-1}$ x h^{-1}	Hydrogen ion conc.	x E-8 M
Glutamate uptake	" " "	Redox potential	mV
Ethylene production	nM x g. dry wt.$^{-1}$ x h^{-1}		
Denitrification	" " "		
CO_2 production	" " "		

Table 30. Changes observed in sediment samples exposed to crude oil for 11 months.

	Trays and Aquaria				
	Control	Oil	Dif.	%	p<
Variable	$\bar{Y}$	$\bar{Y}$	Δ	Δ	
Acetate Uptake	54	14	40	-74	0.02
Glucose Uptake	36	18	18	-50	0.05
Glutamate Uptake	246	90	156	-63	0.002
Glutamate Vmax	237	89	148	-62	0.02
Glutamate Kt+Sn	33	24	9	-27	NS
Glutamate Tt	74	205	131	+177	0.01
Acetate % Resp.	23	38	15	+65	0.005
Glucose % Resp.	19	34	15	+79	0.0003
Glutamate % Resp.	49	55	6	+12	NS (0.07)
Ethylene Production	0.06	0.004	0.056	-93	0.0003
Denitrification	3.4	0.9	2.5	-74	0.004
CO_2 Production	5	12	7	+58	0.015
Methane Production	23	54	31	+57	0.04

Under each heading, the mean value measured in the control (non-treated) and the oiled sediments is reported along with the difference between the means and the percent of that difference relative to the control mean. The "p" value is the level of statistical significance between the mean values of all control and treated sediments analyzed.

	Units		Units
Acetate uptake	ng x g. dry wt.$^{-1}$ x h^{-1}	Ethylene production	mM x g dry wt.$^{-1}$ x h^{-1}
Glucose uptake	" " " " "	Denitrification	" " "
Glutamate uptake	" " " " "	CO_2 production	" " "
Glutamate Vmax	" " " " "	Methane production	" " "
Glutamate Kt+Sn	µg x liter^{-1}		
Glutamate Tt	h.		

Table 31. Percent changes in variables in response to 5 month exposure to crude oil and crude oil + Corexit.

Variable	Trays		Aquaria	
	Oil	Oil + Corexit	Oil	Oil + Corexit
A. Uptake				
Glucose Uptake	-60	-84	-69	-61
Glutamate Uptake	-71	-85	-73	-76
Glutamate Vmax	-78	-90	-72	-79
Glutamate Tt	+117	+113	+223	+200
Glutamate Percent Resp.	9	+8	+14	+11
Glucose Percent Resp.	+264	+240	+133	+148
B. Total Adenylates	-74	-87	-65	-68
C. Surface Eh	-380	-336	-576	-561
D. Nitrogenase	-85	-82		

Table 32. Summary of glutamate heterotrophic potential data in Kasitsna Bay sediments (July, 1979).

Sample	Source	Vmax[1]	Kt+Sn[2]	Tt[3]
B209	Control Tray	1.09	62	91
210	Control Tray	1.58	43	44
211	Oil Tray	0.16	15	157
212	Oil Tray	0.45	19	68
213	Oil + Hi Corexit Tray	0.12	4	52
214	Oil + Hi Corexit Tray	0.15	23	238
215	Hi Corexit Only Tray	0.79	52	52
216	Hi Corexit Only Tray	1.16	50	65
217	Low Corexit + Oil Tray	0.20	21	151
218	Low Corexit + Oil Tray	0.31	24	116
219	Low Corexit Only Tray	0.72	22	49
220	Low Corexit Only Tray	0.92	37	60
Aquaria				
286	Control A-I	1.05	25	37
287	Control A-O	1.06	11	15
288	Oil B-I	0.22	14	100
289	Oil B-O	0.54	28	80
290	Corexit+Oil D-I	0.21	15	101
291	Corexit + Oil D-O	0.23	9	55
Mean Values				
	Controls	1.05	38	51
	Oil	0.34	19	101
	Oil + High Corexit	0.18	13	68

[1] µg/g dry wt/hr

[2] µg/l

[3] hours

Table 33. Short-term crude oil and Corexit effects on glucose uptake rates in sediments that were non-oiled and those treated with crude oil or crude oil and Corexit for periods of time greater than 1 1/2 months. These sediments were collected at Kasitsna Bay. The values given are the percent reduction in observed uptake rates compared to controls.

A. Sediments which were not exposed in short-term challenge experiments.

Month	Exposure time in months	Short-term effects caused by Crude Oil	Crude Oil + Corexit	Corexit
April	1.5	33	51	36
July	5	28	68	58
Nov.	8	49	-	-
Jan.	11	30	-	-

B. Sediments that were challenged with crude oil.

Month	Exposure time in months	Short-term effects caused by Crude Oil	Crude oil + Corexit	Corexit
April	1.5	0	56	57
July	5	2	79	72
Nov.	8	25	-	-
Jan.	11	23	-	-

C. Sediments that were challenged with crude oil and Corexit.

Month	Exposure time in months	Short-term effects caused by Crude Oil	Crude oil + Corexit	Corexit
April	1.5	4	61	58
July	5	7	71	72
Nov.	8	-	-	-
Jan.	11	-	-	-

Table 34. Long-term effects of crude oil on glucose and glutamate uptake in the Elson Lagoon sediments.

		Uptake				
		Glucose		Glutamate		
Date	Exposure	uptake[1]	Δ%[2]	uptake[1]	Δ%[2]	Vmax[3]
Jan. 1978	Control	3.4		23		0.14
	"	0.2		3		0.25
	"	1.2		6		–
	Oil-6 months	5.7	NS	44	NS	0.09
	"	7.9		15		0.10
	"	2.0		15		–
Apr. 1978	Control	12.2		479		3.5
	Oil-3 months	3.2	NS	393	NS	8.2
Aug. 1978	Control	2.7		19		–
	"	13.2		161		–
	Oil-8 months	6.9	NS	96	NS	–
	Oil-4 months	1.5		9		–
Jan. 1979	Control	2.4		7		–
	"	3.7		8		–
	Oil-12 months	0.8	-74	2	-73	–
	Oil-9 months	0.8	-74	3	-60	–
Aug. 1979	Control	172		1025		0.8
	Oil-20 months	25	-85	152	-85	0.1
	Oil-16 months	38	-72	283	-72	0.2
Jan. 1980	Control	12		208		–
	"	32		75		–
	Oil-24 months	8	-64	75	NS	–

[1] ng/g dry wt/hr.

[2] Percent change relative to control; NS - not significant if ranges overlap.

[3] μg/g dry wt/hr.

Table 35. Long-term effects of crude oil on Percent respiration in Elson Lagoon sediments.

	Treatment	% Resp. Glucose	Δ%	% Resp. Glutamate	Δ%
Jan., 1978	Control	25		44	
	Control	20		41	
	Control	23		51	
	Oil 6 months	20	+9	45	+4
	" "	27		49	
	" "	27		48	
April, 1978	Control	19		44	
	Oil 3 months	29	+53	62	+41
Aug. 1978	Control	29		54	
	Control	-		46	
	Oil 8 months	23	-21	-	
	Oil 4 months	57	+97	54	+8
Jan., 1979	Control	49		78	
	Control	47		7.4	
	Oil 12 months	71	+48	90	+18
	Oil 9 months	59	+23	81	+ 7
Aug., 1979	Control	20		48	
	Oil 20 months	38	+90	49	+ 2
	Oil 16 months	33	+65	53	+10
Jan., 1980	Control	32		53	
	Control	23		46	
	Oil 2 years	32	+16	63	+12

Table 36. Effects of fuel oil on relative microbial activity and respiration percentages in Glacier Bay, AK sediments. Each sample was exposed to crude oil in a short-term challenge experiment.

Sediment type			Glucose Uptake[1] no oil	oil	Percent change	Percent respiration
A.	Coarse sand	Non-oiled	138	42	-70	23
		Oiled	156	83	-47	47
			+13*			+104*
B.	Sand-silt	Non-oiled	30	15	-47	17
		Oiled	18	11	-39	30
			-40*			+76*
C.	Glacial flour	Non-oiled	6.8	5.3	-22	36
		Oiled	2.4	7.1	198	29
			-65*			-26*

1 = Glucose uptake in units of ng/g/hr.

* = These values are the percent change relative to the controls.

Table 37. Long-term effects of crude oil on nitrogen fixation rates in Elson Lagoon.

	Exposure time	Control[1]	Oil[1]
Apr. 1978	0	1.3	1.2
Aug. 1978	1 day	0.3	0.4
Aug. 1978	4 mo.	0.3	0.4
Aug. 1978	8 mo.	0.3	0.4
Apr. 1978	3 mo.	1.4	1.9

[1] ngN fixed/g dry wt/hr.

Table 38. Effects of crude oil on nitrogen fixation in sediments augmented with organic (non-petroleum) carbon.

Treatment[2]	Nitrogen Fixation[1] -Oil	+Oil	Percent change
None	4.03	0.14	-97
"	4.13	0.23	-94
"	2.16	0.22	-90
"	2.09	0.05	-98
Cerophyl[3]	0.48	0	-100
Chitin	0.50	0	-100
Starch	52.8	5.09	-90

[1]ngN fixed/g dry wt/hr.

[2]Carbon supplements at rate of 5% w/v.

[3]Cerophyl is a dried cereal grass product.

Table 39. Long-term crude oil effects on denitrification rates.

Date	Sample	Amendment	Denitrification[1]	
			-Oil	+Oil
Nov. 1979	Tray 2 week	$+NO_3$	3.0	3.1
		"	2.9	2.6
	Tray 8 month	$+NO_3$	3.4	2.7
		"	3.1	1.8
			4.6	1.9
	Aquaria 8 month	$+NO_3$	2.5	0.9
		"	3.0	0.8
	C-source trays 8 month	$+NO_3$		
	Control	"	2.8	1.3
	Cerophyl	"	0.6	0.6
	Chitin	"	0.7	0.2
	Starch	"	0.2	0
Jan. 1980	Tray 11 months	$+NO_3$	3.7	0
		"	3.7	0
		"	3.8	0
		$-NO_3$	3.7	1.9
		"	3.7	1.1
		"	3.9	0.7
	Aquaria 11 months	$+NO_3$	2.5	0
		"	3.2	0
		$-NO_3$	0.1	0.3
		"	3.1	0.5

[1]nM N_2O produced/g dry wt/hr.

Table 40. Long-term effects of crude oil on carbon dioxide and methane production.

Location	Experiment	CO_2 production[1]		CH_4 production[2]	
		Control	Oil	Control	Oil
Kasitsna Bay Nov. 1979	8 month trays	1.1	3.7	-	-
		0.8	2.3	-	-
		0.94	3.2	-	-
		0.93	3.4	-	-
	8 month aquaria	2.6	1.5	-	-
		1.4	1.7	-	-
	C-source trays				
	Control	0.93	3.4	-	-
	Cerophyl	8.7	15.0	-	-
	Chitin	5.2	15.9	-	-
	Starch	2.4	1.1	-	-
Jan. 1980	11 month trays	4.9	22.5	20	40
		5.9	16.8	15	20
		6.2	13.0	10	52
	11 month aquaria	2.8	4.4	28	77
		4.5	4.1	42	80
Elson Lagoon Jan. 1980	2 yr tray	4.1	4.5	-	105
	0 time tray	6.1	1.1	8	-
	Control tray			53	-
				18	-

[1] nM CO_2/g dry wt/hr.
[2] pM CH_4/g dry wt/hr.

Table 41. Adenylate measurements made on sediment samples collected during the summer, 1978 Beaufort Sea study and in the Elson Lagoon in January, 1979.

A. Beaufort Sea, August, 1978

Sample Number	Treatment	*ATP	*ADP	*AMP	Total Adenylate	Energy Charge
BB601	Control	245	6	7	263	0.95
BB602	Oiled 4 mo	3.2	0	0	3.2	1.00
BB605	Oiled 8 mo	3.6	0.2	0	3.7	0.98
BB607	Control	211	19	0	227	0.96
BB619	Offshore	337	7	10	352	0.96
BB624	Offshore	13.7	1.3	0.6	15.7	0.92
BB262	Offshore	23.7	0.3	0	24.6	0.99
BB627	Offshore	62.7	1.9	0.3	65.3	0.98

B. Elson Lagoon, January, 1979

Sample Number	Treatment	*ATP	*ADP	*AMP	Total Adenylate	Energy Charge
BB701	Oiled 9 mo	0.2	0.08	0.28	0.4	0.24
BB702	Oiled 12 mo	0.33	0.54	1.01	1.9	0.38
BB703	Control	0.28	4.0	13	17	0.25
BB703	+ oil	0.63	5.4	17	23	0.27
BB704	Control	0.07	1.9	11	13	0.15
BB705	Oil 0 time	0.17	3.0	11	14	0.19

*nMoles of adenylate/g dry wt.

Table 42. Direct bacterial concentrations in treated and non-treated Kasitsna Bay sediments.

Date	Source	Exposure to oil	Cells (x 10^9/g) Control mean	Control range	Oil mean	Oil range
Apr 1979	Trays	1.5 months	3.9	3.2-4.6	2.5	0.6-3.8
	Aquaria	1.5 months	3.2	3.1-3.3	2.4	1.7-3.4
Jul 1979	Trays	5 months	3.9	1.6-6.4	2.2	1.8-2.4
	Aquaria	5 months	3.7	-	1.9	1.5-2.4
Nov 1979	Trays	8 months	3.6	2.5-4.5	3.5	2.7-4.4

Table 43. Effect of crude oil on enzyme activities in treated and non-treated sediments.

Enzyme	Percent Change in Enzyme Activity Relative to Control			
	Apr. 1979	Aug. 1979	Nov. 1979	Jan. 1980
Kasitsna Bay trays				
phosphatase	+17	-3.4	-28.3	-8.4
arylsulfatase	+10.8	+7.0	-17.1	-25.4
amylase	-5.8	-30	+15.2	-34.7
cellulase	-6.1	+5.4	+21.6	+18.2
Aquaria				
phosphatase	+33.6	-39	-21.5	-64.4
arylsulfatase	+41.1	-22	-31.2	-30.2
amylase	+4.3	+14.2	+7.3	+34.8
cellulase	-13.3	+6.6	-6.7	+13.6
Elson Lagoon	(Jan. 1979)	-3.7	-	+46.3
phosphatase	-	-48.1	-	-46.2
arylsulfatase	-	-35.9	-	0
amylase	-			
cellulase	+157	+15.4	-	_45.3

Table 44. Relative microbial activity in waters and sediments of Kasitsna Bay.

Water		Feb. 1979	Apr. 1979	July 1979	Aug. 1979	Nov. 1979	Jan. 1980
*Glucose	$\overline{Y}$	0.7	135	86	29	5	2
	SD	0.3	131	57	20	3	1
	Range	0.4-1.2	11-366	22-181	6-65	1-10	1.2-3.6
	N	7	6	15	15	17	10
*Glutamate	$\overline{Y}$	7	134	101	-	12	6
	SD	6	115	74	-	5	3
	Range	2-18	32-170	21-245	-	3-23	3-14
	N	7	6	15	-	15	10
Sediments							
°Glucose	$\overline{Y}$	25	27		137	57	46
	SD	29	22		94	57	30
	Range	3-112	5-73		20-357	4-183	11-118
	N	17	10		18	15	10
°Glutamate	$\overline{Y}$	338	318		1068	316	376
	SD	425	254		974	328	283
	Range	32-1856	35-936		182-3600	41-1262	112-1113
	N	17	10		18	15	10

*Units used are ng/liter x hr.

°Units used are ng/g dry wt/hr.

Table 45. Summary of nitrogen fixation rates, total adenylate concentrations and energy charge estimates in Kasitsna Bay sediments.

A. Nitrogenase activity[1]

Date	n	$\overline{Y}$	SD	Range
Feb. 1979	9	0.8	0.7	0.2-2.3
Apr. 1979	10	0.8	0.8	0 -2.1
Jul. 1979	14	0.4	0.4	0 -1.0
Oct. 1979	14	1.7	1.9	0 -7.5
Jan. 1980	3	0.7	0.5	0.3-1.2

B. Adenylate determinations

		Total Adenylates[2]			Energy Charge		
Date	n	$\overline{Y}$	SD	Range	$\overline{Y}$	SD	Range
Feb. 1979	9	4.4	2.3	0.1-7.3	0.31	0.16	0.18-57
Apr. 1979	4	6.9	1.9	4.2-8.5	0.29	0.04	0.24-0.35
Jul. 1979	14	7.7	3.1	3.3-12.9	0.26	0.08	0.12-0.38

[1]nmoles C_2H_4/g dry wt/hr.

[2]nmoles/g.

