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OUTER CONTINENTAL SHELF
ENVIRONMENTAL ASSESSMENT PROGRAM

FINAL REPORTS OF PRINCIPAL INVESTIGATORS

VOLUME 28

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Outer Continental Shelf Environmental Assessment Program

Final Reports of Principal Investigators

VOLUME 28 DECEMBER 1984

C O N T E N T S

MICROBIAL PROCESSES AS RELATED TO TRANSPORT IN THE NORTH ALEUTIAN SHELF AND ST. GEORGE BASIN LEASE AREAS

by

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Final Report
Outer Continental Shelf Environmental Assessment Program
Research Unit 595

30 September 1981

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I. Summary of Objectives, Conclusions, and Implications with Respect to OCS Oil and Gas Development

A. Objectives

Our main objective during this project was to provide data on the sources and sinks of methane which are associated with microbial processes. This study was designed to provide the North Aleutian Shelf Transport Experiment with data with which they can assess more accurately the transport processes in the perspective lease areas of the North Aleutian Shelf and St. George Basin. This information is required so that the most likely routes for spilled crude oil transport can be determined.

A secondary objective was to obtain data on levels of microbial function in these two lease areas. These data make it possible for us to locate regions which may be particularly sensitive to crude oil perturbation.

B. Conclusions and Implications

1. The data presented in this report do not provide information about the transport processes in the North Aleutian Shelf (NAS) and St. George Basin (SGB) lease areas. The information that we have accumulated on methane production and oxidation rates have been given to Dr. Cline (RU #153) of PMEL to be used in his transport model. This model will generate predictions of vertical and horizontal transport rates in these regions.

2. By measuring rates of nitrogen fixation, CO_2 evolution, relative microbial activity, and the activity of the enzymes phosphatase, arylsulfatase, amylase, and laminarinase, we were able to locate regions where microbial function would most severely be impacted

if sediments were exposed to crude oil. In the St. George Basin, the most sensitive area was near station PL7 (55° 48'N, 166° 22'W) which is shown in Figure 53. Microbial variables measured during both the January and May cruises showed highest activities in this region. All of the microbial variables studied are known to be altered when sub-Arctic marine sediments are impacted with fresh crude oil and CO_2 evolution and relative microbial activity are shown to be affected by weathered crude oil in the same sediments. For these reasons we recommend that no drilling take place within a 22 km radius of station PL7.

3. In the North Aleutian Shelf area, the major bays of the Alaska Penninsula are probably the sites which would be most severely impacted as the result of a major oil spill. All of the microbial variables that we studied showed elevated rates of activity in the Port Moller-Herendeen Bay area. These rates were generally significantly higher than those observed in the St. George Basin and in many cases were higher by a factor of 10. These data suggest that this is a region of high biological activity which could be adversely affected for a long period of time if impacted with crude oil. High biological activity has also been documented in another major bay in this region (Izembek Lagoon) by McRoy (1970). In this bay, the main carbon source appears to be the eel grass beds which are some of the most extensive known. It thus seems quite likely that most of the major bays of this area are important biologically and could be particularly sensitive to crude oil perturbation. In the event of an oil spill, it would be very important to prevent contamination of those bays.

Our study of relative microbial activity in the waters of the North Aleutian Shelf and St. George Basin suggests that there is a biologically active water layer at the water-sediment interface. It is known that crude oil tends to accumulate with the flocculant layer at the water-sediment interface. At this time it is not known how the presence of crude oil in this zone might affect the dynamics of the detrital food chain. Because of the close proximity of the crude oil and the biologically active portion of the water column, there is a potential for impact in this area.

II. Introduction

A. General Nature and Scope of Study

This study was designed to determine transport processes in the North Aleutian Shelf and St. George Basin lease areas. This study included observations on the physical, chemical, and biological factors which were relevant to the problem of predicting the horizontal and vertical transport of crude oil in the event of a spill. This information is essential for predicting the potential impact of crude oil production and transport in these two lease areas. There were two microbiological components of this study; the biodegradation rates of petroleum hydrocarbons conducted by Dr. Atlas (RU #29), and the biological sources and sinks of methane in the study area which was the objective of our study.

B. Specific Objectives

1. To measure <u>in situ</u> rates of methane production in these two lease areas with particular emphasis on the main methane source areas that have been identified by the chemists. The major emphasis of this study was to measure methane production rates in marine sediments.

2. To measure rates of methane oxidation in the waters that were being studied by the chemists. Both the uptake rates at one methane concentration and uptake kinetics were to be determined.

3. To determine the regions within the study area where crude oil perturbation of marine sediments would have the greatest impact on microbial processes.

C. Relevance to Problems of Petroleum Development

Without a comprehensive model for oil transport within the lease areas, there is no way that the link between potential spill sites and the most sensitive impact areas can be established. The purpose of this study is to provide that link.

III. Current State of Knowledge

To our knowledge, the only data on the in situ rates of methane oxidation and methanogenesis that have been collected in these two lease areas are those which we have presented in this report.

A. Methane oxidation

Prior to this study, there were reports available on the physiology and ecology of methane oxidizing bacteria (methanotrophs) from fresh water environments (Rudd and Taylor, 1980; Ribbons et al., 1970). Unfortunately, very little is known about methanotrophs in the marine environment. In the late 1940's and early 50's, Hutton and ZoBell (1949,1953) studied the distribution and physiology of methanotrophs in marine sediments using the methods available at that time. More recently, Reeburgh (1976 and 1977) has deduced methane oxidation rates in marine sediments by analyzing methane and O_2 concentrations profiles in marine sediments. In only one case has there been an attempt to measure methane oxidation rates in the marine environment using C^{14} radiotracer techniques (Iversen and Blackburn, 1981) and this was conducted in sediments. There

are two studies where methane oxidation rates in marine waters have been estimated (Scranton and Brewer, 1978; and Sansone and Martens, 1978). Sansone and Martens (1978) calculated methane oxidation rates by observing the reduction in methane concentrations with incubation time using gas chromatography. Scraton and Brewer (1978) used existing methane and O_2 concentrations in deep waters to calculate methane oxidation rates; no direct analyses were conducted.

Although radiotracer techniques have been used to measure oxidation rates in fresh waters (Rudd and Hamilton, 1975, Rudd et al., 1974 and Belyaev et al., 1975), these techniques have not been applied to salt waters and, with the exception of one experiment by Rudd and Hamilton (1975), no attempt has been made to measure the kinetics of methane oxidation. Thus, this study is the first reported where in situ methane oxidation rates have been estimated from methane oxidation kinetic determinations and known methane concentrations.

B. Methanogenesis

Although more is known about methane production than oxidation in marine systems, there have been no studies in which methane production rates have been directly observed in offshore marine sediments. Oremland (1975) observed the accumulation of methane in chambers over shallow sediments in seagrass beds and coral reefs but no attempt was made to conduct similar studies in deeper marine sediments. Most of the estimates of methane production in marine sediments have been calculated from the observed concentration gradients with core depth (Reeburgh, 1976; Barnes and Goldbers, 1976). There have been no reports where total in situ methane

production rates have been estimated in marine sediments using radioactive tracers.

IV. Study Area

Figures 1 and 2 show the station locations where samples were collected during the first NASTE cruise conducted in August-September 1980. Figures 3 and 4 show the station locations where samples were collected during the second NASTE cruise conducted in January-February, 1981. Figures 5, 6, and 7 show the station locations during the May 1981 cruise.

V. Methods and Materials

A. August-September NASTE cruise

1. Sample Collection

With the exception of the sediment samples taken by hand or with a small grab sampler in Port Moller during small boat operations, all sediment samples were collected using a Smith-MacIntyre grab sampler. Subsamples of the sediments collected with this grab were taken with plastic core liners measuring 6 cm in diameter and 25 cm in length. The core liner was slowly pushed into the grab sample. The core liner was then removed slowly and sealed at each end with a #12 rubber stopper. The sediments taken in this way measured ca. 10 cm in length.

All water samples were collected using a Niskin "butterfly" sampler fitted with a sterile plastic bag. The bottom water samples were taken within 10 cm of the water-sediment interface by using a specially modified inverted Niskin "butterfly" sampler mounted on the Smith-MacIntyre grab.

2. Assay for Methanogenesis

After the cores were taken, they were placed in an anaerobic glove bag in which the head space over the cores, and all glassware

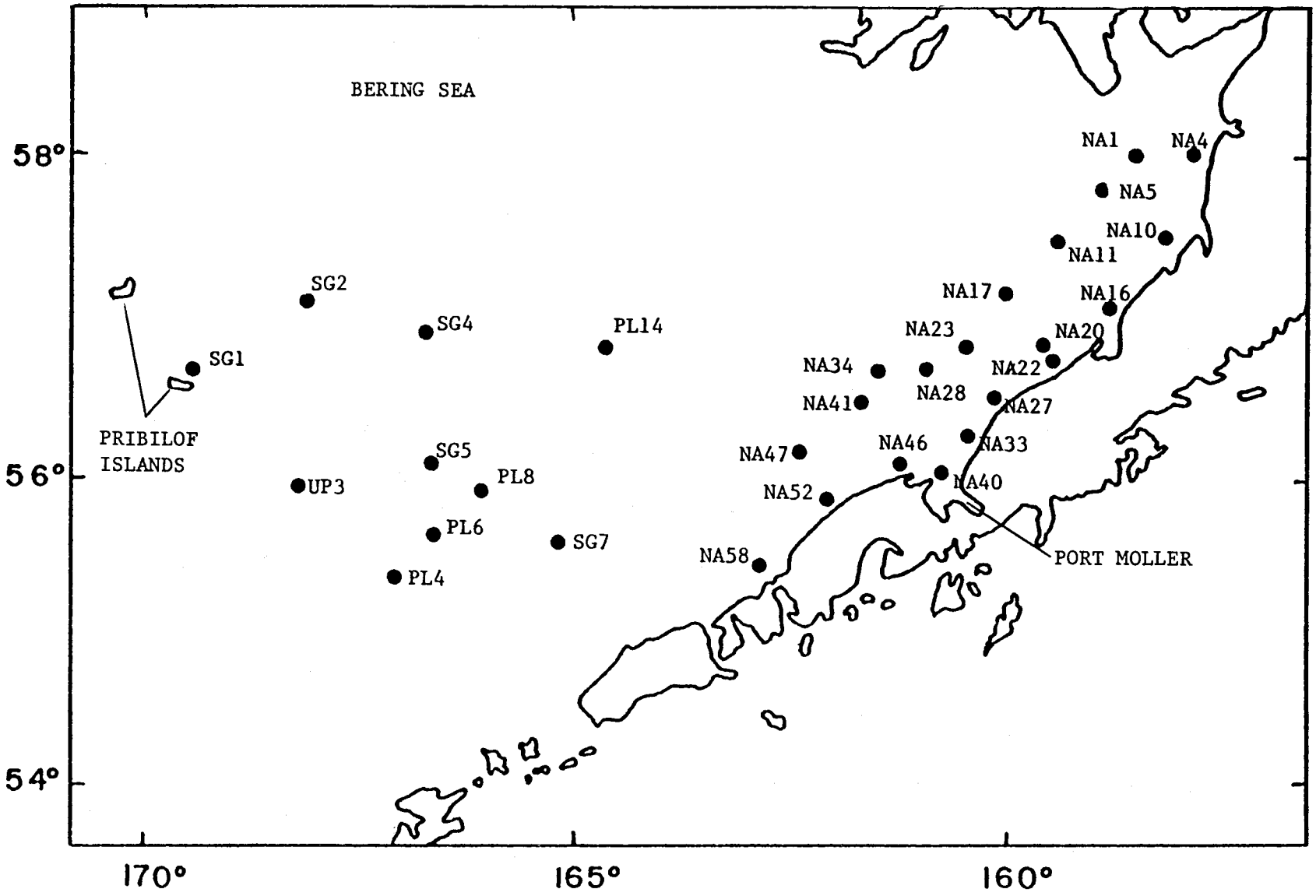

Figure 1. Positions and station designations for locations sampled during the August 1980 North Aleutian Shelf Transport Study cruise.

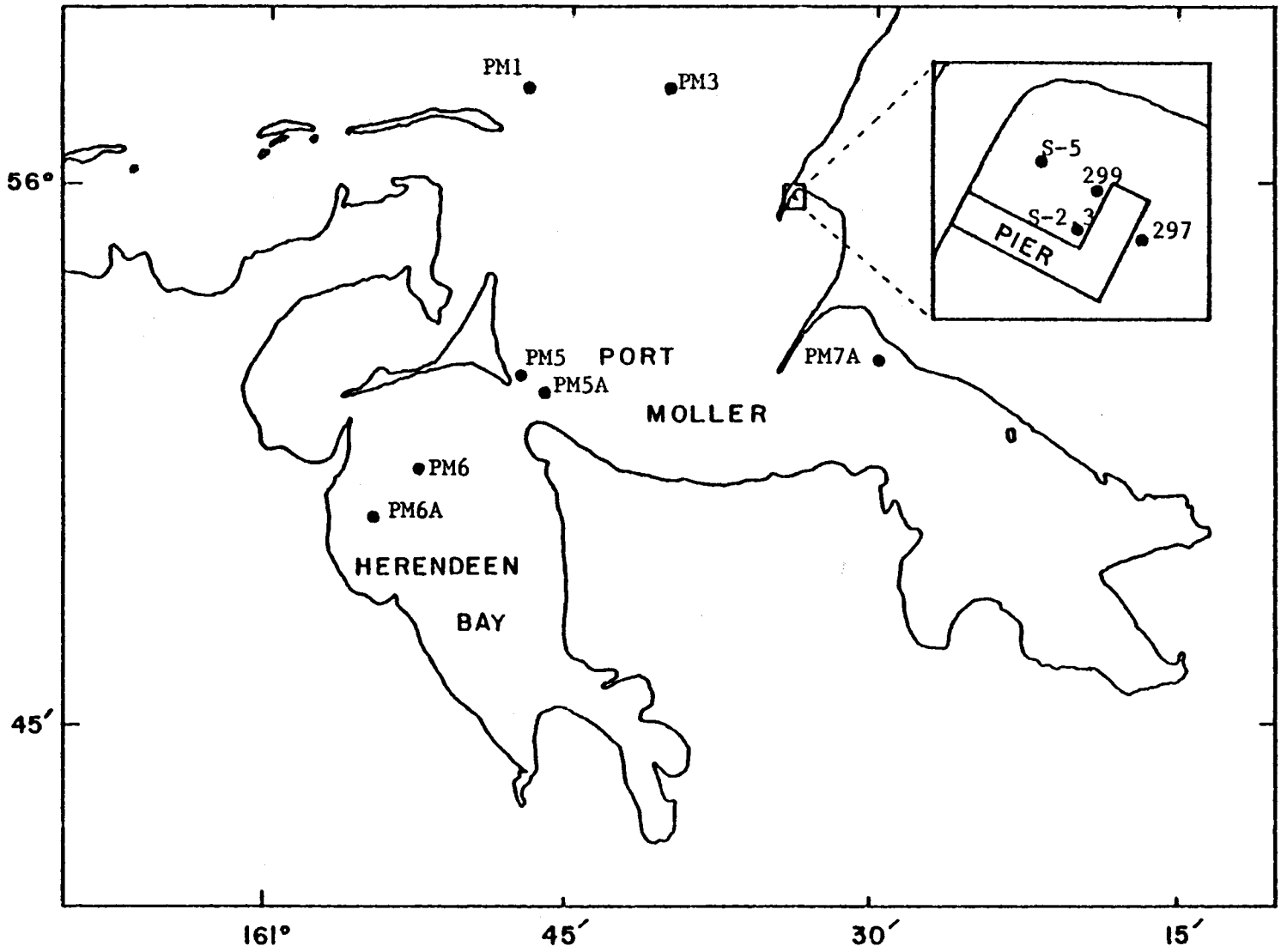

Figure 2. Positions and station designations for locations sampled in Port Moller during the August 1980 North Aleutian Shelf Transport Study.

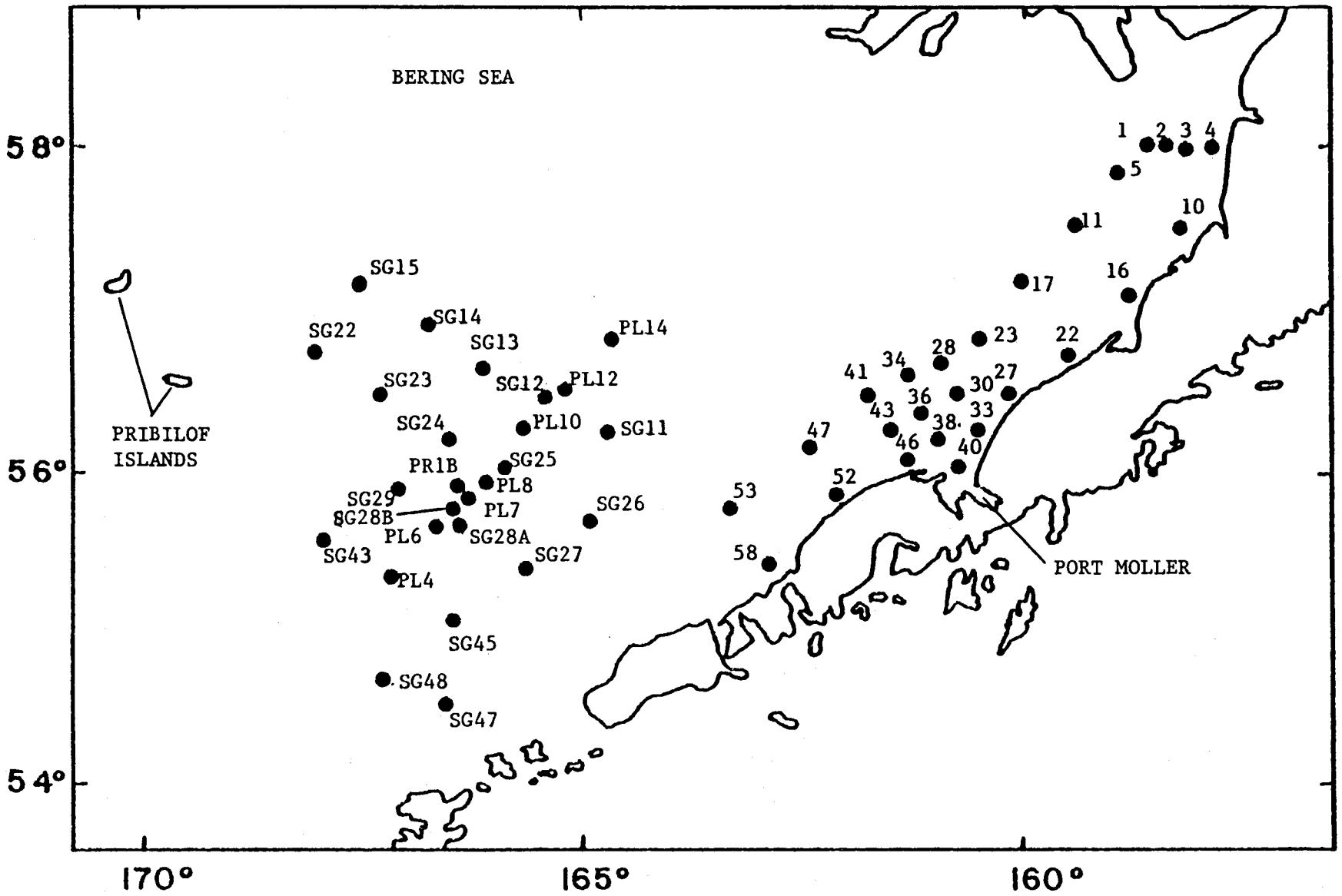

Figure 3. Positions and station designations for locations sampled during the January 1981 North Aleutian Shelf Transport Study (excluding Port Moller stations). Stations without letter designators are "NA" stations.

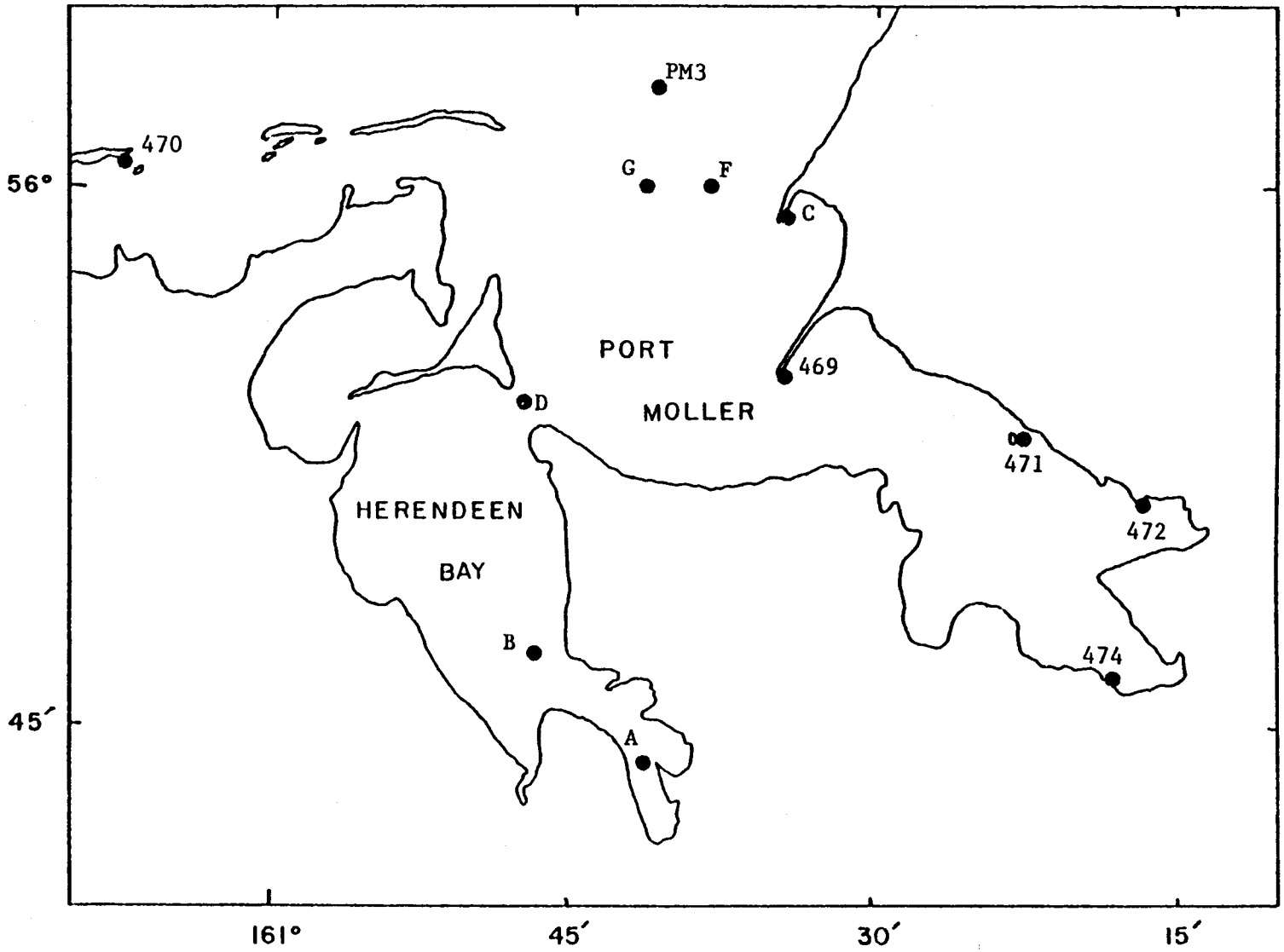

Figure 4. Positions and station designations for locations sampled in Port Moller during the January 1981 cruise.

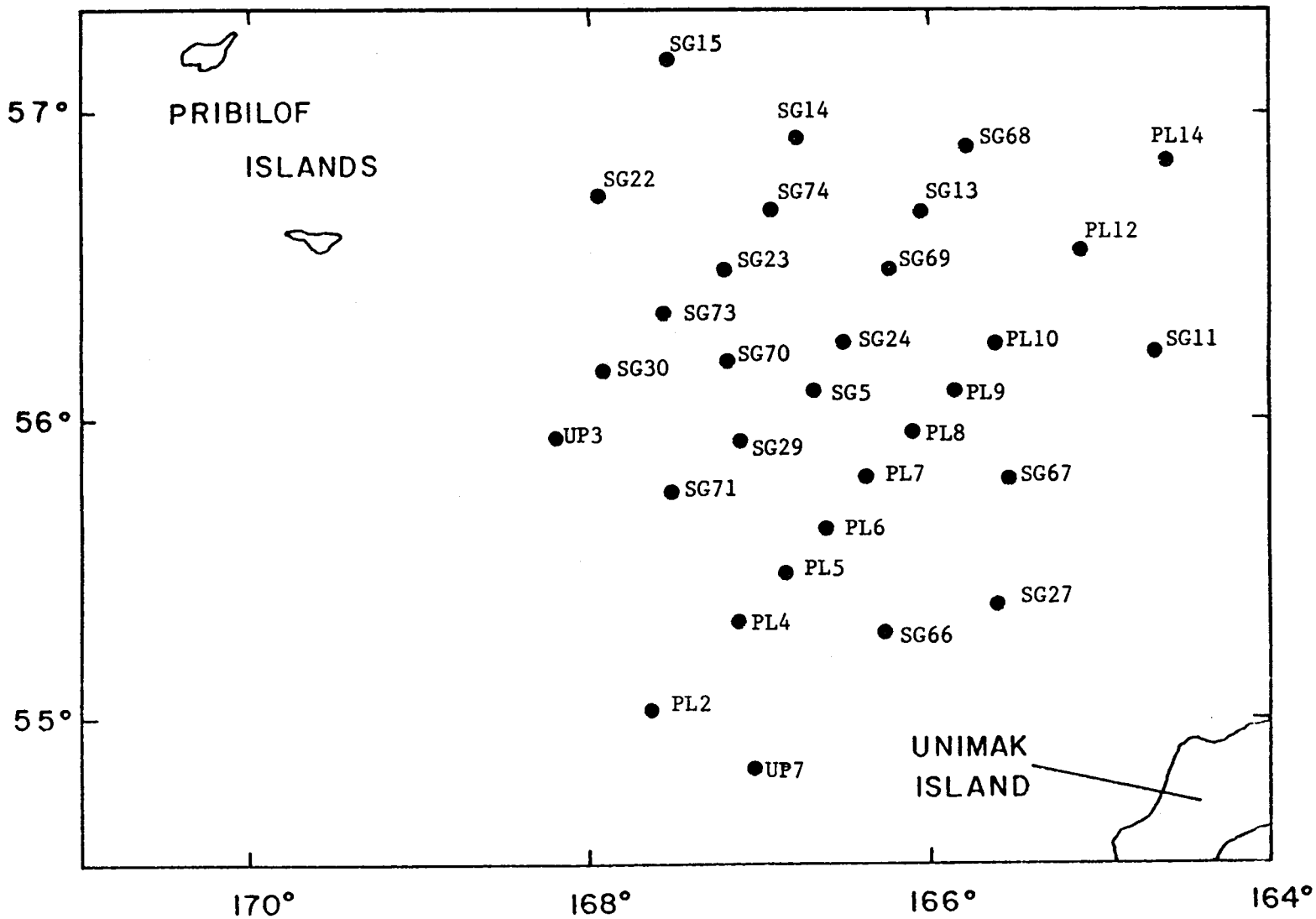

Figure 5. Positions and station designations for locations sampled in the Saint George Basin during the May 1981 North Aleutian Shelf Transport Study cruise.

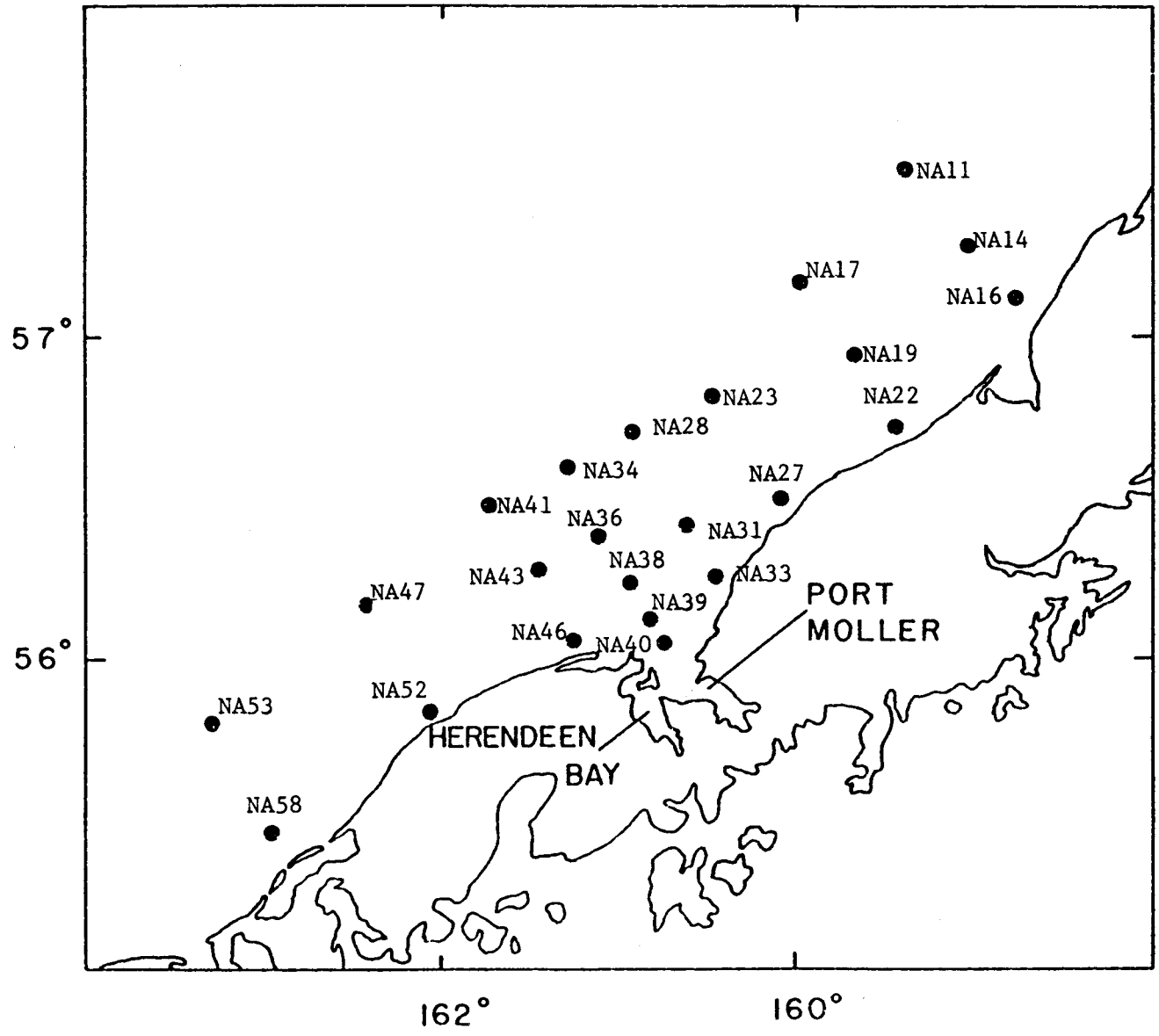

Figure 6. Positions and station designations for locations sampled in the North Aleutian Shelf during the May 1981 North Aleutian Shelf Transport Study cruise.

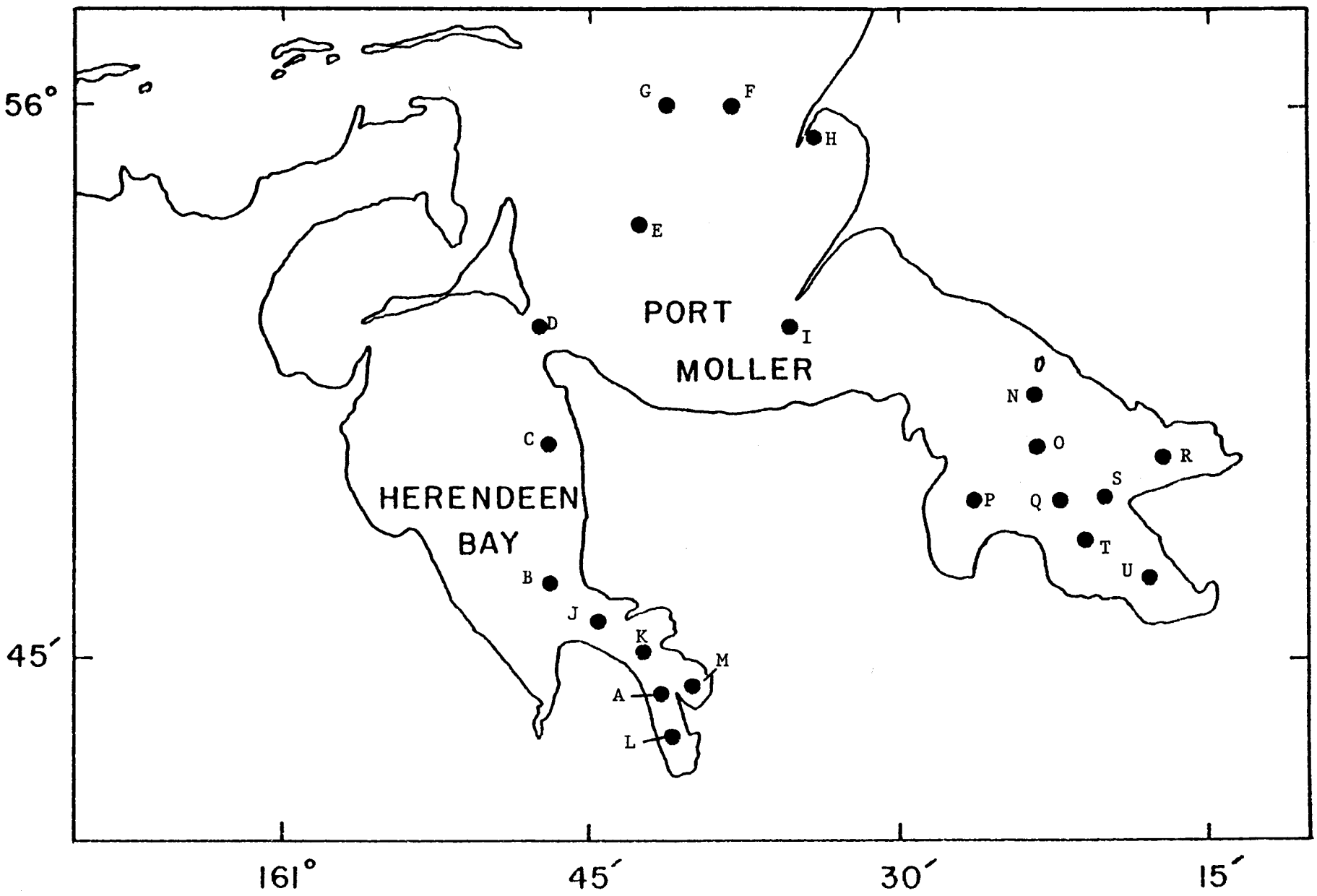

Figure 7. Positions and station designations for locations sampled in Port Moller during the May 1981 North Aleutian Shelf Transport Study cruise.

were purged with oxygen-free nitrogen. After these items were sufficiently purged with oxygen-free nitrogen, the glove bag was purged with the same and then sealed.

a. Assay using [2-^{14}C] acetic acid (Method 1)

After the subcores were taken, they were placed into glass roll tubes measuring 1.6 x 6.0 cm which have a volume of 8 ml when stoppered with special 00 butyl rubber stoppers (A. H. Thomas Co., Philadelphia, PA). Before the roll tubes were placed into the glove bag, the labeled substrate was added to them. In most experiments, duplicate subsamples at each of four substrate concentrations were used. The substrate used was (2-^{14}C) acetic acid, sodium salt, from Amersham with a specific activity of 59 mCi/mmole. The substrate was prepared by diluting the material received from Amersham with distilled water to give a solution containing 1.04 μCi/ml. This was filter sterilized through a 0.45 μm Millipore membrane filter and dispensed into sterile 2 ml ampules which were then sealed and frozen. These were kept frozen until just prior to their use. In the concentration series, 0.025, 0.05, 0.10, and 0.20 ml were used. No attempt was made to keep these solutions anaerobic prior to use because it was assumed that after these small volumes were gassed vigorously with oxygen-free nitrogen while in the roll tubes, very little oxygen would remain in solution.

Before and after the sediment was added to the roll tubes, the tubes were purged with O_2 free N_2. At this point one of two techniques was used. In some cases, two sets of tubes from the same sediment sample were treated using both approaches. In the first approach, nothing else was added. The tubes were mixed with a vortex mixer for

15 seconds. The tubes were then incubated at the in situ temperature ± 1°C and analyzed for labeled methane as described below. In the second procedure, we removed 2 ml of gas with a syringe and replaced this with 2 ml of cysteine water. The cysteine water was made up of 3% NaCl, 0.05% cysteine-HCl, 0.0001% resazurin at pH 7.0. Any overpressure remaining in the tubes was removed by the syringe. When the cysteine water was used, controls were also run using the cysteine water containing 0.1% w/v sodium azide. In our preliminary experiments, we found that azide solution was very effective in stopping methanogenesis.

After incubation time of 1 to 14 days, the tubes were assayed for the presence of ^{14}C methane using a Packard model 894 gas proportional counter. One ml of head space was withdrawn from each sample with a one ml gas-tight syringe and injected into a model 5734 Hewlett Packard gas chromatograph. A helium carrier gas was used at a flow rate of 60 ml per min. The gas chromatograph was fitted with a 6 meter x 1/8 inch stainless steel column packed with Porapak R. (80/100 mesh). The temperatures used were; injection port 150°C, detector 300°C, oven ambient with the oven open. These conditions permitted a 3 minute separation between the labeled CO_2 and methane peaks as detected by the gas proportional counter. The signal from the gas proportional counter was analyzed and calibrated on a Hewlett Packard model 3380 integrator.

Labeled bicarbonate and methane of known specific activities and concentrations were used to calibrate the gas proportional counter. By counting the radioactivity of methane at varying concentrations, we determined that the limit of detection for one ml of gas was between 200 and 400 DPM (1.5-3.0 pmole). We also

determined that the response of the instrument was linear between 200 and 1000 DPM.

Although most of the samples were analyzed within the first two days incubation, some were allowed to incubate for periods up to 14 days.

b. Assay using gas chromatography (Method 2).

Using a 3 ml plastic disposable syringe with the end of the barrel removed, a total of 6 subscores were taken by holding the plunger steady while the barrel was slowly pushed into the sediment. Six subcores containing 3 ml each were taken from the bottom half of the core. Of the 6 subsamples taken, 3 were used as controls and 3 were the active samples. The cores were treated in the same way as that described in method 1 except no labeled acetate was added.

Two techniques were used to prepare the sediments for the assay. In the first technique, there was nothing added to the sediment and in the second, 2 ml of headspace was removed and 2 ml of cysteine water was added using a syringe. In the first technique, the sediments were poisoned with 1 ml of acetylene and in the second technique, the sediments were poisoned with 0.1 w/v sodium azide. In samples where both techniques were used, the same methanogenesis rate was observed.

After the roll tubes had been incubated in the dark for varying lengths of time (up to 14 days), they were assayed for methane concentrations between 4 to 6 times during the course of an experiment. During the assay itself, 0.3 ml of the headspace was removed using a 0.5 ml gas-tight syringe.

The concentration of methane in all tubes was determined by gas chromatography. The instrument used was a Hewlett Packard model 5734A gas chromatograph fitted with a dual flame ionization detector. The resulting detector output was analyzed using a Hewlett Packard model 3380 recording integrator. The column used was a 2 meter x 1/8 inch stainless steel column filled with Porapak R. A helium carrier gas with a flow rate of 40 cc/min was used. The following temperatures were used in the GC: injection port 150°C, oven 50°C, and detector 300°C.

The integrator was calibrated using a gas standard of 100 ppm methane in nitrogen prior to each set of determinations.

c. Water samples

Methanogenesis was measured in water samples using two techniques. In the first technique, 1 liter of water was filtered through a 0.45 μm Millipore membrane filter (47 mm in diameter) to trap the particulate fraction. The membranes were then placed into a roll tube which had been purged with oxygen-free nitrogen as described previously. When the roll tubes were removed from the glove bag, 2 ml of cysteine water was added. Three filters per sample were observed for methane production. The amount of labeled acetate solution added to each tube was 0.2 ml.

The second method used to measure methanogenesis in water samples was to add 55 ml of seawater to a 60 ml serum bottle. These samples were removed from a Niskin sterile water sample bag using a 50 ml syringe which had been flushed with oxygen-free nitrogen and sealed with a rubber stopper on the end of the syringe needle. Care was taken to prevent gas from entering the sample

bag before and during the time the samples were taken. Before the water sample was placed into the serum bottle, the bottle was purged with gas in the glove bag and then the sample was transferred under an anaerobic atmosphere. After the samples had been incubated, the head space was analyzed for methane as previously described.

3. Methane Oxidation Assay

Water samples were taken with a Niskin-sterile bag water sampler. After sample collection, the water samples were placed in 500 ml glass bottles and shaken. A 50 ml plastic syringe fitted with a cannula was used to transfer the water sample from the larger glass bottle into the serum bottles. When methane oxidation rates in sediments were assayed, 50 ml of a 1:10 dilution of sediment in sterile seawater were added to a 60 ml serum bottle and sealed with a serum bottle stopper.

Six subsamples were used in each methane oxidation rate determination (duplicate at each of three methane concentrations). The ^{14}C-methane used in this study was specially prepared by Amersham with a specific activity of 58 μCi/μmole. The final methane concentration was 1 μCi/ml in the gas that was added to the serum bottles. Three volumes of the gas mixture (^{14}C-methane in nitrogen) were used (0.25, 0.5 and 1.0 ml). This resulted in a final concentration of 0.29, 0.58, and 1.16 μl of methane/liter of seawater respectively. This is within the natural methane concentration range normally encountered in the study area (0.1-2.5 μl/liter).

In some cases methane oxidation rates were measured using one concentration of labeled methane (0.58 μl/liter). In these samples, triplicate subsamples were assayed. Before the labeled methane was added to the serum bottle, an equal volume of head space was removed

so that there was no net pressure change when the methane was injected with the syringe. One set of control subsamples was run with every third sample processed. Methane oxidation was stopped in the controls by adding 1.0 ml acetylene to the head space.

Once the methane was added, the samples were incubated in the dark on a rotary shaker at the in situ temperature ± 1°C for 48 hours. At the end of the incubation period, the reaction was terminated by adding 1.0 ml of 10 N NaOH with a syringe through the stopper. The serum bottle was then shaken for 1 hr at room temperature to permit the labeled CO_2 to be absorbed into solution. The rubber stopper was then removed and the sample transferred to a 125 ml serum bottle using a 50 ml syringe fitted with a cannula. This procedure removed the labeled methane while the labeled CO_2 remained in solution. The serum bottle was then stoppered with a serum bottle stopper fitted with a plastic bucket containing a fluted strip of filter paper. Two ml of 10 N H_2SO_4 was then added to the sample to release the labeled CO_2. The sample was then shaken for 1 hr at room temperature to release the labeled CO_2 which was then trapped on 0.2 ml of β-phenylethylamine which was placed in the filter paper using a 1 ml syringe. The CO_2 trapping procedure and the assay for radioactivity in the cell fraction is essentially the same as that which we have used in the past (Griffiths et al., 1978).

After the CO_2 was trapped, the sample was filtered through a 0.45 μm membrane filter which trapped the cells so that the amount of label incorporated into cell material could be determined. These filters were then dried and assayed using a liquid scintillation

counter (Beckman model LS100). The external standard method of determining channel ratios was used to calculate counting efficiencies.

4. Microbial Activity Determinations

The procedure used in these studies involved adding a U-^{14}C compound to identical 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 (Knotes Glass Co., Vineland, N.J.: K-882320) containing 25 x 50 mm strips of fluted Whatman #1 chromatography paper. The samples were incubated in the dark within 1.0°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 absorbent β-phenylethylamine was injected onto the filter paper. The bottles were then shaken on a rotary shaker at 200 rpm for at least 45 minutes at room temperature to facilitate the absorption 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 Filmware (Nalge) scintillation bags containing 2 ml of the above mentioned fluor. The vials were counted in a 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.

U-^{14}C L-glutamic acid with a specific activity of 285 mCi/mmole (Amersham-Searle) was used in all water samples at a final concentration of 5.4 µg/liter. Glutamic acid with a lower specific activity (10 mCi/mmole) was used in all sediment samples. U-^{14}C D-glucose with a specific activity of 291 mCi/mmole (Amersham-Searle) was used in all water and sediment samples with a final concentration of 3.8 µg/liter).

Triplicate subsamples were analyzed for each sample and the results reported in Figures 29 to 43 are the means of the observed values. 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 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.

B. January-February and May 1981 NASTE Cruises

1. Methanogenesis Assay

During these cruises, no radioactive tracer experiments were conducted. Methane production rates generated from sediment subcores were estimated by assaying the headspace in roll tubes or by assaying the water column over an intact core. In the latter case, the methane was stripped from the water and collected in a liquid nitrogen cold trap before assaying on a gas chromatograph. The

water analyses of the water over intact cores were conducted by Mr. Chuck Katz of PMEL. During the January cruise, the roll tube experiments were conducted using 2 controls and 4 methane-producing subsamples. During the May cruise, 6 methane producing subsamples were used for each sediment analyzed. Because of the statistical analytical procedures used, no controls were necessary. In these experiments, no cysteine water was used. Methanogenesis in the controls was terminated by the addition of 1 ml 37% buffered formaldehyde. All other procedures were the same as that described for the August cruise. The headspace of the roll tubes was assayed for methane after 1,3,5 and 7 days incubation.

When we returned to Oregon State University after the cruise, the methane data was analyzed by three different methods. In all methods, the amount of methane in the headspace removed during the course of the experiment was corrected for in the calculations. The first approach used was to subtract the mean methane concentration found in the two controls from the methane concentration observed in the headspace after 7 days incubation. The rate was the mean of those values divided by 7. By knowing the volume of the headspace, the volume of sediment and the incubation time, the amount of methane produced per ml of sediment per day was calculated.

The second approach was to assume that it took one day to allow the sediment to come to equilibrium. This assumption was based on the observation that methane production rate observed during the first day was generally greater than that observed from the first day to the seventh day of incubation. This increase in apparent methane production was undoubtedly due to the establishment of a new equilibrium between methane in the sediment and methane in the

headspace. The mean of the control methane concentration was subtracted from the concentration observed in sediments that had been incubated 1 and 7 days. The concentration of methane observed after 1 day incubation was then subtracted from the concentration observed after 7 days for each of the 4 subsamples. The net mean production was calculated using an incubation period of 6 days. The third method consisted of running a linear regression on the observed methane concentration for all methane-producing subsamples at all times that the methane concentrations were determined up through 7 days. In essentially all cases where longer incubation times were used, the rate observed between 7 and 9 days incubation was greater than observed during any other time period. The 9 day values were therefore not used in any of the above calculations.

In this last method, the methanogenesis rate was the slope of the best fitting line through all points. Surprisingly, the rates calculated using all three approaches produced very similar results. Of the three methods used, we feel that the last one mentioned is the most valid statistically and therefore it was the one that was adapted to estimate methanogenesis rates reported for all cruises. The methanogenesis rates and the observed correlation coefficients measured using this method during the January cruise are given in Table 1.

Table 1. Methane production rates in ml x m^{-2} x day^{-1} and the corresponding correlation coefficients observed in sediments collected during the January NASTE cruise. These calculations were made assuming an active methanogenesis depth of 10 cm.

Station	Methane Production	Correlation Coefficient	Station #	Methane Production	Correlation Coefficient
NA40	0.07	0.3	PL6	0.12	0.7
NA46	0	0.6	PL8	0.24	0.5
SG11	0.04	0.5	PL10	0.03	0.6
SG12	0.10	0.3	PL12	0.03	0.4
PL7	0.02	0.2	PL14	0.08	0.6
SG28	0	0.7	SG13	0.22	0.8
NA52	0.02	0.4	SG14	0.08	0.7
PR1B	0	0.5	SG15	0.06	0.7
PR1B	0.07	0.4	SG22	0.18	0.7
NA16	0.02	0.5	SG23	0.04	0.5
PM3	0.17	0.7	SG24	0.12	0.7
297	0.77	0.9	SG25	0.10	0.8
A	49	0.5	SG26	0.04	0.5
B	1.7	0.8	SG27	0.07	0.6
NA40	0.07	0.8	SG45	0	0.5
NA46	0.06	0.4	SG28	0.04	0.7
PL4	0.21	0.6	SG29	0.01	0.2
			SG48	0.02	0.2
			SG47	0	0.4

The relatively low correlation coefficients were caused by sample heterogeneity for methane production. The correlation coefficients for individual subsamples were much greater indicating that the rates observed with time in a given subsample were consistent.

The production rate units that are the most useful for the chemists is the amount of methane produced per m^2 per day. This conversion was made by assuming that the active depth for net methane production was 10 cm. In most cases, subcores were made of the sample core from the surface down to a depth of 5 cm. In some samples, two sets of cores were taken, one from the top and the other from the bottom of the core. These subcores were taken parallel to the length of the original core. Since most cores were 10 cm in length, subcores taken from both the top and bottom

represented the total length of the core. When both top and bottom cores were taken at a given location, the mean value for rates observed in both cores was used to estimate rates for the total core. Where only top subcores were taken, the rates estimated for the total core is double the 5 cm estimate.

During the first NASTE cruise, two methods of determining methanogenesis rates were used; radioactive tracer and GC analysis of the headspace (methods 1 and 2). During the second cruise, a third method was employed along with method 2. In this method, four identical cores were taken using the Pamatmat multiple coring device. This sampler produced four undisturbed cores with each cast. The corers are made out of a plastic material that is impervious to O_2 and have an inside diameter of 5.7 cm. When the sampler was brought on board after a sample had been taken, the cores were removed from the sampler and stoppered at each end with number 12 rubber stoppers. The cores were taken into the laboratory where the water overlaying the cores was removed and replaced with surface water which contained a low concentration of methane (ca. 225 µl/liter). The core tubes were resealed and placed into an incubation chamber designed to hold the core tubes vertically. The incubator was plumbed into the ship's seawater system to maintain a constant flow of fresh seawater, thus keeping the cores within 0.5°C of the bottom water temperature. The water level within the incubator was kept at a constant level so that the entire core was submerged in the water.

After the cores had incubated for 1 day, the water from one core was removed and assayed. This methane concentration was considered the time 0 control although determinations of the methane

in the surface water used to cover the cores was also determined. The core water samples in the remaining three cores were assayed after 4, 6, and 8 days. From these observations, a rate of methanogenesis was generated using the observed concentrations, the core length and the total water volume over the core. These calculations were made by Mr. Katz of PMEL. In the cases where there were only three good cores available from sampling, water samples were assayed after 1, 4, and 8 days incubation.

The method used to assay methane oxidation was the same during all cruises.

2. Nitrogen fixation

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. In most cases, one control and three subsamples were used for each analysis. After the bottles were sealed with a rubber stopper, the samples were gassed for one minute 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 the first GC analysis was conducted. A second determination was made after 48 hrs. The analysis for ethylene was made using the same GC and analytical conditions used in the methane concentration determinations. The resulting levels of ethylene were normalized using incubation times and gram dry weight conversion. All rates were calculated in terms of ng nitrogen fixed per gram dry weight of sediment per hour using a factor of 0.33 to convert the amount of ethylene measured to the theoretical amount of nitrogen fixed.

3. CO_2 determinations

CO_2 evolution rates in sediments were determined by adding 10 ml of sediment to a 25 ml flask fitted with a serum bottle stopper. Triplicate subsamples of each sediment were assayed using gas chromatography. The samples were incubated in the dark for periods up to 40 hr. The headspace of the flasks was assayed for CO_2 concentrations by injecting 0.3 ml of headspace into a GC fitted with a thermal conductivity detector. The conditions for GC analysis were the same as that used for methane assays. The CO_2 concentrations were determined after at least two incubation periods. The incubation periods used were 8, 20, 32, and 40 hr. The CO_2 evolution rates were calculated by measuring the net production between two incubation periods and dividing the amount by the number of hours between observations.

4. Enzyme Assays

Sediment samples used for enzyme assays and dry weight measurements were frozen on shipboard and returned to OSU for analysis.

a. Arylsulfatase and phosphatase activities were measured using modifications of techniques described by Tabatabai and Bremer (1969,1970). These assays are based on the enzymatic release of p-nitrophenol from the appropriate chromogenic substrate. To one ml of sediment slurry was added: one ml 30 ppt Rila Marine Mix - 0.05 M Tris buffer, pH 7.5; and one ml of either 0.006 M p-nitrophenylphosphate or 0.006 M-p-nitrophenylsulfate (Sigma) in buffer. Substrate and sediment blanks were run by omitting either the sediment slurry or the substrate. The reaction mixture was incubated at 25°C for one hour and then terminated with two ml 0.5 N NaOH and 0.5 ml of 0.5 M $CaCl_2$. The sample was then centrifuged

and the optical density of the clear supernatant at 410 nm was measured to determine the amount of p-nitrol released. A calibration curve was prepared using dilutions of 10 μM per ml p-nitrophenol (Sigma). Final results were calculated as μmoles p-nitrophenol released x gram dry weight^{-1} x hour^{-1}.

b. Polysaccharide hydrolase assays were based on the colorimetric determination of reducing sugars enzymatically released from appropriate substrates. Three ml of sediment slurry were added to three ml 30 ppt Rila Marine Mix - 0.05 M Tris buffer containing one of the following: 1% soluble starch, 1% carboxymethylcellulose, 1.0% laminarin or 1.0% xylan. Three tenths ml of toluene were added to limit microbial metabolism during the assay. Substrate and sediment blanks were run by omitting either the sediment slurry or the substrate. The reaction mixture was incubated at 25°C for 24 hours and then centrifuged. Two ml of the resulting supernatant were added to two ml of DNSA reagent. (This reagent was prepared by dissolving one gram of 2,5-dinitrosalicylic acid (Sigma) in 20 ml 2 N NaOH, adding 30 ml distilled water followed by 30 grams of sodium potassium tartrate, and then bringing the solution to a final volume of 100 ml with distilled water). The DSNA-supernatant mix was developed by placing the reaction vessels in a boiling water bath for 10 minutes and then cooling. The mixture was centrifuged to remove precipitants and the optical density at 540 nm measured to determine the reducing sugar (as glucose) content. A calibration curve was prepared using dilutions of one mg per ml glucose solution. Final results were calculated as μg glucose (or equivalent) released x gram dry weight^{-1} x hour^{-1}.

VI. Results

A. Methane Oxidation

1. North Aleutian Shelf

During the August cruise, we measured rates of methane oxidation in 93 water and 5 sediment samples. In the water samples, relative methane oxidation rates were measured in all samples analyzed and the kinetics of methane oxidation were measured in 61 samples. Along the North Aleutian Shelf (NAS), the methane oxidation rates at one substrate concentration were generally higher in the samples collected inshore than those collected offshore (Figs. 8 and 9). In the surface water samples, the mean rate was 1.2 nl x liter^{-1} x day^{-1} for the offshore samples and the mean rate in the inshore samples was 1.7 nl x liter^{-1} x day^{-1}; however, this difference was not statistically significant. In the bottom samples, the difference was greater with the mean values for offshore and inshore samples being 1.6 and 3.3 nl x liter^{-1} x day^{-1} respectively. The level of significance for this difference was $p = 0.005$. These statistical analyses were conducted on the methane oxidation rates reported in the figures.

During the January cruise, the same trend was observed. In surface waters, the mean rates for offshore and inshore samples were 0.3 and 0.6 nl x liter^{-1} x day^{-1} respectively (Figs. 10 and 11). In the bottom waters, the mean values were 0.6 and 2.0 respectively for offshore and inshore waters. The significance of these differences were $p = 0.002$ and 0.09 respectively. During the May cruise, a similar trend was seen but the differences were not statistically significant. The mean uptake in the surface waters

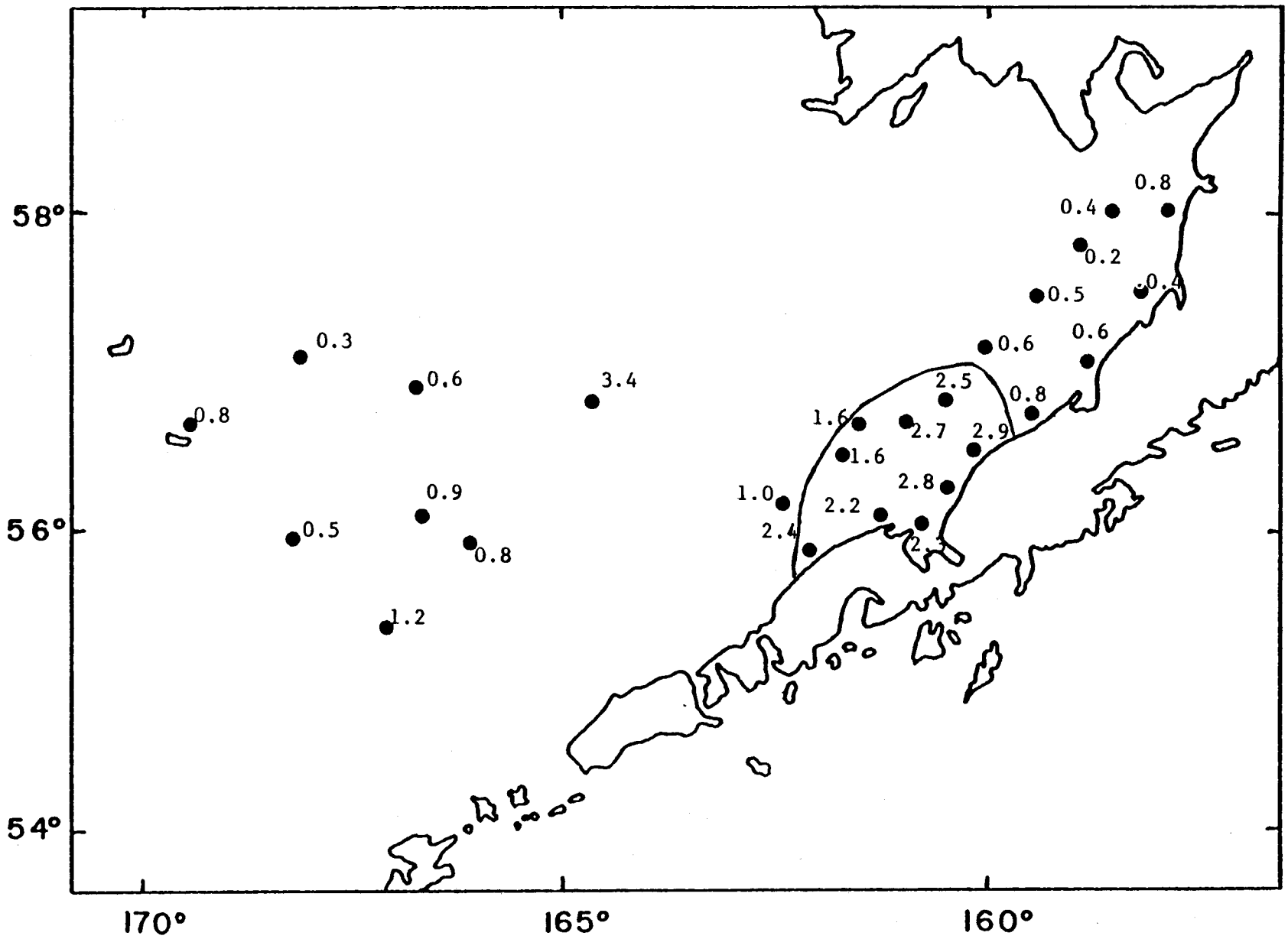

Figure 8. Methane oxidation rates in nl x liter^{-1} x day^{-1} observed in surface water samples from the August 1980 cruise. The enclosed area on all figures indicates areas with rates $\geq$ the mean.

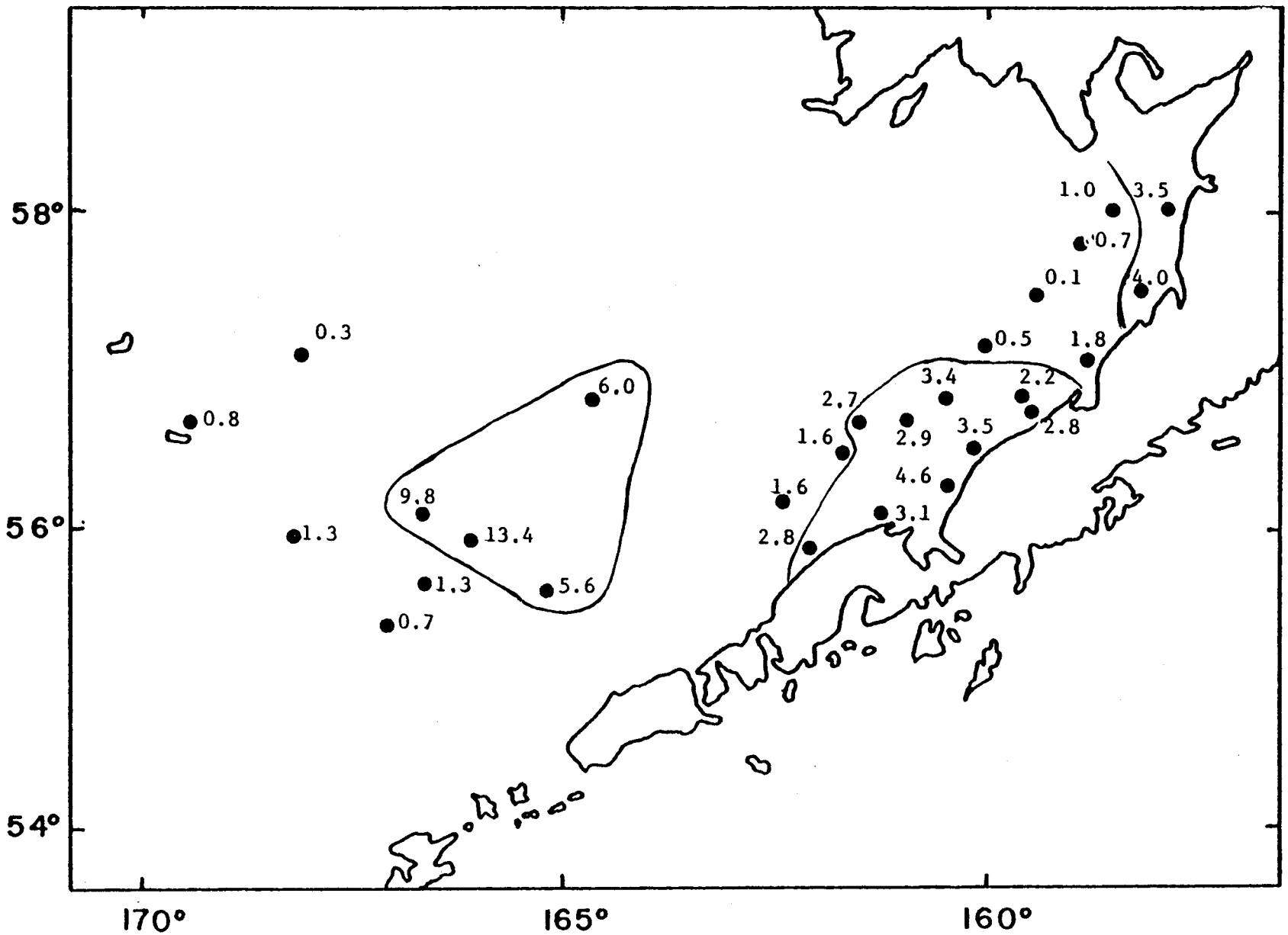

Figure 9. Methane oxidation rates in nl x liter^{-1} x day^{-1} observed in bottom water samples from the August 1980 cruise.

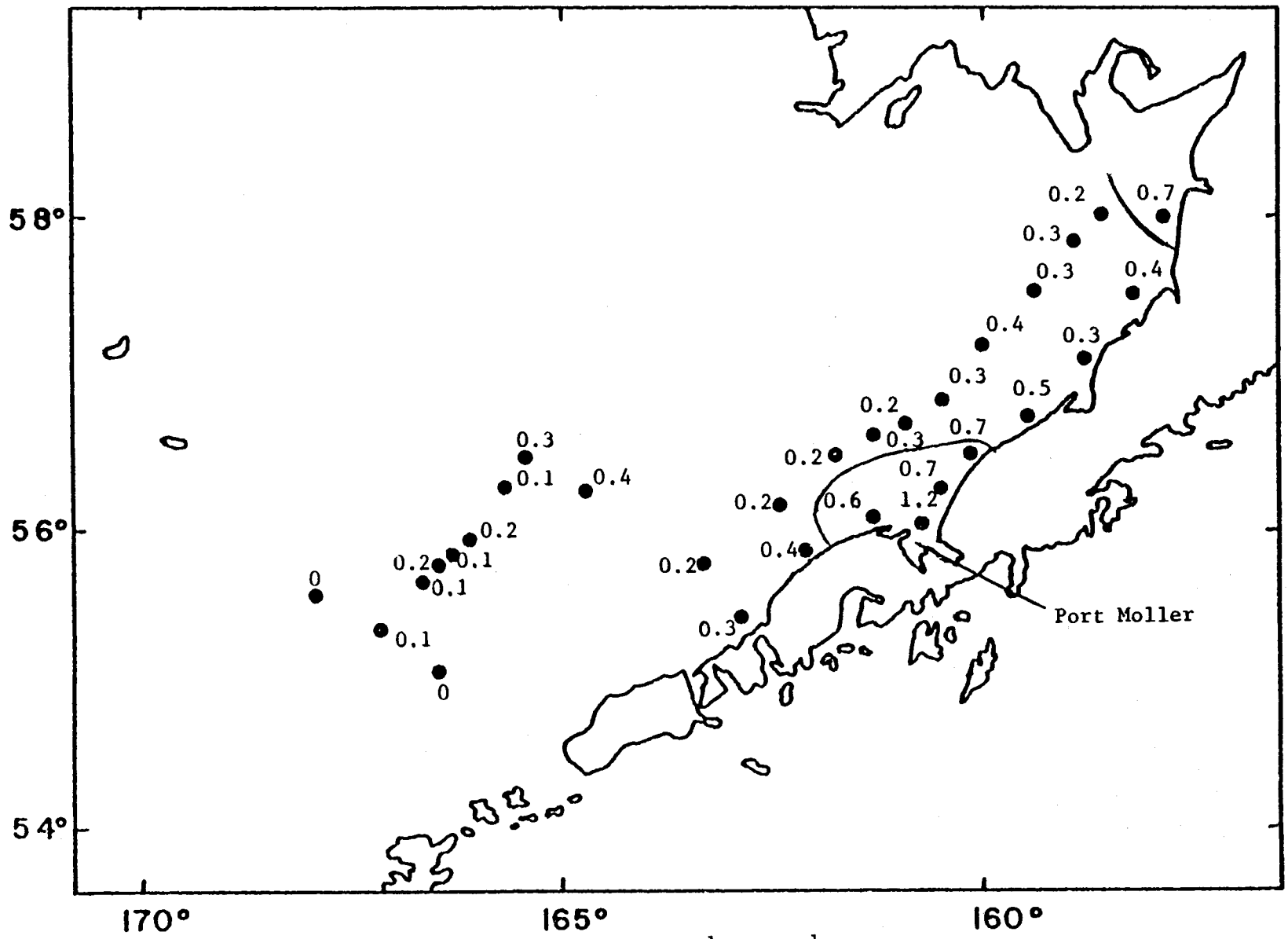

Figure 10. Methane oxidation rates in nl x liter^{-1} x day^{-1} observed in surface water samples from the January 1981 cruise.

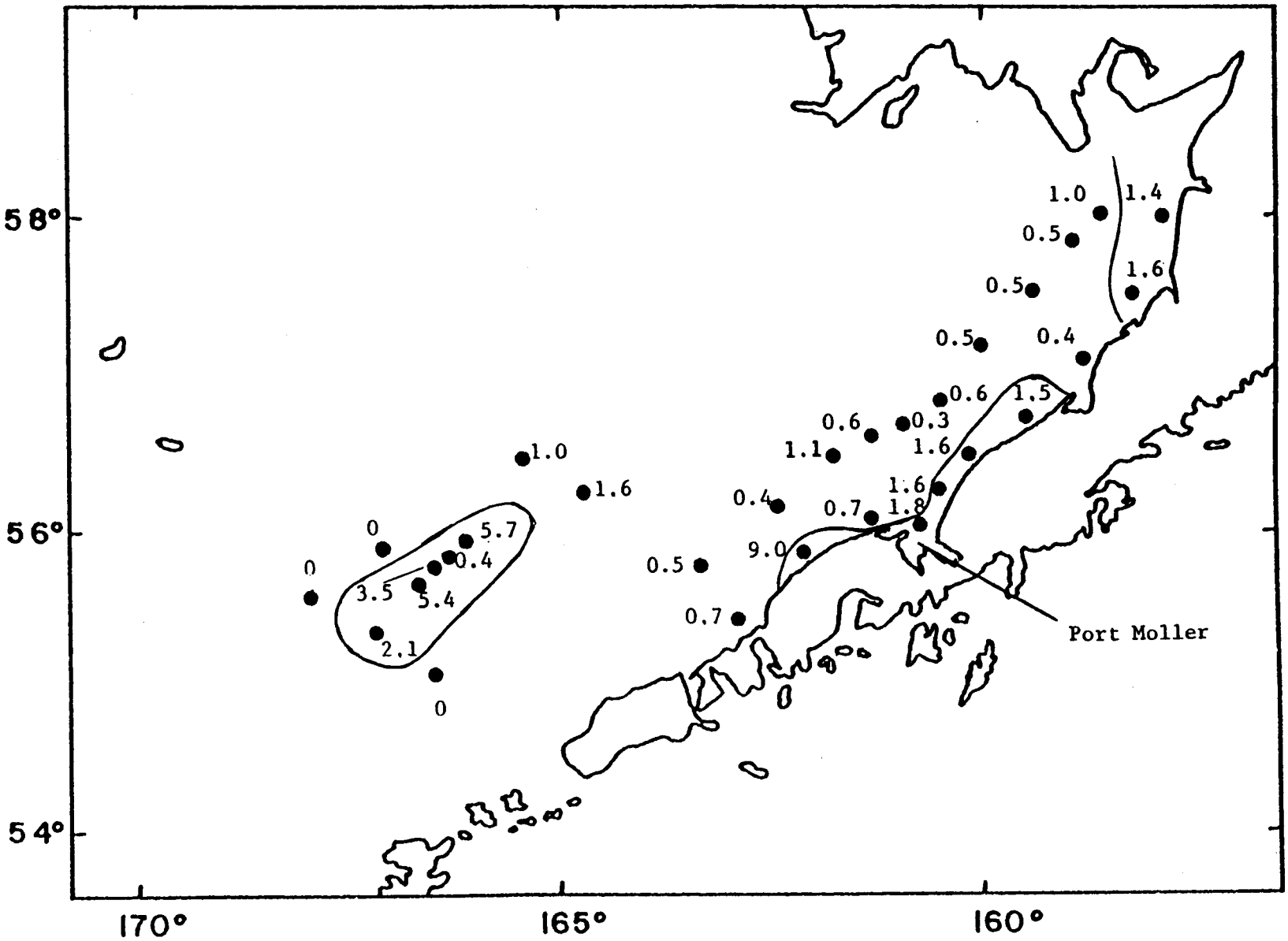

Figure 11. Methane oxidation rates in nl x liter^{-1} x day^{-1} observed in bottom water samples from the January 1981 cruise.

was 0.8 and 1.5 nl x liter^{-1} x day^{-1} in the offshore and inshore samples respectively and 1.0 and 1.8 nl x liter^{-1} x day^{-1} in the bottom waters (Figs. 12 and 13).

During all cruises, the methane oxidation rates in the bottom waters were generally greater than those observed at the surface at the same location (Figs. 8-13). During the August cruise, the mean rates observed in surface and bottom waters were 1.5 and 2.2 nl x liter^{-1} x day^{-1} respectively. During the January cruise, the mean methane oxidation rates in the surface and bottom waters were 0.4 and 1.3 nl x liter^{-1} x day^{-1} respectively. During the May cruise, the mean rates in the surface and bottom waters was 1.0 and 1.5 nl x liter^{-1} x day^{-1} respectively. The significance of these differences between surface and bottom waters for the cruises was $p = 0.02$, 0.05 and 0.11 respectively for August, January and May.

In the surface waters analyzed during the August and January cruises, there was a plume of high activity in the area around Port Moller (the enclosed areas in Figs. 8 and 10). This same pattern was observed in the bottom waters (Figs. 9 and 11) but high rates were also observed along the coast in the inshore stations to the east of Port Moller. The geographical patterns of methane oxidation were somewhat different during the May cruise. In the surface waters, methane oxidation rates were still elevated in the area near Port Moller but other areas also showed elevated rates (Fig. 12). In the bottom waters, elevated rates were observed both inshore and offshore in the regions to both sides of Port Moller (Fig. 13). In these and all other figures used in this report, the enclosed areas represent regions where the observed values were $\geq$ the mean value for all samples tested.

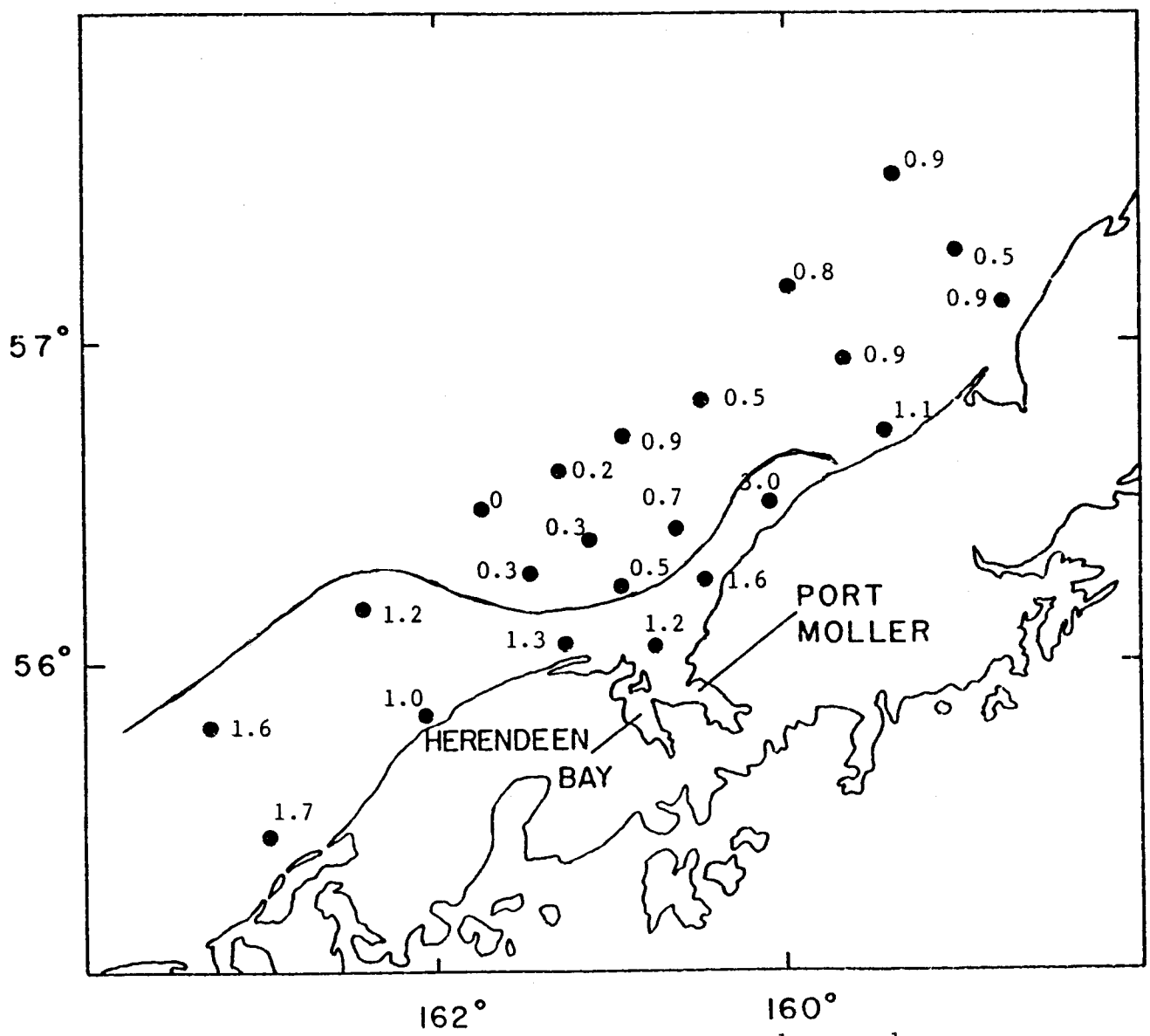

Figure 12. Methane oxidation rates in nl x liter^{-1} x day^{-1} observed in surface water samples from the May 1981 cruise.

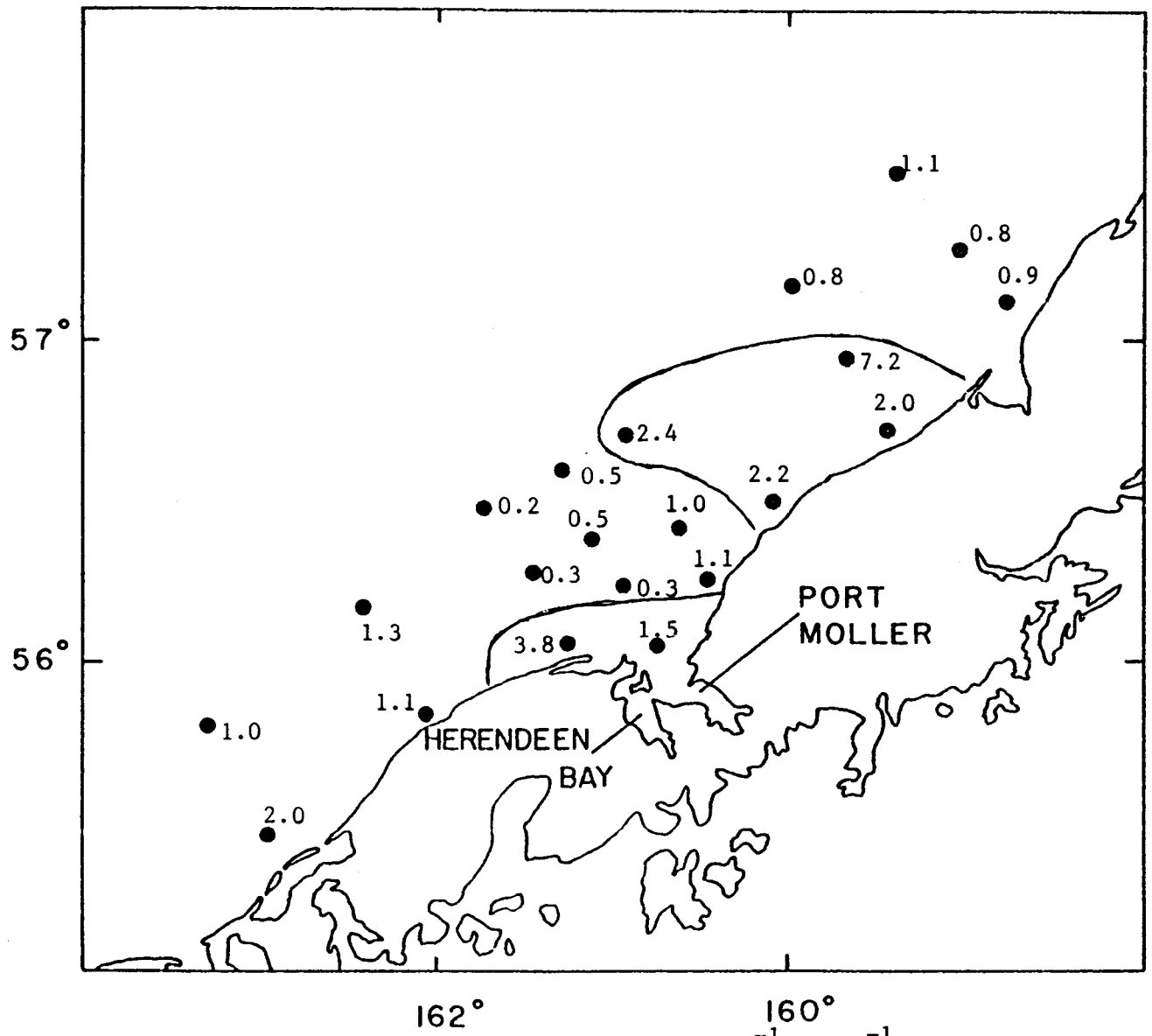

Figure 13. Methane oxidation rates in nl x liter^{-1} x day^{-1} observed in bottom water samples from the May 1981 cruise.

2. Port Moller

During the August cruise, we were able to take samples only near the mouth of Port Moller. The methane oxidation rates observed were generally higher than the mean methane oxidation rate observed in the North Aleutian Shelf samples but they were not high relative to those found in the stations near Port Moller (Fig. 14). During the January and May cruises, we were able to sample more intensively in Port Moller. The methane oxidation rates observed there were greater than those observed at any other location (Figs. 15 to 18). The highest rates were observed at the head of Port Moller and Herendeen Bay during January. The rates observed in the samples collected near the mouth were lower in January than the rates observed in the same general area during the August and May.

3. St. George Basin

In the St. George Basin (SGB), we observed the same trend that was seen in the North Aleutian Shelf region. The methane oxidation rates in the bottom waters were higher than those observed in surface waters. During the August cruise the mean methane oxidation rates in the surface and bottom waters were 1.1 and 4.4 nl x liter^{-1} x day^{-1} respectively. During the January cruise, the rates for surface and bottom waters were 0.1 and 2.0 nl x liter^{-1} x day^{-1} respectively. During the May cruise, the rates for surface and bottom waters was 2.0 and 4.5 nl x liter^{-1} x day^{-1} respectively (Figs. 19 and 20). The significance of these differences was $p = 0.07$, 0.001 and 0.001, respectively for August, January, and May.

In May, we also measured methane oxidation throughout the water column on four occasions at three locations (Fig. 21). In all four cases, there was a minimum methane oxidation rate observed in the

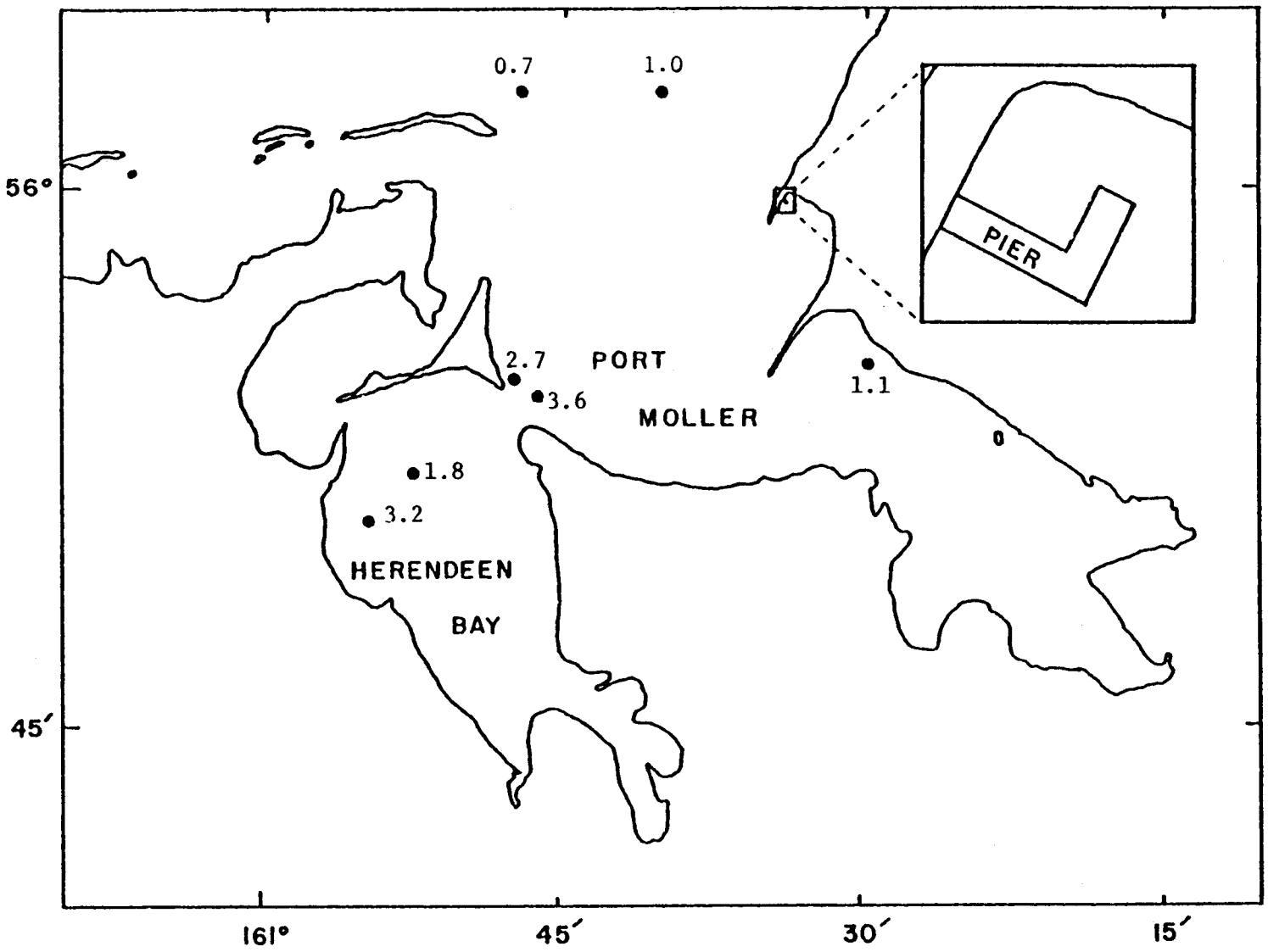

Figure 14. Methane oxidation rates in nl x liter^{-1} x day^{-1} for water samples collected in Port Moller during the August 1981 cruise.