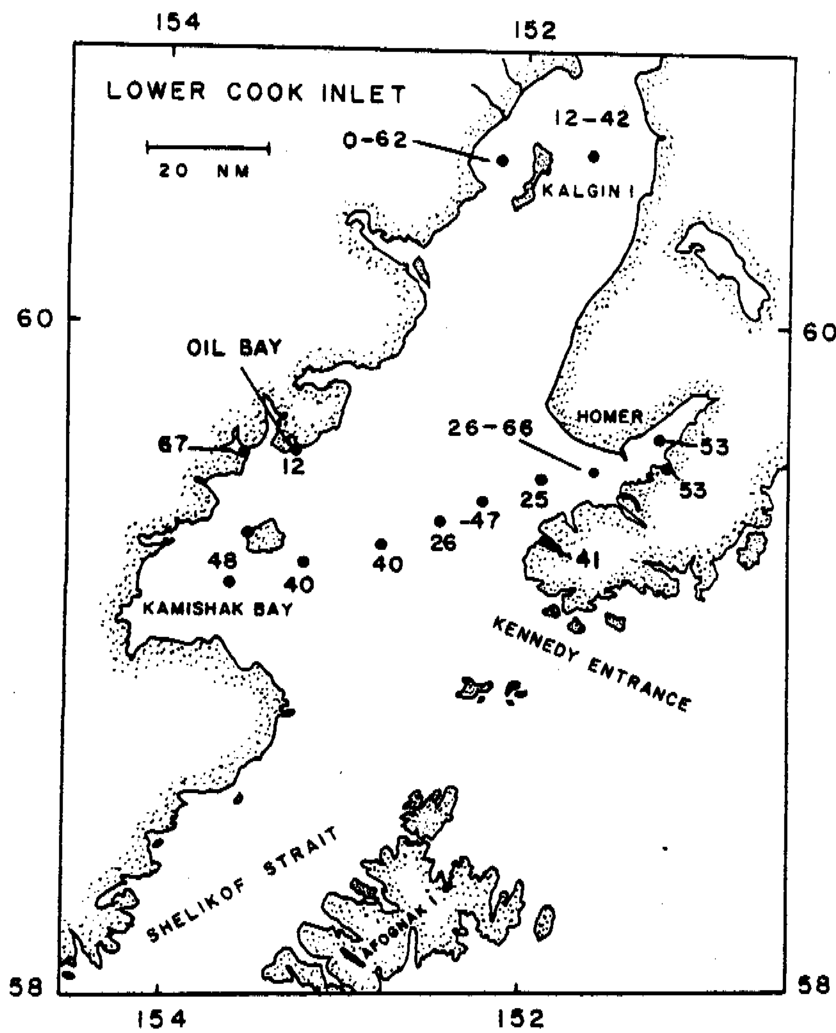

Figure 37. Percent reduction in glucose uptake in water samples exposed to crude oil during the April, 1978 cruise.

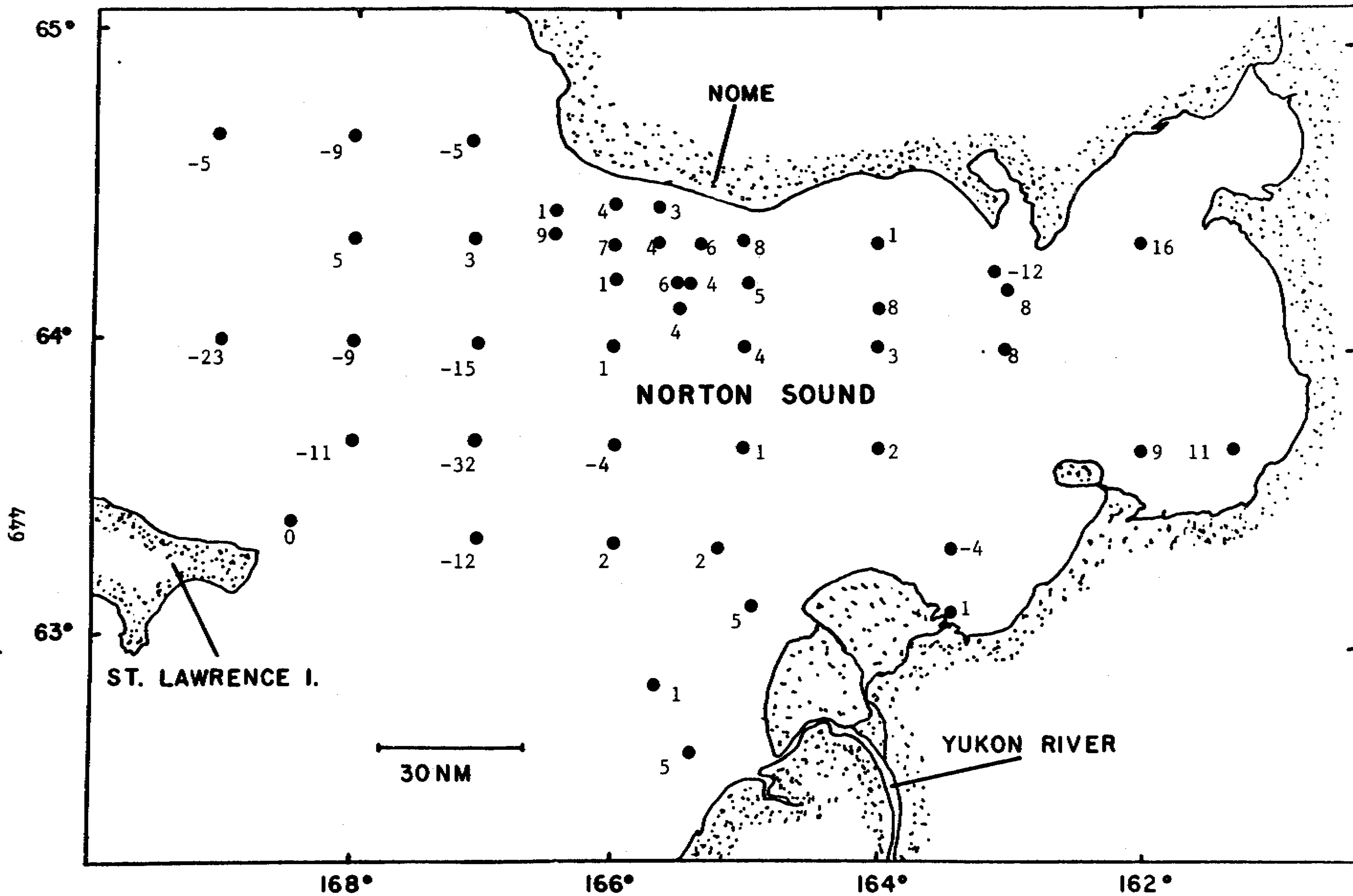

Figure 38. Change in percent respiration of glucose in water samples exposed to 50 ppm Corexit.

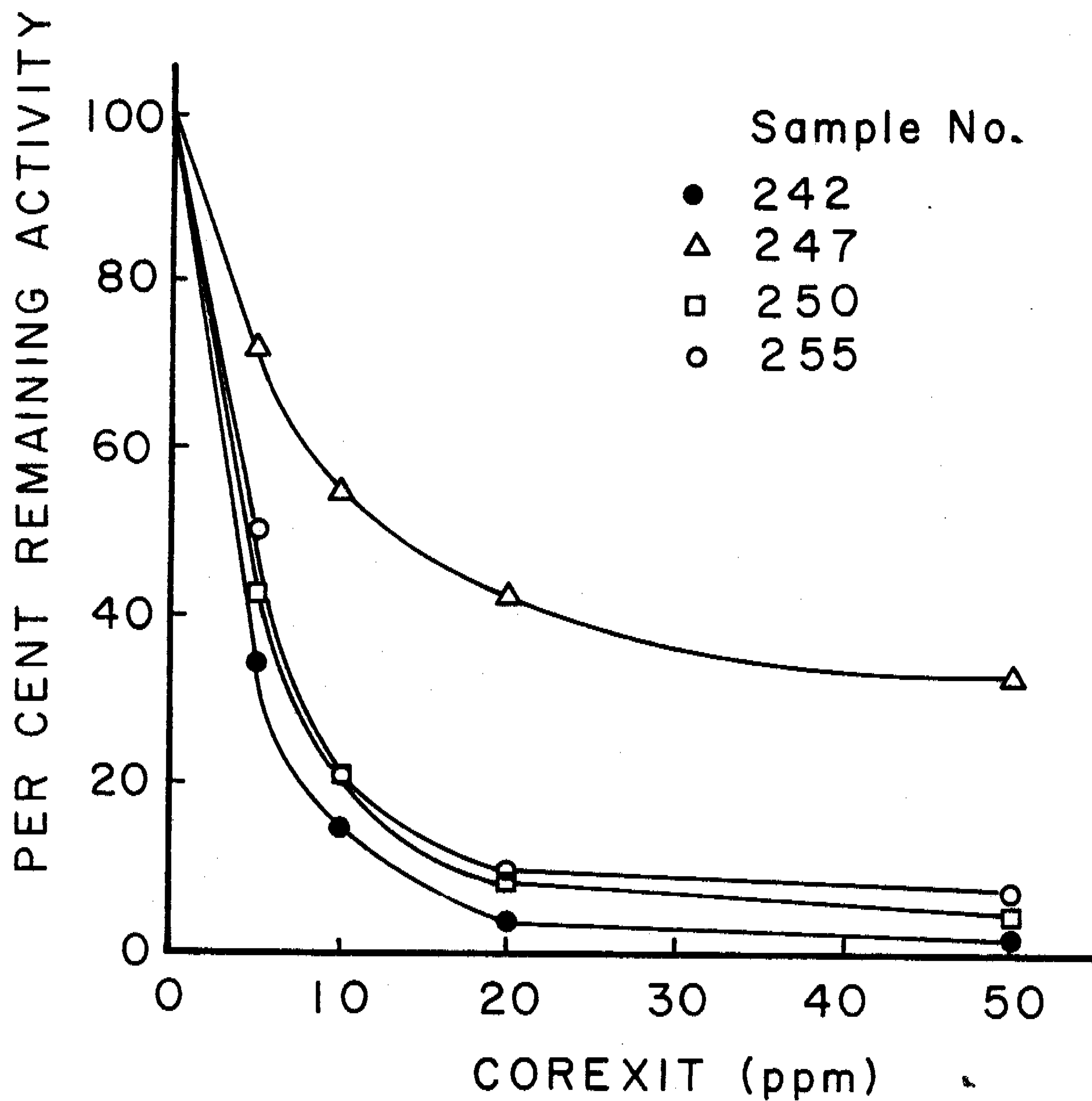

Figure 39. Changes in glucose uptake relative to controls in four water samples exposed to increasing concentrations of Corexit.

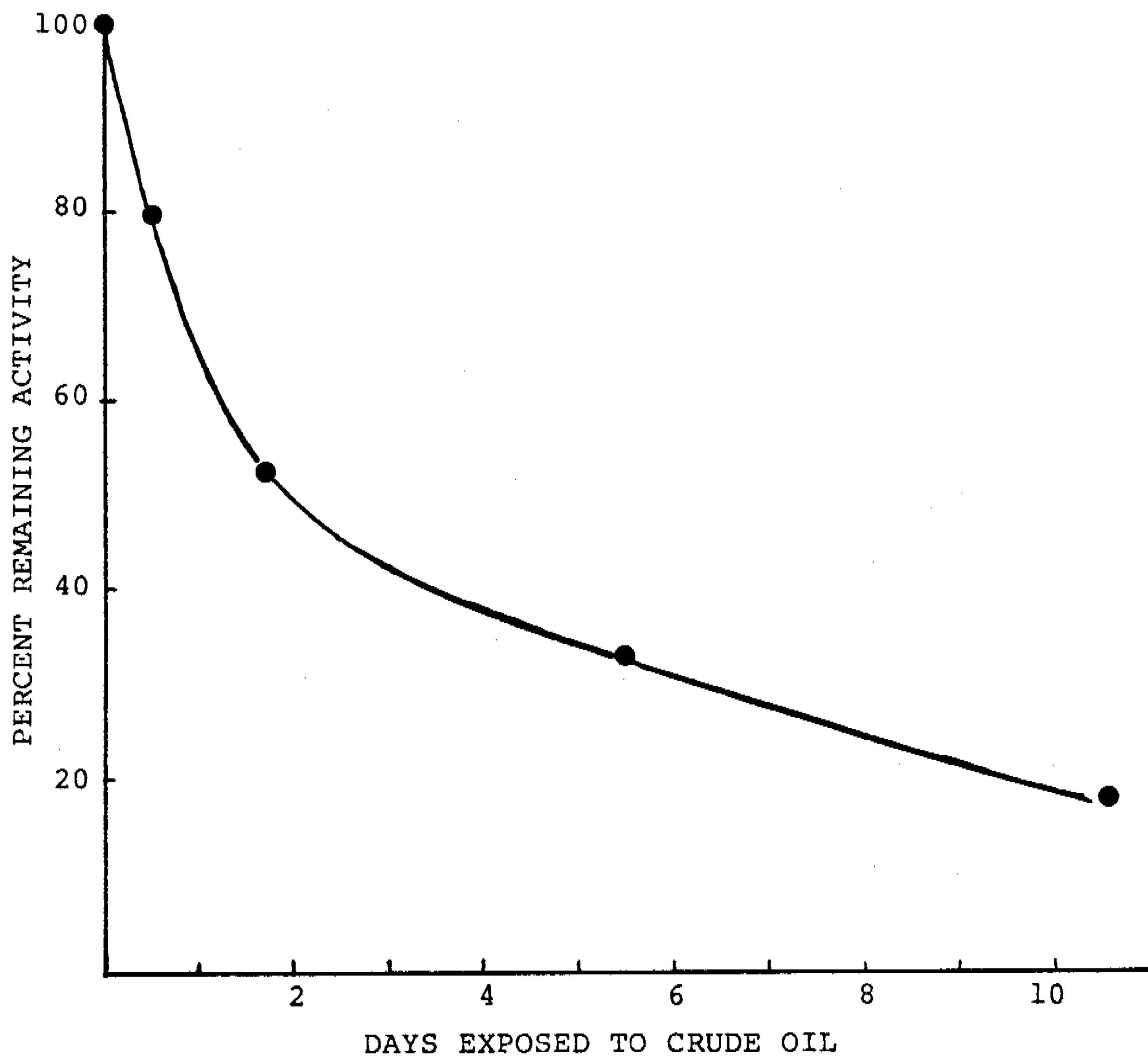

Figure 40. Change in nitrogenase activity in a sediment sample exposed to crude oil. Percent activity is calculated relative to a control.

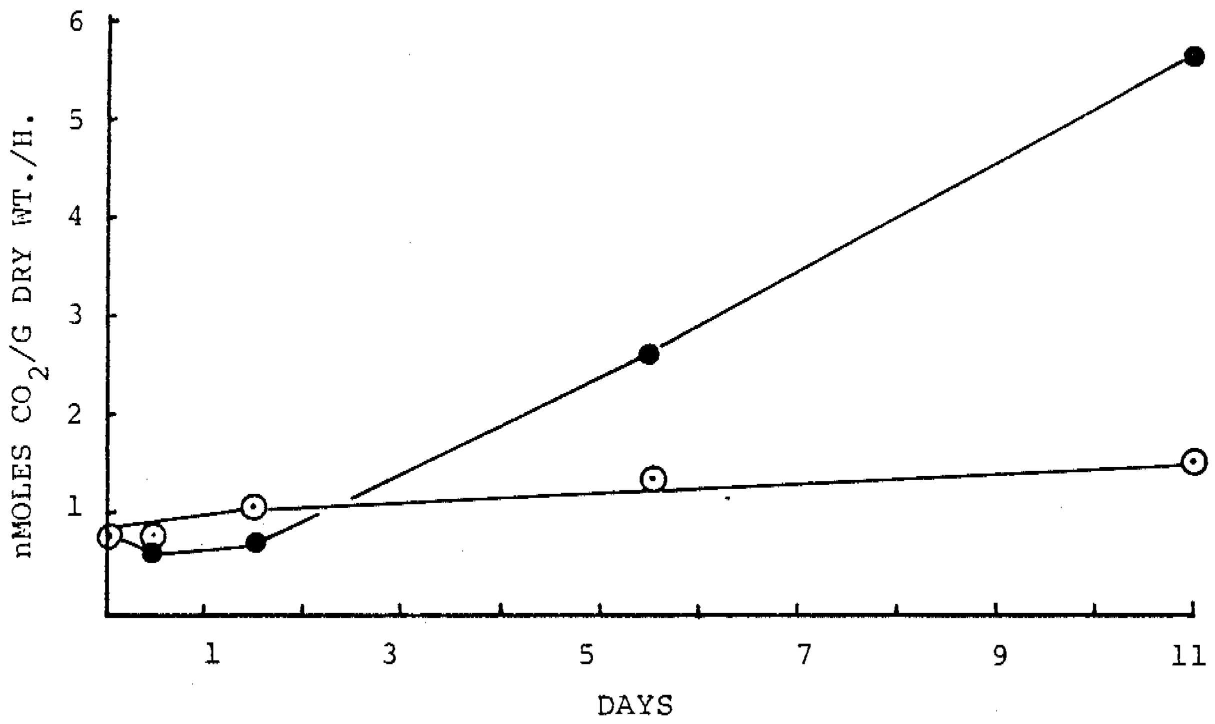

Figure 41. CO_2 evolution in oiled (●) and non-oiled (⊙) sediments.

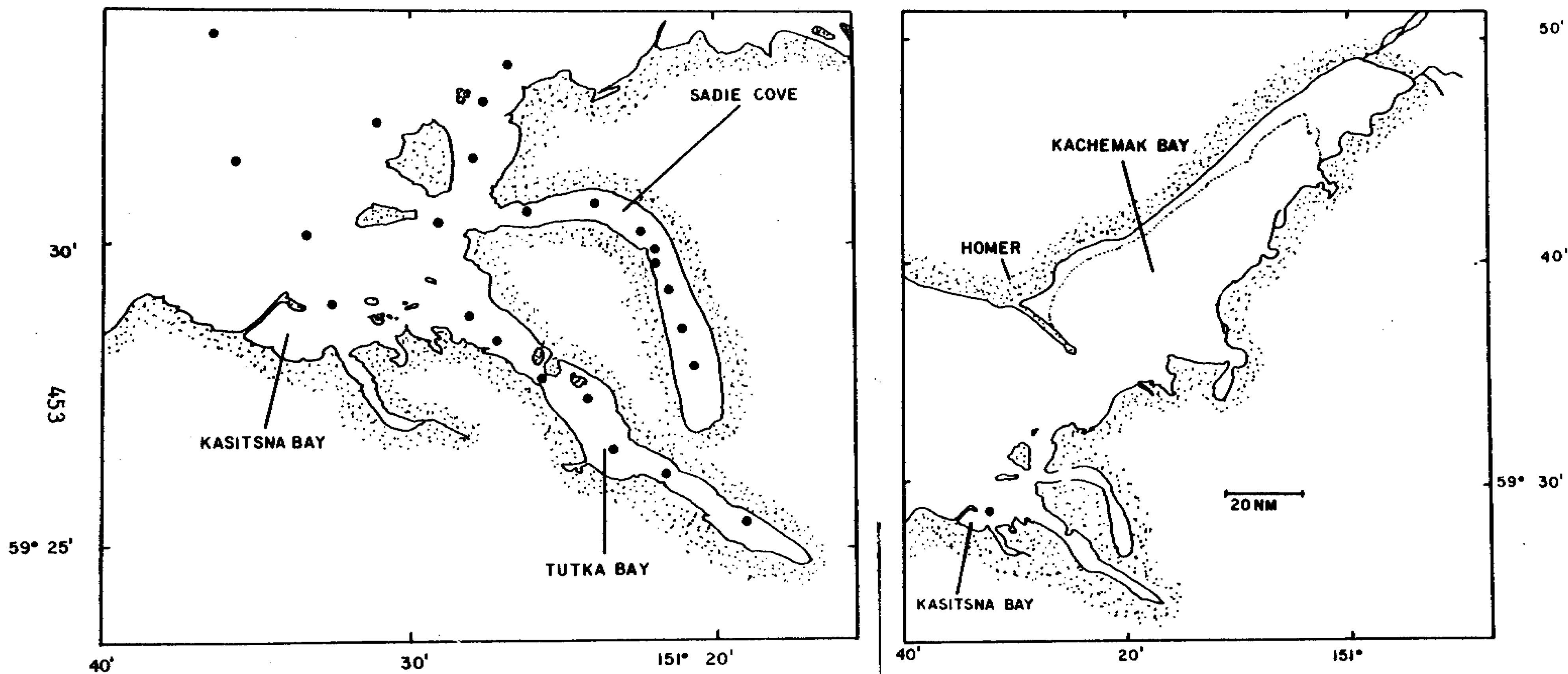

Figure 42. Location of sampling sites in the Kasitsna Bay area.

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VIII. Glossary of Terms

Acute effects -- These are the effects that are observed in samples that had been exposed to the substance being tested for 24 hours or less.

Adenylates -- A group of compounds found in all living things that act as energy transporters within the cell. These include the compounds, ATP, ADP, and AMP.

Amylase -- This is an enzyme that hydrolyzes starch into simple sugars.

Anaerobic fermentation -- This is the degradation of organic nutrients under anoxic conditions. The typical products of anaerobic fermentation are: hydrogen sulfide, methane, ammonia, and organic acids.

Anoxic -- This is a condition where oxygen is absent.

ATP, ADP, AMP -- These are adenylates which are responsible for energy transfer within living cells. Adenosine triphosphate (ATP) contains the most energy, adenosine diphosphate (ADP) contains less energy, and adenosine monophosphate (AMP) is the lowest energy level.

Bacteriovore -- Any organisms that consume bacteria as a food source.

Benthic microorganisms -- Those microorganisms that live in sediments.

Biosynthesis -- This is the process by which new cell material is made.

Challenge experiments -- These are the acute effects experiments where samples are exposed to the substance to be tested for 24 hours or less.

Chitinase -- This is the enzyme that hydrolyses chitin into simple sugars. Chitin is one of the major structural components of crab and shrimp exoskeletons.

Detrital food chain (web) -- This is the food chain which is based on food from detritus rather than from food that is directly consumed as living plant material.

Detritus -- All non-living organic material (including soluble organics) found in the ecosystem.

Direct cell counts -- This is the procedure used to determine the concentration of bacteria by counting them directly under a microscope.

Diversity index -- This is a measure of the number of different types of organisms in a sample.

Epifluorescent microscopy -- This is a procedure that we use to make direct bacterial cell counts. The cells are tagged with a fluorescent stain which can be seen under an ultraviolet light source to distinguish cells from detritus.

Eucaryotic organisms -- Organisms that have cells containing nuclei; i.e., organisms that are higher than the bacteria.

Glucose or glutamic acid uptake studies -- These are studies in which we measure the rate of substrate that is taken up and respired (mineralized) by natural microbial populations. The higher the rate, the more active the population. This is often expressed as "relative microbial activity".

Herbivore -- An organism that consumes plants to obtain food.

Heterotrophic potential studies -- Those studies where we measure the uptake and respiration of organic substrates by natural microbial populations at various substrate concentrations. From these data, we can calculate kinetic variables such as V_{max}, T_t and $K_t + S_n$. Heterotrophic potential = the maximum potential rate at which the test substrate can be taken up and utilized by the microorganisms.

Hydrolase -- Any enzyme that catalyzes a hydrolytic reaction. The hydrolases that we are concerned with (amylase and cellulase) break down large molecules into smaller ones that can be readily utilized by bacteria.

Infauna -- Any organisms that live in marine sediments.

Kinetic data -- Variables that have been calculated from data generated from heterotrophic potential studies.

$K_t + S_n$ -- This is a single value that is estimated during heterotrophic potential studies which includes both the transport constant (K_t) and the natural substrate concentration (S_n); the natural substrate concentration of the same substrate that is added during the experiment. This value can be used in a comparative sense when the S_n value is known not to differ between the samples being compared. Under these conditions, any change in $K_t + S_n$ can be attributed to changes in K_t. If this value increases for some reason, it can then be said that the population will take up the substrate at a lower rate at a given substrate concentration.

Long-term effects -- These are effects that are observed in samples that had been exposed to the substance to be tested for more than 24 hours.

Macrophytes -- A plant that is large enough to be seen by the unaided eye. As used in this context, it means large marine algae that are normally attached to some hard surface.

Mineralization -- This is the process by which organic molecules are converted to inorganic molecules.

Nitrogen fixation -- The process by which atmospheric nitrogen (N_2) is converted to fixed nitrogen; i.e., NH_4, NO_2, NO_3. This is a reaction that requires a great deal of energy.

p value -- This represents the statistical significance of the difference between a set of mean values. A p value of 0.05 means that there is a 95% probability that the difference between the two mean values being compared is not due to chance.

Pelagic microorganisms -- Those microorganisms associated with the water column.

Percent respiration -- This is the percent of the total amount of substrate taken up by the microorganisms that is respired as CO_2. It is calculated by dividing the amount of labeled carbon associated with the CO_2 fraction by the total amount of substrate taken up by the cells (both cell and CO_2 radioactivity) and multiplying this ratio by 100.

Phosphatase -- This is the enzyme that converts organic phosphate to inorganic phosphate.

Primary productivity -- This is the process by which atmospheric CO_2 is converted to organic carbon. This new organic carbon can be in the form of new plant material or soluble organic material released by the plants during photosynthesis.

Relative microbial activity -- This is a relative estimate of how metabolically active a natural microbial population is. This is determined by the rate at which the microorganisms take up a simple organic compound which is labeled with a radioactive tracer. This is done using two methods; uptake at one substrate concentration or uptake at a series of different substrate concentrations from which kinetic parameters can be calculated. In the multi-concentration method, the kinetic parameter that we use in the determination of relative microbial activity is the maximum potential rate at which the substrate can be taken up (V_{max}).

Secondary productivity -- This is a poorly defined term which generally means the generation of organic nutrients in a form that can be used by animals (not including living plant material). This is a food source that would not be useful to that animal in its original form.

Short-term effects studies -- Effects studies conducted for 24 hours or less.

Suspended particulate matter (also suspended matter) -- This is anything in the water column that will not pass through a membrane filter with a pore size of 0.45 nm.

Transport constant (T_t) -- This is a variable that is calculated from the data generated during a heterotrophic potential determination. This value is the time in hours required for the natural microbial population to utilize the natural substrate concentration of the substrate being tested.

Uptake rates -- See relative microbial activity.

V_{max} -- This is the kinetic variable calculated from heterotrophic potential determinations which is used as an indicator of relative microbial activity. V_{max} = the maximum potential rate at which the tested population could possibly take up the test substrate.

RECEPTORS-ECOLOGICAL PROCESS STUDIES

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FOODWEB AND NUTRIENT DYNAMICS STUDIES IN NEARSHORE ALASKAN BEAUFORT SEA WATERS

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HIGHLIGHTS OF 1979 RESULTS AND CONCLUSIONS

Ice algae

The distribution and productivity of ice algae was estimated from chlorophyll and standing stock measurements in the Beaufort Sea lease area and over a flight line extending 100 miles to sea. Biomasses were most variable in the nearshore region due primarily to areas of turbid ice which prevented light penetration. Offshore populations were uniformly high at the locations sampled although no data was obtained from pressure fields or in the vicinity of pressure ridges.

Biomass estimates in the Stefansson Sound area ranged from undetectable in areas of turbid ice to a maximum of approximately 3 grams dry weight/m^2 (silica-free) in Mikkelsen Bay. Simpson Lagoon was characterized by clearer ice but ice algae densities were less than 0.5 g dry weight/m^2 at the two sample locations. The high salinities ($\sim$ 44°/oo) and correspondingly low temperatures in the lagoon waters may be the cause of these low populations. Offshore ice algae biomass ranged between 0.6 and 2.7 g dry wt/m^2 with the highest concentrations found at the station 100 miles out indicating active growth beneath the continental shelf ice cover. Sampling capabilities should be improved in 1980 through the deployment of an in situ fiber optic sensor for measuring chlorophyll fluorescence and thereby estimating algal biomass. Through the conversion of estimated standing stocks to estimated annual production (based upon turnover rates derived from primary production measurements of ice algae in Stefansson Sound) ice algae carbon fixation was found to range from near zero to 2.5 g/m^2 with an average annual production rate of 1.4 g C/m^2. Average offshore annual production for ice algae is estimated at 1.9 g C/m^2 and in Simpson Lagoon, 0.2 g C/m^2.

Energy flow and trophic relationships

Carbon isotope tracer techniques have revealed that the foodweb pathways of energy transfer into the fish and birds of the nearshore Beaufort Sea are almost totally based upon marine primary production in

spite of the large quantities of terrestrial carbon available to the nearshore ecosystem.

The organic matter transported by the Colville and Kuparuk Rivers was found to be strongly depleted in ^{14}C and the depletion increased as breakup progressed. Thus the major fraction of fluvially transported carbon is derived from eroded peat from river banks and the input of surficial vegetative material is pronounced only during the initial stages of breakup in the rivers. The Kuparuk River organic matter contained the lowest quantity of ^{14}C, approximately 71% of the 1950 standard radiocarbon activity compared to 65% for the mean radiocarbon content of Simpson Lagoon soil sections.

Refined estimates of the mean ^{14}C content of the allochthonous carbon sources and the determination of ^{14}C activity in several additional species of marine and freshwater fauna of the coastal zone and rivers has revealed startling variations with regard to the utilization of eroded peat carbon. The anadromous fish of the Colville River (arctic cisco, least cisco, humpback whitefish) are almost entirely dependent upon the marine ecosystem for their nutrition as evidenced by C^{13}/C^{12} isotope ratios, but apparently also consume a small quantity of freshwater food based upon freshwater primary production with a resulting small elevation in ^{14}C content. There is no evidence of significant detrital production based upon peat in any of the anadromous fish. In contrast, two obligate freshwater fishes, arctic grayling and round whitefish, yielded nearly identical ^{13}C depletions typical of freshwater production but varied in ^{14}C content by nearly 20% with the grayling containing the highest radiocarbon content of any fish sampled to date, and the round whitefish, the lowest. The two species are apparently feeding upon differing prey organisms with a detrital food chain sustaining a large fraction of the energy requirements of the round whitefish. This finding is not supported by a literature review of the feeding habits of these species which lists many identical insect larvae in gut contents taken from both species. An immature round whitefish yielded a radiocarbon content approximately halfway between the grayling and adult round whitefish values, which, if representative, may indicate that the food habits of round whitefish change during growth with an

increasing dependence upon detritus based foodwebs. Additional samples are currently being analyzed for confirmation of ^{14}C content in these species. We plan further sampling in the Colville River in 1980 with the purpose of identifying food source materials and acquiring well-documented specimens of fish and other aquatic biota for radiocarbon activity determination.

Marine detrital based production

Laboratory experiments in cooperation with Dr. David Schneider (RU 356) were conducted at the Naval Arctic Research Laboratory in July 1979 to determine the cellulose oxidation capabilities of in situ populations of marine microflora and resident populations of amphipods. The experiments confirmed previous findings that active microbial oxidation occurs in the water column but that *Onisimus sp.* amphipods do not have the ability to utilize cellulose (or peat) directly.

In contrast, experiments run with *Gammarus setosus* showed rapid cellulose uptake and metabolism. At 8°C, the average adult *Gammarus setosus* oxidized approximately 1.6 μg peat/hr to carbon dioxide. Microbial oxidation rates of peat in seawater yielded turnover times of about 1050 days at 0°C and 640 days at 8°C. This turnover time implies that the fate of most eroded peat is microbial oxidation within the lagoon-nearshore system.

The collection problems associated with acquiring sufficient specimens of *Gammarus setosus* from the lagoon environment to perform radiocarbon activity determinations (for high precision, 5 g of carbon is required) have prevented the acquisition of isotopic data on these animals. However, baited traps set in Simpson Lagoon during November 1979 yielded sufficient adult amphipods of *Atylus*, *Gammaracanthus*, and *Weyprechtia spp.* to obtain radiocarbon activities. These adult organisms contained the lowest radiocarbon activities of all faunal specimens determined to date corresponding to body compositions of 16-23% peat carbon. These organisms are apparently heavily dependent upon detrital foodwebs and reflect the high inputs of terrestrial detritus to the nearshore environment.

I. SUMMARY OF OBJECTIVES, CONCLUSIONS AND IMPLICATIONS WITH RESPECT TO OCS OIL AND GAS DEVELOPMENT

Objectives

The overall objective of RU 537 is to describe the principal processes supplying energy (i.e. fixed carbon) to the biota of the Beaufort Sea coastal zone and to follow transfer efficiencies of this energy through the foodwebs of the nearshore zone. In addition this research unit is investigating the nutrient dynamics of coastal waters and relating the nutrient regimes observed to this production of energy. Both terrestrially derived and offshore derived nutrient sources are considered. The information obtained is for integration into the overall structure of the LGL Barrier Island study group, RU 467. Their efforts will relate this information to: 1) description of the overall ecosystem, 2) possible OCS direct impacts on the nearshore biota by offshore oil and gas development, and 3) possible impacts on the near-shore biota caused by "upstream effects" land which would change the character of terrestrial input of nutrients and/or carbon to the marine ecosystem via erosional processes or runoff.

In addition, this research unit is cooperating with units 356 (Dr. Carter Broad) and 359 (Dr. Rita Horner) with the following objectives: 1) to seek information on the rates of macrofloral primary production in the "boulder patch" of Stefansson Sound (RU 356); 2) investigate the ability of indigenous species of amphipods to utilize peat carbon through symbiotic intestinal cellulose degrading microfloral populations, (RU 356) and 3) cross calibrate estimation techniques for ice algae biomass and seasonal production (RU 359) with the goal of developing methods that would allow estimation of annual ice algae production based on observed standing stocks at particular intervals of the spring season.