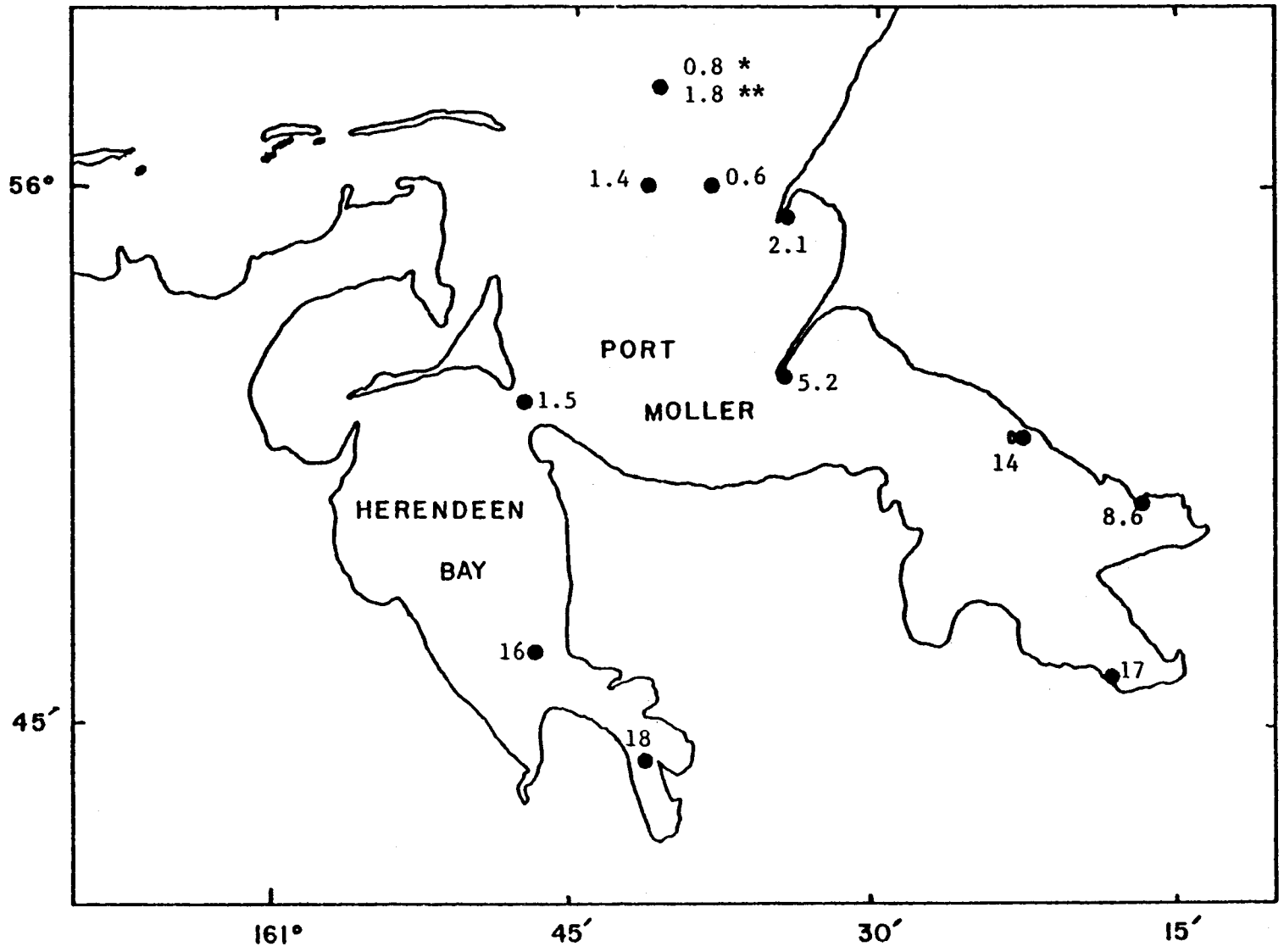

Figure 15. Methane oxidation rates in nl x liter^{-1} x day^{-1} for surface waters in Port Moller during the January 1981 cruise. * Denotes rate at high slack water; ** rate at low slack water.

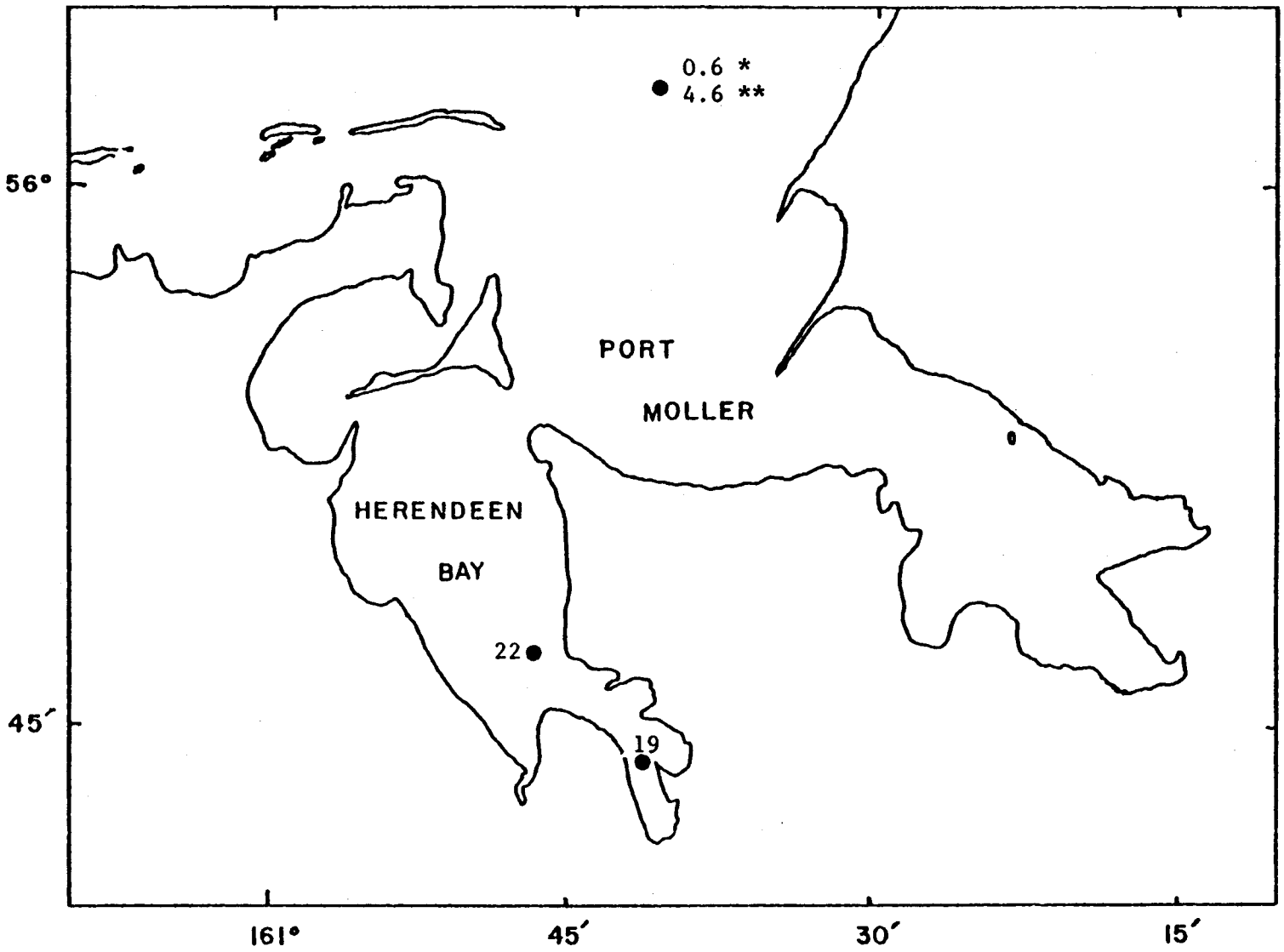

Figure 16. Methane oxidation rates in nl x liter^{-1} x day^{-1} for bottom waters in Port Moller during the January 1981 cruise. * Denotes rate at high tide, ** rate at low tide.

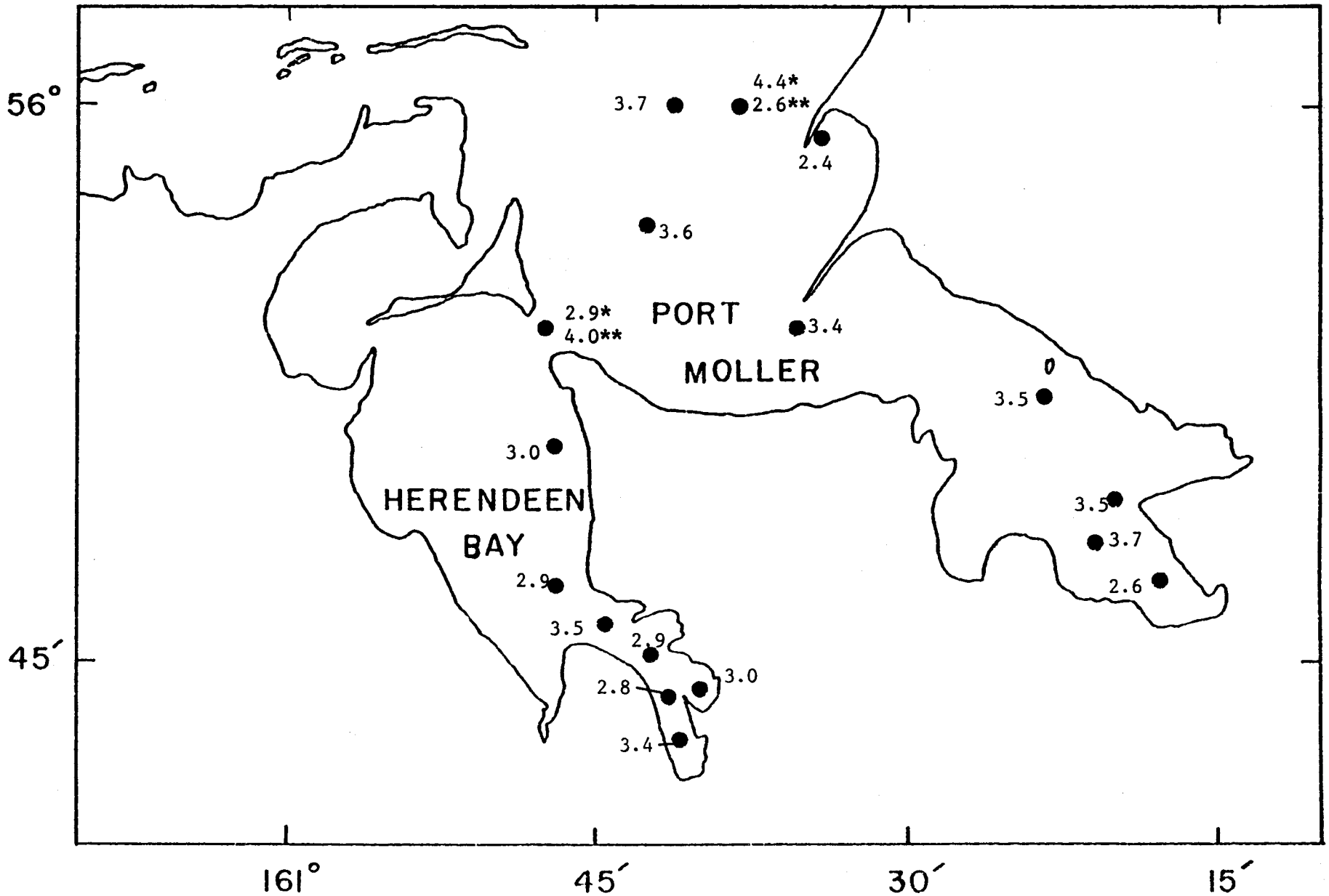

Figure 17. Methane oxidation rates in nl x liter^{-1} x day^{-1} for surface water samples from the May 1981 cruise. * Denotes rate at high tide; ** rate at low tide.

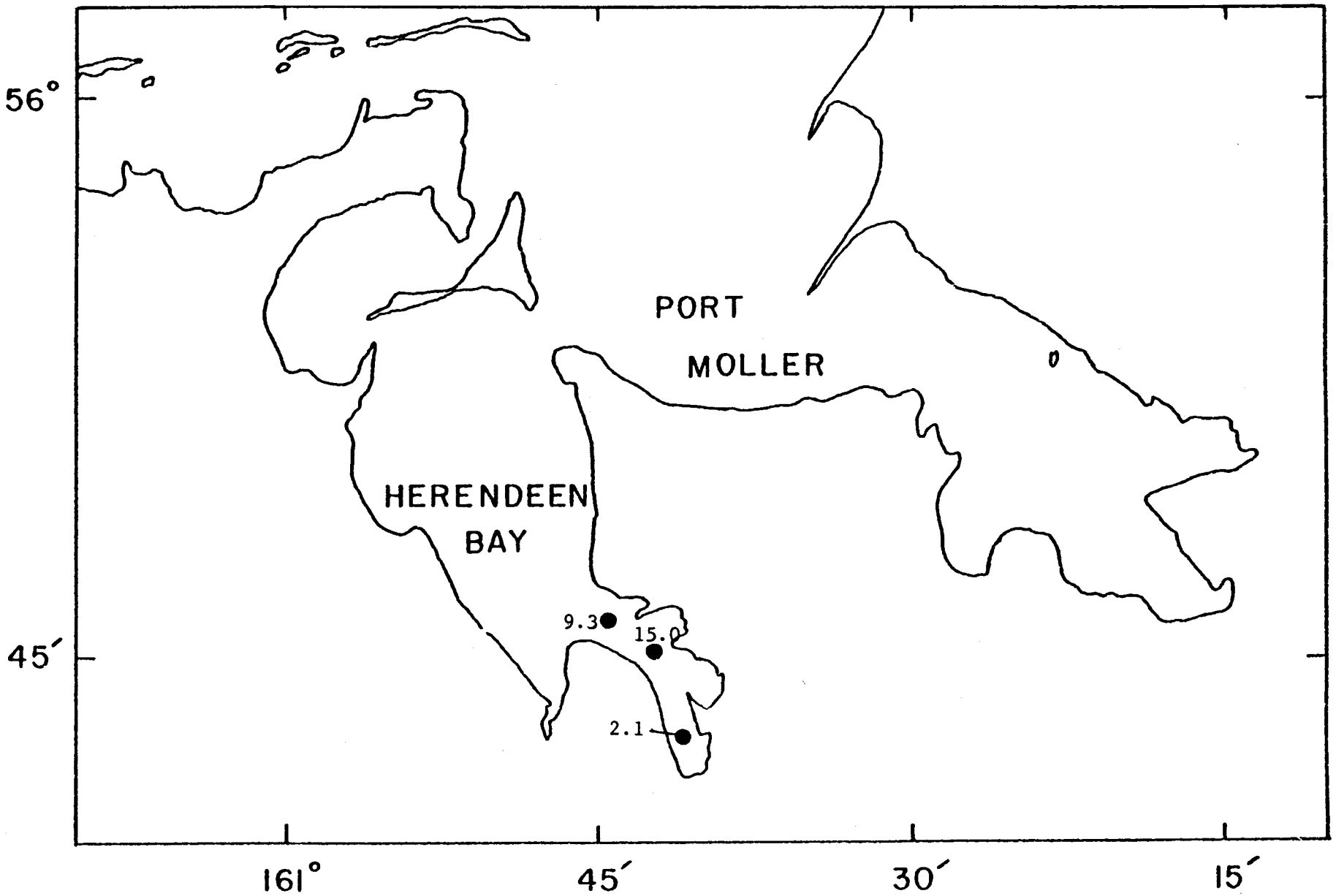

Figure 18. Methane oxidation rates in nl x liter^{-1} x day^{-1} observed in bottom waters during the May 1981 cruise.

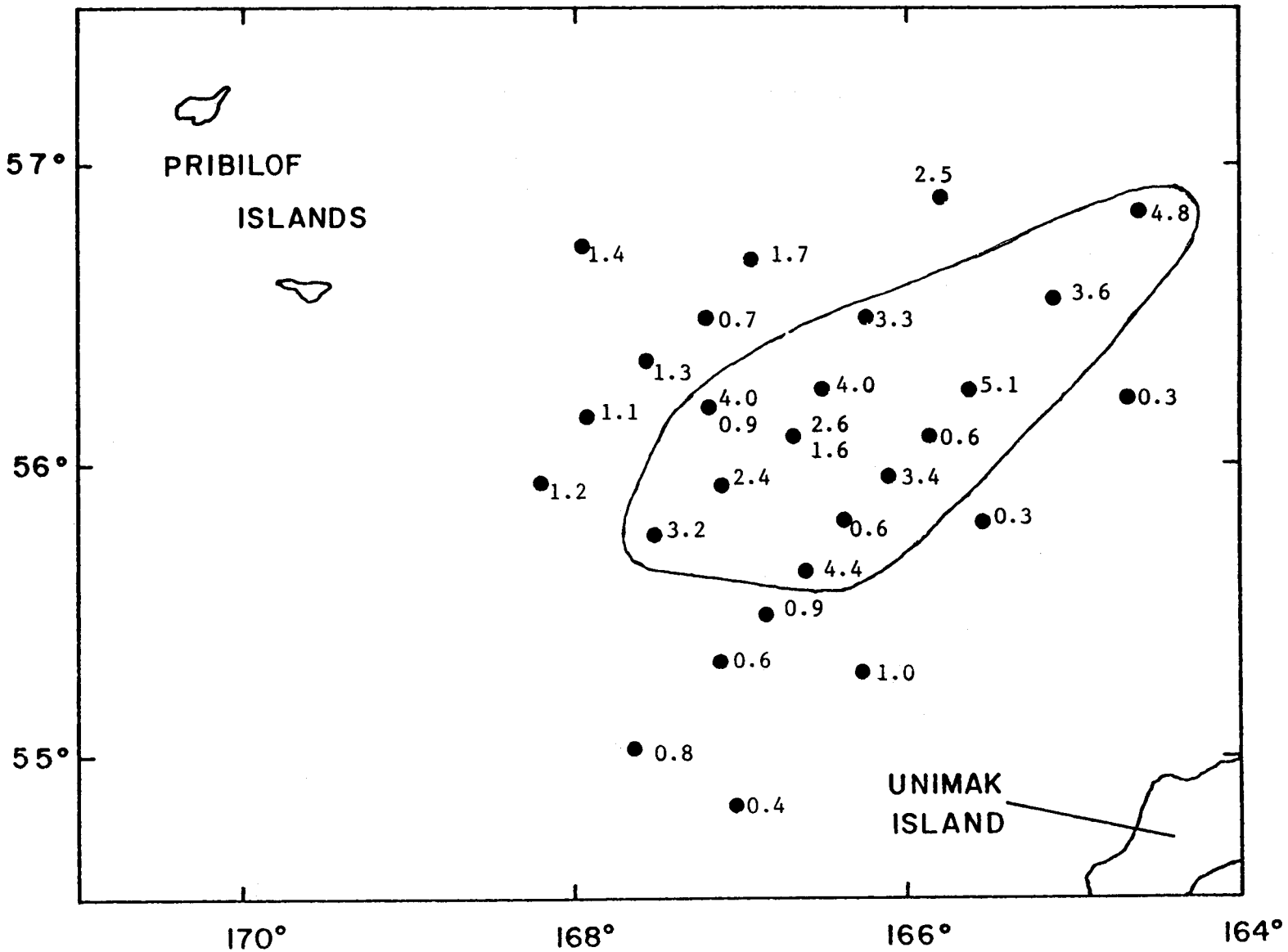

Figure 19. Methane oxidation rates in nl x liter^{-1} x day^{-1} for surface waters from the May 1981 cruise.

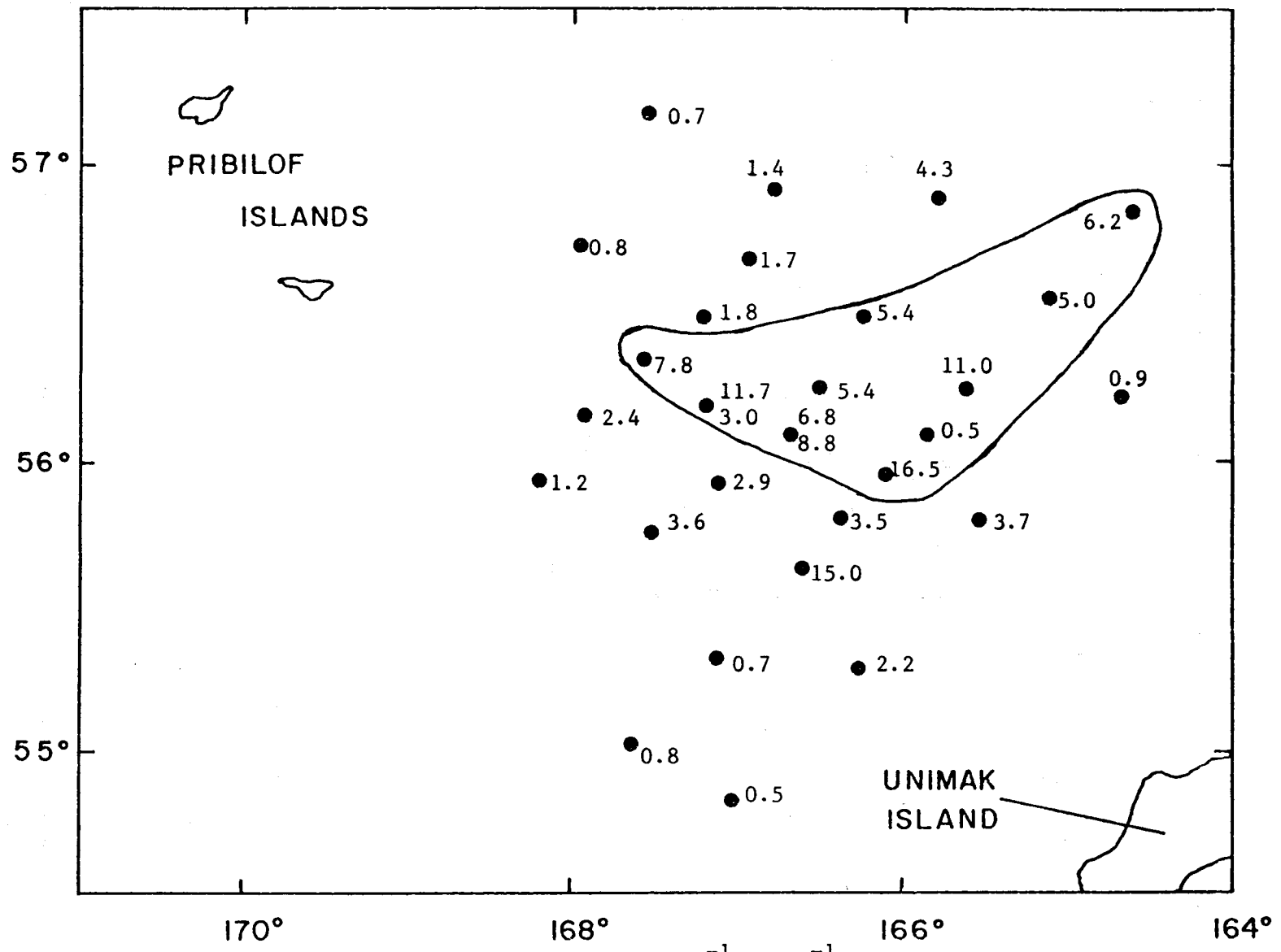

Figure 20. Methane oxidation rates in nl x liter^{-1} x day^{-1} in bottom water samples from the May 1981 cruise.

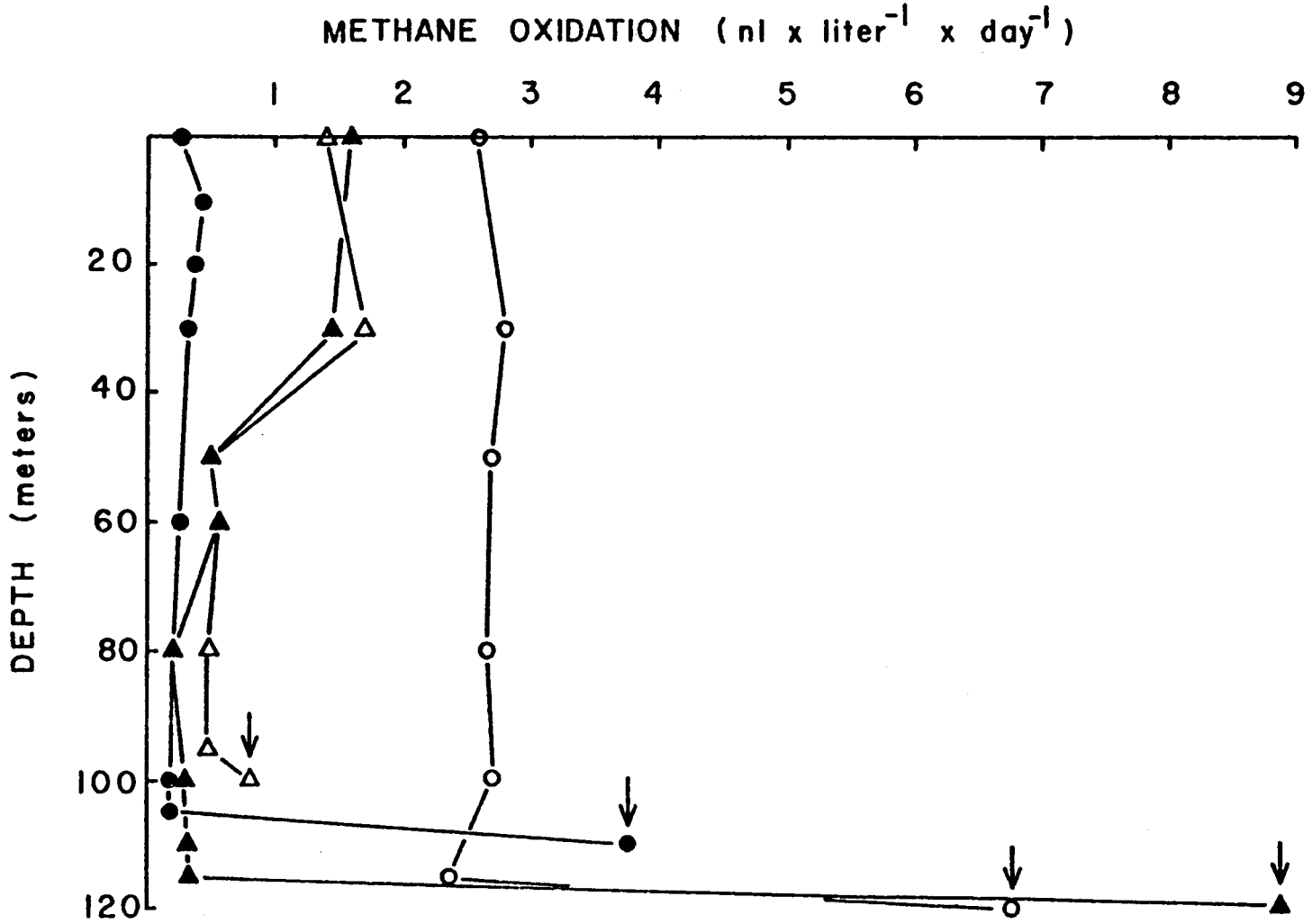

Figure 21. Methane oxidation in waters at decreasing depth. Arrows indicate bottom waters. ● = SG67, ○ = SG5-1, △ = SG22, ▲ = SG5-2.

samples taken five meters from the bottom. There was also an interesting trend observed in two of the water columns analyzed. At station SG22 and once at station SG5, the methane oxidation rates in the top 30 meters were greater than that observed further down the water column. This was an unexpected finding since, during past cruises, the rate observed at the surface was usually the same as that observed at five meters from the bottom. During the May cruise, we measured methane oxidation rates in the surface, bottom and bottom minus five meter (B-5 m) water samples on 23 occasions. In 19 of these water columns, the methane oxidation rates observed in the sample taken five meters from the bottom were lower than those taken either on the surface or at the bottom (Figs. 19, 20 and 22). This difference was significant at the $p < 0.001$ level for both comparisons. This difference is also reflected in the mean methane oxidation rates for samples collected at the surface, bottom and B-5 m which were 2.0, 4.5, and 1.4 nl x liter^{-1} x day^{-1} respectively.

During the May cruise the methane oxidation data also suggest that microbial function may alter substantially at a given location after a realtively short time interval. This is graphically illustrated in Fig. 21 where different methane oxidation patterns were observed at station SG5 after an interval of 3 days. At station SG 70, the methane oxidation rate in the bottom waters decreased from 11.7 to 3.0 nl x liter^{-1} x day^{-1} in the same time period. The even numbered PL (PROBES line) stations were sampled on May 17 and 18. The methane oxidation rates observed in the surface waters were 0.6, 4.4, 3.4, and 5.1 nl x liter^{-1} x day^{-1} respectively for stations 4, 6, 8 and 10. Six days later, stations 5, 7, and 9 were sampled and the observed surface water methane

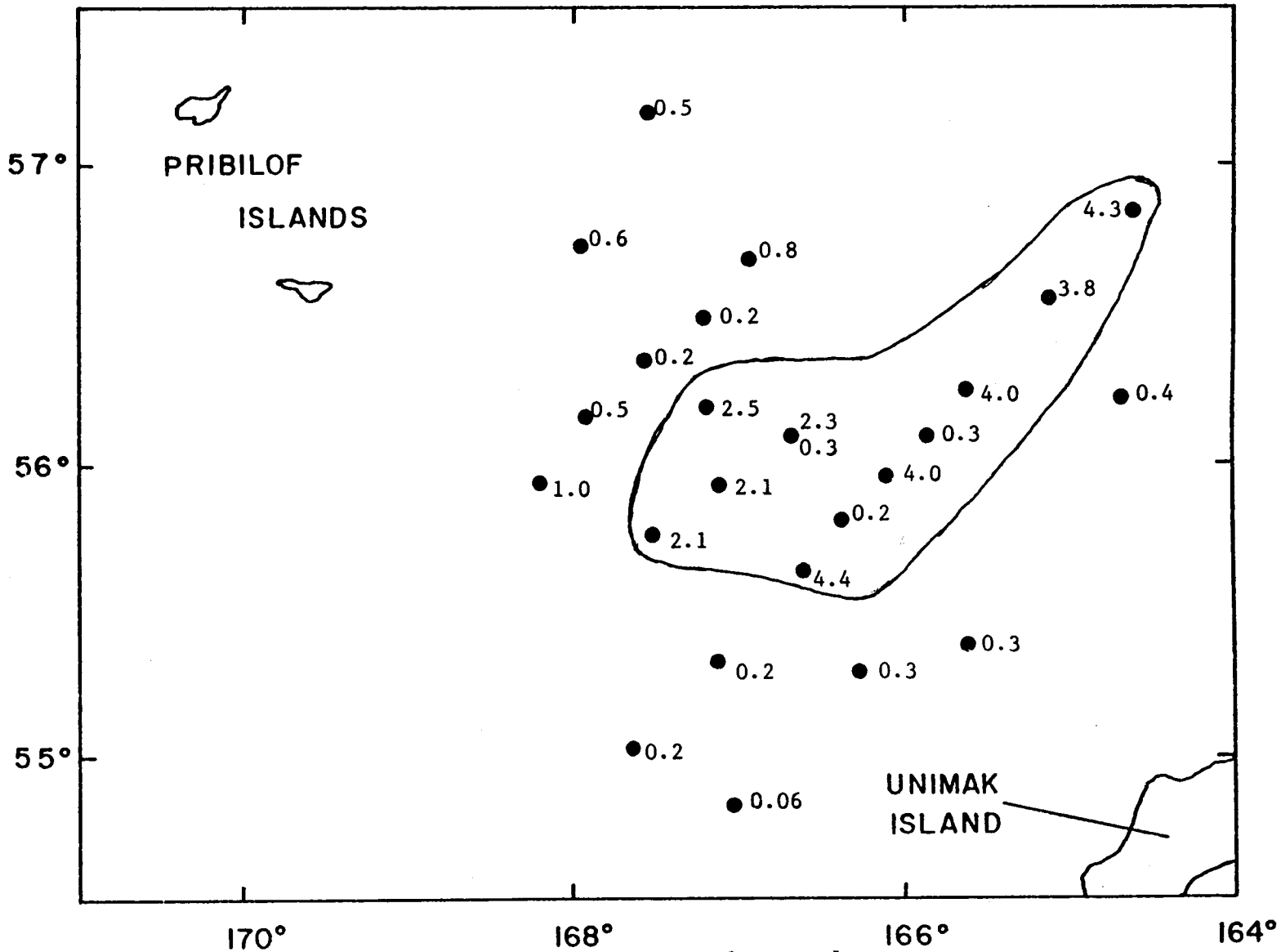

Figure 22. Methane oxidation rates in nl x liter^{-1} x day^{-1} for B-5 waters during the May 1981 cruise.

oxidation rates were 0.9, 0.6, and 0.16 nl x liter^{-1} x day^{-1} respectively. A similar trend was observed in the bottom water samples.

There was not a consistent pattern to the geographical distribution of methane oxidation rates in the surface water samples collected during the August and January cruises in SGB. During the May cruise, elevated methane oxidation rates were observed in the center of the study area (the encircled region on Fig. 19). In the bottom sediments, elevated methane oxidation rates were observed in approximately the same area during all three cruises. This pattern is most clearly defined in the May data set (Fig. 20). During this cruise, the highest rates were observed in a set of samples collected in the center of the sample grid (encircled area).

4. Seasonal differences

When the methane oxidation rates observed in water samples collected from the same areas are compared seasonally, there were many differences noted. The mean methane oxidation rate in bottom water samples collected along the NAS dropped from 2.2 nl x liter^{-1} x day^{-1} in August to 1.3 in January (a 41% reduction). The mean rate then increased slightly to 1.5 nl x liter^{-1} x day^{-1} in May. When the surface water samples from the same area were compared seasonally, there was a 73% reduction from August to January. A similar trend was noted in the surface waters of the North Aleutian Shelf where the mean methane oxidation rates were 1.5, 0.4, and 1.0 nl x liter^{-1} x day^{-1} for August, May and January respectively.

In the SGB, a seasonal change was also observed. The mean methane oxidation rate observed in the surface waters was 1.1, 0.1, and 2.0 nl x liter^{-1} x day^{-1} respectively for the August, January,

and May cruises. The bottom waters showed mean rates of 4.4, 2.0, and 4.5 nl x liter^{-1} x day^{-1} for the same cruises. All of the seasonal differences observed in the NAS area were significant; of these comparisons, the highest p value was 0.01. In the SGB area, the seasonal differences in the surface waters were highly significant but those observed in the bottom waters were not statistically significant.

5. Methane oxidation rates in sediments

During the May cruise, the methane oxidation rates were measured in both bottom waters and sediments at seven locations (Table 2). When these rates are compared on a equal volume basis, it is evident that the observed methane oxidation rates are much greater in the sediments. The most active waters showed rates that were only 7% of that found in the sediments.

Table 2. The comparison of methane oxidation rates measured at one methane concentration in bottom water and sediment samples collected at the same location. The units used in this comparison are nl x l^{-1} x day^{-1}.

Station Number	Methane Oxidation Rate Sediment	Water	Percent Activity in Water Compared to Sediment
SG67	150	4	3
PL6	210	15	7
PL8	260	17	7
SG15	10	0.7	7
U	5,240	3	0.05
A	130	3	2
B	490	3	0.6

B. Methanogenesis

1. Methanogenesis in water

Methanogenesis rates were measured in 10 water samples during the August cruise. Even with incubation times of up to 14 days, no detectable methane production was observed. The theoretical lower limit of detection in this system was 0.25 nl x liter^{-1} x day^{-1}. The samples were collected from the following stations: NA4A, NA22, NA40, SG7, UP3, PM3, PL4, PL6, PL14 and the pier at Port Moller.

During the May cruise, methanogenesis in water samples was again assayed in seven water samples (Table 3). In these samples, incubation times of up to 22 days were used. The rates observed ranged from 0 to 95 nl x liter^{-1} x day^{-1}.

Table 3. Methanogenesis in water samples collected during the May cruise.

Station Number	Methanogenesis nl x liter^{-1} x day^{-1}
PL4	95
SG70	0
SG5	41
PL7	0
PL9	27
PL8	68
SG70	14

2. Methanogenesis in sediments

During the August cruise, methane production rates were measured in 11 sediments using method #2 (gas chromatographic analysis of headspace). The results of these measurements are shown in Figs. 23 and 24. The highest methane production rates were observed in the SGB at stations PL8 and PL14. High rates were also observed

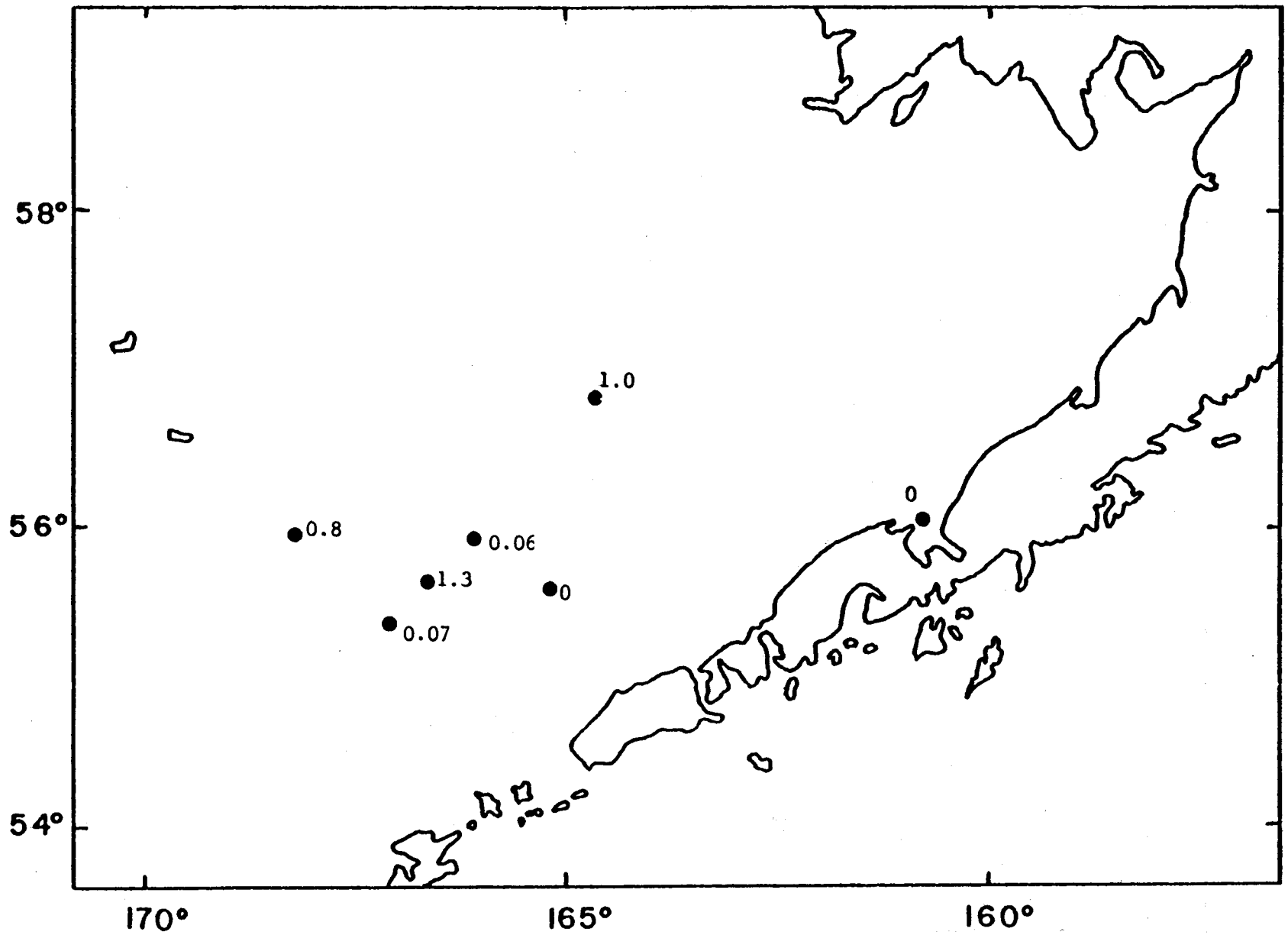

Figure 23. Methane production rates in ml x m^{-2} x day^{-1} for top 10 cm of sediment from August 1980 cruise.

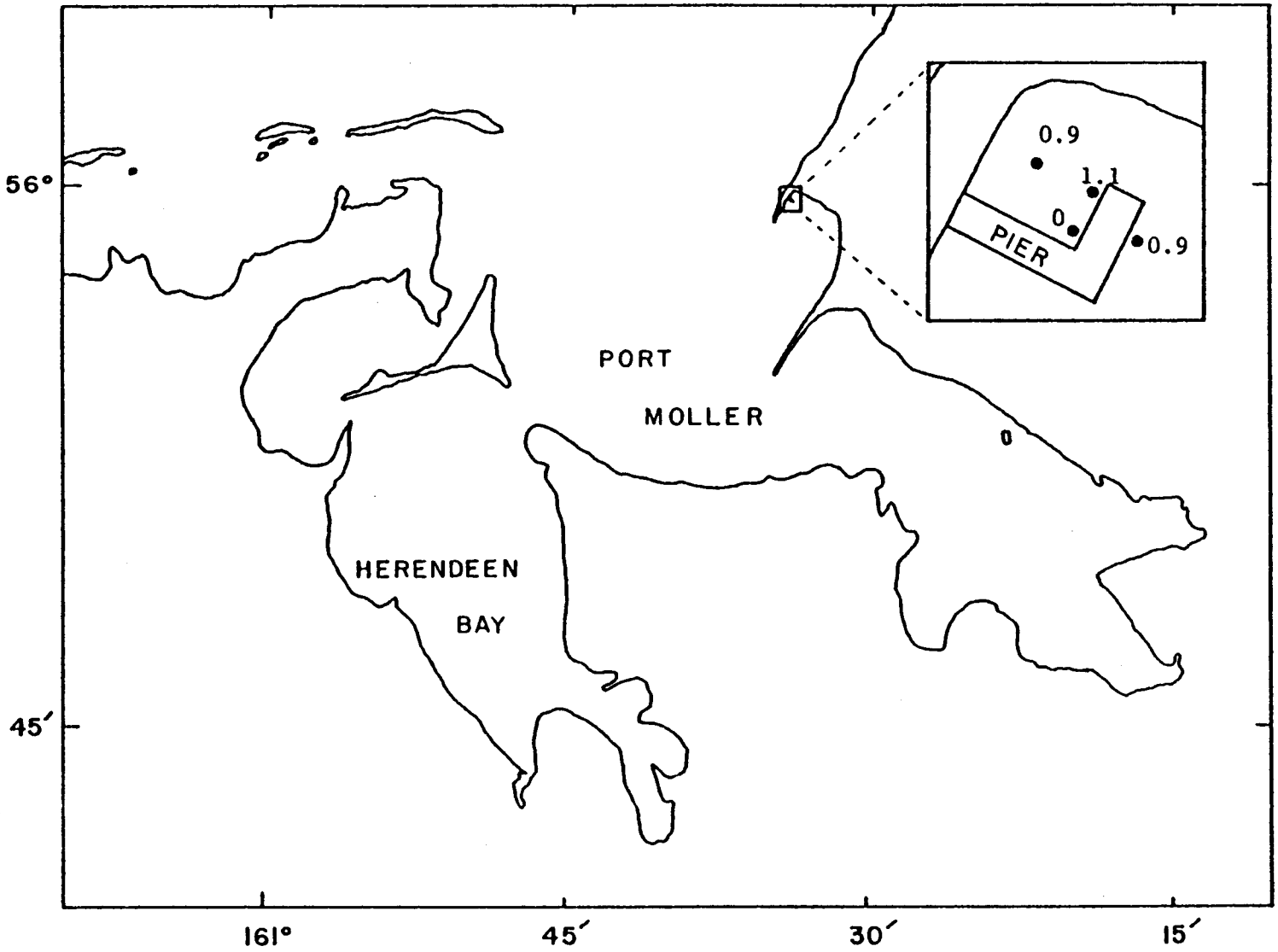

Figure 24. Methane production rates in ml x m^{-2} x day^{-1} for top 10 cm in Port Moller from August 1980 cruise.

in the sediments near the Port Moller cannery pier. During this cruise, methane production rates were measured in 19 sediment samples using method #1 (radioactive tracers). No methane production was observed in any of the samples tested even though we know that some of these sediments were producing methane as determined by method #2.

During the January NASTE cruise, methanogenesis rates were measured in 35 sediments using method #2 (Figs. 25 and 26). In Herendeen Bay we were able to collect samples in an area that we were not able to sample during the previous cruise. At the head of this bay, we observed methane production rates much greater than that observed anywhere else during either cruise. The mean production rate for all other samples analyzed was 0.08 ml x m^{-2} x day^{-1}. The rate observed in the sediment collected at the head of Herendeen Bay (49 ml x m^{-2} x day^{-1}) was over 600 times that rate. A high methanogenesis rate was also observed in the other sediment collected in this bay and in the sediment collected at the cannery pier. The rates observed in the other sediments analyzed from the NAS were very close to the detection limits of the technique used (ca. 0.01 ml x m^{-2} x day^{-1}). In the SGB, there were three areas where the methanogenesis rates were 0.1 ml x m^{-2} x day^{-1} or greater (enclosed areas in Fig. 25). The highest rate observed was that in the sediment collected at station PL8 (0.24 ml x m^{-2} x day^{-1}).

During the May cruise, methanogenesis rates were analyzed in 42 sediments using method #2 (Figures 27 and 28). The mean methane production rate in the SGB was 0.14 ml x m^{-2} x day^{-1} based on an active methane production depth of 10 cm. In 10 sediments, both the top and bottom 5 cm of the 10 cm sediment cores were analyzed.

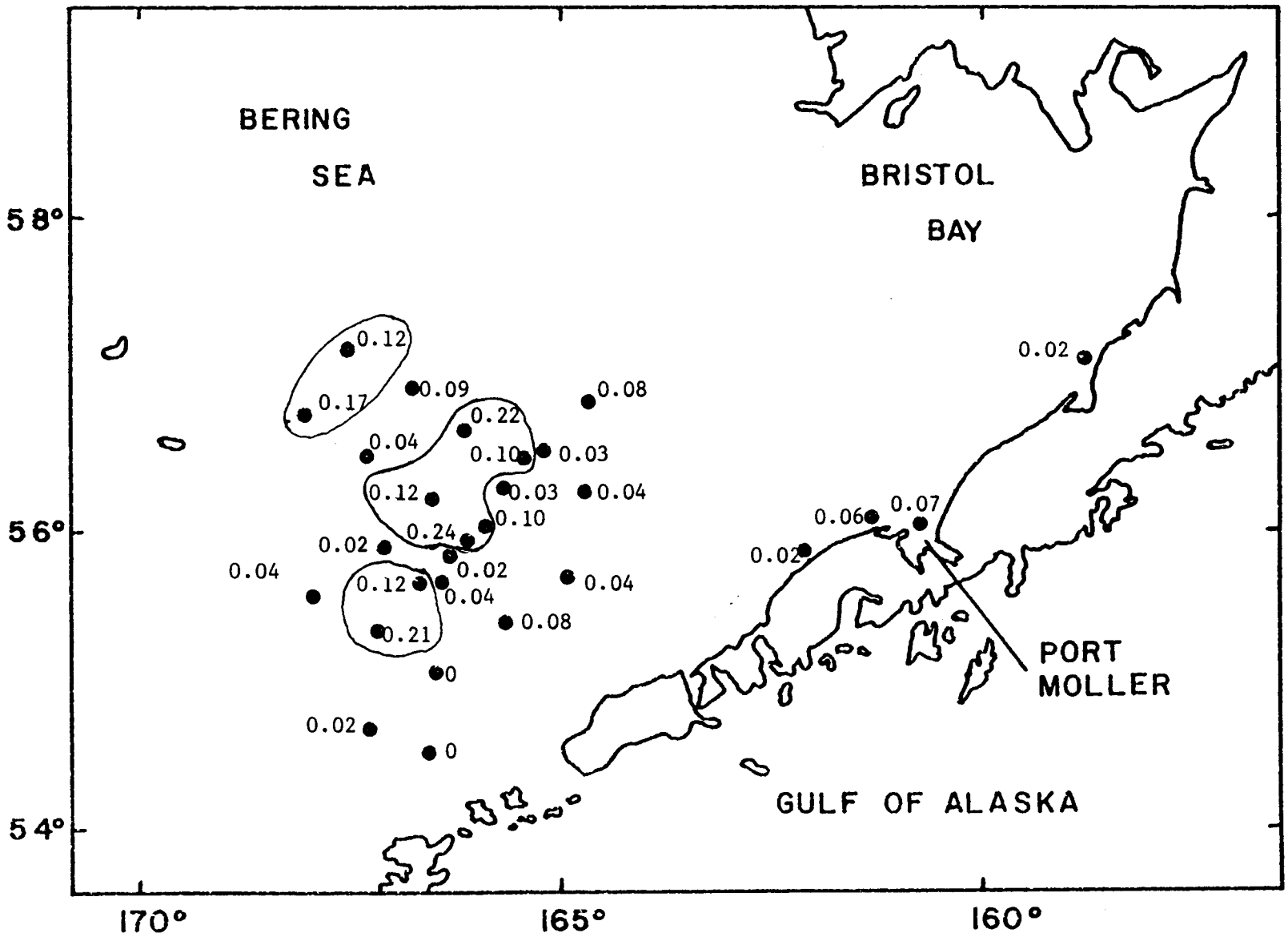

Figure 25. Methane production rates in ml x m^{-2} x day^{-1} observed in top 10 cm of sediment from January 1981 cruise (excluding Port Moller).

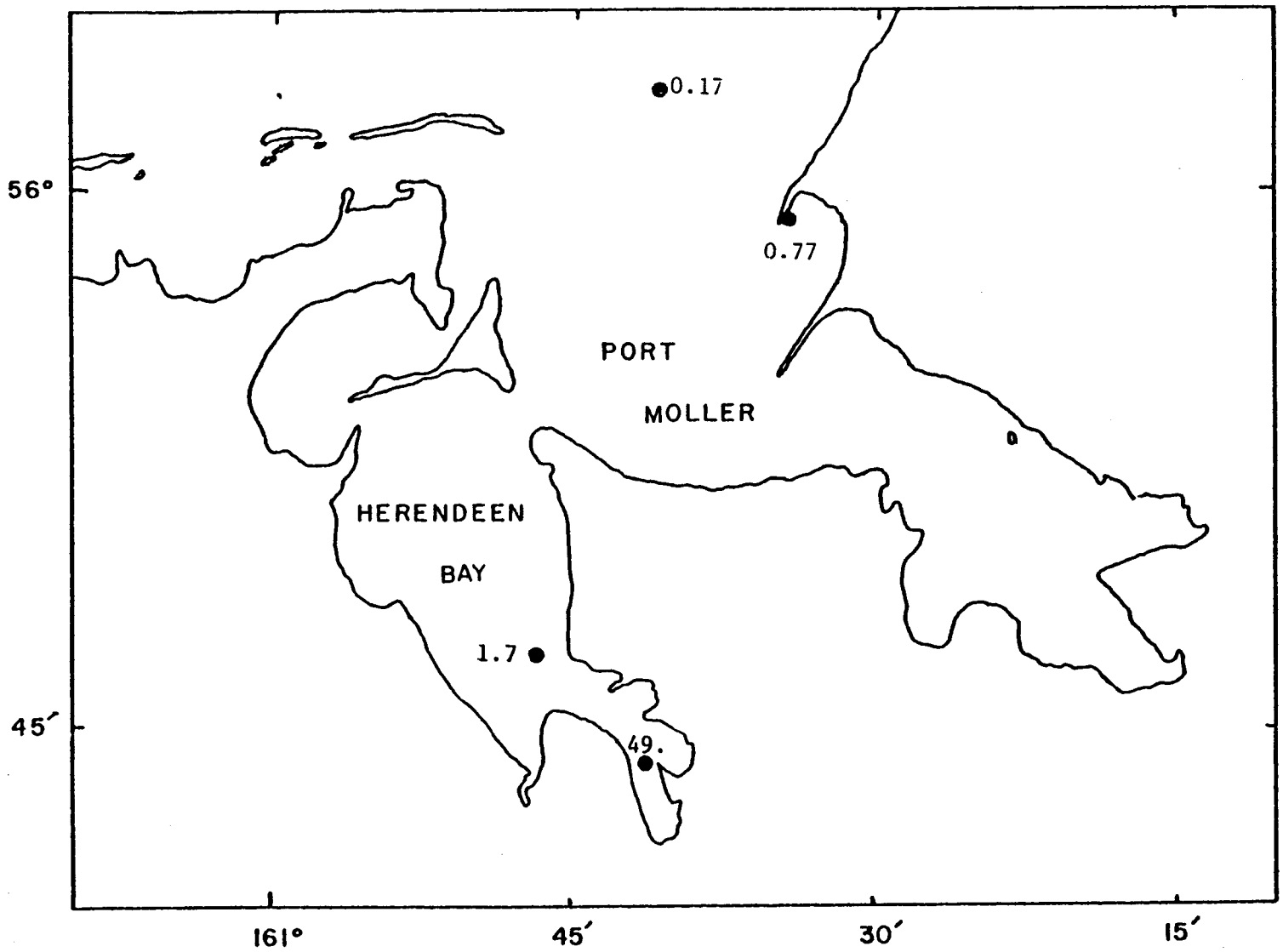

Figure 26. Methane production rates in ml x m^{-2} x day^{-1} observed in top 10 cm of sediment during January 1981 cruise.

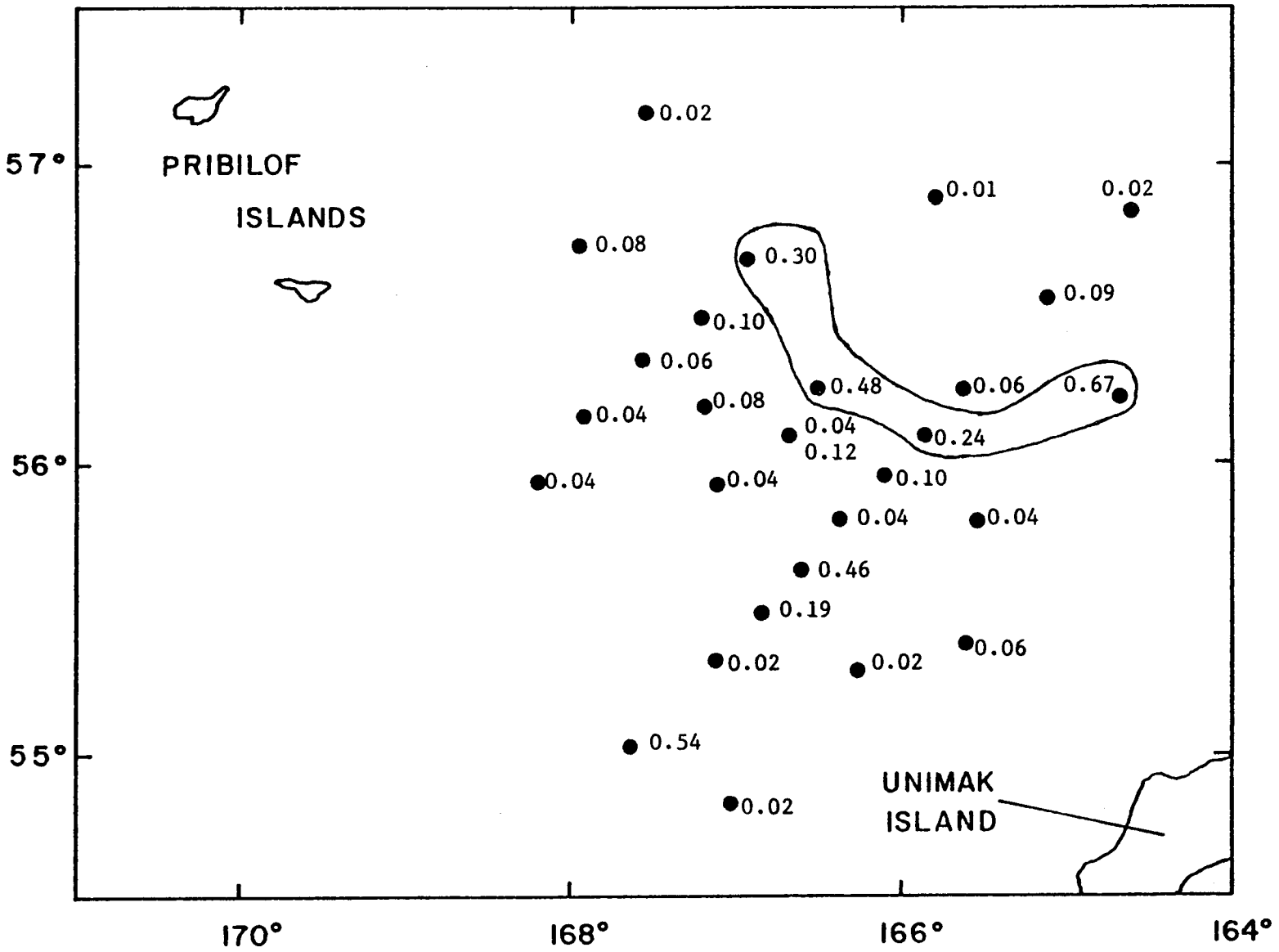

Figure 27. Methane production rates in ml x m^{-2} x day^{-1} for top 10 cm of sediment from the May 1981 cruise.

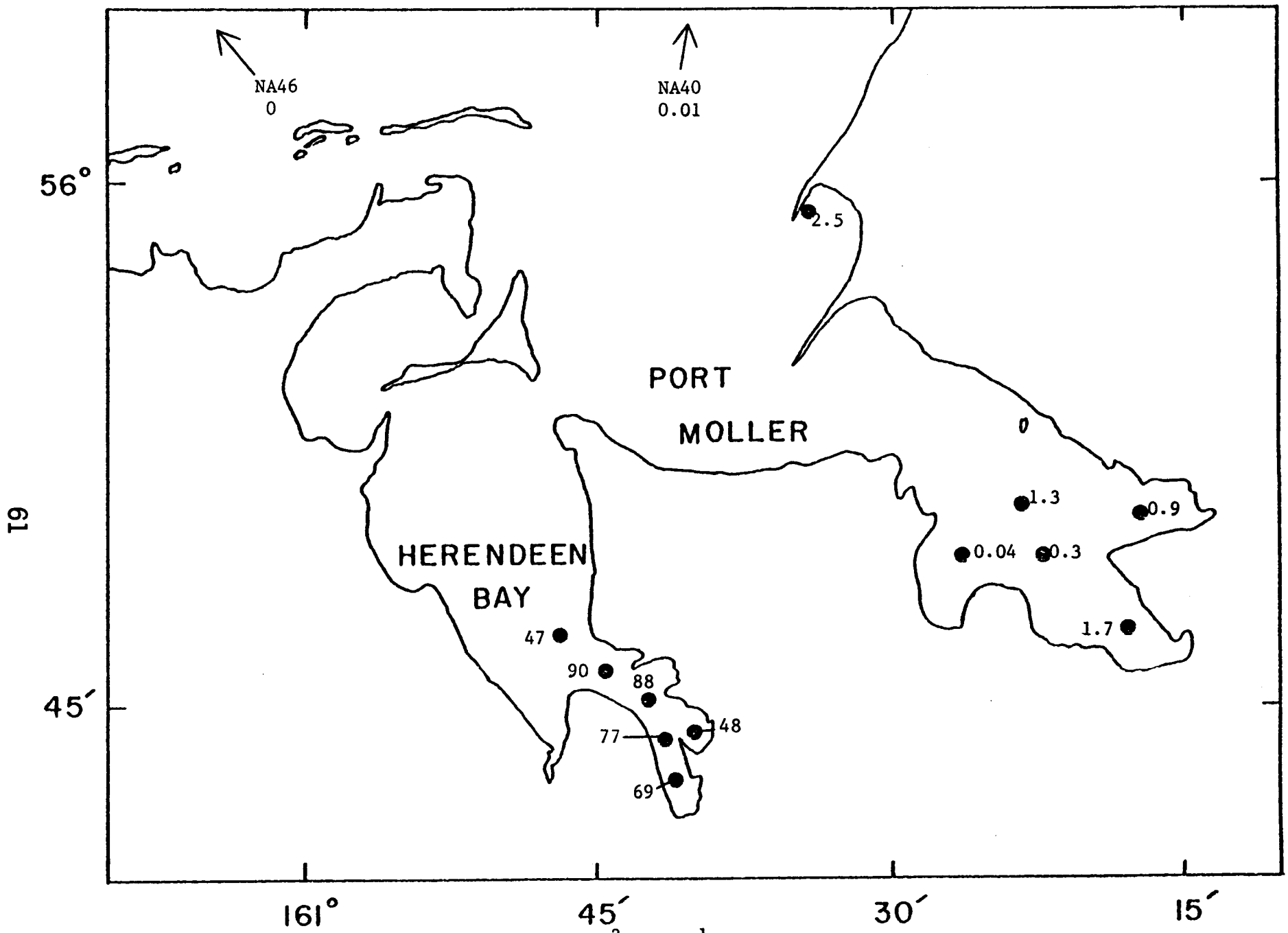

Figure 28. Methane production rates in ml x m^{-2} x day^{-1} for top 10 cm of sediment from the May 1981 cruise.

These values were compared and although the mean value in the bottom 5 cm was slightly higher (0.07 as compared to 0.05 ml x m^{-2} x day^{-1} found in the top 5 cm.), this difference was not statistically significant. In the remaining 32 sediments, methane production rates were observed only in the top 5 cm and the results multiplied by 2 to give the rate for the 10 cm core. In the SGB, there was an area which showed elevated methane production (illustrated by the enclosed area on Fig. 27). The enclosed area represents the region where methane production rates were $\geq$ the mean. As was the case during the January cruise, the highest methane production rates were observed in the Port Moller area (Fig. 28). In this region, the highest rates were found at the head of Herendeen Bay.

Table 3. Methane production rates in 7 sediment samples collected at the same locations during the August and January cruises. The units are ml x m^{-2} x day^{-1}. These values were calculated assuming an active methane production depth of 10 cm.

Location	August	January
PL14	1.0	0.8
PL8	0.6	1.0
PL6	1.3	1.2
PL4	0.4	2.1
SG7/SG26/SG27	0	0.04
NA40	0	0.07
Cannery Pier	1.1	0.8
Mean (all samples)	0.6	0.9

There were 7 stations where methane production rates were measured in sediments collected during both the August and January cruises (Table 3).

With the exception of the sediments collected at station PL4, there is good agreement between the rates observed during the August and January cruises indicating little seasonal change between these two sample periods. This also shows that this technique produces methanogenesis rates which are reproducible from one sampling period to the next. The observed rates can thus be considered internally consistant. When a linear regression analysis is performed on these data, the correlation coefficient is high when the PL4 data are omitted from the analysis ($r = 0.9$; $r^2 = 0.8$; $p = 0.01$). The difference in the mean values observed between the two sets of data is not statistically significant.

Table 4. Methane production rates observed at 20 common locations during the January and May cruises. The units and method of calculation was the same as that described in Table 3.

Location	January	May
PL4	2.1	0.02
PL8	1.0	0.10
PL10	0.03	0.06
PL12	0.03	0.09
PL14	0.8	0.02
PL7	0.02	0.04
SG27/SG26	0.04	0.06
SG15	0.12	0.02
SG11	0.04	0.67
SG22	0.17	0.08
SG23	0.04	0.10
SG24	0.12	0.48
SG29	0.02	0.04
SG48/UP7	0.02	0.04
NA40	0.07	0.02
NA46	0.03	0
H (cannery pier)	0.8	2.5
A	49	77
B	1.7	47

Direct comparisons can also be made in methanogenesis rates observed at 20 common locations during the January and May cruises (Table 4). When all common stations except those in Port Moller are compared, the mean methanogenesis rate observed in January was higher than that observed in May (0.3 and 0.1 ml x meter^{-2} x day^{-1} respectively). This difference, however, was not statistically significant. When the observed rates were compared on a location by location basis, the correlation coefficient (r) was only 0.15. Thus, even though there was a good correlation between the August and January data sets, this was not the case when the January and May data sets were compared. When all SGB rates were compared seasonally, there was little difference in rates (0.08 and 0.1 ml x m^{-2} x day^{-1} for January and May respectively.

During the January cruise, the cores were taken with a Pamatmat multiple coring device (method #3). Cores were taken at four locations along the PROBES line (PL stations). The rates observed using this method were approximately 2 orders of magnitude lower than those observed at the same location as measured using method #2 (Table 5).

Table 5. Comparison of methane production rates as measured using methods #2 and #3. The units used in both sets of data are μl x m^{-2} x day^{-1}.

Location	Method #2	Method #3
PL6	120	0
PL8	240	0.05
PL10	30	0.14
PL12	30	0.16

C. Relative microbial activity in waters

During all cruises, we measured relative microbial activity in water samples using ^{14}C labeled glutamic acid. The trends

observed in relative microbial activity paralleled those observed in the methane oxidation data (Figs. 29 to 36). In the NAS area, the level of microbial activity in the offshore waters was lower than that observed in the waters collected from inshore locations. During the August cruise, the mean uptake rate in the inshore waters and offshore waters was 7.7 and 4.0 ng x liter^{-1} x hr^{-1} respectively in the surface waters and 81 and 10 ng x liter^{-1} x hr^{-1} respectively in the bottom waters (Figs. 29 and 30). The level of significance for these differences was $p = 0.07$ in the surface waters and $p = 0.12$ in the bottom waters.

In January, the same trend was noted. The mean uptake rates in the inshore and offshore waters were 10 and 1.5 ng x liter^{-1} x hr^{-1} respectively for surface waters and 42 and 7.4 respectively for the bottom waters (Figs. 31 and 32). The level of significance for these differences was $p = 0.01$ in the surface waters and $p = 0.11$ in the bottom waters. This trend was observed again in May where the mean uptake rates in the inshore and offshore waters were 15 and 7 ng x liter^{-1} x hr^{-1} respectively, in the surface waters and 35 and 28 ng x liter^{-1} x hr^{-1} respectively in the bottom waters (Figs. 33 and 34). The level of significance for the difference in the surface waters was $p = 0.014$ but the difference in the bottom samples was not significant.

There was also a pattern in the microbial activity in the bottom vs. the activity in the surface water samples. During the August cruise, the mean uptake rates in bottom and surface samples collected at the same locations were compared. The mean rates for surface and bottom waters were 8 and 65 ng x liter^{-1} x hr^{-1} ($p = 0.04$) respectively. During the January and May cruises, there

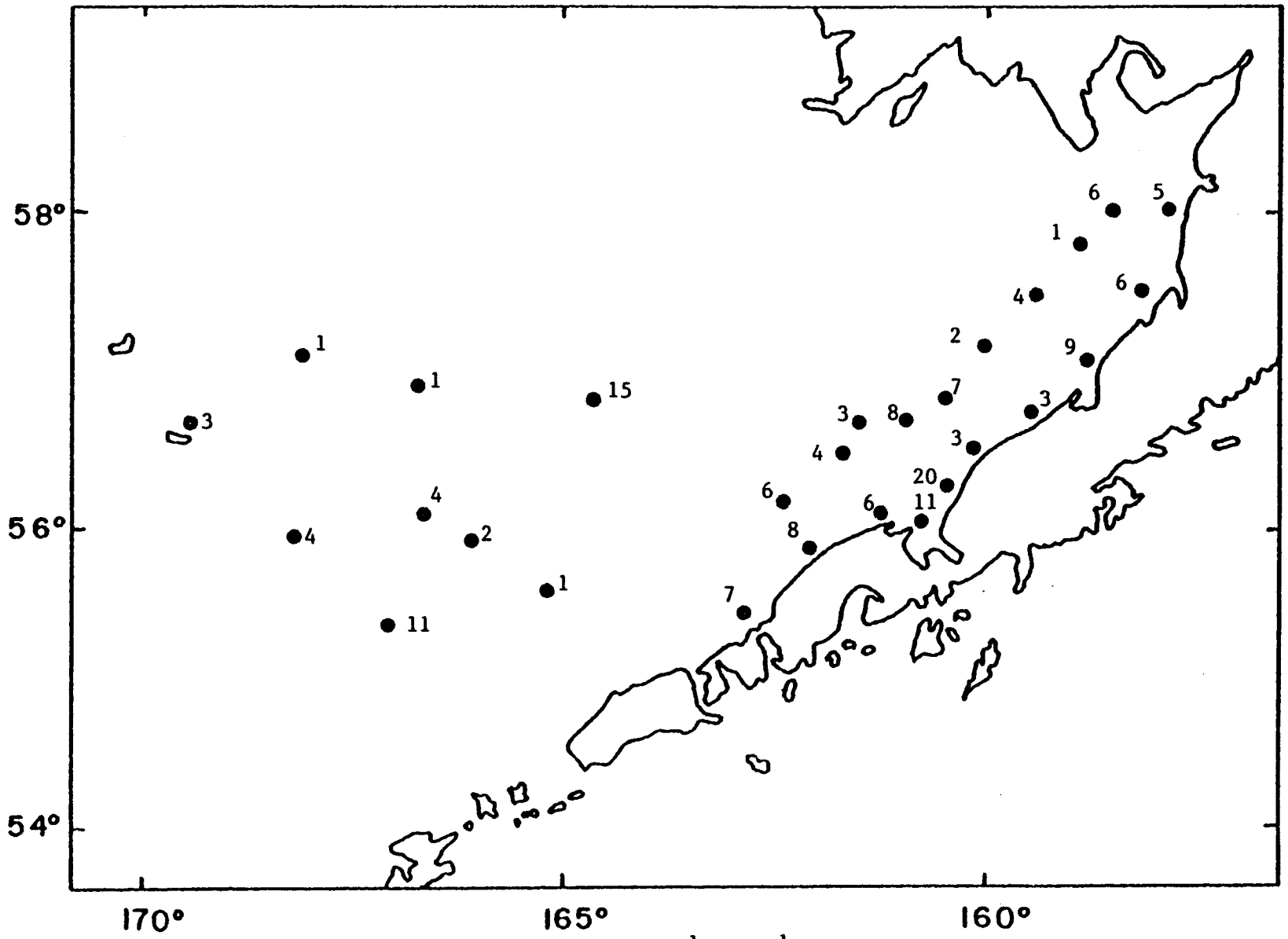

Figure 29. Glutamate uptake rates in ng x liter^{-1} x hr^{-1} in surface water samples observed during the August 1980 cruise.

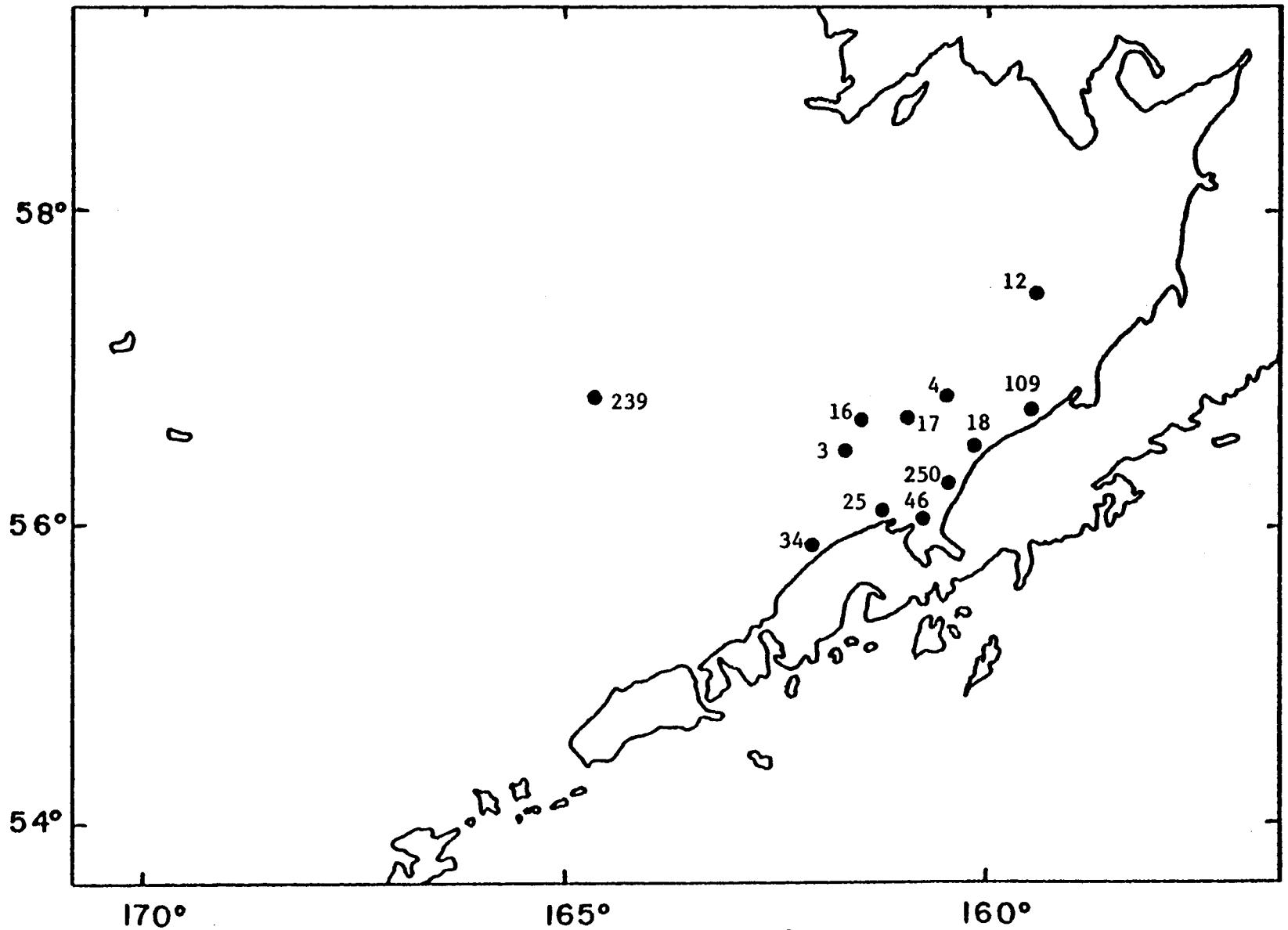

Figure 30. Glutamate uptake rates in ng x liter^{-1} x hr^{-1} in bottom water samples observed during the August 1980 cruise.

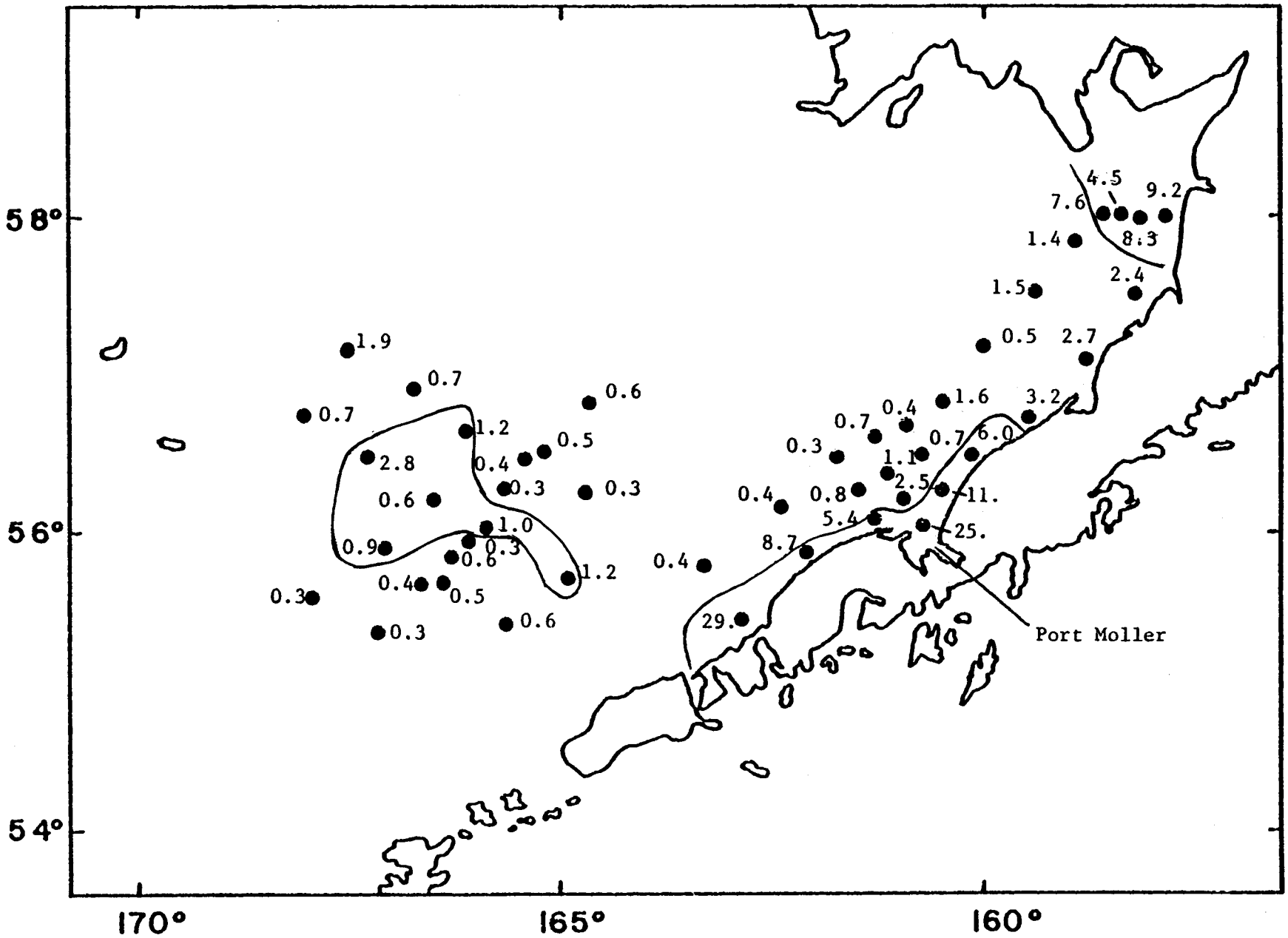

Figure 31. Glutamate uptake rates in ng x liter^{-1} x hr^{-1} from surface water samples during the January 1981 cruise.

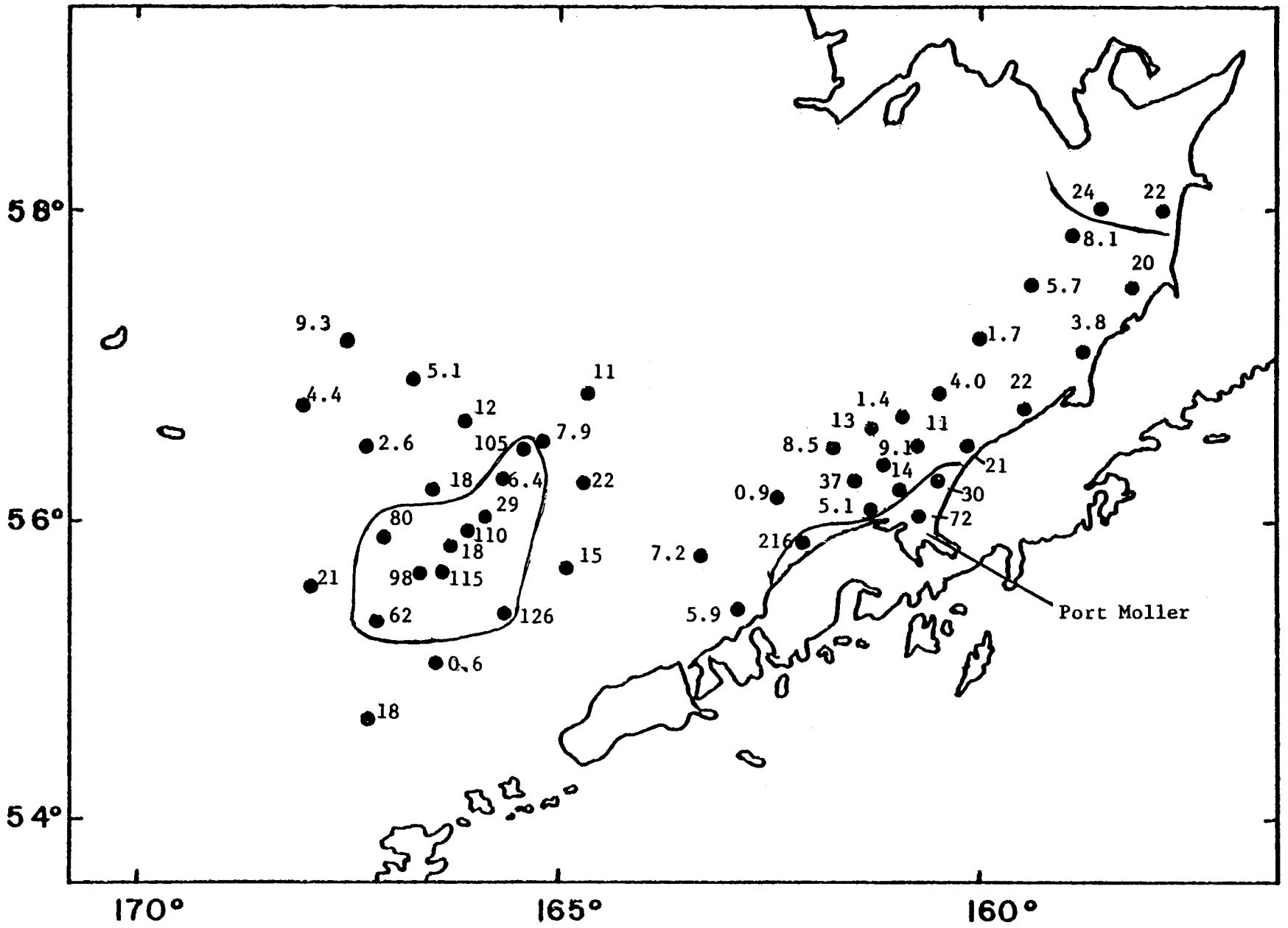

Figure 32. Glutamate uptake rates in ng x liter^{-1} x hr^{-1} from bottom water samples during the January 1981 cruise.

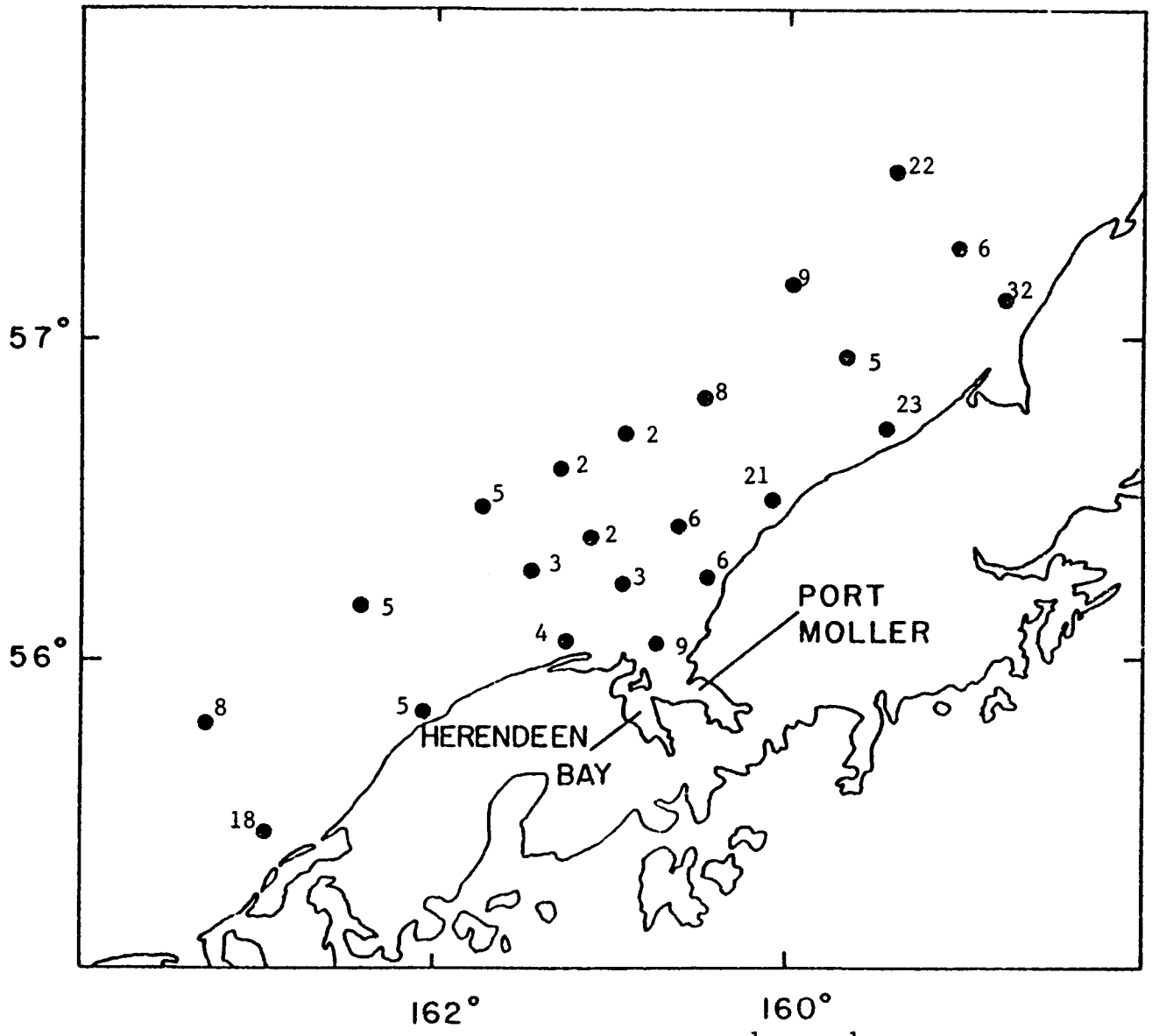

Figure 33. Glutamate uptake rates in ng x liter^{-1} x hr^{-1} in surface water samples observed during the May 1981 cruise.