Conclusions

The conclusions to date give evidence that the nearshore Beaufort Sea is decidedly different from more temperate Alaskan coastal zones

with regard to the energy sources supporting the biota. The estimates of energy input to nearshore waters have been revised as new data obtained by this research unit and RU 530 are utilized to appraise the various input sources. These estimates show that over 50% of the carbon input to nearshore marine biota (within approximately 10 km of shore) is terrestrially derived from two primary processes, fluvial transport and coastline erosion. The rivers carry large quantities of organic matter during spring breakup and coastal erosion occurs during summer months, resulting in the deposition of large amount of organic carbon along the shoreline. This carbon is composed primarily of peat-like material that has been accumulating on land for up to 12,000 years. Thus the nearshore marine biome is apparently a "fossil fuel" subsidized ecosystem wherein the meager annual primary production by ice algae and phytoplankton is supplemented by organic carbon eroded from coastal peat bluffs and transported by river flow into the coastal zone. The distinctive carbon isotopic signatures of the terrestrial and marine source materials allow detection and quantitation of the source fractions in the tissues of the fauna comprising the foodwebs built upon these sources.

Implications with respect to oil and gas development

Detrimental impacts on the primary producers and detritivores within the lease area could result from either spills of hydrocarbons or petroleum related developmental activities. Since the range of specific insults to the environment is extreme, I will deal with the direct and indirect effects of developmental activities in a general sense. The research results of this RU have shown that 1) most of the higher organisms including the larger invertebrates are dependent primarily upon marine primary production for their food sources; 2) primary production in the nearshore zone is very low by most coastal standards; 3) extreme variability exists in the nearshore aquatic environment due to such natural phenomena as river breakup, daylight extremes, ice turbidity, solute exclusion during ice formation and strong winds. It is reasonable to expect therefore that the microflora and fauna living in this

environment are some of the most opportunistic and resilient organisms extant for their living conditions demand the flexibility. There are probably exceptions. The boulder patch floral and faunal densities and assemblages are anomalous and very likely unique due to the protected environment they enjoy and the paucity of similar areas free of ice gouging in deep (6 m) water with rocky substrate along the Alaskan Beaufort Sea coast. These same rigorous environmental conditions also imply, however, that the organisms living therein are periodically stressed and the fact that this coastline is a range limit for several also implies that the stresses can be severe and in some cases, limiting. In this context the implications of petroleum development acquire a greater potential for impact. If the environmental stresses are increased through the addition of man-produced insults such as might result from toxic waste inputs, increased turbidity, radical changes in circulation patterns, or oil spills, the impacts on some species could be direct with food web dependencies passing on the interspecific change. For example, an impact such as shoreline stabilization along the lease area would radically change the gross energetic input to the nearshore ecosystem by eliminating erosion of the peat bluff shorelines. This impact might not become apparent in the higher organisms such as fish due to their almost exclusive dependency upon marine primary production foodwebs. Some invertebrates, however, could be adversely impacted insofar as detrital food chains have been found to supply over 20 percent of the energy requirements of the amphipod *Weyprechtia heuglini* taken from Simpson Lagoon.

Major impacts would result from perturbations that altered the summer primary production by phytoplankton and ice algae. Oil spills, phytotoxic chemical discharges or other impacts that decreased primary production in a significant fraction of the coastal zone would be felt to the top of the food chain including all the anadromous fish. Other direct effects on primary producers such as uptake of toxic substances from low ambient concentrations could indirectly produce severe impact at the higher trophic levels through biomagnification of concentration in foodweb transfers. Toxic heavy metals or halogenated organic compounds are potential problems in the limited circulation situation which occurs during winter months in the lease area.

II. INTRODUCTION

General Nature and Scope of Study

This research unit originally proposed to look at a very specific aspect of the nearshore primary production regime, namely, the contribution of ice algae, and the effects of thermohaline convection in supplying nutrients to ice algae populations. Since that time the importance of terrestrial detritus as an energy source became more evident and this research unit began to consider the possible magnitude of its significance through the use of data collected previously by the author. These results, which have been refined and presented below, (See Figure 1) show the approximate magnitudes of carbon inputs to the Simpson Lagoon ecosystem and set forth an outline of isotopic techniques that allow determination of the relative inputs of peat carbon versus modern primary production carbon (Figure 2). By using carbon isotope techniques, this research unit has undertakne the task of allocating significances to each of the carbon sources to the fauna in the marine environments of the coastal Beaufort Sea.

Specific Objectives

The specific objectives of this research unit currently include:

1. Determine the role of terrestrial allochthonous carbon versus marine primary production in the trophic energetics of marine organisms in the nearshore Beaufort Sea through use of carbon isotope tracer techniques.
2. Determine the total inputs of energy to the coastal ecosystem including allochthonous carbon and nitrogen entering the system via terrestrial runoff and coastal erosion.
3. Compare standing stocks of epontic algae in relation to under-ice water circulation.
4. Collect data delineating temporal and spatial variability in ice algae blooms in the nearshore Beaufort Sea.

5. Relate the observed patterns in nutrient availability over the annual cycle to the heterotrophic utilization of detrital carbon within the coastal ecosystem.

Relevance to Problems of Petroleum Development

An ecosystem with a substantial detrital base along large portions of the Beaufort Sea could be readily altered directly or indirectly through OCS-related petroleum development. A summary of the specific impacts that might affect the various sources of energy into the ecosystem would include, by type:

Ice algae Productivity:

1. Oil spills on or under the spring ice cover would diminish primary production through either phytotoxic effects or by attenuation of light passing through the ice sheet.
2. Alteration of bottom topography by dredging channels or constructing causeways could alter ice algae production by changing patterns of thermohaline convective flow beneath the ice cover. Prevention of brine drainage by closing off deeper channels would lead to brine accumulation on the bottom which could seriously impact both fauna and flora.

Phytoplankton Production: Open water primary production would be most sensitive to such impacts as phytotoxicity resulting from oil spills. The rapid lateral flushing of water along the Beaufort Sea coast may, however, serve to minimize this aspect of potential impact.

Detrital-based production and heterotrophic productivity: Impacts upon the heterotrophic organisms that depend upon eroded and transported peat materials as their energy source would occur primarily through OCS-related developments that impinged upon the sources of detritus. Such procedures as shoreline stabilization could alter the food base by eliminating eroded materials. Causeway construction could change wave energy regimes and thus decrease shoreline erosion. Stabilization or

channelizing of streambeds might add to or subtract from the total organic load carried by runoff waters. As knowledge is gained concerning the role of detrital-based production in the overall foodweb of the Beaufort Sea assessment of the potential impacts becomes more feasible.

III. CURRENT STATE OF KNOWLEDGE

Primary Production

In comparison to the warmer waters along the more southern Alaskan coastlines, the Beaufort Sea supports a relatively sparse biota. No appreciable harvests of renewable marine resources are made, with the exception of small commercial fisheries operated principally by native communities in the estuaries along the coast, and seasonal harvesting of bowhead whales. The zone of maximum biological productivity is confined to a relatively narrow strip along the coast wherein the interaction of terrestrial influences ameliorates and somewhat enhances the sparse oceanic regime.

The primary production supporting the pelagic community occurs in two distinct phases in the Beaufort Sea (and other polar waters). The initial algal bloom in the spring occurs well before the > 2 m ice cover has begun to melt, and after the returning daylight reaches critical intensities sufficient to supply the necessary energy beneath the ice (Appolonio, 1965; Bunt, 1963). Attached, or epontic algal populations grow on the ice-water interface and thrive until the melt begins around the beginning of June. Estimates of the carbon fixed during this period range from about 1 gm/m^2-yr in the shallow Prudhoe Bay area (Horner et al., 1974) to 5 gm/m^2-yr off Point Barrow (Clasby et al., 1976). Little is known about spatial variability in ice algae production along the Beaufort Sea coast.

As the ice cover melts, phytoplankton production assumes the major role in energetic input, although the stability of the water column caused by the melting of the nutrient-poor ice cover hinders the advection of deep water nutrients to the photic zone. Only in limited areas

near Barter Island has Hufford (1974) identified possible upwelling of deep waters. As a result, primary production by phytoplankton is low and estimates range from < 10 g C/m^2-yr in the central Arctic Ocean (English, 1961) to about 20 g C/m^2-yr on the coastal zone near Barrow (Alexander et al., 1974).

Input of Terrestrial Carbon to the Nearshore Coastal Zone

The enhancement of biological activity in the proximity of land has been long attributed to various factors among which are the provision of suitable habitat for both benthic flora and fauna, substrate for macrophytes and input of terrigenous nitrogen, phosphorus and carbon via runoff from land. The arctic coastline provides very limited habitat for macrophytes or benthic infauna due to the 2 m freeze depth, which effectively eliminates the shallow nearshore zone as a year-round environment for marine organisms. Again, in the deeper water, ice scouring creates sufficient habitat disturbance to account for the paucity of observed infauna. The exception to this generalization is the area of dense macrophytic algae that has been described by RU356 in the central region of Stefansson Sound. This area has relatively deep lagoon waters (6 m) with protection from ice scouring afforded by the offshore barrier islands. In addition, a rocky substrate is available on the sea bottom in the form of relict cobbles and boulders. Dense stands of Laminaria are present and this research unit is participating with RU 356 in assessing the contributions of this biologically rich community to the surrounding coastal waters.

The shallow lagoon areas with mud bottoms are characterized by low infaunal densities and a dearth of macroflora. Below the 2 m contour in the bays and lagoons, however, large standing stocks of invertebrates - amphipods, mysids and isopods - are common and the LGL-Barrier Island Study (RU 467) personnel are documenting this biomass in Simpson Lagoon. These invertebrates are commonly found in close association with eroded organic material from the shoreline. Studies by Broad (RU 356) have shown that certain gammarid amphipods and isopods do ingest and degrade

the peat. This ingestion is probably accompanied by the removal and digestion of heterotrophic microflora and microfauna that are attached to the peat particles.

During the past year this research unit, in cooperation with RU 356, has shown that some species of amphipods can utilize cellulosic material directly and other carbon isotope data have shown high peat carbon content in adult *Gammaracanthus*, *Atylus* and *Weyprechtia* amphipods as described in detail below. In contrast, the predatory *Boeckosimus* and *Onisimus* amphipods have been shown by the author to be unable to directly utilize peat carbon and the carbon isotope data acquired support this finding.

By using data obtained by Lewellen (1973) and the author during an earlier study of the Simpson Lagoon shoreline, (Schell, 1975) erosion rates and the resulting quantities of carbon and nitrogen washed into the lagoon were estimated for the shoreline between Oliktok Point and Beechey Point. These estimates have been expanded by Cannon and Rawlinson (RU 530) to include all of Simpson Lagoon and are presented in Section VI. Further estimates on the total input of allochthonous carbon to the Beaufort Sea have been made by the author and S. Rawlinson (RU 530), showing that most of the total carbon input is terrestrially derived. The implications of this compartmentalization of the energy input to the marine ecosystem are discussed in Section VI.

IV. STUDY AREA - BEAUFORT SEA - 100%

The study area for this project has been shifted from the originally proposed Elson Lagoon-Dease Inlet area near Pt. Barrow to the Stefansson Sound - Simpson Lagoon area approximately 60 km west of Prudhoe Bay. This shift in siting was made to allow integration with the tasks being undertaken by the LGL-Barrier Island Study group and RU 356. The principal data collection effort and detailed analyses on primary production and heterotrophic production have been made in this area. However, in conjunction with RU 530, estimates of terrestrial input of carbon along the entire Beaufort Sea coast via runoff and erosion will be undertaken on a much less detailed program.

Laboratory experiments seeking to determine the mechanisms by which trophic movement of peat carbon occurs were conducted at the Naval Arctic Research Laboratory at Point Barrow. The ready availability of amphipods and isopods in Elson Lagoon allowed controlled laboratory experiments on cellulose biooxidation with freshly collected animals.

V. SOURCES, METHODS, AND RATIONALE OF DATA COLLECTION

Primary Production by Epontic Ice Alage

The sampling program for ice algae production and spatial distribution occurs during the spring months and involves sampling the ice-water interface before and after the ice algae bloom. The first sampling period is during early April and yields water chemistry data representing the maximum nutrient concentrations and salinities of the annual cycle.

The second sampling trip of the spring, in late May, coincides with the maximum standing stocks of ice algae. Ice cores, water samples and nutrient samples are taken at this time to estimate ice algae and phytoplankton biomasses in the water column. In order to expedite sampling and handling of collected ice cores, the ice algae biomasses were estimated via indirect techniques. Total in vivo fluorescence measurements were made on the melted core samples (or on diluted aliquots) and converted to dry weight by cross calibration with laboratory cultures of known algal populations. Since the silica content of the algae is variable and does not contribute to nutrition, the biomasses shown represent the estimated silica-free weight in mg/m^2. Since fluorescence is dependent upon many factors among which physiological state, algal morphology, and past light history are very important, the algal biomasses shown are primarily for comparison and secondarily as an indication of quantitative amounts of biomass. Actual carbon content will be available pending analyses of collected aliquots of the populations, but these samples have not yet been processed. These data are useful in projecting primary production estimates for the lease area.

Annual primary production by ice algae was also estimated by indirect methods based upon algal biomass and short term primary productivity measurements and the results summarized in Table 2 below are semi-quantitative at best (see RESULTS AND DISCUSSION Section). The estimates by Horner, et al., 1974, McRoy, 1979, Horner, 1979, and this study are all based upon the extrapolation of standing stock data and short primary productivity experiments. Errors due to grazing, diurnal variations in light, loss of standing stock during sampling, etc. were approximated but the paucity of data does not allow realistic error bars. This research unit is cooperating closely with Dr. Horner of RU 359 to improve the ice algae productivity data by conducting a seasonal monitoring of the ice algal bloom in spring, 1980.

Analytical methods employed for nitrate, ammonia and phosphate analyses are similar to those utilized by Alexander et al. (1974) for their ice algae studies. Particulate nitrogen analyses were run on glass fiber filters containing particulate material from melted ice cores or underlying water. The filters were burned and the evolved nitrogen gas measured using a Coleman Nitrogen Analyzer.

By establishing detailed nitrogen budgets for the water column and ice column before and after the epontic algal bloom, it is possible to determine two important facets of the nearshore productivity regime. First, the total standing stocks of ice algae (and assimilated nitrogen) can be quantitatively described for the nearshore zone, and the validity of extrapolating primary production measurements obtained by Clasby et al. (1976) at Point Barrow in include other areas of the Beaufort Sea coast can be determined. Second, by establishing budgets of nitrogen in the dissolved and particulate phase, the importance of thermohaline convective flow as a nutrient input in the nearshore zone can be estimated. This hypothesis states that enhanced primary production by ice algae can be expected in the 2-5 m depth zone in the late spring and the measurement of total particulate and inorganic nitrogen in the ice/water column offers the most direct test of whether or not this enhancement occurs.

Utilization of Detrital Carbon and Transfer of Detrital Carbon in the Foodweb

The magnitude of detrital carbon input to the nearshore zone of Simpson Lagoon (Fig. 1) required that the effects of this energy source be evaluated in respect to the inputs of primary production. Detrital input occurs through essentially two sources - coastal erosion and runoff from the tundra. Thus assessment of these inputs becomes a geomorphological problem for the former source and a hydrological problem for the second. Chemical data on the eroded tundra have been previously obtained by Schell (1975) and have been refined through additional work since beginning this study. Vertical profiles of newly exposed permafrost shoreline bluffs at Milne Point and Pingok Island were obtained in August 1978. Fluvially transported particulate carbon in the Colville and Kuparuk river was sampled during the May-June break-up season of 1979. A 120 micrometer mesh plankton net was suspended beneath the river surface and allowed to filter river flow until sufficient sample had been collected to provide approximately 5 g of carbon for radioactivity determination. Samples were dried at 80°C in vacuo prior to shipping to the radiocarbon assay laboratory.

Refinements in shoreline erosion rates along the Beaufort coast were determined by Cannon and Rawlinson (RU 530). Total organic carbon data for the Colville River waters have been kindly provided by the U.S. Geological Survey (Charles Sloan, personal communication) and flow data are available from the literature (Arnborg et al., 1967; Walker, 1974).

The utilization of detrital organic carbon by heterotrophs and the further transfer of this carbon into the foodweb has been investigated through the use of carbon isotope ratios in the various coastal marine living and nonliving organic materials. Figure 2A shows the three fractions that would comprise the organic carbon of a detritivore or the predators of detritivores. The analytical techniques employed to identify these fractions are shown in Figure 2B. If the carbon in the eroded peat materials of the shoreline is incorporated to a significant extent in the foodweb of heterotrophic microorganisms and these are then consumed and assimilated by benthic invertebrates such as amphipods,

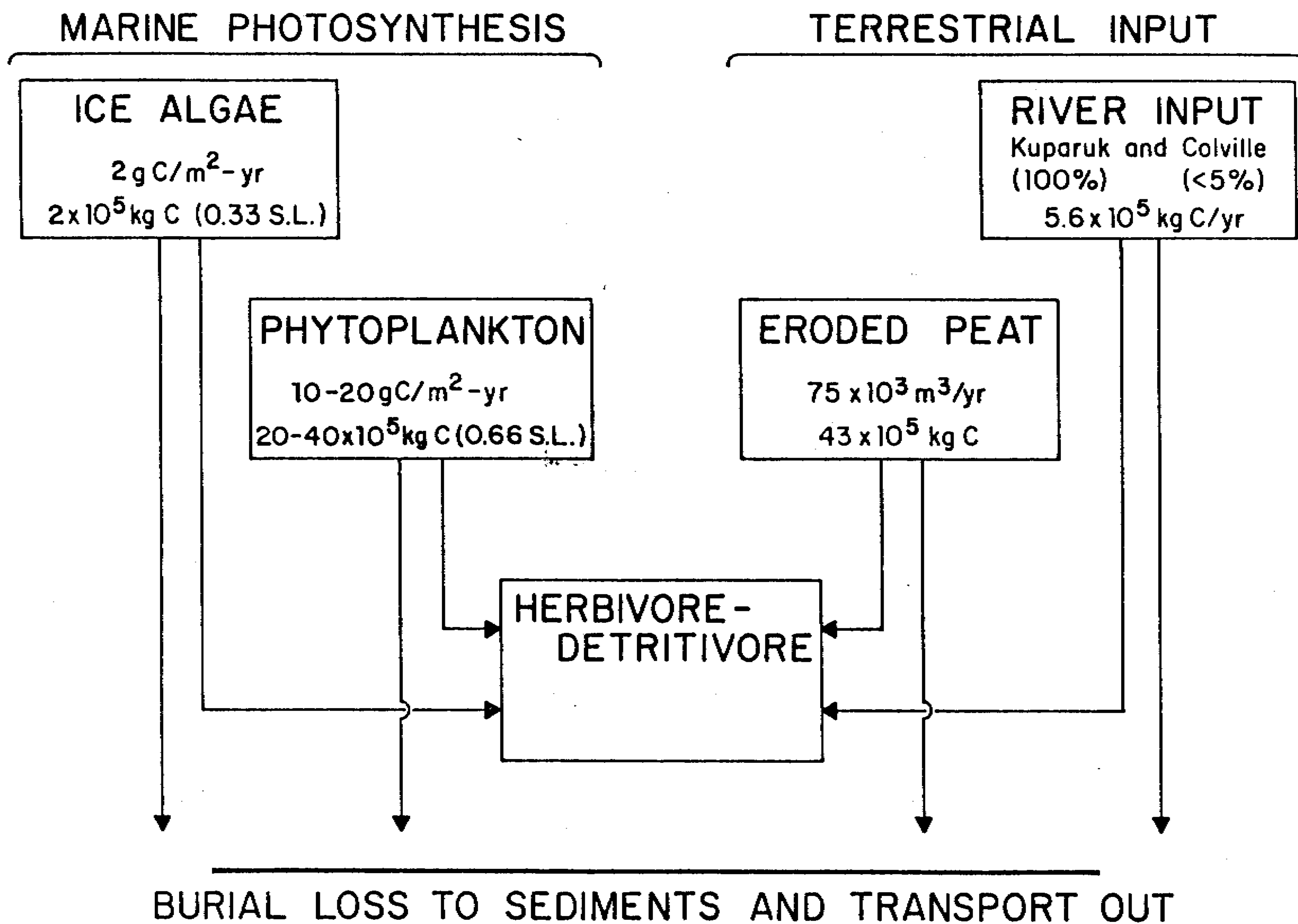


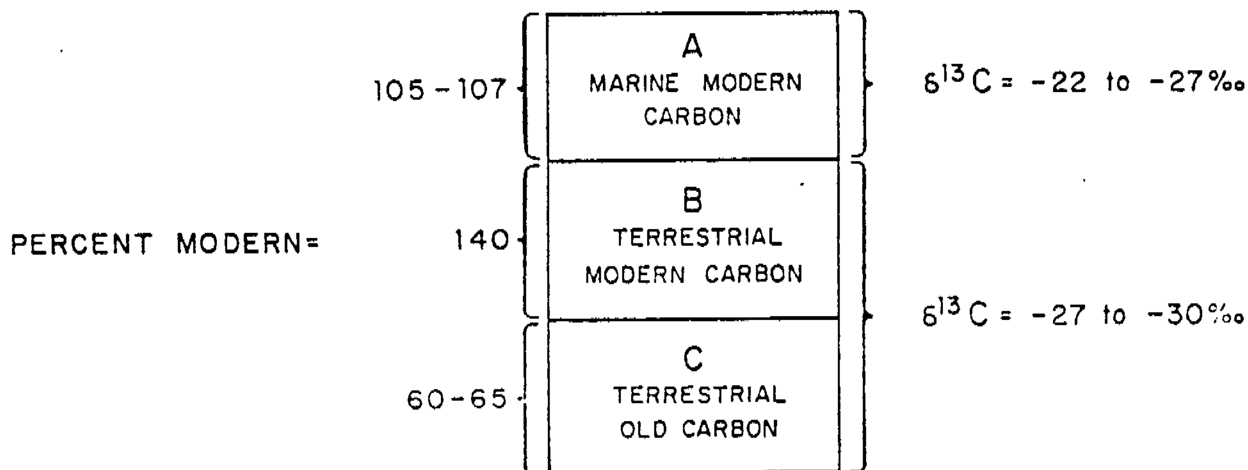
Figure 1: Energy input model for Simpson Lagoon.

DETERMINATION OF HERBIVORE-DETRITIVORE CARBON SOURCE

IS CARBON SOURCE RECENT OR OLD ?

HERBIVORE-DETRITIVORE BIOMASS

IS CARBON SOURCE MARINE OR TERRESTRIAL ?



RADIOCARBON DATING

$$\% C = \left[\frac{\text{Mean peat age} - \text{Sample age}}{\text{Mean peat age}} \right] \times 100$$

$$\% A+B = 100 - C$$

= percent modern carbon (marine and terrestrial)

STABLE ISOTOPE RATIOS

$$\% A = \frac{{}^{12}C/{}^{13}C(\text{terr.}) - {}^{12}C/{}^{13}C(\text{sample})}{{}^{12}C/{}^{13}C(\text{terr.}) - {}^{12}C/{}^{13}C(\text{marine})} \times 100$$

$$\% B = 100 - (A+C)$$

= carbon derived from modern terrestrial sources.

Figure 2: Determination of herbivore-detritivore carbon source.
a) sources comprising Beaufort Sea fauna.
b) techniques for delineating carbon fractions.

isopods and mysid shrimp, the isotopic abundances in the higher organisms should reflect the food source with some variation (< 1 o/oo) due to biochemical fractionation. Indeed, the data described below support this premise.

Stable isotope techniques allow the discrimination of food sources in ecosystems where the source materials (primary producers) have significantly different $^{13}C/^{12}C$ ratios. By comparing $^{13}C/^{12}C$ ratios of organisms at different trophic levels, the food sources of the higher orgamisms can be apportioned. This technique has been used by McConnaughey (1978) to study the detrital input of eelgrass beds in Izembek Lagoon to the fauna of the lagoon and nearshore Bering Sea. Application of this technique is shown in Figure 2B and was used as a method to separate terrestrial and marine contributions to the nearshore fauna. Although the method is acknowledged to be less sensitive than ^{14}C dating, the applicability to modern carbon sources increases its desirability. Analytical cost is low compared to ^{14}C dating.

Process studies to measure the rate of cellulose biodegradation in the under-ice waters of the Beaufort Sea were undertaken at the Naval Arctic Research Laboratory at Barrow. Water samples were collected at stations in Elson Lagoon, Smith Bay and Dease Inlet. A big-tired truck was used for transportation on Elson Lagoon and a Cessna 180 ski-equipped aircraft was used to sample the latter two locations. In July 1979, hand netting of amphipods was employed to collect specimens of *Gammarus setosus*.

A baited trap was set overnight in Elson Lagoon and, upon retrieval, approximately 200 *Onisimus* and *Boeckosimus* amphipods were captured. These animals were used to investigate their ability to digest cellulose through a series of experiments employing ^{14}C-labeled cellulose mixed and peat samples obtained from Simpson Lagoon. Similarly, the microbial acitivity in the detrital peat was studied by measuring the production of radiolabeled CO_2 from added ^{14}C-labeled cellulose. Samples of seawater were incubated with ^{14}C-labeled cellulose and aliquots were taken at approximately 12-hour intervals. These aliquots were acidifed and stripped with nitrogen to remove the carbon dioxide fraction, which was subsequently absorbed in phenethylamine liquid scintillation cocktail.

These experiments, conducted at 0° and 20°, showed active microbial decomposition and oxidation of the labeled cellulose, with the fastest rates occuring at 20°.

To test the hypothesis that *Onisimus* and *Bocekosimus spp.* possessed intestinal microflora which would be active cellulose degraders (and thus be able to symbiotically contribute to the nutrition of the amphipods), an experiment was conducted using freshly captured animals fed on radiolabeled cellulose. Animals were offered both carrier-free and a mix of labeled cellulose and peat aged in seawater. A control of peat plus labeled cellulose without amphipods was used to determine the oxidation rate due to microflora alone. At 12 hour intervals, animals were sampled and aliquots of seawater stripped for radiolabeled carbon dioxide as described above.

Similar experiments were repeated in July 1979 at the NARL in cooperation with Dr. David Schneider (RU 356) to test if the known detritvorous amphipod, *Gammarus setosus* could utilize cellulose. Experiments were conducted as described above using both *G. setosus* and *Onisimus spp.* at 0° and 8°C. The intestinal tracts of the *G. setosus* amphipods were surgically removed and the radioactivity of the guts and bodies determined separately. Each sample of tissue was oxidized with a Harvey Biological Oxidizer to carbon dioxide and the labeled gas collected quantitatively in phenethylamine cocktail for scintillation counting.

RESULTS AND DISCUSSION

Ice Algae Distribution and Production

The major field effort in spring 1979 was directed toward obtaining a representative number of ice algae samples such that standing crop estimates could be projected for the lease area. Sixteen stations were occupied with triplicate cores obtained from each site. One transect was run approximately 100 miles north of the lease area to attempt to delineate trends in distribution with distance from shore. In addition, visual observations were made along lead and pressure ridge systems to

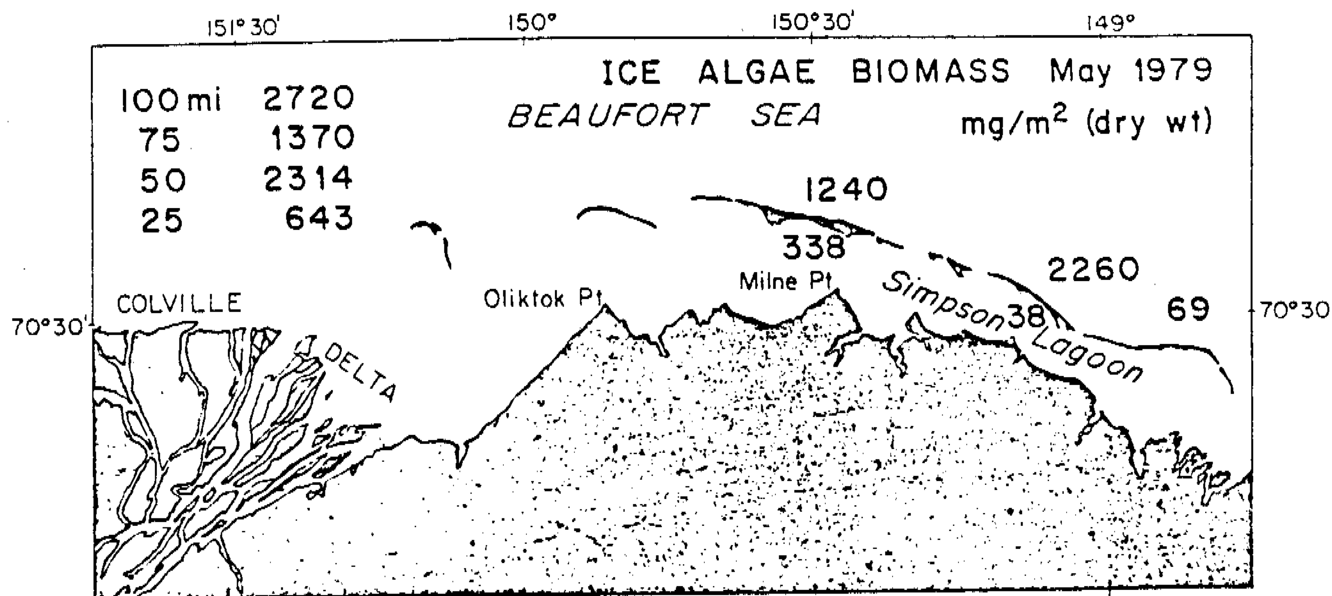
obtain qualitative information regarding ice conditions and ice algae presence. The cores obtained were used to estimate standing stocks as described above and the data obtained are shown in Figure 3. The Stefansson Sound area showed the greatest variability in standing crops as might be expected from the large patches of turbid ice.

Offshore, the ice algae biomasses generally increased although patches of turbid ice were visible as far as 25 miles offshore. Along lead systems, overturned pieces of annual sea ice were often heavily discolored by algal populations. A general observation was that algae were present on ice of sufficient thickness (> 1.0 m) such that the sheet represented all or most of the winter freezing season. No ice algae were visible on thin sheets which represented ice less than 2 months old. Cores cut from the fast-ice sheet near the open leads off Harrison Bay had dense populations and may represent the largest area of uniformly high populations.

Closer to shore, the variability in algal biomass sharply increased. Simpson Lagoon was sampled at only two locations but in both cases algal populations were much lower than immediately outside of the barrier islands (see Figure 3). The ice in the lagoon was clear but salinities in the underlying water were over 40°/oo which may have been partially responsible for the low plant densities. Assuming that the water was at or near the freezing temperature this would indicate ambient temperatures of about -2°C which may also in part inhibit growth.

Stefansson Sound yielded cores with plant biomasses ranging from near undetectable to almost 3 g/m^2 in Mikkelsen Bay beneath clear ice. Most of the central area of Stefansson Sound and Prudhoe Bay had less than 1 g/m^2 biomass beneath the ice.

The light measurements listed in Table 1 are taken at the same stations as the ice algae samples, but a month earlier and not at identical locations. They do not therefore describe light conditions at the point of ice algae sampling, but serve to illustrate the range of variability in light penetration through the ice. The presence of winter snow cover is sufficient to decrease incident light to about 0.5-1.0% of that striking the snow surface but the presence of even small amounts of particulate organic matter in the ice causes the rapid extinction of



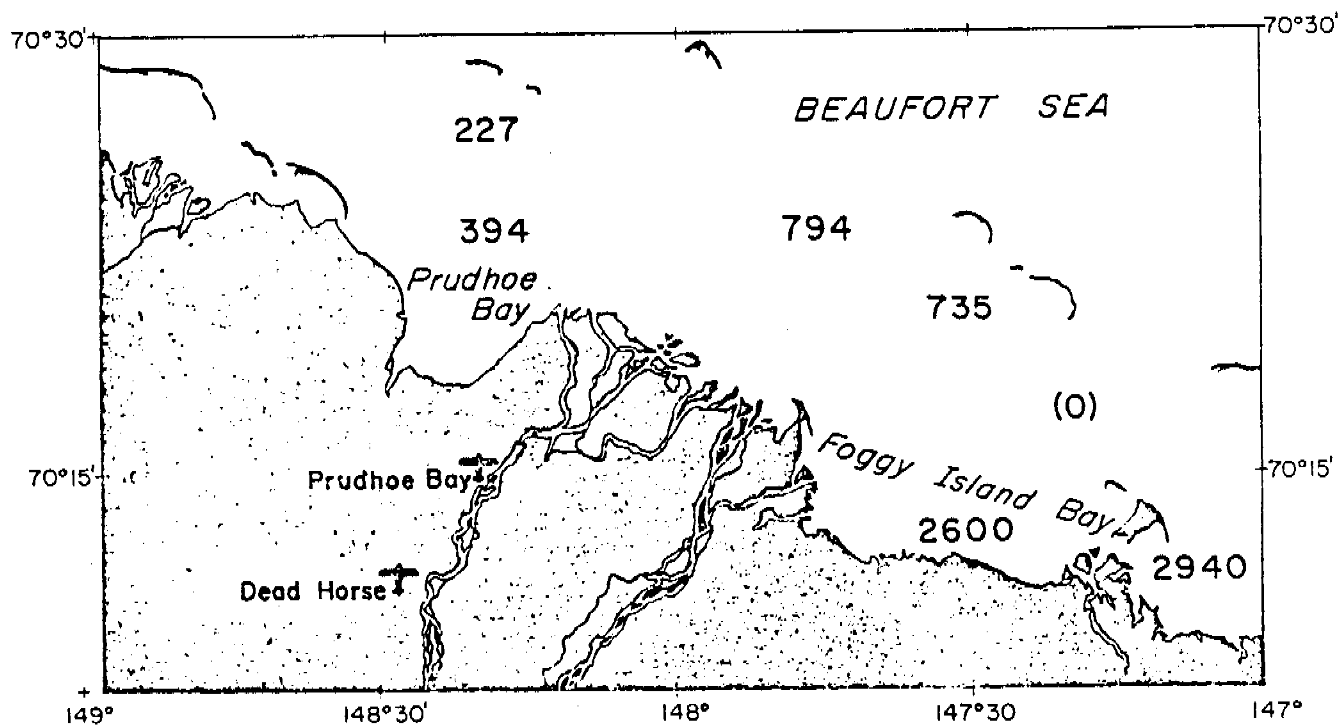


Figure 3: Ice algae biomass estimates (silica-free). Values shown are averages of triplicate cores column of values at upper left represent biomasses at 100, 75, 50 and 25 nautical miles north of Oliktok Point.