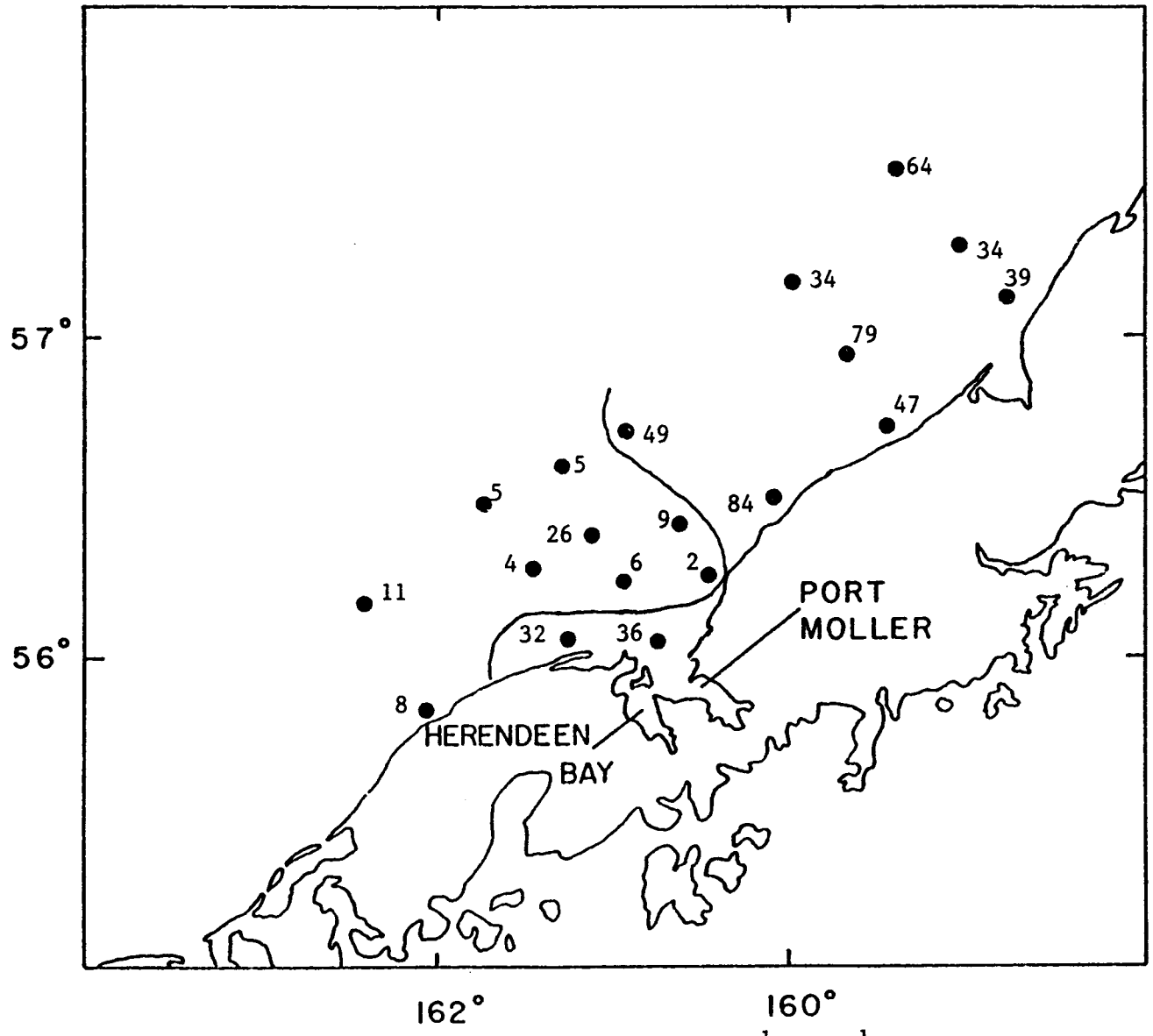

Figure 34. Glutamate uptake rates in ng x liter^{-1} x hr^{-1} in bottom water samples observed during the May 1981 cruise.

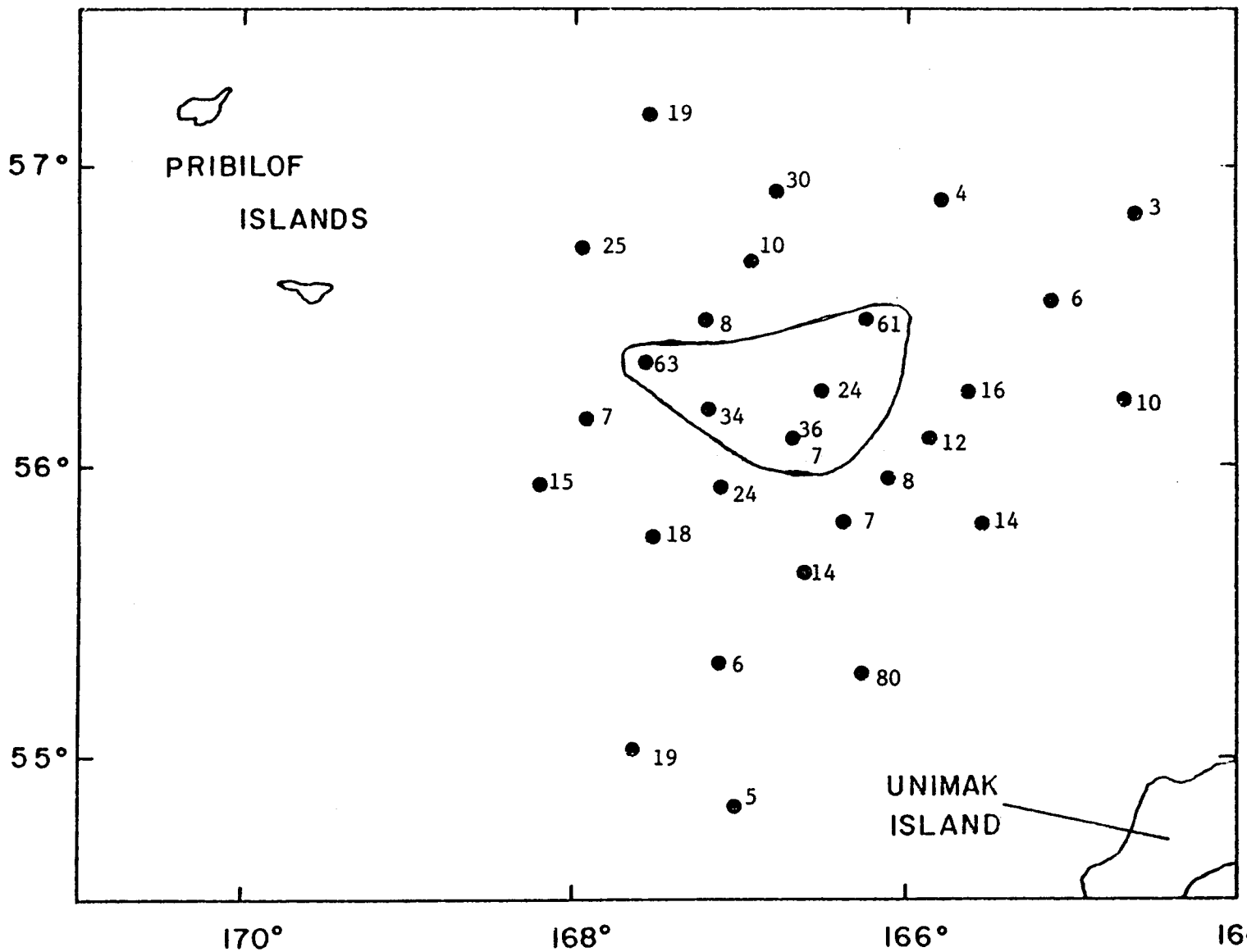

Figure 35. Glutamate uptake rates in ng x liter^{-1} x hr^{-1} in surface water samples observed during the May 1981 cruise.

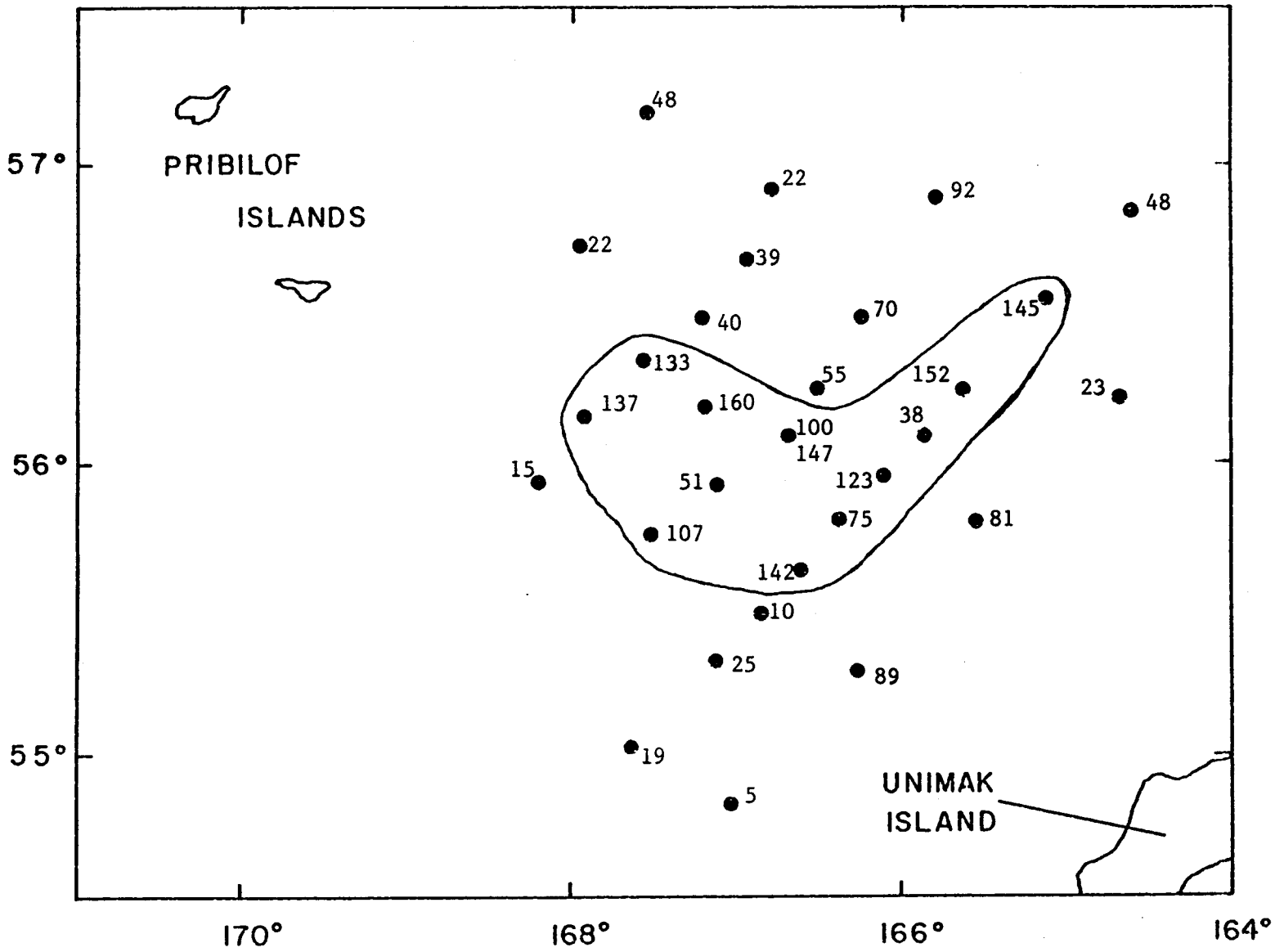

Figure 36. Glutamate uptake rates in ng x liter^{-1} x hr^{-1} in bottom water samples observed during the May 1981 cruise.

were enough samples collected in both the SGB and in the NAS areas that uptake rates in surface and bottom waters could be analyzed separately. In the NAS area the mean rate in the surface and bottom waters was 5 and 23 ng x liter^{-1} x hr^{-1} respectively in January and 9 and 30 ng x liter^{-1} x hr^{-1} respectively in May (Figs. 31 to 34). The significance of these differences was $p = 0.03$ and 0.001 respectively for January and May. In the SGB, the mean rate in the surface and bottom waters was 0.8 and 25 ng x liter^{-1} x hr^{-1} respectively in January and 20 and 74 ng x liter^{-1} x hr^{-1} respectively in May (Figs. 31, 32, 35, and 36). The significance of these differences was $p = 0.002$ and 0.00004 respectively for January and May.

As was the case with the methane oxidation data, the glutamate uptake rates were higher in waters collected during the August cruise than those collected during the January cruise. When comparing the January with the August uptake rates there was a 63% reduction in surface waters and a 55% reduction in the bottom waters. This reduction was not statistically significant at the $p < 0.1$ level in the surface waters of the NAS area but it was at the $p = 0.09$ level in the SGB. The reduction in uptake rates in the January bottom waters was not statistically significant. There was also an increase observed in glutamate uptake rates from January to May (66% and 63% increase in surface and bottom waters respectively). In the NAS area, this difference was significant at the $p = 0.06$ level in the surface waters but was not significant in the bottom waters. In the SGB area, the differences were $p = 0.003$ and 0.01 in the surface and bottom waters, respectively.

During the January and May cruises, geographical patterns of glutamate uptake were noted as shown by the enclosed areas in Figs. 31, 32, 34, 35 and 36. The enclosed areas are those where observed uptake rates were $\geq$ the mean value observed in the region shown (the NAS and SGB regions were treated separately). The January uptake rates showed high levels along the coast to either side of Port Moller, to the northeast near the Kvichak River and in the center of the SGB (Figs. 31 and 32). This pattern was observed in both surface and bottom waters. During the May cruise, no pattern was observed in the surface waters of the NAS area (Fig. 33) but there were elevated levels observed in the inshore areas near Port Moller and to the northeast (Fig. 34). In the SGB area, the glutamate uptake rates in both surface and bottom waters were generally higher in the center of the region (Figs. 35 and 36).

The microbial activities observed in Port Moller and Herendeen Bay were essentially the same as the rates observed in the waters just offshore from Port Moller (Figs. 37 to 39). The highest rates measured in this area were found in the waters collected at the head of Port Moller in January (Fig. 37).

D. Relative microbial activity in sediments

During the January and May cruises, studies were conducted on the relative microbial activity of marine sediments using glucose or glutamate uptake rates (Figs. 40 to 42). The mean glucose uptake rate in January was 11 ng x g dry wt.$^{-1}$ x hr^{-1} and the mean glutamate uptake rate was 130 ng x g dry wt.$^{-1}$ x hr^{-1}. The highest rates were observed in the sediments collected along the PL line from

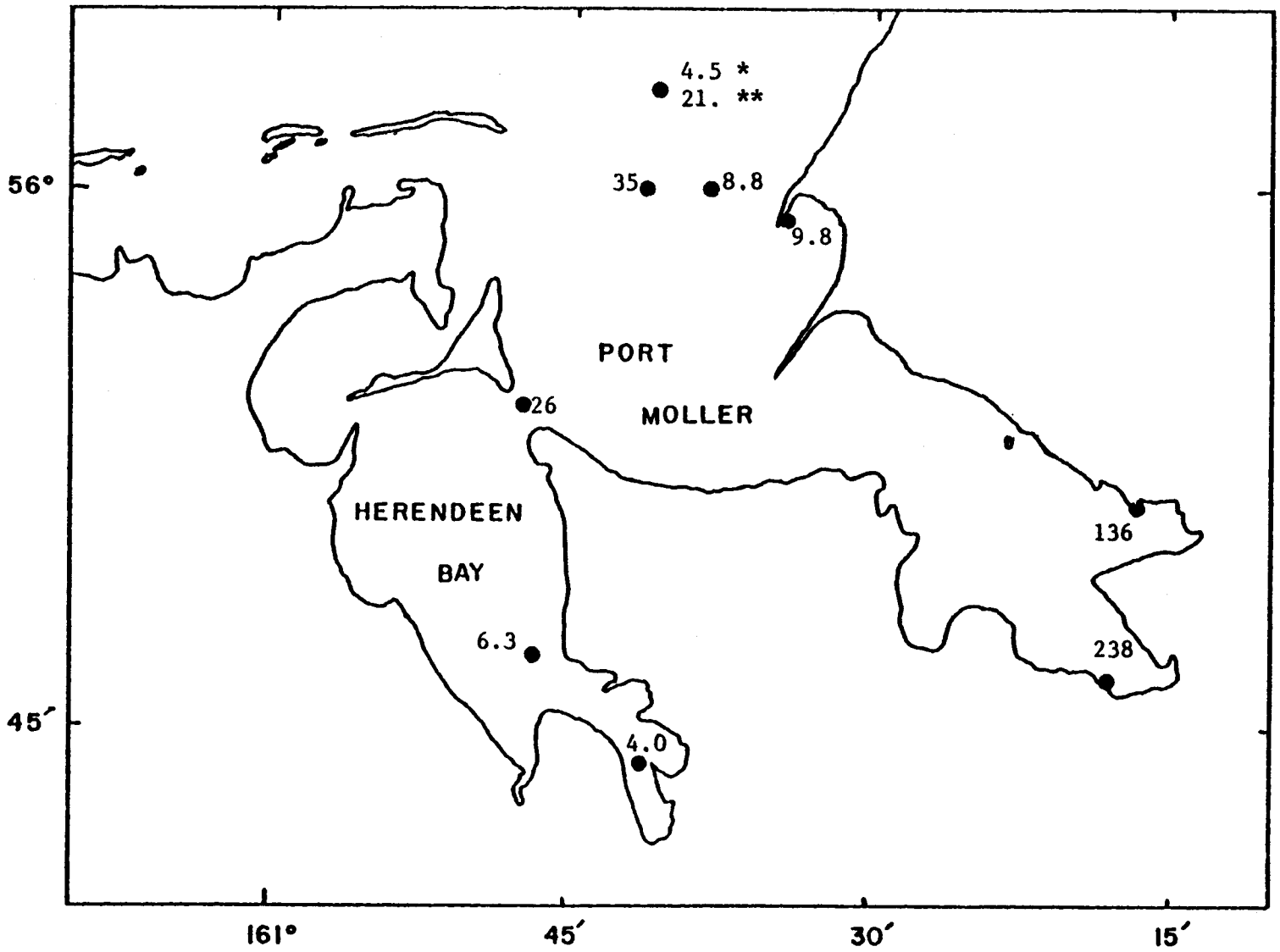

Figure 37. Glutamate uptake rates in ng x liter^{-1} x hr^{-1} from surface waters in Port Moller during the January 1981 cruise. * Denotes rate at high tide, ** rate at low tide.

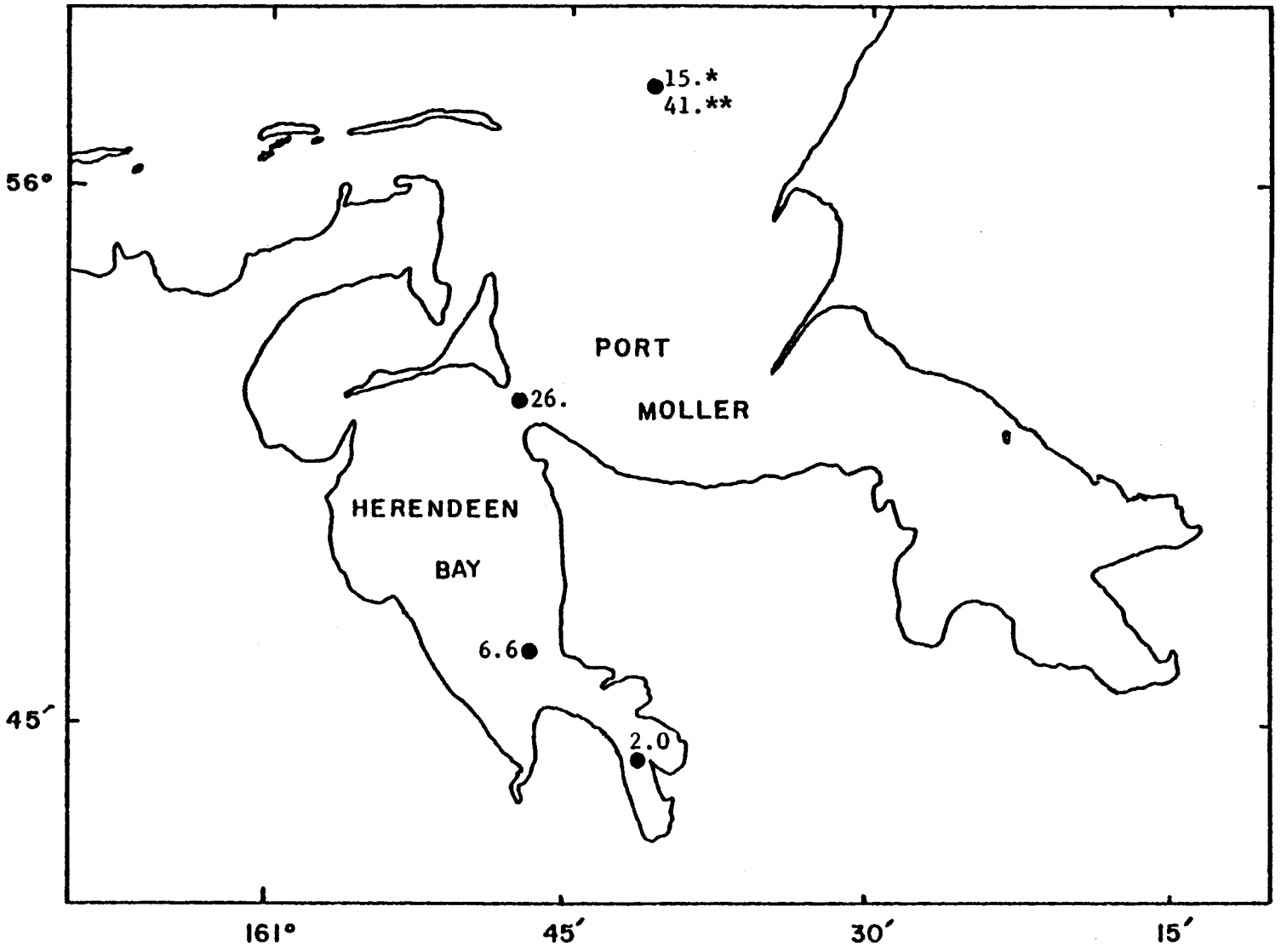

Figure 38. Glutamate uptake rates in ng x liter^{-1} x hr^{-1} from bottom waters in Port Moller during the January 1981 cruise. * Denotes rate at high tide, ** rate at low tide.

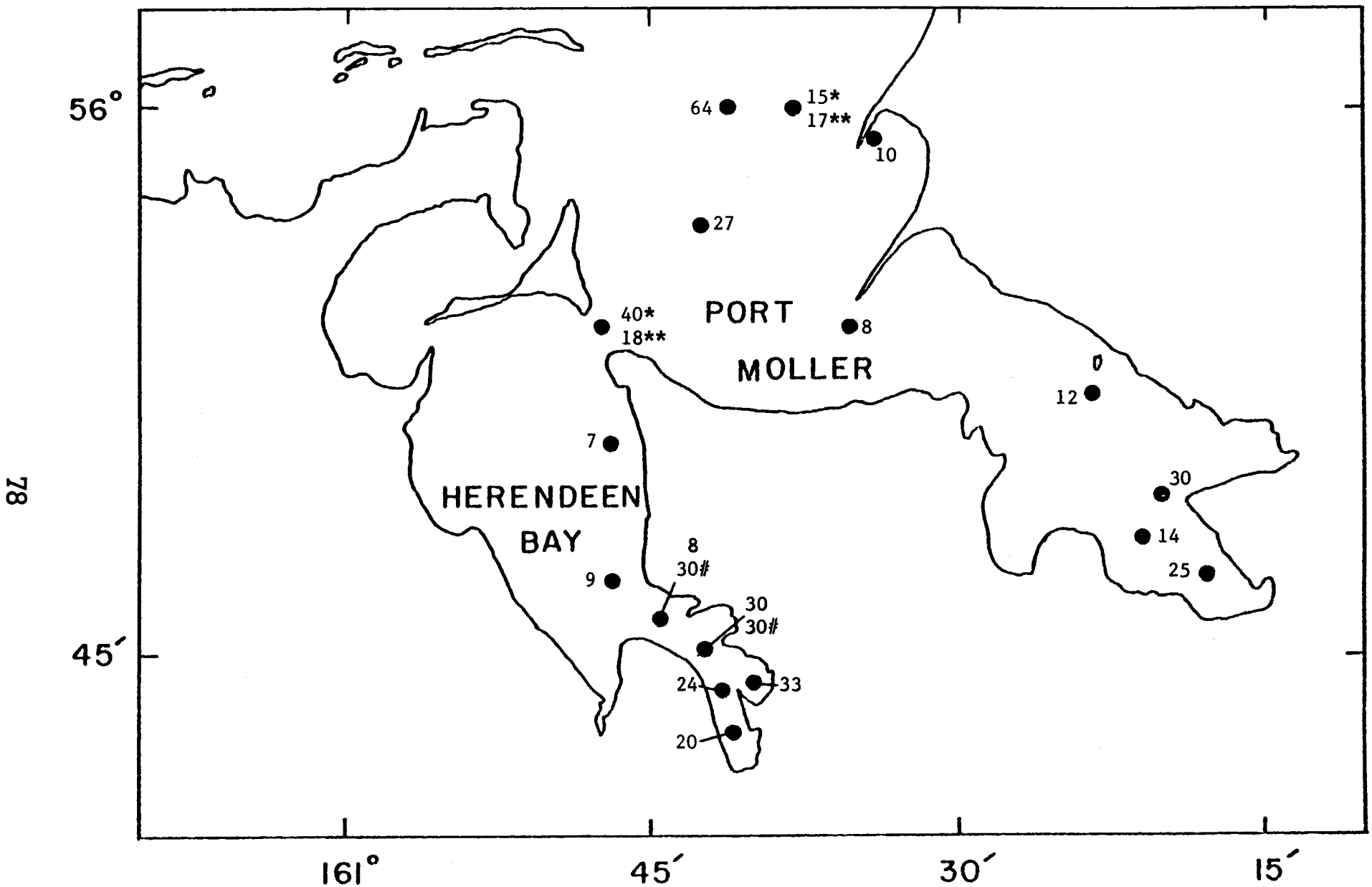

Figure 39. Glutamate uptake rates in ng x liter^{-1} x hr^{-1} in surface and bottom water samples from the May 1981 cruise. * Denotes high tide, ** low tide, # bottom waters.

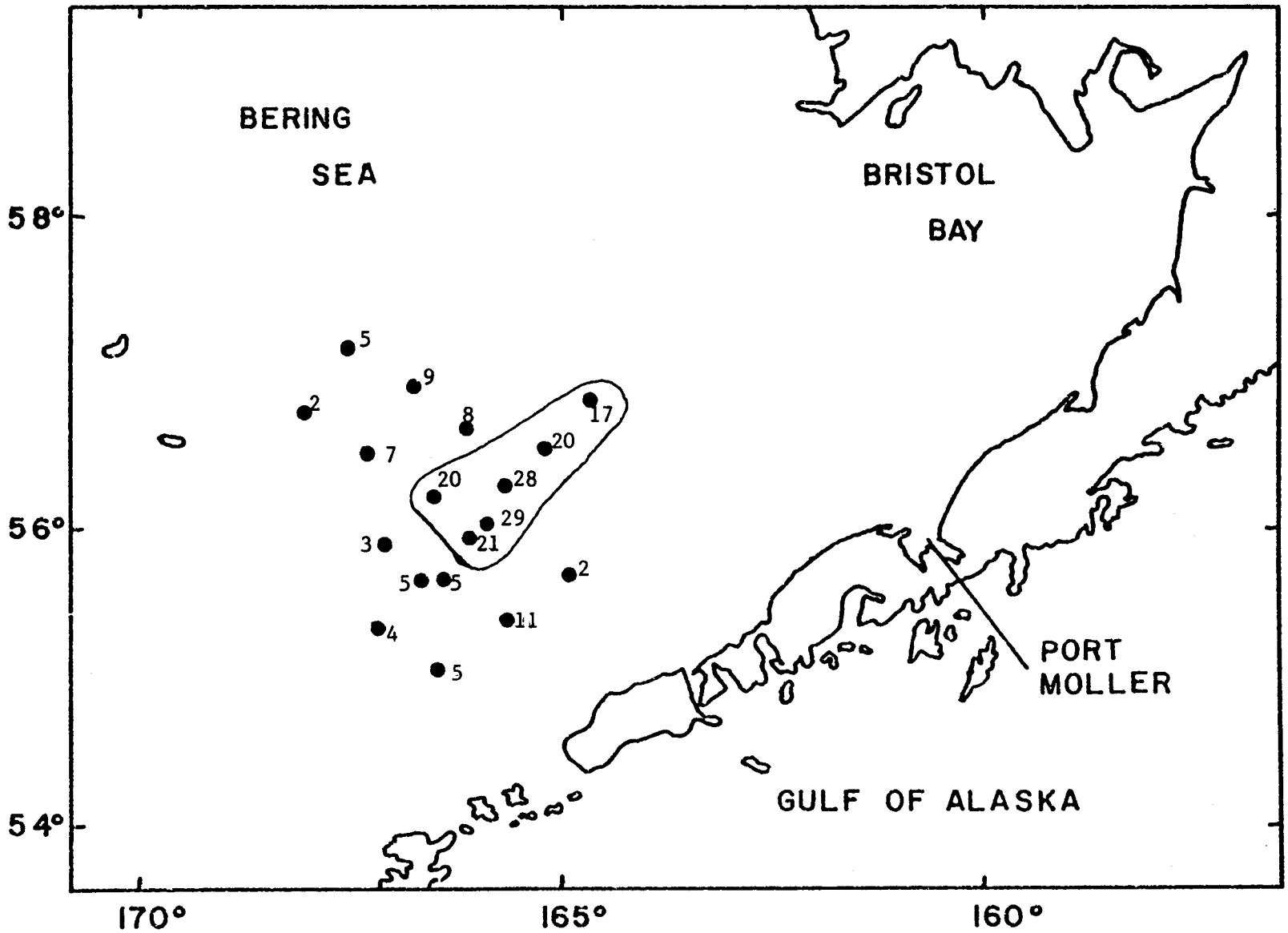

Figure 40. Glucose uptake rates in ng x g dry weight^{-1} x hr^{-1} for sediment samples observed during the January 1981 cruise.

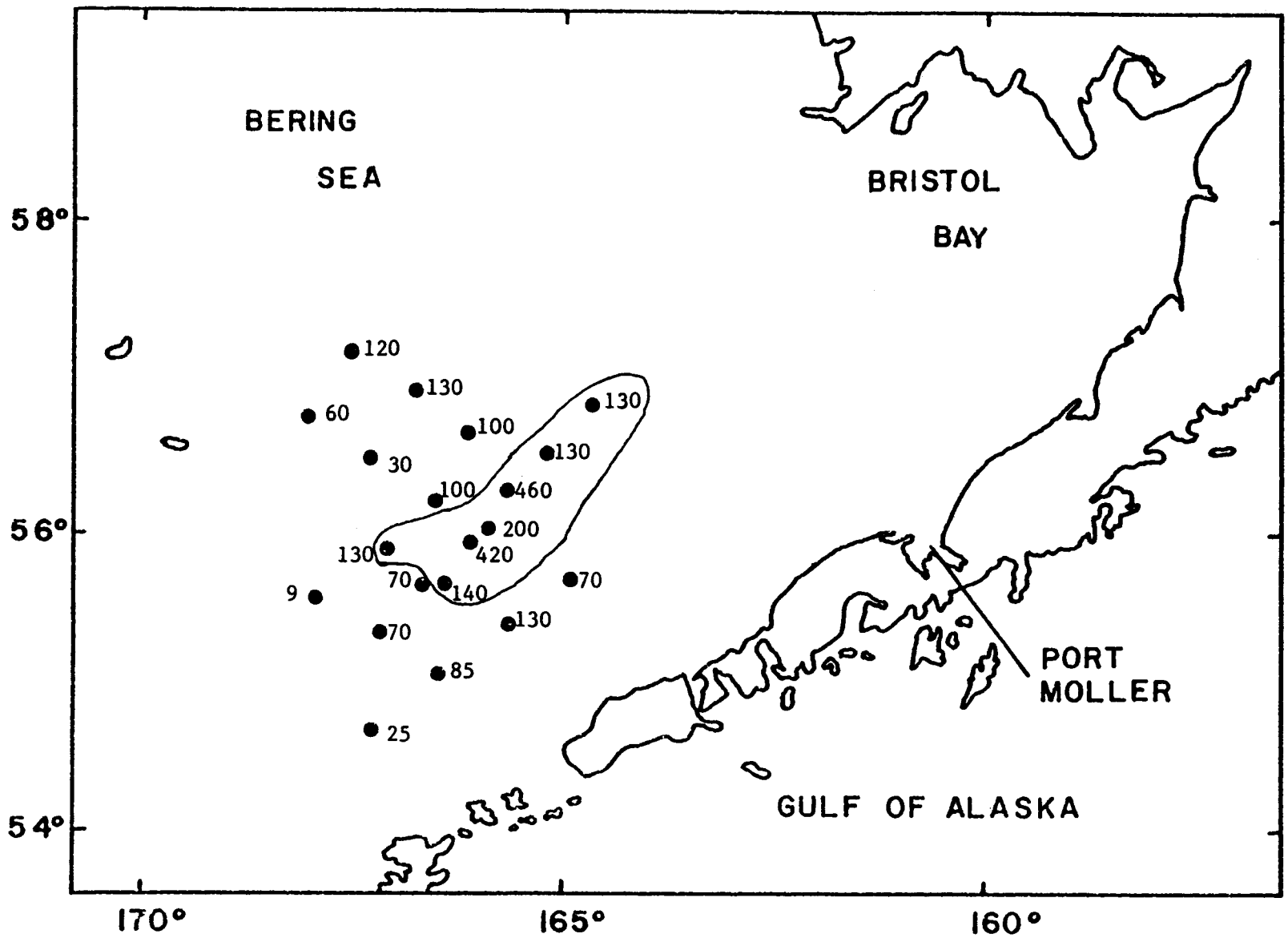

Figure 41. Glutamate uptake rates in ng x g dry weight^{-1} x hr^{-1} for sediment samples observed during the January 1981 cruise.

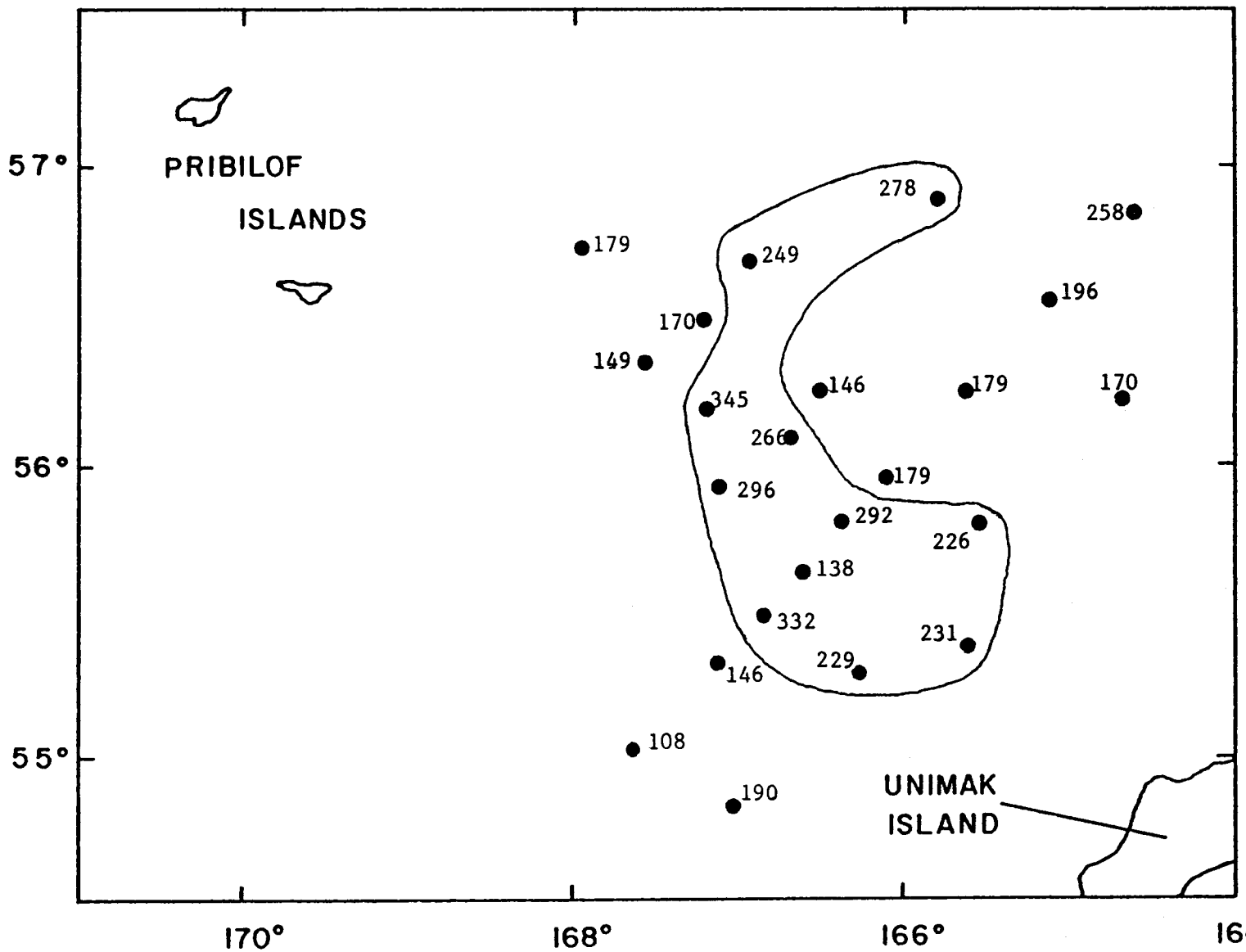

Figure 42. Glutamate uptake rates in ng x g dry weight^{-1} x hr^{-1} for sediment samples observed during the May 1981 cruise.

PL6 to PL14. During the May cruise, the pattern of glutamate uptake was different as shown in Figure 42. The mean glutamate uptake rate was also 65% higher in May (215 ng x g dry wt^{-1} x hr^{-1}). When the data from the common stations were compared, this seasonal difference was significant at the $p = 0.009$ level. During this same cruise, microbial activity was also measured in sediments of Port Moller and Herendeen Bay (Fig. 43). The mean glutamate uptake rate in these sediments was 1700 ng x g dry wt^{-1} x hr^{-1}. This is almost 8 times the mean rate observed in the SGB.

E. Nitrogen fixation rates

During the January and May cruises, nitrogen fixation rates were also measured in the SGB (Figs. 44 and 45). The mean nitrogen fixation rates for these cruises were 0.1 and 0.2 ng x g dry $wt.^{-1}$ x hr^{-1}. When compared on a station by station basis, this difference was not statistically significant.

F. CO_2 production

Rates of CO_2 production were assayed in the sediments of SGB (Fig. 46) and Port Moller (Fig. 47) during the May cruise. The mean rate in SGB was 2.4 nmole x g dry $wt.^{-1}$ x hr^{-1} and 20 nmole x g dry $wt.^{-1}$ x hr^{-1} in Port Moller. The significance of the difference was $p = 0.0003$.

G. Enzyme activities

During the May cruise, rates of phosphatase, arylsulfatase, amylase and laminarinase were assayed in the sediment of SGB (Figs. 48 to 51). The mean activities observed for these enzymes were: phosphatase 0.53 µmole and arylsulfatase 0.63 µmole p-nitrophenol released x g dry $wt.^{-1}$ x hr^{-1} and amylase 35.2 µg and laminarinase

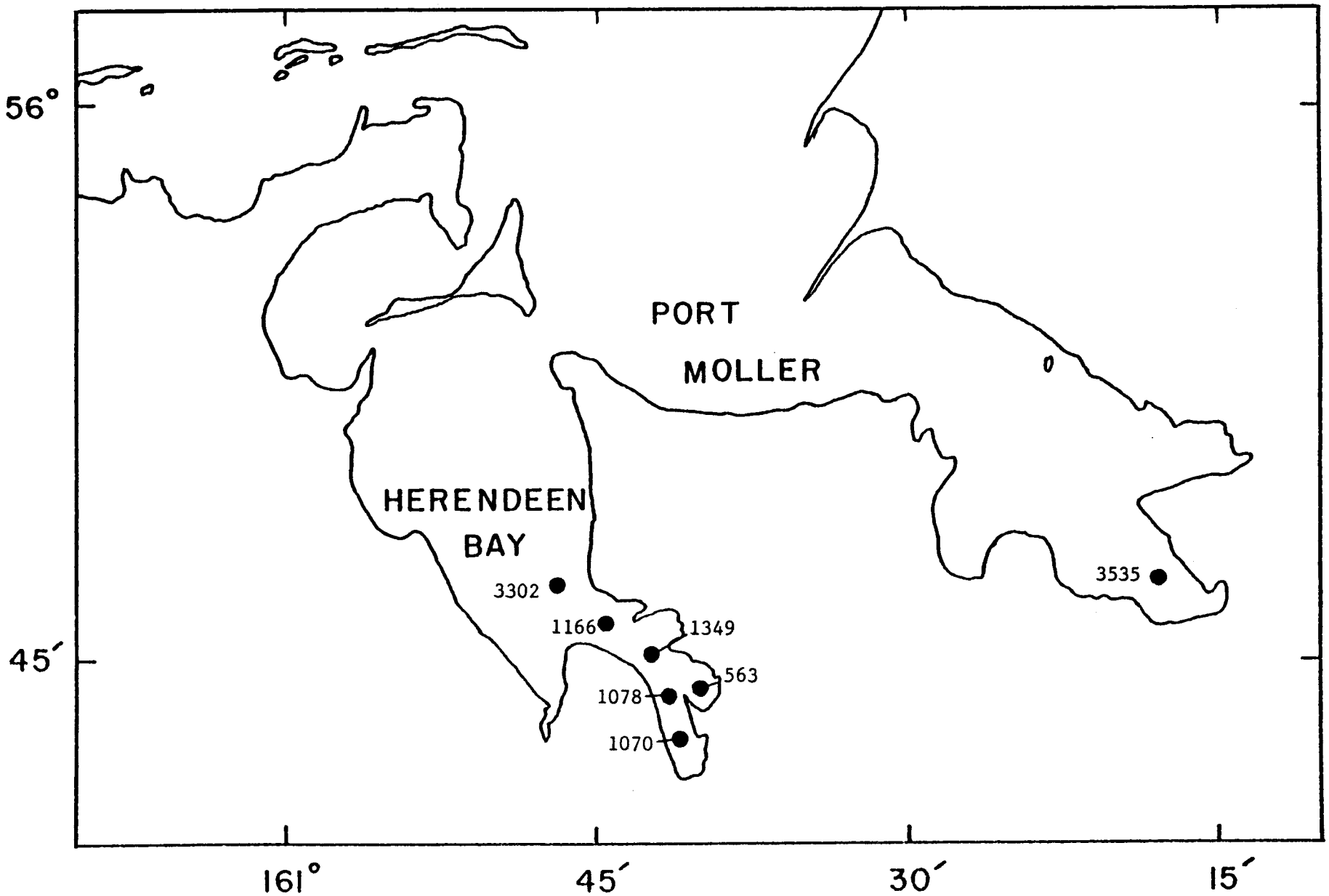

Figure 43. Glutamate uptake rates in ng x g dry weight^{-1} x hr^{-1} for sediment samples observed during the May 1981 cruise.

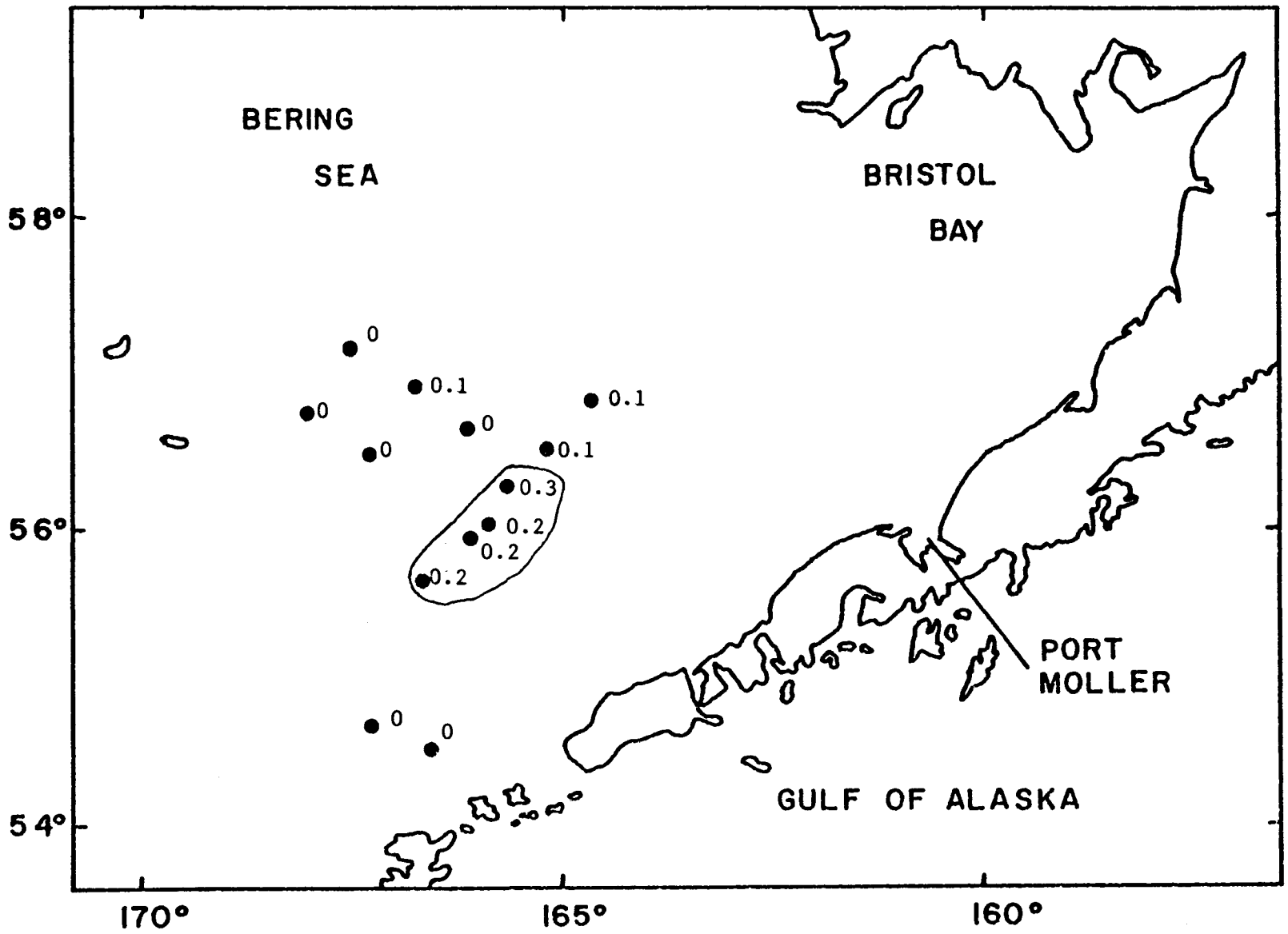

Figure 44. Nitrogen fixation rates in ng N_2 fixed x g dry weight^{-1} x hr^{-1} for sediment samples observed during the January 1981 cruise.

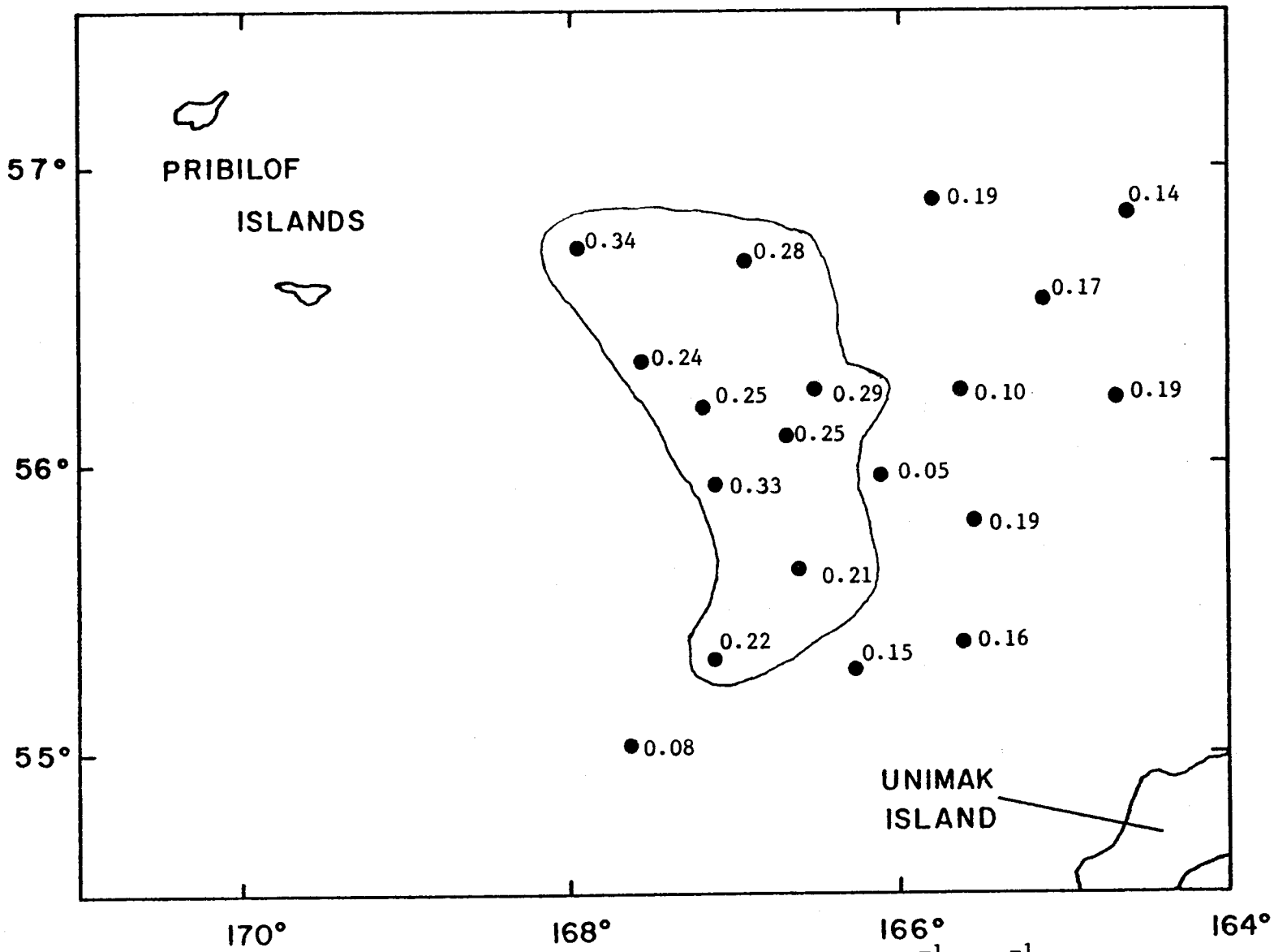

Figure 45. Nitrogen fixation rates in ng N_2 fixed x g dry weight^{-1} x hr^{-1} for sediment samples observed during the May 1981 cruise.

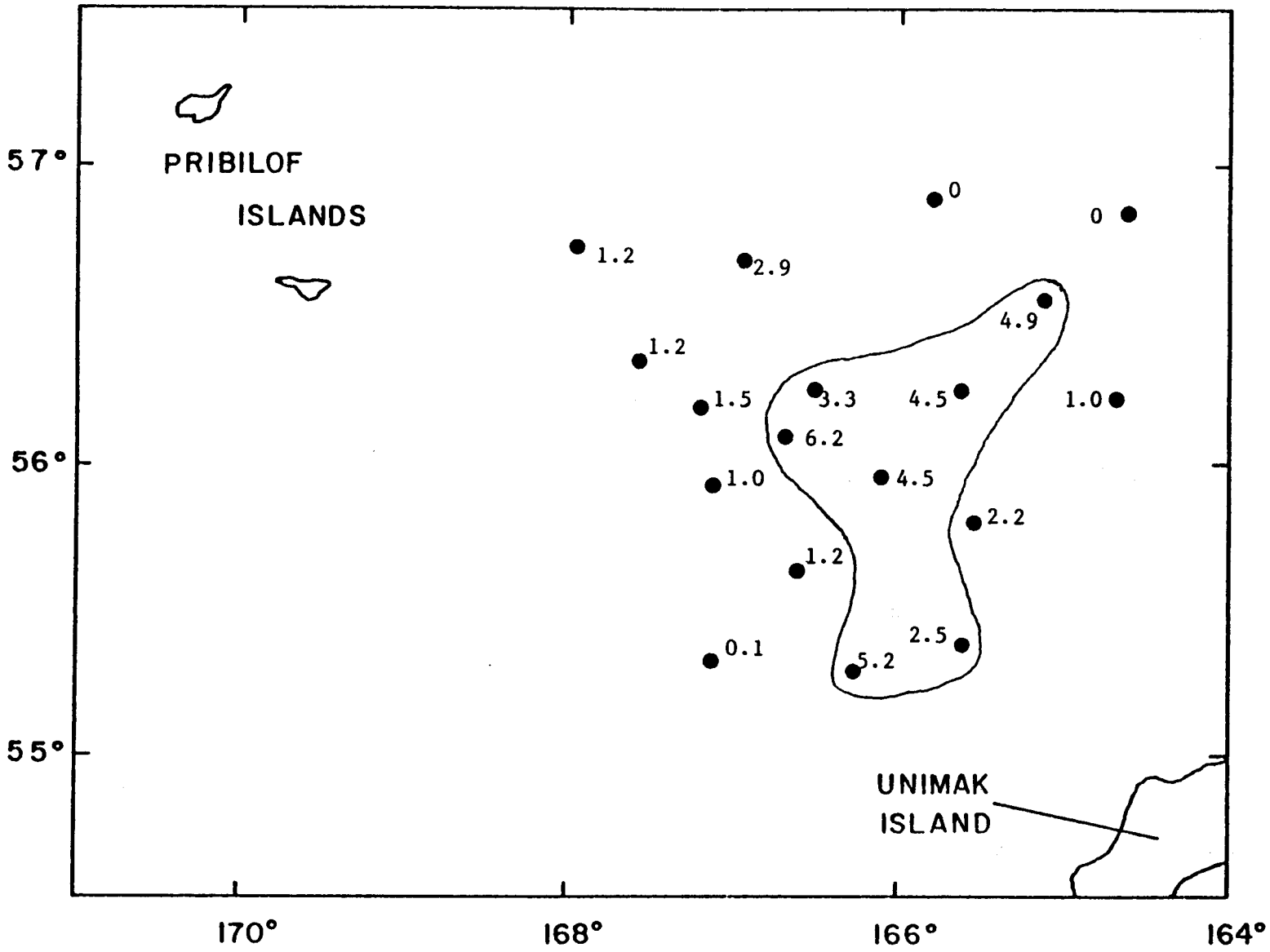

Figure 46. CO_2 production rates in nmoles x g dry weight^{-1} x hr^{-1} for sediment samples observed during the May 1981 cruise.

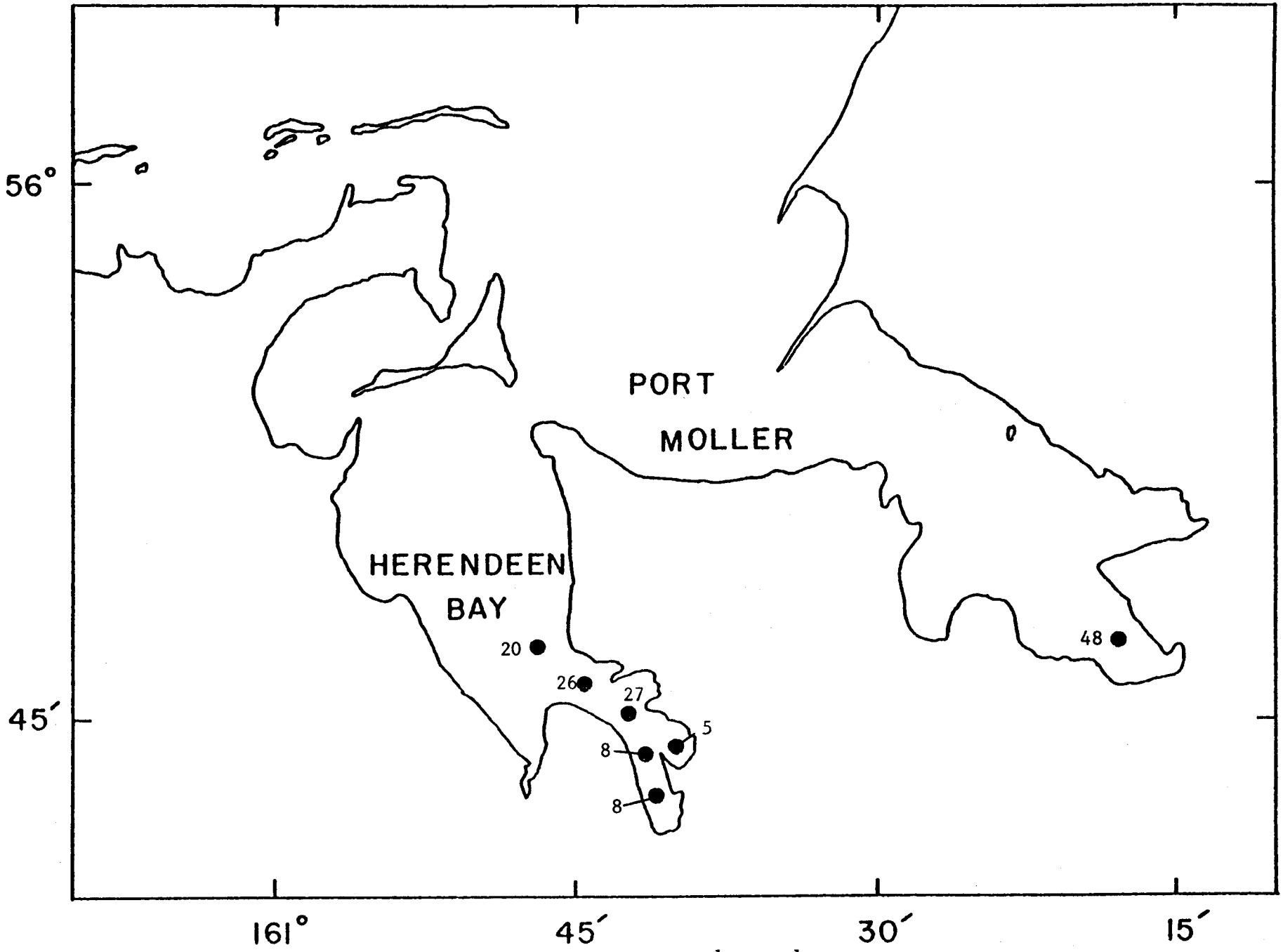

Figure 47. CO_2 production rates in nmoles x g dry weight^{-1} x hr^{-1} for sediment samples observed during the May 1981 cruise.

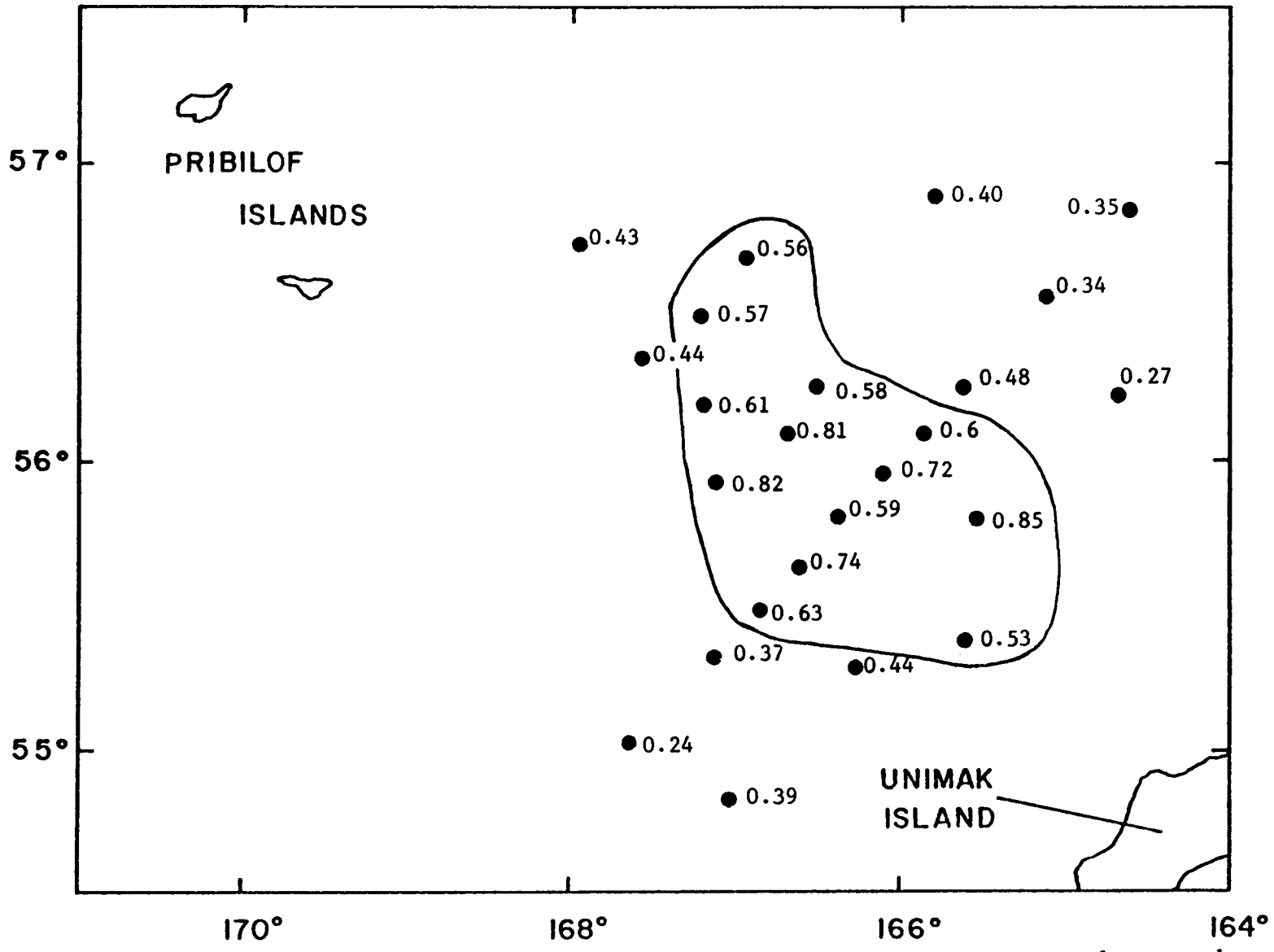

Figure 48. Phosphatase activity in µmole p-nitrophenol released x g dry weight^{-1} x hour^{-1} for sediment samples collected during the May 1981 cruise.

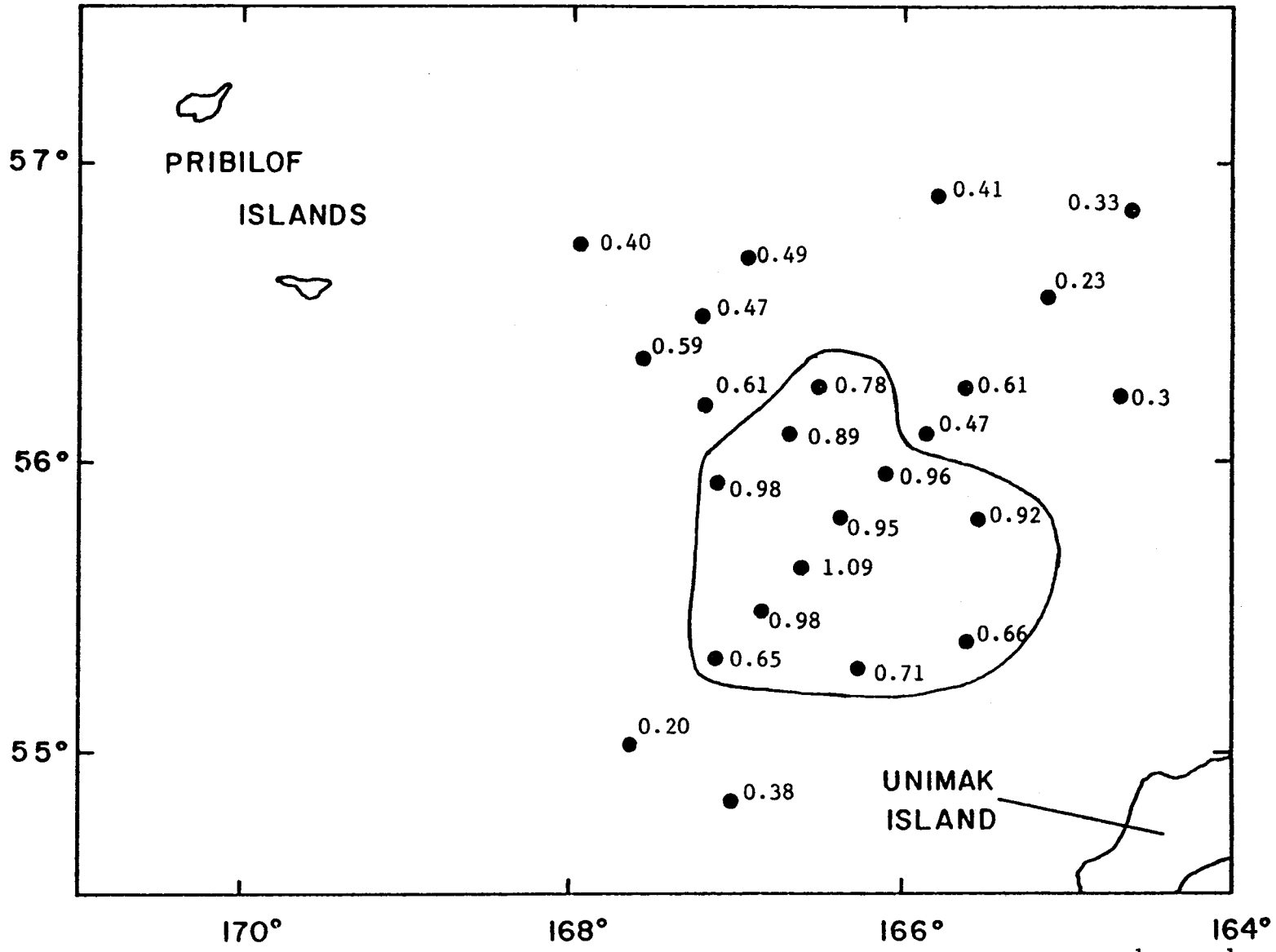

Figure 49. Arylsulfatase activity in µmole p-nitrophenol released x g dry weight^{-1} x hr^{-1} for sediment samples collected during the May 1981 cruise.

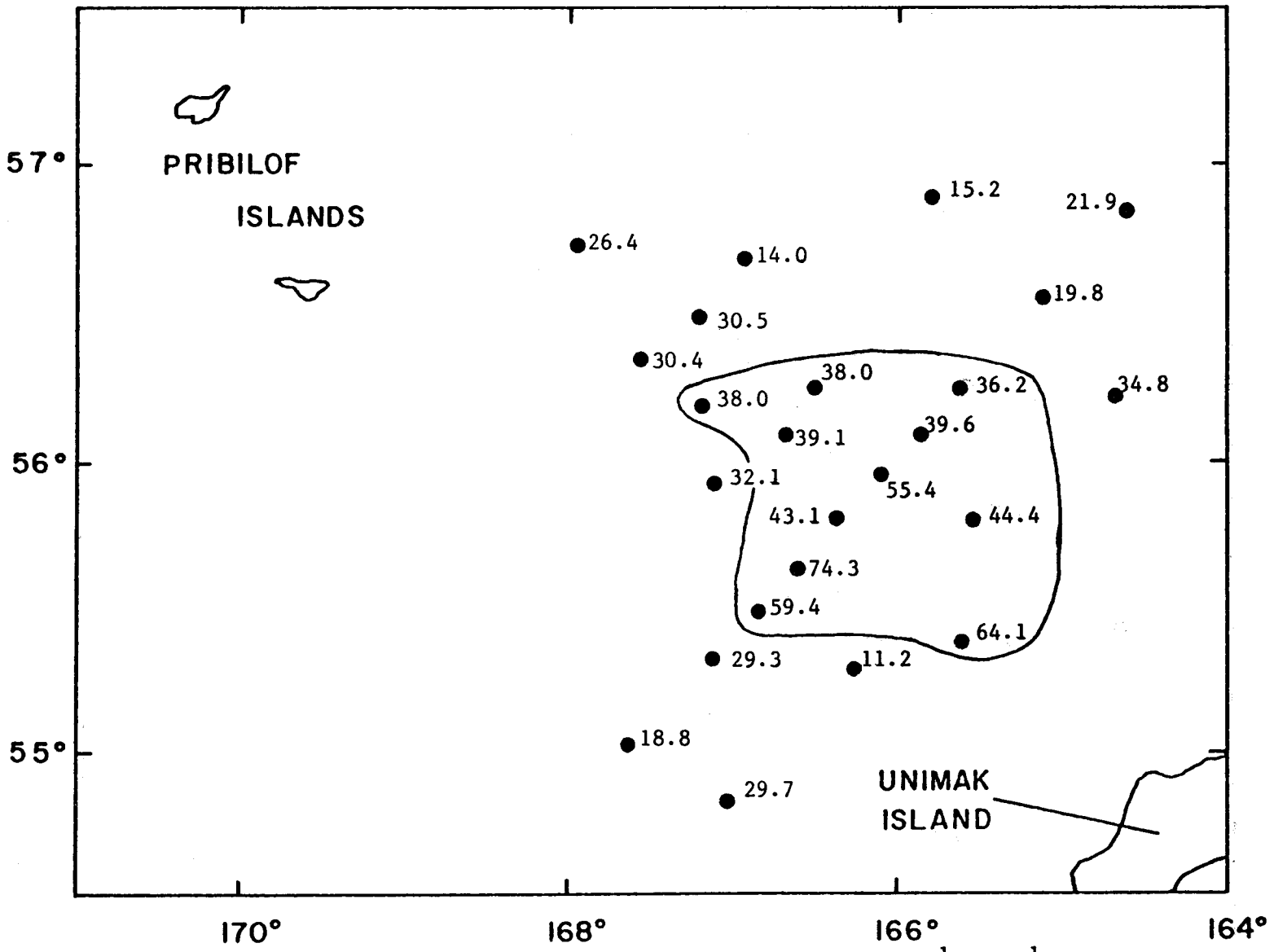

Figure 50. Amylase activity in µg glucose released x g dry weight^{-1} x hr^{-1} for sediment samples collected during the May 1981 cruise.

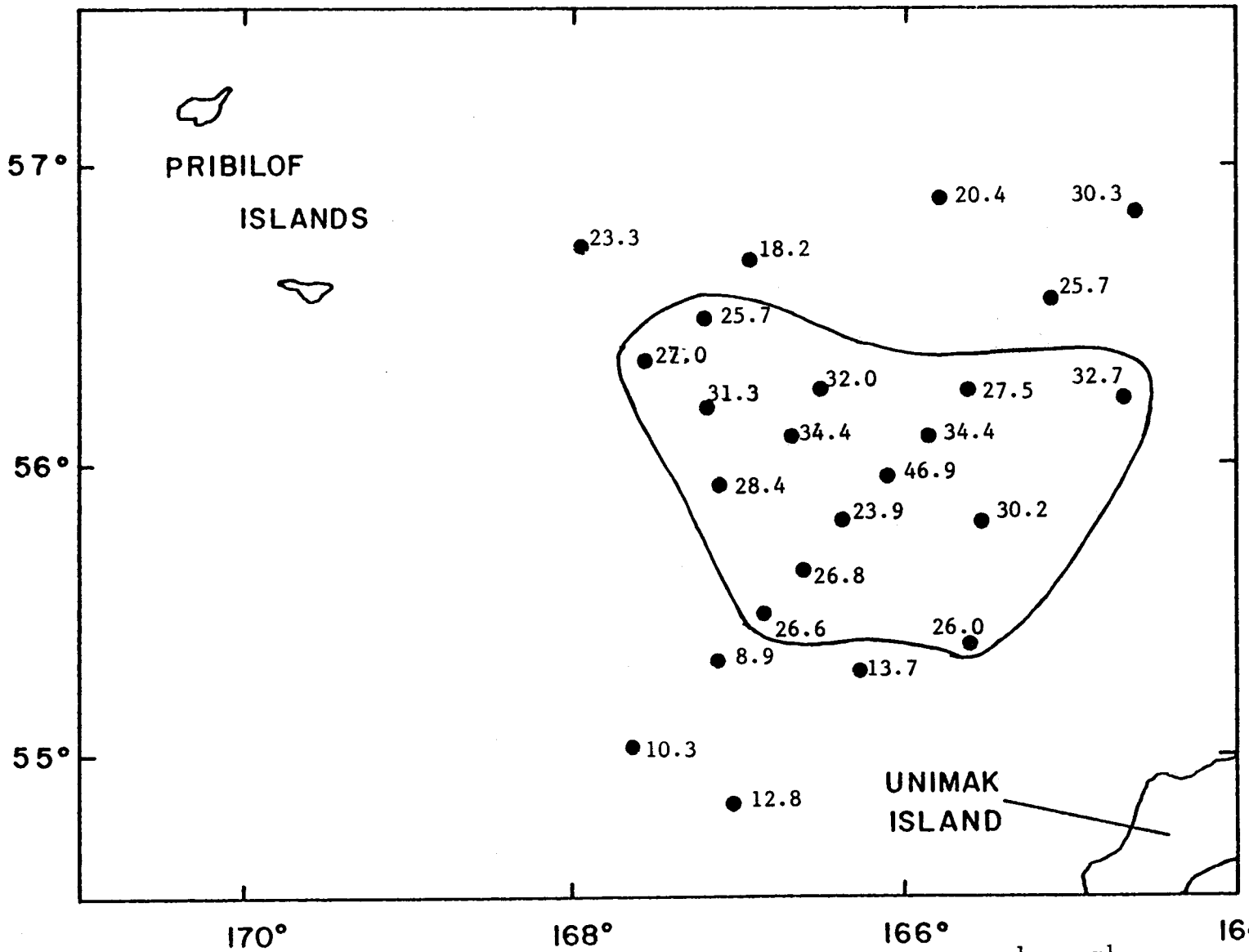

Figure 51. Laminarinase activity in µg glucose released x g dry weight^{-1} x hr^{-1} for sediment samples collected during the May 1981 cruise.

25.7 μg glucose released x g dry wt^{-1} x hr^{-1}. The regions that showed elevated activities were approximately the same for all of these enzymes.

H. Data storage

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, water column depth, methanogenesis rates, methane oxidation rates, relative microbial activity, nitrogen fixation rates and CO_2 evolution rates. The data collected for each cruise are located in a different file. The file numbers for these cruises are as follows:

	Sample # series	File #
August, 1980	AW/B 200-300	344
January, 1981	AW/B 400-500	345
May, 1981	AW/B 600-800	346

VII. Discussion

A. Methane oxidation

1. Observed rates compared with those reported by other investigators

The methane oxidation rates that we have reported in the "Results" section of this study were the actual rates observed with a methane concentration of 0.58 µl x liter^{-1} of seawater. While this information is very useful for comparative purposes within this study, this should not be confused with an in situ rate calculated from methane oxidation kinetics and the actual methane concentrations observed in the same samples. In order to compare our data set with the methane oxidation rates reported by others, we have estimated in situ methane oxidation rates using the preliminary methane concentration data given to us by Dr. Cline of PMEL. For illustrative purposes, we have chosen the May data set since it is the most comprehensive set that was collected during the three NASTE cruises (Table 6).

Table 6. In situ rates of methane oxidation observed during the May$_1$ NASTE cruise. All rates are given in units of nl x liter^{-1} x day^{-1}.

Region	Sample Depth	Mean	S.D.	Range	Number of Samples
St. George Basin	surface	0.3	0.2	0.03-0.9	29
	bottom	2.5	3.9	0.03-18	30
North Aleutian Shelf	surface	0.4	0.4	0.01-4	28
	bottom	0.4	0.3	0.03-3	29
Port Moller	surface	16	12	2.4-49	15

Scranton and Brewer (1978) estimated that the initial rates of methane oxidation in the deep sea waters they studied were on the order of 0.01 nl x liter^{-1} x day^{-1}. This value is lower than any observed by

us. The overall mean rate in all samples except those in Port Moller was 1 nl x liter^{-1} x day^{-1}. The mean rate observed by Sansone and Martens (1978) was 0.22 nl x liter^{-1} x day^{-1} in the Cape Lookout Bight, North Carolina waters that they examined. While measuring methane oxidation rates in an African rift lake, Jannasch (1975) observed methane oxidation rates near 0.11 nl x liter^{-1} x day^{-1}. Although these values are all within the range that we observed, they are lower than the mean value. Rates of methane oxidation measured with radioisotopes have produced rates which are considerably higher than those reported above. In a recent study by Harris and Hanson (1980), radioactive methane was used to measure methane oxidation at one substrate concentration in an eutrophic lake. They observed rates in bottom waters ranging from approximately 640 nl x liter^{-1} x day^{-1} in May to a high of 640 µl x liter^{-1} x day^{-1} in September. These rates are much higher than any that we observed. Welch et al. (1980) observed methane oxidation rates in an Arctic lake which produced a mean methane oxidation rate for the lake of 7.2 µl x liter^{-1} x day^{-1}. This rate is approximately 7,000 times the mean value we observed in May. There is thus a large range of values that have been observed in aquatic and marine systems. Our values seem reasonable when compared to the few data that are available for marine waters.

2. Factors affecting methane oxidation rates in marine waters

There is currently no information in the literature concerning environmental factors affecting methane oxidation rates in marine waters. Essentially everything that is known about these factors has come from the study of small fresh water lakes. These data have recently been summarized in a review article by Rudd and Taylor (1980). Their studies have stressed the importance of O_2 and dissolved inorganic nitrogen (DIN) on the patterns of methane oxidation observed in these

lakes. When DIN was not limiting, methane concentrations became a dominant factor at levels below 224 µl x liter^{-1} (Rudd and Hamilton, 1975). They also concluded that temperature and pH were not normally controlling factors in the system they studied. There is also information that suggests that the presence of particles in the water column might increase methane oxidation rates by some unknown mechanism (Weaver and Dugan, 1972).

If the system that we studied behaved in a similar fashion to that observed in fresh water systems, we would anticipate a correlation between methane oxidation rates and methane concentrations since the concentrations normally encountered in our study area were at least two orders of magnitude lower than the methane limiting concentration listed above. We would also predict that methane oxidation rates would be highest in regions of high suspended particulate concentrations. In general, the areas where there were elevated concentrations of suspended particles, the methane oxidation rates were also elevated; i.e. in bottom waters of both lease areas and the inshore stations along the North Aleutian Shelf (NAS). However, during the January cruise, Dr. Baker (PMEL) assayed the levels of suspended particles in a number of our samples and we found no linear correlation between these variables.

The same thing can be said for the correlation between methane oxidation rates and methane concentrations. In general, the areas where we observed the highest rates of methane oxidation were also the areas where the chemists (Dr. Cline and Mr. Katz, PMEL) observed the highest methane concentrations. The highest methane concentrations observed during the cruises were in the heads of Port Moller and Herendeen Bay. We also observed high rates of methane oxidation in this same area (Figs. 14 to 18). In the NAS area during the August and January cruises,

the highest methane concentrations were observed near Port Moller and along the coast to the east of Port Moller with the highest concentrations in the nearshore stations. Again, this parallels the pattern observed in methane oxidation rates (Figs. 8 to 11).

During the January cruise, we conducted a time study of methane oxidation rates over one tide cycle. We observed a much higher methane oxidation rate in the water that was coming out of Port Moller on an outgoing tide than in water that was flooding into this embayment (Figs. 15 and 16). This same trend was observed in the methane concentrations (higher concentrations on the ebbing tide [Mr. Chuck Katz, personal communication]).

In St. George Basin (SGB), the methane concentrations were generally higher in the bottom waters than in surface waters. The highest methane concentrations were observed in the vicinity of stations PL6 and SG28 during the August and January cruises and near station SG70 during the May cruise. This is similar to the pattern of methane oxidation rates found in this region. We found relatively low methane oxidation rates in the surface waters during all cruises (Figs. 8, 10 and 19). The methane oxidation rates were consistently higher in the bottom waters with the highest rates observed near the areas where the highest methane concentrations were observed (Figs. 9, 11, and 22). When these data are compared seasonally, there is also good agreement between the August and January data sets. The methane concentration in the bottom waters of the SGB was reduced approximately 50% in January relative to the higher values observed in August. The same trend was also observed in methane oxidation rates. On a large scale, these data appear to be loosely correlated; however, this apparent correlation breaks down significantly when comparing the data collected in January with that collected in May. In the SGB,

the mean methane oxidation rate increased from 2.0 to 4.5 nl x liter^{-1} x day^{-1} although this increase was not statistically significant. The methane concentrations remained about the same or even decreased during the same period.

To check the validity of this correlation, we conducted a statistical analysis of linear regression between these two variables. There was essentially no correlation when the data were compared on a sample by sample basis. The correlation coefficient (r) between methane oxidation rates and methane concentration was 0.14, 0.7 and 0.03 for the August, January and May cruises respectively. The January data were heavily biased by the high methane oxidation rates observed in 7 Port Moller samples. When these are excluded from the analysis, the r value is 0.02. From these data we have concluded that there is not a strong linear relationship between these two variables.