TABLE 1: LIGHT PENETRATION THROUGH ANNUAL SEA ICE COVER, BEAUFORT SEA, 1-3 APRIL, 1979

Station	Location	Surface Illumination	Under-ice Illumination	Percent of Surface Illumination
		(micro-Einsteins/sec-m^2)		
Spy	70° 34.7'N 149° 51.5'W	1400	2.21	0.16
Thetis I	70° 33.3'N 150° 21.6'W	1358	1.77	0.13
Reindeer	70° 26.4'N 148° 19.0'W	1400	3.91	0.28
Long	70° 31.0'N 148° 55.0'W	1129	9.8	0.85
Cottle	70° 31.7'N 149° 04.4'W	752	0.07	0.009
Mikkelson	70° 11.0'N 147° 03.5'W	1170	1.63	0.14
Cross	70° 26.4'N 148° 19.0'W	731	0.1	0.013
Dinkum Sands	70° 26.2'N 147° 56.1'W	418	2.40	0.57
Boulder	70° 18.0'N 147° 32.3'W	334	< 0.01	
Stockton	70° 16.7'N 147° 17.3'N	155	0.06	0.037

ANNUAL ICE ALGAE PRODUCTIVITY ESTIMATES

ALEXANDER, HORNER, AND CLASBY, 1974	CHUKCHI SEA	5 G C/M^2
McROY, 1976	BERING SEA SHELF	24 "
HORNER, COYLE, AND REDBURN, 1974	PRUDHOE BAY	1 "
HORNER, 1979	STEFANSSON SOUND	0.9 "
THIS STUDY	SIMPSON LAGOON	0.18 "
	STEFANSSON SOUND	1.4 "
	OFFSHORE	1.7 "

Table 2: Estimates of annual ice algae productivity.

light to intensities below the limit of detection. Since the particulate matter is often dispersed over much of the upper ice column, the associated drastic light attenuation can be expected to persist almost as long as the ice cover lasts. Thus primary production by ice algae in April and May and by phytoplankton in June and even early July is effectively prevented in areas of sediment-laden ice. Based upon the observed presence of sediment-laden ice in the Stefansson Sound-Simpson Lagoon area, this condition probably reduced the 1979 ice algae production and possibly phytoplankton production to as low as 25-30 percent of the maximum possible if snow cover alone were attenuating light penetration. At this time very little data exists on year-to-year variability of sediment-laden ice and on the length of persistence of the opaque ice into the summer season. Duration estimates based on satellite imagery indicate that approximately 40-50 percent of the Stefansson Sound area is ice free by the first week of July followed by rapid dispersal of the remainder during the month. Thus sediment-laden ice could potentially prevent light penetration into the water column for two or more weeks past the solar solstice with a consequent large decrease in annual primary production. More detailed documentation on the ice retreat characteristics in relation to light penetration is currently being assembled using satellite imagery and field observation.

Ice algae primary production in the nearshore Beaufort Sea is a small fraction of the total annual primary production and is small relative to epontic algae production in the Bering and Chukchi Seas although very little data is available on the latter region. Table 2 compares the estimates of total annual production of epontic ice algae in other continental shelf waters of Alaska with data obtained in the Beaufort Sea. Only the study by Alexander, Horner and Clasby (1974) represents integrated ^{14}C uptake measurements determined over the spring season. The remaining estimates are extrapolations made based on either limited ^{14}C-uptake measurements or on standing stock measurements converted to production numbers by cross calibration with instantaneous production measurements and standing stocks estimated from chlorophyll concentrations.

The estimates of ice algae production for this study in Table 2 are probably high since sampling is unavoidably biased by the choice of

helicopter landing spots which are flat. Due to the roughness of pressure fields and the inaccessability of the bottom surface of pressure ridges, I have no data on ice algae from these areas. Light attenuation would be greater in pressure fields due to the increased snow cover and/or thicker ice would be expected to preclude ice algae growth. However, the estimates of annual production in Table 2 do not take ice morphology into account but are based on the actual samples taken which may result in a systematic error. In the context of total annual primary production and when considering the effects of sediment-laden ice, this error would probably have little effect.

Nutrient Dynamics and Under-ice Circulation

Nutrient concentrations rise steadily in the under-ice waters of the nearshore Beaufort Sea following the cessation of plant uptake in the Fall. The increase is due to three additive factors - regeneration in situ by microbiological activity, advective transport, and freeze concentration of the solutes in under-lying water as the ice thickens. By November, samples taken in the nearshore area showed nitrate concentrations of approximately 1-2 μg-atoms NO_3-N/liter although the phosphate concentrations remained very low in Simpson Lagoon. The low phosphate concentrations in the presence of nitrate may indicate that bacterial nitrification is active in these waters following mineralization of organic N in eroded peat. Microbial uptake of phosphate has been demonstrated in eroded peat samples from Simpson Lagoon and high nitrification rates were evident in the Colville delta channels in earlier studies (Schell, 1974).

By Spring, thermohaline convection has replaced nearshore waters from all areas where density-driven circulation is possible. The generalized movement of the under-ice water as shown in Figure 4 was hypothesized as a possible supply of nutrients to ice algae by this research unit in 1977 and the combination of nutrient data, ice algae biomass in Stefansson Sound and the current data obtained by Brian Matthews (RU 526) now strongly support this concept. Matthews is now

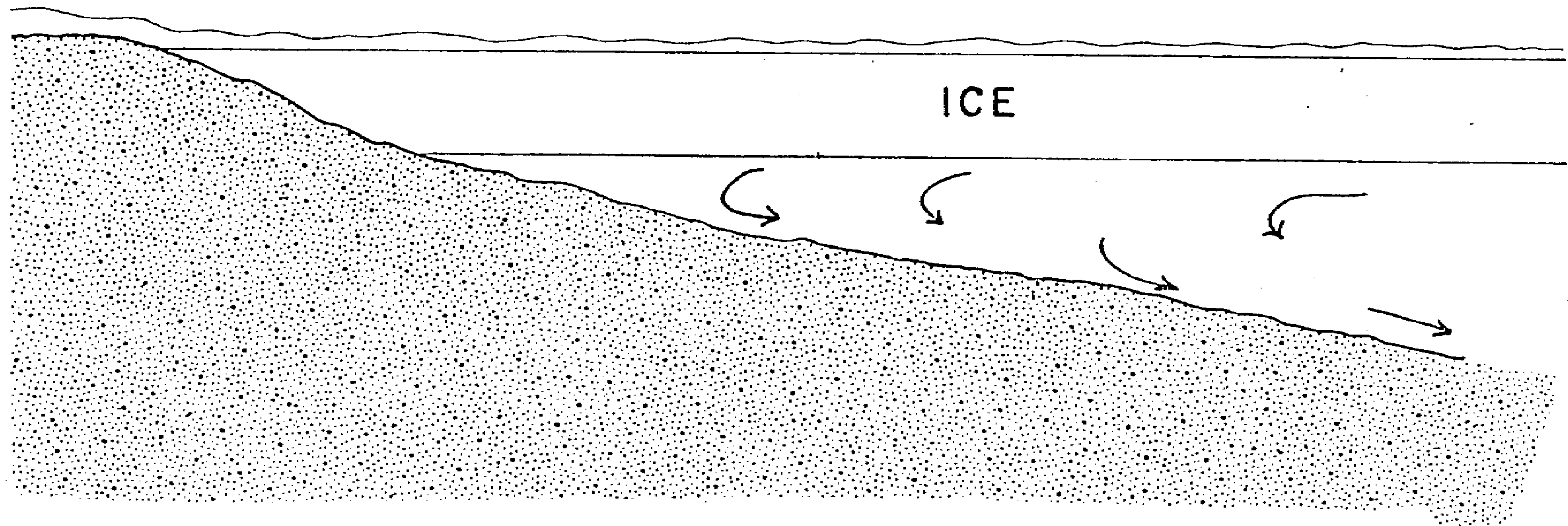


Figure 4: Generalized circulation patterns due to solute exclusion during sea ice formation.

developing models based on observed salinities that should allow estimation of the turnover time of the under-ice water in the nearshore zone and from nutrient data acquired offshore we can calculate the uptake efficiency of the epontic algae as the water moves shoreward at the ice-water interface. Table 3 lists the environmental conditions in Stefansson Sound at the peak of the ice algae bloom (late May). Note that there is no indication of nutrient limitation at this late stage in the development of the epontic algal community. Instead, as described above, light is the predominant factor restraining primary production. No data are available on the rate of nutrient utilization in June but this may be the time of maximum production beneath areas of clear ice. Melting and sloughing off of the ice algal layer should result in greatly increased light penetration which when coupled with 24 hour sunlight, should provide near optimum conditions for photosynthesis. A detailed study of the early June phytoplankton response is planned for 1980.

Foodweb Transfer Efficiencies

The transfer of carbon derived from marine photosynthesis through marine foodwebs can be separated from the foodweb transfer of detrital peat carbon by analysis of the carbon isotope composition of the source materials and the predators at the apex of the foodweb. Originally, this research unit sought to determine the role of terrestrial detritus in supplying energy to the Simpson Lagoon ecosystem and in particular, the epibenthic invertebrates and anadromous fish which utilize the lagoon waters intensively during the summer months. The carbon-14 abundances in lagoon invertebrates and the anadromous fish showed that in spite of large inputs of detrital carbon to the lagoon, the transfer efficiencies of this carbon was so low that less that 10 percent of the anadromous fish and lagoon invertebrates was derived from terrestrial carbon sources. Over the past year several additional samples of biota have been analyzed for ^{14}C and $^{13}C/^{12}C$ isotope ratios. The results which are described below, have been very revealing and indicate that this technique can be a very powerful tool in trophic system analysis.

ENVIRONMENTAL CONDITIONS
STEFANSSON SOUND - MAY 1979

SALINITY	32 − 50 ‰	ICE	1.6 − 2.0 m
WATER TEMP.	−1.8 to −2.9°	AIR TEMP.	−30 to 0°
SNOW COVER	10 − 50 cm	DAYLIGHT	> 20 hrs

LIGHT PENETRATION, UNDER-ICE - 0.0 - 0.90 % surface

MAXIMUM LIGHT INTENSITY, UNDER-ICE ~ 1000 lux
~20 $\mu E\ m^{-2} s^{-1}$

NUTRIENT CONCENTRATIONS

NITRATE + AMMONIA - N	4 - 10 µg - at/l
PHOSPHATE - P	0.5 - 1.1 "
SILICATE - Si	20 - 50 "

Table 3: Late winter environmental conditions in the nearshore Beaufort Sea.

Marine Carbon Isotope Abundances

The abundance of carbon-14 in detritus and living organisms from the nearshore Beaufort Sea are listed in Table 4 along with the ^{13}C depletions observed relative to the PDB carbonate standard expressed as parts per thousand. The ^{14}C abundances are expressed as percent modern relative to the 1950 NBS standard (= 100%). The values in excess of 100% represent the burden of ^{14}C in modern plants due to the additions of radiocarbon following atmospheric testing of thermonuclear weapons in the 1950's and 60's. Modern plants growing in equilibrium with atmospheric carbon dioxide have a ^{14}C abundance of approximately 1.4 times the 1950 standard of which the tundra plants clipped at Milne Point (sample MIL-78-4) serve as an example. To account for biochemical fractionation of the heavier carbon isotopes during photosynthesis and metabolism, all ^{14}C contents are normalized to a $\delta^{13}C$ of -25°/oo. Thus data from marine, terrestrial, and freshwater environments are directly comparable in Table 4. For ease in comparing ^{14}C abundances, the data of Table 4 are plotted on a scale of radiocarbon activity in Figures 5A and 5B. Figure 5B is an expanded scale of the region between 95 and 123 percent modern. Data are shown without error bars which range from 0.5 to 1.6 percent (90% confidence) and thus the exact position of a point relative to another within the error bar is uncertain. Figure 5A shows that in spite of the broad spectrum of ^{14}C abundances that are present in the various source materials, the marine fauna and anadromous fish show a very limited range of ^{14}C content. Also apparent is the fact that the anadromous fish have a higher ^{14}C content than the invertebrates of Simpson Lagoon although the ^{13}C abundances indicate that virtually all of the nutrition of these fish is derived from marine waters. The cause of this ^{14}C enrichment is not yet established, but the evidence suggests that during the period in which the anadromous fish enter freshwater, they feed upon organisms containing much higher concentrations of ^{14}C. Although the quantity assimilated is not sufficient to alter the $^{13}C/^{12}C$ ratio of the fish noticeably, the ^{14}C signal is sufficient to be observed. Stomach contents of anadromous fish netted in Simpson Lagoon are mainly epibenthic invertebrates from the lagoon waters and predominantly mysid shrimp. Unfortunately, capturing

TABLE 4: SUMMARY OF CARBON ISOTOPE DATA - BEAUFORT SEA

Sample Identification	$\delta^{13}C$	% Modern (corr. to $\delta^{13}C$ = -25°/oo)
<u>Vegetative</u>		
MIL 78-3; GX5822 Sagavanirktok River Basal peat	-28.5	65.5
MIL 78-2; GX58321 Pingok Is. Basal peat	-28.3	35.0
MIL 78-4; GX5824 Tundra plants, modern	-28.2	141.1 ± .5
MIL 78-1; GX5820 Milne basal peat	-28.7	32.4
I-6838 Oliktok basal peat	--	34.5
I-6839 Kavearak Pt. basal peat	--	20.8
79-5; GX6274 Laminaria, whole plants	-15.4	105.7
79-7; UM-1738 Laminaria stipes	-17.47	105.0 ± .8
79-8; UM-1739 Laminaria blades	-15.04	107.1 ± 1.4
79-12; UM-1803 Colville R. particulate C., 2 June 79	-26.39	89.4 ± 1.6
79-14; UM-1804 Kuparuk R. particulate C., 31 May 79	-26.89	71.6 ± 1.1
79-11; Beta 1027 Colville R. particulate C, 12-14 June 79	-27.01	74.4 ± .9
<u>Invertebrates</u>		
MIL-78-6; GX5825 Mysids (Aug 78)	-23.6	105.9
MIL-78-8; GX5827 Saduria (Aug 78)	-18.5	103.1
MIL-78-9; GX5828 Onisimus amphipods (Aug 78)	-18.3	103.7
79-18; Beta 1030 Saduria (Nov. 79 S.L.)	-20.43	102.9 ± .9
79-19; Beta 1031 Gammaracanthus (amphipods)(Nov 79)	-20.91	99.8 ± .8
79-20; Beta 1032 Weyprechtia (amphipods)(Nov 79)	-20.71 ± .05	98.4 ± 1.5
79-21; Beta 1033 Arctic cisco *Coregonus autumnalis* stomach contents (Nov 79)	-22.70 ± .02	97.4 ± 0.6
<u>Fish</u>		
MIL-78-5; GX5824 Four horn sculpin, *Myoxocephalus quadricornis* (Aug 78)	-20.5	105.3
MIL-78-7; GX5826 Humpback whitefish, *C. pidschian*, (Colville delta, fall 77)	-20.4	109.5
GX5388; Arctic cisco, *C. autumnalis* (Colville delta, fall 77)	-21.8	109.6

TABLE 4: SUMMARY OF CARBON ISOTOPE DATA - BEAUFORT SEA (cont.)