The lack of correlation between methane oxidation rates and methane concentrations is graphically illustrated in the comparison of these two variables in the same water columns (Figs. 21 and 52). In all three water columns, the lowest methane concentrations were observed at the surface with little increase at depths less than 30 meters. At depths below 30 meters, the methane concentrations generally increased until they reached maximum values in water samples taken 5 meters from the bottom (B-5 m). The methane concentrations in the bottom waters were generally slightly lower than those observed at B-5M. The methane oxidation rates by contrast, were highest at the bottom, and lowest at B-5m. The elevated methane oxidation rates in the bottom water samples may be due, in part, to contamination of these samples by sediment particles resuspended during the sampling process. All water columns showed a secondary

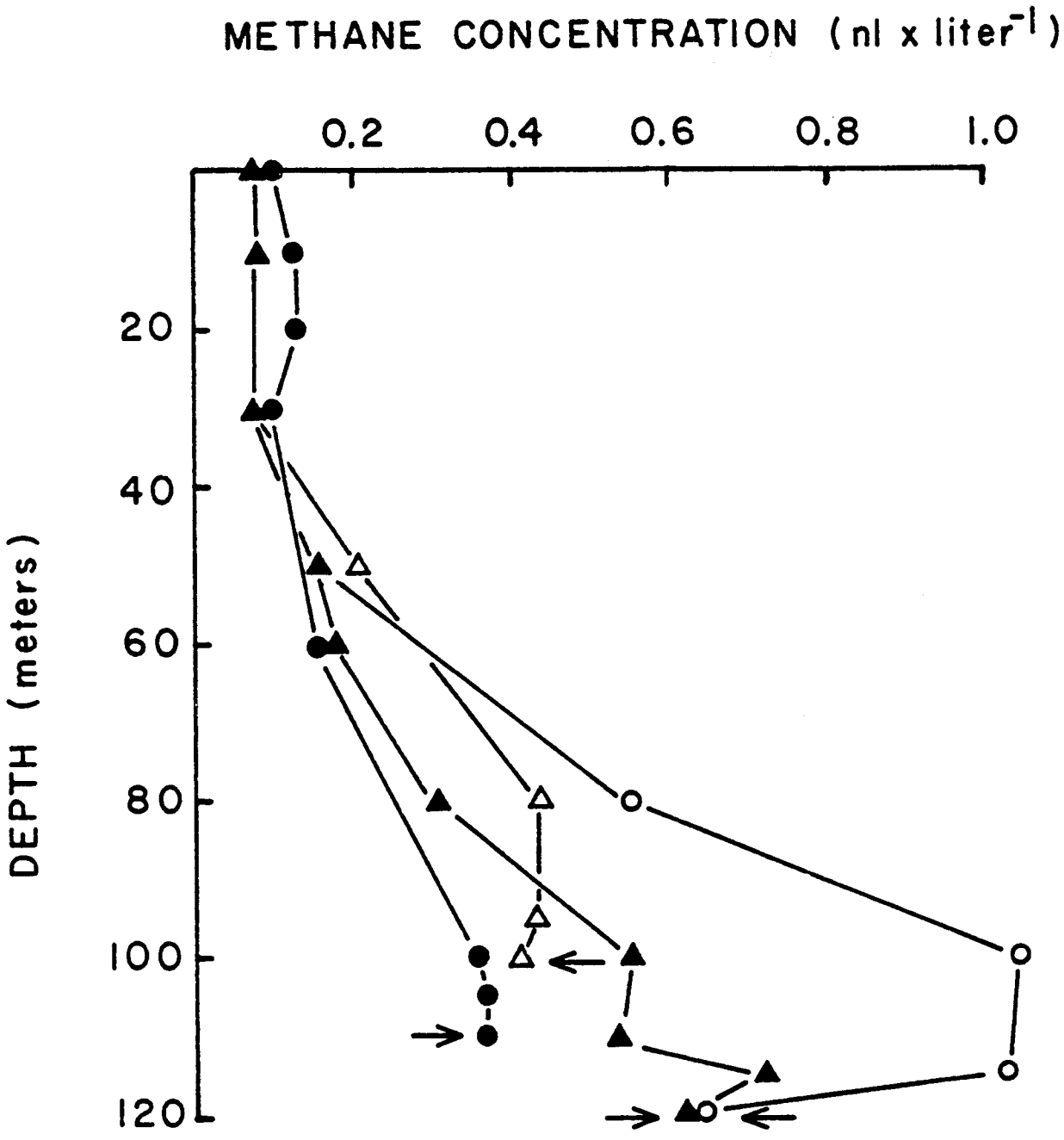

Figure 52. Methane concentration in waters at decreasing depth. Arrows indicate bottom waters. ● = SG67, ○ = SG5-1, △ = SG22, ▲ = SG5-2.

methane oxidation maximum within 30 meters of the surface where the methane concentrations were all at the lowest values.

Oxygen levels have also been shown to affect methane oxidation rates. If DIN is limiting, high O_2 levels can apparently limit methane oxidation (Rudd et al., 1976). During the January and May cruises, Mr. Katz of PMEL measured O_2 levels in selected samples from the entire study area (including Port Moller). The raw data suggested that the O_2 levels were essentially constant from the surface to the bottom of the water column in most cases (C. Katz, personal communication). It thus seems unlikely that O_2 levels would greatly affect the methane oxidation rate patterns that we observed. Thus, the factors that limit methane oxidation rates in freshwater systems do not appear to affect methane oxidation in the system that we studied.

Although the above mentioned variables do not directly correlate with methane oxidation, there are several sets of data that suggest another correlation. That is the correlation between methane oxidation and microbial heterotrophic activity. The differences observed seasonally in methane oxidation may give a clue to this correlation. We observed a significant seasonal difference in the methane oxidation rates observed in the surface waters but not in the bottom waters of the SGB. If it is assumed that methane oxidation rates are closely related to overall microbiological activity, the above correlations make sense.

Our studies and those of others (Griffiths and Morita, 1981; Fuhrman et al., 1980) have shown that microbial heterotrophic activity is linked with phytoplankton productivity. Since phytoplankton activity takes place in the surface waters one would expect a reduction in phytoplankton activity in surface waters in the winter and higher activities in the summer. The water column in the SGB is well-structured with little vertical mixing. Thus, changes due to differences in phytoplankton

activity should accurately reflect these seasonal changes. The seasonal changes in the bottom waters should be much less. This same seasonal trend was observed in the methane oxidation rates (Table 7).

Table 7. Summary of methane oxidation rates measured at one substrate concentration, during the three NASTE cruises.

Cruise Date	Area	Sample Depth	Methane Oxidation $nl \times liter^{-1} \times day^{-1}$
8/80	NAS	surface	1.5
		bottom	2.2
	SGB	surface	1.1
		bottom	4.4
1/11	NAS	surface	0.4
		bottom	1.3
	SGB	surface	0.1
		bottom	2.0
5/81	NAS	surface	1.0
		bottom	1.5
	SGB	surface	2.0
		bottom	4.5

Further evidence also comes from the study of methane oxidation rates in waters at the same location. During all cruises, we measured methane oxidation rates at the surface, bottom and waters 5 meters from the bottom (B-5m). During August and January cruises, the rates observed in the B-5m were usually the same as that observed at the surface. In the May SGB data set, the B-5m methane oxidation rates were lower than that observed at either the bottom or surface in 19 out of 23 water columns studied (Figs. 19, 20 and 22). This trend is illustrated in four locations where detailed analyses of the water columns were conducted (Fig. 21). The elevated methane oxidation rates observed at stations SG5 and SG22 within the top 50 meters may well reflect a relationship with increased phytoplankton productivity in the same water column.

In general terms, the methane oxidation data and the relative microbial activities seem to fit well in the SGB and NAS area; i.e., both were highest in the nearshore stations in the NAS and both were highest in the bottom waters. When these two data sets were compared by cruise, the following correlation coefficients (r) values were observed 0.6, 0.9 and 0.7 for the August, January and May cruises respectively. This means that for these three cruises, the variation in methane oxidation rates could be explained by 36%, 81% and 49% of the variation in glutamate uptake rates for the August, January, and May cruises respectively.

Although there has been extensive work conducted on the characterization of methanotrophs, most of these have been isolated from fresh water and soils. At this time, very little is known about the biology of marine water methanotrophs. Most of these organisms that have been isolated prefer single carbon nutrients sources and may lack the enzymes required to break carbon - carbon bonds (Ribbons et al., 1970). More recent studies however, have shown that some methanotrophs are able to metabolize a wide variety of organic compounds (Higgins et al., 1980). It is quite possible therefore, that methane oxidation may be a consistent feature of general heterotrophic microbial populations and may not be tied to the exclusive utilization of methane. If methane oxidizers depended exclusively on methane as a carbon/energy source in the waters that we studied, we would have expected a much higher correlation with methane concentrations.

There is one feature of our results that does appear to be unique. With the exception of some of the samples that were examined from Port Moller, none of the water samples studied in NAS and SGB showed significant (i.e, measurable) incorporation of the methane carbon into cell material. Wherever natural fresh water populations have been studied with ^{14}C

methane, methanogrophs have incorporated up to 50% of the methane carbon into cell material (Rudd and Taylor, 1980). The only report where most of the methane carbon was respired as CO_2 was in anaerobic lake sediments (Panganiban et al., 1979). Under aerobic conditions, the organisms in aerobic sediments of the same lake incorporated 30-60% of the methane carbon into cell material. It thus appears that the organisms that we have studied are different from those studied before in freshwater systems. It is quite possible that these organisms are utilizing methane only as an electron source (energy production) and that they are either fixing CO_2 or utilizing another organic carbon source for cellular carbon.

B. Methanogenesis

1. Observed rates compared with those reported by other investigators

The methanogenesis rates given in this report were calculated from the actual methane production rates observed in subsamples taken from 10 cm cores. All of our estimates of methane production are calculated assuming an active methane producing layer of 10 cm. This assumption is based on a conclusion reached by Reeburgh (1976) which was that methane produced in aerobic sediments below this depth was probably oxidized before it reaches the surface.

The methane production rates that we observed are summarized in Table 8. Unfortunately, there have been no studies in marine sediments in which the same method was used. The only study that measured methane production directly in marine sediments was that reported by Oremland (1975). In seagrass beds, he observed rates of 0.01 to 0.25 ml x m^{-2} x day^{-1} and in coral reef sediments, he observed rates of 0.08 to 1.0 ml x m^{-2} x day^{-1}. These rates are very close to those observed in our work in the SGB (Table 8).

Table 8. Summary of methane production rates observed during the NASTE cruises. These rates are reported in units of ml methane produced x meter^{-2} x day^{-1}. The calculations used assumed an active methane production layer of 10 cm.

Region	Cruise Date	Mean	S.D.	Range	Number Samples
St. George Basin	Aug	0.5	0.5	0-1.3	6
Port Moller	"	0.7	0.5	0-1.1	4
St. George Basin	Jan	0.08	0.07	0-0.2	25
Port Moller	"	13	24	0.2-49	4
St. George Basin	May	0.1	0.2	0.01-0.7	29
Port Moller	"	35	38	0.04-90	12

There have also been estimations made of methane rates in marine sediments by indirect means which generally are much greater. These rates were calculated on the basis of sediment methane concentrations. Reeburgh (1976) estimated a net methane production rate of about 10 ml x m^{-2} x day^{-1} from samples taken at two locationsin the Cariaco Trench off the coast of Venezuela. Barnes and Goldberg (1976) estimated that the methane production rate in anoxic sediments of the Santa Barbara Basin to be about 6.5 ml x m^{-2} x day^{-1}. In both of the latter studies, the sediments studied were anoxic and would most closely resemble the sediments that we studied in Port Moller. These values were in fact within the same order of magnitude as those that we observed in Port Moller.

The methane production rates reported by other investigators suggest that the rates that we observed in the Pamatmat cores (Table 5) were unrealistically low. This fact plus the relatively low rates of methane oxidation that we observed in these sediments leads us to conclude that there was a loss of methane from the cores during the course of the experiment.

2. Methanogenesis in water samples

Studies of methane concentrations in the world's oceans have indicated that under certain conditions, there appears to be a net production of methane in some water columns which are not anoxic (Rudd and Taylor, 1980). The source of this methane is not known although there is some documentation for the fact that methane might be produced in the intestinal tract of zooplankton (Oremland, 1979). This same conclusion was reached by Scranton and Brewer (1977) as the result of their studies on methane production in aerobic marine waters.

During the August and May cruises, we measured methane production rates in bottom water samples (Table 3). Ten samples were tested in August with none of the samples showing methane production after 7 days incubation. During the May cruise, however, methane production was observed in 5 of the 7 bottom water samples assayed. The samples from this cruise were incubated for up to 22 days and showed reduced oxygen levels at the end of the incubation period. It is quite possible that the methane production that we observed was induced by the long incubation period and did not reflect in situ methane production in these samples. Since we did not observe measurable increases in methane until most samples had been incubated for over 8 days, we have concluded that these are unnaturally high rates.

3. Seasonal variation in sediment methanogenesis

The mean methanogenesis rates observed during the August, January, and May cruises were 0.5, 0.08, and 0.1 ml x m^{-2} x day^{-1} respectively in SGB. Although these differences were not statistically significant, they do generally reflect the methane concentrations observed in the water column during these cruises. The highest methane concentrations

were observed in August, with January and May concentrations being much lower. These results were not surprising in light of the seasonal studies conducted at Kasitsna Bay, AK (Griffiths and Morita, 1981). In that study, we observed that the microbial activity in the sediments did not reflect the increase in carbon input from the spring phytoplankton bloom until several months had elapsed. The elevated chlorophyll concentrations and the particulate size distributions indicated that there was a spring bloom in progress during the May cruise (Dr. E. Baker, PMEL, personal communication). The input of this carbon may have also been reflected in the elevated rates of methane oxidation and microbial activity observed in the May water samples.

As far as the methane production was concerned, the data suggest that the carbon being converted to methane in May came into the system the previous summer. If the study had been continued through the summer of 1981, we would expect to find the methanogenesis rates increasing as more carbon was incorporated into the sediments. The rates observed in August were probably close to the maximum for the year since this would be the time when the sediment temperatures and readily degradable carbon should be at the highest levels.

The Port Moller data are more difficult to interpret. Unfortunately, the only time that we were able to obtain a complete sampling of the Port Moller area was in May so there are very few common data points that can be used in a seasonal comparison. The methane production rates observed at the Port Moller cannery pier were about the same in August and January but increased by a factor of two in May. Stations A and B were both sampled in January and May. The observed methane oxidation rate increased from 2 to 47 and 49 to 77 ml x m^{-2} x day^{-1} respectively

for stations A and B from January to May. Some of this increase undoubtedly reflects the higher incubation temperature used in May. The increase observed may also reflect the observed increase in the in situ temperature; the sediment temperature at station B increased from 1.5 C to 6.5 C during the same period of time methane production rate increased from 2 to 47 ml x m^{-2} x day^{-1}.

From the results of the methane concentration data, we would have anticipated the reverse seasonal pattern for methane production in the Port Moller sediments. The chemists observed the highest concentrations at the mouth of Port Moller in August and January and the lowest concentrations in May. It is possible that the few locations where methane production was observed seasonally were not representative of methane production rates for the area. The transport mechanisms responsible for releasing methane from the Port Moller area may have changed and/or the methane oxidation rates may have changed. The latter alternative seems unlikely since the observed methane oxidation rates in the Port Moller waters were fairly constant seasonally (Figs. 14 to 18).

4. Geographical patterns of methanogenesis

Prior to this study, it was known from previous methane concentration data that there was a signficant source of methane associated with the region around Port Moller and the center of the SGB. Our studies have shown that it is very unlikely that there is a significant methane source in the offshore waters of NAS and that the major source of methane in this region is Port Moller/Herendeen Bay. The extremely high methane production rates observed in this region strongly suggests that this is the source of methane which is used by the chemists to calculate transport rates in the NAS lease area. As far as the transport model is concerned,

it was very important to establish that there were no major secondary sources of methane outside of Port Moller. With the possible exception of a relatively insignificant input of methane from Port Heiden, it has now been documented that there are no such sources outside of Port Moller.

In the SGB, the results are more equivocal. In general, the methane production rates were highest in the center of the SGB sample grid. This is also the region where the methane concentrations were the highest in the water column. When these data sets are compared on a station by station basis however, the correlation is not good. Most of the chemistry data shows elevated methane concentrations in the region of stations SG5, SG70 and SG73 in May, SG28, SG29, PL6 and P18 in January and SG5, SG2, PL6 and PL8 in August.

The best sampling resolution was obtained by both the chemists and ourselves during the May cruise. During that cruise, the region where we found the highest methane production rates was just to the north of the region where the highest methane concentrations were observed in the water column. The poor correlation between these two data sets may, in part, be due to the nature of methane production in this region.

These sediments were not neatly layered with the surface being aerobic and the sediment becoming reduced with depth. When the cores were taken, numerous small reduced black zones could be seen in many of the subcores. This feature was even more pronounced in sediments that we stored for extended periods of time at our home laboratory. It appears that organic carbon is being trapped underneath the surface of the sediments in small pockets which become anaerobic and then produce methane. For this reason, the levels of methanogenesis usually varied

greatly from one subsample to the next. The rates observed with time in a given subsample was generally very consistent with r values usually 0.9 or above. When the rates in all subsamples were analyzed (either 4 or 6 subsamples per sample), the r value usually dropped to about 0.4. This suggests to us that the method used produced consistent results but that there was a great deal of heterogeneity between subsamples. This heterogeneity was again illustrated during the May cruise when we analyzed sediments from the same location on two different occasions. At one time we observed a rate of 0.04 and at the next we observed a rate of 0.12 ml x m^{-2} x day^{-1} at station SG5 (Fig. 27).

Another possible explanation for the lack of correlation between methane production and water column methane concentrations is that there is another source of methane other than from recent marine sediments. We did observe methane production in bottom water samples collected in SGB. It is our opinion however, that this is most probably due to the conditions of the experiment where oxygen levels were unnaturally low after extended incubation.

The other possible source of methane might be gas vents which are introducing methane into the system from old sediments; a situation similar to that reported by Cline and Holmes (1977) in the Norton Sound.

C. Potential impact of crude oil on biological productivity

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

We would like to define the "detrital food chain" or web as the movement of all organic carbon (organic nutrients) from one trophic level to the next where the carbon originates from sources other than the direct ingestion and direct utilization of plant material. Thus the nutrients that form the basis for the detrital food chain includes all 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 Pomeroy (1974) that the ocean's food web is detrital". Pomeroy 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. In a recent study by Fuhrman and Azam (1980), natural rates of bacterial biomass production were estimated in water samples collected from very different coastal marine locations. They concluded that bacterial biomass production was a quantitatively important component of coastal marine food webs.

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 recalcitrant compounds such 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%.

2. Crude oil effects on the detrital food chain.

There now exists in the literature, extensive documentation on the effects of crude oil on benthic organisms (Boucher, 1980; Carr and Reish, 1977; Elmgreen et al., 1980; 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 burrowing activity, which is now known to have an important function in the overall metabolism of the sediment by increasing the total oxidized surface area, is reduced. Burrowing activity is also responsible for the turning over of the sediments. 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 study of long-term crude oil effects on subArctic marine sediments (Griffiths and Morita, 1981) 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 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 burrowing activity.

During this same study, it was shown that there was a reduction in total adenylates indicating 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 bacteriovorous.

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; however, 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 the 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 are being cropped, this would mean a greatly reduced efficiency in converting detrital carbon into usable carbon for secondary productivity.

An additional major function of microorganisms in the detrital food chain is to produce enzymes which breakdown organic compounds which would otherwise accumulate in the sediments and thus would not be utilized by higher trophic levels. Our studies have shown that crude oil reduces the activity of some of these enzymes. More specifically, we have observed that the enzymes that breakdown cellulose and chitin (two structural polysaccharides) are inhibited. Actually, our data suggests that the enzyme itself may not be inhibited, but that the formation of the enzyme and/or the growth of the organisms that produce these enzymes are inhibited. Regardless of the mechanism involved, the results of our effects studies suggest that the degradation of chitin (the structural material in invertebrate shells), cellulose (the structural material in plants) and macrophytic material is reduced in the presence of crude oil.

Under normal conditions, such enzymes would solubilize complex compounds into simple molecules which are, in turn, taken up by the

bacteria to form new biomass. This biomass would then be utilized as a food source by higher trophic levels. It appears from our data that crude oil interferes with this process at all of these levels; from the enzymes that breakdown the complex organic molecules, to the formation of bacteria biomass, to the utilization of bacterial biomass as a food source.

3. The importance of long-term crude oil effects relative to overall productivity in NAS and SGB.

Iverson et al. (1979) concluded from their study of the SGB that in the middle shelf area, most of the organic carbon produced there is cycled through the detrital food chain. In the sub-Arctic marine environment, the total amount of available food cycled through the detrital food chain may range from 50 to 90%. Our studies on crude oil effects indicate that at a concentration of 1 ppt, the production of bacterial biomass (the basis for this food chain) is reduced by at least 50% in sediments exposed for as long as one year. Even if this was the only adverse effect caused by crude oil, we would expect to find a reduction of 25 to 45% in the amount of food available to the higher trophic levels in the impacted area. In the SGB it is very likely that many commercially valuable species are directly dependent on food from the detrital food chain. Therefore an extensive oil spill in this region could have a serious impact on the commercial fishing industry particularly if crude oil became associated with the sediments of the middle shelf area.

At this time, we have no good estimate on how much of the inorganic nutrients present in the SGB are generated locally by microbial mineralization. From what is known about the circulation patterns in this region, it is quite likely that most of the inorganic nutrients present are regenerated

locally (J. Schumacher, PMEL, personal communication). If this is an important source of inorganic nitrogen and phosphorous for primary production we would anticipate a further reduction in overall productivity if this area were extensively perturbed by crude oil.

D. Regions of high potential impact in NAS and SGB.

1. St. George Basin

During a recent NOAA sponsored synthesis meeting on the St. George Basin, it was concluded that it was possible to transport large quantities of crude oil into the sediments of this region. This would occur primarily during storm surges when the entire water column is disturbed. Since crude oil production is planned in this important Alaskan fishery, it is important to determine if there are any regions where crude oil impact would do the most damage in case of a major accident. We feel that we have located such a region within SGB.

This was done by measuring rates of several microbial processes which reflect levels of microbial metabolism in marine sediments. The variables studies were CO_2 evolution, nitrogen fixation, relative microbial activity (glutamic acid uptake) and the activity of the enzymes phosphatase, arylsulfatase, amylase and laminarinase. The CO_2 evolution rates, enzyme rates and relative microbial activity were chosen because they all reflect key biological processes in carbon cycling and/or processes associated with mineralization. They also are all known to be functions that are altered by the impact of crude oil (Griffiths and Morita, 1981). Nitrogen fixation was chosen since it is very sensitive to crude oil perturbation and it can be a key component of the detrital food chain.

During the January cruise relative microbial activity and nitrogen fixation rates were measured in sediments of the SGB (Figs. 40, 41 and

44). The relative microbial activity was measured by using both glucose and glutamic acid. The resulting activity pattern was essentially the same regardless of the substrate used to measure relative microbial activity. The area where nitrogen fixation and relative microbial activity were higher than the mean is defined by the enclosed area "A" in Fig. 53.

During the May cruise, a much more comprehensive study of microbial function was conducted on the sediments of SGB. The region of high enzyme activity was well defined by all of the enzymes tested (Figs. 48 to 51). If these four data sets are compared, the region of highest activity for all enzymes ($\geq$ the mean) is defined by a circle with a radius of approximately 54 km and a center at station PL7 (Fig. 53, enclosure "B"). If the same comparison is made with the CO_2 production, nitrogen fixation and relative microbial activity data (Figs. 42, 44 and 46), the area of high activity overlap is defined by enclosed area "C" in Fig. 53. Since this area is within the circle of high enzyme activity, it represents a region where all variables studied showed elevated activities. It is our opinion that the secondary productivity of this region would be significantly impacted by crude oil concentrations of 1 ppt or greater. This opinion is based on the long-term crude oil effects studies that we conducted in Cook Inlet (Griffiths and Morita, 1981).

If the results of both cruises are combined, there is a region surrounding station PL7 which was found to have elevated activities in both January and May. Although the May data set is much more comprehensive and probably more accurately reflects the pattern of microbial function in this region, it is important to note that there was an overlap of high activity in both of these data sets. We strongly recommend that no drilling be conducted in the 22 km radius around station PL7 (the approximate

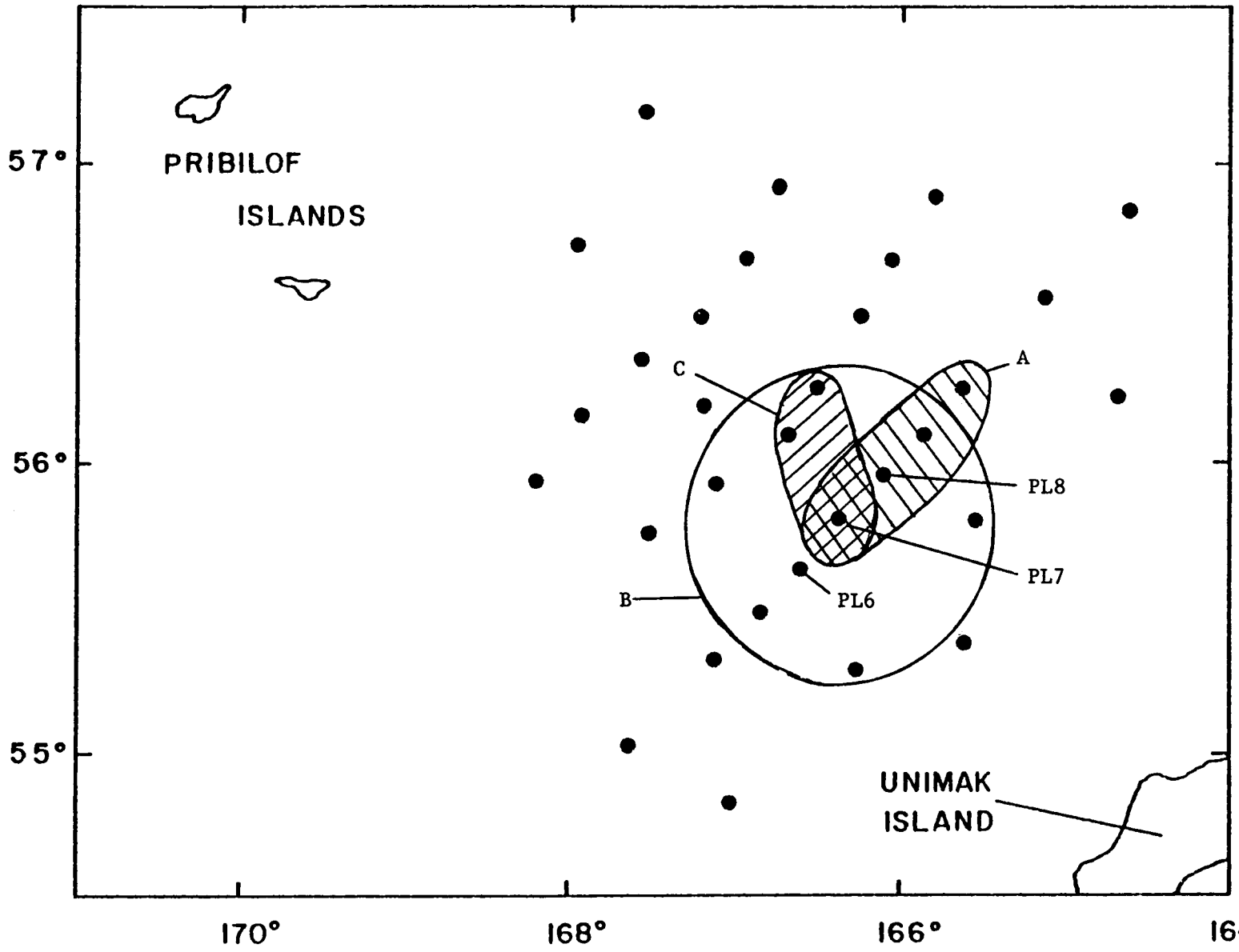

Figure 53. Areas of high microbial activity in sediments of the St. George Basin. A = area of elevated activity during January cruise. B = area of high enzyme activity during May cruise. C = area of elevated activity as defined by nitrogen fixation, glutamate uptake and CO_2 evolution rates in May.

radius of the overlap area around station PL7). Not only is this a very sensitive region but it is located in an area that would impact the sensitive area to the north in the event of a blow-out. From what is known of the hydrology of the area, it is thought that the prevailing near bottom water current moves to the north in this area (J. Schumacher and J. Cline, PMEL, personal communication). If there was a break in an oil production pipe at the sediment surface, the prevailing bottom currents could carry the crude oil in the direction of the most sensitive area as defined by the May data set (Fig. 53).

It should also be noted that this is also a region where elevated relative microbial activities were also observed in the bottom waters (Fig. 36). These bottom water samples were taken just above the sediment and should reflect the activities of microbially active and particulate laden waters in this portion of the water column. Although the effects of crude oil on these biologically active waters are not known, this is the link between the water column and the sediments in which changes are known to occur.

2. North Aleutian Shelf

Most of the waters in this region are relatively shallow and physically dynamic. The fact that no fine grained sediments were found in the sediments outside of the major bays suggest that the bottom currents keep this matter in suspension rather than depositing them into the sediments of the region. If there were a major oil spill in the NAS area, crude oil could become associated with the suspended matter in the well-mixed inshore waters. The oil could then be transported directly into the major bays of the Aleutian Penninsula. A much greater threat to the major bays would come from direct oiling via a surface slick.

During the May cruise, we measured relative microbial activity, methanogenesis CO_2 evolution rates, and enzyme activities in the Port Moller/Herendeen Bay area. These rates were all significantly higher than those found in SGB and in some cases, the rates were more than 100 times greater than those observed anywhere in SGB. In terms of carbon respired to CO_2, the mean rate in Port Moller was 0.29 g carbon x m^{-2} x day^{-1} and in the SGB the mean was 0.035. Thus the release of carbon from Port Moller was 8 times than that observed in SGB. The mean glutamate uptake was also 8 times higher in Port Moller than that observed in the SGB. The elevated rates observed in Port Moller suggest that there is a great deal of biological activity at the heads of this Bay. Although Port Moller was the only bay in the NAS that we studied, there is no reason to believe that the other major bays should be any less active and presumably biologically important. It is therefore our recommendation that all possible precautions be taken to keep crude oil from entering these bays.

VIII. Conclusions

The methane oxidation and methanogenesis data that we have collected in the St. George Basin and North Aleutian Shelf lease areas during the NASTE cruises have been useful in determining the biological sources and sinks of methane in these areas. The implications of these observations relative to the problem of transport processes in these lease areas are still being analyzed by the chemists at PMEL. The conclusions that can be drawn from these data at this point in the analysis will be discussed in Dr. Cline's final report for RU #153.

Our studies of microbial function in this study area have indicated that the regions that would be most sensitive to crude oil perturbation would be the major bays along the Alaskan Penninsula and the center of the St. George Basin in the vicinity of 55°48'N, 166°22'W. If fresh crude oil concentrations $\geq$ 1.0 ppt accumulated in the marine sediments of these areas as the result of an oil spill, we predict that significant reductions in biological productivity would result. This could ultimately result in a long-term reduction in the fisheries productivity in the southern Bering Sea.

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X. Literature Cited

Barnes, R. O., and E. D. Goldberg. 1976. Methane production and consumption in anoxic marine sediments. Geology 4:297-300.

Belyaev, S. S., K. S. Laurinavichus, and M. V. Ivanov. 1975. Determination of the intensity of the process of microbial oxidation of methane using $^{14}CH_4$. Microbiol. 44:478-481.

Boucher, G. 1980. Impact of Amoco Cadiz oil spill on intertidal and sublittoral meifauna. Mar. Pollut. Bull. 11:95-101.

Carr, R. S., and D. J. Reish. 1977. The effect of petroleum hydrocarbons on the survival and life history of polychaetous annelids. 168-173. In D. A. Wolfe (ed.), Fate and Effects of Petroleum Hydrocarbons in Marine Organisms and Ecosystems. Pergamon Press, New York.

Cline, J. D., and J. L. Holmes. 1977. Submarine seepage of natural gas in Norton Sound, Alaska. Science 198:1149-1153.

Cole, J. J., and G. E. Likens. 1979. Measurements of mineralization of phytoplankton detritus in an oligotrophic lake. Limnol. Oceanogr. 24:541-547.

Elmgren, R., S. Hansson, U. Larsson, and B. Sundelin. 1980. Impact of an oil on deep soft sediments. 97-126. In J. J. Kineman, R. Elmgren, S. Hansson (eds.). The Tsesis Oil Spill. U.S. Dept. Commerce.

Fenchel, T. M., and B. B. Jørgensen. 1977. Detritus food chains of aquatic ecosystems: The role of bacteria. In: Advances in Microbial Ecology 1:1-58.

Fuhrman, J. A. and F. Azam. 1980. Bacterioplankton secondary production estimates for coastal waters of British Columbia, Antarctia, and California. Appl. Environ. Microbiol. 39:1085-1095.

Fuhrman, J. A., J. W. Ammerman, and F. Azam. 1980. Bacterioplankton in the coastal euphotic zone: Distribution, activity and possible relationships with phytoplankton. Mar. Biol. 60:201-207.

Giere, O. 1979. The impact of oil pollution on intertidal meiofauna. Field studies after the La Coruna-spill, May 1976. Cashiers De Biol. Mar. 20:231-251.

Griffiths, R. P., and R. Y. Morita. 1981. Study of microbial activity and crude oil-microbial interactiosn in the waters and sediments of Cook Inlet and the Beaufort Sea. Final Report to NOAA/OMPA.

Griffiths, R. P., S. S. Hayasaka, T. M. McNamara, and R. Y. Morita. 1978. Relative microbial activity and bacterial concentrations in water and sediment samples taken in the Beaufort Sea. Can. J. Microbiol. 24:1217-1226.

Harrison, G. P., and K. H. Mann. 1975. Detritus formation from eelgrass (Zostera marina L.): the relative effects of fragmentation, leaching and decay. Limnol. Oceanogr. 20:924-934.

Harrits, S. M., and R. S. Hanson. 1980. Stratification of aerobic methane oxidizing organisms in Lake Mendota, Madison, Wisconsin. Limnol. Oceanogr. 25:412-421.

Higgins, I. J., and D. J. Best, and R. C. Hammond. 1980. New findings in methane oxidizing bacteria highlight their importance in the biosphere and their commercial potential. Nature 286:561-564.

Hutton, W. E., and C. E. ZoBell. 1949. The occurrence and characteristics of methane oxidizing bacteria in marine sediments. J. of Bacteriol. 58:463-473.

Hutton, W. E., and C. E. ZoBell. 1953. Production of nitrite from ammonia by methane oxidizing bacteria. J. of Bacteriol. 65:216-219.

Iversen, N., and T. H. Blackburn. 1981. Seasonal rates of methane oxidation in anoxic marine sediments. Appl. Environ. Microbiol. 41:1295-1300.

Jannash, H. W. 1975. Methane oxidation in Lake Kiva (Central Africa). Limnol. Oceanogr. 20:860-864.

Larrson, U., and A. Hagstrom. 1979. Phytoplankton exudate release as an energy source for the growth of pelagic bacteria. Mar. Biol. 52: 199-206.

Mann, K. H. 1972. Macrophyte production and detritus food chains in coastal waters. In: Detritus and Its Ecological Role in Aquatic Ecosystems. Proc. IBP-UNESCO Symp. Pallanza. It. pp. 353-383.

McRoy, C. P. 1970. Standing stocks and other features of eelgrass (Zostera marine) populations on the coast of Alaska. J. Fish. Res. Bd. Canada. 27:1811-1821.

Naiman, R. J., and J. R. Sibert. 1979. Detritus and Juvenile salmon production in the Nanaimo estuary: III. Importance of detrital carbon to the estuarine ecosystem. J. Fish. Res. Board Can. 36: 504-520.

Oremland, R. S. 1975. Methane production in shallow-water tropical marine sediments. Appl. Microbiol. 30:602-608.

Oremland, R. S. 1979. Methanogenic activity in plankton samples and fish intestines: A mechanism for in situ methanogenesis in oceanic surface waters. Limnol. Oceanogr. 24:1136-1141.

Paerl, H. W. 1978. Microbial organic carbon recovery in aquatic ecosystems. Limnol. Oceanogr. 23:927-935.

Panganiban, A. T., Jr., T. E. Patt, W. Hart, and R. S. Hanson. 1979. Oxidation of methane in the absence of oxygen in lake water samples. Appl. Environ. Microbiol. 37:303-309.

Pomeroy, W. M. 1974. The ocean's food web, a changing paradigm. Bioscience 24:499-504.

Reeburgh, W. S. 1976. Methane consumption in Cariaco Trench waters and sediments. Earth and Planetary Science Letters 28:337-344.

Reeburgh, W. S., and D. T. Heggie. 1977. Microbial methane consumption reactions and their effects on methane distribution in freshwater and marine environments. Limnol. Oceanogr. 22:1-9.

Ribbons, D. W., J. E. Harrison, and A. M. Wadzinski. 1970. Metabolism of single carbon compounds. Ann. Rev. Microbiol. 24:135-158.

Rudd, J. W. M., and R. D. Hamilton. 1975. Factor controlling rates of methane oxidation and the distribution of the methane oxidizers in a small stratified lake. Archiv. fur Hydrobiologie 75:522-538.

Rudd, J. W. M., and C. D. Taylor. 1980. Methane cycling in aquatic environments. Adv. Aquatic Microbiol. 2:77-150.

Rudd, J. W. M., R. D. Hamilton, and N. E. R. Campbell. 1974. Measurement of microbial oxidation of methane in lake water. Limnol. Oceanogr. 19:519-524.

Sansone, F. J., and C. S. Martens. 1978. Methane oxidation in Cape Lookout Bight, North Carolina. Limnol. Oceanogr. 23:349-355.

Scranton, M. I., and P. G. Brewer. 1977. Occurrence of methane in the near-surface waters of the western subtropical North Atlantic. Deep-Sea Res. 24:127-138.

Scranton, J. T., and P. G. Brewer. 1978. Consumption of dissolved methane in the deep ocean. Limnol. Oceanogr. 23:1207-1213.

Smith, D. F., and W. J. Wiebe. 1976. Constant release of photosynthate from marine phytoplankton. Appl. Environ. Microbiol. 32:75-79.

Stewart, W. D. P., G. P. Fitzgerald, and R. H. Burns. 1967. In situ studies on N_2 fixation using the acetylene reduction technique. Proc. Nat. Acad. Sci. (US) 59:2071-2078.

Swedmark, M., A. Granmo, and S. Kollgerg. 1973. Effects of oil dispersants and oil emulsions on marine animals. Water Res. 7:1649-1672.

Tabatabai, M. A., and J. M. Bremner. 1969. Use of p-nitrophenyl phosphate for assay of soil phosphatase activity. Soil Biol. Biochem. 1: 301-307.

Tabatabai, M. A., and J. M. Bremner. 1970. Arysulfatase activity of soil. Soil Sci. Soc. Ann. Proc. 34:225-229.

Taylor, T. L., and J. F. Karinen. 1977. Response of the clam, Macoma balthica (Linnaeus), exposed to Prudhoe Bay crude oil as unmixed oil, water-soluble fraction, and oil-contaminated sediment in the laboratory. 299-237. In: D. A. Wolfe, (ed.), Fate and Effects of Petroleum Hydrocarbons in Marine Organisms and Ecosystems. Pergamon Press, New York.

Thayer, G. W., D. W. Engel, and M. W. LaCroix. 1977. Seasonal distribution and changes in the nutritive quality of living, dead, and detrital fractions of Zostera marina L. J. Exp. Mar. Biol. Ecol. 30:109-127.

Weaver, T. L., and P. R. Dugan. 1972. The eutrophication implications of interactions between naturally occurring particulates and methane oxidizing bacteria. Water Res. 6:817-828.

Weaver, T. L., and P. R. Dugan. 1972. Enhancement of bacterial methane oxidation by clay minerals. Nature 237:518.

Welch, H. E., J. W. M. Rudd, and D. W. Schindler. 1980. Methane addition to an arctic lake in winter. Limnol. Oceanogr. 25:100-113.

BIODEGRADATION OF AROMATIC COMPOUNDS BY HIGH LATITUDE PHYTOPLANKTON

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SUMMARY

It was the purpose of the work undertaken to bring into pure culture representative diatoms from the Cook Inlet and the ice-edge in the Bering Sea and to examine their capacity for the oxidation of aromatic compounds using naphthalene as a model substrate. Three diatoms from the Cook Inlet (Kasitsna Bay) were shown to metabolize naphthalene at 6 or 12°C to 1-naphthol and other unidentified ethyl acetate and water-soluble products. Likewise, three diatoms isolated from samples collected at the ice-edge in the Bering Sea also formed small amounts of 1-naphthol from naphthalene when incubated in the light at 0 or 10°C.

We have not been able to rigorously prove that any algal cell, be it a blue-green alga, a green alga, or a diatom can metabolize (1-^{14}C) naphthalene far enough to produce $^{14}CO_2$. However, if we assume a stoichiometery of one 1-naphthol in the algae equivalent to one CO_2 in bacteria, then for mesophilic algae, the rate of 1-naphthol production is roughly estimated as 10% of the *in situ* marine potential, and perhaps higher if only the photic zone is considered. We have as yet, no corresponding values for rate of 1-naphthol formation from naphthalene by cold-adapted or psychrophilic diatom cultures, however, it seems reasonable to suggest that algal aromatic transformations may also be a significant fraction of bacterial activity in cold environments. In addition to studies on the oxidation of naphthalene we have also examined the sensitivity of the Bering Sea psychrophilic diatoms to crude oil samples from Cook Inlet and Prudhoe Bay. The results with pure cultures indicate that the toxicity of crude oil was enhanced in psychrophilic diatoms growing at 0°C or 10°C as compared to previous studies with mesophilic forms.

There are several important consequences of the results for Alaskan OCS oil and gas development. It is now clear that pure cultures of diatoms isolated from either the lower Cook Inlet or from the ice-edge in the Bering Sea can oxidize aromatic compounds such as naphthalene. Whether the metabolites persist through the food chain and will be more or less toxic than naphthalene itself is not known. The results with naphthalene also imply that the photic zone can be an important sink for aromatic hydrocarbon transformations. There are certainly differences among microalgae in the capacity to oxidize naphthalene. It seems prudent, therefore, to insure, via monitoring, that accidental introduction of aromatic compounds in Alaskan waters does not cause a selective or enrichment effect on existing phytoplankton populations.

A second area of environmental concern is the suggestion of an enhanced crude oil toxicity in slower growing psychrophilic diatoms as compared to their mesophilic cousins. Crude oil spills near or under the sea ice may severely impact primary productivity, and thereby higher tropic level.

INTRODUCTION

The results are presented in two sections, one dealing with the Cook Inlet isolates and the other with the isolates from the ice-edge in the Bering Sea. Each section has an introduction, description of materials and methods, results and discussion section, and references.

THE OXIDATION OF NAPHTHALENE BY DIATOMS ISOLATED FROM THE KACHEMAK BAY REGION OF ALASKA

Aromatic hydrocarbons have been found to be widely distributed in open ocean waters (Brown and Huffman, 1976). Many of these compounds and/or their metabolites have toxic properties which include initiation of tumor formation and cancer (Miller and Miller, 1976). In studies of the fate of hydrocarbons in aquatic ecosystems, a considerable amount of information is available on the bacterial and fungal degredation of these compounds and their derivatives (Atlas, 1981; Cerniglia, 1981). In view of the fact that cyanobacteria and microalgae are widely distributed in many aquatic environments and may be important in the catabolism of hydrocarbons, we initiated a research program on the algal oxidation of aromatic hydrocarbons (Cerniglia et al. 1979, 1980 a,b,c).

Most of the studies on the microbial oxidation of hydrocarbons have been conducted at temperatures between 20°C to 30°C. Since there has been increased activities of oil exploration and transport of petroleum in Alaskan waters, there has been recent interest in the microbia degradation of crude oil at low water temperatures (Atlas, 1981).

In this investigation, we report on three diatoms isolated from the Kachemak Bay region of Alaska which have the ability to metabolize the aromatic hydrocarbon, naphthalene at low temperatures.

MATERIALS AND METHODS

Organisms and Growth Conditions. The diatoms K1A (Navicula sp.), K8A (Nitzschia sp.) and 4D (Synedra sp.) were isolated via enrichment culture at 6 to 10°C from oblique net (20 μm Nitex nylon) tows made during August 1979 and April, 1980 in the Kachemak Bay region, south of Homer, Alaska. The enrichment medium was local sea water plus 5, 20, or 50% ASP-2 medium (Van Baalen, 1962). Pure cultures were obtained by repeated streaking or by several minutes treatment with ultraviolet radiation (254 nm, 15 W germicidal lamp) and subsequent pour plates. Organism N-1 (Cylindrotheca sp.) was isolated from a water sample taken from the Pass adjacent to the Port Aransas Marine Laboratory (Estep et al., 1978). The organisms were grown on ASP-2 medium containing 125 mg l^{-1} $Na_2SiO_3.9H_2O$, 4 μg l^{-1} vitamin B_{12} and 250 μg l^{-1} thiamine in 22 x 175 mm Pyrex test tubes at 12°C. The growth tubes were illuminated with fluorescent lamps F20T12-WWX two on each side of the water bath, 8 cm from the front edge of the lamp to the tube center. The cultures were continuously aerated with 1 ± 0.1% CO_2 enriched air. The generation times under these conditions for the four organisms were about 24 hours.

Naphthalene Biotransformation Experiments. [1-^{14}C]-Naphthalene experiments were conducted in order to determine the amount of naphthalene oxidized by each organism. Cells (0.5 to 0.8 mg) were pooled from several growth tubes and placed in 22 x 175 mm screw cap tubes, final volume 10 ml. [^{14}C]-Naphthalene (1 μCi in 20 μl ethanol, 6.9 mg/liter) was added just before closing the tube with a plastic top lined with a chromatography septum, aluminum foil and 1 ml Teflon film. Carbon dioxide was added

through a small hole in the plastic top with a gas tight syringe to an initial concentration of 1%. The screw cap tubes were clamped to a glass rod and rotated slowly in the same illuminated water bath used for growing the cultures. The tubes were incubated at either 6°C or 12°C.

After 22 hr incubation, cells were removed by centrifugation and each supernatant extracted with five thirty ml volumes of ethyl acetate. The organic extracts were dried over anhydrous sodium sulfate and the solvent was removed in vacuo at 42°C. Each residue was redissolved in methanol and analyzed by high pressure liquid chromatography. The ratio of ethyl acetate soluble metabolites to water soluble metabolites was determined by taking each organic soluble extract and redissolving in 50 ul of acetone and 10 ul aliquots was added to vials containing 10.0 ml of scintillation fluid. The radioactivity present was determined in a liquid scintillation counter. Corrections were made for machine efficiency and quenching.

An experiment with unlabeled naphthalene was conducted with organism K8A in order to obtain sufficient material for the isolation and structure elucidation of the naphthalene biotransformation products. Four 10 ml samples of organism K8A were incubated in crew cap tubes as described above with 6.9 mg/liter naphthalene at 12°C. After 22 hr the cells were centrifuged and the supernatant was extracted and concentrated as described above. The residue was redissolved in methanol and analyzed by gas-chromatography and mass spectrometry.

Analysis of Metabolic Products. High pressure liquid chromatography (hplc) was used for the separation of metabolites. All hplc analysis were

performed on a Beckman Model 332 hplc and Model 155-10 variable wavelength absorbance detector (Beckman Instruments, Inc., Berkeley, CA, USA) operated at 254 nm. An Altex Ultrasphere-ODS Column (25 cm x 4.6 mm id) [Altex Scientific, Inc., Berkeley, CA, USA] was used for the separation of naphthalene metabolites, which was achieved with a programmed methanol/water gradient (50 to 95%, v/v, 30 min.) with a flow rate of 1 ml/min. In experiments with [^{14}C]-naphthalene, 0.5 ml fractions were collected at 0.5 min. intervals in scintillation vials and 5.0 ml of Aquasol-2 (New England Nuclear Corp., Boston, MA, USA) was added to each vial. The radioactivity present in ach fraction was determined in a Beckman LS-250 liquid scintillation counter.

Gas chromatographic and mass spectral analysis (GC-MS) of naphthalene metabolites was performed on a Finnigan Model 3100 mass spectrometer coupled to a gas chromatograph equipped with a glass column (2 m x 1.5 mm id) packed with 3% OV-1 on Chromosorb Q. The injection temperature was 50°C with a temperature program of 100-250°C at 8°C/min. The carrier gas was helium, with a flow rate of 30 ml/min. The following conditions were used for mass spectrometry: molecular separator temperature 350°C; ion source temperature 100°C ionization beam 70 eV; and ionization current 200 uA.

Chemicals: Naphthalene (99.9%) was from Aldrich Chemical Co., Milwaukee, Wis., USA. [1(4,5,8)-^{14}C]-Naphthalene [5 mCi/mmol] was from Amersham Searle, Arlington Heights, Il., USA. All naphthalene derivatives were purified as described previously (Cerniglia and Gibson, 1977). Solvents for hplc were purchased from Burdick and Jackson Laboratories, Muskegon, Mich., USA.

RESULTS AND DISCUSSION

Three pure cultures of diatoms isolated from Alaskan waters (strains K8A, 4D and K1A) were incubated with [^{14}C]-naphthalene at either 6°C or 12°C. The hplc elution profile of the ethyl-acetate soluble naphthalene metabolites formed by each diatom is shown in Fig. 1. For comparative purposes the chromatographic properties of synthetic naphthalene derivatives is shown in Fig. 1A. All of the organisms oxidized naphthalene to a compound which co-chromatographed with 1-naphthol. These results are similar to our earlier studies on the oxidation of naphthalene by cyanobacteria and microalgae (Cerniglia et al., 1980b).

In order to confirm that 1-naphthol was the major metabolite in the oxidation of naphthalene, cells of Nitzschia sp. strain K8A were incubated for 22 hr. in the presence of naphthalene and the ethyl acetate soluble extract analyzed by GC-MS. The GC-MS analysis of the ethyl acetate extract of the metabolism of naphthalene by Nitzschia sp. strain K8A showed a compound that had a similar retention time (9.5 min.) and mass spectrum (m/e 144) to that of authentic 1-naphthol.

Table 1 shows that these diatoms oxidized naphthalene to both organic soluble and water soluble derivatives. The amount of naphthalene oxidized ranged from 0.7 to 1.2%. It is also interesting to note that Cylindrotheca sp. strain N-1 when grown at 12°C, wherein it had a similar rate as oranism 4D gave less total naphthalene oxidation (Table 1). This data suggests that cold-water adapted microalgae may prove to be more metabolically active than is implied by their slow growth rates.

In an earlier study we showed that the cyanobacterium Oscillatoria sp. strain JCM oxidized 4.8% of the added naphthalene. The

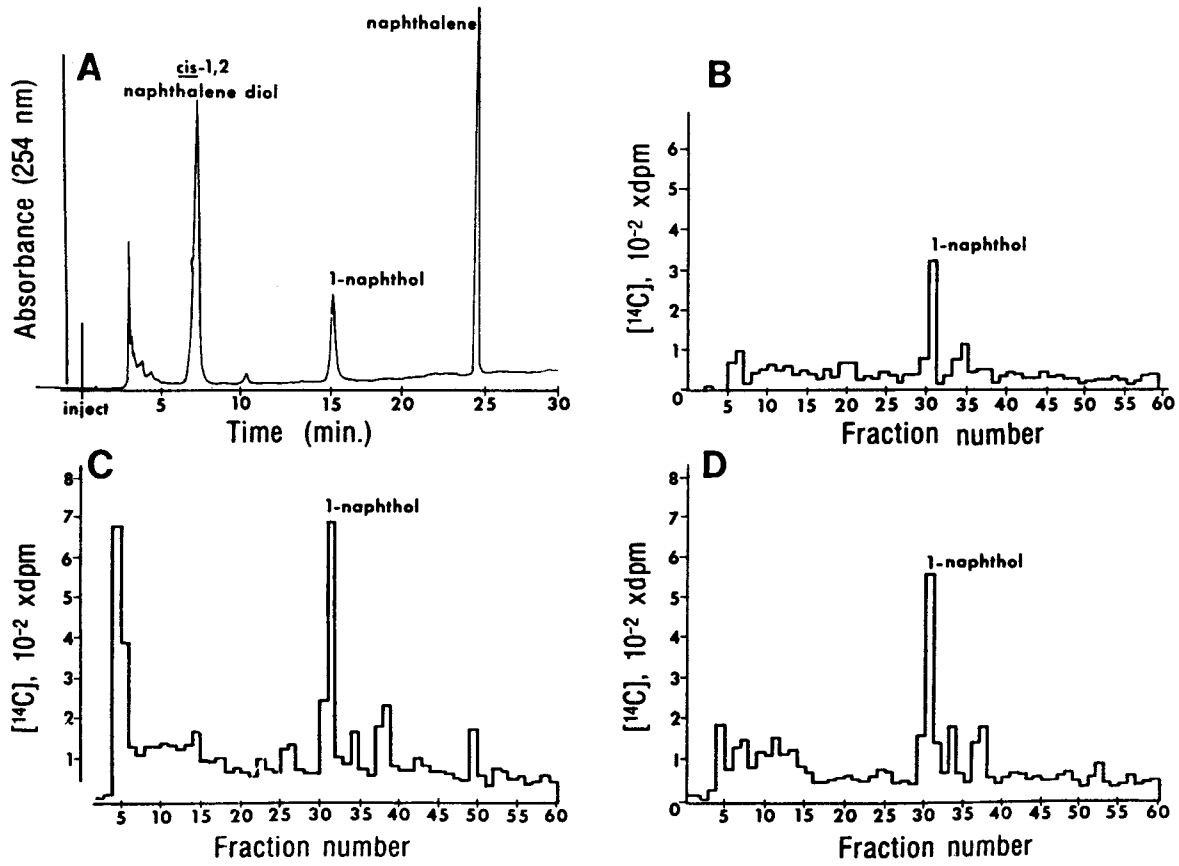

Figure 1. Hplc elution profile of metabolites formed from [^{14}C]-naphthalene by different diatoms. A, resolution of a mixture of synthetic naphthalene derivatives. B, Nitzschia sp. strain K8A; C, Synedra sp. strain 4D; D, Navicula sp. strain K1A.

Hplc conditions were as described in Methods.

Table 1. Distribution of Radioactivity in the Ethyl Acetate Soluble and Water-Soluble Metabolites Formed from [^{14}C]-Naphthalene by Diatoms.

Organism	d.p.m. mg dry wt^{-1}		Total Radioactivity	Percentage Metabolism of Naphthalene
	Organic-Soluble	Water-Soluble		
Nitzschia sp. strain K8A	8,965 (49)*	9,311 (51)	18,276	0.7
Synedra sp. strain 4D	18,044 (58)	13,332 (42)	31,376	1.2
Navicula sp. strain K1A	9,658 (55)	7,987 (45)	17,645	0.7
Cylindrotheca sp. strain N-1	6,550 (43)	8,784 (57)	15,334	0.6

* percent of total metabolites

ratio of ethyl acetate soluble metabolites to water-soluble metabolites was 41:59. Table 1 shows that all of the diatoms formed water-soluble products. The identification of these products remains to be determined but these results suggest that diatoms may have the ability to oxidize naphthalene to ring cleavage or conjugated products.

The results herein extend the original observations on the oxidation of naphthalene by temperate forms (Cerniglia et al., 1980b) to cold-adapted diatoms and reinforce the view that the capacity for oxidation of aromatic compounds is a general metabolic feature in the microalgae. Algal rates of aromatic oxidation versus rates for the aerobic heterotrophic microbial populations in the photic zone are unknown. However, the photic zone in the sea may prove to be a major sink for transformations of aromatic compounds in nature. Whether this will increase or decrease their toxicity for zooplankton and higher trophic levels is unknown.

ACKNOWLEDGEMENTS

We are very indebted to Dr. Robert P. Griffiths for his invaluable help with the phytoplankton tows. We are grateful to Drs. Rita Horner and Robert A. Gibson for their help with identification of the cultures.

REFERENCES

Atlas, R.M. (1981). Microbial Degradation of Petroleum Hydrocarbons: An Environmental Perspective. Microbiological Reviews, 45, 180-209.

Brown, R.A. and Huffman, H.L., Jr. (1976). Hydrocarbons in Open Ocean Waters. Science, 191, 847-849.

Cerniglia, C.E. (1981). Aromatic Hydrocarbons: Metabolism by Bacteria, Fungi and Algae. In Reviews in Biochemical Toxicology, 3, pp. 321-361. Edited by E. Hodgson, J.R. Bend and R.M. Philpot. Elsevier/North Holland, New York, U.S.A.

Cerniglia, C.E., Gibson, D.T. and Van Baalen, C. (1979). Algal Oxidation of Aromatic Hydrocarbons: Formation of 1-Naphthol from Naphthalene by Agmenellum quadruplicatum, Strain PR-6. Biochemical and Biophysical Research Communications, 88, 50-58.

Cerniglia, C.E., Gibson, D.T. and Van Baalen, C. (1980a). Oxidation of Naphlhalene by the Cyanobacterium Oscillatoria sp. Strain JCM. Journal of General Microbiology, 116, 485-494.

Cerniglia, C.E., Gibson, D.T. and Van Baalen, C. (1980b). Oxidation of Naphthalene by Cyanobacteria and Microalgae. Journal of General Microbiology, 116, 495-500.

Cerniglia, C.E., Van Baalen, C., and Gibson, D.T. (1980c). Oxidation of Biphenyl by the Cyanobacterium Oscillatoria sp. Strain JCM. Archives of Microbiolgoy, 125, 203-209.

Estep, M.F., Tabita, F.R. and Van Baalen, C. (1978). Purification of Ribulose 1,5-Bisphosphate Carboxylase and Carbon Isotope Fractionation by Whole Cells and Carboxylase from Cylindrotheca sp. (Bacillariophyceae). Journal of Phycology, 14, 183-188.

Miller, E.C. and Miller, J.A. (1976). The Metabolism of Chemical Carcinogens to Reactive Electrophiles and Their Possible Mechanisms of Action in Carcinogenesis. In Chemical Carcinogens, pp 737-762. Edited by C.E. Searle. ACS Monograph 173, Washington, D.C.: American Chemical Society.

Van Baalen, C. (1962). Studies on Marine Blue-Green Algae. Botanica Marina, 4, 129-139.

BERING SEA DIATOMS: GROWTH CHARACTERISTICS, OXIDATION OF NAPHTHALENE, AND SENSITIVITY TO CRUDE OIL

It has been known for many years that a rich assemblage of microalgae, primarily diatoms and small flagellates, is associated with the underside of the sea ice, the so-called ice algae (1). These ice forms are believed to contribute importantly to the primary production of the polar regions (2,3). In the Bering Sea the ice algae comprised the first spring bloom well preceeding blooms that occurred in the open water further south (3). We herein describe the photoautotrophic growth characteristics of pure cultures of psychrophilic diatoms isolated from water and ice samples taken at the ice edge in the Bering Sea, February-March, 1981 (4). In addition, because of the very real impact that petroleum exploration and production in the Arctic may have on the ice microflora (5) we have measured growth rates in the presence of two representative crude oils from the Cook Inlet and Prudhoe Bay.

Samples were collected from water pumped from the bow intake system of the R/V Surveyor, from melted ice cores, or from small pieces of floating, brown colored ice (Table 2). The samples were submitted to enrichment culture within one hour of collection. The medium for the enrichment cultures was composed of filtered (0.4 μm) 1/2 local sea water plus 1/2 of a synthetic algal medium, KASP-2 (6). Medium KASP-2 contained per liter of glass distilled water: 18 g NaCl; 5 g $MgSO_4.7H_2O$; 0.60 g KCl; 0.37 g $CaCl_2.2H_2O$; 1 g $NaNO_3$; 0.05 g KH_2PO_4; 1 g tris(hydroxymethyl)-aminomethane; 0.03 g ethylenediaminetetraacetic acid, disodium salt,

Table 2. Water and ice samples; Leg II Surveyor S132, 22 Feb. to 14 Mar., 1981

GMT	Date	Latitude	Longitude	Type
1800	Feb. 23	55°44.4'	157°24.1'	Shipboard pumped sea water filtered through 10 µm Nitex nylon net
1900	Feb. 24	55°4.3'	162°11.1'	Pumped sea water and 10 µm net
1830	Feb. 25	55°57.5'	168°45.3'	Pumped sea water and 10 µm net
1730	Feb. 26	58°7.5'	173°17.4'	Two ice cores, courtesy of Seelye Martin, bottom 3-4 cm thawed and filtered through 0.2 µm sterile filter
1830	Feb. 26	58°13.4'	173°8.6'	Pumped sea water and 10 µm net, sea water temp. 1.0°C[a]
1745	Feb. 27	58°26.6'	172°51.2'	Piece of "brown ice" thawed in 10 µm net
1930	Feb. 27	58°29.0'	172°37.1'	Pumped sea water and 10 µm net, sea water temp. 0°C
1900	Feb. 28	58°07.6'	173°17.8'	Pumped sea water and 10 µm net
1830	Mar. 1	58°39.9'	172°19.6'	Pumped sea water and 10 µm net
1800	Mar. 3	59°15.5'	171°12.0'	"Brown ice" thawed in 10 µm net, sea water temp. -1.5°C
1800	Mar. 4	59°18.2'	171°29.2'	Pumped sea water and 10 µm net
0400	Mar. 5	59°17.0'	171°41.8'	Surface tow, 10 µm net in clearings around ice
1900	Mar. 5	59°08.8'	171°55.0'	Pumped sea water and 10 µm net, sea water temp. -1.2°C
2300	Mar. 5	59°07.7'	171°50.4'	Pumped sea water 10 µm net
1830	Mar. 7	58°43.0'	172°15.4'	Pumped sea water 10 µm net
1930	Mar. 8	58°46.3'	172°51.7'	Pumped sea water 10 µm net, sea water temp. 0.6°C
1900	Mar. 9	58°32.9'	173°29.4'	Pumped sea water 10 µm net

[a] Not precise temperatures, listed only to give some idea of the prevailing temperatures at the time the algal samples were collected.

dihydrate; 0.004 g $FeCl_3.6H_2O$; 0.034 g H_3BO_3; 0.004 g $MnCl_2.4H_2O$; 670 g $ZnSo_4.7H_2O$; 38 g $Na_2MoO_4.2H_2O$; 12 g $CoCl_2.6H_2O$; 0.3 g $CuSo_4.5H_2O$ and supplemented with 0.125 g $Na_2SiO_3.9H_2O$, 300 g thiamine; 8 g vitamin B_{12}; 30 g biotin. The enrichment cultures were incubated in continuous light at -1 to 0°C in the shipboard flowing water system (one overhead fluorescent fixture) or at 5 to 7°C in a refrigerator (one 40W tungsten lamp). The cultures were frequently examined microscopically and transferred to fresh medium as appropriate. Unialgal cultures were purified by repeated streaking on petri dishes containing agarized (1% low gelling temperature agar, No. A4018, Sigma Chemical Co., St. Louis, MO) medium composed of ½ offshore Gulf of Mexico sea water plus ½ medium KASP-2. The dishes were incubated in continuous fluorescent or tungsten light in sealed plastic containers in an atmosphere of 0.5 to 1% CO_2-in-air at 5 or 10°C. After 5 to 15 days suitable micro-colonies were excised, transferred to agar slants, and examined for purity microscopically and in the basal medium supplemented with complex organic materials; 0.1% each of yeast extract, trypticase, and soytone (all products of Difco Laboratories, Detroit, MI). Stock cultures were routinely maintained as slants in a refrigerator at 5-10° with illumination provided by one 40W tungsten lamp 25 to 40 cm from the cultures.

The cultures were kindly identified by Professor Qi Yu-zao of the Department of Biology, Jinan University, Guangzhou, P.R.C. as; Thalassiosira sp. (our notation D1-2), Navicula sp. (J-4), Nitzschia sp. (K3-3), Chaetocerous sp. (K3-10) and KD-50). Organisms K3-10 and KD-50 isolated from two different samples may be the same species, tentatively

C. laciniosus Schutt, but they were sufficiently different in physiology to warrant experimentally being considered two different organisms. It should be noted that these diatoms isolated from the enrichment cultures, while certainly not all the organisms present, were common in numerous fresh samples examined on shipboard.

The light-temperature gradient plate (7) was used to survey the general growth characteristics of the isolates from 6 to 22°C (Fig. 2). All the cultures were clearly cold-adapted. Only one strain, the Chaetocerous sp. (K3-10), grew well at 18°C. The optimum temperatues were from 10 to 14°C. It was not practical to operate the light-temperature gradient plate below 6°C nor was the plate useful for measuring growth rates. Growth rates were therefore measured in liquid cultures at 0 or 10°C (Table 3). Four of the isolates, KD-50, K3-10, K3-3, and J-4 maintained reproducible generation times at 0°C of from 5 to 7 days. At 10°C the growth rates were 1 to 2½ days. The Thallassiosira sp. (D1-2) grew at such a slow rate even at 10°C as to preclude useful experimental work. Organism K3-3 was found to require vitamin B_{12}, organism J-4 was stimulated by vitamin B_{12}. The other cultures grew without added vitamins. Of particular interest were the exceedingly slow growth rates, especially at 0°C. We have looked for chemical or physical factors having significant effect on the growth rate. Light and dark cycles (18L:6D) or addition of reduced nitrogen, NH_4Cl or organic nitrogen in the form of casamino acids, had little effect. The choice of lamps, deluxe warm-white phosphor fluorescent lamps shielded by one screen to cut intensity, was made on the basis of extensive early screening of different combinations of phosphors

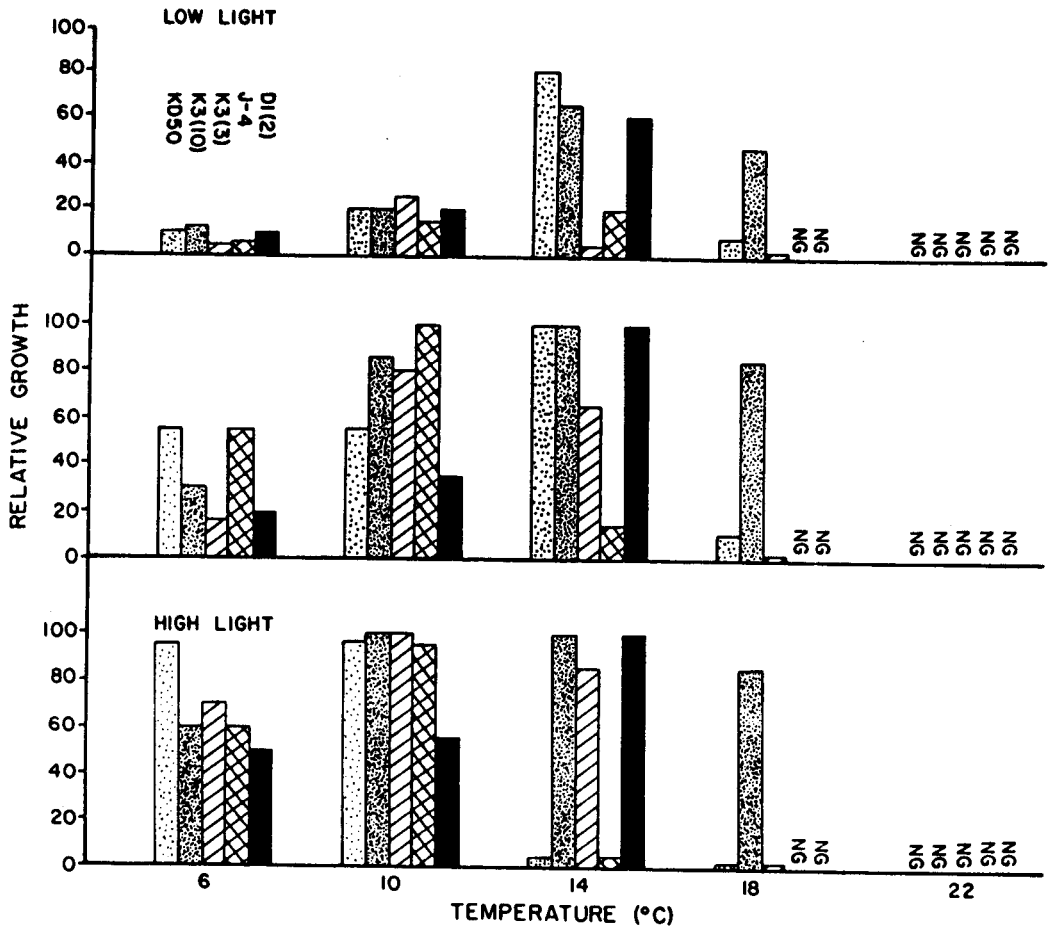

Fig. 2. Relative growth of ice edge diatoms as a function of temperature and light intensity. The aluminum light-temperature gradient plate was 46x63.5x1.27 cm. It was illuminated by two rows (2 lamps per row placed end to end) of F20T12-WWX lamps placed 34 cm above the front edge. The light intensity over the front edge of the plate was 420µw/cm^2 (Model 65 Radiometer, YSI Co., Yellow Springs, OH). Pyrex petri dishes, 60x15 mm, containing 10 ml of medium (1/2 KASP-2 plus 1/2 sea water) plus inoculum, were placed at desired locations on the plate. Growth was judged visually or optically, if dense enough. For each organism the data were recorded relative to the position on the plate which gave the best growth. The experiments were purposely terminated after 9-12 days at relatively low cell densities to avoid severe CO_2 or light limitations on growth. The notation NG means no growth.

Table 3. Growth rates, as generation times in hours, of diatoms isolated from the ice edge in the Bering Sea and inhibition of their growth by two crude oils.

	Temperature (°C)									
	0			10			0		10	
Strain No.	Cook Inlet Crude (ppm)						Prudhoe Bay Crude (ppm)			
	0	50	500	0	50	500	50	500	50	500
KD-50	144	144	0	30	33	36	0	0	42	72
K3-10	120	120	0	30	30	39	120	0	36	55
K3-3	144	0	0	48	48	NG*	0	0	45	NG
J-4	170	ND†	ND	60	60	60	ND	ND	72	103

*NG means no growth. † ND means not determined. Continuous illumination was provided by two F20T12/WWX fluorescent lamps 10 cm from the lamp center to the growth tube center. Lamp output was cut to approximately 60% by one copper screen inserted between the lamps and the growth bath. Temperatures were held to ± 0.2 at 1°C and ± 0.5 at 10°C. The growth tubes were continuously bubbled with 1 ± 0.1% CO_2 in air, cell concentration was measured turbidimetrically or by collecting cells on a 0.4 µm filter and drying at 45°C in a vacuum oven over P_2O_5. The crude oils were sterilized by filtration with pressure (N_2) through 0.45 µm silver membranes (Selas Corp., Dresher, PA). The crude oil was absorbed onto washed 12.7 mm filter paper discs and the discs placed directly into the culture tubes. Crude oils presented in this manner remain absorbed on the discs and in contact with the algae (15). The generation times shown are conservatively good to ± 15%.

and intensities. Moreover short-time photosynthesis measurements ($^{14}CO_2$ fixation) carried out under these same lighting conditions gave linear and saturated rates of CO_2 uptake over several hours. By several fundamental criteria of algal culture, cell density and elementary analysis, these cultures are behaving as expected. Cell yields of 0.5mg dry weight ml^{-1} were routinely achieved. The elemental analysis of organism KD-50 grown at 0° or 10°C was: %C, 32.29 and 32.91; %H, 4.99 and 4.98; %N, 5.16 and 5.42, %residue, 34.9 and 30.9. On an ash-free basis these values compare very favorably with a variety of algal cells (8).

There are, then, two very interesting features which emerge from the characterization of growth in these ice edge diatoms. First, these organisms fit the textbook definition of obligate psychrophiles, micro-organisms that can grow well at 0°C and that do so optimally below 20°C (9). In other words these are not just mesophilic forms capable of growth at 0°C but with optimum temperatures above 20°C, but rather strains restricted to temperatures below 18°C (Fig. 2). Their second significant characteristic was their exceedingly slow measured generation times, 5 to 7 days at 0°C. Such very slow generation times are not anticipated from the existing large body of information primarily on mesophilic microalgae (10). Indeed a theoretical treatment of algal growth rates versus temperature predicted generation times approaching 1 day at 0°C (11). In work with unialgal (bacterized) cultures of four Arctic ice diatoms at 5°C generation times of 1 to 2 days were found (12). A unialgal strain of Skeletonema costatum, a typical mesophilic form, had an estimated generation time of approximately 2 days at 0°C (13).

The generation times measured herein at 0°C with pure cultures of cold-adapted diatoms appear to be the first of their kind. The very slow growth rates at 0°C may perhaps be a reflection of one or several enzymes with unavoidably low turnover times at 0°C. However, the very marked increase in the solubility of oxygen at low temperatures may cause special problems for a photosynthetic cell, for example, with the oxygenase reaction catalyzed by ribulose 1,5-bisphosphate carboxylase (14). If generation times approaching one week are typical under supposedly optimum conditions in the lab for ice edge algae then their turnover times in situ may be much lower. These unique Arctic (probably Antarctic as well) ice phytoplankton and hence these ecosystems may truly merit the appellation of fragile.

Notwithstanding the slow growth rates of these psychrophilic diatoms, we have been able to grow enough cells to examine their capacity for oxidation of aromatic hydrocarbons using naphthalene as a model substrate. Figures 3 and 4 demonstrate that 1-naphthol was formed from (1-^{14}C) naphthelene at 0 or 10°C. The amounts were very small but are real and suggest that cold-adapted microalgae can oxidize aromatic hydrocarbons as is now well-described in mesophilic forms (see page 1).

The observations on the toxicity of crude oils (Table 3) also suggest that cold-adapted diatoms will generally prove more sensitive to any accidental crude oil spills in or around the ice edge in the Bering Sea. Lethality was evident in two of the diatoms, KD-50 and K3-3 at 50 ppm at 0°C, while 500 ppm was lethal to all four organisms. At 10°C toxicity was lessened. For comparison the same Prudhoe Bay crude had no effect at

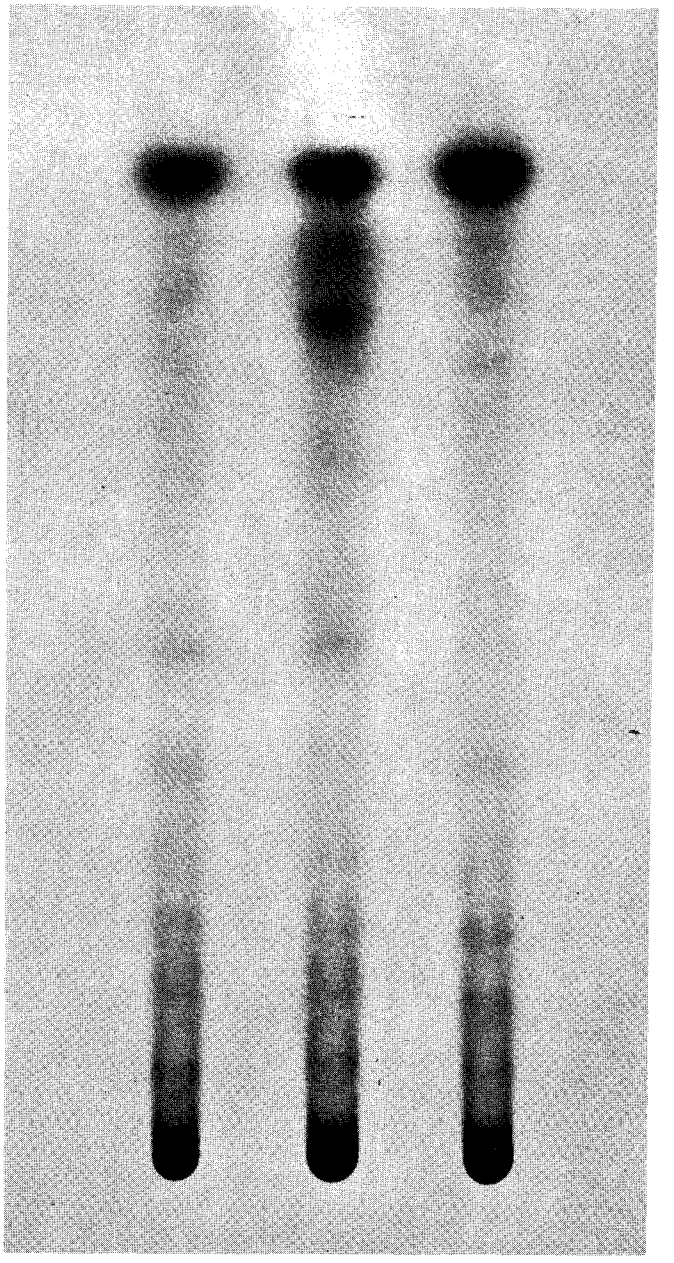

Fig. 3. Radioautogram of products formed from (1-^{14}C) naphthalene by psychrophilic diatoms grown and incubated with naphthalene at 0 ± 0.1°C. The organisms are identified in the text. Naphthalene, 1μCi (specific activity 1μCi/μmol) was added to 10ml of diatom culture (approx. 0.5mg dry weight/ml) in a screw cap tube. After incubation for 24 hours in the same bath as used for growing the cells, the cells were removed by centrifugation and the supernatants from 3 tubes (30ml total) were extracted with ethyl acetate. The ethyl acetate extract was dried over Na_2SO_4, evaporated, and the whole sample chromatographed on silica gel plates using chloroform-acetone (4:1). The region marked A on the radioautogram is naphthalene, region B is 1-naphthol.

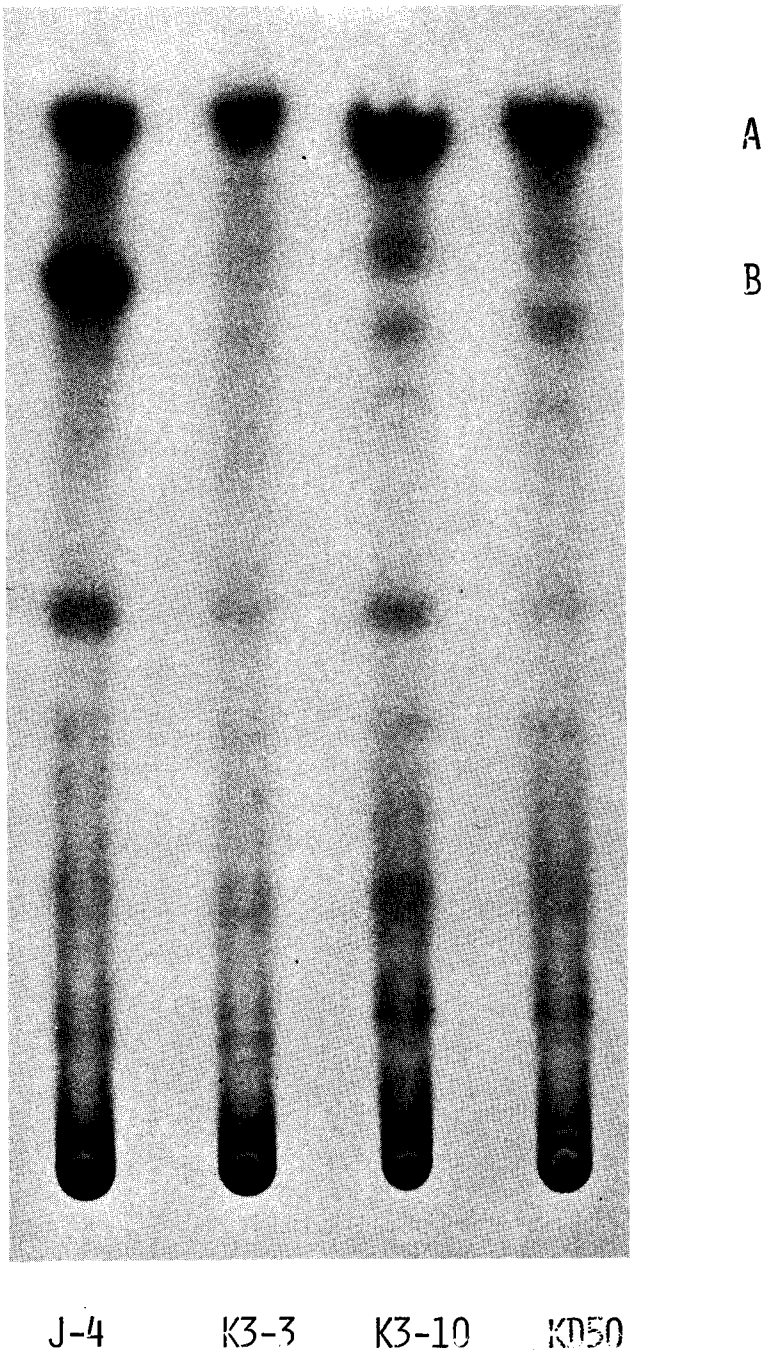

Figure 4. Radioautogram of products formed from (1-^{14}C) naphthalene by psychrophilic diatoms grown and incubated with naphthalene at 10 ± 0.5°C. Experimental details were the same as in Figure 3.

500 ppm and caused only slight lags in growth at 1500 ppm when tested at 30°C against three mesophilic algae, a blue-green alga, a green alga, or a diatom (15). In work with four unialgal cultures isolated from the southern Beaufort Sea growth of diatoms and a green flagellate was markedly inhibited by crude oil concentrations higher than 100 ppm but diatoms seemed more sensitive than the green flagellate (16). Curiously, in this work greater inhibition was observed with longer exposure at temperatures between 5 to 10°C than at 0°C.

The capacity for oxidation of aromatic hydrocarbons and enhanced toxicity of crude oil in psychrophilic diatoms may, in the case of an oil spill, be important to maintenance of primary production levels and therefore to higher trophic levels in the Bering Sea. These observations need broader confirmation both in laboratory and field studies.

With the enrichment and isolation in pure culture of these psychrophilic Arctic diatoms, especially with the easily cultivated Nitzschia sp. (K3-3) and the Chaetocerous sp. (K3-10, KD-50) as experimental tools, we should now gain further understanding of regulation of photosynthetic and biosynthetic pathways in cold-adapted microalgae.

REFERENCES AND NOTES

1. J.S. Bunt and E.J.F. Wood, Nature 199, 1254 (1963); M.J. Dunbar, J. Fish. Res. Board Can. 32, 2276 (1975); R.S. Horner, Oceanogr. Mar. Biol. Ann. Rev. 14, 167 (1976); K. Saito and A. Taniguchi, Asarte 11, 27 (1978).

2. J.S. Bunt, Nature 199, 1255 (1963);

3. C.P. McRoy and J.J. Goering, in Oceanography of the Bering Sea, D.W. Hood and E.J. Kelley, Eds. (Institute of Marine Science, Fairbanks, 1974), p. 403.

4. C.H. Pease and R.D. Muench, Coastal Oceangr. Climatology News 3, 43 (1981).

5. W.J. Campbell and S. Martin, Science 181, 56 (1973); L.S. Wolfe and D.P. Hoult, J. Glaciol. 13, 473 (1974); D.W. Hood and J.A. Calder, in The Eastern Bering Sea Shelf: Oceanography and Resources, D.W. Hood and J.A. Calder Eds. (Office of Marine Pollution Assessment, NOAA) p. 1299 (1981).

6. C. Van Baalen, Botanica Mar. 4, 129, (1962). Medium KASP-2 derived from medium ASP-2 described in this paper.

7. C. Van Baalen and P. Edwards, in Handbook of Phycological Methods, Culture Methods and Growth Measurements Methods, J.R. Stein, Ed. (University Press, Cambridge, 1973), p. 267.

8. B. Kok, Acta Bot. Neerl. 1, 445 (1952).

C. Van Baalen and J.E. Marler, J. Gen. Microbiol. 32, 457 (1962).

C. Van Baalen, D.S. Hoare, E. Brandt, J. Bacteriol. 105, 685 (1971).

P.J. Bottomley and C. Van Baalen, J. Gen. Microbiol. 107, 309 (1978).

The microanalyses were done by Galbraith Laboratories, Inc., Knoxville, TN.

9. J.L. Ingraham and J.L. Stokes, Bact. Revs. 23, 97 (1959);

W.E. Inniss, Ann. Rev. Microbiol. 29, 445 (1975);

R.Y. Morita, Bact. Revs. 39, 144 (1975).

10. C. Van Baalen, in Handbook of Microbiology, A.I. Laskin and H.A. Lechevalier Eds. (CRC Press, Cleveland, OH, 1974), vol IV, p. 21.

11. R.W. Eppley, Fishery Bull. 70, 1063 (1972).

12. W.S. Grant and R.A. Horner, J. Phycol. 12 180 (1976).

13. J.A. Yoder, J. Phycol. 15, 362 (1979).

14. G.H. Lorimer, Ann. Rev. Plant Physiol. 32, 349 (1981).

15. J.C. Batterton, K. Winters, C. Van Baalen, Mar. Environ. Res. 1, 31 (1978).

16. S.I.C. Hsiao, Environ. Pollut. 17, 93 (1978).

17. We are grateful to Captain Bruce Williams and the crew of the R/V SURVEYOR for their invaluable help in collecting the samples. We thank Seelye Martin for providing several ice cores. The sample of Cook Inlet crude oil was kindly provided by James R. Payne and the Prudhoe Bay sample by C.P. Falls.

RATE STUDIES OF 1-NAPHTHOL FORMATION IN MESOPHILIC ALGAE

To determine if microalgae can degrade naphthalene to CO_2 we have incubated a blue-green alga, a green alga, and a diatom in closed flasks at 30°C with (1-^{14}C) naphthalene and recovered CO_2 from the gas phase by precipitation as $BaCO_3$. The $BaCO_3$ was carefully washed with water, ethanol and again with water, then acidified and any radioactivity trapped in 5 ml of 0.1N NaOH. Part of the NaOH solution was added to scintillation cocktail and counted. The above procedure completely eliminated any carry over of naphthalene. Recoveries using $NaH^{14}CO_3$ carried through the precipitation, washing, acidification and trapping in NaOH steps were 90% or better. We have not found any evidence that the above cultures can metabolize naphthalene to $^{14}CO_2$. We have examined the time course of 1-naphthol formation in the blue-green alga, Oscillatoria sp. our strain JCM (Fig. 5). We estimate from such data that strain JCM can form 20 nmol of 1-naphthol per mg dry weight of cells in 24 hours. If we assume that the experimentally measured algal rate formation of 1-naphthol can be equated with bacterial hydrocarbon biodegradation rates (Bartha and Atlas, 1977) then at the reasonable level of 1 µg chlorophyll a/liter in a natural system algal hydrocarbon oxidation can amount to 10% of the "in situ" marine potential.

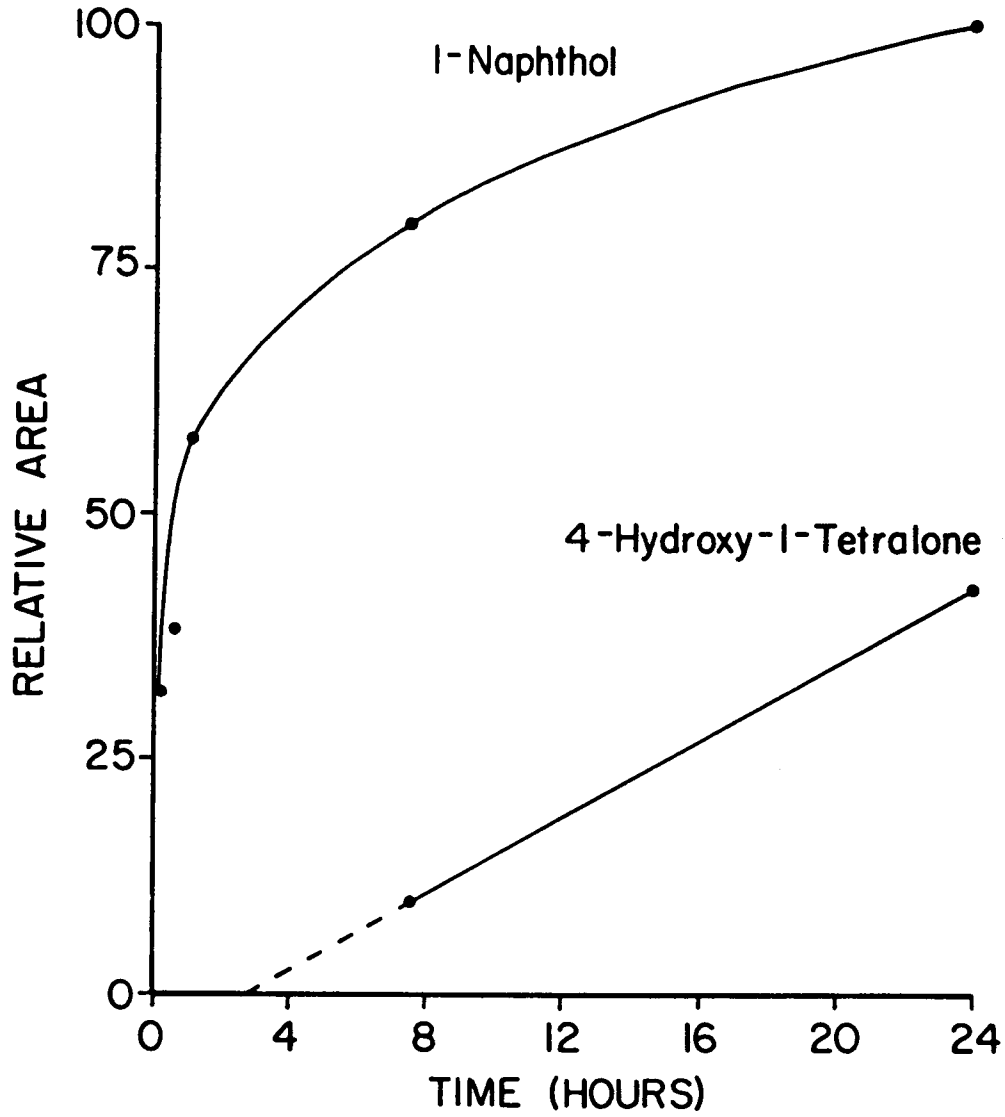

Figure 5. Time course of formation of 1-naphthol and 4-hydroxy-1-tetralone by the blue-green alga, Oscillatoria sp. strain JCM.

REFERENCE

Bartha, R. and Atlas, R.M. (1977). The Microbiology of Aquatic Oil Spills. Adv. Appl. Microbiol. 22, 225-266.

ASSESSMENT OF POTENTIAL INTERACTIONS OF MICROORGANISMS AND POLLUTANTS RESULTING FROM PETROLEUM DEVELOPMENT ON THE OUTER CONTINTENTAL SHELF OF ALASKA

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Final Report
Outer Continental Shelf Environmental Assessment Program
Research Unit 29

31 December 1982

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I Objectives

To examine the potential interactions of microbial populations with pollutants that may be produced as a result of oil and gas development in Alaskan outer continental shelf regions. Specifically to determine population levels of microorganisms indigenous to surface waters and sediments in proposed oil and gas lease areas of the Gulf of Alaska, Cook Inlet, Bering Sea and Beaufort Sea; to characterize the microbial populations in these Alaskan outer continental shelf regions with respect to the taxonomic placement of indigenous bacteria; to characterize the microbial communities with respect to diversity of bacterial populations; to determine the potential denitrifying activities of indigenous sediment microorganisms; to determine the potential of water and sediment microbial populations for biodegrading petroleum hydrocarbons; and to examine the weathering of petroleum with respect to the chemical modification of the oil due to microbial hydrocarbon biodegradation.

II Introduction

This study was conducted in 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 using numerical taxonomy to determine the diversity of the microbial community and an inventory of the dominant microbial taxa within a given geogrophic area, to determine the potential denitrifying activities of microorganisms, and to determine the biodegradation potentials of indigenous microbial populations for petroleum hydrocarbons. To this end intensive surveys of proposed Alaskan OCS oil and gas lease areas were conduted. In addition to surveys to determine hydrocarbon

biodegradation potentials, intensive studies were carried out in the Beaufort Sea to follow the chemical changes in crude oil as it undergoes biotic (biodegradation) and abiotic (physical and chemical) weathering in sediment.

III Current State of Knowledge--Review of the Literature Concerning Hydrocarbon Biodegradation

In 1946, Claude E. ZoBell reviewed the action of microorganisms on hydrocarbons (ZoBell, 1946). He recognized that many microorganisms have the ability to utilize hydrocarbons as sole sources of energy and carbon and that such microorganisms are widely distributed in nature. He further recognized that the microbial utilization of hydrocarbons was highly dependent on the chemical nature of the compounds within the petroleum mixture and on environmental determinants.

Twenty-one years after ZoBell's classic review, the supertanker Torrey Canyon sank in the English Channel. With this incident, the attention of the scientific community was dramatically focused on the problems of oil pollution. After this event, several studies were initiated on the fate of petroleum in various ecosystems. The expansion of petroleum development into new frontiers, such as deep offshore waters and ice-dominated Arctic environments, and the apparently inevitable spillages which occur during routine operations and as a consequence of acute accidents have maintained a high research interest in this field.

The chemically and biologically induced changes in the composition of a polluting petroleum hydrocarbon mixture are known collectively as weathering. Microbial degradation plays a major role in the weathering process. Biodegradation of petroleum in natural ecosystems is complex. The evolution of the hydrocarbon mixture depends on the nature of the oil, on the nature of the

microbial community, and on a variety of environmental factors which influence microbial activities.

Attention has been focused on marine environments since the world's oceans are the largest and ultimate receptors of hydrocarbon pollutants. Most previous reviews concerning the microbiology of petroleum pollutants have been concerned with the marine environment (Atlas, 1977a; Atlas and Bartha, 1973c; Atlas and Schofield, 1975; Bartha and Atlas, 1977; Colwell and Walker, 1977; Crow et al., 1974; Floodgate, 1972a, 1972b, 1973, 1976; Jordan and Payne, 1980; Karrick, 1977; National Academy of Sciences, 1975; Van der Linden, 1978; ZoBell, 1946, 1964, 1969, 1973). This review expands the scope to include consideration of the fate of petroleum hydrocarbons in freshwater and soil ecosystems. It also discusses several case histories relevant to the role of microbial degradation in determining the fate of petroleum pollutants from major oil spills.

CHEMISTRY OF PETROLEUM BIODEGRADATION

Degradation of Individual Hydrocarbons

Petroleum is an extremely complex mixture of hydrocarbons. From the hundreds of individual components, several classes, based on related structures, can be recognized. The petroleum mixture can be fractionated by silica gel chromatography into a saturate or aliphatic fraction, an aromatic fraction, and an asphaltic or polar fraction (Brown et al., 1969a). Several studies have been performed to determine the metabolic pathways for degradation of these compounds, and there have been a number of reviews on this subject (Donoghue et al., 1976; Foster, 1962a, 1962b; Gibson et al., 1968, 1971; Hopper, 1978; Markovetz, 1971; McKenna and Kallio, 1965; National Academy of Sciences, 1975; Perry, 1977, 1979; Pirnik, 1977; Rogoff, 1961; Stirling et al., 1977; Trudgill, 1978; Van der Linden and Thijsse, 1965).

Hydrocarbons within the saturate fraction include n-alkanes, branched alkanes, and cycloalkanes (naphthenes). The n-alkanes are generally considered the most readily degraded components in a petroleum mixture (Davies and Hughes, 1968; Kator et al., 1971; McKenna and Kallio, 1964; Treccani, 1964; ZoBell, 1946). Biodegradation of n-alkanes with molecular weights up to n-C_{44} has been demonstrated (Haines and Alexander, 1974). The biodegradation of n-alkanes normally proceeds by a monoterminal attack; usually a primary alcohol is formed, followed by an aldehyde and a monocarboxylic acid (Foster, 1962a, 1962b; McKenna and Kallio, 1965; Miller and Johnson, 1966; Ratledge, 1978; Van der Linden and Thijsse, 1965; Van Eyk and Bartels, 1968; ZoBell, 1950). Further degradation of the carboxylic acid proceeds by β-oxidation with the subsequent formation of two-carbon-unit shorter fatty acids and acetyl coenzyme A, with eventual liberation of CO_2. Fatty acids, some of which are toxic, have been found to accumulate during hydrocarbon biodegradation (Atlas and Bartha, 1973d; King and Perry, 1975). Omega (diterminal) oxidation also has been reported (Jurtshuk and Cardini, 1971). Subterminal oxidation sometimes occurs, with formation of a secondary alcohol and subsequent ketone, but this does not appear to be the primary metabolic pathway utilized by most n-alkane-utilizing microorganisms (Markovetz, 1971). A new pathway recently was elucidated by W. R. Finnerty (personal communication), who found that an Acinetobacter species can split a hydrocarbon at the number 10 position, forming hydroxy acids. The initial steps appear to involve terminal attack to form a carboxylic acid, subterminal dehydrogenation at the number 10 position to form an unsaturated acid, and splitting of the carbon chain to form a hydroxy acid and an alcohol.

Highly branched isoprenoid alkanes, such as pristane, have been found to undergo omega oxidation, with formation of dicarboxylic acids as the major degradative pathway (McKenna and Kallio, 1971; Pirnik, 1977; Pirnik et al., 1974). Methyl branching generally increases the resistance of hydrocarbons to microbial attack (Fall et al., 1979; McKenna and Kallio, 1964; Pirnik, 1977; Schaeffer et al., 1979). Schaeffer et al. (1979), for example, found that terminal branching inhibits biodegradation of hydrocarbons. Methyl branching at the beta position (anteiso-terminus) blocks β-oxidation, requiring an additional strategy, such as alpha oxidation (Beam and Perry, 1973; Lough, 1973), omega oxidation (Pirnik, 1977), or beta alkyl group removal (Cantwell et al., 1978; Seubert and Fass, 1964).

Cycloalkanes are particularly resistant to microbial attack (Donoghue et al., 1976; Ooyama and Foster, 1965; Perry, 1979; Stirling et al., 1977; Trudgill, 1978). Complex alicyclic compounds, such as hopanes (tripentacyclic compounds), are among the most persistent components of petroleum spillages in the environment (Atlas et al., 1981). There have been several reports of the direct oxidative and co-oxidative degradation of both substituted and unsubstituted cycloalkanes. The microbial metabolism of cyclic hydrocarbons and related compuonds has been reviewed by Perry (Austin et al., 1977b). Up to six-membered condensed ring structures have been reported to be subject to microbial degradation (Cobet and Guard, 1973; Walker et al., 1975a). Several unsubstituted cycloalkanes, including condensed cycloalkanes, have been reported to be substrates for co-oxidation with formation of a ketone or alcohol (Beam and Perry, 1973, 1974a, 1974b; Perry, 1979). Once oxygenated, degradation can proceed with ring cleavage. Degradation of substituted cycloalkanes appears to occur more readily than the degradation of the unsubstituted forms, particularly if there is an n-alkane substituent of

adequate chain length (Perry, 1979; Soli, 1973). In such cases, microbial attack normally occurs first on the substituted portion, leading to an intermediate product of cyclohexane carboxylic acid or a related compound. A novel pathway for the degradation of cyclohexane carboxylic acid involves formation of an aromatic intermediate (Perry, 1979) followed by cleavage of the aromatic ring structure.

The degradation of aromatic hydrocarbons has been reviewed by Gibson and others (Cripps and Watkinson, 1978; Gibson, 1968, 1971, 1976, 1977; Gibson and Yeh, 1973; Hopper, 1978; Rogoff, 1961). The bacterial degradation of aromatic compounds normally involves the formation of a diol followed by cleavage and formation of a diacid such as cis,cis-muconic acid. In contrast, oxidation of aromatic hydrocarbons in eukaryotic organisms has been found to form a trans-diol. For example, fungi have been shown to oxidize naphthalene to form trans- 1,2-dihydroxy-1, 2-dihydronaphthalene (Cerniglia and Gibson, 1977, 1978; Cerniglia et al., 1978b; Ferris et al., 1976). The results indicate that only one atom of molecular oxygen is incorporated into the aromatic nucleus, as has been found for mammalian aryl hydrocarbon hydroxylase systems. Cerniglia and Gibson (1980a, 1980b) and Cerniglia et al. (1978a, 1978b) have investigated the fungal oxidation of polynuclear aromatic hydrocarbons. They found evidence for formation of trans-7,8-dihydroxy-7,8-dihydrobenzo(a)pyrene by Cunnninghamella elegans from the oxidation of benzo(a)pyrene. Cerniglia and Gibson (1979) and Cerniglia et al. (1980a, 1980b, 1980c) also investigated the metabolism of naphthalene by cyanobacteria. They found that naphthalene was oxidized in the light but not in the dark. Scenedesmus strains also were shown to utilize n-heptadecane in the light (mixotrophic growth), but were unable to utilize this alkane in the dark (Masters and Zajic, 1971). The major product formed by Agmenellum and Oscillatoria species was 1-naphthol (Cerniglia and Gibson,

1979). These organisms also formed cis-1,2-dihydroxy-1,2-dihydronaphthalene and 4-hydroxy-1-tetralene (Cerniglia et al., 1980b). These results suggest that cyanobacteria have a variety of mechanisms for initiating the oxidation of naphthalene. The Oscillatoria species also has been found to oxidize biphenyl, indicating that a wider range of aromatic hydrocarbons are subject to oxidation by cyanobacteria (Cerniglia et al., 1980c).

Light aromatic hydrocarbons are subject to evaporation and to microbial degradation in a dissolved state (Kappeler and Wuhrmann, 1978a, 1978b). Extensive methyl substitution can inhibit initial oxidation (Atlas et al., 1981; Cripps and Watkinson, 1978). Initial enzymatic attack may be on the alkyl substituent or, alternatively, directly on the ring (Gibson, 1971). Condensed ring aromatic structures are subject to microbial degradation by a similar metabolic pathway as monocyclic structures (Cripps and Watkinson, 1978; Dean-Raymond and Bartha, 1975; Gibson, 1975; ZoBell, 1971); condense ring aromatic hydrocarbons, however, are relatively resistant to enzymatic attack; for example, Lee and Ryan (1976) found that biodegradation rates were over 1,000 times higher for naphthalene than for benzopyrenes. Structures with four or more condensed rings have been shown to be attacked, in some cases, by co-oxidation or as a result of commensalism (Barnsley, 1975; Cripps and Watkinson, 1978; Gibson, 1975; Walker and Colwell, 1974b; Walker et al., 1975d, 1976b).

The metabolic pathways for the degradation of asphaltic components of petroleum are probably least well understood. These are complex structures which are difficult to analyze with current chemical methodology. The degradation of various sulfur-containing components of petroleum has bee examined (Hou and Laskin, 1976; Kodama et al., 1970; Nakatani et al., 1968; Walker et al., 1976b; Yamada et al., 1968), but no uniform degradative pathway,

comparable to the pathways established for aliphatic and aromatic hydrocarbons, has yet emerged for the asphaltic petroleum components. Advances in determining degradative pathways for asphaltic petroleum components are dependent on improved chemical analytical methodology. The elucidation of the biochemical fate of asphaltic petroleum compounds is a major challenge for future research on petroleum biodegradation.

Another important future research need involves determining the importance in the environment of the various pathways for hydrocarbon biodegradation. It is clear that various biochemical strategies exist for the microbial utilization of petroleum hydrocarbons. What remains to be done is to detect intermediate products in natural environments that receive petroleum hydrocarbons to determine which pathways are actively used by microbial populations in natural ecosystems. It is likely, but as yet unproven, that different pathways will be active under different conditions, e.g., at different hydrocarbon concentrations.

Degradation of Hydrocarbons Within Petroleum Mixtures

The qualitative hydrocarbon content of the petroleum mixture influences the degradability of individual hydrocarbon components. Walker et al. (1976c) examined the susceptibility to microbial degradation of hydrocarbons in weathered crude and fuel oils. They reported far less degradation in a heavy no. 6 fuel oil (Bunker C oil) than in a light no. 2 fuel oil (heating oil and diesel fuel) and less degradation in a heavy Kuwait crude oil than in a light south Louisiana crude oil. They reported major differences in the susceptibility to degradation of each of the components (identical compounds) within the context of the different hydrocarbon mixtures of the oils tested.

Mulkins-Phillips and Stewart (1974b) found that n-alkanes within a Venezuelan crude oil were degraded less than the same n-alkanes within an

Arabian crude oil. Westlake et al. (1978) examined the effect of crude oil composition on petroleum biodegradation. The ability of mixed microbial populations to utilize the hydrocarbons in four crude oils as the sole carbon source was found to depend not only on the composition of the unsaturated fraction but also on that of the asphaltic fraction. Using an oil which lacked a normal n-alkane component, they demonstrated that the aromatic fraction of oil was capable of sustaining bacterial growth. Horowitz et al. (1975) used the technique of sequential enrichment to isolate organisms which could utilize progressively more complex (i.e., resistant to microbial degradation) compounds.

Several investigators have examined the potential activities of hydrocarbon-degrading bacteria by using ^{14}C-radiolabeled hydrocarbons. Caparello and LaRock (1975) described an enrichment method for quantifying the activity of hydrocarbon-oxidizing bacteria in water and sediment that used [^{14}C]hexadecane. They found that the hydrocarbon-oxidizing potential of environmental samples reflects the hydrocarbon burden of the area and the ability of the indigenous microorganisms to utilize hydrocarbons. Walker and Colwell (1976c) observed that rates of mineralization were greater for hexadecane than for naphthalene, which were greater than those for toluene, which were greater than those for toluene, which were greater than those for cyclohexane. Greater rates of uptake and mineralization were observed for bacteria and samples collected from an oil-polluted harbor than for samples from a relatively unpolluted region. They reported turnover times of 15 and 60 min for the polluted and unpolluted areas, respectively, using [^{14}C]hexadecane. Roubal and Atlas (1978) found that biodegradation potentials follow the order hexadecane > naphthalene >> pristane > benzanthracene. Lee (1977b) found that alkanes and low-molecular-weight aromatics (benzene, toluene, naphthalene, and

methylnaphthalene) were degraded to CO_2 by microorganisms in river water, but that higher-molecular-weight aromatics were relatively resistant to microbial degradation. Herbes and Schwall (1978) found that polyaromatic hydrocarbon turnover times in petroleum-contaminated sediments increased from 7.1 h for naphthalene to 400 h for anthracene, 10,000 h for benz(a)anthracene, and more than 30,000 h for benz(a)pyrene. Polynuclear aromatic compounds tended to be only partially, rather than completely, degraded to CO_2.

Two processes which need be considered in the metabolism of petroleum hydrocarbons are co-oxidation and sparing. Both processes can occur within the context of a petroleum spillage. LePetit and Tagger (1976), for example, found that acetate, an intermediate product in hydrocarbon degradation, reduced the utilization of hexadecane. A diauxic phenomenon has been reported for the degradation of pristane, in which pristane was not degraded in the presence of hexadecane (Pirnik et al., 1974). The basis for this sparing effect was not defined, and it is not known whether this is an example of classical catabolite repression. Similar sparing effects undoubtedly occur for other hydrocarbons. Such diauxic phenomena do not alter the metabolic pathways of degradation, but rather determine whether the enzymes necessary for metabolic attack of a particular hydrocarbon are produced or active. These sparing effects have a marked influence on the persistence of particular hydrocarbons within a petroleum mixture and thus on the evolution of the weathered petroleum hydrocarbon mixture.

The phenomenon of co-oxidation has been referred to several times in this section. Compounds which otherwise would not be degraded can be enzymatically attacked within the petroleum mixture due to the abilities of the individual microorganisms to grow on other hydrocarbons within the oil (Horvath, 1972). A petroleum hydrocarbon mixture, with its multitude of potential primary

substrates, provides an excellent chemical environment in which co-oxidation can occur. Many complex branched and cyclic hydrocarbons undoubtedly are removed as environmental contaminants after oil spills as a result of co-oxidation (Perry, 1979; Raymond and Jamison, 1971; Raymond *et al.*, 1967). Jamison et al. (1976) found that the degradation of hydrocarbons within a high-octane gasoline was not in agreement with the degradation of individual hydrocarbons by pure cultures. They concluded that co-oxidation played a major role in the degradation of the hydrocarbon mixture within gasoline. Horowitz and Atlas (1977a) found, using chromatographic and mass spectral analysis, that residual oils recovered after exposure in Arctic coastal waters contained similar percentages of the individual components in classes of hydrocarbons regardless of the amount of degradation, indicating that most hydrocarbon components of the oil were being degraded at similar rates. This study is in contrast to most, which show preferential utilization of *n*-alkane hydrocarbons. Co-oxidation was hypothesized to be responsible for the degradation of a number of compounds in the oil to account for the similar rates of disappearance of compounds which are normally easily degraded and those which are normally resistant. Herbes and Schwall (1978) also postulated that co-oxidation led to the accumulation of relatively large amounts of partially oxidized products of polynuclear aromatic hydrocarbon degradation in sediments and only limited amounts of CO_2 production. Assessing the role of co-oxidation in natural environments is difficult since multiple microbial populations are present. In the above-mentioned mixed-population studies, synergism could be an alternative hypothesis to explain the observed results. Future studies are needed to clarify the role of co-oxidation in determining the fate of petroleum hydrocarbons in natural ecosystems.

An interesting and as yet unexplained, but consistent, process which occurs during the biodegradation of petroleum hydrocarbons is the enrichment of compounds within the "unresolved envelope" which is run during gas chromatographic analysis of petroleum hydrocarbons. This envelope is due to a mixture of several compounds which are not resolved into individually defined peaks even by glass capillary gas chromatography. Since these compounds cannot currently be analytically resolved, they cannot be identified. It has been hypothesized that, during the biodegradation of petroleum hydrocarbons, microorganisms are producing (synthesizing) hydrocarbons of different molecular weights or chemical structures. Walker and Colwell (1976b) found that a wax was produced during microbial degradation of Altamont crude oil but not during abiotic weathering of oil. The high-boiling n-alkanes in the wax were associated with microbial degradation of the oil and appeared to be similar to components of tar balls found in the open ocean. The possible production of such high-molecular-weight alkanes during petroleum biodegradation also has been reported by several other investigators (Pritchard et al., 1976; Seesman et al., 1976). The biochemical mechanism for formation of such hydrocarbons during petroleum biodegradation is unknown. Sexstone et al. (Sexstone and Atlas, 1978; Sexstone et al., 1978) found that oil biodegradation in tundra soils was accompanied by accumulation of polar lipoidal compounds in the soil column that were not present in fresh oil and were not detected in unoiled soils; the identities of the compounds, however, were not determined. Jobson et al. (1972) reported an increase in the polar nitrogen-sulfur-oxygen fraction during oil biodegradation in soil.

The role of microorganisms in producing complex products from hydrocarbon metabolism which may persist in the environment requires further investigation. Of particular importance is the possible involvement of microorganisms in the

formation of tar balls. The synthesis of complex high-molecular-weight hydrocarbons would suggest that microorganisms can play a role in prolonging the impact of petroleum pollutants as well as in abating the impact of such environmental contaminants through biodegradative removal. It is difficult to separate the importance of photochemical and biochemical processes in the formation of oxygenated and polymeric compounds in the environment. It is apparent that the fate of the component hydrocarbons is extremely complicated and requires further research efforts.

TAXONOMIC RELATIONSHIPS OF HYDROCARBON-UTILIZING MICROORGANISMS

The ability to degrade petroleum hydrocarbons is not restricted to a few microbial genera; a diverse group of bacteria and fungi have been shown to have this ability. ZoBell (1946) in his review noted that more than 100 species representing 30 microbial genera had been shown to be capable of utilizing hydrocarbons. In a previous review, Bartha and Atlas (1977) listed 22 genera of bacteria, 1 algal genus, and 14 genera of fungi which had been demonstrated to contain members which utilize petroleum hydrocarbons; all of these microorganisms had been isolated from an aquatic environment. The most important (based on frequency of isolation) genera of hydrocarbon utilizers in aquatic environments were Pseudomonas, Achromobacter, Arthrobacter, Micrococcus, Nocardia, Vibrio, Acinetobacter, Brevibacterium, Corynebacterium, Flavobacterium, Candida, Rhodotorula, and Sporobolomyces (Bartha and Atlas, 1977). Bacteria and yeasts appear to be the prevalent hydrocarbon degraders in aquatic ecosystems. In polluted freshwater ecosystems, bacteria, yeasts, and filamentous fungi all appear to be important hydrocarbon degraders (Cooney and Summers, 1976). Jones and Eddington (1968) found that isolates representing 11 genera of fungi and 6 genera of bacteria were the dominant microbial genera

responsible for hydrocarbon oxidation in soil samples. They found that fungi played an important role in the hydrocarbon-oxidizing activities of the soil samples. Cerniglia and Perry (1973) found that several fungi (Penicillium and Cunninghamella spp.) exhibited greater hydrocarbon biodegradation than bacteria (Flavobacterium, Brevibacterium, and Arthrobacter spp.). Recent studies continue to expand the list of microbial species which have been demonstrated to be capable of degrading petroleum hydrocarbons. In one such study, Davies and Westlake (1979) examined 60 fungal isolates for their ability to grow on n-tetradecane, toluene, naphthalene, and seven crude oils of various compositions. Forty cultures, including 28 soil isolates, could grow on one or more of the crude oils. The genera most frequently isolated from soils were those producing abundant small conidia, e.g., Penicillium and Verticillium spp. Oil-degrading strains of Beauveria bassiana, Mortierella spp., Phoma spp., Scolecobasidium obovatum, and Tolypocladium inflatum also were isolated.

Walker et al. (1975) compared the abilities of bacteria and fungi to degrade hydrocarbons. The following genera were included in their study: Candida, Sporobolomyces, Hansenula, Aureobasidium, Rhodotorula, Cladosporium, Penicillium, Aspergillus, Pseudomonas, Vibrio, Acinetobacter, Leucothrix, Nocardia, and Rhizobium. Bacteria and yeasts showed decreasing abilities to degrade alkanes with increasing chain length. Filamentous fungi did not exhibit preferential degradation for particular chain lengths. Patterns of degradation, i.e., which hydrocarbons could be utilized, were similar for bacteria and fungi, but there was considerable variability among individual isolates.

Komagata et al. (1964) examined almost 500 yeasts for their ability to degrade hydrocarbons and found 56 that could utilize hydrocarbons, almost all of which were in the genus Candida. The fermentation industry has considered

using hydrocarbon-utilizing Candida species for producing single-cell protein. Ahearn and co-workers (Ahearn et al., 1971; Cook et al., 1973) have examined yeasts that can utilize hydrocarbons and have isolated strains of Candida, Rhodosporidium, Rhodotorula, Saccharomyces, Sporobolomyces, and Trichosporon, which are capable of doing so. Cladosporium resinae has been isolated from soil (Cooney and Walker, 1973; Walker et al., 1973) and has repeatedly been found as a contaminant of jet fuels (Bailey and May, 1979; Cooney et al., 1968; Hill, 1978; Hill and Thomas, 1976). The organism can grow on petroleum hydrocarbons and creates problems in the aircraft industry by clogging fuel lines.

Nyns et al. (1968) examined the "taxonomic value" of the property of fungi to assimilate hydrocarbons, i.e., whether the ability of fungi to utilize hydrocarbons was a useful diagnostic test for defining different fungal genera or species. They found that the ability to utilize hydrocarbons occurred mainly in two orders, the Mucorales and the Moniliales. They found that Aspergillus and Penicillium are rich in hydrocarbon carbon-assimilating strains. They concluded that the property of assimilating hydrocarbons is relatively rare and that it is a property of individual strains and not necessarily a characteristic of particular species or related taxa. Llanos and Kjoller (1976) examined changes in fungal populations in soil after oil waste application. They found that oil application favored growth of Graphium and Paecilomyces. In their study, strains of Graphium, Fusarium, Penicillium, Paecilomyces, Acremonium, Mortierella Gliocladium, Trichoderma, and Sphaeropsidales were found to be important groups of soil fungi capable of utilizing crude oil hydrocarbons. In a similar study, Jensen (1975) studied the bacterial flora of soil after application of oily waste and found that the

most important species of oil degraders belonged to the genera Arthrobacter and Pseudomonas.

Cundell and Traxler (1974) studied 15 isolates from an asphaltic flow near a natural seepage at Cape Simpson, Alaska. The isolates were psychrotrophic and utilized paraffinic, aromatic, and asphaltic petroleum components. The isolates belonged to the bacterial genera Pseudomonas, Brevibacterium, Spirillum, Xanthomonas, Alcaligenes, and Arthrobacter. In northwest Atlantic coastal waters and sediment, Mulkins-Phillips and Stewart (1974a) reported finding hydrocarbon-utilizing bacteria of the genera Nocardia, Pseudomonas, Flavobacterium, Vibrio, and Achromobacter.

Walker et al (1976a) isolated Vibrio, Pseudomonas, and Acinetobacter species from oil-contaminated sediment and Pseudomonas and coryneform species from oil-free sediment. Microorganisms from the oil-free sediment produced greater quantities of polar compounds (asphaltics) after degradation, whereas microorganisms from the oil-contaminated sediment provided greater degradation of saturated and aromatic hydrocarbons. Walker et al. (1975) also examined bacteria from water and sediment for their ability to degrade petroleum. Water samples contained a greater variety of bacterial species capable of degrading petroleum than sediment samples. Cultures from both water and sediment contained Pseudomonas and Acinetobacter species. Bacteria present in the water samples yielded significantly greater degradation of two-, three-, four-, and five-ring cycloalkanes and mono-, di-, tri-, tetra-, and penta-aromatics compared with bacteria from sediment samples.

Both temperature and chemical composition of a crude oil have been shown to have a selective influence on the genera of hydrocarbon utilizers. Cook and Westlake (1974) isolated Achromobacter, Alcaligenes, Flavobacterium, and Cytophaga at 4°C on a substrate of Prudhoe Bay crude oil; Acinetobacter,

Pseudomonas, and unidentified gram-negative cocci at 4°C on a substrate of Atkinson Point crude oil; Flavobacterium, Cytophaga, Pseudomonas, and Xanthomonas at 4°C with Norman Wells crude oil as substrate; and Alcaligenes and Pseudomonas on Lost Horse crude oil at 4°C. At 30°C, the major genera isolated on Prudhoe Bay crude oil were Achromobacter, Arthrobacter, and Pseudomonas; on Atkinson Point crude oil, the major genera were Achromobacter, Alcaligenes, and Xanthomonas; on Norman Wells crude oil, the major genera were Acinetobacter, Arthrobacter, Xanthomonas, and other gram-negative rods; and on Lost Horse crude oil, they were Achromobacter, Acinetobacter, and Pseudomonas.

Several thermophilic hydrocarbon-utilizing bacteria have been isolated, including species of Thermomicrobium and other, yet unidentified genera (Merkel et al., 1978). Both gram-negative and gram-positive thermophilic bacteria have been demonstrated to be capable of hydrocarbon utilization. Some isolated thermophiles are obligate hydrocarbon utilizers and cannot grow on other carbon sources. The possible existence of obligate hydrocarbon utilizers is intriguing but perplexing, since the biochemical degradative pathways indicate that hydrocarbon utilizers must also be capable of metabolizing fatty acids and alcohols.

A large number of Pseudomonas species have been isolated which are capable of utilizing petroleum hydrocarbons. The genetics and enzymology of hydrocarbon degradation by Pseudomonas species has been extensively studied (Chakrabarty, 1972; Chakrabarty et al., 1973; Dunn and Gunsalus, 1973; Friello et al., 1976; Williams, 1978). The genetic information for hydrocarbon degradation in these organisms generally has been found to occur on plasmids. Pseudomonas species have been used for genetic engineering, and the first successful test case in the United States to determine whether genetically

engineered microorganisms can be patented involved a hydrocarbon-utilizing Pseudomonas which was "created" by Chakrabarty (Diamond v. Chakrabarty, 1980).

Numerical taxonomy has been used to examine petroleum degrading bacteria (Austin et al., 1977a, 1977b). Austin et al. (1977a) examined 99 strains of petroleum-degrading bacteria, isolated from Chesapeake Bay water and sediment, by numerical taxonomy procedures. Eighty-five percent of the petroleum-degrading bacteria examined in this study were defined at the 80 to 85% similarity level with 14 phenetic groups. The groups were identified as actinomycetes (mycelial forms, four clusters), coryneforms, Enterobacteriaceae, Klebsiella aerogenes, Micrococcus spp. (two clusters), Nocardia spp. (two clusters), Pseudomonas spp. (two clusters), and Sphaerotilus natans. It was concluded that degradation of petroleum is accomplished by a diverse range of bacterial taxa. Of particular note was the finding that some enteric bacteria can utilize petroleum hydrocarbons; the suggestion has been made that some of these enteric bacteria may have acquired this ability through plasmid transfer.

Some cyanobacteria and algae have been found to be capable of hydrocarbon degradation. Walker et al. (1975b) described a hydrocarbon-utilizing achlorophyllous strain of the alga Prototheca. Cerniglia et al. (1980a) tested nine cyanobacteria, five green algae, one red alga, one brown alga, and two diatoms for their ability to oxidize naphthalene. They found that Oscillatoria spp., Microcoleus sp., Anabaena spp., Agmenellum sp., Coccochloris sp., Nostoc sp., Aphanocapsa sp., Chlorella spp., Dunaliella sp., Chlamydomonas sp., Ulva sp., Cylindretheca sp., Amphora sp., Porphyridium sp., and Petalonia all were capable of oxidizing naphthalene. Their results indicate that the ability to oxidize aromatic hydrocarbons is widely distributed among the cyanobacteria and algae.

It is now abundantly clear that the ability to utilize hydrocarbons is widely distributed among diverse microbial populations. Hydrocarbons are naturally occurring organic compounds, and it is not surprising that microorganisms have evolved the ability to utilize these compounds. When natural ecosystems are contaminated with petroleum hydrocarbons, the indigenous microbial communities are likely to contain microbial populations of differing taxonomic relationships which are capable of degrading the contaminating hydrocarbons.

DISTRIBUTION OF HYDROCARBON-UTILIZING MICROORGANISMS

Hydrocarbon-degrading bacteria and fungi are widely distributed in marine, freshwater, and soil habitats. The literature on actual numbers of hydrocarbon utilizers is confusing because of methodological differences used to enumerate petroleum-degrading microorganisms. A number of investigators have used hydrocarbons incorporated into an agar-based medium (Atlas and Bartha, 1973a; Horowitz and Atlas, 1978; Horowitz *et al.*, 1978; Sexstone and Atlas, 1977b; Stewart and Marks, 1978). This approach has been criticized (Atlas, 1978b; Colwell, 1978; Mills *et al.*, 1978; Walker and Colwell, 1976a); in some cases, a high correlation has been found between growth on agar media containing hydrocarbons as the sole carbon source and the ability to rigorously demonstrate hydrocarbon utilization of isolates from these media in liquid culture; in other studies, only a low percentage of isolates from agar-based media could be demonstrated to be capable of hydrocarbon utilization. The inclusion of organic contaminants in agar media and the growth of oligotrophic bacteria probably result in the counting of non-hydrocarbon utilizers in some cases when plate counts are used for enumerating hydrocarbon utilizers.

The use of silica gel as a solidifying agent has been shown to improve the reliability of procedures for enumerating hydrocarbon utilizers (Seki, 1976).

Walker and Colwell (1976a) reported that a medium containing 0.5% oil and 0.003% phenol red was best for enumerating petroleum-degrading microorganisms. They also found that addition of Amphotericin B permitted selective isolation of hydrocarbon-utilizing bacteria and that addition of either streptomycine or tetracycline permitted selective isolation of yeasts and fungi. Washing the inoculum to remove contaminating organic compounds did not improve the recovery of petroleum degraders in this study. These authors specifically recommended the use of a silica gel-oil medium for enumerating petroleum-degrading microorganisms; they also suggested that counts of petroleum degraders be expressed as a percentage of the total population rather than as total numbers of petroleum degraders per se.

Buckley et al. (1976) characterized the distribution of microorganisms in an estuary relative to ambient hydrocarbon concentrations. Although counts were performed on non-hydrocarbon-based media, at all but two stations most of the species isolated were able to grow on hydrocarbons, indicating that the ability to utilize hydrocarbons is widespread, even in environments not subjected to high levels of hydrocarbon pollution. Crow et al. (1976) examined the distribution of hydrocarbon utilizers in surface ocean layers and in the underlying water column. They found that populations of hydrocarbonoclastic microorganisms occurred in concentrations 10 to 100 times greater in the surface layer than at a 10-cm depth.

Mulkins-Phillips and Stewart (1974a) examined the distribution of hydrocarbon-utilizing bacteria in northwestern Atlantic waters and coastal sediments. The fraction of the total heterotrophic bacteria represented by the hydrocarbon utilizers ranged up to 100%, depending on the area's previous history of oil spillage; most values were less than 10%. They found that the location, numbers, and variety of the microbial hydrocarbon utilizers

illustrated their ubiquity and that the broad enzymatic capacity for hydrocarbon degradation indicated the microbial potential for removal or conversion of oil in the environments examined. The presence of hydrocarbon-utilizing microorganisms was demonstrated in sediments and adjacent waters taken from Bermuda, Canadian Northwest Atlantic, and eastern Canadian Arctic marine shorelines.

Bunch and Harland (1976) found that numbers of hydrocarbon utilizers occurred in similar concentrations in Arctic and temperate marine samples; i.e., quantitative differences in the distribution of hydrocarbon utilizers were relatively unimportant over large geographic distances. Indeed, hydrocarbon utilizers have been found to be widely distributed even in cold marine ecosystems (Atlas, 1978a; Cundell and Traxler, 1973, 1976; Robertson et al., 1973a, 1973b; Tagger et al., 1976; Walker and Colwell, 1976a).

Most-probable-number (MPN) procedures have been suggested as a substitute for plate court procedures for enumerating hydrocarbon-utilizing microorganisms, since such procedures eliminate the need for a solidifying agent and permit direct assessment of the ability to actually utilize hydrocarbons (Atlas, 1978b; Colwell, 1978). The use of liquid media for MPN procedures permits removal of trace organic contaminants and allows for the chemical definition of a medium with a hydrocarbon as the sole source of carbon. Enumeration methods which incorporate the specificity for counting only hydrocarbon utilizers and which eliminate the problem of counting organisms growing on other trace organic contaminants represents a significant improvement in the accuracy with which numbers of hydrocarbon utilizers can be determined. Higashihara et al. (1978) reported that plate counts, using either agar or silica gel solidifying agents, were unsuitable for enumerating hydrocarbon-utilizing microorganisms since many marine bacteria grow and

produce microcolonies even on small amounts of organic matter. They recommended the use of an MPN procedure, with hydrocarbons as the source of carbon and trace amounts of yeast extract for necessary growth factors, for accurate enumerations of microbial populations which degrade hydrocarbons in marine environments. Mills et al. (1978) compared several media designed for use in an MPN determination of petroleum-degrading microorganisms. The best results, i.e., largest numbers, were obtained with a buffered (32 mM phosphate) liquid medium containing 1% hydrocarbon substrate. In this study, turbidity was used as the criterion for establishing positive results. Counts of petroleum degraders obtained with a liquid medium and an MPN procedure are usually higher than those obtained on silica gel medium with oil added as the carbon source.

^{14}C-radiolabeled hydrocarbons have been used in MPN procedures for determining the distribution of hydrocarbon-utilizing microorganisms. Atlas (1978b) has described a technique that uses [^{14}C]hexadecane-spiked crude oil to enumerate petroleum-degrading microorganisms. This method uses the conversion of a radiolabeled hydrocarbon to radiolabeled carbon dioxide for establishing positive results in the MPN procedure. Placing the radiolabeled hydrocarbon within a crude oil mimics the availability of hydrocarbons to the microbial community, as would occur in an actual oil spill. Lehmicke et al. (1979) used low concentrations of radiolabeled hydrocarbons in MPN determinations; in their studies, the concentrations of radiolabeled hydrocarbons were adjusted to reflect actual concentrations which might be present in soluble form.

Roubal and Atlas (1978) studied the distribution of hydrocarbon-utilizing microorganisms in Alaskan Continental Shelf regions, using an MPN procedure based on the mineralization of ^{14}C-labeled hydrocarbons. They reported that hydrocarbon utilizers were ubiquitously distributed, with no significant

overall concentration differences between Arctic and subarctic sampling regions nor between surface water and sediment samples. There were, however, significant seasonal differences in numbers of hydrocarbon utilizers. In a study in a temperate region, Raritan Bay, N.J., Atlas and Bartha (1973a), using oil-agar plate enumerations, also found that numbers of hydrocarbon-utilizing microorganisms were lower during winter than summer. Walker and Colwell (1976d) similarly found seasonal variations in numbers of hydrocarbon utilizers in Chesapeake Bay.

It is clear from a number of studies that the distribution of hydrocarbon-utilizing microorganisms reflects the historical exposure of the environment to hydrocarbons. A large number of laboratory studies have demonstrated sizable increases in populations of hydrocarbon-utilizing microorganisms when environmental samples are exposed to petroleum hydrocarbons (Atlas and Bartha, 1972b; Calomiris et al., 1976; Davis, 1956; Kator, 1973; Perry and Cerniglia, 1973; Pritchard and Starr, 1973; Soli, 1973; Traxler, 1973; ZoBell, 1973).

Mironov (1970) and Mironov and Lebed (1972) found highly elevated populations of hydrocarbon-utilizing microorganisms in the oil tanker shipping channels of the Indian Ocean and the Black Sea. Polyaka (1962) found high numbers of hydrocarbon-oxidizing microorganisms in Neva Bay, U.S.S.R., in association with petroleum inputs. ZoBell and Prokop (1966) reported that numbers of hydrocarbon utilizers in sediment of Baritaria Bay, La., were correlated with sources of oil pollutants. Similarly, Atlas and Bartha (1973a) for Raritan Bay and Colwell et al. (1973) and Walker and Colwell (1975) for Chesapeake Bay found that distributions of hydrocarbon utilizers correlated highly with sources of oil pollutants entering the bays. The distribution of hydrocarbon utilizers within Cook Inlet was also positively correlated with

the occurrence of hydrocarbons in the environment (Roubal and Atlas, 1978). LePetit et al. (1977) reported that bacteria utilizing a gas-oil as the sole carbon source represented 10% of the heterotrophic bacteria in the area of a refinery effluent compared with 4% in an area not directly polluted by hydrocarbons. The degradation potential was highest in areas in chronic discharge (Tagger et al., 1979).

Several studies have shown a rise in populations of hydrocarbon-utilizing microorganisms after oil spills. Kator and Herwig (1977) found that, within a few days after spillage of South Louisiana crude oil in a coastal estuary in Virginia, levels of petroleum-degrading bacteria rose by several orders of magnitude. The elevated levels of hydrocarbon utilizers were maintained for over 1 year. Raymond et al. (1976) found significant increases in hydrocarbon-utilizing microorganisms in soils receiving hydrocarbons; increased populations were maintained throughout the year. Pinholt et al. (1979) examined the microbial changes during oil decomposition in soil. They found an increase from 60 to 82% in oil-utilizing fungi and an increase from 3 to 50% in oil-degrading bacteria after a fuel oil spill. Oppenheimer et al. (1977) found a tendency toward higher ratios of hydrocarbon-utilizing bacteria to total viable heterotrophs in the active Ekofisk oil field of the North Sea, probably due to the occurrence of hydrocarbons in the sediments of this region. Gunkel et al. (1980) confirmed the occurrence of high numbers of hydrocarbon-utilizing microorganisms in the vicinity of the North Sea oil fields and found a high correlation between concentrations of hydrocarbons and oil-utilizing bacteria in the North Sea.

High numbers of fungi have been found in association with the Cape Simpson, Alaska, oil seeps (Barsdate, 1973). Numbers of filamentous fungi 0.2 m from the edge of the seep were reported to be three times higher than those

50 m from the seep; bacterial populations in ponds in contact with the Cape Simpson oil seeps were found to be higher than in unstressed ponds; bacterial populations in soils adjacent to the asphaltic sections of the seeps were higher than those 50 m from the seep.

In experimental field studies in the Arctic, Atlas and co-workers have found large increases in hydrocarbon-utilizing microorganisms in marine (Atlas, 1978a; Atlas and Busdosh, 1976; Atlas et al., 1978; Atlas and Schofield, 1975; Atlas et al., 1976; Horowitz and Atlas, 1978), freshwater (Atlas et al., 1976; Horowitz and Atlas, 1977), and soil (Sexstone and Atlas, 1977b Sexstone et al., 1978) ecosystems; concentrations of hydrocarbon-utilizing microorganisms have been found to rise rapidly and dramatically in response to acute inputs of petroleum hydrocarbons. Bergstein and Vestal (1978), however, found a lack of elevated microbial populations in an oil-treated tundra pond unless phosphate also was added. Horowitz and Atlas (1977b), using a continuous-flow-through model system, found large increases and shifts to a high percentage of hydrocarbon utilizers in Arctic coastal water when nitrogen and phosphorus were added to oil slicks. Sexstone and Atlas (1977b) found that addition of crude oil to Arctic tundra soils resulted in large increases in total numbers of heterotrophs and of oil-utilizing microorganisms. The response of microbial populations to contaminating oil was found to depend on soil type and depth. Increases in microbial populations in subsurface soils parallel downward migration of the oil (Sexstone and Atlas, 1977a).

Sparrow et al. (1978) found a rise in oil-utilizing bacterial populations in taiga soils which were experimentally contaminated with hot Prudhoe Bay crude oil. Studies in the Swan Hills area of north-central Alberta, Canada, by Cook and Westlake (1974) showed slightly increased bacterial populations 308 and 433 days after treatment with Swan Hills oil at an application rate of 6.5

liters/m^2. Increases in numbers of bacteria were significantly higher when the plots were also treated with urea-phosphate fertilizer. Similar results were obtained at Norman Wells 321 and 416 days after treatment with 6.5 liters of Norman Wells crude per m^2. As with the Swan Hills spill, slight increases in bacterial numbers occurred when oil alone was added, and significantly higher increases occurred when fertilizer was also added.

Gunkel (1968a, 1968b) reported that populations of hydrocarbon utilizers were elevated in sediments affected by the Torrey Canyon spill. Stewart and Marks (1978) found higher numbers of hydrocarbon utilizers in sediment affected by the Arrow spill Chedabucto Bay, Nova Scotia; 5 years after the spill, only a few site examined had significant concentrations of residual petroleum and elevated counts of hydrocarbon utilizers. Significantly elevated numbers of hydrocarbon utilizers (several orders of magnitude above normal) were found after the Amoco Cadiz spill in Brittany (Atlas and Bronner, 1980) and the XTOC-I well blowout in the Bay of Campeche, Gulf of Mexico (Atlas et al., 1980b). In the case of the Amoco Cadiz spill, the numbers of hydrocarbon utilizers in intertidal sediments were positively correlated with the degree of hydrocarbon contamination; during recovery after the spillage, the numbers of hydrocarbon utilizers returned at most sites to background levels as the oil disappeared due to biodegradative removal. Counts of hydrocarbon utilizers associated with an oil-in-water emulsion (mousse) from the XTOC-I well blowout were three to five orders of magnitude higher than in surface water samples not contaminated with oil (Atlas et al., 1980b). In the sediment of an Arctic lake that had been contaminated with a leaded refined gasoline, populations of hydrocarbon-utilizing microorganisms were found to be significantly elevated within a few hours of the spill (Horowitz and Atlas, 1977b) through 1 year after the spill (Horowitz and Atlas, 1978). This degree of elevation in

numbers of microbial hydrocarbon utilizers correspond with the degree of contamination.

In general, population levels of hydrocarbon utilizers and their proportions within the microbial community appear to be a sensitive index of environmental exposure to hydrocarbons. In unpolluted ecosystems, hydrocarbon utilizers generally constitute less than 0.1% of the microbial community; in oil-polluted ecosystems, they can constitute up to 100% of the viable microorganisms. The degree of elevation above unpolluted compared reference sites appears to quantitatively reflect the degree or extent of exposure of that ecosystem to hydrocarbon contaminants.

ENVIRONMENTAL FACTORS INFLUENCING BIODEGRADATION OF PETROLEUM HYDROCARBONS

The fate of petroleum hydrocarbons in the environment is largely determined by abiotic factors which influence the weathering, including biodegradation of the oil. Factors which influence rates of microbial growth and enzymatic activities affect the rates of petroleum hydrocarbon biodegradation. The persistence of petroleum pollutants depends on the quantity and quality of the hydrocarbon mixture and on the properties of the affected ecosystem. In one environment petroleum hydrocarbons can persist indefinitely, whereas under another set of conditions the same hydrocarbons can be completely biodegraded within a relatively few hours or days.

Physical State of Oil Pollutants

The physical state of petroleum hydrocarbons has a marked effect on their biodegradation. At very low concentrations hydrocarbons are soluble in water, but most oil spill incidents release petroleum hydrocarbons in concentrations far in excess of the solubility limits (Boylan and Tripp, 1971; Frankenfeld, 1973; Harrison et al., 1975; McAuliffe, 1966). The degree of spreading determines in part the surface area of oil available for microbial colonization

by hydrocarbon-degrading microorganisms; in aquatic systems, the oil normally spreads, forming a thin slick (Berridge et al., 1968a). The degree of spreading is reduced at low temperatures because of the viscosity of the oil. In soils, petroleum hydrocarbons are absorbed by plant matter and soil particles, limiting its spreading.

Wodzinsky and LaRocca (1977) found that liquid aromatic hydrocarbons were utilized by bacteria at the water-hydrocarbon interface but that solid aromatic hydrocarbons were not metabolized. At 30°C diphenylmethane is a liquid and could be degraded, but at 20°C the solid form of diphenylmethane could not be utilized by a Pseudomonas sp. They also found that naphthalene could not be utilized in the solid form but could be utilized if dissolved in a liquid hydrocarbon. Atlas (unpublished data) similarly found that hexadecane supported only marginal bacterial growth at 5°C when the compound was in the solid form, but if hexadecane was dissolved in another liquid hydrocarbon or crude oil, extensive degradation of the liquid hexadecane occurred at 5°C. The role of temperature in determining the physical state of a hydrocarbon and the influence of the physical state on rates of microbial hydrocarbon degradation are apparent in these studies.

Hydrocarbon-degrading microorganisms act mainly at the oil-water interface. Hydrocarbon-degrading microorganisms can be observed growing over the entire surface of an oil droplet; growth does not appear to occur within oil droplets in the absence of entrained water. Availability of increased surface area should accelerate biodegradation (Gatellier, 1971; Gatellier et al., 1973). Not only is the oil made more readily available to microorganisms, but movement of emulsion droplets through a water column makes oxygen and nutrients more readily available to microorganisms.

In aquatic ecosystems, oil normally forms emulsions. This has been termed "pseudosolubilization" of the oil (Gutnick and Rosenberge, 1977). The water-in-oil emulsion which occurs in seawater after oil spills is referred to as "chocolate mousse" or simply "mousse." The processes involved in the formation of mousse have been examined by a number of investigators (Berridge et al., 1968b; Burwood and Speers, 1974; Dean, 1968). Mousse is chemically and physically heterogeneous. Photooxidation (Burwood and Speers, 1974) and microbial oxidation (Berridge et al., 1968b) have been reported to play a role in mousse formation under different environmental conditions; both abiotic and microbial processes appear to be capable of initiating mousse formation under appropriate environmental conditions; both abiotic and microbial processes appear to be capable of initiating mousse formation under appropriate environmental conditions. In some cases, a fine emulsion is formed with small droplets of mousse. In these cases, the hydrocarbons in the mousse probably are more susceptible to microbial degradation, and their fate is similar to that of "dissolved" hydrocarbons. Mousse can also refer to large accumulations of emulsified oil in "globs" up to 1 m in diameter. Such large "mousse plates" have limited surface areas, and hydrocarbons internal to the mousse may be spared from microbial degradation. Davis and Gibbs (1975) found that large accumulations of "mousse" weathered extremely slowly with no net loss of hydrocarbons over 2 years. Atlas et al. (1980b) proposed that degradation of hydrocarbons released into the Gulf of Mexico by the XTOC-I blowout was limited in part by the physical properties of the mousse accumulations. Colwell et al. (1978) postulated that degradation of oil from the Metula spill was restricted by the formation of tar balls and aggregates of oil which restricted accessibility of the hydrocarbons to microorganisms. Microbial degradation was

ineffective when oil was deposited on the beach and subsequently buried or when the oil formed asphalt layers or tar balls.

Dispersants have been used to treat oil spills. In some cases the use of toxic dispersants probably has resulted in greater ecological impact than the oil spill itself; such was the case in the Torrey Canyon incident (Cowell, 1971; Smith, 1968). Some dispersants may contain chemicals which are inhibitory to microorganisms. Without toxicity, however, dispersion can enhance petroleum biodegradation. Mulkins-Phillips and Stewart (1974c) found that some dispersants enhanced n-alkane degradation in crude oil, but that other dispersants had no effect. Gatellier et al. (1971, 1973) and Robichaux and Myrick (1972) likewise found that some dispersants inhibited hydrocarbon-oxidizing populations, whereas others enhanced hydrocarbon-degrading microorganisms. Atlas and Bartha (1973b) tested several dispersants and found that all increased the rate but not the extent of hydrocarbon mineralization.

A number of hydrocarbon-degrading microorganisms produce emulsifying agents (Abbott and Gledhill, 1971; Guire et al., 1973; Reisfeld et al., 1972; ZoBell, 1946). Some of these bioemulsifiers have been considered for use in cleaning oil storage tanks, such as on supertankers (Gutnick and Rosenber, 1977). Reisfeld et al. (1972) have studied an Arthrobacter strain which extensively emulsifies oil when growing on hydrocarbons. Zajic and co-workers (1974) have characterized the the emulsifying agents produced by strains of Pseudomonas and Corynebacterium. In some cases, the emulsifying agents appear to be fatty acids or derivatives of fatty acids; in other cases, more complex polymers are the active emulsifying agents. Although the production of emulsifying agents should increase the susceptibility of hydrocarbons in an oil to microbial degradation, microbial strains which effectively emulsify oil

often do not extensively degrade the hydrocarbons in the oil. It is not clear yet why extensive emulsification does not permit greater hydrocarbon degradation by these organisms.

After extensive weathering, petroleum hydrocarbons often occur in the environment as tar balls. Hydrocarbons in tar are quite resistant to microbial degradation. Many of the hydrocarbons in tar have chemical structures which are not readily attacked by microbial enzymes. The surface area-to-volume ratio of a tar ball is not favorable for microbial growth on this insoluble substrate. Tar balls often accumulate on beaches where microbial activities are limited by available water, which is needed to support microbial growth and enzymatic hydrocarbon-degrading activities.

From the point of view of microbial hydrocarbon degradation, dissolution and emulsification of hydrocarbons appear to have a positive effect on degradation rates. If there are no adverse toxic effects, dispersion of oil should accelerate microbial hydrocarbon degradation. This is an important consideration when determining whether dispersants should be added to oil spills. Increased toxicity must remain, however, a major concern when considering the use of such chemical dispersants.

Temperature

Hydrocarbon biodegradation can occur over a wide range of temperatures, and psychrotrophic, mesophilic, and thermophilic hydorcarbon-utilizing microorganisms have been isolated. ZoBell (1973) and Traxler (1973) reported on hydrocarbon degradation at below 0°C; Klug and Markovetz (1967a, 1967b) and Mateles et al. (1967) reported on hydrocarbon degradation at 70°C.

Temperature can have a marked effect on the rates of hydrocarbon degradation. The effects of temperature on the physical state of hydrocarbons was discussed in the previous section. ZoBell (1969) found that hydrocarbon

degradation was over an order of magnitude faster at 25°C than at 5°C. Very low rates of hydrocarbon utilization were found by Gunkel (1967) at low water temperatures. Ludzack and Kinkead (1956) found that motor oil was rapidly oxidized at 20°C but not at 5°C. Mulkins-Phillips and Stewart (1974b) found that, 9 months after the spillage of Bunker C fuel oil into Chedabucto Bay, the bacterial populations isolated from contaminated areas showed rates of degradation at 5°C that were 21 to 70% less during 14 days of incubation than during 7 days at 10°C.

There are seasonal shifts in the composition of the microbial community which can be reflected in the rates of hydrocarbon metabolism at a given temperature. Atlas and Bartha (1973a) found that higher numbers of hydrocarbon utilizers capable of growth at 5°C were present in Raritan Bay, N.J., during winter than during other seasons. Rates of hydrocarbon mineralization measured at 5°C were significantly higher in water samples collected in winter than in summer. The evidence suggests a seasonal shift to a microbial community capable of low-temperature hydrocarbon degradation.

Gibbs and Davis (1976) studied the degradation of oil in beach gravel at temperatures from 6 to 26°C. They found a Q_{10} (6 to 16°C) of 3.3 and a Q_{10} (11 to 21°C) of 2.05. The average Q_{10} values of 2.7 was the same as the value found in other studies, by Gibbs et al. (1975), on the effects of temperature on the degradation of oil in seawater. Atlas and Bartha (1972a) found a Q_{10} of approximately 4, using seawater over a temperature range of 5 to 20°C.

Atlas and Bartha (1972a) found that the effects of temperature differ, depending on the hydrocarbon composition of a petroleum mixture. Low temperatures retard the rates of volatilization of low-molecular-weight hydrocarbons, some of which are toxic to microorganisms. The presence of such toxic components was found to delay the onset of oil biodegradation at low

temperatures (Atals and Bartha, 1972a). In a subsequent study, Atlas (1975) examined the biodegradability of seven different crude oils and found biodegradation to be highly dependent on the composition and on incubation temperature. At 20°C, lighter oils had greater abiotic losses and were more susceptible to biodegradation than heavier oils; rates of oil mineralization for the heavier oils were significantly lower at 20°C than for the lighter ones. The light crude oils, however, had toxic volatile components which evaporated only slowly, inhibiting microbial degradation of these oils at 10°C. A significant lag phase before the onset of hydrocarbon biodegradation was found for the lighter oils. No toxic volatile fractions were subject to biodegradation. Some preference was shown for paraffin degradation, especially at low temperatures. Horowitz and Atlas (1977a) found that during summer, in Arctic surface waters, different structural classes of hydrocarbons were degraded at similar rates. They postulated that at low temperatures cometabolism played an important role in determining the rates of disappearance of hydrocarbons in the mixture.

Walker and Colwell (1974a), using a model petroleum incubated with estuarine water collected during winter, found that slower but more extensive biodegradation occurred at 0°C than at higher temperatures. Decreased toxicity of hydrocarbons at lower temperatures was hypothesized to explain the more extensive growth at the lower temperature.

Ward and Brock (1976) studied the influence of environmental factors on the rates of hydrocarbon oxidation in temperate lakes. Rates of hydrocarbon oxidation were assessed by using the conversion of ^{14}C-radiolabeled hexadecane to $^{14}CO_2$. They found that a lag phase preceded hydrocarbon oxidation and that the length of the lag phase depended on population density or on factors influencing growth rate. Hydrocarbon oxidation was coincident with growth and

was presumed to occur only under conditions of development of indigenous hydrocarbon-degrading microorganisms. They found that hydrocarbon-degrading microorganisms persisted during the year, but that there were seasonal variations in the rates of hydrocarbon oxidation. Rates of petroleum hydrocarbon biodegradation were correlated with temperature. During winter, spring, and fall, temperature was a major limiting factor. Dibble and Bartha (1979c) found that the rates of disappearance of hydrocarbons from an oil-contamination field in New Jersey showed a definite correlation with mean monthly temperature.

Arhelger et al. (1977) compared Arctic and subarctic hydrocarbon biodegradation. In situ [^{14}C]dodecane oxidation rates based on $^{14}CO_2$ production were: Port Valdez, 0.7 g/liter per day; Chuckchi Sea, 0.5 g/liter per day; and Arctic Ocean, 0.001 g/liter per day. This study indicates that rates of hydrocarbon degradation show a definite climatic shift and are lower in the Arctic Ocean than in more southerly Alaskan regions.

Atlas et al. (1977b, 1978) examined the degradation of Prudhoe Bay crude oil in Arctic marine ice, water, and sediment ecosystems. Petroleum hydrocarbons were degraded slowly. They found that ice greatly restricted losses of light hydrocarbons and that biodegradation of oil on the surface of ice or under sea ice was negligible. They concluded that petroleum hydrocarbons will remain in cold Arctic ecosystems for long periods of time after oil contamination. In these studies, however, temperature was not specifically elucidated as a major factor limiting hydrocarbon degradation, except as it related to the occurrence of ice.

Colwell et al. (1978) reported greater degradation of Metula crude oil at 3°C than at 22°C with mixed microbial cultures in beach sand samples; when 0.1% oil was added, 48% of the added hydrocarbons were degraded at an incubation

temperature of 3°C, compared with only 21% degraded at 22°C with cultures adapted at the same temperatures as the incubation temperature. They found that under in situ conditions oil degradation proceeded slowly, but concluded that temperature does not seem to be the limiting factor for petroleum degradation in the Antarctic marine ecosystem affected by the Metula spill.

A number of studies have been conducted on the fate of oil in cold Arctic soils. Sexstone and colleagues (Sexstone and Atlas, 1978; Sexstone et al., 1978a, 1978b) have reported very long persistence times for oil in tundra soils. It appears that degradation of hydrocarbons ceases during winter when tundra soils are frozen. Westlake and colleagues (Cook and Westlake, 1974; Jobson et al., 1972; Westlake et al., 1978) found that the microbial populations in northern soils were able to degrade hydrocarbons at the ambient temperatures found during the warmer seasons. Several aspects of these studies were discussed earlier in this review.

It is apparent that the influence of temperature on hydrocarbon degradation is more complex than simple consideration of Q_{10} values. The effects of temperature are interactive with other factors, such as the quality of the hydrocarbon mixture and the composition of the microbial community. Hydrocarbon biodegradation can occur at the low temperatures (<5°C) that characterize most of the ecosystems which are likely to be contaminated by oil spills. Temperature often is not the major limiting factor for hydrocarbon degradation in the environment except as it relates to other factors such as the physical state of the oil or whether liquid water is available for microbial growth. Concern must be expressed, however, regarding the rates of microbial oil degradation in Arctic and subarctic regions. These are areas of new petroleum development and the data gathered to date suggest that rates of

microbial degradation in these cold ecosystems may not be adequate to rapidly remove hydrocarbon contaminants.

Nutrients

There is some confusion and considerable apparent conflict in the literature regarding the limitation of petroleum biodegradation by available concentrations of nitrogen and phosphorus in seawater. Several investigators (Atlas and Bartha, 1972c; Bartha and Atlas, 1973; Floodgate, 1973, 1979; Gunkel, 1967; Lehtomake and Barthelemy, 1968; LePetit and N'Guyen, 1976) have reported that concentrations of available nitrogen and phosphorus in seawater are severely limiting to microbial hydrocarbon degradation. Other investigators (Kinney et al., 1969), however, have reached the opposite conclusion, i.e., that nitrogen and phosphorus are not limiting in seawater. The difference in results is paradoxical and appears to be based on whether the studies are aimed at assessing the biodegradation of hydrocarbons within an oil slick or the biodegradation of soluble hydrocarbons. When considering an oil slick, there is a mass of carbon available for microbial growth within a limited area. Since microorganisms require nitrogen and phosphorus for incorporation into biomass, the availability of these nutrients within the same area as the hydrocarbons is critical. Extensive mixing can occur in turbulent seas, but in many cases the supply of nitrogen and phosphorus is dependent on diffusion to the oil slick. Rates of diffusion may be inadequate to supply sufficient nitrogen and phosphorus to establish optimal C/N and C/P ratios for microbial growth and metabolism. Researchers examining the fate of large oil spills have thus properly concluded in many cases that concentrations of N and P are limiting with respect to rates of hydrocarbon biodegradation. When considering soluble hydrocarbons, nitrogen and phosphorus are probably not limiting since the solubility of the hydrocarbons is so low as to preclude

establishment of an unfavorable C/N or C/P ratio. Investigators considering the fate of low-level discharges of hydrocarbons (soluble hydrocarbons) have, thus, properly concluded that available nutrient concentrations are adequate to support hydrocarbon biodegradation.

Floodgate (1973), in considering the limitations of nutrients to biodegradation of hydrocarbons in the sea, proposed the concept of determining the "nitrogen demand," analogous to the concept of biochemical oxygen demand. Based on Kuwait crude oil at 14°C, the nitrogen demand was found to be 4 nmol of nitrogen per ng of oil. Bridie and Bos (1971) found that addition of 3.2 mg of ammonium nitrogen and 0.6 mg of phosphate permitted maximal rates of degradation of Kuwait crude in seawater at a concentration of 70 mg of oil per liter. Atlas and Bartha (1972c) found that concentrations of 1 mg of nitrogen and 0.07 mg of phosphorus per liter supported maximal degradation of Sweden crude oil in New Jersey coastal seawater at a concentration of 8 g of oil per liter. Reisfeld et al. (1972) reported optimal concentrations of nitrogen and phosphorus of 11 and 2 mg per liter for biodegradation of 1 g of Iranian crude oil per liter in Mediterranean seawater.

Colwell et al. (1978) concluded that Metula oil is degraded slowly in the marine environment, most probably because of limitations imposed by the relatively low concentrations of nitrogen and phosphorus available in seawater.

Ward and Brock (1976) reported that although temperature was the main limiting factor much of the year, during summer nutrient deficiencies limited oil biodegradation in temperate lakes. Higher rates of oil biodegradation could be obtained by addition of nitrogen and phosphorus. High rates of hydrocarbon degradation were found only during 1 month of the year when temperature and nutrient supplies were optimal. They concluded that

environmental factors limited hydrocarbon-utilizing microorganisms within the indigenous microbial community was not a limiting factor.

LePetit and N'Guyer (1976) found that the artificial stimulation of bacterial hydrocarbon degradation requires the addition of phosphorus to seawater. They reported optimal concentrations of phosphorus to support hydrocarbon degradation of between 20×10^{-4} and 8×10^{-4} M for seawater and between 1.5×10^{-3} and 3×10^{-3} M for coastal waters receiving a significant supply of fresh water. Inhibition of bacterial development was observed with higher phosphate concentrations. Gibbs (1975) calculated that 1 m^3 of Irish Sea water provides sufficient nitrogen to degrade 30 g of oil per year at summer temperatures and 11 g of oil per year at winter temperatures.

Bergstein and Vestal (1978) studied the biodegradation of crude oil in Arctic tundra ponds. They concluded that oleophilic fertilizer may provide a useful tool to enhance the biodegradation of crude oil spilled on such oligotrophic waters. Without addition of nitrogen and phosphorus, hydrocarbon biodegradation was limited. Atlas and Bartha (1973e) described an oleophilic nitrogen and phosphorus fertilizer which could overcome limitations of nitrogen and phosphorus in seawater and stimulate petroleum biodegradation in seawater. The fertilizer consisting of paraffinized urea and octylphosphate supported degradation of oil in seawater. Optimal C/N and C/P ratios were 10:1 and 100:1, respectively. In conjunction with the U.S. Office of Naval Research, they obtained a patent for use of fertilizers for stimulating oil degradation in seawater (Bartha and Atlas, 1976).

Olivieri et al. (1976) described a slow-release fertilizer containing paraffin-supported magnesium ammonium phosphate as the active ingredient for stimulating petroleum biodegradation. They reported that the biodegradation of Sarir crude oil in seawater was considerably enhanced by addition of the

paraffin-supported fertilizer. After 21 days, 63% of the oil had disappeared when fertilizer was added compared with 40% in a control area. Kator et al. (1972) suggested the use of paraffinized ammonium and phosphate salts for enhancing oil biodegradation in seawater. Raymond et al. (1976) were able to stimulate the microbial degradation of hydrocarbons in contaminated groundwater by procedures which included the addition of nitrogen and phosphorus nutrients.

Dibble and Bartha (1976) examined the effect of iron on the biodegradation of petroleum in seawater. Biodegradation of south Louisiana crude oil and the effects of nitrogen, phosphorus, and iron supplements on this process were compared in polluted and relatively clean littoral seawater collected along the New Jersey coast. Without supplements, the biodegradation of south Louisiana crude oil was negligible in both seawater samples. Addition of nitrogen and phosphorus allowed very rapid biodegradation; up to 73% of the oil was degraded with 3 days in polluted seawater. Total iron in the seawater sample was high (5.2 mM), and the addition of iron did not increase biodegradation rates. In less polluted and less iron-rich (1.2 mM Fe) seawater samples, biodegradation of south Louisiana crude oil was considerably slower (21% in 3 days), and addition of chelated iron had a stimulating effect. Ferric octoate was shown to have a stimulating effect on south Louisiana crude oil biodegradation, similar to that of chelated iron. Ferric octoate in combination with paraffinized urea and octylphosphate is suitable for treatment of floating oil slicks. The authors concluded that spills of south Louisiana crude oil and similar oils can be cleaned up rapidly and efficiently by stimulated biodegradation, provided that water temperatures are favorable.

Dibble and Bartha (1979a) examined the effect of environmental factors on the biodegradation of oil sludge. They conducted a laboratory study aimed at evaluating and optimizing the environmental factors of land farming, i.e.,

disposal by biodegradation in soil of oily sludges generated in the refining of crude oil. They found that oil sludge biodegradation was optimal at a soil water-holding capacity of 30 to 90%, a pH of 7.5 to 7.8, a C/N ratio of 60:1, and a C/P ratio of 800:1. Optimal temperatures were 20°C or above. They reported that an application rate of 5% (by weight) oil sludge hydrocarbon to soil, i.e., 100,000 liters/hectare, gave a good compromise between high biodegradation rates and efficient land use and resulted in the best overall biodegradation rate of oil hydrocarbon classes. Frequent small applications resulted in higher biodegradation rates than single large applications.

Fedorak and Westlake (personal communication) found that, without added nutrients, aromatic hydrocarbons were more readily attacked than saturated hydrocarbons by soil and marine microbes; addition of nitrogen and phosphorus nutrients stimulated degradation of saturated hydrocarbons more than of aromatic hydrocarbons.

Westlake et al. (1978) examined the in situ degradation of oil in a soil of the boreal region of the northwest territories of Canada. Where fertilizer containing nitrogen and phosphorus was applied to the oil, there was a rapid increase in bacterial numbers, but no increase in fungal propagules. This was followed by a rapid disappearance of n-alkanes and isoprenoids and a continuous loss of weight of saturated compounds in the recovered oil. The seeding of oil slick plots with oil-degrading bacteria had no effect on the composition of the recovered oil. Jobson et al. (1974) similarly found that nitrogen and phosphorus addition stimulated hydrocarbon degradation in oil applied to soil but that seeding did not stimulate degradation. Hunt et al. (1973) found that fertilizer application to subarctic soils enhanced microbial hydrocarbon degradation. They found, however, in laboratory tests that nitrogen addition caused an initial negative response in microbial activity which was followed by

enhanced biodegradation; microbial activity also responded positively to phosphorus addition.

Raymond et al. (1976) studied oil biodegradation in soil. Greater oil degradation was found in soils receiving fertilizer application and rototilling than in untreated soils. They did not find any leaching of hydrocarbons into groundwater. Dibble and Bartha (1979b) studied the leaching aspect of oil sludge biodegradation in soil. They added fertilizer to oil sludges in soils and examined the leachate for phosphate and undegraded hydrocarbons. There was a modest increase in total organic carbon in the leachate, presumably due to hydrocarbon biodegradation, but no undegraded hydrocarbons or phosphorus was recovered in the leachate. The results support the concept that oil sludge application to soil can be used for biodegradation removal of these materials.

The above studies indicate that the available concentrations of nitrogen and phosphorus severely limit the extent of hydrocarbon degradation after most major oil spills. Rates of nutrient replenishment generally are inadequate to support rapid biodegradation of large quantities of oil. The addition of nitrogen- and phosphorus-containing fertilizers can be used to stimulate microbial hydrocarbon degradation.

Oxygen

As with nutrients, there has been controversy over whether oxygen is absolutely required for hydrocarbon biodegradation or whether hydrocarbons are subject to anaerobic degradation. The current evidence supports the view that anaerobic degradation by microorganisms at best proceeds at negligible rates in nature (Bailey et al., 1973; Ward et al., 1980). The existence of microorganisms which are capable of anaerobic hydrocarbon metabolism has not, however, been excluded. In fact, there have been several reports of isolated microorganisms which are capable of alkane dehydrogenation (Chouteau and Senez,

1962; Iizuka et al., 1969; Parekh et al., 1977; Senez and Azoulay, 1961; Traxler and Bernard, 1969) under anaerobic conditions. These organisms have an enzymatic mechanism which should permit addition of water across the double bond, forming a secondary alcohol, and therefore permit anaerobic growth. Although there have been preliminary reports (Traxler and Bernard, 1969) on the ability of isolated organisms to grow on n-alkanes anaerobically, these findings generally have not been adequately repeated upon further testing (R. W. Traxler, personal communication). In the case of the Pseudomonas strain studied by Senez and Azoulay (1961), the organisms consumed oxygen when growing on heptane even though it had an n=heptane dehydrogenase enzyme.

There have been few reports on the anaerobic degradation of hydrocarbons in natural ecosystems (Bailey et al., 1973; Brown et al., 1969a, 1969b; Pierce et al., 1975; ZoBell and Prokop, 1966). These reports suggested that nitrate or sulfate could serve as an alternate electron acceptor during anaerobic respiration using hydrocarbon substrates. This mechanism has not been biochemically confirmed for hydrocarbon utilization in pure cultures, and the criteria used for assessing anaerobic hydrocarbon degradation in the above-mentioned studies generally were inadequate to establish definite results; either there was a lack of exhaustive evidence for the complete exclusion of oxygen or there was a lack of chemical evidence needed to establish that hydrocarbons were in fact degraded. In the study by Shelton and Hunter (1975), there was an 11% decrease in hexane-extractable material under anaerobic conditions compared with only 4% under aerobic conditions in oiled sediments during 30 weeks of incubation. They concluded, however, that the rapid loss of aliphatic hydrocarbons under anaerobic conditions could not be accounted for by microbial degradation.

Hambrick et al. (1980) found that, at pH values between 5 and 8, mineralization of hydrocarbons in estuarine sediments was highly dependent on oxygen availability. Rates of hydrocarbon degradation decreased with decreasing oxygen reduction potential , i.e., with increasing anaerobiosis. They concluded that hydrocarbons would persist in reduced sediments for longer periods of time than would hydrocarbon contaminants in aerated surface layers. Some mineralization of alkanes (about 10 to 20%) was reported during 35 days of incubation under anaerobic conditions, but mineralization of naphthalene was insignificant (about 0.4% under these incubation conditions). Naphthalene mineralization increased from 0.6 to 22.6% when the redox potential was gradually increased from -220 mV to +130 mV over an additional 35-day incubation period (Delaune et al., 1980). Ward and Brock (1978a) similarly found that hexadecane was rapidly mineralized in freshwater lake sediments under aerobic conditions but that almost no hydrocarbon mineralization occurred under anaerobic conditions. Addition of nitrate and sulfate, in this study, failed to increase hydrocarbon mineralization under anaerobic conditions.

In a recent study, Ward et al. (1980) compared rate of hydrocarbon oxidation in sediments affected by the Amoco Cadiz spillage under aerobic and anaerobic conditions. With ^{14}C-labeled hydrocarbons, $^{14}CO_2$ production from heptadecane and toluene, but not from hexadecane, was found during anaerobic incubation. Methanogenesis could be demonstrated in these tests, indicating rigorous anaerobic conditions. Although measurable degradation rates under anaerobic conditions were found, rates of $^{14}CO_2$ production were orders of magnitude lower under anaerobic than under aerobic conditions. In the absence of oxygen, less than 5% of added hydrocarbon was oxidized to $^{14}CO_2$ during 233 days compared with over 20% during 14 days under aerobic conditions. In this study, petroleum was found in a relatively unweathered state in anaerobic

sediments oiled by the Amoco Cadiz spill, indicating that hydrocarbons are indeed preserved from microbial attack under anaerobic conditions in the environment.

The importance of oxygen for hydrocarbon degradation is indicated by the fact that the major degradative pathways for both saturated and aromatic hydrocarbons, discussed earlier, involve oxygenases and molecular oxygen. The theoretical oxygen demand is 3.5 g of oil oxidized per g of oxygen (Floodgate, 1979; ZoBell, 1969). ZoBell (1969) calculated that the dissolved oxygen in 3.2 X 10^5 liters of seawater therefore would be required for the complete oxidation of 1 liter of oil. Within anoxic basins, the hypolimnion of stratified lakes and benthic sediments, oxygen may severely limit biodegradation.

Johnston (1970) examined the consumption of oxygen in sand columns containing Kuwait crude oil. The oxygen concentration in the interstitial water decreased rapidly. The mean rate of oxygen consumption over 4 months was 0.45 g/m^2 per day at 10°C, corresponding to an oil degradation rate of 90 mg of oil/m^2 per day. Biodegradation of oil in sediments has been found to be stimulated by bioturbation (Gordon, et al., 1978; Lee, 1977). The introduction of oxygen by burrowing animals such as polychaete worms is apparently very important in determining the rate of biodegradation of hydrocarbons in oil-contaminated sediments.

Jamison et al. (1975) used forced aeration to supply oxygen for hydrocarbon biodegradation in a groundwater supply which had been contaminated by gasoline. Nutrient addition without aeration failed to stimulate biodegradation, but when both nutrients and oxygen were supplied, it was estimated that up to 1,000 barrels of gasoline was removed by stimulated microbial degradation. Such manipulations to supply oxygen probably are not feasible in open systems where natural forces such as wind and wave action will

have to be relied upon for turbulent mixing and resupply of oxygen to support biodegradation of oil.

Regardless of whether hydrocarbon degradation can occur at all under anaerobic conditions, the environmental importance of anaerobic hydrocarbon biodegradation can be discounted. Rapid biodegradation of hydrocarbons does not occur in anaerobic environments. Hydrocarbons which enter anaerobic environments such as anoxic sediments are well preserved and persist indefinitely as environmental contaminants.

Salinity and Pressure

The influence of several other environmental factors on hydrocarbon biodegradation has been studied. Typically these factors are specific features of particular ecosystems such as saline lakes or deep seas (high hydrostatic pressure), which represent specialized environments that may be contaminated by petroleum hydrocarbons.

Ward and Brock (1978b) examined hydrocarbon biodegradation in hypersaline environments. When hydrocarbons were added to natural samples of various salinities (from 3.3 to 28.4%) from salt evaporation ponds of Great Salt Lake, Utah, rates of metabolism of these compounds decreased as salinity increased. Rate limitations did not appear to relate to low oxygen levels or to availability of organic nutrient. Gas chromatographic examination of hexane-soluble components of tar samples from natural seeps at Rozel Point in Great Salt Lake demonstrated no evidence of biological oxidation of isoprenoid alkanes which are subject to degradation in normal environments. Attempts to enrich for microorganisms, in saline waters, able to use mineral oil as a sole source of carbon and energy were successful below, but not above, approximately 20% salinity. The study strongly suggests a general reduction of metabolic

rate at extreme salinities and raises doubts about the biodegradation of hydrocarbons in hypersaline environments.

Hydrocarbon pollutants in the world's oceans may eventually sink; some petroleum components may contaminate deep benthic zones. Microbial degradation of organic matter in the deep sea has been found to be greatly restricted (Jannasch et al., 1971). Hydrocarbons do not appear to be an exception. Schwarz et al. (1974a, 1974b, 1975) examined the growth and utilization of hydrocarbons at ambient and in situ pressure for deep-sea bacteria. The rate of hydrocarbon utilization under high pressure and ambient temperatures and atmospheric pressure. Whereas 94% of hexadecane was utilized within 8 weeks at 1 bar, at 500 bars it took 40 weeks for similar degradation. It appears that oil which enters deep-ocean environments will be degraded very slowly and persist for long periods of time.

Case Histories

The fate of petroleum hydrocarbons in the environment from various actual environmental oil contamination incidents has now been examined. It is extremely complex to study the weathering of a mixture such as petroleum in natural, variable environments. Patchiness of oil distribution and uncertainty about localized environmental variations make definitive scientific conclusions difficult to reach. Determining quantitatively the specific role of microorganisms in the fate of polluting oil is difficult, but changes in an environmentally contaminating oil can be viewed in light of the enzymatic degradative capacity of the indigenous hydrocarbon-degrading microbial populations. Environmental factors known to influence rates of microbial hydrocarbon degradation can be examined to estimate limitations of the biodegradative contribution to the removal of petroleum pollutants.

In February 1970, the tanker Arrow ran aground and spilled a large portion of its 108,000-barrel cargo into Chedabucto Bay, Nova Scotia. An estimated 300 km of shoreline was affected. Rashid (1974) described the changes in the oil 3.5 years after the spillage. Degradation of oil depended largely on environmental factors, especially wave energy. Degradation was greatest in high-wave-energy environments and lowest in protected embayment areas. In the high-energy environments, there was a substantial loss of n-alkanes, which was believed to be due to microbial degradation. Presumably, oxygen and nutrients replenished by wave-driven mixing permitted more extensive degradation. Six years after the spill, it was impossible to estimate the amount of oil remaining in Chedabucto Bay from the spillage due to the patchy distribution of the oil, contributions of more recent spillages, and the absence of adequate control sites (Keizer et al., 1978).

In January 1973, the Irish Stardust ran aground near Vancounver Island, B.C. Approximately 180 metric tons of fuel oil was spilled. Cretney et al. (1978) examined the long-term fate of the heavy fuel oil from the spill that contaminated a British Columbia, Canada, coastal bay. They reported that biodegradation accounted for almost complete removal of n-alkanes during the first year after the spill. Pristane and phytane were biodegraded more slowly, but were almost completely gone after 4 years. The non-n-alkane components of the C_{28} to C_{30} range appeared to be the most resistant to degradation of all the components resolved by gas chromatography.

During March 1971, a pipeline rupture allowed JP-4 jet fuel and no. 2 fuel oil to enter the intertidal zone of a cove at Searsport, Maine. The spill was approximately 13 metric tons, but only a fraction of that amount probably entered the cove. Mayo et al. (1978) examined the weathering characteristics of petroleum hydrocarbons deposited in fine-clay marine sediments of the cove.

They found that petroleum residues isolated from the spill gave the appearance of weathering particularly slowly in the cold anoxic sediment. In 1976, they found that the average area contained roughly 20% less hydrocarbon than in 1971, when the spill occurred. At a number of sites, there appeared to be no decline in gross hydrocarbon concentrations and essentially no weathering of the aliphatic portions of the petroleum residues. The data indicated that although microbial degradation of the aliphatic linear chain systems had a measurable impact on the residues contained in upland sediments, this action was greatly suppressed in the residues absorbed on the anoxic cold clay silt of the cove. Microbial transport of the oil from the upland spill location to the marine sediment, but within the marine sediments rates of microbial degradation must have been near zero. It is likely that a lack of available oxygen in the contaminated sediments severely limited rates of biodegradation.

The tanker Metula grounded in the Straits of Magellan in August 1974. Approximately 46,000 metric tons of oil was lost, contaminating a cold marine environment. Colwell et al. (1978) examined the biodegradation of petroleum from the Metula spill in the Straits of Magellan region. They found from biodegradation studies that oil degradation under in situ conditions proceeded relatively slowly, with marked persistence of Metula oil in the Straits of Magellan 2 years after the spill. They reported that the slow rates of oil degradation most probably were due to limitations imposed by relatively low concentrations of nitrogen and phosphorus available in seawater, as well as restricted accessibility to degradable compounds within aggregated oils or tar balls. Temperature did not seem to be a limiting factor for petroleum degradation in the cold marine environment. There was an indigenous cold-adapted microbial community capable of utilizing hydrocarbons. Microbial degradation was not effective in attacking buried oil or oil that had formed

asphalt layers on beaches. Microbial action may have contributed significantly to the formation of polar material and contributed to the extensive removal of aliphatic hydrocarbons in favorable environments. It was concluded that the oil from the Metula spill would persist for a long period of time.

Two major spillages of no. 2 fuel oil into Buzzards Bay, Mass., have been studied. The Florida created the West Falmouth spill in 1969, and the Bouchard was the source of a second spill in 1974. Blumer et al. (1972, 1972a, 1972b) examined the disappearance of oil from the West Falmouth spill. They found that the disappearance of petroleum hydrocarbons was slow and that bacterial degradation contributed to the removal of n-paraffins. Teal et al. (1978) examined the aromatic hydrocarbons contaminating the sediments of Buzzards Bay resulting from both spillages. Microbial degradation was believed to contribute to the disappearance of naphthalenes with zero to three alkyl substituents and phenanthrenes with zero to two substituents from surface sediments. The more substituted aromatics decreased relatively less and probably were more resistant to biodegradation.