Sample Identification	$\delta^{13}C$	% Modern (corr. to $\delta^{13}C$ = -25°/oo)
MIL-78-10; GX5829 Least cisco, *C. sardinella*	-21.2	109.2
79-17; Beta 1029 Grayling, *Thymallus arcticus*, Colv. R.	-26.4 ± .02	120.9 ± .7
79-15; Beta 1028 Round whitefish, *Prosopium cylindraceum*	-26.9 ± .02	101.4 ± .8
79-2; GX6271 Arctic cod	-21.5	107.3
79-6; GX6275 Arctic cisco, *C. autumnalis*	-20.7	112.7
79-10; UM-1741 Arctic cod	-21.60	103.7
79-9; UM-1740 Four horn sculpin, *M. quadricornis*, (Apr 79)	-20.82	104.4
80-1; Beta 1075 Round whitefish, Immature *P. cylindraceum*	-27.50 ± .05	109.0 ± 7
<u>Birds</u>		
79-1; GX6270 Oldsquaw, *Clangula hyemalis*, adult female	-20.7	104.9

RADIOCARBON CONTENT OF SURFACE ORGANIC MATTER
BEAUFORT SEA COASTLINE

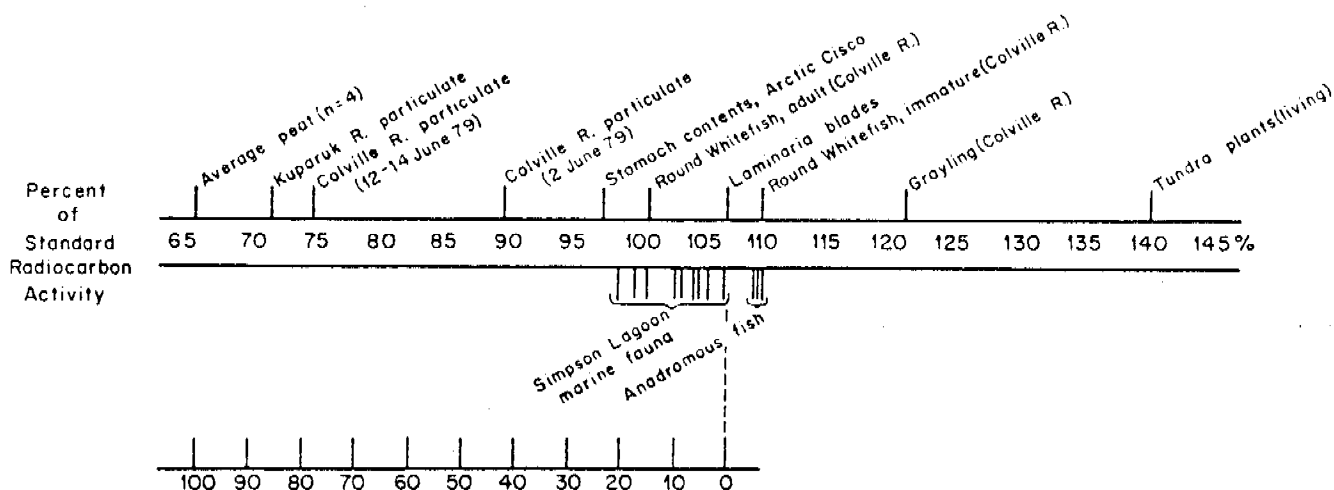


Figure 5A: Radiocarbon activities of organic matter in the nearshore Beaufort Sea and terrestrial environment.

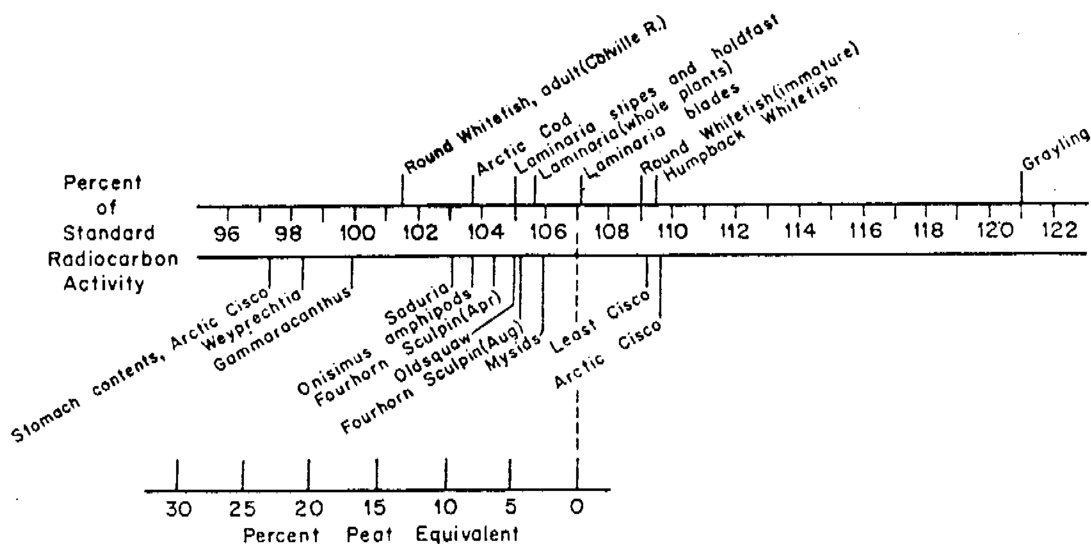


Figure 5B: Radiocarbon activities of fauna and flora from the marine ecosystem. *Laminaria* blades are representative of marine modern carbon pool. Lower activities in *Laminaria* stipes and holdfasts are due to lower contributions from bomb effects.

sufficient mysids for radiocarbon analysis is difficult and only one sample of sufficient size has been available to data (MIL-78-6). Replication of this sample is highly desirable and will be a priority item in the 1980 field season agenda. Also desirable would be a sample of the various large copepods resident off Prudhoe Bay in that being filter feeding herbivores they would serve as an accurate representative of the marine radiocarbon activity in surface water. The other excellent modern marine reference standard is the kelp, *Laminaria solidungula*. The blades from this organism represent photosynthetically fixed carbon derived from the midsummer bicarbonate pool and are easily obtained in large quantities and free from contamination. I appreciate the cooperation of K. Dunton (RU 356) in obtaining these specimens.

The invertebrates of Simpson Lagoon are also predominately marine carbon, indicating that terrestrially derived detritus in not important to their nutrition on a total biomass basis. It may be important, however, to note that approximately ten percent of the animal's composition is derived from peat and that this source is not seasonal as are phytoplankton and ice algae. Thus the detrital based foodwebs may be important for winter survival of some invertebrates and thereby very significant from an ecological standpoint. Further support for this hypothesis results from the much higher peat carbon content in two samples of amphipods captured in November from Harrison Bay. These samples were composed of large adult specimens of *Weyprechtia heuglini* and *Gammaracanthus loricatus* (samples 79-19, 79-20) and contained the lowest ^{14}C activity of any organisms sampled to data, corresponding to approximately 16-21% peat carbon. I feel that this probably results from the high input of organic matter from the Colville River over the course of break-up and summer flow. The prevailing easterly winds cause accumulation of the detritus in western Harrison Bay and this high detrital input relative to primary production is reflected in the fractions of carbon transfered up the foodwebs.

Freshwater Foodwebs

Only two obligate freshwater fish have been analyzed for ^{14}C content to date and the results are evidence of a wide dissimilarity in

energy dependencies. Personnel from LGL-USA (RU 467) kindly provided samples of round whitefish (*Prosopium cylindraceum*) and grayling (*Thymallus arcticus*) taken below Umiat on the Colville River during September 1979. Radiocarbon activity in the grayling (sample 79-17) was 120 percent of standard activity, by far the highest activity of all fish tested to data and approaching the activity of terrestrial plants. In contrast, a mature round whitefish had the lowest ^{14}C content of all fish sampled to date indicating a wide diversity in energy sources. An immature round whitefish contained about 109 percent of modern activity. Investigations into the literature on food habits of arctic fishes was unrewarding since both fish are described as consuming the identical food items - aquatic insect larvae, snails, aerial insects and bivalves (Scott and Crossman, 1973; Bendock, 1979; and McCart, Craig, and Bain, 1972). Grayling are perhaps more opportunistic and predatory and the whitefish more of a bottom feeder. In view of the observed radiocarbon contents of these fish, the overall ^{14}C abundance in the organic matter present in the rivers becomes important. Tethered plankton net samples for suspended particulate matter in the Colville River showed that peat detritus comprises the bulk of the organic load. The initial spring break-up flow is higher in ^{14}C and reflects the surficial run-off from the tundra and the associated modern vegetative matter. As the season progresses, however, radiocarbon content drops (see Figure 5A) and approaches the value for the average peat sections along Simpson Lagoon, indicating that river bank erosion becomes the source of most detrital material once the break-up flooding subsides and thermoerosional nicheing and collapse of riverbanks becomes active. The biological implications of the observed radiocarbon contents in the fish are interesting: grayling apparently select from a matrix of organic matter only those insects bearing a high radiocarbon content which in turn requires that the insect prey are selecting as food only substances derived from recent primary production. Whether this primary production is aquatic or terrestrial is unknown. Round whitefish are apparently much more inclined to food substances that derive from detrital foodwebs and reflect this in their radiocarbon content. It may be possible that microflora in the intestines of round whitefish degrade cellulosic detritus ingested by the fish and allow direct assimilation of detrital

matter. The intermediate radiocarbon activity present in the immature round whitefish may reflect food habits in the juvenile that are closer akin to those of the grayling with a shift to heavier dependence on detritus foodwebs with maturity.

The ^{13}C depletions shown in Figure 6 verify that both the grayling and round whitefish are composed of terrestrially (or fluvially) derived carbon. Similarly the arctic cisco, humpback whitefish, and least cisco are of similar isotopic composition as the marine invertebrates of Simpson Lagoon confirming the marine dietary requirements of these fish and indicating that the freshwater environment is not contributing a significant fraction of their food. Figure 7 depicts the generalized result of foodweb efficiencies in transferring the carbon inputs to the nearshore marine ecosystem up trophic steps to the epifauna invertebrates and anadromous fish of the Simpson Lagoon-Stefansson Sound area. The "faunal composition" shown is the average of estimated peat compositions of the resident fish and invertebrates of Simpson Lagoon and does not reflect the *Gammaracanthus* and *Weyprechtia* samples taken from Harrison Bay. The energy source fractions are based upon carbon input estimates developed by this research unit in cooperation with RU 530 and which were reported in the 1978 Annual Report (RU 537). Further research along these lines is planned for 1980.

Cellulose Biodegradation in Marine Waters

Oxidation of cellulose by Elson Lagoon water microbial populations was demonstrated in 1978 in a series of laboratory experiments performed at the Naval Arctic Research Laboratory at Barrow. During 1979, in cooperation with D. Schneider (RU 356) these experiments were expanded to test the ability of the amphipods *Gammarus setosus* and *Onisimus sp.* to utilize peat detritus as a food source and to acquire additional data on microbial oxidation by insitu populations at temperatures typical of summer and winter environments. The 1978 experiemnts had revealed active microbial oxidation in peat exposed to seawater and in the water column. Figure 8 shows the temperature effects on cellulose oxidation

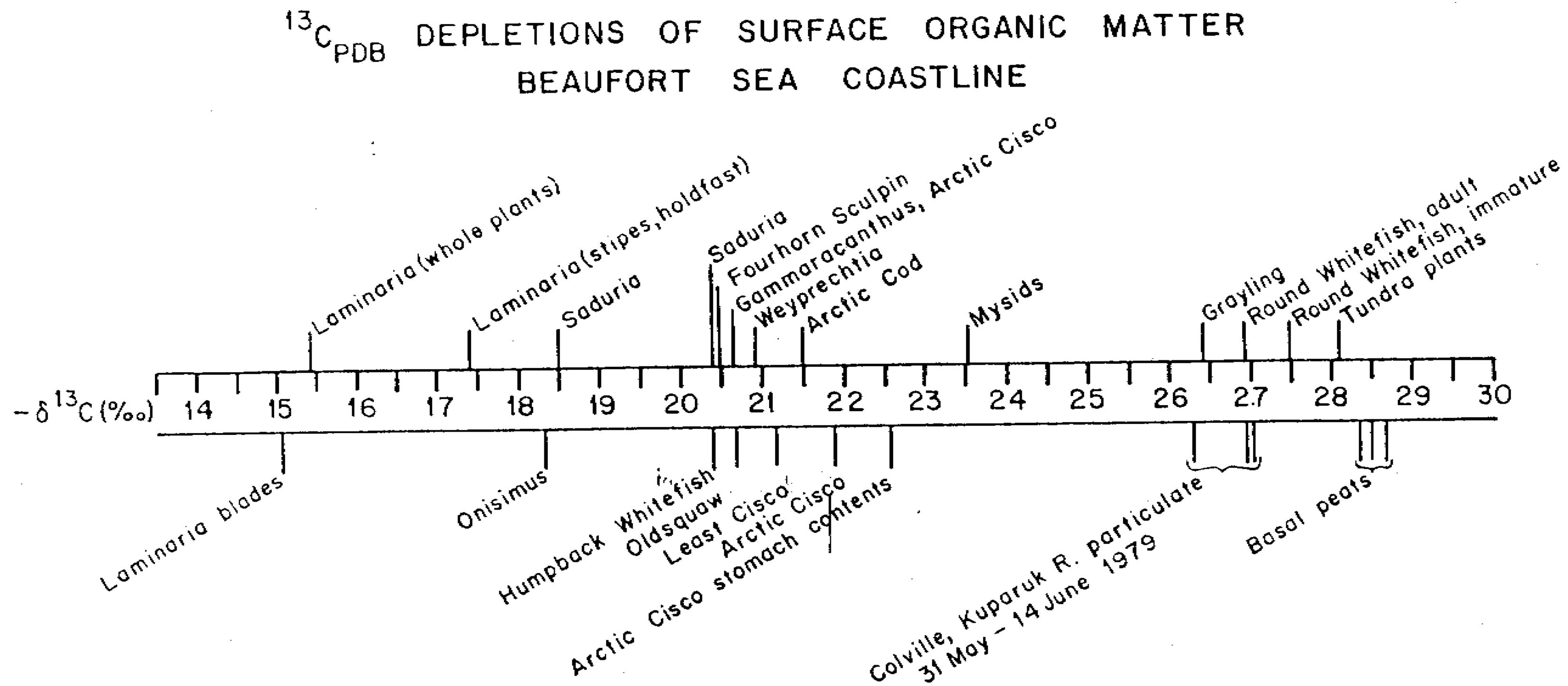


Figure 6: Carbon-13 depletions in Beaufort Sea organic matter.

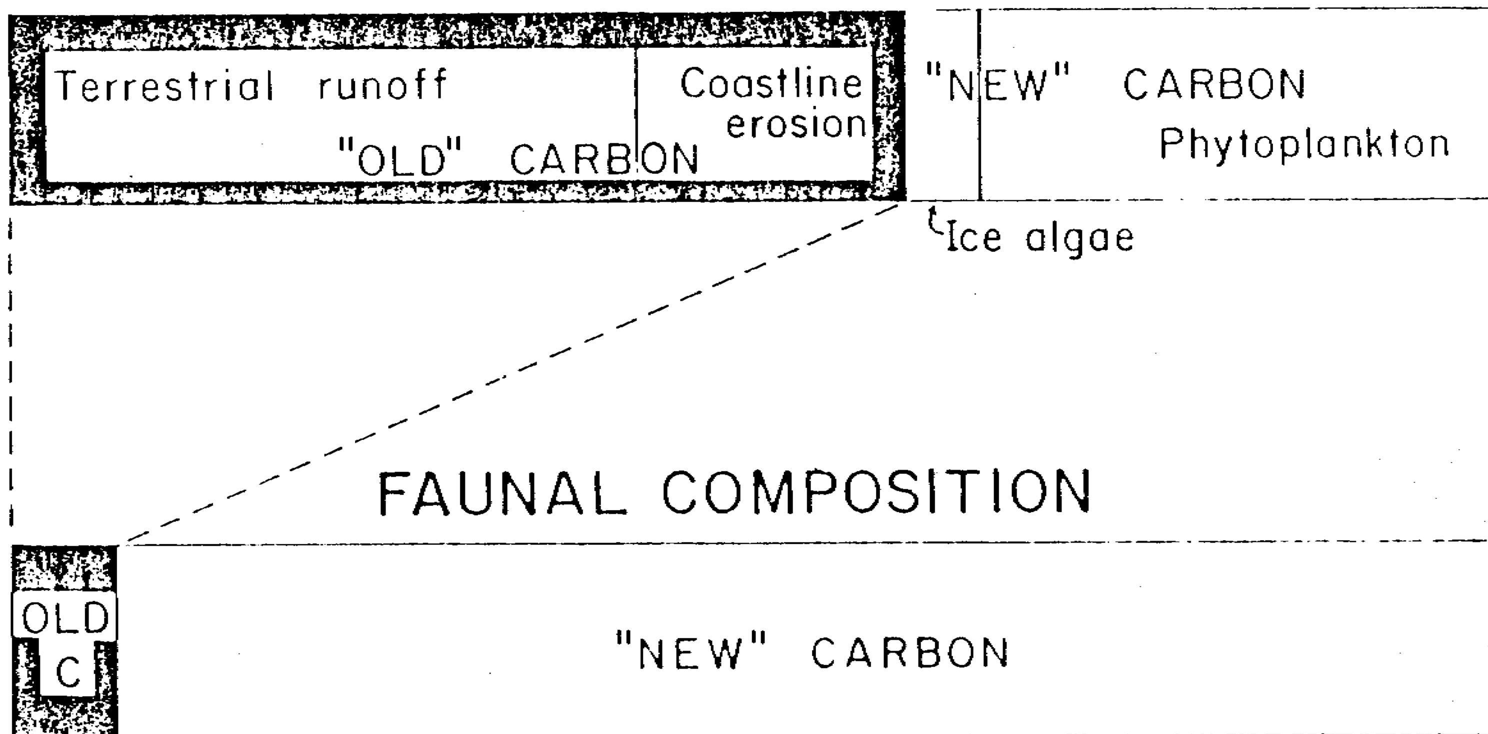


Figure 7: Fauna carbon composition of Simpson Lagoon invertebrates and fish relative to carbon source inputs.

using water from two stations in Elson Lagoon. The cellulose was suspended in the seawater and subsamples stripped for radiolabeled carbon dioxide to yield the plot shown. The active degradation of cellulose at 0°C indicates that the microbial populations have adapted to the cold environment and it is reasonable to assume that the influx of eroded peat contributes to the active populations of microorganisms in the water column.