Pierce et al. (1975) examined the persistence and biodegradation of fuel oil on an estuarine beach which came from the spillage of 90,000 gal (ca. 342,000 liters) of no. 6 fuel oil into Narragansett Bay, R.I., in 1973. The concentrations of hydrocarbons in the midtide region declined simultaneously with an increase in populations of hydrocarbon-utilizing bacteria. During the winter months, hydrocarbon biodegradation was apparent at rates of less than 1 µg of hydrocarbon per g (dry weight) of sediment per day. McAuliffe et al. (1975) examined the fate of 65,000 barrels of crude oil spilled in 1970 from a Chevron platform 11 miles (ca. 17.6 km) east of the Mississippi River delta. They found that only 1% of the oil entered the sediments; much of the oil dissipated. One week after the spill, there was evidence for biodegradation of

the oil in the sediment as shown by an alteration in the the ratio of n-paraffins to isoprenoid hydrocarbons. Within 1 year, most of the oil was gone and rapid biodegradation appeared to contribute to the removal of contaminating hydrocarbons.

Atlas et al. (1978) studied petroleum biodegradation in various coastal Arctic ecosystems which had been experimentally contaminated with Prudhoe crude oil. Hydrocarbon biodegradation potentials were lower in ice than in water or sediment. Natural rates of degradation were slow, and maximal losses from experimental oil spills were less than 50% during the Arctic summer due to combined abiotic and biodegradative losses. Rates of biodegradation were found to be limited by temperature and concentration of available nitrogen and phosphorus. Residual oil had similar percentages of hydrocarbon classes as fresh oil; i.e., biodegradation of all oil component classes, including paraffinic and aromatic fractions, apparently proceeded at similar rates. In March 1977, there was a spill from the Potomac into the ice-laden waters of Melville Bay in the northeastern part of Baffin Bay, off western Greenland. About 107,000 gal (ca. 406,600 liters) of Bunker C fuel oil was lost. The fate of the oil was investigated by a team of scientists (Grouse et al, 1979). Biodegradation of the oil at the low water temperatures was found to proceed very slowly if at all. There was no significant increase in numbers of hydrocarbon utilizers within a few weeks after the spill. During this period there was also almost no change in the C_{17}/pristane ratio in the oil, indicating that biodegradation was not occurring at a significant rate.

The spill of the supertanker Amoco Cadiz in March 1978 resulted in the largest oil spill to that date. In excess of 190,000 metric tons of oil was released into the marine environment during 2 weeks. A variety of intertidal sites off the Brittany coast was affected. Aminot (1980) examined the fate of

the oil in the water column before reaching the shoreline. He found a depletion of N. P, and O_2 in the water column beneath the oil, which apparently resulted from microbial degradation of petroleum hydrocarbons. The in situ deficits of N, P, and O_2 converted to a hydrocarbon biodegradation rate of 0.03 mg of oil degraded per liter per day in the water column under the oil. Aminot estimated that 9,000 metric tons of oil was biodegraded in the water column during the 2 weeks following the spill. The fate of the Amoco Cadiz oil within the intertidal zone was studied by several investigators (Atlas et al., 1981; Atlas and Bronner, 1980; Boehm and Fiest, 1980a; Calder and Boehm, 1980; Vandermeulen and Traxler, 1980; Ward et al., 1980). Microbial degradation appears to have played a very important role in the weathering of oil stranded within the littoral zone. Atlas and Bronner (1980) estimated a biodegradation rate of 0.5 µg of hydrocarbon per g (dry weight) of sediment per day within the affected intertidal zone. The onset of extensive changes in the oil appears to have occurred more rapidly after the wreck than was anticipated, extensive biodegradation even preceding complete evaporation and dissolution of volatile aromatics (Atlas et al., 1981; Calder and Boehm, 1980); there was a rapid change in the n-alkane/isoprenoid hydrocarbon ratio within days to weeks. The isoprenoid alkanes, C_{27} to C_{31} n-alkanes, hopanes, alkylated dibenzothiophenes, and alkylated phenanthrenes were the classes of hydrocarbons most resistant to biodegradation. Despite the rapid rates of biodegradation, the magnitude of the spill was such that the oil will persist within the littoral zone for a prolonged period. Oil that was buried, oil within anoxic sediments, and oil within embayments appears to be most persistent (Atlas et al., 1981; Boehm and Fiest, 1980a; Walker and Colwell, 1976d). Conditions which enhance aeration and resupply nutrients, such as high-energy wave action, favor biodegradation.

The magnitude of the Amoco Cadiz spill was surpassed by the spill form the IXTOC-I well blowout. In June 1979, oil began spilling into the Bay of Campeche, Gulf of Mexico. The oil flowed for 10 months before the well was capped. Some of the oil washed onto the coastal beaches of Texas, but for the most part the current carried the oil away from U.S. waters. The oil from the IXTOC-I well formed a mousse. Boehm and Fiest (1980b) found little evidence for biological weathering of the hydrocarbons in the mousse. Atlas and co-workers (1980a, 1980b) found that biodegradation of mousse was greatly restricted, probably due to nutrient limitations and limited surface area for microbial attack. During a 6-month laboratory incubation under simulated natural conditions, 2 to 5% of the mousse (Cobet and Guard, 1973) was converted to CO_2. Despite favorable temperatures and high populations of hydrocarbon utilizers in association with the mousse, changes in n-alkane/isoprenoid ratios took months rather than days to weeks. The contribution of biodegradation to weathering of oil from the IXTOC-I well was notably slower and of less magnitude than was found for the Amoco Cadiz. Pfaender and co-workers (Buckley and Pfaender, 1980; Pfaender et al., 1980) examined the degradation of hydrocarbons within the water column affected by the IXTOC-I oil. They found relatively rapid turnover times for hydrocarbons which had become dissolved in the water column. Rates of degradation ranged from 0.01 to 44 µg of aliphatic hydrocarbon respired per liter per h with turnover times of 30 to 266 h.

In contrast to the cited studies on large marine oil spills, there have been few studies on freshwater ecosystems occurs frequently, but the spillages are generally of small magnitude. Unless a special resource such as a drinking water supply is contaminated, such "minor" spillages are often neglected. Jamison et al. (1975, 1976) did examine the degradation of gasoline in a contaminated groundwater supply. They used stimulated biodegradation to

enhance removal of hydrocarbons from the contaminated water supply. Roubal et al. (1979) followed the disappearance of hydrocarbons from the Ohio River after a major spillage of gasoline. They found that the hydrocarbons were rapidly removed. The microbial community was found to be capable of contributing to the disappearance of the contaminating hydrocarbons; the biodegradative potential was capable of responding within 1 to 2 days. Horowitz and Atlas (1977b) examined the fate of 55,000 gal (ca. 209,000 liters) of gasoline which had contaminated an Arctic lake that served as a drinking water supply. In situ measurement of gasoline degradation showed that, if untreated, sediment retained even "volatile" light hydrocarbons. Nutrient addition was found to enhance biodegradative losses.

Several studies have examined the fate of oil in soil ecosystems. Some of these studies involved experimental contamination of soil to examine the feasibility of using land farming for removal of oily wastes (Dibble and Bartha, 1979a; Francke and Clark, 1974; Gudin and Syratt, 1975; Kincannon, 1972; Lehtomake and Niemela, 1975; Maunder and Waid, 1973; Raymond et al., 1976). Concern has been expressed about the leaching of oil applied to soil into groundwater supplies. There have been some reports on mobilization of oil into the soil column (Verstraete et al., 1975), but in most cases there has been little evidence for significant downward leaching of oil (Dibble and Bartha, 1979a; Raymond et al., 1976). Kincannon (1972) applied residual oil from a refinery tank, Bunker C fuel oil, and a waxy raffinate to soils and found a degradation rate of 8.3 $m^3/4 \times 10^3 m^2$ per month. Francke and Clark (1974) reported a degradation rate of 11.9 $m^3/4 \times 10^3 m^2$ per month for used crankcase oil applied to soil. They found that rates of degradation did not exceed 2.4 $m^3/4 \times 10^3 m^2$ per month.

Dibble and Bartha (1979c) examined the rehabilitation of a New Jersey wheat field which had been contaminated with approximately 1.9 million liters of kerosene over 1.5 hectares. A rehabilitation program consisting of liming, fertilization, and frequent tilling was initiated, and the decrease of hydrocarbon contaminants was monitored for a 2-year period. During the 2 years of the study, the hydrocarbon content of the surface oil decreased to an insignificant level. Seasonal differences were found in the rate of hydrocarbon disappearance. Within 1 year after the spillage, the field returned to a near-normal productive state. Odu (1972) reported evidence for microbial degradation of oil spilled on a sandy soil in Nigeria from an oil well blowout. Several Arctic terrestrial oil spills have been examined. Cook and Westlake (1974) found evidence for extensive utilization of n-alkanes in oils applied in the Norman Wells area of the northwest territories and in the Swan Hill area of northern Alberta. They also found evidence for biodegradation of oil of the Nipisi spill in northern Alberta. The spill was on a sphagnum bog. Sexstone et al. (1978a), in contrast, found evidence for greatly restricted rates of biodegradation in northern soils. They found that hydrocarbons were still present in soils at Fish Creek, Alaska, 28 years after contamination by spillage of refined oil.

SUMMARY OF CURRENT STATE OF KNOWLEDGE

The rates of biodegradation of hydrocarbons from oil spills appear to be highly dependent on localized environmental conditions. It is apparent that the microbial degradation of oil pollutants is a complex process and that environmental factors have a great influence on the fate of spilled oil. The fate of many components in petroleum, the degradative pathways which are active in the environment, the importance of co-oxidation in natural ecosystems, and the role of microorganisms in forming persistent environmental contaminants

from hydrocarbons such as the compounds found in tar balls are unknown and require future research. Although a number of rate-limiting factors have been elucidated, the interactive nature of microorganisms, oil, and environment still is not completely understood, and further examination of case histories is necessary to improve predictive understanding of the fate of oil pollutants in the environment and the role of microorganisms in biodegradative environmental decontamination. With an understanding of the microbial hydrocarbon degradation process in the environment, it should be possible to develop models for predicting the fate of hydrocarbon pollutants and to develop strategies for utilizing microbial hydrocarbon-degrading activities for the removal of hydrocarbons from contaminated ecosystems.

IV Study Area

Regions studied during the course of this study included: the northeastern Gulf of Alaska, the northwestern Gulf of Alaska, Cook Inlet, the north Aleutian shelf region of the Bering Sea, the St. George basin region of the Bering Sea, the Navarin Basin Region of the Bering sea, Norton Sound, and the Beaufort Sea.

V Methods and Materials

SAMPLING

Water samples were collected using a Niskin sterile water sampler. Water samples were transferred to sterile containers and processed as soon as possible. Sediment samples were collected using a Smith-MacIntyre or Sutar-Van Veen grab sampler in water depths of greater than 5 m. A relatively undisturbed surface sediment was collected from each of the grab sampler. In shallow waters a Kahl mud snapper was used to collect sediment samples. Each sediment was mixed with a small portion of overlying seawater to produce a slurry to facilitate handling of the sediment. At the time of sampling the location, temperature, and salinity were determined. The sampling sites are shown in Figures 1a - 1s. A comprehensive list of samples collected in this study including sampling locations, dates of collection, sampling depths, temperatures, and salinities is given in Table 1.

ENUMERATION OF MICROBIAL POPULATIONS

Total Numbers of Microorganisms

Enumeration of total bacterial populations was performed using a direct count procedure. Serial dilutions of the samples were preserved 1:1 with formaldehyde. Samples were filtered through 0.2 µm cellulose nitrate black filters and stained with acridine orange according to the procedures of Daley and Hobbie (1975). The black filters were examined with an Olympus epifluorescence microscope with a BG-12 exciter filter and a O-530 barrier filter. Ten fields per filter and two filters per sample were enumerated and the counts averaged.

Viable Plate Counts

Serial dilutions of samples were made in Rila marine salts solution (Rila Products, Teaneck, N.J., U.S.A.) and plated on marine agar 2216 (Difco). (The

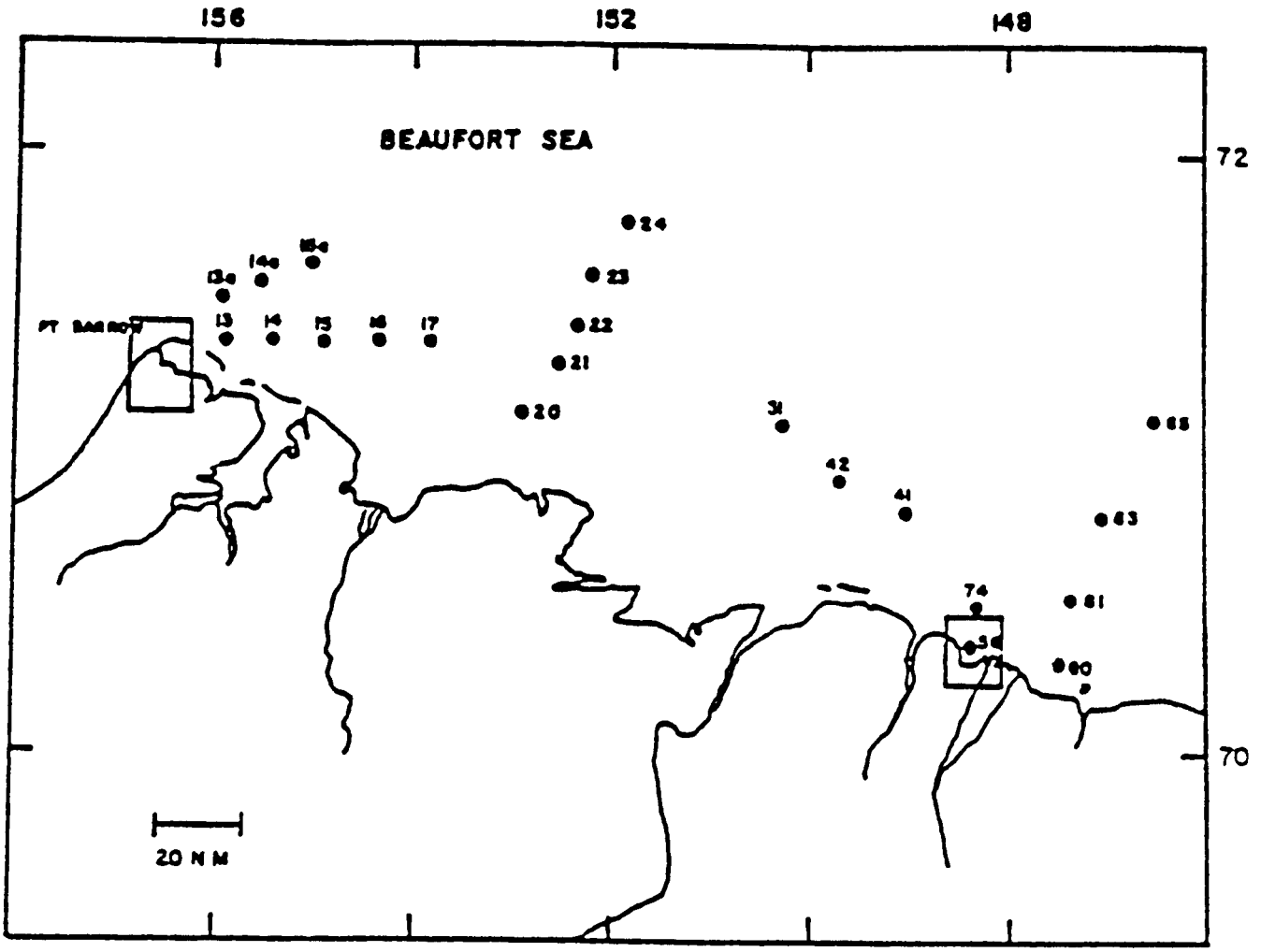

Figure 1a. Stations sampled in the Beaufort Sea during April and August 1976.

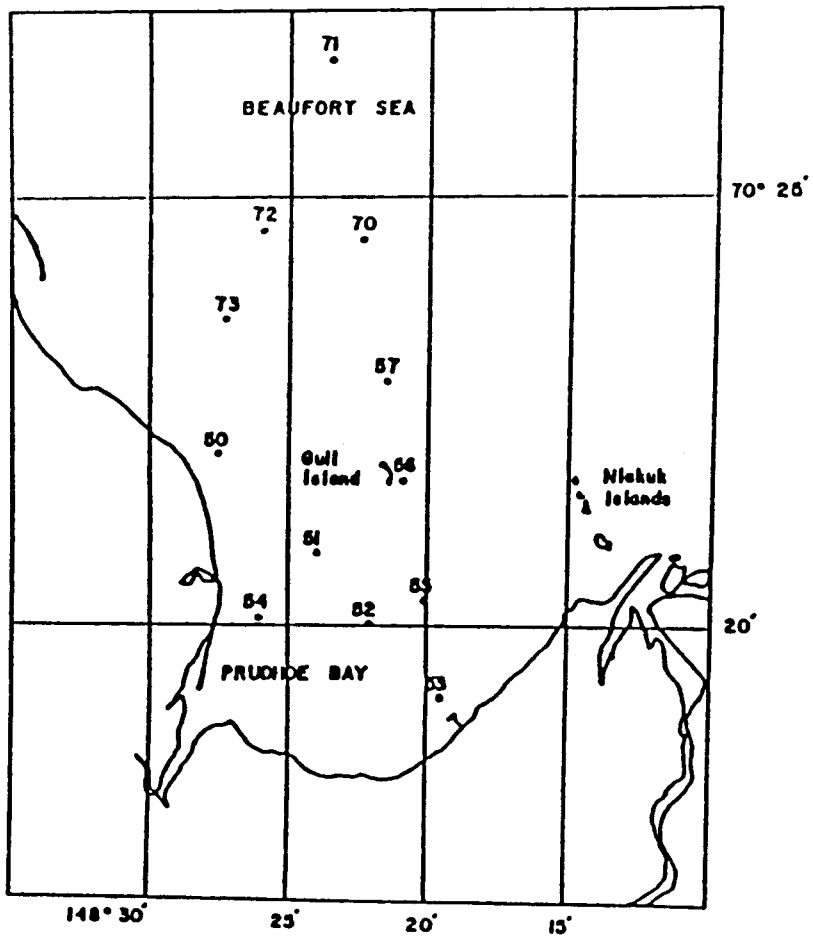

Figure 1b. Stations sampled in the Prudhoe Bay area during April 1976.

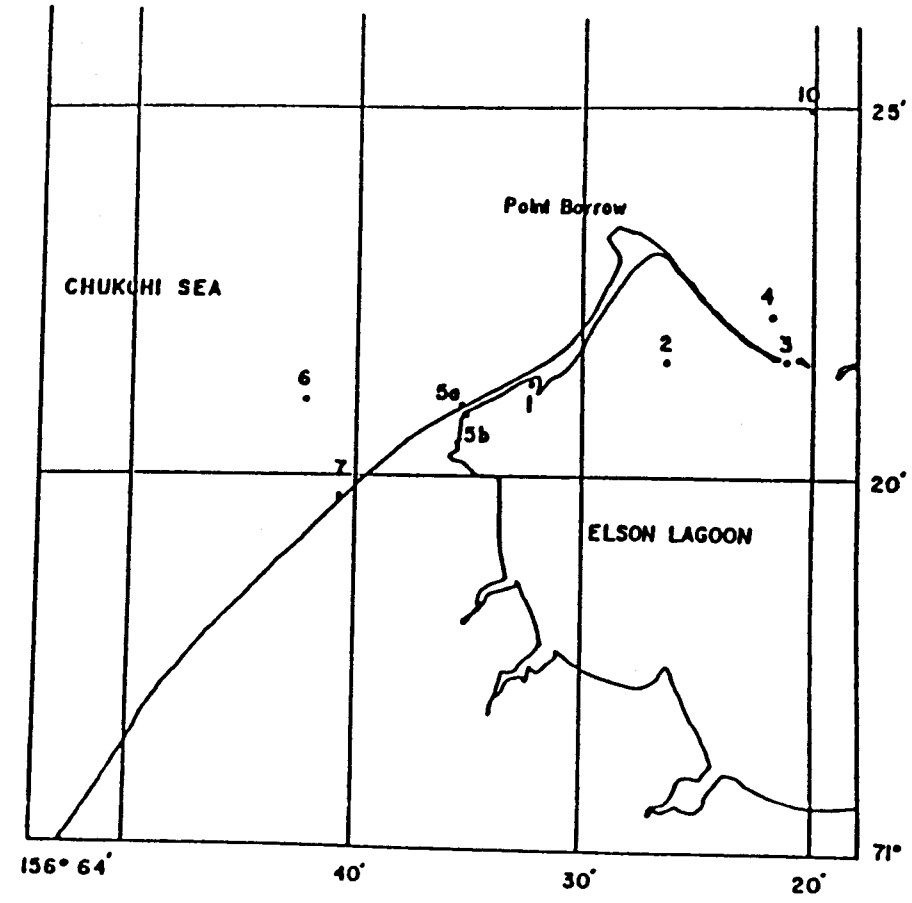

Figure 1c. Stations sampled in the Barrow area during April 1976.

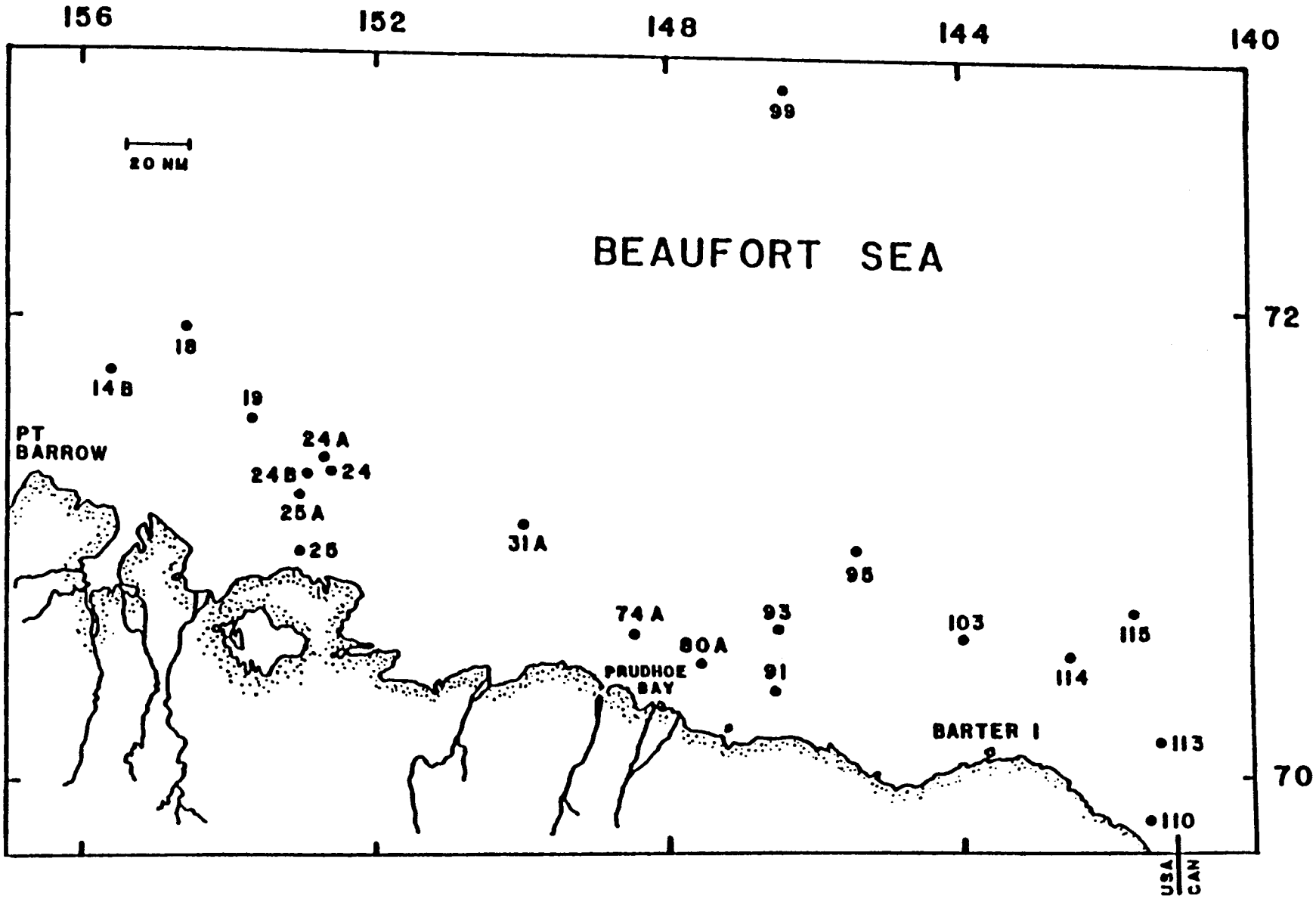

Figure 1d. Stations sampled in the Beaufort Sea during the September 1977 cruise.

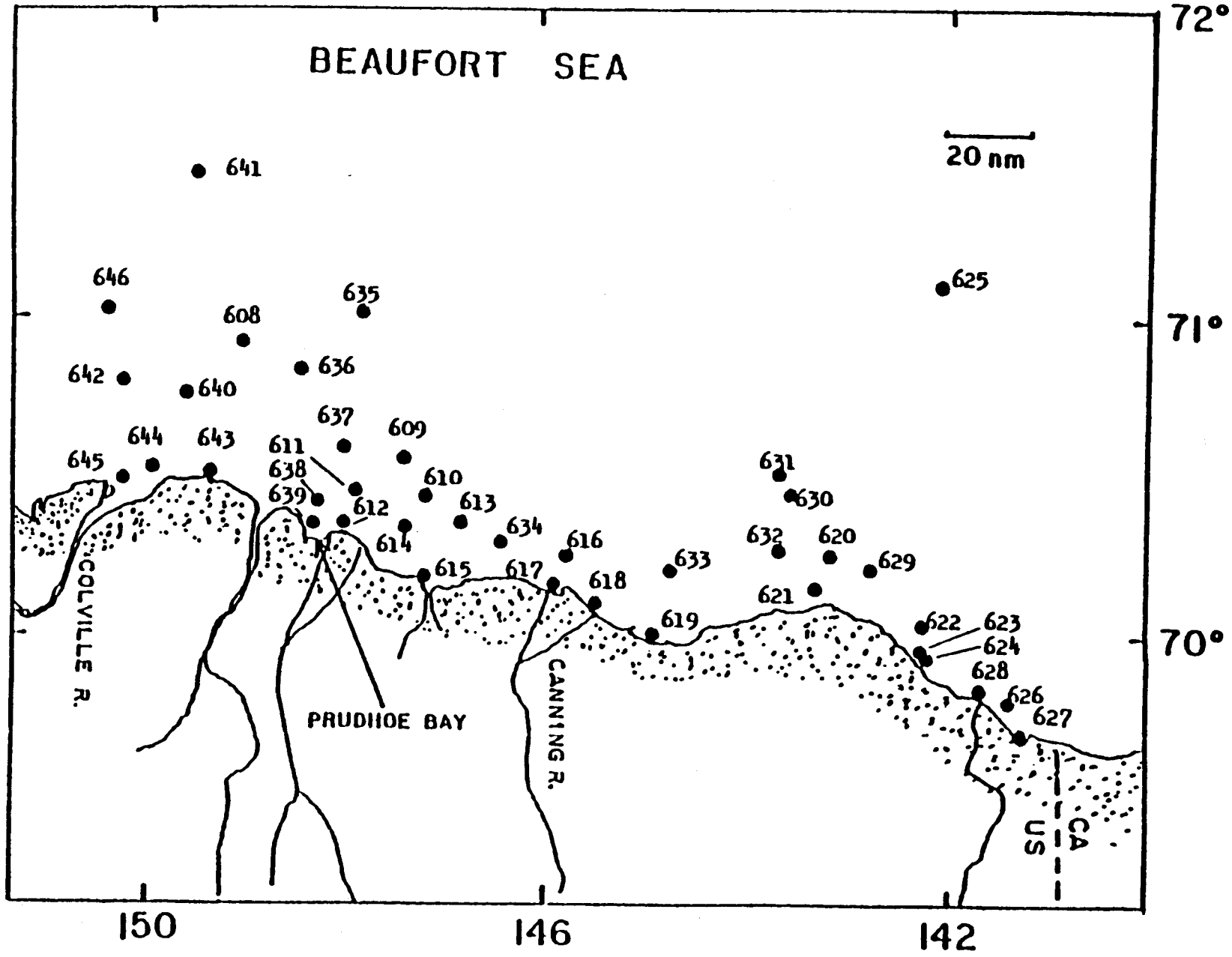

Figure 1e. Stations sampled in the Beaufort Sea during the August 1978 cruise.

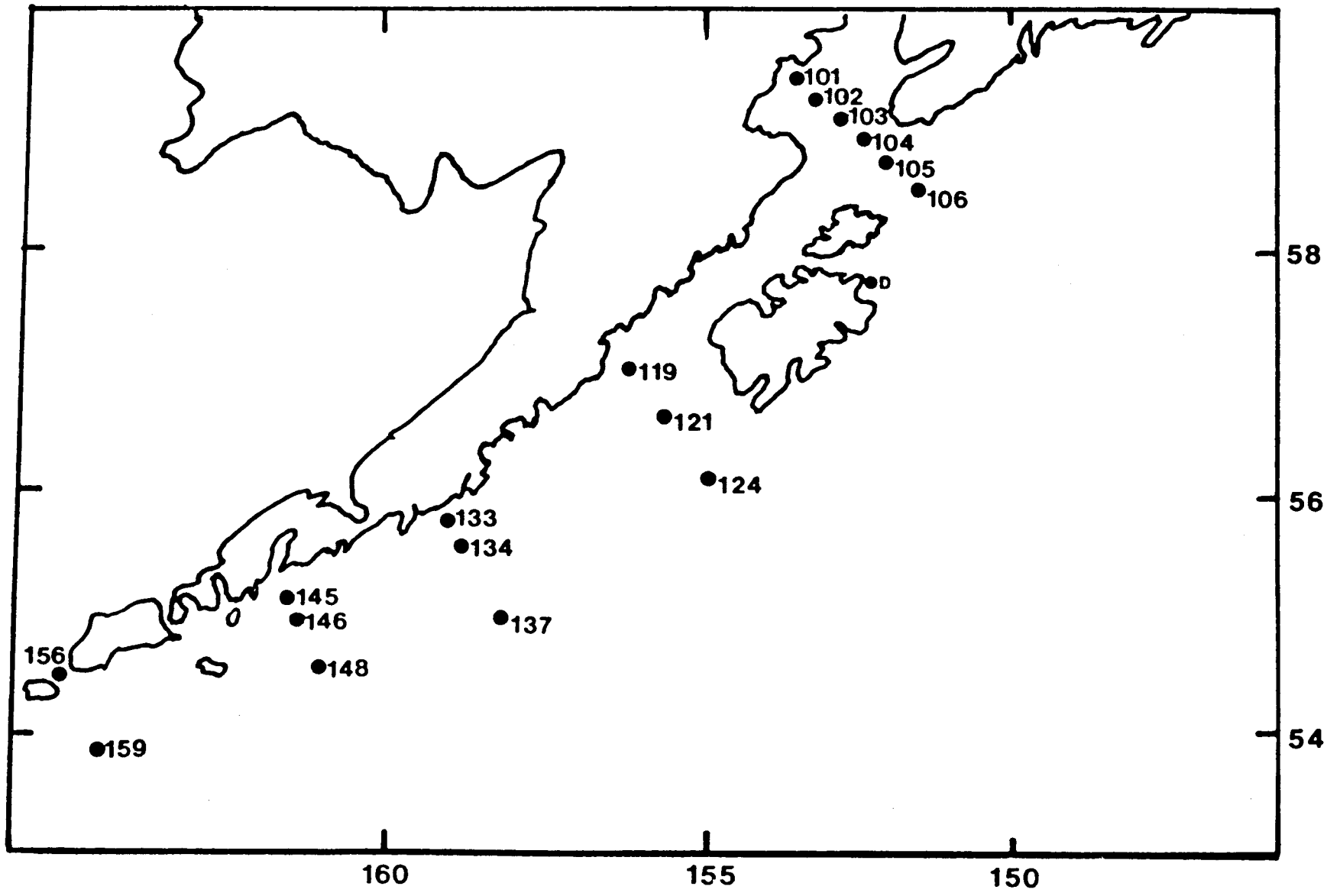

Figure 1f. Stations sampled in northwest Gulf of Alaska, September 1975.

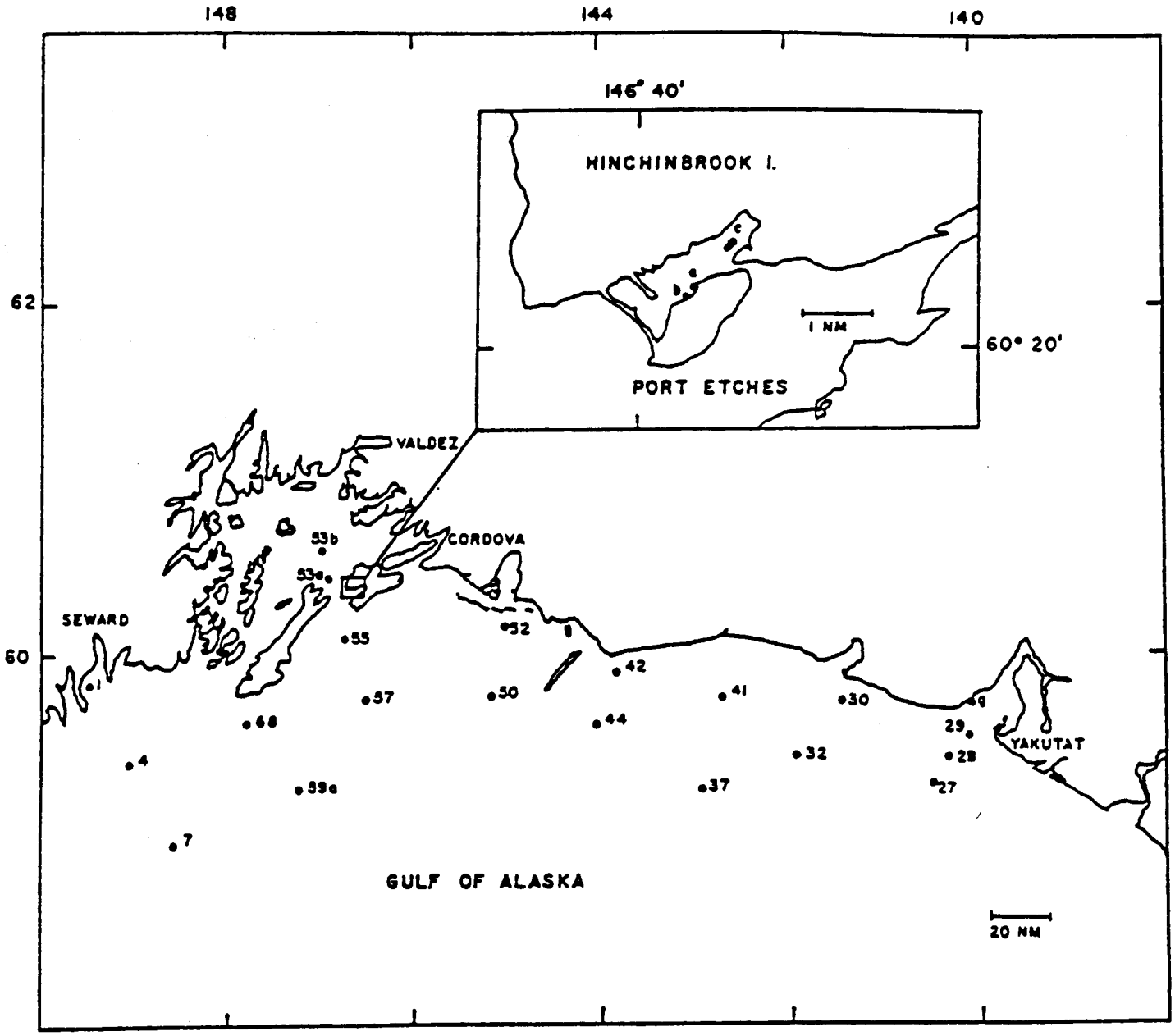

Figure 1g. Stations sampled in the Gulf of Alaska during the March 1976 cruise.

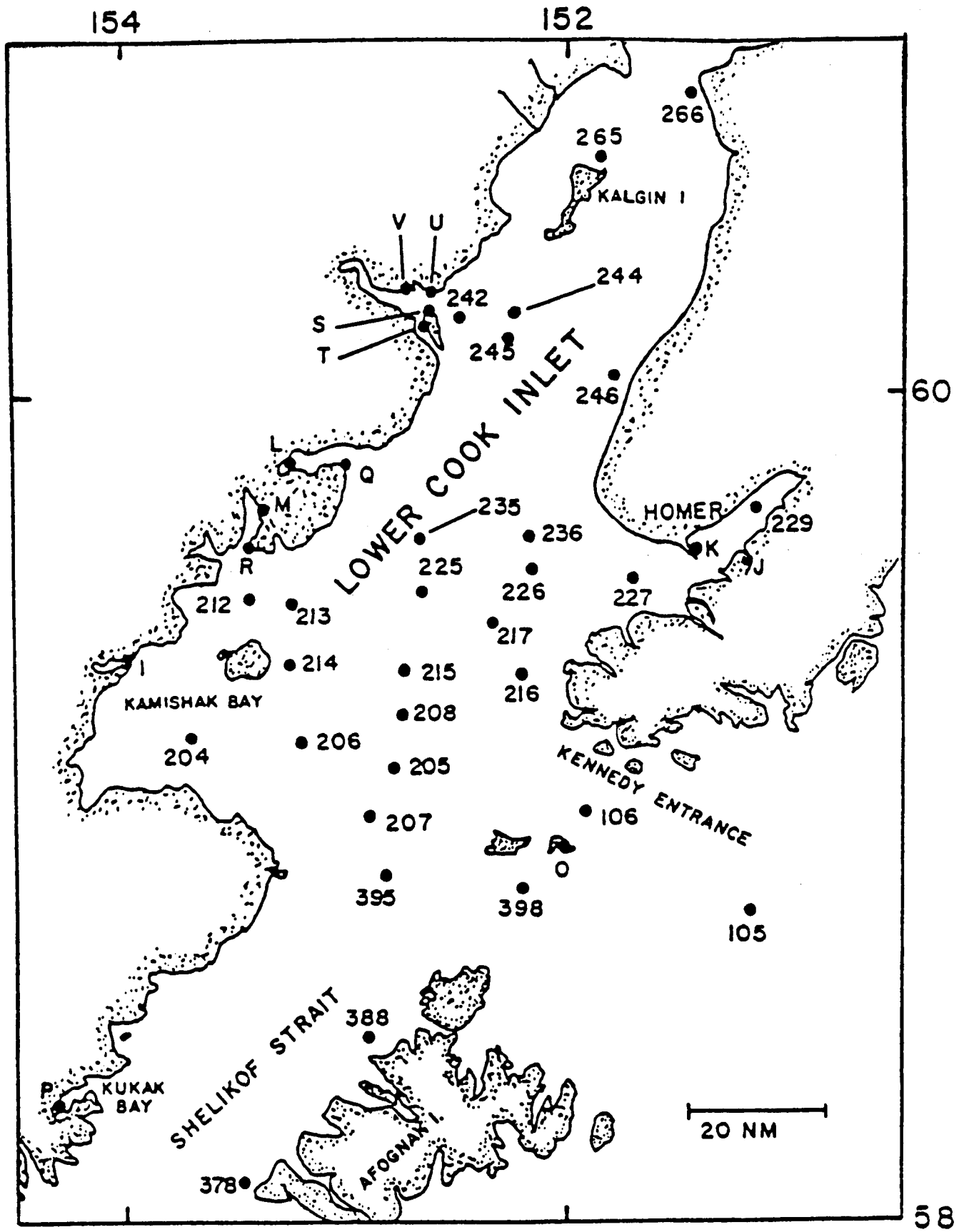

Figure 1h. Stations sampled in Cook Inlet during the April 1977 cruise.

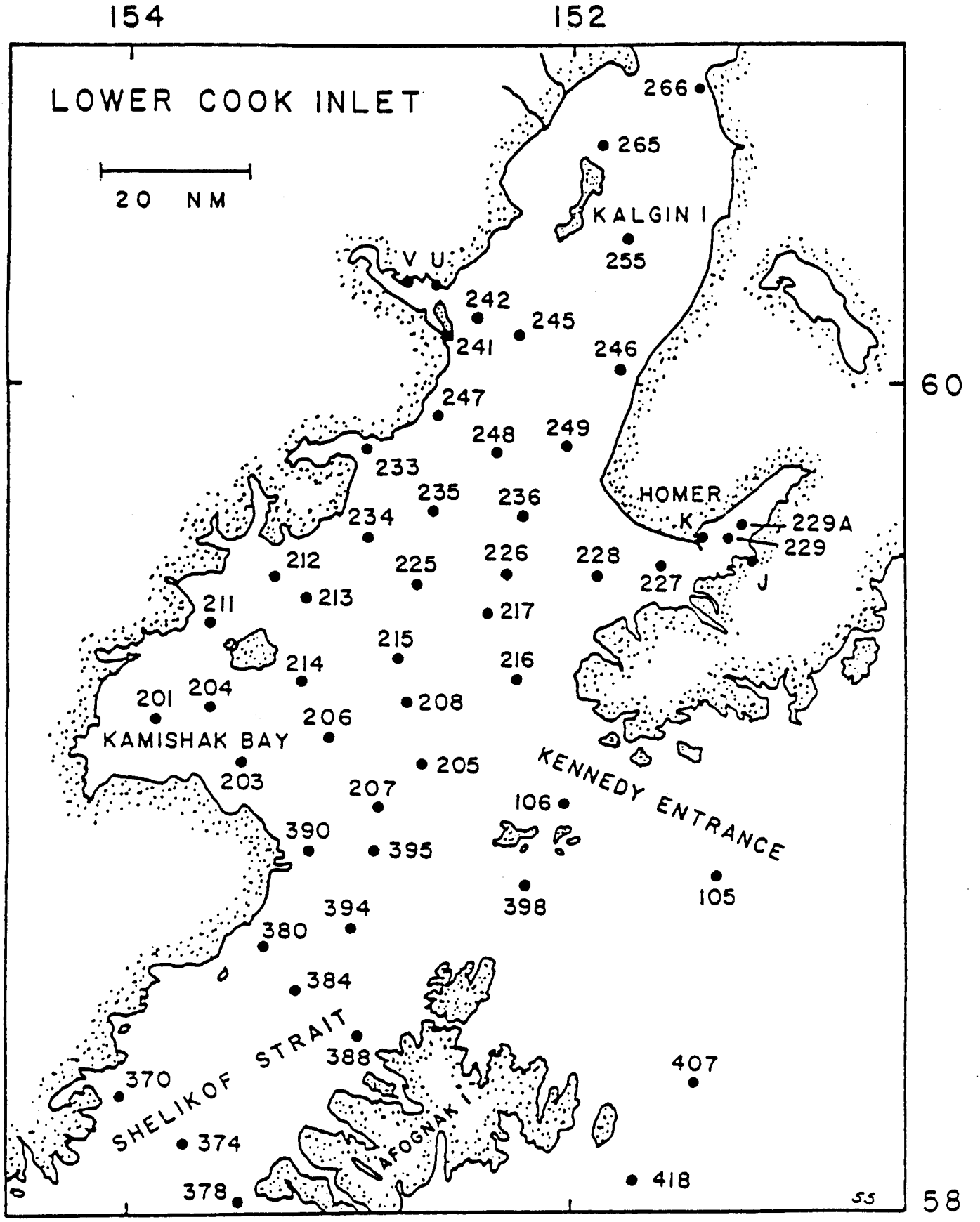

Figure 1i. Stations sampled in Cook Inlet during the November 1977 cruise.

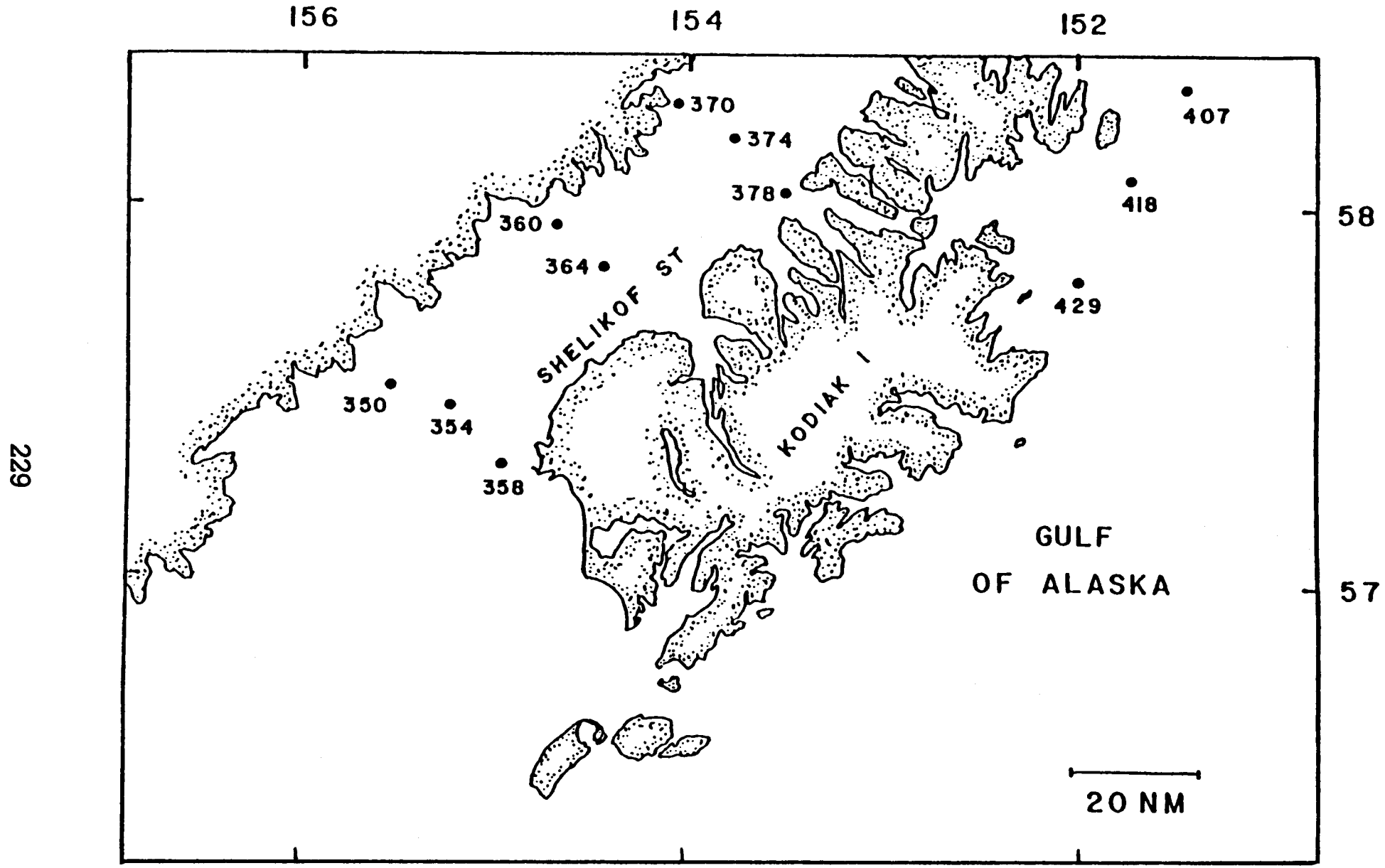

Figure 1j. Stations sampled near Kodiak Island during the November 1977 cruise.

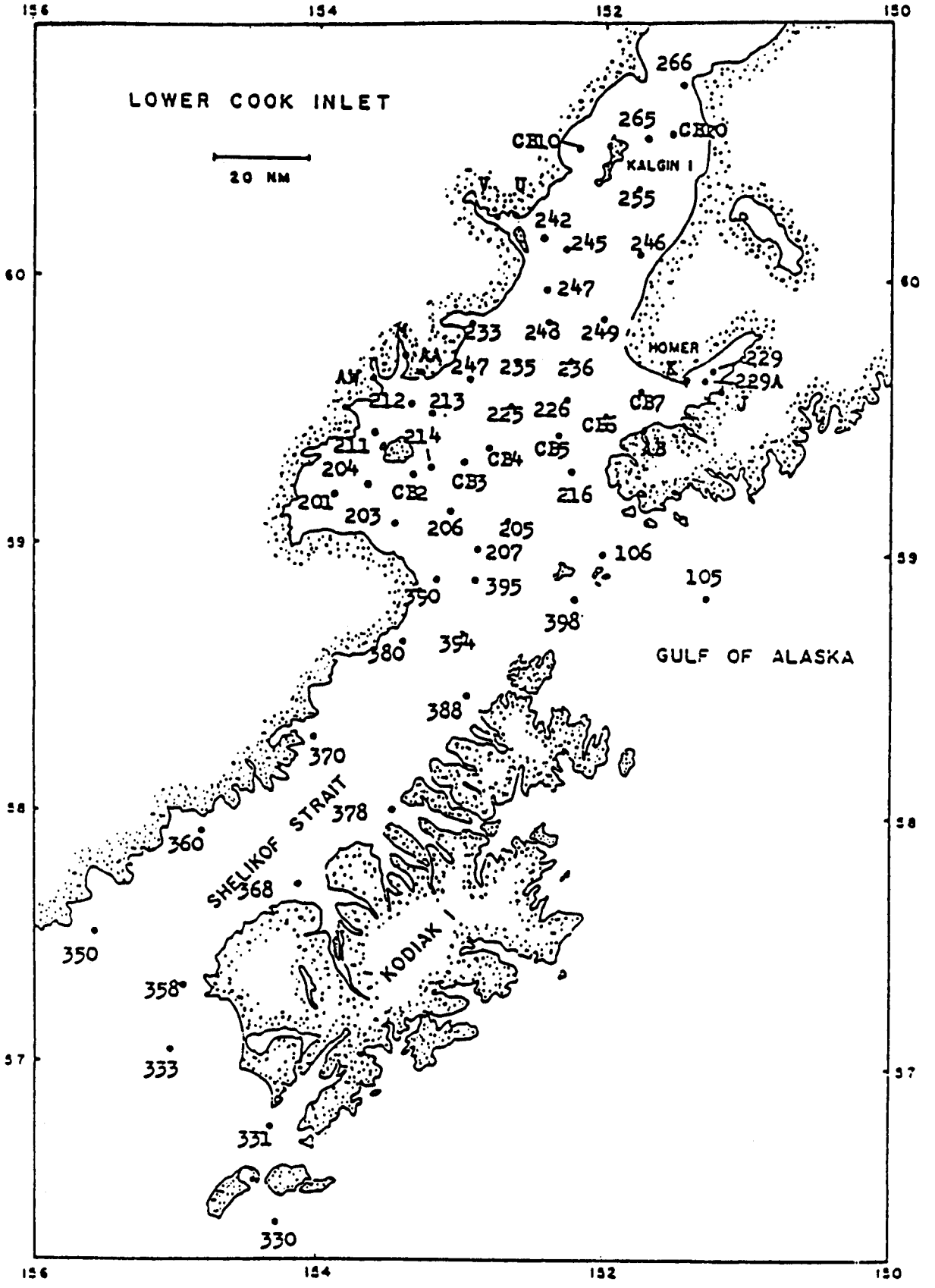

Figure 1k. Location of stations sampled during the April 1978 Cook Inlet cruise.

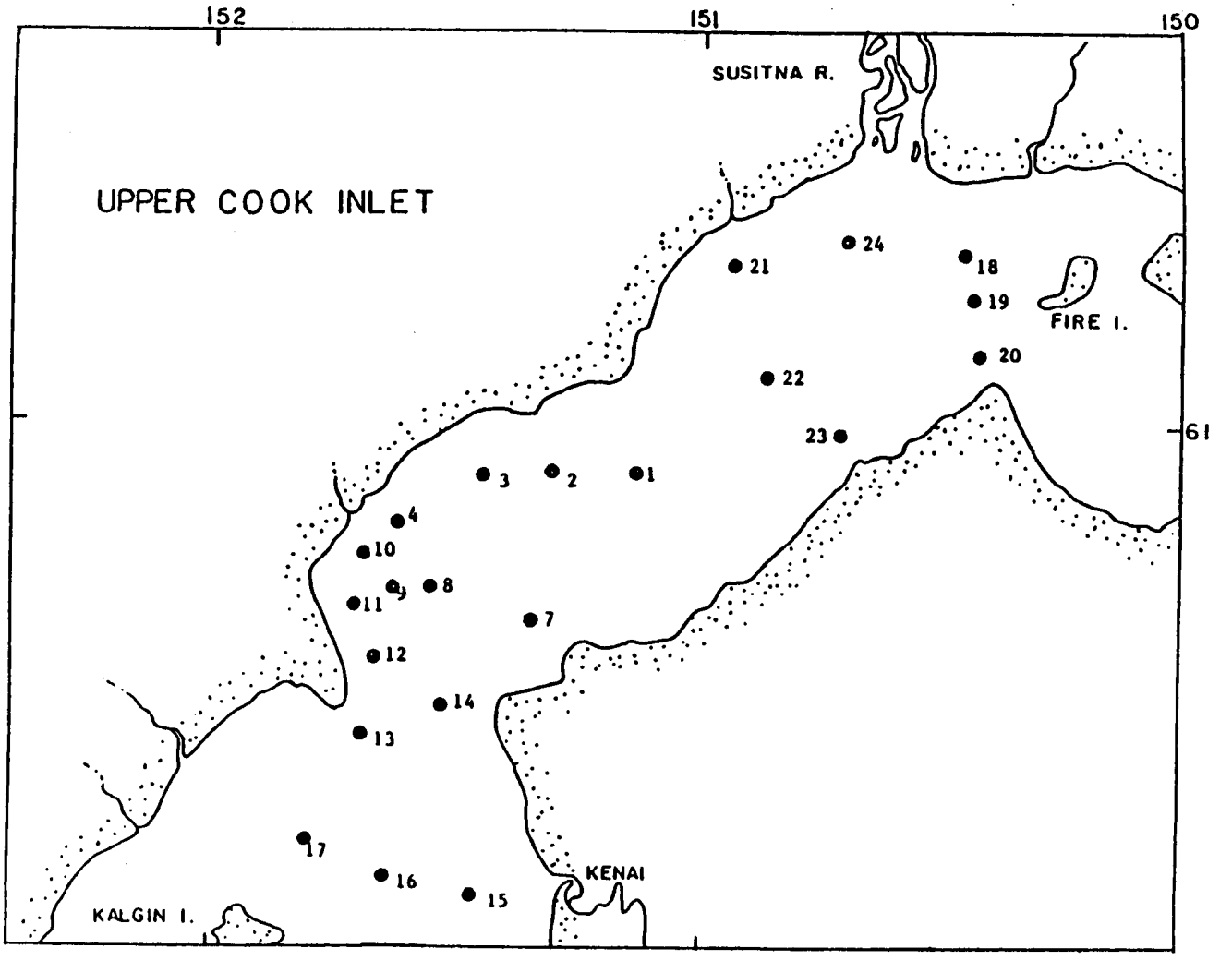

Figure 11. Stations sampled in upper Cook Inlet during the April 1979 cruise.

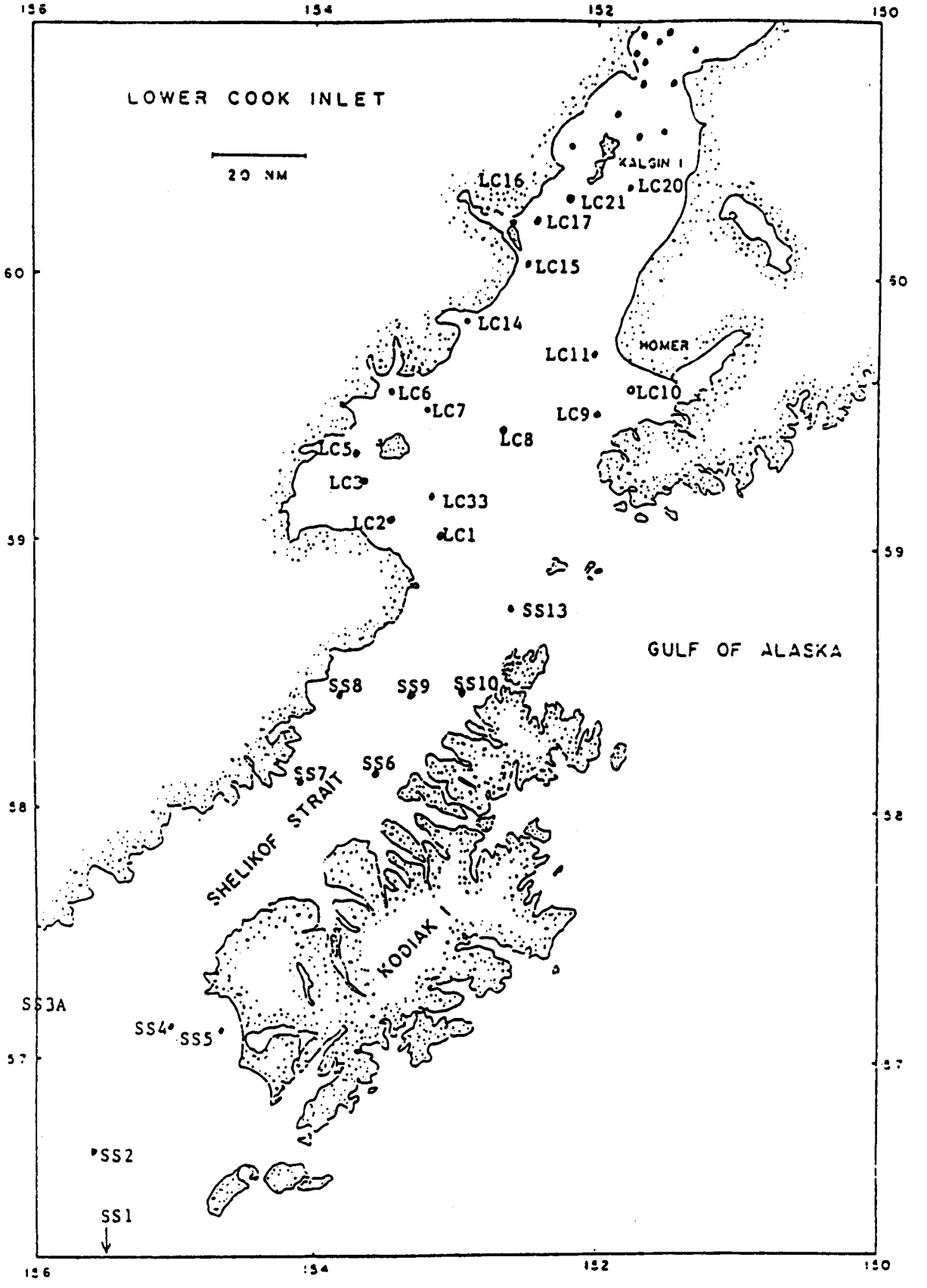

Figure 1m. Stations sampled in Cook Inlet during the April 1979 cruise.

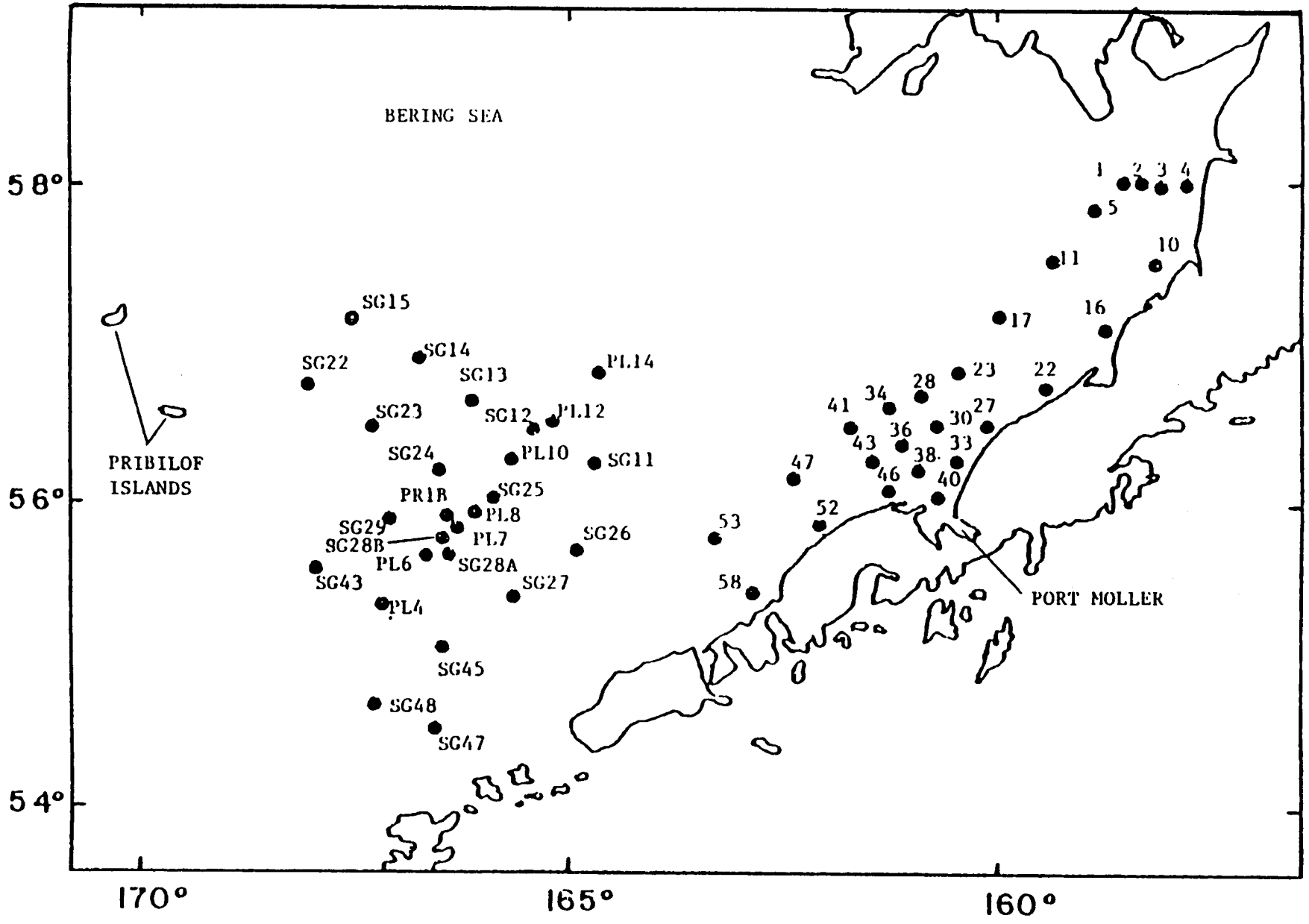

Figure 1n. Positions and station designations for locations sampled during January 1981 North Aleutian Shelf Transport Study (excluding Port Moller stations). Stations without letter designations are "NA" stations.

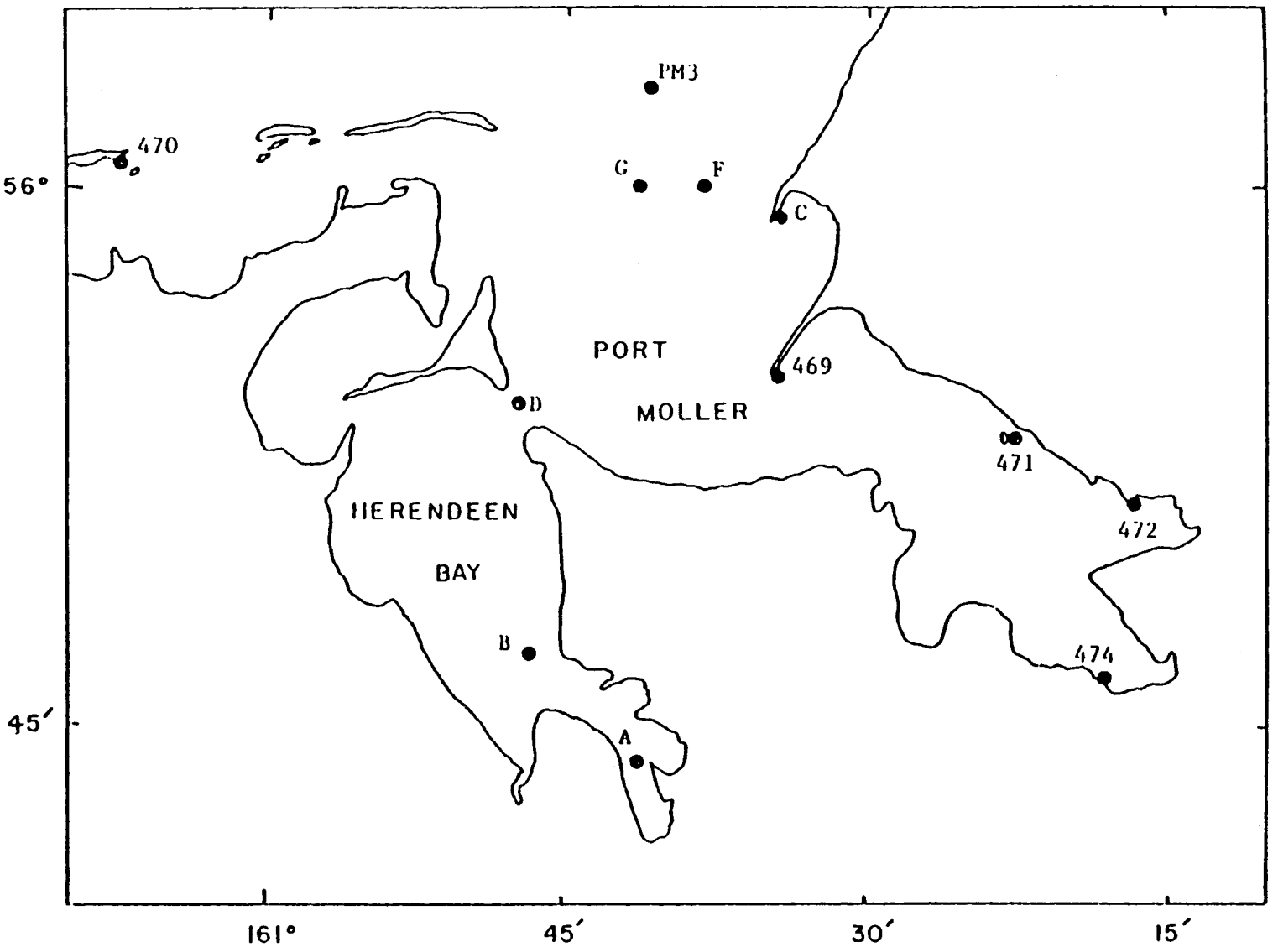

Figure 1o. Positions and station designations for locations in Port Moller during the January 1981 cruise.

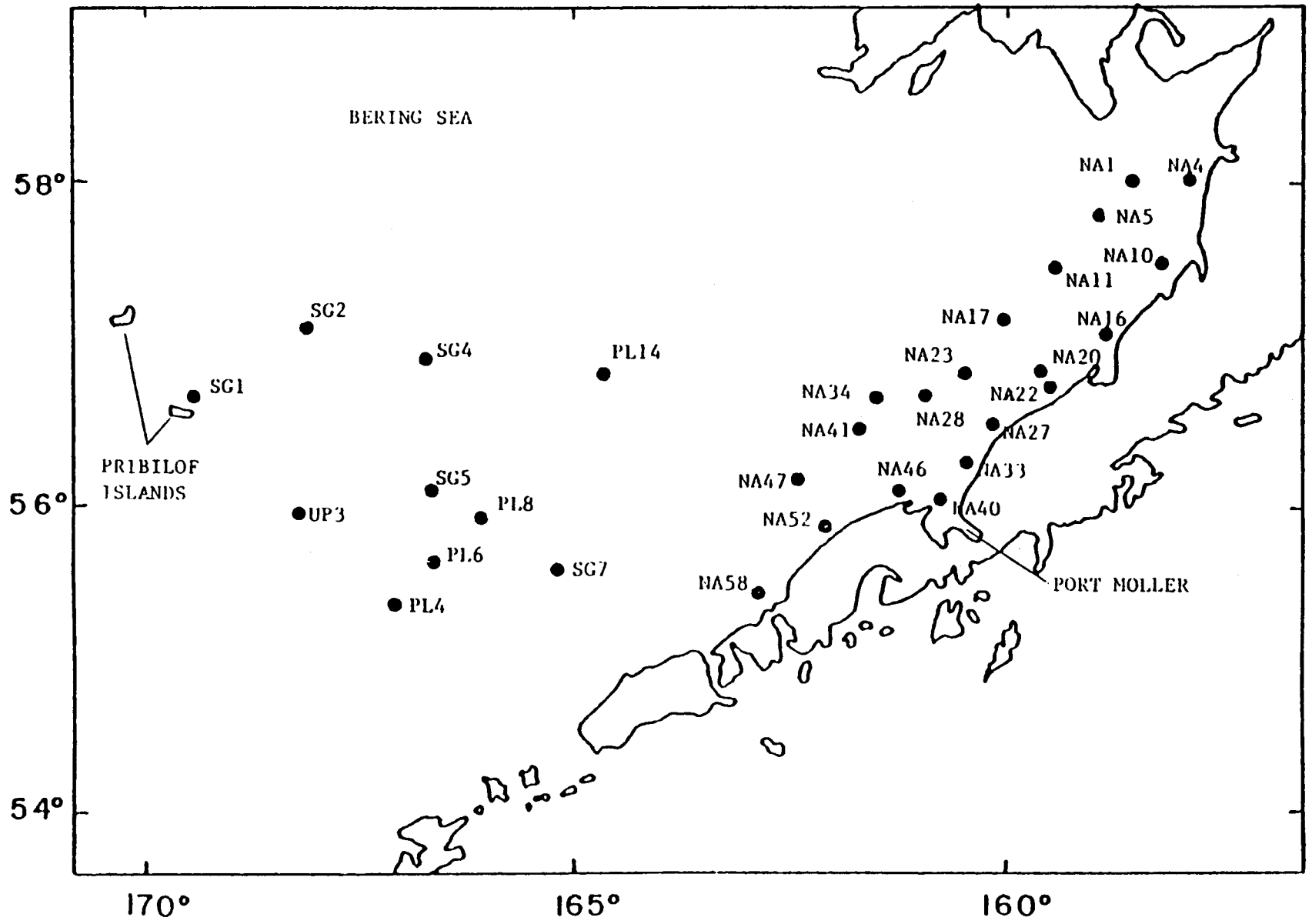

Figure 1p. Positions and station designations for locations sampled during the August 1980 North Aleutian Shelf Transport Study cruise.

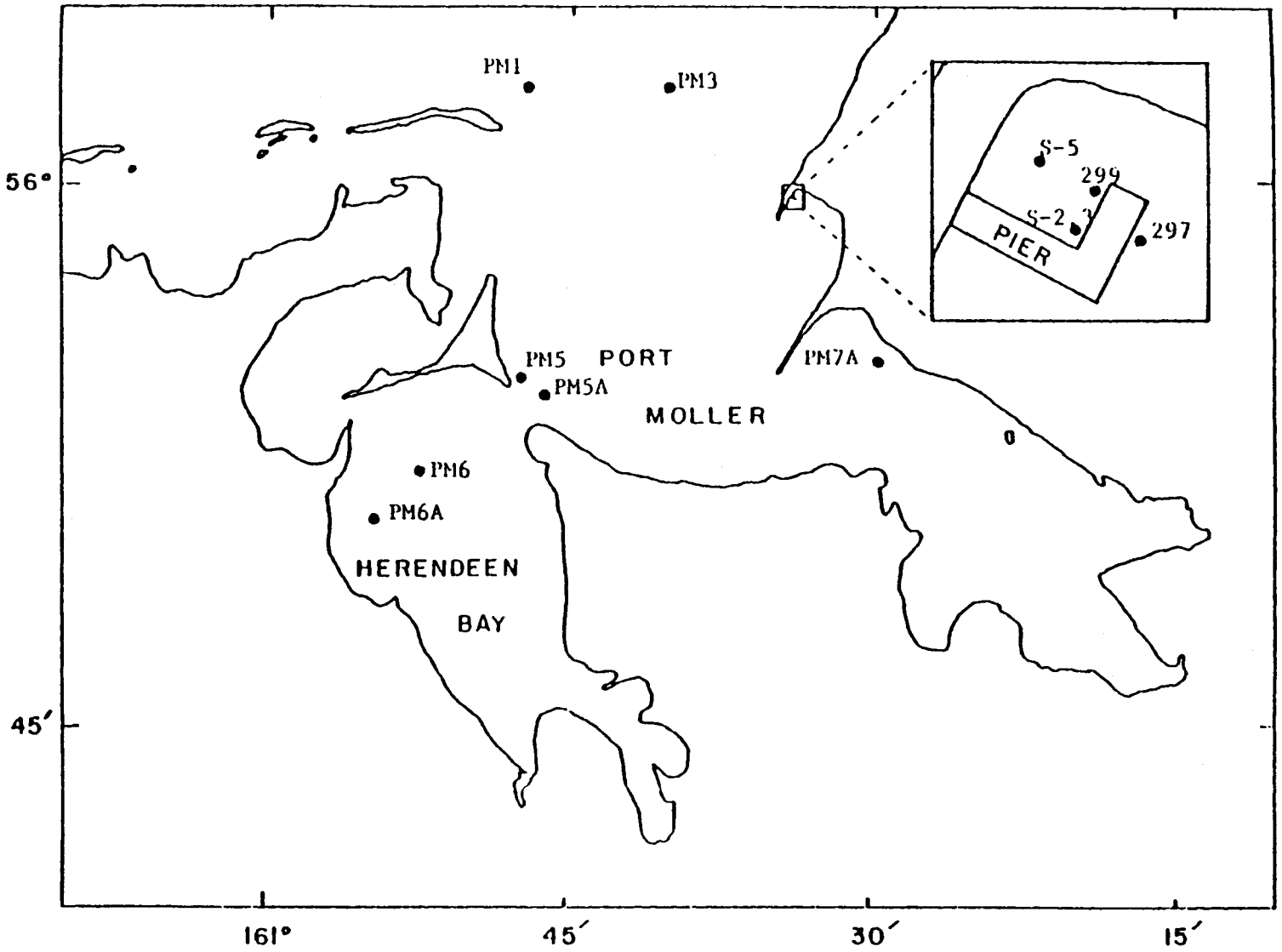

Figure 1q. Positions and station designations for locations sampled in Port Moller during the August 1980 North Aleutian Shelf Transport Study.

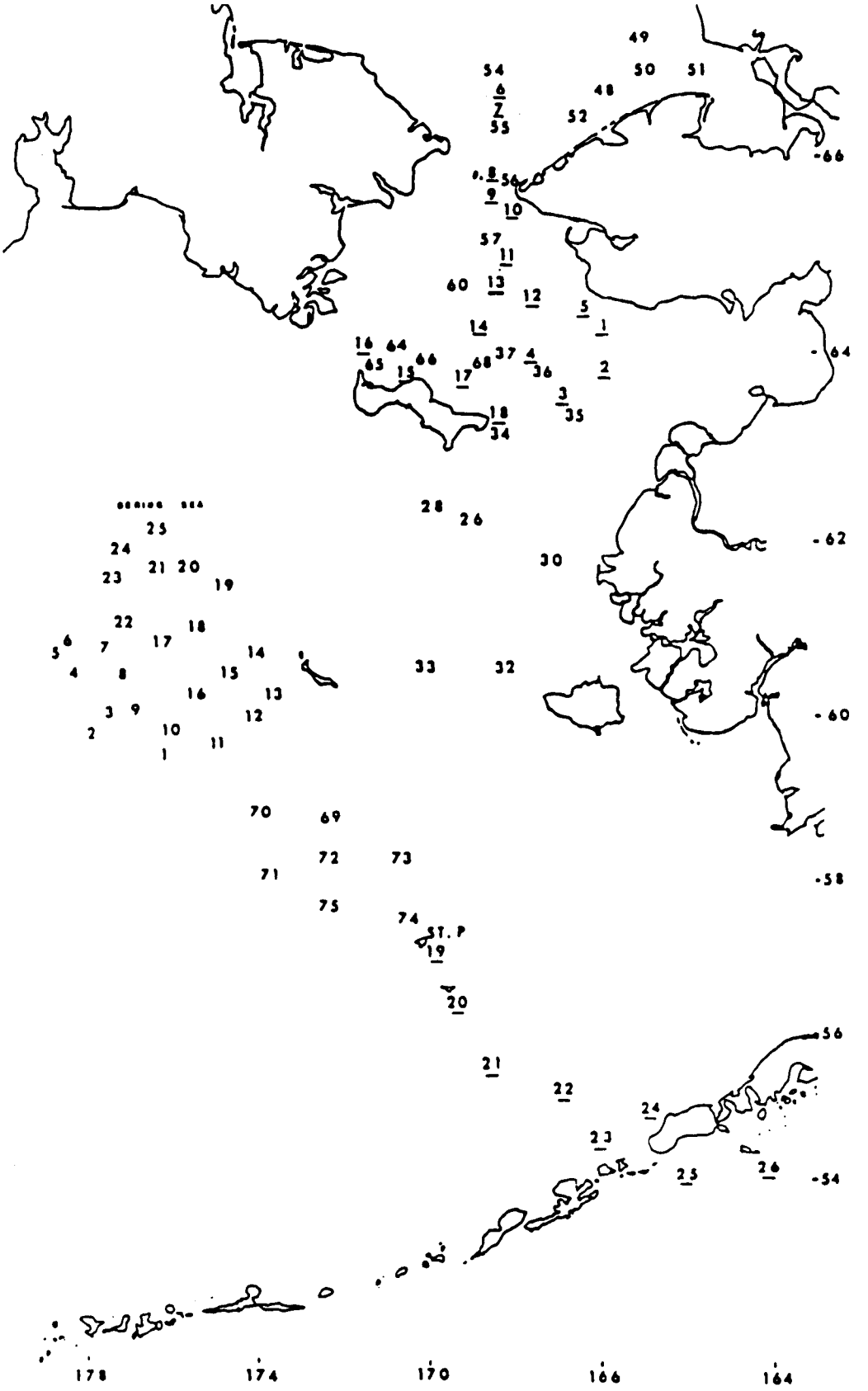

Figure 1r. Station locations for Polar Sea (April 1979) and Polar Star (June 1980) cruises. Polar Sea stations are underlined.

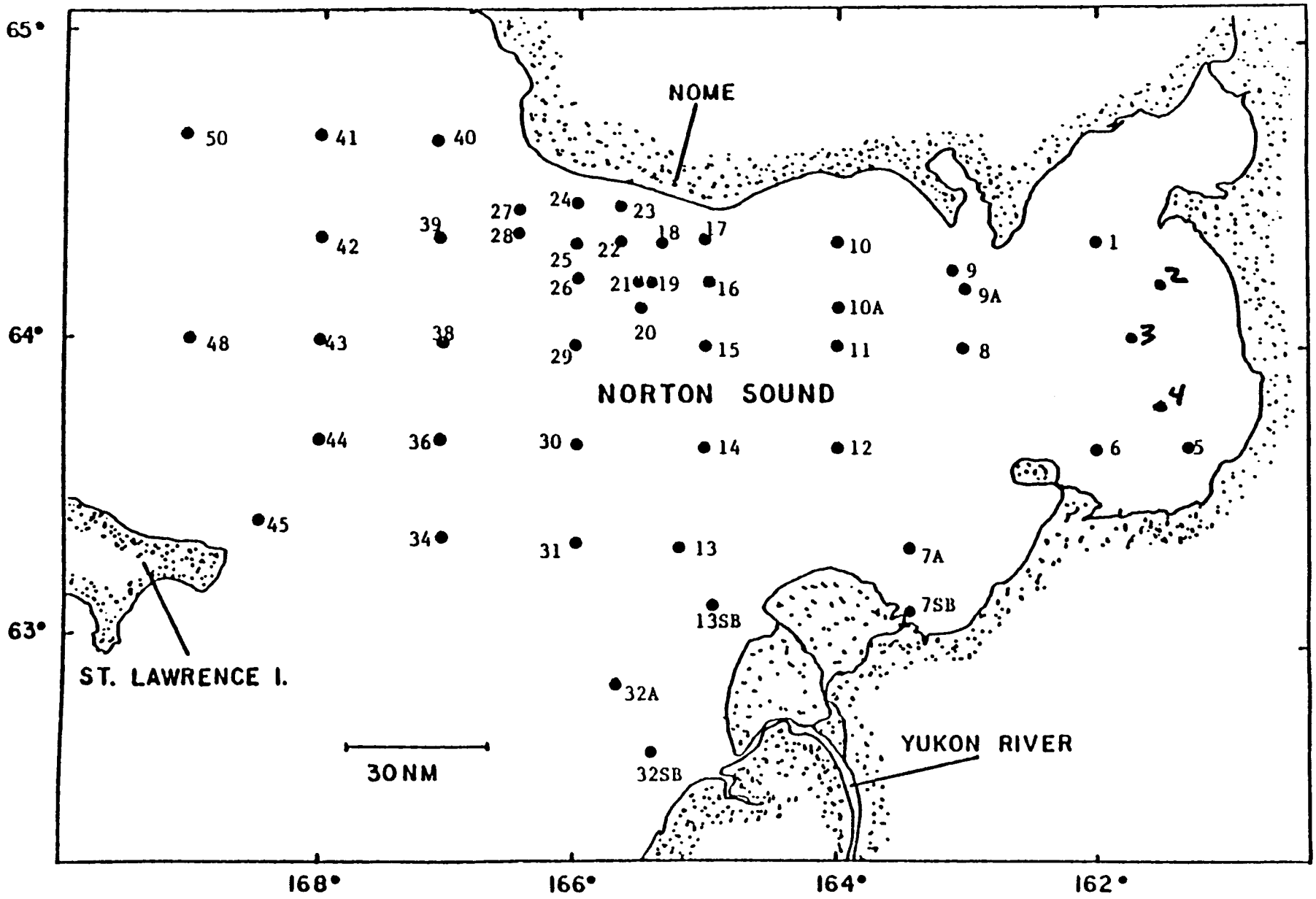

Figure 1s. Stations sampled during the Norton Sound cruise in July 1979.

Table 1. Sample descriptions.

SAMPLE	DEG LAT	MIN LAT	DEG LONG	MIN LONG	DATE	DEPTH	TEMP	SALINITY	SOURCE
BBP1					04/16/76				BEAUFORT BEACH ELSON STA.03A
BB0001	71	21.22	156	32.17	08/20/75	3.70	3.0		BEAUFORT ELSON LAGOON ST01 SEDIMENT
BB0002	71	21.22	156	32.17	08/20/75	2.30	3.2		BEAUFORT ELSON LAGOON ST01 SEDIMENT
BB0003	71	25.00	156	20.00	08/28/75	9.30			BEAUFORT ELSON LAGOON ST10 SEDIMENT
BB0004	71	15.70	156	0.00	08/28/75	2.00		22.8	BEAUFORT PT BARROW ST12 SEDIMENT
BB0005	71	19.00	156	15.79	08/31/75	3.30	2.0	17.0	BEAUFORT ELSON LAGOON ST11 SEDIMENT
BB0006	71	15.70	156	0.00	08/31/75	3.30	1.5		BEAUFORT PT BARROW ST12 SEDIMENT
BB0007	71	21.55	156	21.08	09/05/75	11.30			BEAUFORT ELSON LAGOON ST03 SEDIMENT
BB0008	71	22.13	156	21.74	09/05/75	8.00			BEAUFORT PLOVER PT ST04 SEDIMENT
BB0009	70	30.60	149	34.21	09/05/75	2.00	2.9		BEAUFORT OLIGTOK PT ST30 SEDIMENT
BB0010	70	26.50	149	3.15	09/05/75	2.00	1.8		BEAUFORT PRUDHOE BAY ST40 SEDIMENT
BB0011	70	19.14	148	19.35	09/08/75	2.00	-0.8	19.2	BEAUFORT PRUDHOE BAY ST53 SEDIMENT
BB0012	70	20.31	148	20.02	09/08/75	2.30	-0.4	19.8	BEAUFORT PRUDHOE BAY ST55 SEDIMENT
BB0013	70	20.87	148	23.95	09/08/75	2.70	-0.2	19.8	BEAUFORT PRUDHOE BAY ST51 SEDIMENT
BB0014	70	20.11	148	26.05	09/08/75	1.70	-0.2	17.5	BEAUFORT PRUDHOE BAY ST54 SEDIMENT
BB0015	71	21.22	156	32.17	09/11/75	2.30			BEAUFORT ELSON LAGOON ST01 SEDIMENT
BB0016	71	21.49	156	26.19	09/11/75	2.00			BEAUFORT ELSON LAGOON ST02 SEDIMENT
BB0017	71	21.55	156	21.08	09/11/75	11.30			BEAUFORT ELSON LAGOON ST03 SEDIMENT
BB0018	71	22.13	156	21.74	09/11/75	8.00			BEAUFORT ELSON LAGOON ST04 SEDIMENT
BB0019	70	19.14	148	19.35	09/12/75	2.70	1.0	19.5	BEAUFORT PRUDHOE BAY ST53 SEDIMENT
BB0020	70	20.31	148	20.02	09/12/75	3.30	1.5	20.0	BEAUFORT PRUDHOE BAY ST55 SEDIMENT
BB0021	70	24.53	148	22.29	09/12/75	3.00	1.0	21.0	BEAUFORT PRUDHOE BAY ST70 SEDIMENT
BB0022	70	26.57	148	23.47	09/12/75	7.70	0.0	22.5	BEAUFORT PRUDHOE BAY ST71 SEDIMENT
BB0023	70	21.78	148	27.53	09/13/75	1.70	2.3	17.5	BEAUFORT PRUDHOE BAY ST50 SEDIMENT
BB0024	70	20.87	148	23.95	09/13/75	2.30	2.2	19.3	BEAUFORT PRUDHOE BAY ST51 SEDIMENT
BB0025	70	20.00	148	22.06	09/13/75	2.70	2.2	18.5	BEAUFORT PRUDHOE BAY ST52 SEDIMENT
BB0025A	71	21.20	156	32.20	09/02/75	1.00	0.4	20.0	STA 1 OLD SAMPLE 25 LOCATION B
BB0026	70	22.90	148	21.45	09/13/75	1.70	1.8	19.2	BEAUFORT PRUDHOE BAY ST57 SEDIMENT
BB0027	70	24.53	148	22.29	09/13/75	6.70	0.3	21.5	BEAUFORT PRUDHOE BAY ST70 SEDIMENT
BB0027A	71	21.50	156	26.20	09/05/75	2.00	-0.5	20.5	STA 2 OLD SAMPLE 27 LOCATION B
BB0028	70	26.57	148	23.47	09/13/75	6.30	0.0	21.3	BEAUFORT PRUDHOE BAY ST71 SEDIMENT
BB0029	70	24.64	148	25.96	09/14/75	5.00	0.6	19.2	BEAUFORT PRUDHOE BAY ST72 SEDIMENT
BB0030	70	23.59	148	27.26	09/14/75	2.30	1.5	20.2	BEAUFORT PRUDHOE BAY ST73 SEDIMENT
BB0031	71	21.22	156	32.17	09/17/75	2.00	-0.5		BEAUFORT ELSON LAGOON ST01 SEDIMENT
BB0032	71	21.49	156	26.19	09/17/75	2.30	-0.5		BEAUFORT ELSON LAGOON ST02 SEDIMENT
BB0033	71	21.55	156	21.08	09/17/75	2.30	-0.5		BEAUFORT ELSON LAGOON ST03 SEDIMENT
BB101	71	23.00	155	56.00	04/07/76	6.00	0.0		BEAUFORT SEDIMENT BARROW STA.13
BB102	71	23.00	155	26.00	04/07/76	6.00	0.0		BEAUFORT SEDIMENT BARROW STA.14
BB103	71	23.00	154	54.00	04/07/76	7.00	0.0		BEAUFORT SEDIMENT BARROW STA.15
BB104	71	21.40	156	27.00	04/10/76	5.00	0.0		BEAUFORT SEDIMENT ELSON STA.02

BB105	71	21.60	156	21.00	04/10/76	5.00	0.0		BEAUFORT SEDIMENT ELSON STA.03
BB106	70	32.00	148	22.00	04/12/76	8.00	0.0		BEAUFORT SEDIMENT PRUDHOE STA.74
BB107	70	31.00	147	24.00	04/12/76	14.00	0.0		BEAUFORT SEDIMENT PRUDHOE STA.81
BB108	70	28.00	147	30.00	04/12/76	3.50	0.0		BEAUFORT SEDIMENT PRUDHOE STA.80
BB109	70	47.00	147	0.00	04/14/76	26.00	0.0		BEAUFORT SEDIMENT PRUDHOE STA.83
BB110	70	22.50	148	20.00	04/14/76	2.00	0.0		BEAUFORT SEDIMENT PRUDHOE STA.56
BB111	71	26.00	152	22.00	04/16/76	50.00	0.0		BEAUFORT SEDIMENT PITT PT STA.22
BB112	71	19.00	152	33.00	04/16/76	30.00	0.0		BEAUFORT SEDIMENT PITT PT STA.21
BB113	71	8.00	152	55.00	04/18/76	9.00	0.0		BEAUFORT SEDIMENT PITT PT STA.20
BB114	71	23.00	153	50.00	04/18/76	22.00	0.0		BEAUFORT SEDIMENT BARROW STA.17
BB115	71	23.00	154	22.00	04/18/76	13.00	0.0		BEAUFORT SEDIMENT BARROW STA.16
BI101	71	31.00	156	6.00	04/05/76	0.00	0.0		BEAUFORT ICE BARROW STA.13A
BI102	71	34.50	155	35.00	04/05/76	0.00	0.0		BEAUFORT ICE BARROW STA.14A
BI103	71	39.00	155	4.00	04/05/76	0.00	0.0		BEAUFORT ICE BARROW STA.15A
BI104	71	21.60	156	21.00	04/07/76	0.00	0.0		BEAUFORT ICE ELSON STA.03
BI105	71	23.00	155	56.00	04/07/76	0.00	0.0		BEAUFORT ICE BARROW STA.13
BI106	71	23.00	155	26.00	04/07/76	0.00	0.0		BEAUFORT ICE BARROW STA.14
BI107	71	23.00	154	54.00	04/07/76	0.00	0.0		BEAUFORT ICE BARROW STA.15
BI108	71	21.40	156	27.00	04/10/76	0.00	0.0		BEAUFORT ICE ELSON STA.02
BI109	71	21.60	156	21.00	04/10/76	0.00	0.0		BEAUFORT ICE ELSON STA.03
BI110	71	8.00	146	30.00	04/14/76	0.00	0.0	3.2	BEAUFORT ICE PRUDHOE STA.85
BI111	70	32.00	148	22.00	04/14/76	0.00	0.0		BEAUFORT ICE PRUDHOE STA.74
BI112	70	31.00	147	24.00	04/14/76	0.00	0.0		BEAUFORT ICE PRUDHOE STA.81
BI113	70	28.00	147	30.00	04/14/76	0.00	0.0		BEAUFORT ICE PRUDHOE STA.80
BI114	70	47.00	147	0.00	04/14/76	0.00	0.0	23.8	BEAUFORT ICE PRUDHOE STA.83
BI115	70	22.50	148	20.00	04/14/76	0.00	0.0	1.0	BEAUFORT ICE PRUDHOE STA.56
BI116	71	36.00	152	12.00	04/16/76	0.00	0.0	6.0	BEAUFORT ICE PITT PT STA.24
BI117	71	46.00	151	52.00	04/16/76	0.00	0.0	2.5	BEAUFORT ICE PITT PT STA.23
BI118	71	26.00	152	22.00	04/16/76	0.00	0.0	11.0	BEAUFORT ICE PITT PT STA.22
BI119	71	19.00	152	33.00	04/16/76	0.00	0.0	8.5	BEAUFORT ICE PITT PT STA.21
BI120	71	8.00	152	55.00	04/18/76	0.00	0.0	3.5	BEAUFORT ICE PITT PT STA.20
BI121	71	23.00	153	50.00	04/18/76	0.00	0.0	5.0	BEAUFORT ICE BARROW STA.17
BI122	71	23.00	154	22.00	04/18/76	0.00	0.0	5.0	BEAUFORT ICE BARROW STA.16
BI123	71	34.50	155	35.00	04/18/76	0.00	0.0	4.0	BEAUFORT ICE BARROW STA.14A
BW0001	71	21.22	156	32.17	08/20/75	1.00	3.0	26.0	BEAUFORT ELSON LAGOON ST01 WATER
BW0002	71	21.22	156	32.17	08/20/75	1.00	3.2	26.5	BEAUFORT ELSON LAGOON ST01 WATER
BW0003	71	25.00	156	20.00	08/28/75	1.00	-0.5	23.8	BEAUFORT ELSON LAGOON ST10 WATER
BW0004	71	15.70	156	0.00	08/28/75	1.00	2.0	22.5	BEAUFORT POINT BARROW ST12 WATER
BW0005	71	20.81	156	35.17	08/30/75			0.0	CHUKCH1 BARROW ST05B ICE
BW0006	71	19.00	156	15.79	08/31/75	1.00	2.0	17.0	BEAUFORT ELSON LAGOON ST11 WATER
BW0006A	71	21.20	156	32.20	08/21/75	1.00	3.0	20.1	STA 1 OLD SAMPLE 6 LOCATION B
BW0007	71	15.70	156	0.00	08/31/75	1.00	1.5	21.0	BEAUFORT POINT BARROW ST12 WATER
BW0007A	71	21.50	156	26.20	08/21/75	1.00	2.5	21.0	STA 2 OLD SAMPLE 7 LOCATION B
BW0008	71	21.01	156	42.18	08/31/75	1.00	2.0	25.5	CHUKCH1 BARROW ST06 WATER
BW0009	71	21.55	156	21.08	09/05/75	1.00	-0.2	20.0	BEAUFORT ELSON LAGOON ST03 WATER
BW0009A	71	21.50	156	21.10	08/21/75	1.00	2.0	20.0	STA 3 OLD SAMPLE 9 LOCATION B
BW0010	71	22.13	156	21.74	09/05/75	1.00	-0.2	20.5	BEAUFORT PLOVER PT. ST04 WATER
BW0011	70	30.60	149	34.21	09/05/75	1.00	1.9	12.1	BEAUFORT OLIGTOK PT. ST30 WATER
BW0011A	71	19.70	156	40.90	08/24/75	1.00			STA 7 OLD SAMPLE 11ALOCATION B
BW0011B	71	22.10	156	21.70	08/21/75	1.00	3.0	25.0	STA 4 OLD SAMPLE 11 LOCATION B
BW0012	70	26.50	149	3.15	09/05/75	1.00	1.8	18.8	BEAUFORT PRUDHOE BAY ST40 WATER
BW0012A	71	19.70	156	40.90	08/26/75	1.00	3.0	31.0	STA 7 OLD SAMPLE 12 LOCATION B
BW0013	70	20.00	148	22.06	09/05/75	1.00	1.5	20.0	BEAUFORT PRUDHOE BAY ST52 WATER
BW0013A	71	19.70	156	40.90	08/27/75	1.00		0.5	STA 7 OLD SAMPLE 13ALOCATION B
BW0013B	71	19.70	156	40.90	08/28/75	1.00	2.0	23.0	STA 7 OLD SAMPLE 13 LOCATION B

BW0014	70	19.14	148	19.35	09/08/75	1.00	-0.8	11.8	BEAUFORT PRUDHOE BAY ST53 WATER
BW0014A	71	19.70	156	40.90	08/27/75	1.00	3.0	23.0	STA 7 OLD SAMPLE 14 LOCATION B
BW0015	70	20.31	148	20.02	09/08/75	1.00	-0.8	11.1	BEAUFORT PRUDHOE BAY ST55 WATER
BW0016	70	20.31	148	20.02	09/08/75	2.30	-0.4	19.8	BEAUFORT PRUDHOE BAY ST55 WATER
BW0017	70	20.87	148	23.95	09/08/75	1.00	-0.5	11.4	BEAUFORT PRUDHOE BAY ST51 WATER
BW0018	70	20.11	148	26.05	09/08/75	1.00	-0.4	9.0	BEAUFORT PRUDHOE BAY ST54 WATER
BW0019	71	21.22	156	32.17	09/11/75	1.00	-0.5	18.3	BEAUFORT ELSON LAGOON ST01 WATER
BW0019A	71	20.80	156	35.20	08/30/75	1.00	2.0	25.0	STA 5B OLD SAMPLE 19 LOCATION B
BW0020	71	21.49	156	26.19	09/11/75	1.00	0.0	18.2	BEAUFORT ELSON LAGOON ST02 WATER
BW0021	71	21.55	156	21.08	09/11/75	1.00	0.0	18.5	BEAUFORT ELSON LAGOON ST03 WATER
BW0022	71	22.13	156	21.74	09/11/75	1.00	0.0	18.8	BEAUFORT ELSON LAGOON ST04 WATER
BW0023	70	19.14	148	19.35	09/12/75	1.00	1.0	14.5	BEAUFORT PRUDHOE BAY ST53 WATER
BW0024	70	20.31	148	20.02	09/12/75	1.00	1.5	16.0	BEAUFORT PRUDHOE BAY ST55 WATER
BW0024A	71	21.00	156	42.20	09/02/75	1.00	2.0	25.5	STA 7 OLD SAMPLE 24 LOCATION B
BW0025	70	21.74	148	20.84	09/12/75	1.00	1.5	19.5	BEAUFORT PRUDHOE BAY ST56 WATER
BW0026	70	24.53	148	22.29	09/12/75	1.00	1.0	20.0	BEAUFORT PRUDHOE BAY ST70 WATER
BW0026A	71	21.20	156	32.20	09/05/75	1.00	0.4	20.0	STA 1 OLD SAMPLE 26 LOCATION B
BW0027	70	26.57	148	23.47	09/12/75	1.00	0.5	21.5	BEAUFORT PRUDHOE BAY ST71 WATER
BW0028	70	21.78	148	27.53	09/13/75	1.00	2.5	17.8	BEAUFORT PRUDHOE BAY ST50 WATER
BW0028A	71	21.50	156	26.20	09/05/75	1.00	-0.5	20.5	STA 2 OLD SAMPLE 28 LOCATION B
BW0029	70	20.00	148	22.06	09/13/75	1.00	1.9	18.1	BEAUFORT PRUDHOE BAY ST52 WATER
BW0030	70	22.90	148	21.45	09/13/75	1.00	2.2	16.2	BEAUFORT PRUDHOE BAY ST57 WATER
BW0031	70	24.53	148	22.29	09/13/75	1.00	1.5	18.5	BEAUFORT PRUDHOE BAY ST70 WATER
BW0032	70	26.57	148	23.47	09/13/75	1.00	0.3	20.2	BEAUFORT PRUDHOE BAY ST71 WATER
BW0033	70	24.64	148	25.96	09/14/75	1.00	1.9	16.0	BEAUFORT PRUDHOE BAY ST72 WATER
BW0034	70	23.59	148	27.26	09/14/75	1.00	2.3	15.8	BEAUFORT PRUDHOE BAY ST73 WATER
BW0035	71	21.22	156	32.17	09/17/75	1.00	-0.5	22.0	BEAUFORT ELSON LAGOON ST01 WATER
BW0036	71	21.49	156	26.19	09/17/75	1.00	-0.5	22.2	BEAUFORT ELSON LAGOON ST02 WATER
BW0037	71	21.55	156	21.08	09/17/75	1.00	-0.5	22.2	BEAUFORT ELSON LAGOON ST03 WATER
BW0038	71	17.23	156	48.48	09/23/75			0.0	CHUKCH1 BARROW ST09A ICE
BW0039	71	17.90	156	46.30	09/23/75			0.0	CHUKCH1 BARROW ST09B ICE
BW0040	71	18.85	156	43.37	09/23/75			0.0	CHUKCH1 BARROW ST09C ICE
BW0041	71	20.94	156	35.22	09/23/75	0.00	-1.0	27.0	BEAUFORT ELSON LAGOON ST05A WATER
BW0042	71	20.81	156	35.17	09/23/75	0.00	-1.0	25.5	CHUKCH1 BARROW ST05B WATER
BW0043	71	23.15	156	29.24	09/25/75	0.00	-1.2	26.0	CHUKCH1 BARROW ST08 WATER
BW0079	71	19.70	156	40.90	09/16/75	1.00	2.0	25.5	STA 7 OLD SAMPLE 79 LOCATION B
BW0080	71	20.80	156	35.20	09/16/75	1.00	2.0	25.2	STA 5B OLD SAMPLE 80 LOCATION B
BW0081	71	20.80	156	35.20	09/17/75	1.00	1.5	25.0	STA 5B OLD SAMPLE 81 LOCATION B
BW0082	71	20.80	156	35.20	09/16/75	1.00		1.2	STA 5B OLD SAMPLE 82 LOCATION B
BW0083	71	20.80	156	35.20	09/16/75	1.00		0.5	STA 5B OLD SAMPLE 83 LOCATION B
BW0084	71	20.80	156	35.20	09/16/75	1.00		2.0	STA 5B OLD SAMPLE 84 LOCATION B
BW0091	71	20.90	156	35.20	09/20/75	1.00	-1.0	25.0	STA 5A OLD SAMPLE 91 LOCATION B
BW0092	71	20.80	156	35.20	09/20/75	1.00	-1.0	26.0	STA 5B OLD SAMPLE 92 LOCATION B
BW0093	71	20.80	156	35.20	09/20/75	1.00		9.0	STA 5B OLD SAMPLE 93 LOCATION B
BW0094	71	20.80	156	35.20	09/20/75	1.00		6.0	STA 5B OLD SAMPLE 94 LOCATION B
BW0095	71	20.80	156	35.20	09/21/75	1.00	2.0	26.0	STA 5B OLD SAMPLE 95 LOCATION B
BW101	71	30.00	156	6.00	04/05/76	1.00	-1.6	28.0	TIME1400 STA 13A
BW102	71	34.50	155	35.00	04/05/76	1.00	-2.0	22.0	TIME1500 STA 14A
BW103	71	39.00	155	4.00	04/05/76	1.00	-2.0	19.2	TIME1600 STA 15A
BW104	71	21.60	156	21.00	04/05/76	1.00	-2.0	24.5	BEAUFORT WATER ELSON STA.03
BW105	71	23.00	155	56.00	04/07/76	1.00	-2.0	25.0	BEAUFORT WATER BARROW STA.13
BW106	71	23.00	155	26.00	04/07/76	1.00	-2.0	22.0	BEAUFORT WATER BARROW STA.14
BW107	71	23.00	154	54.00	04/07/76	1.00	-2.0	17.0	BEAUFORT WATER BARROW STA.15
BW107A	71	21.60	156	21.00	04/09/76	1.00	-2.0	25.0	TIME1400 STA 3
BW108	71	21.40	156	27.00	04/10/76	1.00	-2.0	31.0	BEAUFORT WATER ELSON STA.02

BW109	71	21.60	156	21.00	04/10/76	1.00	-2.0	29.0	BEAUFORT WATER ELSON STA.03
BW110	71	8.00	146	30.00	04/14/76	1.00	-2.0	23.0	BEAUFORT WATER PRUDHOE STA.85
BW111	70	32.00	148	22.00	04/12/76	1.00	-1.5	28.0	BEAUFORT WATER PRUDHOE STA.74
BW112	70	31.00	147	24.00	04/12/76	1.00	-2.0	28.0	BEAUFORT WATER PRUDHOE STA.81
BW113	70	28.00	147	30.00	04/12/76	1.00	-1.5	29.0	BEAUFORT WATER PRUDHOE STA.80
BW114	70	47.00	147	0.00	04/14/76	1.00	-2.0	17.0	BEAUFORT WATER PRUDHOE STA.83
BW116	71	36.00	152	12.00	04/16/76	1.00	-2.0	24.0	BEAUFORT WATER PITT PT STA.24
BW117	71	46.00	151	52.00	04/16/76	1.00	-2.0	20.5	BEAUFORT WATER PITT PT STA.23
BW118	71	26.00	152	22.00	04/16/76	1.00	-2.0	19.0	BEAUFORT WATER PITT PT STA.22
BW119	71	19.00	152	33.00	04/16/76	1.00	-2.0	19.8	BEAUFORT WATER PITT PT STA.21
BW120	71	8.00	152	55.00	04/18/76	1.00	-2.0	25.5	BEAUFORT WATER PITT PT STA.20
BW121	71	23.00	153	50.00	04/18/76	1.00	-2.0	28.0	BEAUFORT WATER BARROW STA.17
BW122	71	23.00	154	22.00	04/18/76	1.00	-2.0	29.0	BEAUFORT WATER BARROW STA.16
BW123	71	34.50	155	35.00	04/18/76	1.00	-1.5	28.0	BEAUFORT WATER BARROW STA.14A
GB0101	59	18.60	152	23.48	10/14/75	91.00	9.3	30.8	GULF OF ALASKA ST101 SEDIMENT
GB0121	56	43.64	155	27.98	10/13/75	230.50	4.7	33.5	GULF OF ALASKA ST121 SEDIMENT
GB0134	55	33.58	158	39.95	10/12/75	154.00			GULF OF ALASKA ST134 SEDIMENT
GB0137	54	55.03	157	58.54	10/12/75	99.00	7.7	31.9	GULF OF ALASKA ST137 SEDIMENT
GB0146	54	49.50	161	11.77	10/11/75	75.00	7.8	31.7	GULF OF ALASKA ST146 SEDIMENT
GB0148	54	23.69	160	49.24	10/11/75	109.70	5.5	32.5	GULF OF ALASKA ST148 SEDIMENT
GB201					03/15/76				GULF ALASKA SEDIMENT KODIAK BEACH
GB202	59	50.30	149	30.40	03/18/76	190.00	4.3	32.4	GULF ALASKA SEDIMENT STATION 01
GB203	59	24.60	149	4.20	03/18/76	183.00	4.6	32.8	GULF ALASKA SEDIMENT STATION 04
GB204	58	58.80	148	39.50	03/18/76	217.00	5.0	33.8	GULF ALASKA SEDIMENT STATION 07
GB206	59	17.20	147	15.60	03/19/76	251.80	5.3	33.8	GULF ALASKA SEDIMENT STATION 59
GB207	59	45.50	146	31.00	03/20/76	70.50	4.3	32.3	GULF ALASKA SEDIMENT STATION 57
GB210	59	23.30	146	54.50	03/21/76	302.30	4.9	32.5	GULF ALASKA SEDIMENT STATION 53A
GB211	59	32.50	147	0.60	03/21/76	308.60	5.1	32.7	GULF ALASKA SEDIMENT STATION 53B
GB212	60	8.80	145	2.00	03/21/76	45.80	3.7	31.9	GULF ALASKA SEDIMENT STATION 52
GB213	59	47.00	145	10.20	03/22/76	158.10	4.6	32.3	GULF ALASKA SEDIMENT STATION 50
GB214	59	54.30	143	52.30	03/22/76	75.90	4.4	32.1	GULF ALASKA SEDIMENT STATION 42
GB216	59	16.70	142	56.90	03/23/76	2282.00	2.1	34.6	GULF ALASKA SEDIMENT STATION 37
GB217	59	45.90	142	44.00	03/23/76	127.80	4.8	32.2	GULF ALASKA SEDIMENT STATION 41
GB218	59	44.20	141	29.00	03/24/76	40.30	4.2	31.9	GULF ALASKA SEDIMENT STATION 30
GB219	59	27.10	141	48.50	03/24/76	174.90	5.8	33.2	GULF ALASKA SEDIMENT STATION 32
GB220					03/25/76				GULF ALASKA SEDIMENT NYAKITAT BEACH
GB223	59	26.70	140	19.00	03/25/76	226 40	5.8	33.1	GULF ALASKA SEDIMENT STATION 28
GB224	59	18.40	140	29.60	03/26/76	124.00	5.6	32.8	GULF ALASKA SEDIMENT STATION 27
GB225					03/26/76				GULF ALASKA SEDIMENT HILDENBACH BCH
GB226					03/26/76				GULF ALASKA SEDIMENT PRINCEWILL BCH
GW0101	59	18.60	152	23.48	10/14/75	2.00	9.1	30.8	GULF OF ALASKA ST101 WATER
GW0102	59	10.10	152	4.45	10/14/75	2.00	9.2	29.5	GULF OF ALASKA ST102 WATER
GW0103	59	0.55	151	48.15	10/14/75	2.00	9.3	28.7	GULF OF ALASKA ST103 WATER
GW0104	58	50.00	151	26.15	10/15/75	2.00	8.4	32.0	GULF OF ALASKA ST104 WATER
GW0105	58	40.11	151	7.32	10/15/75	2.00	8.4	32.0	GULF OF ALASKA ST105 WATER
GW0106	58	28.74	150	49.28	10/15/75	2.00	7.7		GULF OF ALASKA ST106 WATER
GW0119	57	6.01	156	0.58	10/14/75	2.00	8.6	29.6	GULF OF ALASKA ST119 WATER
GW0124	56	7.01	154	39.93	10/13/75	2.00	8.3	31.8	GULF OF ALASKA ST124 WATER
GW0133	55	44.82	158	49.33	10/12/75	2.00	8.8	30.8	GULF OF ALASKA ST133 WATER
GW0137	54	55.03	157	58.54	10/12/75	2.00	8.6	31.3	GULF OF ALASKA ST137 WATER
GW0145	55	1.00	161	19.80	10/11/75	2.00	8.0	31.4	GULF OF ALASKA ST145 WATER
GW0148	54	23.69	160	49.24	10/11/75	2.00	7.8	31.9	GULF OF ALASKA ST148 WATER
GW0156	54	29.00	165	11.40	10/10/75	2.00	6.4	31.7	GULF OF ALASKA ST156 WATER
GW0159	53	51.98	164	33.71	10/10/75	2.00	7.5	31.9	GULF OF ALASKA ST159 WATER
GW201					03/15/76	0.00	2.0	31.4	GULF ALASKA WATER KODIAK BEACH

GW202	59 50.30	149 30.40	03/18/76	2.00	3.0	31.7	GULF ALASKA WATER STATION 01
GW203	59 24.60	149 4.20	03/18/76	2.00	3.5	32.2	GULF ALASKA WATER STATION 04
GW204	58 58.80	148 39.50	03/18/76	2.00	3.5	32.3	GULF ALASKA WATER STATION 07
GW205	59 38.30	147 48.00	03/18/76	1.00	3.5	32.1	TIME2145 STA 68
GW206	59 17.20	147 15.60	03/19/76	2.00	4.0	32.2	GULF ALASKA WATER STATION 59
GW207	59 45.50	146 31.00	03/20/76	2.00	4.0	32.1	GULF ALASKA WATER STATION 57
GW208	60 4.70	146 44.40	03/19/76	1.00	4.0	31.9	TIME1940 STA 55
GW209			03/20/76	0.00	3.0	31.1	GULF ALASKA WATER SEWARD BEACH
GW210	59 23.30	146 54.50	03/21/76	2.00	3.0	31.8	GULF ALASKA WATER STATION 53A
GW211	59 32.50	147 0.60	03/21/76	2.00	3.0	31.8	GULF ALASKA WATER STATION 53B
GW212	60 8.80	145 2.00	03/21/76	2.00	3.2	31.8	GULF ALASKA WATER STATION 52
GW213	59 47.00	145 10.20	03/22/76	2.00	3.4	31.8	GULF ALASKA WATER STATION 50
GW214	59 54.30	143 52.30	03/22/76	2.00	4.1	32.1	GULF ALASKA WATER STATION 42
GW215	59 38.10	144 2.00	03/22/76	1.00	3.0	32.2	TIME1130 STA 44
GW216	59 16.70	142 56.90	03/23/76	2.00	4.5	32.5	GULF ALASKA WATER STATION 37
GW217	59 45.90	142 44.00	03/23/76	2.00	4.5	32.1	GULF ALASKA WATER STATION 41
GW218	59 44.20	141 29.00	03/24/76	2.00	3.8	31.7	GULF ALASKA WATER STATION 30
GW219	59 27.10	141 48.50	03/24/76	2.00	4.5	32.2	GULF ALASKA WATER STATION 32
GW220	59 44.70	140 7.50	03/25/76	0.00	3.0	30.8	GULF ALASKA WATER N YAKITAT BEACH
GW221	59 33.20	139 48.80	03/25/76	0.00	4.0	30.8	GULF ALASKA WATER S YAKITAT BEACH
GW222	59 34.50	140 6.60	03/25/76	2.00	4.2	32.0	GULF ALASKA WATER STATION 29
GW223	59 26.70	140 19.00	03/25/76	2.00	4.7	32.2	GULF ALASKA WATER STATION 28
GW224	59 18.40	140 29.60	03/26/76	2.00	4.8	32.2	GULF ALASKA WATER STATION 27
GW225	60 20.80	146 38.40	03/26/76	0.00	5.0	31.1	GULF ALASKA WATER HILDENBACH BEACH
GW226	60 20.60	146 38.50	03/26/76	0.00	5.0	30.7	GULF ALASKA WATER PRINCE WILLBEACH
GW227	60 21.40	146 37.40	03/26/76	0.00	3.2	31.3	GULF ALASKA WATER PRINCE WILLBEACH
BB201	70 36.00	148 12.00	08/24/76	16.00	-1.4	30.3	BEAUFORT PRUDHOE BAY SEDIMENT STA 74
BB203	70 32.00	147 33.00	08/25/76	25.00	0.6	31.7	BEAUFORT PRUDHOE BAY SEDIMENT STA 80
BB204	70 39.00	147 37.00	08/26/76	25.00	-1.6	39.2	BEAUFORT PRUDHOE BAY SEDIMENT STA 81
BB205	70 57.00	149 33.00	08/27/76	30.00			BEAUFORT COLVILLE R. SEDIMENT STA 41
BB206	70 57.00	149 33.00	08/27/76	30.00	-1.1	31.6	BEAUFORT COLVILLE R. SEDIMENT STA 41
BB207	71 8.00	151 19.00	08/28/76	34.00	-0.6	30.7	BEAUFORT COLVILLE R. SEDIMENT STA 31
BB208	71 43.00	151 47.00	08/29/76	1700.00	-0.2	34.8	BEAUFORT PITT PT. SEDIMENT STA 24
BB212	71 22.00	152 20.00	08/30/76	74.00	-0.9	32.2	BEAUFORT PITT PT. SEDIMENT STA 22
BB214	71 19.00	152 32.00	08/31/76	52.00	-0.3	31.5	BEAUFORT PITT PT. SEDIMENT STA 21
BB215	71 8.00	152 57.00	09/01/76	40.00	0.3	30.1	BEAUFORT PITT PT. SEDIMENT STA 20
BB216	71 23.00	154 21.00	09/02/76	22.00	2.1	29.4	BEAUFORT PT. BARROW SEDIMENT STA 16
BB217	71 36.00	155 32.00	09/02/76	171.00	0.2	32.0	BEAUFORT PT. BARROW SEDIMENT STA 15
BB219	71 21.00	156 32.00	09/08/76	2.00	2.0	24.5	BEAUFORT ELSON LAGOONSEDIMENT STA 01
BB220	71 21.00	156 26.00	09/08/76	2.00	2.0	25.5	BEAUFORT ELSON LAGOONSEDIMENT STA 02
BW201	70 36.00	148 12.00	08/23/76	1.00	-0.2	10.0	BEAUFORT PRUDHOE BAY WATER STA74
BW202	70 36.00	148 12.00	08/24/76	1.00	-0.2	10.0	BEAUFORT PRUDHOE BAY WATER STA74
BW203	70 32.00	147 33.00	08/25/76	1.00	-1.3	6.0	BEAUFORT PRUDHOE BAY WATER STA80
BW204	70 39.00	147 37.00	08/26/76	1.00	0.3	5.0	BEAUFORT PRUDHOE BAY WATER STA81
BW205	70 57.00	149 33.00	08/27/76	1.00	0.3	10.8	BEAUFORT COLVILLE R. WATER STA41
BW206	70 57.00	149 33.00	08/27/76	1.00	0.4	7.6	BEAUFORT COLVILLE R. WATER STA41
BW207	71 8.00	151 19.00	08/28/76	1.00	0.7	19.0	BEAUFORT COLVILLE R. WATER STA31
BW208	71 43.00	151 47.00	08/29/76	1.00	0.3	10.2	BEAUFORT PITT PT. WATER STA24
BW209	71 43.00	151 47.00	08/29/76	15.00	-1.0	29.5	BEAUFORT PITT PT. 15 WATER STA24
BW210	71 33.00	152 3.00	08/30/76	1.00	0.4	13.0	BEAUFORT PITT PT. WATER STA23
BW211	71 33.00	152 3.00	08/30/76	15.00	0.4	22.0	BEAUFORT PITT PT. 15 WATER STA23
BW212	71 22.00	152 20.00	08/30/76	1.00	0.5	15.7	BEAUFORT PITT PT. WATER STA22
BW213	71 22.00	152 20.00	08/30/76	15.00	2.7	28.8	BEAUFORT PITT PT. 15 WATER STA22
BW214	71 19.00	152 32.00	08/31/76	1.00	0.3	17.4	BEAUFORT PITT PT. WATER STA21
BW215	71 8.00	152 57.00	09/01/76	1.00	1.8	20.5	BEAUFORT PITT PT. WATER STA20

BW216	71	23.00	154	21.00	09/02/76	1.00	1.2	17.4	BEAUFORT PT. BARROW WATER STA16
BW217	71	36.00	155	32.00	09/02/76	1.00	0.1	8.3	BEAUFORT PT. BARROW WATER STA15A
BW218	71	36.00	155	32.00	09/02/76	15.00	-0.7	28.5	BEAUFORT PT. BARROW15WATER STA15A
BW219	71	21.00	156	32.00	09/08/76	1.00	2.0	24.5	BEAUFORT ELSON LAGOONWATER STA01
BW220	71	21.00	156	26.00	09/08/76	1.00	2.0	25.5	BEAUFORT ELSON LAGOONWATER STA02
GB301	59	6.00	152	47.00	10/18/76	148.00	8.5		COOK INLET SEDIMENT STA.205
GB303	59	20.00	152	44.00	10/19/76	73.00	10.0	27.0	COOK INLET SEDIMENT STA.215
GB304	59	33.00	153	24.00	10/20/76	28.00	8.4	26.0	COOK INLET SEDIMENT STA.212
GB308	59	44.00	153	21.00	10/20/76	0.00	6.5		COOK INLET SEDIMENT STA. M BEACH
GB311	59	15.00	153	40.00	10/21/76	31.00	9.1	23.2	COOK INLET SEDIMENT STA.204LOW TIDE
GB312	59	38.00	152	33.00	10/22/76	75.00	10.0	26.0	COOK INLET SEDIMENT STA.225
GB313	59	42.00	151	9.00	10/22/76	28.00	9.3	26.0	COOK INLET SEDIMENT STA.229
GB318	59	34.00	151	44.00	10/22/76	88.00	9.5	28.0	COOK INLET SEDIMENT STA.227
GB319	59	33.00	152	9.00	10/23/76	45.00	9.5	28.0	COOK INLET SEDIMENT STA.226
GB320					10/76				COOK INLET SEDIMENT STA.245
GB324									
GB325	59	50.00	153	16.00	10/24/76	0.00	6.0		COOK INLET SEDIMENT STA. L BEACH
GB327	59	21.00	153	15.00	10/25/76	44.00	9.0	26.0	COOK INLET SEDIMENT STA.214
GB328	59	10.20	153	8.50	10/25/76	84.00	9.0	27.0	TIME0130 STA 206
GB329	59	15.00	153	40.00	10/25/76	34.00	9.8	26.5	COOK INLET SEDIMENT STA.204HIGH TIDE
GB333	58	59.70	152	52.80	10/27/76	170.00	7.0	22.5	TIME2200 STA 207
GB334									
GB335	58	46.00	151	10.00	10/28/76	120.00	7.5	28.0	COOK INLET SEDIMENT STA.105
GW301	59	6.00	152	47.00	10/18/76	1.00	9.0	27.5	COOK INLET WATER STA.205
GW302	59	20.00	152	12.00	10/19/76	1.00	9.5	27.0	COOK INLET WATER STA.216
GW303	59	20.00	152	44.00	10/19/76	1.00	9.5	27.0	COOK INLET WATER STA.215
GW304	59	33.00	153	24.00	10/20/76	1.00	8.4	25.0	COOK INLET WATER STA.212
GW305	59	38.80	153	25.00	10/20/76	1.00	8.0	23.3	TIME1130 STA R
GW306	59	38.80	153	25.00	10/20/76	1.00	8.0	23.3	TIME1130 STA R
GW307	59	44.20	153	21.50	10/20/76	1.00	5.5	22.5	TIME1245 STA M
GW308	59	44.00	153	21.00	10/20/76	0.00	6.5	22.0	COOK INLET WATER STA. M BEACH
GW309	59	22.00	153	59.00	10/21/76	0.00	12.0	21.0	COOK INLET WATER STA. I BEACH ON
GW310	59	22.00	153	59.00	10/21/76	0.00	12.0	20.5	COOK INLET WATER STA. I BEACH OFF
GW311	59	15.00	153	40.00	10/21/76	1.00	5.5	28.0	COOK INLET WATER STA.204LOW TIDE
GW312	59	38.00	152	33.00	10/22/76	1.00	9.5	26.2	COOK INLET WATER STA.225
GW313	59	42.00	151	9.00	10/22/76	1.00	8.5	24.0	COOK INLET WATER STA.229
GW314	59	34.70	151	11.10	10/22/76	1.00	10.0	25.0	TIME1000 STA J
GW315	59	35.00	151	11.00	10/22/76	0.00	9.5	24.0	COOK INLET WATER STA. J BEACH
GW316	59	36.40	151	24.00	10/22/76	1.00	12.0	24.0	TIME1130 STA K
GW317	59	36.00	151	25.00	10/22/76	0.00	12.0	23.0	COOK INLET WATER STA. K BEACH
GW318	59	34.00	151	44.00	10/22/76	1.00	9.5	27.0	COOK INLET WATER STA.227
GW319	59	33.00	152	9.00	10/23/76	1.00	9.5	27.0	COOK INLET WATER STA.226
GW320	60	0.00	152	10.00	10/23/76	1.00	9.0	26.0	COOK INLET WATER STA.245
GW321	60	2.30	151	45.90	10/23/76	1.00	9.0	20.5	TIME1245 STA 246
GW322	60	40.00	151	26.00	10/24/76	1.00	8.5	21.0	COOK INLET WATER STA.266
GW323	60	31.00	151	50.00	10/24/76	1.00	9.0	19.5	COOK INLET WATER STA.265
GW324	59	50.30	153	15.00	10/24/76	1.00	6.5	16.3	TIME1420 STA L
GW325	59	50.00	153	16.00	10/24/76	0.00	6.0	17.0	COOK INLET WATER STA. L BEACH
GW326	59	50.40	152	59.50	10/24/76	1.00	8.0	23.0	TIME1524 STA Q
GW327	59	21.00	153	15.00	10/25/76	1.00	9.0	26.0	COOK INLET WATER STA.214
GW328	59	10.20	153	8.50	10/25/76	1.00	9.0	27.5	TIME0130 STA 206
GW329	59	15.00	153	40.00	10/25/76	1.00	9.5	25.0	COOK INLET WATER STA.204HIGH TIDE
GW330	59	20.80	152	43.70	10/25/76	1.00	9.5	28.0	TIME1000 STA 215
GW331	58	15.90	154	16.40	10/26/76	1.00	2.0	18.0	TIME1530 STA P
GW332	58	15.90	154	16.40	10/26/76	1.00	-1.5	18.0	TIME1530 STA P

GW333	58 59.70	152 52.80	10/27/76	1.00	8.0	23.7	TIME2200 STA 207	
GW334	59 2.70	151 58.70	10/28/76	1.00	7.5	24.0	TIME0230 STA 106	
GW335	58 46.00	151 10.00	10/28/76	1.00	9.0	25.5	COOK INLET WATER STA.105	
GW336	58 50.20	151 21.30	10/28/76	1.00	8.0	27.5	TIME1030 STA 0	
GW337	58 55.30	152 0.10	10/28/76	1.00	5.0	27.5	TIME1030 STA 0	
NB002	64 11.20	161 25.70	07/79	18.00	10.4	21.2	NORTON SOUND STA 02 SEDIMENT	
NB003	63 59.80	162 0.60	07/79	20.00	7.2	31.2	NORTON SOUND STA 03 SEDIMENT	
NB004	63 48.40	161 23.00	07/79				NORTON SOUND STA 04 SEDIMENT	
NB007	63 39.20	163 14.60	07/79	16.50	9.8	26.1	NORTON SOUND STA 07 SEDIMENT	
NB016	64 11.60	165 1.70	07/79	17.00	8.6	30.0	NORTON SOUND STA 16 SEDIMENT	
NB021	64 12.10	165 30.50	07/79	20.00	7.2	29.6	NORTON SOUND STA 21 SEDIMENT	
NB024	64 28.00	166 6.10	07/79				NORTON SOUND STA 24 SEDIMENT	
NB027	64 25.50	166 26.90	07/79	27.00	8.8	29.8	NORTON SOUND STA 27 SEDIMENT	
NB031	63 20.30	166 0.30	07/79				NORTON SOUND STA 31 SEDIMENT	
NB036	63 40.00	167 1.80	07/79				NORTON SOUND STA 36 SEDIMENT	
NB040	64 40.30	167 1.80	07/79	26.00	5.0	31.3	NORTON SOUND STA 40 SEDIMENT	
NB045	63 23.00	168 29.00	07/79				NORTON SOUND STA 45 SEDIMENT	
NB047	62 59.90	168 0.90	07/79	24.00	5.1	31.7	NORTON SOUND STA 47 SEDIMENT	
NB049	64 20.10	169 0.30	07/79	40.00	5.1	31.8	NORTON SOUND STA 49 SEDIMENT	
NB101	64 12.10	166 27.70	07/79	26.00	6.3	30.1	NORTON SOUND STA 28 SEDIMENT	
NB103	64 12.00	165 59.70	07/79	19.00	6.6	30.0	NORTON SOUND STA 26 SEDIMENT	
NB107	64 6.60	165 30.40	07/79	19.00	7.2	29.7	NORTON SOUND STA 20 SEDIMENT	
NB111	64 14.10	165 16.60	07/79	18.00	8.1	30.0	NORTON SOUND STA 19 SEDIMENT	
NB113	64 19.70	165 1.00	07/79	31.00	9.1	30.1	NORTON SOUND STA 17 SEDIMENT	
NB115	64 19.90	165 23.20	07/79	26.00	9.1	30.1	NORTON SOUND STA 18 SEDIMENT	
NB117	64 19.90	165 42.40	07/79	22.00	8.5	30.1	NORTON SOUND STA 22 SEDIMENT	
NB119	64 20.00	165 59.80	07/79	21.00	8.0	29.6	NORTON SOUND STA 25 SEDIMENT	
NB124	64 26.80	165 41.70	07/79	26.00	9.5	29.5	NORTON SOUND STA 23 SEDIMENT	
NB126	63 59.60	164 58.50	07/79	18.00	9.1	29.0	NORTON SOUND STA 15 SEDIMENT	
NB127	63 50.30	165 41.70	07/79	20.00			NORTON SOUND STA A29 SEDIMENT	
NB128			07/79	27.00			NORTON SOUND STA 29B SEDIMENT	
NB129	63 20.20	165 12.40	07/79	15.00	8.6	29.5	NORTON SOUND STA 13 SEDIMENT	
NB130	64 0.30	163 4.00	07/79	20.00	8.2	30.7	NORTON SOUND STA 08 SEDIMENT	
NB131	64 19.20	162 0.50	07/79	17.00	9.7	25.7	NORTON SOUND STA 01 SEDIMENT	
NB132	63 39.90	161 17.80	07/79	12.80	10.4	21.0	NORTON SOUND STA 05 SEDIMENT	
NB133	63 40.10	161 58.90	07/79	13.00	10.0	26.3	NORTON SOUND STA 06 SEDIMENT	
NB134	64 14.70	163 6.10	07/79	20.00	9.6	27.1	NORTON SOUND STA 09 SEDIMENT	
NB135	64 11.00	163 1.70	07/79	24.00	8.1	30.2	NORTON SOUND STA 9A SEDIMENT	
NB137	64 0.20	164 0.10	07/79	17.00	8.6	30.2	NORTON SOUND STA 11 SEDIMENT	
NB138	63 40.30	163 59.10	07/79	13.00	9.8	27.0	NORTON SOUND STA 12 SEDIMENT	
NB139								
NB140	63 8.00	163 16.00	07/79	2.00			NORTON SOUND STA S7 SEDIMENT	
NB141	64 8.20	164 0.00	07/79	22.00	8.2	30.8	NORTON SOUND STA 10A SEDIMENT	
NB143	64 20.20	164 1.30	07/79	17.00	10.2	27.9	NORTON SOUND STA 10 SEDIMENT	
NB145	63 40.00	165 0.50	07/79		9.1	28.9	NORTON SOUND STA 14 SEDIMENT	
NB147	63 19.90	165 11.60	07/79	1.00			NORTON SOUND STA S13 SEDIMENT	
NB149	62 39.80	165 40.10	07/79	13.00	9.2	28.8	NORTON SOUND STA A32 SEDIMENT	
NB151	63 20.00	167 1.50	07/79	26.00	5.1	31.4	NORTON SOUND STA 34 SEDIMENT	
NB152								
NB153	64 0.10	169 0.90	07/79	35.00	5.1	32.0	NORTON SOUND STA 48 SEDIMENT	
NB154	63 59.50	168 1.30	07/79	36.00	5.1	31.9	NORTON SOUND STA 43 SEDIMENT	
NB156			07/79				NORTON SOUND STA 38 SEDIMENT	
NB157	63 39.70	165 59.10	07/79	26.00	5.2	31.5	NORTON SOUND STA 30 SEDIMENT	
NB158	64 0.20	165 59.70	07/79	22.00	6.4	30.4	NORTON SOUND STA 29 SEDIMENT	
NB161	64 40.20	168 0.80	07/79	38.00	5.0	31.8	NORTON SOUND STA 41 SEDIMENT	

NB162	64	39.60	168	59.80	07/79	44.00	5.0	32.1	NORTON SOUND STA 50 SEDIMENT
NB163	64	20.00	167	59.60	07/79	36.00	5.1	31.8	NORTON SOUND STA 42 SEDIMENT
NWA29	63	50.30	165	41.70	07/79				NORTON SOUND STA A29 WATER
NWB29	63	46.10	166	7.20	07/79				NORTON SOUND STA B29 WATER
NW002	64	11.20	161	25.70	07/79	1.50	11.1	19.3	NORTON SOUND STA 02 WATER
NW003	63	59.80	162	0.60	07/79	1.30	10.8	23.1	NORTON SOUND STA 03 WATER
NW004	63	48.40	161	23.00	07/79	1.30	11.2	22.2	NORTON SOUND STA 04 WATER
NW007	63	39.20	163	14.60	07/79	1.00	10.4	21.0	NORTON SOUND STA 07 WATER
NW013	63	20.20	165	12.40	07/79	1.40	8.6	29.4	NORTON SOUND STA 13 WATER
NW047	62	59.90	168	0.90	07/79	2.00	6.3	31.6	NORTON SOUND STA 47 WATER
NW049	64	20.10	169	0.30	07/79	1.10	4.6	31.6	NORTON SOUND STA 49 WATER
NW101	64	12.10	166	27.70	07/79	1.00	6.9	30.0	NORTON SOUND STA 28 WATER
NW102									
NW103	64	12.00	165	59.70	07/79	1.80	7.8	29.4	NORTON SOUND STA 26 WATER
NW104									
NW105	64	12.10	165	31.00	07/79	1.90	8.2	28.7	NORTON SOUND STA 21 WATER
NW106	64	16.10	165	45.70	07/79	18.00	7.2	29.6	NORTON SOUND STA Z21 BOTTOM WATER
NW107	64	7.10	165	30.00	07/79	2.30	7.2	29.7	NORTON SOUND STA 20 WATER
NW108									
NW109	64	11.60	165	1.70	07/79	1.00	8.9	27.9	NORTON SOUND STA 16 WATER
NW110									
NW111	64	14.10	165	16.60	07/79	1.00	8.8	28.0	NORTON SOUND STA 19 WATER
NW112									
NW113	64	19.70	165	1.00	07/79	1.00	13.4		NORTON SOUND STA 17 WATER
NW114									
NW115	64	20.20	165	21.70	07/79	1.10	10.4	27.4	NORTON SOUND STA 18 WATER
NW116									
NW117	64	19.90	165	42.40	07/79	2.70	9.0	28.1	NORTON SOUND STA 22 WATER
NW118									
NW119	64	20.00	165	59.80	07/79	1.00	8.8	28.0	NORTON SOUND STA 25 WATER
NW120									
NW121	64	25.50	166	26.90	07/79	1.50	9.4	28.5	NORTON SOUND STA 27 WATER
NW122	64	28.00	166	6.10	07/79	1.90	10.4	28.3	NORTON SOUND STA 24 WATER
NW123									
NW124	64	26.80	165	41.70	07/79	1.20	10.6	21.4	NORTON SOUND STA 23 WATER
NW125					07/79				NORTON SOUND STA 23 WATER
NW129					07/79				NORTON SOUND STA 13 WATER
NW130	64	0.30	163	4.00	07/79	1.40	11.7	23.4	NORTON SOUND STA 08 WATER
NW131	64	19.20	162	1.30	07/79	1.20	11.1	20.7	NORTON SOUND STA 01 WATER
NW132	63	39.90	161	17.80	07/79	1.70	11.1		NORTON SOUND STA 05 WATER
NW133	63	40.10	161	58.90	07/79	1.20	10.4	24.6	NORTON SOUND STA 06 WATER
NW134	64	14.70	163	6.10	07/79	1.10	11.6	22.8	NORTON SOUND STA 09 WATER
NW135	64	11.00	163	1.70	07/79	1.50	11.6	22.1	NORTON SOUND STA A9 WATER
NW136									
NW137	64	0.20	164	0.10	07/79	1.30	11.0	26.1	NORTON SOUND STA 11 WATER
NW138	63	40.30	163	59.10	07/79	1.10	9.9	26.8	NORTON SOUND STA 12 WATER
NW139									
NW140	63	8.00	163	16.00	07/79	1.00	10.6	20.0	NORTON SOUND STA S7 WATER
NW141	64	8.20	164	0.00	07/79	1.30	11.9	23.4	NORTON SOUND STA A10 WATER
NW142									
NW143	64	20.20	164	1.30	07/79	1.00	11.6	23.3	NORTON SOUND STA 10 WATER
NW144	63	59.60	164	58.50	07/79	1.00	9.1	28.6	NORTON SOUND STA 15 WATER
NW145	63	40.00	165	0.50	07/79	1.10	9.3	28.7	NORTON SOUND STA 14 WATER
NW146	63	20.30	166	0.30	07/79	1.00	7.5	30.7	NORTON SOUND STA 31 WATER
NW147	63	7.20	164	46.50	07/79	1.70	9.6	27.8	NORTON SOUND STA S13 WATER

NW148								
NW149	62 39.80	165 40.10	07/79	1.20	10.1	21.9	NORTON SOUND STA A32 WATER
NW150	63 23.00	168 29.00	07/79	1.60	4.2	31.5	NORTON SOUND STA 45 WATER
NW151	63 20.00	167 1.50	07/79	2.20	6.3	31.4	NORTON SOUND STA 34 WATER
NW152							
NW153	64 0.10	169 0.90	07/79	2.40	6.4	30.5	NORTON SOUND STA 48 WATER
NW154	63 59.50	168 1.30	07/79	2.90	6.7	31.7	NORTON SOUND STA 43 WATER
NW155	63 40.00	167 1.80	07/79	3.90	6.8	31.9	NORTON SOUND STA 36 WATER
NW156							
NW157	63 39.70	165 59.10	07/79	2.30	9.1	30.4	NORTON SOUND STA 30 WATER
NW158	64 0.20	165 59.70	07/79	2.50	10.0	28.9	NORTON SOUND STA 29 WATER
NW159							
NW160	64 40.30	167 1.80	07/79	1.00	9.7	29.3	NORTON SOUND STA 40 WATER
NW161	64 40.20	168 0.80	07/79	1.70	7.5	31.1	NORTON SOUND STA 41 WATER
NW162	64 39.60	168 59.80	07/79	1.00	4.2	31.5	NORTON SOUND STA 50 WATER
NW163	64 20.00	167 59.60	07/79	1.50	7.0	30.6	NORTON SOUND STA 42 WATER
NW164							
NW165			07/79				NORTON SOUND STA 28 WATER
NZS13	63 7.20	164 46.50	07/79				NORTON SOUND STA S13 BOTTOM WATER
NZ024	64 28.00	166 26.90	07/79	22.00	9.3	29.8	NORTON SOUND STA 13 BOTTOM WATER
GB401	57 39.00	152 31.00		0.00	6.5	16.5	SEDIMENT STA.D
GB410	60 9.50	152 25.00	04/06/77	39.00	2.3	30.2	COOK INLET SEDIMENT STA.242
GB411	60 12.70	152 36.50	04/07/77	3.00	6.0	15.0	COOK INLET SEDIMENT STA.U OFF
GB412	60 13.70	152 46.80		0.00	4.0	22.0	SEDIMENT STA.V
GB420	59 33.50	151 36.40	04/09/77	89.00	4.4	31.5	COOK INLET SEDIMENT STA.227
GB421	59 36.10	151 25.00	04/09/77	3.00			COOK INLET SEDIMENT STA.K
GB425	59 37.60	151 18.00	04/09/77	67.00	4.1	31.3	COOK INLET SEDIMENT STA.229
GB428	59 33.50	152 18.70	04/10/77	60.00	4.7	31.5	COOK INLET SEDIMENT STA.226
GB429	59 31.40	152 41.50	04/10/77	39.00	4.4	31.5	COOK INLET SEDIMENT STA.225
GB430	59 30.00	153 13.20		33.00	3.1	31.0	SEDIMENT STA.213
GB431	59 18.20	153 14.30	04/10/77	49.00	3.3	31.1	COOK INLET SEDIMENT STA.214
GB432	59 14.20	153 39.70		36.00	2.9	30.9	SEDIMENT STA.204
GB434	59 32.40	153 21.80	04/11/77	27.00	2.5	30.7	COOK INLET SEDIMENT STA.212
GB435	59 21.90	152 48.70	04/11/77	76.00	4.6	31.5	COOK INLET SEDIMENT STA.215
GB436	59 33.40	153 24.50		22.00	2.4	30.7	SEDIMENT STA.212
GB437							SEDIMENT STA. 208
GB438	59 6.30	152 43.10	04/12/77	148.00	5.4	32.1	COOK INLET SEDIMENT STA.205
GB440	58 53.00	152 54.00	04/13/77	172.00			COOK INLET SEDIMENT STA.395
GB442	58 50.00	151 20.00	04/14/77	118.00	7.5	32.6	COOK INLET SEDIMENT STA.105
GB444	58 28.60	153 10.00	04/14/77	168.00			COOK INLET SEDIMENT STA.388
GB445	58 2.00	153 29.00	04/14/77	95.00	5.0	32.0	COOK INLET SEDIMENT STA.378
GW401	57 39.00	152 31.00	04/03/77	0.00		16.5	TIME1400 STA D
GW402	60 41.20	151 25.00	04/05/77	1.00	1.0	21.0	COOK INLET WATER STA.266
GW403	60 34.30	151 51.40	04/05/77	1.00	1.0	27.4	COOK INLET WATER STA.265
GW404	60 9.60	152 15.00	04/06/77	56.00	2.2	30.2	TIME0700 STA 244
GW405	60 6.80	152 14.00	04/06/77	1.00	2.9	26.0	COOK INLET WATER STA.245
GW406	60 10.70	152 36.00	04/06/77	1.00	2.8	24.5	COOK INLET WATER STA.S OFF
GW407	60 10.70	152 36.00	04/06/77	0.00	4.5	24.0	COOK INLET WATER STA.S
GW408	60 9.30	152 38.00	04/06/77	0.00	2.8	25.0	TIME1500 STA T
GW409	60 9.30	152 38.00	04/06/77	0.00	4.0	25.0	TIME1500 STA T
GW410	60 9.50	152 25.00	04/06/77	0.00	2.3	30.2	TIME1315 STA 242
GW411	60 12.70	152 36.50	04/07/77	1.00	6.0	15.0	COOK INLET WATER STA.U OFF
GW412	60 13.70	152 46.80	04/07/77	0.00	4.0	22.0	TIME1412 STA V
GW413	60 13.70	152 46.80	04/07/77	0.00	2.0	23.0	TIME1410 STA V
GW414			04/ 7/7				TIME STA

GW415	60	9.50	152	25.00	04/07/77	1.00	2.3	30.1	COOK INLET WATER STA.242
GW416	60	9.50	152	25.00	04/07/77	33.00	2.3	30.1	TIME1905 STA 242
GW417	60	3.00	151	46.20	04/08/77	1.00	2.5	25.5	COOK INLET WATER STA.246
GW418	59	40.80	152	38.60	04/09/77	40.00	2.8	30.6	TIME1325 STA 235
GW419	59	40.90	151	14.10	04/10/77	48.00	4.1	31.4	TIME0340 STA 236
GW420	59	33.50	151	36.40	04/09/77	1.00	4.4	31.4	COOK INLET WATER STA.227
GW421	59	36.10	151	25.00	04/09/77	1.00	5.3	25.3	COOK INLET WATER STA.K OFF
GW422	59	36.10	151	25.00	04/10/77	0.00	5.0	26.0	TIME0950 STA K
GW423	59	35.30	151	10.70	04/10/77	0.00	4.6	26.8	TIME1050 STA J
GW424	59	35.30	151	10.70	04/10/77	0.00	5.0	25.0	TIME1115 STA J
GW425	59	37.60	151	18.00	04/09/77	1.00	4.2	31.3	COOK INLET WATER STA.229
GW426	59	18.20	152	14.10	04/10/77	1.00	5.0	31.5	COOK INLET WATER STA.216
GW427	59	27.00	152	23.20	04/11/77	74.00	4.6	31.5	TIME0315 STA 217
GW428	59	33.50	152	18.70	04/10/77	1.00	4.7	31.5	COOK INLET WATER STA.226
GW429	59	31.40	152	41.50	04/10/77	1.00	3.7	31.3	COOK INLET WATER STA.225
GW430	59	30.00	153	13.20	04/11/77	33.00	3.1	31.0	TIME1200 STA 213
GW431	59	18.20	153	14.30	04/10/77	1.00	3.3	31.1	COOK INLET WATER STA.214
GW432	59	14.20	153	39.70	04/10/77	1.00	2.7	30.8	COOK INLET WATER STA.204
GW433	59	9.80	153	8.20	04/10/77	1.00	4.9	31.5	COOK INLET WATER STA.206
GW434	59	32.40	153	21.80	04/11/77	1.00	2.3	30.6	COOK INLET WATER STA.212
GW435	59	21.90	152	48.70	04/11/77	1.00	3.4	31.2	COOK INLET WATER STA.215
GW436	59	33.40	153	24.50	04/12/77	22.00	2.4	30.7	TIME1100 STA 212
GW437	59	14.70	152	45.50	04/12/77	104.00	4.9	31.6	TIME1445 STA 208
GW438	59	6.30	152	43.10	04/12/77	1.00	5.0	31.6	COOK INLET WATER STA.205
GW439	58	59.90	152	52.00	04/12/77	1.00	4.8	31.7	COOK INLET WATER STA.207
GW440	58	53.00	152	54.00	04/13/77	1.00	4.6	26.5	COOK INLET WATER STA.395
GW441	59	0.40	152	0.00	04/13/77	1.00	4.7	31.5	COOK INLET WATER STA.106
GW442	58	50.00	151	20.00	04/14/77	1.00	4.6	27.8	COOK INLET WATER STA.105
GW443	58	48.80	152	11.90	04/12/77	122.00	4.7	31.7	TIME2045 STA 398
GW444	58	28.60	153	10.00	04/14/77	1.00	4.7	24.4	COOK INLET WATER STA.388
GW445	58	2.00	153	29.00	04/14/77	1.00	4.5	26.3	COOK INLET WATER STA.378
GB501	57	47.20	151	48.80	11/77	75.00	7.1	32.2	COOK INLET SEDIMENT ST.429
GB502	58	4.80	151	42.00	11/77	166.00	6.0	32.9	COOK INLET SEDIMENT ST.418
GB503	58	19.00	151	25.70	11/04/77	75.00			TIME0010 STA407
GB506	59	36.50	151	25.50	11/77	1.50	3.5	29.0	COOK INLET SEDIMENT ST. K BEACH
GB507	58	39.50	153	23.50		75.00	7.4	31.4	COOK INLET SEDIMENT ST 380
GB508	58	17.20	154	2.30	11/77	128.00	7.3	32.3	COOK INLET SEDIMENT ST.370
GB509	57	57.00	154	41.30		225.00	5.4	33.1	COOK INLET SEDIMENT ST 360
GB510	57	31.40	155	32.80	11/77	185.00	5.9	32.9	COOK INLET SEDIMENT ST.350
GB511	57	27.50	155	14.50	11/77	234.00	5.0	33.5	COOK INLET SEDIMENT ST.354
GB512	57	18.40	154	57.00	11/77	175.00	5.4	33.1	COOK INLET SEDIMENT ST.358
GB513	57	50.10	154	25.00	11/77	211.00	5.0	33.4	COOK INLET SEDIMENT ST.364
GB514	58	1.60	153	29.00	11/77	85.00	7.0	32.3	COOK INLET SEDIMENT ST.378
GB515	58	10.80	153	45.00	11/77	189.00	5.5	33.1	COOK INLET SEDIMENT ST.374
GB516	58	27.00	152	57.50		214.00	5.8	32.9	COOK INLET SEDIMENT ST 388
GB517	58	33.40	153	14.30		178.00	6.4	32.7	COOK INLET SEDIMENT ST 384
GB518	58	42.40	152	59.70	11/77	99.00	6.6	32.5	COOK INLET SEDIMENT ST.394
GB519	58	53.30	153	11.50		163.00	6.7	32.6	COOK INLET SEDIMENT ST 390
GB520	58	53.30	152	54.00	11/77	159.00	6.1	32.8	COOK INLET SEDIMENT ST.395
GB523	59	32.50	153	21.20	11/77	17.00	5.7	30.1	COOK INLET SEDIMENT ST.212
GB524	59	26.10	153	37.30	11/77	16.00	5.6	30.3	COOK INLET SEDIMENT ST.211
GB525	59	29.40	153	12.70		37.00	6.0	29.9	COOK INLET SEDIMENT ST 213
GB526	59	17.80	153	14.00		50.00	6.6	30.5	COOK INLET SEDIMENT ST 214
GB527	59	6.20	153	29.10		44.00	5.5	30.5	COOK INLET SEDIMENT ST 203
GB528					11/77				COOK INLET SEDIMENT ST.201

GB529	59	14.30	153	38.50	11/77	33.00		
GB532	58	59.80	152	52.90		167.00	6.4	32.7
GB536	59	33.30	152	18.60	11/11/77	49.00		
GB538	59	33.50	151	36.10	11/77	75.00	7.4	31.1
GB545	59	42.10	152	38.00		36.00	6.1	30.1
GB549	60	12.80	152	36.10	11/77	3.00	2.0	27.0
GB550	60	13.70	152	45.70	11/77	1.50	2.0	28.0
GB552	60	9.00	152	25.50	11/77	31.00	5.4	29.4
GB554	59	50.40	152	56.50		26.00		
GB557	59	40.40	151	14.30	11/77	16.00	5.6	30.8
GB558	59	37.50	151	17.80	11/77	72.00	7.0	31.1
GW501	57	47.20	151	58.80	11/77	1.00	7.1	31.7
GW502	58	4.80	151	42.00	11/77	1.00	6.4	32.0
GW503	58	17.50	151	25.80	11/77	1.00	6.4	32.2
GW504	58	48.90	152	11.60	11/77	1.00	7.0	32.0
GW505	59	35.50	151	10.50	11/77	1.00	7.5	29.0
GW506	59	36.50	151	25.50		1.00	3.5	29.0
GW507	58	39.50	153	23.50		1.00	4.8	29.9
GW508	58	17.20	154	2.30		1.00	5.2	30.2
GW509	57	57.00	154	41.30	11/77	1.00	7.0	30.7
GW510	57	31.40	155	32.80	11/77	1.00	6.7	30.5
GW511	57	27.50	155	14.50	11/77	1.00	6.1	31.8
GW512	57	18.40	154	57.00	11/77	1.00	6.3	31.8
GW513	57	50.10	154	25.00	11/77	1.00	6.1	31.9
GW514	58	1.60	153	29.00	11/77	1.00	6.4	31.2
GW515	58	10.80	153	45.00	11/77	1.00	7.0	31.3
GW516	58	27.00	152	57.50	11/77	1.00	7.3	31.3
GW517	58	33.40	153	14.30		1.00	7.3	30.9
GW518	58	42.40	152	59.70	11/77	1.00	7.4	31.0
GW519	58	53.30	153	11.50	11/77	1.00	5.1	30.2
GW520	58	53.30	152	54.00	11/77	1.00	7.1	31.9
GW521	59	6.20	152	41.30	11/77	1.00	7.8	31.0
GW522	59	9.40	153	7.10	11/77	1.00	6.6	30.3
GW523	59	32.50	153	21.20	11/77	1.00	5.7	30.1
GW524	59	26.10	153	37.30	11/77	1.00	5.6	30.3
GW525	59	29.40	153	12.70	11/77	1.00	6.0	29.9
GW526	59	17.80	153	14.00	11/77	1.00	6.2	30.0
GW527	59	6.20	153	29.10	11/77	1.00	5.1	30.3
GW528	59	12.80	153	52.40	11/77	1.00		
GW529	59	14.30	153	38.50	11/77	1.00	6.7	
GW530	59	21.10	153	48.80	11/77	1.00	6.5	30.6
GW531	59	15.00	152	44.90	11/77	1.00	7.0	31.0
GW532	58	59.80	152	52.90	11/77	1.00	7.0	31.4
GW533	59	18.00	152	15.00	11/77	1.00	7.8	31.4
GW534	59	27.70	152	22.90	11/77	1.00	7.6	31.5
GW535	59	31.50	152	41.90	11/77	1.00	7.1	31.3
GW536	59	33.30	152	18.60	11/77	1.00	7.2	31.3
GW537	59	32.90	151	53.40		1.00		
GW538	59	33.50	151	36.10	11/77	1.00	7.2	31.0
GW539	59	51.30	152	2.10	11/77	1.00	6.3	30.8
GW540	60	2.50	151	47.50	11/77	1.00	2.2	30.7
GW541	60	41.20	151	25.60	11/77	1.00	4.4	23.0
GW542	60	33.60	151	51.60	11/77	1.00		
GW543	60	19.90	151	45.90	11/77	1.00	5.4	28.8
GW544	59	50.50	152	56.50		1.00	5.9	29.8

COOK INLET SEDIMENT ST.204
COOK INLET SEDIMENT ST 207
TIME1430 STA226
COOK INLET SEDIMENT ST.227
COOK INLET SEDIMENT ST 235
COOK INLET SEDIMENT ST. U BEACH
COOK INLET SEDIMENT ST. V BEACH
COOK INLET SEDIMENT ST.242
COOK INLET SEDIMENT ST 233
COOK INLET SEDIMENT ST.229A
COOK INLET SEDIMENT ST.229
COOK INLET WATER ST.429
COOK INLET WATER ST.418
COOK INLET WATER ST.417
COOK INLET WATER ST.398
COOK INLET WATER ST. J BEACH
COOK INLET WATER ST K BEACH
COOK INLET WATER ST 380
COOK INLET WATER ST 370
COOK INLET WATER ST.360
COOK INLET WATER ST.350
COOK INLET WATER ST.354
COOK INLET WATER ST.358
COOK INLET WATER ST.364
COOK INLET WATER ST.378
COOK INLET WATER ST.374
COOK INLET WATER ST.388
COOK INLET WATER ST 384
COOK INLET WATER ST.394
COOK INLET WATER ST.390
COOK INLET WATER ST.395
COOK INLET WATER ST.205
COOK INLET WATER ST.206
COOK INLET WATER ST.212
COOK INLET WATER ST.211
COOK INLET WATER ST.213
COOK INLET WATER ST.214
COOK INLET WATER ST.203
COOK INLET WATER ST.201
COOK INLET WATER ST.204
COOK INLET WATER ST.215
COOK INLET WATER ST.208
COOK INLET WATER ST.207
COOK INLET WATER ST.216
COOK INLET WATER ST.217
COOK INLET WATER ST.225
COOK INLET WATER ST.226
COOK INLET WATER ST 228
COOK INLET WATER ST.227
COOK INLET WATER ST.249
COOK INLET WATER ST.246
COOK INLET WATER ST.266
COOK INLET WATER ST.265
COOK INLET WATER ST.255
COOK INLET WATER ST 248

GW545	59	42.10	152	38.10	11/77	1.00	6.1	30.0	COOK INLET WATER ST.235
GW545A	59	42.10	152	38.10	11/77	20.00	6.1	30.1	COOK INLET WATER ST.235 >20M
GW546	59	37.60	152	55.80	11/77	1.00	6.3	30.4	COOK INLET WATER ST.234
GW547	60	6.70	152	14.50	11/77	1.00	5.3	29.4	COOK INLET WATER ST.245
GW548	60	6.80	152	36.00		1.00			COOK INLET WATER ST 241
GW549	60	12.80	152	36.10	11/77	1.00	2.0	27.0	COOK INLET WATER ST. U BEACH
GW550	60	13.70	152	45.70	11/77	1.00	2.0	28.0	COOK INLET WATER ST. V BEACH
GW551	60	6.80	152	36.00		1.00			COOK INLET WATER ST 241
GW552	60	9.00	152	25.50	11/77	1.00	5.1	29.3	COOK INLET WATER ST.242
GW553	59	56.00	152	37.10		1.00			COOK INLET WATER ST 247
GW554	59	50.40	152	56.50		1.00			COOK INLET WATER ST 233
GW555	59	29.40	153	12.70		1.00	5.6	30.3	COOK INLET WATER ST 213
GW556	59	41.30	152	14.10	11/77	1.00	7.0	31.4	COOK INLET WATER ST.236
GW557	59	40.40	151	14.30	11/77	1.00	5.5	30.7	COOK INLET WATER ST.229A
GW558	59	37.50	151	17.80	11/77	1.00	6.2	30.9	COOK INLET WATER ST.229
GW559	59	0.60	152	1.40	11/77	1.00	7.0	31.5	COOK INLET WATER ST.106
GW560	58	49.80	151	17.80	11/77	1.00	6.5	31.2	COOK INLET WATER ST.105
GB801	55	43.90	156	19.70	05/79	247.00	5.1	33.4	UPPER COOK STA SS1 SEDIMENT
GB802	56	41.90	155	55.60	05/79	292.00	4.8	33.2	UPPER COOK STA SS2 SEDIMENT
GB804	57	10.70	155	9.20	05/79	223.80	4.6	33.0	UPPER COOK STA SS4 SEDIMENT
GB805	57	3.40	154	48.10	05/79				UPPER COOK STA SS5 SEDIMENT
GB808	58	32.90	153	47.20	05/79				UPPER COOK STA SS8 SEDIMENT
GB809	58	28.60	153	12.50	05/79				UPPER COOK STA SS9 SEDIMENT
GB810	58	27.80	152	51.00	05/79				UPPER COOK STA SS10 SEDIMENT
GB811	58	47.20	152	40.30	05/79	203.00	5.8		UPPER COOK STA SS13 SEDIMENT
GB812	58	59.10	151	53.50	05/79	181.00	6.5		UPPER COOK STA SS18 SEDIMENT
GB817					05/79				UPPER COOK STA LC1 SEDIMENT
GB818	59	2.30	153	31.10	05/79				UPPER COOK STA LC2 SEDIMENT
GB819	59	13.40	153	40.70	05/79				UPPER COOK STA LC3 SEDIMENT
GB822	59	31.60	153	26.40	05/79	22.00	6.2	31.4	UPPER COOK STA LC6 SEDIMENT
GB842	60	30.20	151	48.80	05/79	28.00	5.5	30.1	UPPER COOK STA LC25 SEDIMENT
GB843	60	12.40	152	25.00	05/79				UPPER COOK STA LC17 SEDIMENT
GB844	60	12.40	152	25.00	05/79	44.00	5.0	30.8	UPPER COOK STA LC17 SEDIMENT
GB863	59	17.90	153	13.20	05/79	1.00	7.5		UPPER COOK STA LC16 SEDIMENT
GW800					05/79	1.00	6.0	30.9	UPPER COOK STA SS0 TOP WATER
GW801	55	43.90	156	19.80	05/79	1.20	5.0	32.2	UPPER COOK STA SS1 TOP WATER
GW802	56	42.00	155	54.90	05/79	2.60	5.6	31.9	UPPER COOK STA SS2 TOP WATER
GW803	57	24.30	155	56.60	05/79	1.60	5.0	31.7	UPPER COOK STA SS3A TOP WATER
GW804	57	10.70	155	9.20	05/79	1.20	5.4	31.8	UPPER COOK STA SS4 TOP WATER
GW805	57	3.10	154	49.20	05/79		5.5	32.4	UPPER COOK STA SS5 WATER
GW806					05/79				UPPER COOK STA SS6 WATER
GW807	58	5.80	154	8.40	05/79		5.0	31.5	UPPER COOK STA SS7 WATER
GW808	58	33.10	153	47.20	05/79		5.0	31.2	UPPER COOK STA SS8 WATER
GW809	58	28.60	153	12.20	05/79		5.5	31.9	UPPER COOK STA SS9 WATER
GW810	58	27.70	152	51.10	05/79		5.8	31.6	UPPER COOK STA SS10 WATER
GW811	58	47.20	152	40.30	05/79	1.00	5.0		UPPER COOK STA SS13 TOP WATER
GW812	58	59.30	151	54.10	05/79	1.00	5.2		UPPER COOK STA SS18 TOP WATER
GW813	59	33.90	152	4.80	05/79		5.0	32.0	UPPER COOK STA LC10 WATER
GW814	59	33.20	152	17.80	05/79		4.8	32.1	UPPER COOK STA LC9 WATER
GW815	59	23.50	152	50.80	05/79		4.4	31.5	UPPER COOK STA LC8 WATER
GW816	59	10.60	153	12.40	05/79		3.9	31.9	UPPER COOK STA LC33 WATER
GW817	58	58.30	153	9.80	05/79		4.6	32.2	UPPER COOK STA LC1 WATER
GW818	59	2.30	153	31.10	05/79		4.8	30.4	UPPER COOK STA LC2 WATER
GW819	59	13.20	153	41.10	05/79		4.6	30.7	UPPER COOK STA LC3 WATER
GW820	59	28.30	153	12.60	05/79		4.2	31.3	UPPER COOK STA LC7 WATER

GW821	59	25.60	153	34.70	05/79		5.0	30.5
GW822	59	31.30	153	26.70	05/79	1.00	4.2	31.1
GW823	59	46.90	152	54.90	05/79		4.5	30.9
GW824	60	3.50	152	29.20	05/79		4.0	
GW825	60	20.70	151	41.00	05/79	1.00	3.9	31.2
GW826	59	44.80	152	1.00	05/79	1.00	5.6	31.2
GW827	60	48.30	151	32.10	05/79	2.30	4.5	26.6
GW828	60	47.50	151	19.80	05/79	1.00	6.5	26.0
GW829	60	47.50	151	19.80	05/79		6.0	26.5
GW830	60	57.20	151	8.80	05/79	1.80	5.7	23.5
GW831	60	57.20	151	8.80	05/79		5.7	24.2
GW832	60	56.80	151	18.30	05/79	2.30	6.0	25.8
GW833	60	56.70	151	26.90	05/79	2.70	6.0	26.0
GW834	60	56.70	151	26.90	05/79		5.0	26.0
GW835	60	45.80	151	40.30	05/79	1.40	5.5	27.3
GW836	60	32.30	151	30.50	05/79		6.0	29.3
GW837	60	32.30	151	30.50	05/79	1.00	6.5	29.3
GW838	60	32.80	151	40.20	05/79		4.8	28.0
GW839	60	32.80	151	40.20	05/79	1.00	5.0	28.0
GW840	60	35.90	151	47.00	05/79		5.0	28.1
GW841	60	35.90	151	47.00	05/79	1.00	5.0	28.1
GW842	60	30.20	151	48.80	05/79			30.1
GW843	60	11.10	152	26.00	05/79	1.00	4.5	30.8
GW844					05/79			
GW845	60	18.30	152	12.20	05/79	2.00	4.5	30.7
GW846	60	53.10	151	35.50	05/79		4.4	25.7
GW847	60	48.30	151	35.40	05/79	3.00	4.3	27.7
GW848	60	48.30	151	35.40	05/79		4.3	27.6
GW849	60	50.60	151	40.80	05/79	2.00	4.3	26.2
GW850	60	47.60	151	42.30	05/79	1.00	4.3	26.6
GW851	60	42.10	151	40.00	05/79	1.00	4.3	27.7
GW852	60	42.30	151	32.80	05/79	1.00		28.1
GW853	60	42.30	151	32.80	05/79			28.1
GW854	61	3.20	150	51.30	05/79	1.00	5.0	24.0
GW855	61	3.20	150	51.30	05/79		5.0	24.0
GW856	60	59.20	150	42.50	05/79	1.00	6.5	23.4
GW857	61	4.00	150	22.70	05/79	1.00	6.0	22.4
GW858	61	8.00	150	23.20	05/79	1.00	6.0	21.5
GW859	61	8.00	150	23.20	05/79		6.0	21.5
GW860	61	9.70	150	23.80	05/79	1.00	6.5	20.3
GW861	61	9.70	150	55.00	05/79	1.00	6.8	16.2
GW862	61	9.70	150	55.00	05/79		6.8	16.2
GW863	59	17.90	153	13.20	05/79	1.00	8.0	
GW865	59	31.50	152	39.60	05/79	1.00	5.0	
GZ800					05/79	1.00	6.0	30.9
GZ806	57	3.40	154	48.10	05/79	208.00	4.5	32.9
GZ825	60	20.70	151	41.00	05/79	43.00		33.4
GZ829	60	47.50	151	19.80	05/79	20.00	6.0	
GZ831	60	57.20	151	8.80	05/79	25.00	5.7	24.2
GZ834	59	40.20	151	14.30	05/79	20.00	5.0	26.0
GZ836	60	32.30	151	30.50	05/79	29.00	6.0	29.3
GZ838	60	32.80	151	40.20	05/79	44.00	4.8	28.0
GZ839	60	56.70	151	26.90		00 5.0	26.0	
GZ840	60	35.90	151	47.00	05/79	20.00	5.0	28.1
GZ844	60	13.20	152	24.30	05/79	44.00	4.5	30.8

UPPER COOK STA LC5 WATER
UPPER COOK STA LC6 TOP WATER
UPPER COOK STA LC14 WATER
UPPER COOK STA LC15 WATER
UPPER COOK STA LC20 TOP WATER
UPPER COOK STA LC11 TOP WATER
UPPER COOK STA UC8 TOP WATER
UPPER COOK STA UC7 TOP WATER
UPPER COOK STA UC7 BOTTOM WATER
UPPER COOK STA UC1 TOP WATER
UPPER COOK STA UC1 BOTTOM WATER
UPPER COOK STA UC2 TOP WATER
UPPER COOK STA UC3 TOP WATER
UPPER COOK STA UC3 BOTTOM WATER
UPPER COOK STA UC12 TOP WATER
UPPER COOK STA UC15 BOTTOM WATER
UPPER COOK STA UC15 TOP WATER
UPPER COOK STA UC16 BOTTOM WATER
UPPER COOK STA UC16 TOP WATER
UPPER COOK STA UC17 BOTTOM WATER
UPPER COOK STA UC17 TOP WATER
UPPER COOK STA LC25 TOP WATER
UPPER COOK STA LC17 TOP WATER
UPPER COOK STA LC17 WATER
UPPER COOK STA LC21 TOP WATER
UPPER COOK STA UC4 WATER
UPPER COOK STA UC9 TOP WATER
UPPER COOK STA UC9 BOTTOM WATER
UPPER COOK STA UC10 TOP WATER
UPPER COOK STA UC11 TOP WATER
UPPER COOK STA UC13 TOP WATER
UPPER COOK STA UC14 TOP WATER
UPPER COOK STA UC14 BOTTOM WATER
UPPER COOK STA UC22 TOP WATER
UPPER COOK STA UC22 BOTTOM WATER
UPPER COOK STA UC23 TOP WATER
UPPER COOK STA UC20 TOP WATER
UPPER COOK STA UC19 TOP WATER
UPPER COOK STA UC19 BOTTOM WATER
UPPER COOK STA UC18 TOP WATER
UPPER COOK STA UC21 TOP WATER
UPPER COOK STA UC21 BOTTOM WATER
UPPER COOK STA LC16 TOP WATER
UPPER COOK STA LC15 TOP WATER
UPPER COOK STA SS0 BOTTOM WATER
UPPER COOK STA SS3 BOTTOM WATER
UPPER COOK STA LC20 BOTTOM WATER
UPPER COOK STA UC7 BOTTOM WATER
UPPER COOK STA UC1 BOTTOM WATER
UPPER COOK STA UC3 BOTTOM WATER
UPPER COOK STA UC15 BOTTOM WATER
UPPER COOK STA UC16 BOTTOM WATER
UPPER COOK STA UC17 BOTTOM WATER
UPPER COOK STA LC17 BOTTOM WATER

GZ848	60	48.30	151	35.40	05/79	50.00	4.3	27.6	UPPER COOK STA UC9 BOTTOM WATER
GZ853	60	42.30	151	32.80	05/79	30.00		28.1	UPPER COOK STA UC14 BOTTOM WATER
GZ855	60	3.20	150	51.30	05/79	30.00	5.0	24.0	UPPER COOK STA UC22 BOTTOM WATER
GZ859	61	8.00	150	23.20	05/79	6.00	6.0	21.5	UPPER COOK STA UC19 BOTTOM WATER
GZ862	61	9.70	150	55.00	05/79	15.00	6.8	16.2	UPPER COOK STA UC21 BOTTOM WATER
GZ864	59	32.20	152	39.10	05/79	151.00	5.0		UPPER COOK STA LC15 BOTTOM WATER
GB601					05/78				COOK INLET SEDIMENT ST 330
GB602	56	46.00	154	20.30	05/78	52.00	3.5	32.1	COOK INLET SEDIMENT ST 331
GB603	57	4.20	155	1.20	05/78	188.00	4.5	32.4	COOK INLET SEDIMENT ST 333
GB604	57	18.10	154	56.00	05/78	148.00	4.5	32.4	COOK INLET SEDIMENT ST 358
GB605	57	31.20	155	33.80	05/78	265.00	4.6	32.5	COOK INLET SEDIMENT ST 350
GB606	57	56.20	154	40.60	05/78	228.00	4.5	32.4	COOK INLET SEDIMENT ST 360
GB607	57	43.90	154	9.00	05/78	53.00	4.4	32.3	COOK INLET SEDIMENT ST 368
GB608					05/78				COOK INLET SEDIMENT ST 378
GB609	58	17.20	154	1.90	05/78	11.20	4.7	32.0	COOK INLET SEDIMENT ST 370
GB610	58	38.90	154	1.90	05/78	166.00	4.0	31.5	COOK INLET SEDIMENT ST 380
GB611	58	40.90	153	0.50	05/78	153.00	4.7	32.3	COOK INLET SEDIMENT ST 394
GB612	58	27.20	152	58.00	05/78	215.00	4.6	32.4	COOK INLET SEDIMENT ST 388
GB613	58	53.20	152	54.90	05/78	166.00	4.7	32.2	COOK INLET SEDIMENT ST 395
GB614	58	59.90	152	53.30	05/78	166.00	4.8	32.1	COOK INLET SEDIMENT ST 207
GB615	58	52.70	153	11.10	04/27/78	170.00			TIME0745 STA390
GB616	59	39.90	151	14.80	05/78	38.00	4.2	31.7	COOK INLET SEDIMENT ST 229
GB617	59	51.30	152	1.60	04/28/78	40.00			TIME0215 249
GB622	60	9.00	152	25.70	04/28/78	36.00			TIME1400 242
GB624	59	58.10	152	34.40	04/28/78	21.00			TIME2000 247
GB625	59	49.70	152	56.00	05/78	15.00	3.9	31.0	COOK INLET SEDIMENT ST 233
GB626	59	50.10	152	24.20	04/29/78	70.00			TIME0150 248
GB627	59	6.30	153	27.80	05/78	41.00	4.0	31.1	COOK INLET SEDIMENT ST 203
GB628	59	12.50	153	52.90	05/78	20.00	4.3	30.7	COOK INLET SEDIMENT ST 201
GB629	59	14.10	153	39.50		34.00		31.2	COOK INLET SEDIMENT ST 204
GB630	59	17.90	153	13.20		53.00		31.7	COOK INLET SEDIMENT ST 214
GB631					05/78				COOK INLET SEDIMENT ST 211
GB632	59	32.70	153	20.90	05/78	26.00	4.0	31.3	COOK INLET SEDIMENT ST 212
GB633	60	13.40	152	45.60	05/78	26.00			COOK INLET SEDIMENT ST V BEACH
GB634	60	12.80	152	36.00	05/78	26.00			COOK INLET SEDIMENT ST U BEACH
GB635	59	37.80	152	55.80	04/30/78	36.00			TIME1600 234
GB636	59	29.60	153	13.90	05/78	33.00	4.2	31.4	COOK INLET SEDIMENT ST 213
GB640	59	32.80	152	18.30	05/01/78	49.00			TIME0115 226
GB644	58	53.40	153	11.60	05/78	62.00	5.0	32.1	COOK INLET SEDIMENT ST 390
GB649	59	38.00	153	37.80	05/78	201.00			COOK INLET SEDIMENT ST AW BEACH
GB650	59	43.30	153	22.60	05/78	201.00			COOK INLET SEDIMENT ST M BEACH
GB652	59	37.80	151	18.40	05/78	64.00	4.3	31.8	COOK INLET SEDIMENT ST CB8
GB653	59	13.70	153	40.10	05/78	31.00	4.4	31.4	COOK INLET SEDIMENT ST CB1
GB654	59	16.60	153	20.30		36.00			COOK INLET SEDIMENT ST CB2
GB660	59	35.40	151	45.90	05/78	51.00	5.1	31.8	COOK INLET SEDIMENT ST CB7 0930 HRS
GB669	59	36.50	151	25.50	05/78	51.00			COOK INLET SEDIMENT ST K BEACH
GB677	60	28.20	152	12.20	05/78	37.00		30.7	COOK INLET SEDIMENT ST CB9 1300 HRS
GW601	56	22.30	154	17.90	05/78	1.00	4.5	32.2	COOK INLET WATER ST 330
GW602	56	46.00	154	20.30	05/78	1.00	3.5	32.1	COOK INLET WATER ST 331
GW603	57	4.20	155	1.20	05/78	1.00	4.9	31.9	COOK INLET WATER ST 333
GW604	57	18.10	154	56.00	05/78	1.00	4.5	32.1	COOK INLET WATER ST 358
GW605	57	31.20	155	33.80	05/78	1.00	4.4	31.9	COOK INLET WATER ST 350
GW606	57	56.20	154	40.60	05/78	1.00	4.4	31.8	COOK INLET WATER ST 360
GW607	57	43.90	154	9.00	05/78	1.00	4.6	32.1	COOK INLET WATER ST 368
GW608	58	1.30	153	29.60	05/78	1.00	4.9	32.1	COOK INLET WATER ST 378