The ability of amphipods to feed upon and digest detrital material directly would be an obvious adaptive advantage in arctic waters where photosynthesis is highly seasonal. Work by RU 356 on feeding preferences of the common amphipods of Elson Lagoon had shown that some amphipods ingested and appeared to assimilate peat detritus. The amphipod *Gammarus setosus* was the most active peat consumer but unfortunately this species was not present among the animals trapped during the December 1978 experiments. Instead, a mix of small amphipods, primarily the predatory amphipods *Boeckosimus sp.* and *Onisimus sp.* were tested for peat ingestion and cellulose biodegradation. Neither species was found to release radiolabeled carbon dioxide into solution indicating there was no assimilation of the cellulose. The presence of amphipods in samples of seawater and peat yielded lower oxidation rates than peat plus seawater alone. Possible grazing of meiofauna and bacteria may have caused this depression in the rate of carbon dioxide evolution but the lack of label appearing in the amphipods did not lend support to this concept.

Although these initial experiments did not support the hypothesis that amphipods were directly utilizing detrital peat, the high rates of microbial biooxidation in the water column and associated with the peat detritus were felt to warrant further investigation to help establish mean lifetimes for peat particles in the nearshore waters. During July 18-20, 1979 the microbial oxidation rates of peat were again measured and compared with the oxidation rates due to the ingestion of the peat by the amphipods *Onisimus sp.* and *Gammarus setosus* at typical summer lagoon temperatures. The results of these experiments which are summarized in Figure 9 show a remarkable contrast between the two amphipods in cellulose oxidation rates. The sample with *Onisimus* amphipods showed

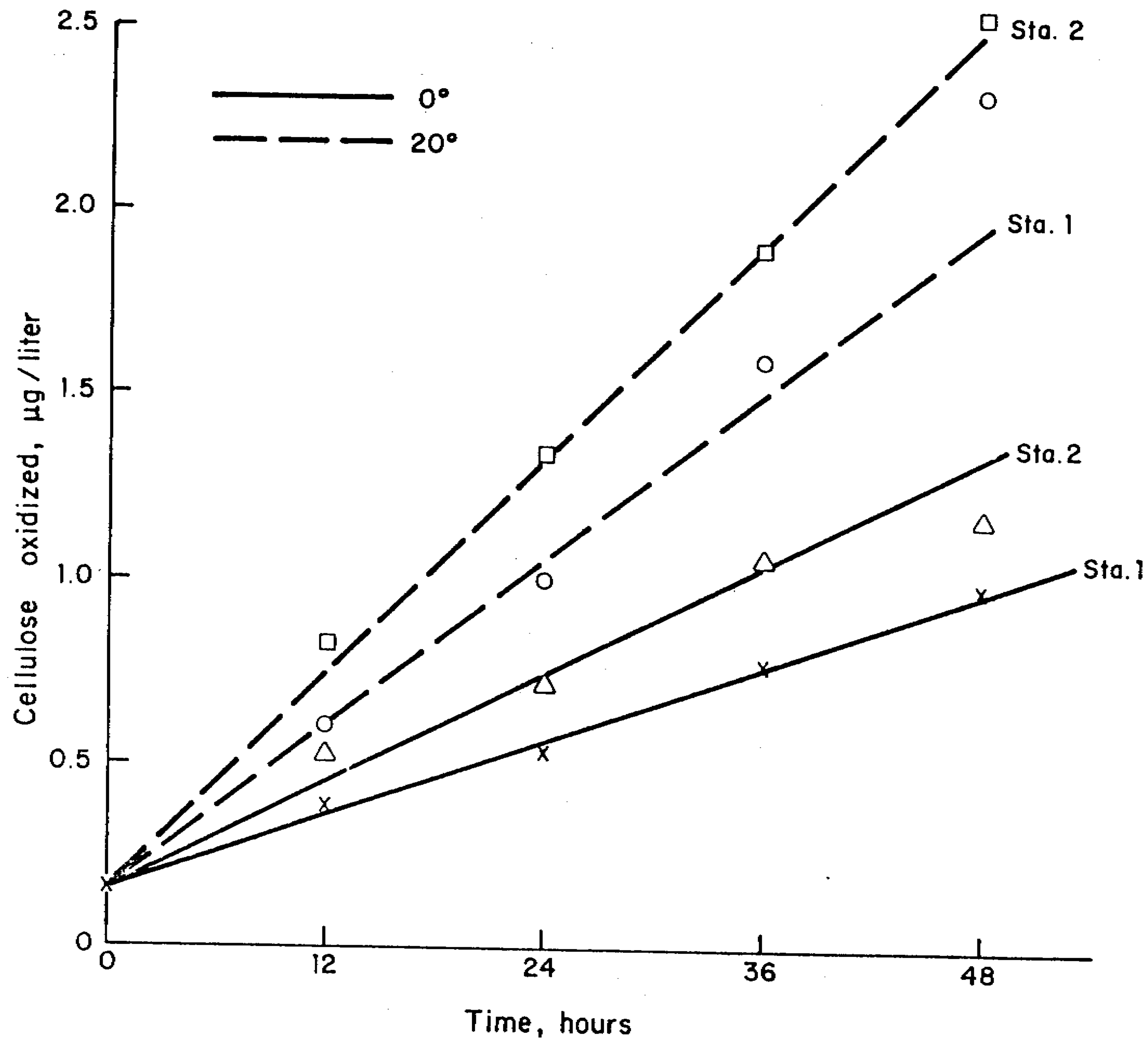


Figure 8: Carbon dioxide evolution from ^{14}C-labeled cellulose in Elson Lagoon water.

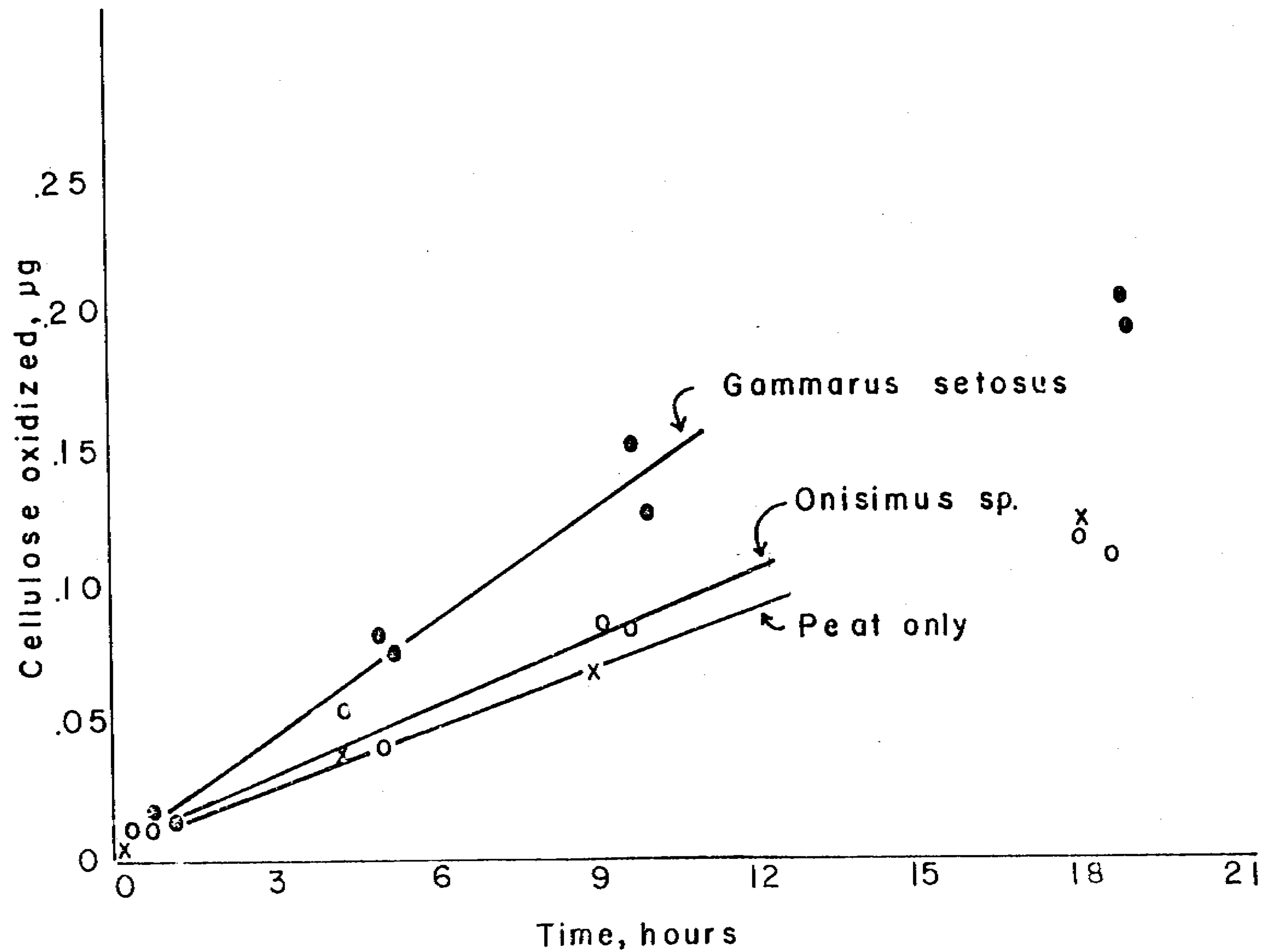


Figure 9: Carbon dioxide evolution from ^{14}C-labeled cellulose mixed with seawater-wet peat and fed to amphipods.

a carbon dioxide evolution rate that closely approximates the background rate due to microflora in the detritus and water confirming the apparent inability of this genus to assimilate peat detritus. In contrast, the sample with *G. setosus* yielded a much higher rate of oxidation and dissection of the animals showed that radioactivity from the cellulose had been incroporated into body tissue, although considerable variability occurred between animals preventing a meaningful estimate of assimilation efficiency. Nevertheless, an average of 0.3 percent of the radiolabeled cellulose had been oxidized to carbon dioxide while mixed with 9 mg peat (dry wt) over the 18 hour experiment. By dividing this oxidation rate into the total weight of peat, a mean consumption (not ingestion) rate of 1.5 μg peat/amphipod-hr is obtained, assuming the peat is assimilated at rates near those of pure cellulose. Incorporation of metabolized ^{14}C-cellulose into body tissue, as measured in the dissected animals, was approximately as great as oxidation to carbon dioxide, indicating utilization of the cellulose for growth. Further experiments are planned to estimate the assimilation efficiencies of *Gammarus* and other invertebrates found to have depressed natural ^{14}C abundances. These invertebrates represent the most likely foodweb pathway by which peat carbon can transferred to higher organisms.

The rate of peat oxidation by microflora and meiofaunal populations was estimated in a similar fashion. During the 18 hr experiment, 0.13% of the label was released as carbon dioxide (8°C) which translates to a turnover time of 6×10^2 days. At 0°C, the turnover time was 4×10^2 days which is somewhat surprising but may only reflect increased populations of microorganisms in the sample of peat which had been held for a day in the laboratory prior to the experiment. These data compare well with data from the 1978 experiments on peat oxidation which gave a turnover time of 6×10^2 days at 0°C using a sample seawater saturated peat from Milne Point. I suspect the agreement is more coincidental than indicative of similarity in oxidation rates as it would be more reasonable to expect wide variations due to differing populations of bacteria, nutrient concentrations, temperature, oxygen concentrations and response to fresh versus degraded peat materials. The turnover times do indicate however that the peat is readily oxidized by microorganisms and contributes to ecosystem energetics in the nearshore zone.

NEEDS FOR FURTHER STUDY

The considerable progress that has been made toward determining the relative importance of terrestrial versus marine energy inputs to the trophic systems of the nearshore Beaufort Sea supports the concept of using natural carbon isotopic abundances as a tracer of foodweb pathways. The similarity in arctic coastal marine environments and nearshore terrestrial environments and their biological components would indicate that the techniques employed to date are suitable for use in other areas such as the eastern Beaufort Sea and the Chukchi and Bering Seas. Specific tasks which can identified as essential toward completing this study of the Simpson Lagoon-Stefansson Sound region are listed below:

1. Are there populations of invertebrates and other organisms in Harrison Bay that are much more dependent upon detritus than those of areas to the east. The low ^{14}C content of amphipods taken off the Colville delta may be indicative of a detritus dependent foodweb of major importance to resident organisms.
2. Kelp from Stefansson Sound is characterized by a distinctive $^{13}C/^{12}C$ isotopic abundance. Organisms which may use kelp as an energy source in the Stefansson Sound area need to be analyzed to ascertain the significance of these primary producers to the regional ecosystem. This task would be accomplished in cooperation with K. Dunton.
3. Additional freshwater isotopic data is needed to identify the sources of food which produce the anomalously high ^{14}C content in the anadromous fish caught in Simpson Lagoon. This task will be undertaken in conjunction with LGL-USA's studies on arctic cisco (RU 467).
4. Validation studies using the ^{14}C and ^{13}C natural abundances as tracers need to be performed at other locations along the Beaufort Sea and Chukchi Sea coastline.
5. Additional areal studies of ice algae and turbid ice cover need to be made to improve the accuracy of ice algae primary production estimates. This work is being continued in the 1980 field season and will be done in cooperation with several research units.

6. Samples of arctic cod need to be analyzed for isotopic abundances to help ascertain the pelagic versus neritic nature of juvenile and adult populations of this critical foodweb species of arctic fish.
7. Additional laboratory experiments need to be performed on seawater-peat-detritus biological interactions including: microbial colonization rates, temperature effects on bio-oxidation, nutrient uptake/regeneration and grazing studies. This study, in cooperation with D. Schneider (RU 356) would provide information on specific pathways by which detrital carbon is recycled and transferred to higher organisms.
8. The persistence of turbid ice cover and the resulting depression of phytoplankton primary production on into the summer months need to be assessed for the Stefansson Sound area and the coastal Beaufort Sea as a whole. This task will be approached by using satellite imagery and ground truth data to determine sediment content in ice and the melting rates over the course of May, June and July.

SUMMARY OF FOURTH QUARTER ACTIVITIES

Field activities. The first 1980 field trip to the Stefansson Sound area is scheduled for 1-7 April. The emphasis of the sampling program will be on collecting water samples for salinity and nutrient determination and obtaining light penetration data on the ice cover at stations between Mikkelsen Bay and Harrison Bay.

Laboratory activities. Processing of salinity samples collected in November and the completion of radiocarbon and $^{13}C/^{12}C$ isotopic ratio determinations on faunal samples comprised the major laboratory activities during this period. Improved analytical capability for nitrogen and phosphorus determinations on peat samples has resulted in plans to re-run these samples for comparision with previously employed techniques. This work is in progress.

Meetings attended. A paper entitled "Ice algae distribution and productivity in the Alaskan Beaufort Sea" was presented at the American Society of Limnology and Oceanography winter meeting in Los Angeles, California on 1 February 1980.

On 19-20 March, the author was chairman of the NOAA-OCSEAP Winter Studies Synthesis Meeting in Fairbanks, Alaska and edited the summaries of that meeting and the "Slush Ice Conference" convened by Larry Larsen, University of Washington, Seattle, Washington on 2 February 1980. The editing of this synthesis volume and field work preparations for April sampling trips contributed to the delay in completing this annual report.

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