GW609	58	17.20	154	1.90	05/78	1.00	4.0	31.5	COOK INLET WATER ST 370
GW610	58	38.90	153	24.70	05/78	1.00	3.9	31.4	COOK INLET WATER ST 380
GW611	58	40.90	153	0.50	05/78	1.00	4.6	31.9	COOK INLET WATER ST 394
GW612	58	27.20	152	58.00	05/78	1.00	4.9	32.0	COOK INLET WATER ST 388
GW613	58	53.20	152	54.90	05/78	1.00	4.7	31.9	COOK INLET WATER ST 395
GW614	58	59.90	152	53.30	05/78	1.00	4.8	32.0	COOK INLET WATER ST 207
GW615	58	52.70	153	11.10	05/78	1.00	3.8	31.3	COOK INLET WATER ST 390
GW616	59	39.90	151	14.80	05/78	1.00	4.7	31.6	COOK INLET WATER ST 229
GW617	59	51.30	152	1.60	05/78	1.00	4.7		COOK INLET WATER ST 249
GW618	60	5.50	151	45.70	05/78	1.00	5.0		COOK INLET WATER ST 246
GW619	60	42.70	151	25.50	05/78	1.00	4.2		COOK INLET WATER ST 266
GW620	60	34.90	151	41.90	05/78	1.00	4.1		COOK INLET WATER ST 265
GW621	60	19.30	151	46.50	05/78	1.00	3.3	30.0	COOK INLET WATER ST 255
GW622	60	9.00	152	25.70	05/78	1.00	3.7	30.8	COOK INLET WATER ST 242
GW623	60	6.30	152	15.50	05/78	1.00	3.8	30.6	COOK INLET WATER ST 245
GW624	59	58.10	152	34.40	05/78	1.00	4.0	30.7	COOK INLET WATER ST 247
GW625	59	49.70	152	56.00	05/78	1.00	3.9	31.2	COOK INLET WATER ST 233
GW626	59	50.10	152	24.20	05/78	1.00	3.8	30.9	COOK INLET WATER ST 248
GW627	59	6.30	153	27.80	05/78	1.00	4.1	30.0	COOK INLET WATER ST 203
GW628	59	12.50	153	52.90	05/78	1.00	4.3	30.7	COOK INLET WATER ST 201
GW629	59	14.10	153	39.50	05/78	1.00		31.2	COOK INLET WATER ST 204
GW630	59	17.90	153	13.20		1.00		31.5	COOK INLET WATER ST 214
GW631	59	26.10	153	37.00	05/78	1.00	4.0	31.3	COOK INLET WATER ST 211
GW632	59	32.70	153	20.90	05/78	1.00	4.0	31.2	COOK INLET WATER ST 212
GW633	60	13.40	152	45.60	05/78	1.00	4.0	30.5	COOK INLET WATER ST V BEACH
GW634	60	12.80	152	36.00	05/78	1.00	4.0	29.0	COOK INLET WATER ST U BEACH
GW635	59	37.80	152	55.80	05/78	1.00	4.1	31.3	COOK INLET WATER ST 234
GW636	59	29.60	153	13.90	05/78	1.00	4.2	31.4	COOK INLET WATER ST 213
GW637	59	31.50	152	39.60	05/78	1.00	4.4	31.7	COOK INLET WATER ST 225
GW638	59	42.60	152	37.30	05/78	1.00	4.1	31.2	COOK INLET WATER ST 235
GW639	59	41.70	152	14.20	05/78	1.00	4.8	31.9	COOK INLET WATER ST 236
GW640	59	32.80	152	18.30	05/78	1.00	4.7	31.9	COOK INLET WATER ST 226
GW641	59	18.10	152	15.40	05/78	1.00	4.8	31.9	COOK INLET WATER ST 216
GW642	59	6.70	152	40.00	05/78	1.00	4.8	31.9	COOK INLET WATER ST 205
GW643	59	8.70	153	5.00	05/78	1.00	4.8	31.9	COOK INLET WATER ST 206
GW644	58	53.40	153	11.60		1.00		31.5	COOK INLET WATEWATERT 390
GW645	58	49.00	152	12.30	05/78	1.00	5.1	32.2	COOK INLET WATER ST 398
GW646	58	49.70	151	19.30	05/78	1.00	5.0	32.1	COOK INLET WATER ST 105
GW647	59	0.60	152	1.20	05/78	1.00	4.9	31.9	COOK INLET WATER ST 106
GW648	59	39.50	153	16.50		1.00		28.0	COOK INLET WATEWATERT AA BEACH
GW649	59	38.00	153	37.80	05/78	1.00	5.5	27.2	COOK INLET WATER ST AW BEACH
GW650	59	43.30	153	22.60	05/78	1.00	8.0	27.6	COOK INLET WATER ST M BEACH
GW651	59	34.60	151	11.00		1.00		31.2	COOK INLET WATEWATERT J BEACH
GW652	59	37.80	151	18.40	05/78	1.00	6.0	31.4	COOK INLET WATER ST CB8
GW653	59	13.70	153	40.10	05/78	1.00	4.4	31.4	COOK INLET WATER ST CB1
GW654	59	16.60	153	20.30	05/78	1.00			COOK INLET WATER ST CB2
GW655	59	19.90	153	58.30	05/78	1.00			COOK INLET WATER ST CB3
GW656	59	23.30	152	38.70	05/78	1.00			COOK INLET WATER ST CB4
GW657	59	25.60	152	19.40	05/78	1.00			COOK INLET WATER ST CB5
GW658	59	29.90	152	0.60	05/78	1.00			COOK INLET WATER ST CB6
GW659	59	35.40	151	45.90	05/78	1.00	5.6	31.8	COOK INLET WATER ST CB7 0330 HRS
GW660	59	35.40	151	45.90	05/78	1.00	5.3	31.8	COOK INLET WATER ST CB7 0930 HRS
GW661	59	35.40	151	45.90	05/78	1.00	5.8	31.8	COOK INLET WATER ST CB7 1530 HRS
GW662	59	27.60	151	43.20	05/78	1.00	5.0	29.5	COOK INLET WATER ST AB BEACH
GW663	59	35.40	151	45.90	05/78	1.00	5.3	31.8	COOK INLET WATER ST CB7 2230

GW664	59	35.40	151	45.90	05/78	1.00	5.8	31.8	COOK INLET WATER ST CB7 0331
GW665	59	35.40	151	45.90	05/78	1.00	5.4	31.7	COOK INLET WATER ST CB7 1015
GW666	59	35.40	151	45.90	05/78	1.00	5.9	31.8	COOK INLET WATER ST CB7 1600
GW667	59	35.40	151	45.90	05/78	1.00	5.4	31.8	COOK INLET WATER ST CB7 2200
GW668	59	35.40	151	45.90	05/78	1.00	5.5	31.8	COOK INLET WATER ST CB7 0400
GW669	59	36.50	151	25.50	05/78	1.00	6.0	29.0	COOK INLET WATER ST K BEACH
GW670	60	31.50	151	30.90	05/78	1.00	4.1	29.5	COOK INLET WATER ST CB10 2100 HRS
GW671	60	31.50	151	30.90	05/78	1.00			COOK INLET WATER ST CB10 0230 HRS
GW672	60	31.50	151	30.90	05/78	1.00	4.2	29.6	COOK INLET WATER ST CB10 0830 HRS
GW673	60	31.50	151	30.90	05/78	1.00	4.4	29.3	COOK INLET WATER ST CB10 1515 HRS
GW674	60	31.50	151	30.90	05/78	1.00	4.2	29.5	COOK INLET WATER ST CB10 2101 HRS
GW675	60	28.20	152	12.20	05/78	1.00	4.7	30.7	COOK INLET WATER ST CB9 2100 HRS
GW676	60	28.20	152	12.20	05/78	1.00	4.7	30.4	COOK INLET WATER ST CB9 0320 HRS
GW677	60	28.20	152	12.20	05/78	1.00	4.9	30.5	COOK INLET WATER ST CB9 0930 HRS
GW678	60	28.20	152	12.20	05/78	1.00		30.5	COOK INLET WATER ST CB9 1530
GW679	60	28.20	152	12.20	05/78	1.00		30.6	COOK INLET WATER ST CB9 2100
GW680	60	28.20	152	12.20	05/78	1.00		30.2	COOK INLET WATER ST CB9 0300 HRS
GW681	60	28.20	152	12.20	05/78	1.00		30.5	COOK INLET WATER ST CB9 0900 HRS
GW682	60	28.20	152	12.20	05/78	1.00		30.4	COOK INLET WATER ST CB9 1500 HRS
GW683	60	28.20	152	12.20	05/78	1.00		30.5	COOK INLET WATER ST CB9 2100 HRS
BB601	71	22.00	156	21.00	08/19/78	3.00			TIME1500 STA3
BB602	71	22.00	156	21.00	08/19/78	3.00			TIME1500 STA3
BB603	71	22.00	156	21.00	08/19/78	3.00			TIME1500 STA3
BB604	71	22.00	156	21.00	08/19/78	3.00			TIME1500 STA3
BB605	71	22.00	156	21.00	08/19/78	3.00			TIME1500 STA3
BB606	71	22.00	156	21.00	08/19/78	3.00			TIME1500 STA3
BB607	71	22.00	156	21.00	08/19/78	3.00			TIME1500 STA3
BB608	71	22.00	156	21.00	08/19/78	3.00			TIME1500 STA3
BB609	70	36.00	147	38.70	09/78	21.00	-0.7	21.3	BEAUFORT SEA SEDIMENT ST 609
BB610	70	29.00	147	23.00	09/78	25.00	-0.3	21.1	BEAUFORT SEA SEDIMENT ST 610
BB611					09/78	5.00	-2.5	25.5	BEAUFORT SEA SEDIMENT ST 611
BB612	70	22.00	148	8.00	09/78	5.00	-2.5	26.2	BEAUFORT SEA SEDIMENT ST 612
BB613	70	21.90	146	51.70	09/78	22.00	-0.1	22.5	BEAUFORT SEA SEDIMENT ST 613
BB614	70	10.00	145	56.00	09/78	3.00	-2.5	26.4	BEAUFORT SEA SEDIMENT ST 614
BB615	70	13.00	147	17.00	09/78	1.00	4.0	25.5	BEAUFORT SEA SEDIMENT ST 615
BB616	70	14.50	145	51.50	09/78	23.00	0.4	23.6	BEAUFORT SEA SEDIMENT ST 616
BB617	70	10.50	145	55.00	09/78	1.00	1.5	26.3	BEAUFORT SEA SEDIMENT ST 617
BB618	70	5.00	145	29.00	09/78	2.00	1.5	24.7	BEAUFORT SEA SEDIMENT ST 618
BB619	69	59.00	144	54.00	09/78	2.50	3.5	27.0	BEAUFORT SEA SEDIMENT ST 619
BB620	70	13.00	143	20.00	09/78	25.00	2.6	26.3	BEAUFORT SEA SEDIMENT ST 620
BB621	70	9.00	143	21.00	09/78	3.00	2.0	29.3	BEAUFORT SEA SEDIMENT ST 621
BB622	69	59.00	142	16.00	09/78	18.00	4.1	28.5	BEAUFORT SEA SEDIMENT ST 622
BB623	69	56.00	142	19.00	09/78	5.00	2.0	28.1	BEAUFORT SEA SEDIMENT ST 623
BB624	69	57.00	142	20.00	09/78	1.00	2.0	27.5	BEAUFORT SEA SEDIMENT ST 624
BB626	69	47.00	141	26.00	09/78	21.00	3.4	29.0	BEAUFORT SEA SEDIMENT ST 626
BB627	69	41.00	141	16.00	09/78	3.00	6.0	26.0	BEAUFORT SEA SEDIMENT ST 627
BB628	69	49.00	141	51.00	09/78	5.00	5.0		BEAUFORT SEA SEDIMENT ST 628
BB629	70	8.00	142	49.00	09/78	19.00	3.0	28.1	BEAUFORT SEA SEDIMENT ST 629
BB630	70	26.00	143	42.00	09/78	54.00	4.4	25.6	BEAUFORT SEA SEDIMENT ST 630
BB631	70	28.00	143	42.00	09/78	60.00	4.2	26.3	BEAUFORT SEA SEDIMENT ST 631
BB632	70	13.00	143	54.00	09/78	30.00	2.9	27.3	BEAUFORT SEA SEDIMENT ST 632
BB633	70	9.00	144	47.50	09/78	20.00	2.3	25.1	BEAUFORT SEA SEDIMENT ST 633
BB634	70	19.30	146	30.30	09/78	22.00	-1.0	26.1	BEAUFORT SEA SEDIMENT ST 634
BB635	71	1.40	147	54.80	09/78	50.00	-0.2	15.9	BEAUFORT SEA SEDIMENT ST 635
BB636	70	46.00	148	34.00	09/78	24.00	0.1	10.3	BEAUFORT SEA SEDIMENT ST 636

BB637	70	35.80	148	4.00	09/78	22.00	-0.5	16.9	BEAUFORT SEA SEDIMENT ST 637
BB638	70	26.00	148	24.00	09/78				BEAUFORT SEA SEDIMENT ST 638
BB639	70	20.50	148	19.00	09/78				BEAUFORT SEA SEDIMENT ST 639
BB640	70	47.00	149	36.50	09/78	24.00	-0.5	10.5	BEAUFORT SEA SEDIMENT ST 640
BB641	71	14.30	149	33.50	09/78	67.00	1.4	27.3	BEAUFORT SEA SEDIMENT ST 641
BB642					09/78	27.00	-0.6	25.7	BEAUFORT SEA SEDIMENT ST 642
BB643	70	31.50	149	34.00	09/78	1.50	2.0		BEAUFORT SEA SEDIMENT ST 643
BB644	70	33.00	150	0.00	09/78	3.50	3.0		BEAUFORT SEA SEDIMENT ST 644
BB645	70	30.00	150	14.00	09/78	2.00	4.0		BEAUFORT SEA SEDIMENT ST 645
BB646	71	1.00	150	25.00	09/78	25.00	-1.0	26.6	BEAUFORT SEA SEDIMENT ST 646
BI009					09/78	1.00			BEAUFORT SEA WATER ST
BW601	71	22.00	156	21.00	08/19/78	3.00	3.0	25.6	TIME1500 STA3
BW604	71	22.00	156	21.00	08/19/78	3.00	2.0	26.0	TIME1500 STA3
BW606	71	22.00	156	21.00	08/19/78	3.00	2.0	25.0	TIME1500 STA3
BW608	71	22.00	156	21.00	08/19/78	3.00	1.0		TIME1500 STA3
BW609	70	36.00	147	38.70	09/78	1.00	-0.7	21.3	BEAUFORT SEA WATER ST 609
BW610	70	29.00	147	23.00	09/78	1.00	-0.3	21.1	BEAUFORT SEA WATER ST 610
BW611					09/78	1.00	-2.5	25.5	BEAUFORT SEA WATER ST 611
BW612	70	22.00	148	8.00	09/78	1.00	-2.5	26.2	BEAUFORT SEA WATER ST 612
BW613	70	21.90	146	51.70	09/78	1.00	-0.1	22.5	BEAUFORT SEA WATER ST 613
BW614	70	10.00	145	56.00	09/78	1.00	-2.5	26.4	BEAUFORT SEA WATER ST 614
BW615	70	13.00	147	17.00	09/78	1.00	4.0	25.5	BEAUFORT SEA WATER ST 615
BW616	70	14.50	145	51.50	09/78	1.00	0.4	23.6	BEAUFORT SEA WATER ST 616
BW617	70	10.50	145	55.00	09/78	1.00	1.5	26.3	BEAUFORT SEA WATER ST 617
BW618	70	5.00	145	29.00	09/78	1.00	1.5	24.7	BEAUFORT SEA WATER ST 618
BW619	69	59.00	144	54.00	09/78	1.00	3.5	27.0	BEAUFORT SEA WATER ST 619
BW620	70	13.00	143	20.00	09/78	1.00	2.6	26.3	BEAUFORT SEA WATER ST 620
BW621	70	9.00	143	21.00	09/78	1.00	2.0	29.3	BEAUFORT SEA WATER ST 621
BW622	69	59.00	142	16.00	09/78	1.00	4.1	28.5	BEAUFORT SEA WATER ST 622
BW623	69	56.00	142	19.00	09/78	1.00	2.0	28.1	BEAUFORT SEA WATER ST 623
BW624	69	57.00	142	20.00	09/78	1.00	2.0	27.5	BEAUFORT SEA WATER ST 624
BW625	71	8.00	142	3.00	09/03/78	2000.00	2.0	24.1	TIME1945 STA625
BW626	69	47.00	141	26.00	09/78	1.00	3.4	29.0	BEAUFORT SEA WATER ST 626
BW627	69	41.00	141	16.00	09/78	1.00	6.0	26.0	BEAUFORT SEA WATER ST 627
BW628	69	49.00	141	51.00	09/78	1.00	5.0		BEAUFORT SEA WATER ST 628
BW629	70	8.00	142	49.00	09/78	1.00	3.0	28.1	BEAUFORT SEA WATER ST 629
BW630	70	26.00	143	42.00	09/78	1.00	4.4	25.6	BEAUFORT SEA WATER ST 630
BW631	70	28.00	143	42.00	09/78	1.00	4.2	26.3	BEAUFORT SEA WATER ST 631
BW632	70	15.00	143	48.00	09/78	1.00	2.9	27.3	BEAUFORT SEA WATER ST 632
BW633	70	9.00	144	47.50	09/78	1.00	2.3	25.1	BEAUFORT SEA WATER ST 633
BW634	70	19.30	146	30.30	09/78	1.00	-1.0	26.1	BEAUFORT SEA WATER ST 634
BW635	71	1.40	147	54.80	09/78	1.00	-0.2	15.9	BEAUFORT SEA WATER ST 635
BW636	70	46.00	148	34.00	09/78	1.00	0.1	10.3	BEAUFORT SEA WATER ST 636
BW637	70	35.80	148	4.00	09/78	1.00	-0.5	16.9	BEAUFORT SEA WATER ST 637
BW638	70	26.00	148	24.00	09/78				BEAUFORT SEA WATER ST 638
BW639	70	20.50	148	19.00	09/78				BEAUFORT SEA WATER ST 639
BW640	70	47.00	149	36.50	09/78	1.00	-0.5	10.5	BEAUFORT SEA WATER ST 640
BW641	71	14.30	149	33.50	09/78	1.00	1.4	27.3	BEAUFORT SEA WATER ST 641
BW642					09/78	1.00	-0.6	25.7	BEAUFORT SEA WATER ST 642
BW643	70	31.50	149	34.00	09/78	1.00	2.0		BEAUFORT SEA WATER ST 643
BW644	70	33.00	150	0.00	09/78	1.00	3.0		BEAUFORT SEA WATER ST 644
BW645	70	30.00	150	14.00	09/78	1.00	4.0		BEAUFORT SEA WATER ST 645
BW646	71	1.00	150	25.00	09/78	1.00	-1.0	26.6	BEAUFORT SEA WATER ST 646
EB101	64	16.70	165	56.30	04/79	22.00	-1.7	31.9	BERING SEA STA 1 SEDIMENT
EB102	63	45.50	165	59.10	04/79	26.00	-1.5	31.7	BERING SEA STA 2 SEDIMENT

EB103	63	32.90	166	53.40	04/79	31.00	-1.6	31.6	BERING SEA STA 3 SEDIMENT
EB104	63	55.70	167	36.50	04/79	34.00	-1.7	31.9	BERING SEA STA 4 SEDIMENT
EB105	64	30.40	166	23.80	04/79	21.00	-1.7	31.5	BERING SEA STA 5 SEDIMENT
EB106	66	36.90	168	24.90	04/79	36.00	-1.7	31.7	BERING SEA STA 6 SEDIMENT
EB107	66	26.30	168	26.50	04/79	52.00			BERING SEA STA 7 SEDIMENT
EB108	65	45.20	168	34.40	04/79	58.00			BERING SEA STA 8 SEDIMENT
EB109	65	37.00	168	37.00	04/79	51.00	-1.7	31.7	BERING SEA STA 9 SEDIMENT
EB110	65	30.50	168	6.20	04/79	45.00	-1.3	31.5	BERING SEA STA 10SEDIMENT
EB111	65	2.30	168	15.90	04/79		-1.4	32.4	BERING SEA STA 11SEDIMENT
EB112	64	31.00	167	41.80	04/79	35.00	-1.2	31.6	BERING SEA STA 12SEDIMENT
EB113	64	38.80	168	26.70	04/79	43.00	-1.6	32.2	BERING SEA STA 13 SEDIMENT
EB114	64	12.80	168	57.40	04/79	36.00	-1.8	32.5	BERING SEA STA 14 SEDIMENT
EB115	63	51.10	170	28.10	04/79	33.00	-1.6	32.0	BERING SEA STA 15 SEDIMENT
EB116	64	1.10	171	24.40	04/79	32.00	-1.7	32.0	BERING SEA STA 16 SEDIMENT
EB117	63	44.20	169	12.10	04/79	35.00	-1.7	32.4	BERING SEA STA 17 SEDIMENT
EB118	63	18.20	168	21.50	04/79	46.00	-1.3	31.5	BERING SEA STA 18 SEDIMENT
EB119	57	6.20	170	0.20	04/79	54.00	-2.7	35.0	BERING SEA STA 19 SEDIMENT
EB120	56	26.40	169	23.80	04/79	119.00	-3.5	32.4	BERING SEA STA 20 SEDIMENT
EB124	54	56.70	164	37.00	04/79	60.00	-4.4	32.4	BERING SEA STA 24 SEDIMENT
EB125					04/79				BERING SEA STA 25 SEDIMENT
EB126	54	12.90	161	53.30	04/79	76.00	-4.0	32.3	BERING SEA STA 26 SEDIMENT
EB127	56	21.60	155	32.70	04/79		-4.7	32.3	BERING SEA STA 27 SEDIMENT
EB128	59	8.60	152	53.60	04/79	151.00	-4.7	32.1	BERING SEA STA 28 SEDIMENT
EB129					04/79	0.50			BERING SEA STA P BEACH SEDIMENT
EW101	64	17.20	165	58.60	04/79	1.00	-1.7	31.8	BERING SEA STA 1 WATER
EW102	63	45.60	165	58.60	04/79	1.00	-1.5	31.7	BERING SEA STA 2 WATER
EW103	63	32.70	166	53.00	04/79	1.00	-1.6	31.6	BERING SEA STA 3 WATER
EW104	63	56.00	167	35.80	04/79	1.00	-1.6	31.9	BERING SEA STA 4 WATER
EW105	64	30.00	166	23.20	04/79	1.00	-1.6	31.2	BERING SEA STA 5 WATER
EW106	66	35.10	168	26.00	04/79	1.00	-1.3	31.6	BERING SEA STA 6 WATER
EW107					04/79				BERING SEA STA 7 WATER
EW108	65	44.10	168	34.20	04/79	1.00			BERING SEA STA 8 WATER
EW109	65	35.60	168	36.00	04/79	5.00	-1.7	31.7	BERING SEA STA 9 WATER
EW110	65	29.60	168	6.20	04/79	1.00	-1.3	31.4	BERING SEA STA 10 WATER
EW111	65	1.20	168	15.50	04/79	1.00	-1.4	32.3	BERING SEA STA 11 WATER
EW112	64	27.80	167	40.10	04/79	1.00	-1.1	31.6	BERING SEA STA 12 WATER
EW113	64	36.60	168	25.20	04/79	1.00	-1.5	32.2	BERING SEA STA 13 WATER
EW114	64	12.20	168	56.20	04/79	1.00	-1.7	32.4	BERING SEA STA 14 WATER
EW115	63	50.90	170	25.90	04/79	1.00	-1.5	32.0	BERING SEA STA 15 WATER
EW116	64	0.50	171	25.50	04/79	1.00	-1.5	32.0	BERING SEA STA 16 WATER
EW117	63	44.80	169	12.20	04/79	1.00	-1.6	32.2	BERING SEA STA 17 WATER
EW118	63	17.90	168	21.10	04/79	1.00	-1.1	31.4	BERING SEA STA 18 WATER
EW119	57	7.10	170	0.60	04/79	1.50	-2.7	32.6	BERING SEA STA 19 WATER
EW120	56	27.60	169	24.70	04/79	1.00	-3.9	32.5	BERING SEA STA 20 WATER
EW121	55	36.40	168	51.20	04/79	1.00			BERING SEA STA 21 WATER
EW122	55	15.50	167	12.40	04/79	1.00			BERING SEA STA 22 WATER
EW123	54	34.20	165	58.90	04/79	1.00	-4.5	32.8	BERING SEA STA 23 WATER
EW124	55	3.90	164	35.20	04/79	1.00	-4.8	32.2	BERING SEA STA 24 WATER
EW125	54	10.50	163	47.70	04/79	1.00	-4.7	31.7	BERING SEA STA 25 WATER
EW126	54	14.70	161	52.50	04/79	1.00	-4.6	31.8	BERING SEA STA 26 WATER
EW127	56	21.50	155	30.70	04/79	1.00	-5.0	32.2	BERING SEA STA 27 WATER
EW128	59	7.00	152	54.50	04/79	1.00	-5.0	32.1	BERING SEA STA 28 WATER
EW129					04/79	0.50			BERING SEA STA P BEACH WATER
EZ125	54	9.10	163	47.70	04/79	80.00	-4.0	32.3	BERING SEA STA 25 BOTTOM WATER
EZ126	54	13.10	161	53.30	04/79	75.00	-4.0	32.3	BERING SEA STA 26 BOTTOM WATER

AA206					08/80				NASTE STA PM7A SEDIMENT
AA228	57	11.40	160	0.30	08/80	60.00	7.6	31.6	NASTE STA NA17 SEDIMENT
AA301	55	8.00	163	30.60	08/80	0.00	9.4	31.5	NASTE STA NA65A WATER
AA302	54	58.20	164	27.60	08/80	0.00	8.1	31.8	NASTE STA NA72 WATER
AA303	54	58.20	164	27.60	08/80	32.00	7.5	32.1	NASTE STA NA72 MID WATER
AA304	55	19.80	164	54.00	08/80	0.00	8.0	32.1	NASTE STA NA67 WATER
AA305	55	19.80	164	54.00	08/80	98.00	5.6	32.9	NASTE STA NA67 MID WATER
AA306	54	46.20	166	34.80	08/80	0.00	7.4	32.6	NASTE STA UP 8 WATER
AA307	54	37.20	166	5.40	08/80	0.00	7.8	32.7	NASTE STA UP 9 WATER
AA308	53	52.20	164	25.80	08/80	0.00	10.1	31.9	NASTE STA UP 19 WATER
AA309	54	3.00	164	28.80	08/80	0.00	9.6	31.8	NASTE STA UP 18 WATER
AA310	54	3.00	164	28.80	08/80	99.00	5.6	32.4	NASTE STA UP18 SEDIMENT
AA311	54	11.40	164	30.00	08/80	0.00	9.4	31.7	NASTE STA UP17 WATER
AB203	55	50.50	160	54.80	08/80				NASTE STA PM 6A SEDIMENT
AB211	58	1.50	157	52.60	08/80	24.00	11.8	28.7	NASTE STA NA 4A SEDIMENT
AB216	57	51.20	158	54.70	08/80	44.00	10.0	30.1	NASTE STA NA 5A SEDIMENT
AB222	57	6.80	158	47.20	08/80	27.00	10.4	31.0	NASTE STA NA16 SEDIMENT
AB238	56	48.90	160	28.60	08/80	65.00	7.3	31.6	NASTE STA NA23 SEDIMENT
AB247	56	2.00	160	44.10	08/80	19.00	10.6	31.1	NASTE STA NA40 SEDIMENT
AB250	56	42.30	161	28.10	08/80	88.00	5.7	31.7	NASTE STA NA34B SEDIMENT
AB253	56	27.80	161	43.30	08/80	96.00	5.9	31.7	NASTE STA NA41 SEDIMENT
AB262	56	9.90	162	24.20	08/80	73.00	5.8	31.8	NASTE STA NA47 SEDIMENT
AB270	55	35.00	165	12.80	08/80	15.00	4.8	32.7	NASTE STA SG 7 SEDIMENT
AB273	56	4.10	166	39.40	08/80	30.00	3.9	32.7	NASTE STA SG 5 SEDIMENT
AB276	56	55.00	166	44.90	08/80	77.00	3.2	32.2	NASTE STA SG 4 SEDIMENT
AB279	57	6.90	168	6.40	08/80	79.00	3.4	32.2	NASTE STA SG 2 SEDIMENT
AB285	55	57.00	168	11.50	03/80	40.00	4.1	33.1	NASTE STA UP 3 SEDIMENT
AB288	55	19.60	167	6.80	08/80	42.00	3.8	33.0	NASTE STA PL 4 SEDIMENT
AB291	55	54.90	166	6.70	08/80	30.00	4.3	32.4	NASTE STA PL 8 SEDIMENT
AB294	56	48.60	164	36.90	08/80	73.00	3.2	31.9	NASTE STA PL14 SEDIMENT
AW202	55	52.10	160	52.30	08/80	0.00			NASTE STA PM 6 WATER
AW206	55	55.00	160	29.70	08/80	0.00			NASTE STA PM 7A BOTTOM WATER
AW211	58	1.50	157	52.60	08/80	0.00	11.8	26.9	NASTE STA NA 4A WATER
AW213	58	0.30	158	32.70	08/80	0.00	11.8	26.4	NASTE STA NA 1A WATER
AW214	58	0.30		32.70	08/80	17.00	11.1	28.7	NASTE STA NA 1A BOTTOM WATER
AW215	58	0.60	158	32.40	08/80	27.00	11.0	28.9	NASTE STA NA 1A MID WATER
AW216	57	51.20	158	54.70	08/80	0.00	11.1	29.1	NASTE STA NA 5A WATER
AW217	57	51.00	158	54.60	08/80	34.00	10.0	30.1	NASTE STA NA 5A MID WATER
AW219	57	32.10	158	11.40	08/80	0.00	10.9	30.7	NASTE STA NA10 WATER
AW220	57	32.40	158	11.40	08/80	18.00	10.9	30.7	NASTE STA NA10 MID WATER
AW221	57	32.10	158	11.40	08/80	28.00	10.9	30.7	NASTE STA NA10 BOTTOM WATER
AW222	57	6.80	158	47.20	08/80	0.00	10.4	30.9	NASTE STA NA16 WATER
AW223	57	6.80	158	47.20	08/80	17.00	10.4	30.9	NASTE STA NA16 MID WATER
AW225	57	29.90	159	24.20	08/80	0.00	10.0	31.2	NASTE STA NA11 WATER
AW226	57	30.00	159	24.00	08/80	43.00	9.2	31.2	NASTE STA NA11 MID WATER
AW227	57	29.90	159	24.20	08/80	53.00	9.2	31.3	NASTE STA NA11 BOTTOM WATER
AW228	57	11.40	160	0.30	08/80	0.00	8.4	31.5	NASTE STA NA17 WATER
AW229	57	11.40	160	0.30	08/80	40.00	7.7	31.6	NASTE STA NA17 MID WATER
AW232	56	43.20	159	28.50	08/80	0.00	10.6	30.8	NASTE STA NA22 WATER
AW233	56	43.20	159	28.50	08/80	15.00	10.5	30.1	NASTE STA NA22 MID WATER
AW234	56	43.20	159	28.50	08/80	25.00	10.5	31.1	NASTE STA NA22 BOTTOM WATER
AW235	56	29.00	160	7.30	08/80	0.00	10.3	31.3	NASTE STA NA27 WATER
AW236					08/80	8.00	10.3	31.3	NASTE STA NA27 MID WATER
AW237	56	29.00	160	7.30	08/80	18.00	10.3	31.3	NASTE STA NA27 BOTTOM WATER
AW238	56	48.90	160	28.60	08/80	0.00	8.3	31.6	NASTE STA NA23 WATER

AW239	56	48.90	160	28.60	08/80	55.00	7.3	31.6	NASTE STA NA23 MID WATER
AW241	56	41.80	160	56.60	08/80	0.00	8.1	31.6	NASTE STA NA28 WATER
AW242	56	41.80	160	56.60	08/80	55.00	7.5	31.6	NASTE STA NA28 MID WATER
AW243	56	41.80	160	56.60	08/80	65.00	7.4	31.6	NASTE STA NA28 BOTTOM WATER
AW244	56	15.30	160	27.70	08/80	0.00	10.3	31.2	NASTE STA NA33 WATER
AW245	56	15.30	160	27.70	08/80	12.00	10.3	31.2	NASTE STA NA33 MID WATER
AW246	56	15.30	160	27.70	08/80	22.00	10.3	31.3	NASTE STA NA33 BOTTOM WATER
AW247	56	2.00	160	44.10	08/80	0.00	10.7	31.1	NASTE STA NA40 WATER
AW248	56	2.00	160	44.10	08/80	9.00	10.6	31.1	NASTE STA NA40 MID WATER
AW250	56	42.30	161	28.10	08/80	0.00	8.3	31.7	NASTE STA NA34B WATER
AW251	56	42.30	161	28.10	08/80	78.00	5.7	31.7	NASTE STA NA34B MID WATER
AW253	56	27.80	161	43.30	08/80	0.00	8.1	31.7	NASTE STA NA41 WATER
AW254	56	27.80	161	43.30	08/80	86.00	5.9	31.7	NASTE STA NA41 MID WATER
AW256	56	2.10	161	16.20	08/80	0.00	11.3	31.1	NASTE STA NA46 WATER
AW257	56	2.10	161	16.20	08/80	18.00	10.5	31.2	NASTE STA NA46 MID WATER
AW258	56	2.10	161	16.20	08/80	28.00	10.4	31.3	NASTE STA NA46 BOTTOM WATER
AW259	55	48.90	162	1.90	08/80	0.00	10.2	31.3	NASTE STA NA52 WATER
AW260	55	48.90	162	1.90	08/80	12.00	10.2	31.3	NASTE STA NA52 MID WATER
AW261	55	48.90	162	1.90	08/80	22.00	10.2	31.3	NASTE STA NA52 BOTTOM WATER
AW262	56	9.90	162	24.20	08/80	0.00	8.3	31.7	NASTE STA NA47 WATER
AW263	56	9.90	162	24.20	08/80	63.00	5.8	31.8	NASTE STA NA47 MID WATER
AW265	55	26.00	162	54.10	08/80	0.00	9.4	31.5	NASTE STA NA58A WATER
AW266	55	26.00	162	54.10	08/80	29.00	8.8	31.7	NASTE STA NA58A MID WATER
AW267	55	26.00	162	54.10	08/80	39.00	8.8	31.7	NASTE STA NA58A BOTTOM WATER
AW270	55	35.00	165	12.80	08/80	0.00	8.2	31.9	NASTE STA SG 7 WATER
AW271	55	35.00	165	12.80	08/80	5.00	4.8	32.7	NASTE STA SG 7 MID WATER
AW273	56	4.10	166	39.40	08/80	0.00	8.4	32.1	NASTE STA SG 5 WATER
AW274	56	4.10	166	39.40	08/80	20.00	3.9	32.7	NASTE STA SG 5 MID WATER
AW276	56	55.00	166	44.90	08/80	0.00	8.2	31.9	NASTE STA SG 4 WATER
AW277	56	55.00	166	44.90	08/80	67.00	3.2	32.2	NASTE STA SG 4 MID WATER
AW279	57	6.90	168	6.40	08/80	0.00	9.1	31.9	NASTE STA SG 2 WATER
AW280	57	6.90	168	6.40	08/80	69.00	3.4	32.2	NASTE STA SG 2 MID WATER
AW282	56	39.70	169	24.50	08/80	0.00	8.1	32.2	NASTE STA SG 1 WATER
AW283	56	39.70	169	24.50	08/80	61.00	4.0	32.4	NASTE STA SG 1 MID WATER
AW284	56	39.70	169	24.50	08/80	71.00	3.8	32.4	NASTE STA SG 1 BOTTOM WATER
AW285	55	57.00	168	11.50	08/80	0.00	8.3	32.8	NASTE STA UP 3 WATER
AW286	55	57.00	168	11.50	08/80	30.00	4.1	33.1	NASTE STA UP 3 MID WATER
AW288	55	19.60	167	6.80	08/80	0.00	8.3	32.3	NASTE STA PL 4 WATER
AW289	55	19.60	167	6.80	08/80	32.00	3.8	33.0	NASTE STA PL 4 MID WATER
AW291	55	54.90	166	6.70	08/80	0.00	8.3	32.1	NASTE STA PL 8 WATER
AW292	55	54.90	166	6.70	08/80	20.00	4.3	32.7	NASTE STA PL 8 MID WATER
AW294	56	48.60	164	36.90	08/80	0.00	7.2	31.8	NASTE STA PL14 WATER
AW295	56	48.60	164	36.90	08/80	63.00	3.2	31.9	NASTE STA PL14 MID WATER

choice of marine agar 2216 as the primary medium for enumeration of viable microorganisms followed testing of several different media that have been recommended for enumeration of marine and estuarine bacteria; marine agar 2216 gave the highest counts of all media tested in these trials.) All materials were cooled to 5°C before plating. For each sample, one set of replicate plates was incubated at 4°C for 3 weeks and another at 20°C for 2 weeks. Colonies were counted with the aid of a stereomicroscope (30x). The mean count from triplicate plates was recorded for each dilution and temperature.

Most Probable Number of Hydrocarbon Utilizers

To determine the Most Probable Number (MPN) of hydrocarbon utilizing microorganisms, dilutions of samples were added to 60 ml stoppered serum vials containing 10 ml of Bushnell Haas broth (magnesium sulfate, 0.2 g; calcium chloride, 0.02 g; monobasic potassium phosphate, 1.0 g; dibasic pottasium phosphate, 1.0 g; ammonium nitrate, 1.0 g; ferric chloride, 0.05 g; distilled water 1 litre, pH 7.0), and crude or refined oil spiked with ^{14}C-radiolabelled hydrocarbon. For these marine studies, 3 percent sodium chloride was added, and the pH was adjusted to 7.5. In some cases 1-10 ml volumes rather than dilutions are used. Poisoned controls were prepared by adding 0.2 ml of concentrated hydrochloric acid to the vials. In the Bering and Beaufort Seas Prudhoe Bay crude oilwas used; in the Gulf of Alaska and Cook Inlet, Cook Inlet crude oil was used. Typically, we used 50 µl of oil per vial, spiked with ^{14}C-*n*-hexadecane (specific activity, 0.4 µCi/ml). A 3-tube MPN procedure was used. After incubation, the solutions were acidified with 0.2 ml of concentrated hydrochloric acid and the radiolabelled carbon dioxide ($^{14}CO_2$) produced was recovered. Incubation for 3 weeks at 5°C was used.

$^{14}CO_2$ was recovered by purging the vials with air and trapping the $^{14}CO_2$ in 10 ml of Oxifluor - CO_2 (New England Nuclear). Counting was

done with a Beckman LS-100 liquid scintillation counter. Counts of greater than or equal to two times the control were considered positive; counts of less than two times the control were considered negative. The MPN of hydrocarbon-degrading microorganisms was determined from the appropriate MPN tables and recorded as the number per ml for water samples or the number per gram of dry weight for sediment samples. The use of two times background for establishing positive tubes was chosen to eliminate false positive tubes, which could result from hdyrocarbon carry-over in the air stream or from impurities in the labelling material. The two times background cutoff was chosen to insure statistical accuracy. In general, the background counts in our studies were less than 100 counts per minute (CPM). With the activities and concentrations of ^{14}C-hydrocarbons that we used, this means that positive tubes represent a conversion of greater than 0.2 percent of substrate to $^{14}CO_2$. This conversion is twice the maximal theoretical impurity of 0.1 percent that chemical reanalysis of the ^{14}C-hydrocarbon assures. Positive tubes almost always yielded counts many times higher than our cutoff limit, but as with all MPN techniques, results are based on the proportions of positive and negative tubes rather than on actual activity levels.

TAXONOMY OF INDIGENOUS MICROBIAL POPULATIONS

Bacterial isolation

Heterotrophic bacteria

Colonies from countable marine agar plates used for enumeration of viable microorganisms were numbered sequentially. Using random number tables, 30-60 bacterial colonies from each sample generally were selected for isolation. After subculturing twice on marine agar to ensure purity and viability, about 25 strains from each sample were randomly selected for numerical taxonomic testing. These strains were maintained on marine agar slants at 4°C and

subcultured monthly. Some strains lost viability during testing (less than 8% from any source) and were discarded. Eleven reference strains were included in this study: Vibrio fisheri ATCC 15381, Psuedomonas coenobios ATCC 14402, Flavobacterium halmephilum ATCC 19717, Flavobacterium marinotypicum ATCC 19260, Alcaligenes pacificus ATCC 27122, Pseudomonas doudoroffi ATCC 27123, Pseudomonas marina ATCC 27129, Pseudomonas nautica ATCC 27132, Arthrobacter citreus ATCC 11624, Beneckea campbelli ATCC 25920 and Vibrio alginolyticus ATCC 17749. In all over 10,000 strains from various Alaskan OCS regions were isolated and examined in these studies.

Low-nutrient bacteria

In addition to taxonomic studies of heterotrophic bacteria capable of growth on marine agar (copiotrophs), isolates were selected from Cook Inlet samples grown on low nutrient media (oligotrophs). Oligotrophs have been postulated to play an important role in low nutrient aquatic ecosystems. For studies comparing oligotrophic with copiotrophic bacteria, samples were collected in Cook Inlet Alaska, during November, 1977 at four sampling locations. Replicates were plated onto marine agar 2216 (Difco) [medium MA], marine agar 2216 + 1% (v/v) Cook Inlet crude oil [medium MO], Bushnell Haas Agar (Difco) [medium BA] and Bushnell Haas Agar + 1% (v/v) Prudhoe Bay crude oil [medium BO]. Marine agar contains 0.5% peptone and 0.01% yeast extract. Bushnell Haas agar contains mineral nutrients but no added organic carbon; some bacteria can grow, however, on the trace organic contaminants in the medium. A similar method of isolating low nutrient bacteria has been used by Moaledi (1978). Oil was added to the media using the procedure of Atlas and Bartha (1973) in which a solidified medium is prepared containing a stable oil emulsion. All materials in our study were cooled to 5°C before plating to

maintain the viability of psycrophilic populations. Platings were performed in triplicate and incubation was at 5°C for 4 weeks.

Using random number tables, approximately 10-50 bacterial colonies were selected for isolation from each of the media for every sample, i.e., 65-135 isolates per sample. These isolates were representative of the major populations of bacteria capable of growth on the various media since they were obtained from countable plates of greatest dilution. The selected organisms were subcultured to ensure purity and viability. A total of 581 isolates were included in the study [163 from medium MA; 138 from medium MO; 120 from medium BA; and 160 from medium BO].

Characterization of Isolates

Approximately 300 phenotypic characteristics were determined for each strain. Unless otherwise indicated, tests on the 4°C and 20°C isolates were incubated at 4°C and 20°C, respectively.

Morphology

Cultures (1 to 4 d depending on growth rate) from marine agar slants overlaid with 1 ml Rila marine salts solution were examined for: cell shape, size and motility (wet mounts); spores, refractile granules of poly-β-hydroxybutyrate (Stanier et al., 1966) and capsules (India ink stain) (phase contrast microscopy); Gram reaction (Hucker modification), acid fastness (Ziehl-Neelsen method) and fat droplets (Burdon method) (Society of American Bacteriologists, 1957). Cultures (10 d) grown on marine agar were examined for colony morphology and size, and for production of diffusible and non-diffusible pigments. Fluorescent pigment formation on marine agar containing 0.15% (w/v) glycerol was assessed daily with ultraviolet light (λ260 nm). Following 10 to 15 min adaptation of the observer to the dark, bioluminescence was tested in the dark daily for 10 d [cultures grown on: Bacto-tryptone, 0.5%; Bacto-yeast

extract, 0.3%; Na_2HPO_4, 0.35%; NH_4NO_3, 0.15%; glycerol, 3.0%; agar, 1.5% (all w/v); in 3/4-strength Rila marine salts solution, pH 7.6].

Physiological and biochemical tests

Tests were read after 14 d incubation unless stated otherwise. Growth on replicate marine agar plates was tested at 5, 10, 15, 20, 25, 37 and 43°C, and at initial pH 3, 4, 5, 6, 7, 8, 9 and 10 (adjusted with HCl or NaOH). Salt tolerance and requirement were tested in the following medium without NaCl and with 0.5, 3, 5, 7.5, 10 and 15% (w/v) NaCl added: Bacto-tryptone, 0.5%; Bacto-yeast extract, 0.1%; $FeCl_3 \cdot 6H_2O$, 0.01%; NH_4NO_3, 0.00016%; Na_2HPO_4, 0.0008%; Bacto-agar, 1.5% (all w/v); pH 8.0. Oxygen relations were determined from stab cultures in marine agar butts.

Distribution of growth, indole production (Kovacs method; Society of American Bacteriologists, 1957) and ammonia production (Nessler's reagent) were determined from 10 d cultures in a medium containing: Bacto-tryptone, 0.3%; Bacto-yeast extract, 0.05%; Tris, 0.6%; KH_2PO_4, 0.01%; $FeCl_3 \cdot 6H_2O$, 0.0005% (all w/v); thiamin, sodium pantothenate, riboflavin, nicotinic acid, choline, pyridoxamine and cyanocobalamin, all 1 μg l^{-1}; folic acid, sodium p-aminobenzoate and biotin, all 0.05 μg l^{-1}.

Cultures (10 d) on marine agar were tested for catalase (with 3% H_2O_2) and cytochrome oxidase production (Gaby and Hadley method, allowing 1 min for the blue colour to develop; Skerman, 1969). Methyl red and Vogest-Proskauer tests (Society of American Bacteriologists, 1957) were done in MR-VP broth (Difco) prepared with full-strength Rila marine salts solution. Alkaline phosphatase was detected (Barber and Kuper, 1951) in cultures (10 d) grown in a medium containing: Bacto-tryptone, 0.5%; Bacto-yeast extract, 0.1%; NH_4NO_3, 0.00016%; $FeCl_3 \cdot 6H_2O$, 0.0005%; phenolphthalein diphosphate, 0.001% (all w/v); in 3/4-strength Rila marine salts solution, pH 7.2.

Arginine, ornithine and lysine decarboxylases were dtected by the Falkow method (Skerman, 1969), modified by replacing distilled water with Rila marine salts solution. These tests measure alkaline end-products and do not distinguish between arginine decarboxylase and arginine dihydrolase.

Nitrate and nitrite reduction were tested in nitrate broth (Difco) with full-strength Rila marine salts solution. Nitrite was detected with naphthylamine-sulphanilic acid reagent and residual nitrate with zinc dust (Skerman, 1969).

Acid production from D-ribose, D-fructose, cellobiose, lactose, sucrose or D-mannitol (all 1%, w/v) was detected in MOF medium (Difco). Oxidation/fermentation tests were done in MOF medium containing 1% (w/v) D-glucose (Leifson, 1963). Gas production from glucose was detected with inverted Durham tubes in the liquid medium used for determining growth distribution, supplemented with 1% (w/v) D-glucose. Substrates were filter-sterilized.

Agar hydrolysis was tested on marine agar; sunken colonies and depressions around colonies were scored as positive. Lipase activity was tested in marine agar containing 0.01% (w/v) $CaCl_2$ and 1% (w/v) Tween 20 or Tween 80 (Sierra, 1957). Starch was tested by flooding plate cultures (7 d) on marine agar containing 0.5% (w/v) potato starch with Lugol's iodine. Gelatin hydrolysis was tested by flooding cultures (7 to 10 d) on marine agar containing 10% (w/v) gelatin with acid $HgCl_2$ (Skerman, 1969). Casein hydrolysis was tested on marine agar overlaid with a double layer of 10% (w/v) skim milk agar. For the last three tests, clear zones around colonies were recorded as positive.

Antibiotic sensitivity tests

Antibiotic sensitivity was tested by spreading bacterial suspensions on marine agar plates and applying BBL antibiotic discs (ampicillin, 2 μg;

colistin, 10 µg; erythromycine, 15 µg; kanamvcin, 30 µg; neomycine, 30 µg; nitrofurantoin, 300 µg; novobiocin, 5 µg; oxytetracycline, 5 µg; penicillin G, 2 units; polymyxin B, 300 units; streptomycin, 2 µg; tetracycline, 5 µg). Zones of inhibition were measured and sensitivity was determined against standard inhibition zones (BBL).

Nutritional tests

Basal medium B used in testing for substrate utilization was prepared as follows. Portion 1: KH_2PO_4, 0.1 g; Tris, 6.0 g; NH_4NO_3, 1.0 g; $FeCl_3 \cdot 6H_2O$, 0.005 g; Rila marine salts solution, 500 ml; pH adjusted with HCl to 8.0. Portion 2: purified agar (Difco), 10 g; distilled water, 500 ml. Portion 3: thiamin, sodium pantothenate, riboflavin, nicotinic acid, choline, pyridoxamine and cyanocobalamin, all 1 µg; folic acid, sodium p-aminobenzoate and biotin, all 0.05 µg; distilled water, 2 ml. Portions 1 and 2 were autoclaved separately. Portion 3 was filter-sterilized. The three portions were mixed at 55°C. Substrates were sterilized by autoclaving or filtration (Stanier et al., 1966), except for hydrocarbons which were sterilized ultrasonically. Substrates were mixed with the basal medium just before pouring to give final concentrations of 0.1% (w/v), except for carbohydrates (0.15%, w/v) and phenol (0.0125%, w/v). A total of 100 substrates was tested. To determine growth factor requirements, two additional basal media were used. Basal medium A was basal medium B without vitamins (portion 3). Basal medium E was basal medium B supplemented with 50 mg Bacto-yeast extract, 50 mg Casamino acids and 10 mg L-tryptophan. Twelve substrates (D-ribose, D-fructose, D-glucose, acetate, fumarate, DL-β-hydroxybutyrate, DL-lactate, pyruvate, 2-ketoglutarate, D-gluconate, glycerol) were used to test the ability of strains to grow on the basal media. Four classes of growth factor requirements were tested: type 1, bacteria able to grow on all basal media (do not require

growth factors); type 2, bacteia able to grow on basal media B and E but not A (require vitamins as growth factors); type 3, bacteria able to grow on basal medium E but not A or B (require complex growth factors such as amino acids); type 4, bacteria unable to grow on any basal medium (require complex unknown growth factors).

Plates were inoculated with a multiple syringe inoculator (Kaneko et al., 1977a). Growth with any substrate was considered positive if within 14 d it exceeded (visually) that on the same basal medium alone.

Data processing and analysis

Data were coded in binary form according to RKC format (Rogosa et al., 1971), punched cards, and verified and proof-read by two people. Errors were also checked by computer with the CREATE program (Krichevsky, 1977). Test reproducibility was checked by periodically retesting selected strains. The estimated total error rate was less than 3%, which would not significantly affect the cluster analyses. The QUERY computer program (Krichevsky, 1977) was used to arrange the data for input to the numerical taxonomy program GTP2 (supplied by R.R. Colwell) or the numerical taxonomy program TAXON. Editing removed strains lacking more than 35% data, and non-differentiating features with more than 99% positive, 99% negative or 90% missing results (MTRXED program; Walczak and Krichevsky, 1977). Similarities were estimated with the Jaccard coefficient (S_J) and cluster analyses were done by single linkage sorting and/or by average linkage sorting (Sokal and Sneath, 1963). Clusters of strains with similarities greater than 75% were designated as taxonomic groupings (Liston et al., 1963). The input data were sorted into the same order as strains in the cluster analysis triangle. The feature frequencies of all characteristics were determined with the feature analysis program FREAK (Walczak et al., 1978). Probabalistic identifications were attempted using the

program IDDNEW and three identification matrices currently being developed at the American Type Culture Collection (Johnson, 1979).

Development of Probabilistic Identification Matrices

Based on the numerical taxonomic analyses of nine data sets, which included data on 4200 strains, groups of four or more strains with a minimal similarity level of 70% using the Jaccard similarity coefficient and single linkage clustering were selected for this study. A total of 1206 strains (representing 108 clusters) were selected. Individual data sets were combined and the data edited to exclude features greater than or equal to 90% positive, 90% negative, or 50% unknown for the entire set, thereby eliminating tests with low discrimininating power. A series of cluster analyses were performed using the Jaccard coefficient (S_J) and unweighted average linkage to assess the integrity of the original clusters. Five overlapping analyses (600 strains each) were performed to include the entire data set. The criterion for inclusion of strain clusters was relaxed to 60% similarity because of the substitution of average linkage for single linkage clustering. Clusters observed to have lost members, such that the number of strains in the new cluster was less than four, were eliminated from the study; original clusters incorporating new members were redefined to include the latter strains.

Of the 1206 strains originally selected from clusters defined in the original studies, 1119 clustered in the same manner when the data from multiple sets were combined and unweighted average linkage was employed. Strains lost from individual clusters, 87 in all, either appeared as outliers linked to members of the same original cluster at levels less than 60% overall similarity, or clustered above the 60% criterion in groups of 3 strains or less. No instances were observed in which an original cluster established using S_J and single linkage clustering algorithm gained member strains of

other clusters initially defined within the same primary study. Based on the results of unweighted average linkage, 1119 strains in 86 clusters were chosen for use in the development of identification matrices.

Character editing and selection

Of the original 320 tests used to characterize bacterial isolates for taxonomic studies, those which were highly reproducible, i.e., ones easy to prepare, to carry out, and to read; those which were most objective; and those that tested for as many different enzymes and biochemical pathways as possible were retained for matrix construction. Features subject to excessive investigator bias and tests requiring extensive time to perform, i.e., those that are unreasonable to include for routine identification, were eliminated from the set of eligible group descriptors for development of probabalistic identification matrices. Additionally, specific descritors of colony pigmentation were reduced to a single test describing whether colonies were pimented or non-pigmented. In this manner 92 tests were eliminated leaving 228 features in the data set, primarily metabolic and physiological features, for potential use as taxa discriminators.

Construction of identification matrices

To develop determinative schema, all 86 clusters were examined for salt requirment, growth at 25°C, and the lack of pigment production (i.e., grey colonies). Six separate sub-matrices were developed to accomplish the probabilitic identification of each of the original 86 clusters. Additionally an inclusive matrix contianing all 86 clusters was developed for comparison. The contents of each sub-matrix were determined by the set of clusters contained in the respective group of the super-matrix and the adequate number of features required to completely separate each cluster pair within the same group. Features were selected based on their discriminatory power using the

CORR program (Walczak and Krichevsky, 1982) to determine the minimal number of features required to completely separate each possible group pair within its respective subset. The CORR program ranks tests in order of decreasing separation value. Correlation and redundancy measures of all appropriate features were calculated for the group combinations contained within each sub-matrix. Features were chosen individually by decreasing rank of group partitioning power such that each group pair combination was separated by at least two tests differing at a minimum of 60% difference in positive feature frequency.

Evaluation technique

The super-matrix, 6 sub-matrices, and the inclusive matrix were incorporated into the identification program IDDNEW (Johnson, 1979). For evaluation of the probability matrices developed in this study, data on the 1119 Alaskan marine strains were used. The threshold identification level set by the IDD Program is 0.999, i.e., for a strain to be identified as a member of a taxon, its identification score must be one thousand times greater than the next most probable taxon. However, for assignment to a sub-matrix, no threshold identification level was set, and the most probable sub-matrix was chosen for each test strain regardless of the probabilities for less likely sub-matrices. The output was examined for each strain such that the number of correct versus incorrect sub-matrix assignments was determined. The final group assignments were determined regardless of whether or not the super-matrix correctly assigned the respective strain to the given sub-matrix. Strains having insufficient data (additional tests suggested when analysed using the super-matrix) were not submitted to sub-matrices as no error rate could be calculated for these strains. The output from the sub-matrices was evaluated for the percent of strains that were properly assigned to the given sub-matrix

which identified with its group of origin. All correct strain-group identifications for which no additional tests were suggested were classed in terms of the actual identification score, i.e., within each matrix the identification scores of correct strain-group identifications were tabulated. In addition to the identification score for evaluation of matrix efficiency, the ratio of the observed likelihood to the best possible likelihood was calculated. This R score value has been described by Wayne, et al. (1980), for the evaluation of probabilistic matrices developed for identification of mycobacteria.

The determination of the efficiency of a matrix for proper identification involved the analysis of the distribution of strains according to the criteria of whether or not they exhibited ID and R scores above or below either or both thresholds of ID = 0.990 and R = 0.01, and ID = 0.970 and R = 0.001. The super-matrix was evaluated in terms of the percent correct sub-matrix assignment of test strains, regardless of identification scores. Sub-matrices were evaluated for the percent identified in the correct group, regardless of ID scores, and for the percent of correct strain-group identifications below or above either or both criteria sets; i.e., ID = 0.90 and R = 0.01, and ID = 0.970 and R = 0.001. Strains having the wrong group identification were examined for identification at these thresholds as well. Positive identification of any strain required that both ID and R scores were both above and equal to the given criteria.

Error rates were calculated for each super-sub-matrix combination (6 in all) and an overall error rate for all matrices as follows:

$$((X + Y + Z)/ Q + R)*100 = \% \text{ ERROR}$$

where

X = total number of strains assigned to the correct

group of origin for which ID and R scores were below threshold levels.

Y = total number of strains assigned to the correct sub-matrix but which identified as being members of the wrong group of origin above identification threshold levels.

Z = total number of strains assigned to the wrong sub-matrix identifying above identification thresholds.

Q = valid number of strains submitted to the correct sub-matrix.

R = valid number of strains submitted to the incorrect sub-matrix.

Microbial populations associated with edible crabs - potential human pathogens

Studies were conducted to determine whether potential human pathogens were associated with edible Alaskan crabs occurring in potential OCS areas. For this purpose Dungeness, Tanner and King crabs were collected near Kodiak Island and in the Southern Bering Sea by the Alaska Department of Fish and Game and by commercial fishermen. Samples of muscle and gill tissue were prepared according to the procedure recommended by the American Public Heath Association (American Public Health Association, 1962). An equal weight by volume mixture of the sample and sterile (1/2 strength) Rila marine salts, pH 8.4 was homogenized in a glass homogenizer for one minute. One-ml of the mixture of each tissue (Hemolymph, muscle and gill) was added to screw capped Falcon tube containing nine-ml Rila marine salts, pH = 8.4. The mixture of each sample with Rila was serially diluted and plated in replicate of three sets of media: marine agar (MA) trypticase soy agar (TSA) TCBS agar, and Salmonella, Shigella

agar (SS). The plates were incubated for 10 days at 5°C. Anaerobic bacteria were isolated by inoculation into thioglycolate broth and also marine agar plates and incubating at room temperature for 7 days using the BBL Gas Pak System. For taxonomic studies 500 isolates, selected at random from muscle and gill tissues, were tested using the API 20E identification system. This system is primarily designed for identification of Gram negative enteric bacteia such as those associated with domestic sewage and those which establisgh human infections via the gastrointestinal system.

In addition to the examination of field samples for possible contamination with bacteria that are human pathogens, a microcosm study was conducted in which crabs were placed into holding tanks and bacteria were added to the water. These microcosm experiments were designed to determine which if any bacterial indicator strains and pathogens survive in seawater and which if any become associated with crab tissues. The microcosm study was conducted using 20 gallon tanks. Dolomite Sand was used as an artificial sediment; the sediment layer in each tank was 8 to 10cm. The tanks were filled with Rila marine salts and kept at 5°C. The tanks were continuously aerated and water was recirculated in each tank through a glasswool and activated charcoal filter. Two crabs were placed into each tank. During the 25 day experiment, oxygen concentration, pH, and temperature were monitored daily. The range of these parameters were: oxygen = 8.5-9.0 PPM, pH = 7.5-8, temp. = 5.5-6°C.

Different species of bacteria (Klebsiella pneumoniae) (ATCC 13883). Pseudomonas fluorescens sp. Vibrio parahaemolyticus sp. Beneckea harvii (ATCC 14126) E. coli (ATCC 25922) Brevibacterium sp. and Bacillus sp., were added to the tanks to yield a final concentration of 200/ml. The tanks were sampled every 48 hours for determination of viable counts of each bacterial type. On

alternate days the tanks were reinoculated with fresh inoculum to preclude artifactual dieoff of the bacteria and/or removal by the filtration system.

Crabs exposed in these experimental tanks were examined using scanning electron microscopy and viable bacteria were isolated and characterized taxonomically. A total of 216 bacteria were selected from enumeration plates for NT examination. Approximately 100 phenotypic characteristics were determined for each isolate. Phenotypic characteristics examined included morphological examination of both cells and colonies, physiological growth ranges including tolerance to temperature, salt, and pH; biochemical tests included determination of a variety of enzymatic activities; and nutritional characteristics included the ability to utilize a variety of biochemically diverse substrates. Data were coded and processed for computer analysis. Data were also subjected to cluster analysis to determine taxonomic groupings (phenotypic clusters) using the Jaccard coefficient (S_j) and unweighted average linkage clustering. The feature frequencies for characteristics were determined for each cluster.

For SEM studies, the tissues (muscle, gill and shell) were prepared and fixed by a double fixation method, glutaraldehyde followed by osmium tetroxide fixation, so that the tissues were exposed to primary fixative (phosphate buffered glutaraldehyde fixative 0.1m, pH - 7.2) for 24 hours. The fixative was changed 4-5 times during this period. Tissues were exposed to the secondary fixative, osmium tetroxide for 1 hour. The tissues were rinsed 4-5 times with distilled water and incubated in 100 ml of distilled water on a rotary shaker overnight, to eliminate excess fixative. The water was then removed from the tissues by gradually increasing the concentration of acetone to 20, 30, 50 and 99.5% and, finally, by placing the samples in a dessicator under an atmosphere of 100% acetone overnight. Specimens in 100% acetone were replaced

with liquid CO_2 (transitional fluid) and then dried by the critical point drying method, using a Polaron Critical Point dryer. Specimens were mounted on aluminum slabs and surface counted with = 60A° gold under the partial pressure of argon gas, using a Polaron Sputter D.C. Coater. The specimens were then viewed using an ISI-40 scanning electron microscope.

DIVERSITY OF MICROBIAL COMMUNITIES

Physiological Tolerance Indices

Indices were developed to describe the capacity of the bacterial community to tolerate (maintain ability to grow) deviations from ambient conditions of temperature, salinity, and pH. Ambient conditions were considered as 5°C, 3% NaCl, and pH 8, which approximate both environmental and isolation conditions. Feature frequencies for the ability to grow at 10, 15, 20, 25, and 37°C; 0, 0.5, 5, 7.5, and 10% NaCl; and pH 5, 6, 7, 9, and 10 were used for calculating physiological tolerance indices. The physiological tolerance index for temperature (P_T) was calculated according to the formula:

$$P_T = \frac{G_{10} + G_{15} + G_{20} + G_{25} + G_{37}}{5}$$

where G_x = the proportion of the populations (represented by the isolates) within the community which are capable of growth at temperature x. According to this calculation, a community composed entirely of true psychrophiles (organisms that cannot grow at 20°C or above, Morita, 1975) would have a P_T of ≤ 0.4. Similarly, a community in which all member populations could grow over the entire range of temperature from 0 to 37°C would have a P_T of 1.

The physiological tolerance index for salinity (P_S) was calculated as:

$$P_S = \frac{G_0 + G_{0.5} + G_5 + G_{7.5} + G_{10}}{5}$$

where G_x = the proportion of the populations within the community capable of growth at NaCl concentration of x percent. A "true marine bacterium" cannot have a $P_S = 1$ since by definition marine bacteria require NaCl and cannot grow at 0% NaCl (ZoBell, 1961, 1963).

The physiological tolerance index for pH (P_H) was calculated as:

$$P_H = \frac{G_5 + G_6 + G_7 + G_9 + G_{10}}{5}$$

where G_x = the proportion of the populations within the community capable of growth at pH value x.

Nutritional Utilization Indices

Indices were calculated to assess the nutritional versatility of bacterial communities. Separate indices were calculated for carbohydrates (N_c), alcohols (N_a), carboxylic acids (N_{ca}), amino acids (N_{aa}), and hydrocarbons (N_h). Each nutritional utilization index was calculated by summing the number of substrates that could be utilized by any member population and dividing by the total number of substrates within that class. A combined nutritional utilization index (N_T) was calculated for all substrates regardless of compositional class. The substrates employed in determining these indices were carbohydrates - arabinose, ribose, xylose, rhamnose, fructose, galactose, glucose, mannose, sorbose, salicin, cellobiose, lactose, maltose, sucrose, trehalose, raffinose; alcohols - 1-butanol, ethanol, 1-propanol, 2-propanol, 1, 2-propanediol, glycerol, arabitol, dulcitol, mannitol, sorbitol, m-inositol, phenol, phenylethanol; carboxylic acids - acetic, butyric, caproic, caprylic, lauric, propionic, valeric, glutaric, malonic, succinic, oleic, fumaric, itaconic, glyceric, β-hydroxybutyric, lactic, tartaric, citric, 2-ketogluconic, pyruvic, α-ketoglutaric, benzoic, m-hydroxybenzoic, p-hydroxybenzoic, o-hydroxybenzoic, ascorbic, galacturonic,

gluconic, stearic; amino acids - alanine, γ-amino butyric, arginine, asparagine, aspartate, cystine, cysteine, glycine, leucine, isoleucine, lysine, methionine, ornithine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, valine; hydrocarbons - n-hexadecane, n-pentadecane, 2-methylnaphthalene, 1-methylnaphthalene, phenyldecane, pristane, pentadecylcyclohexane.

A total of 16 carbohydrates, 13 alcohols, 29 carboxylic acids, 20 amino acids and 7 hydrocarbons (= 85 total substrates) were employed. An $N_x = 1$, for any substrate class x, indicates that all substrates included in that class can be utilized by some member population(s) of the bacterial community. An N_x near 0 indicates a lack of versatility of substrate utilization by the sampled (dominant) bacterial populations of the community. It should be noted that N_x is sensitive to rare populations not selected by the plating and isolation procedures employed in this study.

Taxonomic Diversity

The number of taxonomic groups and the number of individuals within each group, determined by the cluster analyses, were used to calculate the Shannon diversity index, H' (Pielou, 1966; Shannon, 1948; Shannon and Weaver, 1949). The formula $H' = C/N(N \log_{10} N - \Sigma n_i \log_{10} n_i)$ was used, where C = 3.3218, N = total numbers of individuals, and n_i = total numbers of individuals in the ith taxonomic grouping (Lloyd et al., 1968). An H' value near 0 represents a community with low diversity; H' values near 4 represent rather high diversity. Equitability (J') was calculated according to the formula $J' = H'/H_{max}$ where H_{max} = the maximal value of H' for a given sample size (Pielou, 1966, 1975); it assumes that each cluster can be single membered representing taxonomically distinct populations, i.e., $H_{max} = C$

$\log_{10}$ N. A J' value of 1 shows an even distribution; when J' is near 0 there is an uneven distribution of individuals within the taxa of the community.

Effects of oil on bacterial communities associated with Arctic amphipods

Populations of the amphipod Boeckosimus (= Onisimus) affinis were collected in Elson Lagoon, 50 m south of Plover Point near Pt. Barrow, Alaska. Amphipods were captured in wire mesh traps, baited with fish, which were suspended in the water column, beneath the ice. Animals used in this study were between 11 and 16 mm in length.

Scanning electron microcsopy was employed to view the locations of microbial populations associated with Arctic amphipods. Amphipod specimens were placed in buffered formalin at the time of collection. Individual specimens were transferred to 2% glutaraldehyde in 0.1 M phosphate buffer for 12 hours. The specimens were dehydrated with acetone, the acetone was replaced with liquid CO_2 and the specimens were critical point dried using a Polaron apparatus. Following drying the digestive tract was dissected from some of the specimens. Digestive tract segments (including longitudinal and cross sections) and whole amphipods were mounted on aluminum stubs using silver paint. The specimens were coated with gold with a Polaron sputter coater and were observed with an ISI-40 scanning electron microscope.

Besides electron microscopic observations, serial dilutions of the homogenate were plated on marine agar 2216 (Difco) and TCBS agar (Difco). Following 21 days incubation at 5°C the number of colony forming units (CFU) were determined with the aid of a 30x stereo microscope. Approximately 25 colonies from countable marine agar plates were selected at random for numerical taxonomic testing. The bacterial strains were restreaked twice on marine agar to insure purity. One hundred thirty bacterial strains were included in this study. The selected strains were then subjected to extensive

characterization to determine over 300 features each. The procedures used in characterizing the bacterial strains have been previously described (Kaneko et at. 1979).

Cluster analyses were performed to determine the similarity levels of the bacterial strains. The Jaccard Coefficient (S_J) and single linkage clustering algorithm were employed in this study. The GTP-2 computer program (courtesy R. R. Colwell) was used. Phena were defined with similarity levels (S-level) greater than 75%. Feature frequencies were calculated for each phenon. The distribution of strains within phena was used to calculate diversity. The procedure used in calculating bacterial diversity has been previously described (Kaneko et al. 1977). Indices were calculated to describe the capacity of the bacterial community to tolerate deviations from ambient conditons of temperature, salinity and pH. Ambient conditions were considered as 5°C, 3% NaCl and pH 8, which were the isolation conditions. Indices also were calculated to assess the nutritional versatility of bacterial communities.

For animal exposure to petroleum hydrocarbons, seawater containing soluble oil components was prepared by adding fresh Prudhoe Bay crude oil to give a concentration of 1 mg oil/ml water. The water plus oil was mixed with a Teflon-coated magnet stir bar for 6 h at 5°C. The mixture was allowed to stand for 3 h and water was siphoned from beneath the oil slick; no water was collected within 5 cm of the slick. Thirty individuals of B. affinis were added to replicate trays containing 500 ml oil-treated and unoiled (control) seawater. The animals were incubated at 5°C without feeding. The animals had been held for 7 days without feeding before starting this experiment to insure viability of the experimental amphipods. At 7 and 14 days, 10 individuals each from control and oil-treated trays were removed for viable

microbial analyses. There was no mortality during the experimental exposure period.

Actual dissolved or dispersed oil in the water was determined with an AMINCO Model SPF-125 spectrofluorometer, using the methods described by Keizer and Gordon (1973) and Gordon et al. (1974). One liter samples were extracted twice with 10 ml of spectrofluorometric grade methylene chloride. Extracts were combined and the methylene chloride was removed under vacuum at 30°C in a rotary evaporator. The recovered oil was redissolved in hexane. Excitation wavelength was 405 nm, and emission was read at a wavelength of 450 nm. Scans throughout the range of oil fluorescence were made to determine the wavelengths of maximum fluorescence. Oil concentrations were determined from calibration curves prepared from crude oil. Three replicate determinations were made at each time. The mean oil concentrations at 0, 1, 2, 7 and 14 days were found to be 3.4, 2.0, 0.7, 0.4 and 0.2 ppm respectively.

Statistical Analyses

Analyses of variance were performed using the SPSS computer programs (Nie et al., 1975) to determine the levels of statistical significance of differences between grouped data. The Duncan mean comparison test was used to determine if the means of individual groups were significantly different from each other, e.g., was there a significant difference between the taxonomic diversity indices (H') between water and sediment communities. An α value <0.05 was considered necessary for establishing a significant difference.

DENITRIFICATION - POTENTIAL ACTIVITIES

Rates of Denitrification

The acetylene blockage of N_2O reduction method (Balderston et al., 1976; Sorensen, 1978; Yoshinari et al., 1977; Yoshinari and Knowles, 1979) was used for measuring denitrification activities. For denitrification assays, 5-ml

portions of the sediment slurry were used to inoculate previously sterilized, replicate 20-ml serum bottles containing either 5 ml of Rila marine salts mix (40 g/liter) (R), 5 ml of Rila salts plus 0.1% (wt/vol) proteose peptone no. 3 (Difco Laboratories, Detroit, Mich.) (RP), 5 ml of Rila salts plus 0.1% (wt/vol) KNO_3 (RN), or 5 ml of Rila salts plus nitrate broth (Difco) (RNB). After inoculation, all vials were capped with rubber serum stoppers and purged with argon for 15 min. After purging, 5 ml of the headspace gas was withdrawn with a syringe and replaced with C_2H_2 generated from CaC_2. The serum stoppers were then covered with silicone rubber sealant to prevent gas leakage from needle punctures. All vials were incubated at 5°C. Media R, RP, RN, and RNB were used to measure denitrification activity in either unamended sediments or sediments amended with organic carbon and nitrogen, mineral nitrogen, or organic carbon and nitrogen, mineral nitrogen, respectively. A high concentration of NO_3^- was used in media RN and RNB to preclude substrate limitation for measuring potential rates of denitrification. After incubation, the sediment suspension was at a dilution of 1:2 compared with the original sediment. All sample and media combinations were performed in triplicate.

After incubation for 48 to 240 h, 0.5 ml of saturated KOH solution was injected into replicate bottles to stop biological activity. All bottles were resealed with silicone rubber to prevent leakage from needle punctures and maintained at 5°C until the headspace gases could be analyzed in the laboratory.

Analysis of the headspace gases in the sample bottles from the Beaufort Sea were performed with a Hewlett Packard 5700 gas chromatograph equipped with a Carle thermal conductivity detector (TCD) in series with the flame ionization detector (FID). The conditions for chromatography were: injector, 100°C; oven, 50°C; TCD, 50°C, 24 mA; FID, 150°C; carrier gas, 22 ml of helium per min;

and column (6 m by 0.3 cm) of Parapak Q (Supelco Inc., Bellefonte, Pa.), injection size 250 µl. All other samples were analyzed with a Hewlett Packard 5840 gas chromatograph with an electron capture detector (ECD) for N_2O determinations which provided greater sensitivity. Control experiments also were analyzed with the ECD. The instrument was equipped with a column (6 m by 0.3 cm) of Porapak Q. The conditions for chromatography were: injector, 100°C; oven, 40°C; and ECD, 300°C. The carrier for the 5840 gas chromatograph was argon-methane (19:1) at a flow rate of 25 ml/min and injection size of 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 5992A, equipped with a column (6 m by 0.3 cm) of Porapak Q. The carrier gas was helium at 30 ml/min. The conditions for chromatography were: injector, 100°C; and oven, 50°C.

The ECD was found to be linear in response across several orders of magnitude of N_2O concentrations. Experimental results were compared with a standard curve to obtain concentrations of N_2O, and the quantity of N_2O produced per gram (dry weight) of sediment was calculated. The limit of sensitivity with the thermal conductivity detector was approximately 40 nmol of N_2O produced per g (dry weight) sediment (≅90 µmol of N_2O/m^2 per h during 48 h of incubation); with the ECD it was possible to detect production of approximately 51 pmol of N_2O per g (dry weight) of sediment (≅0.01 µmol of N_2O/m^2 per h during 48 h of incubation).

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. Replicates of each of the media were left active, were sterilized by autoclaving, or received 0.5 ml of 6 N HCl.

to N_2O reduction; N_2 in controls was <1% of the quantities of N_2O observed. 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 rates for unamended samples ; the results from the 10-day nitrate-amended experiments can be used to calculate potential rates of denitrification for samples with added NO_3^-.

Effects of oil on denitrifying activity

Surface sediments were collected from Elson Lagoon (4 m in depth) in the Beaufort Sea near Barrow, Alaska, in January 1978. Portions of the sediment were immediately mixed with 0.5% (wt/vol) Prudhoe Bay crude oil, and other portions were left untreated. Treated and untreated sediments were then placed separately in Plexiglas trays (25 by 25 by 5 cm) and placed *in situ* on the bottom of Elson Lagoon. Similar experiments were begun in January 1979 and January 1980. Sediment samples were recovered from replicate trays after 1 week and 5, 8, 18, 24, and 28 months of incubation.

Oil Biodegradation - Potential Activities

One ml portions of water samples or 1/10 g portions of sediment samples were inoculated into sterile serum vials containing 10 ml of a mineral salts medium ($MgSO_4$, $0.2gl^{-1}$; $CaCl_2$, $0.02gl^{-1}$; KH_2PO_4, $1gl^{-1}$; K_2HPO_4, $1gl^{-1}$; NH_4NO_3, $1gl^{-1}$; $FeCl_3$, $0.05gl^{-1}$; NaCl, $30gl^{-1}$) and 30 µl crude oil. All determinations were performed in triplicate. In Beaufort sea and Bering Sea studies Prudhoe Bay crude oil was used; in Gulf of Alaska and Cook Inlet Cook Inlet Crude oil was used. The crude oil was spiked with radiolabelled hydrocarbons, either *n*-1-^{14}C hexadecane; 1-^{14}C-pristane; (1, 4, 5, 8-^{14}C) naphthalene; 9-methyl(U-^{14}C) anthracene; or 12-^{14}C benz(a)anthracene. The specific activity of each hydrocarbon was adjusted to

Replicates of each control medium also did not receive C_2H_2. After 24, 48, 120, and 240 h of incubation at 5°C, 0.5 ml of saturated KOH solution was injected into three replicates each of the active, sterile, or acidified treatments. The purposes of these controls were to determine the effects of acetylene on N_2O production, to determine the extent of spontaneous dismutation of NO_3^- to nitrous oxide under acidic conditions, to check on the possible spontaneous N_2O production from known sterile sediments, and combinations of these aims. These controls were also used to examine the linearity of N_2O production over the the incubation periods up to 240 h. Portions of the same sediment samples also were incubated in serum bottles containing 5 ml of Rila salts plus nitrate broth plus 0.1% KNO_2 (wt/vol). The purpose of this control was to determine whether nitrite was toxic to denitrifying bacteria in marine sediments. Sterile bottles were also incubated with known concentrations of N_2O to determine whether leakage of gases occurred during storage before analysis. The headspace gases were analyzed for various periods of incubation up to 3 weeks.

The control experiments showed that storage of the vials before analysis did not result in signficant loss of N_2O from the headspace. Repetitive analysis of vials over an extended period (in excess of storage times of experimental vials) 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 when incubated with added nitrate in the presence and absence of 0.1% added nitrite. No significant abiotic N_2O production was found. Measurement of pH for selected active sediment samples showed none with pH values less than 6.5, even after 10 days incubation with RNB. Acetylene proved to be an effective block

approximately 10 $\mu Ci g^{-1}$ oil. All hydrocarbons in the oil were considered as potential substrates for the enzymes produced by the mixed microbial community and thus the total concentration of the added oil was used for calculating specific activities. The vials were incubated for 21 days at 5°C in the dark. After incubation microbial hydrocarbon degrading activities were stopped by addition of concentrated KOH. The $^{14}CO_2$ produced was recovered from the vials and quantitated by liquid scintillation counting.

In some cases the residual hydrocarbons and biodegradation products were recovered by solvent extraction with two x 10 ml portions of pentane. The ^{14}C in each extract was determined by liquid scintillation counting after which the mixture was fractionated into degraded and non-degraded and fractions using silica gel column chromatography. A 0.75 cm diameter x 10 cm column packed with 70-230 mesh silica gel 60 was used. The undegraded hydrocarbon fractions were eluted in two 20 ml portions each of pentane and benzene. The degradation product fractions consisted of the eluate collected in 20 ml methanol, the residual material left on the column and the CO_2 (collection described above). Radiolabelled material in each fraction was quantitated by liquid scintillation counting and corrected for recovery and counting efficiencies. Further extraction with methylene chloride routinely failed to recover any additional radiolabelled material. Sterile controls were used to correct for efficiency of recovery and fractionation. Triplicate determinations were made for each sample with radiolabelled hydrocarbons. The percentage hydrocarbon mineralization was calculated as $^{14}CO_2$ produced above sterile control divided by ^{14}C hydrocarbon added. The percentage hydrocarbon biodegradation (which includes mineralization) was calculated as $^{14}CO_2$ produced plus ^{14}C methanol fraction plus ^{14}C residual (all above

sterile control) divided by ^{14}C hydrocarbon added. Carbon balances generally accounted for 90% of the radiolabelled carbon added to the sediment.

Oil Weathering - Microbial Biodegradation of Petroleum Hydrocarbons

Exposure

Sediment (top 5 cm) was collected by divers from the bottom (6 m) of Elson Lagoon (near Point Barrow 71°N latitude and 157°W longitude) and immediately mixed with 5% (v/v) Prudhoe Bay crude oil. The freshly collected sediment and oil were added to a large stainless steel bucket and stirred with a clean wooden paddle until the sediment-oil mixture was a uniform color with no visible oil slick accumulating on the surface. The level of oiling simulated a heavy exposure as might result from a pipeline rupture. The sediment-oil mixture and clean sediment (control) was dispersed into 25 x 25 x 5 cm plexiglas trays and replaced on the bottom of Elson Lagoon for incubation. The experiment was begun during May. After incubation periods of 0, 1/2, 24, 48 and 72 h; 7, 14, 21, and 28 d; 3, 4, and 8 months; 1, 1 1/2, and 2 years, trays were recovered and sampled. The contents of each tray were split into 5 replicate portions of approximately 200 g each, without mixing. The sediment samples were collected with minimal disturbance to the sediment in order to prevent redistribution of the oil. Samples for chemical analyses were frozen and transported to the laboratory.

In addition to the in situ Beaufort Sea studies, the biodegradation of crude oil in Southern Bering Sea (North Aleutian Shelf) sediments was assessed a flow through model ecosystem (laboratory microcosm). Sediments were collected during August - September, 1980 in the southern Bering Sea. Three different types of sediments from three locations on the north Aleutian Shelf were chosen for this experiment including one sand and gravelly sediment (161°12'W, 56°10'N), one silty sediment (165°10'W, 55°40'N), and one fine sand

sediment (164°25'W, 54°05'N). Two hundred g of each sediment and 2 ml of sterile Cook Inlet crude oil were added to 2 liter stainless steel container. Sterilized 200 g portions of each sediment also were added as controls. The vessels containing sediments were attached to a manifold that provided a constant flow of nutrient solution. The nutrient solution was sterile Rila marine salts plus 0.05% (w/v) KNO_3 and KH_2PO_4. Fresh sterile nutrient solution was passed through the incubation vessels continuously. At weekly intervals, up to 6 weeks, sediments remaining hydrocarbons were extracted from the sediments and analyzed as described below.

Hydrocarbon Extraction and Analysis

Samples of sediment were thawed in a 5°C refrigerator. After thawing, 25 g wet sediment was measured into 250 ml Erlenmeyer flasks. Hydrocarbons were extracted by shaking each sample sequentially, twice with 50 ml ethyl ether (Burdick and Jackson) and once with meythylene chloride (Burdick and Jackson).

The solvents were separated from the sediments between each step using separatory funnels. The solvent extracts were combined and reduced in volume using a Vigreaux reflux column. After the volume of the extract had been reduced to about 20 ml, 40 ml pentane (MCB) was added to the flask and the volume reduced again. Addition of pentane and reduciton of the extract volume was repeated twice. The extract was transferred quantitatively to 20 ml glass vials and the volume adjusted to 10 ml with pentane.

The extracts were separated into three fractions using silica gel column chromatography. Silica gel 100 (EM reagents, Darmstadt, W. Germany) was dried overnight at 100°C. The dry silica gel was suspended in CH_2Cl_2 for packing the columns. The columns were 25 ml burets with teflon stopcocks. After rinsing each column with CH_2Cl_2, the silica gel suspension was added slowly to the column until a 15 ml bed volume was achieved. The column bed was rinsed

with 40 ml pentane prior to fractionation of the hydrocarbon extracts. The pentane was drained to the bed surface and 5 ml of each sample was added ot the column and drained into the column bed. After standing for 3-5 min, the alkane fraction (f_1) was eluted from the column with 20 ml pentane and 5 ml 20% (v/v) CH_2Cl_2 in pentane. The aromatic fraciton (f_2) was eluted from the column with 45 ml 40% v/v CH_2Cl_2 in pentane. After elution, each fraction was reduced in volume to approximately 10 ml at 35°C and transferred quantitatively to clean glass vials.

Analysis of fraction f_1 was performed with a Hewlett-Packard 5840 gas chromatograph equipped with a flame ionization detector. Conditions for chromatography were: injector, 240°C; oven, 70 to 270°C at 4°C/min; FID detector, 300°C. The column was a 30 m x 0.2 mm grade AA SE 54 glass capillary column (Supelco, Bellafonte, PA) with 25 cm/sec He flow. Injection size was 2.5 µl.

Fraction f_2 was analysed with a Hewlett-Packard 5992A gas chromatograph-mass spectrometer operated in the selected ion monitor mode. The following molecular ions were monitored: 128 (naphthalene); 142 (C_1-naphthalene); 147 (HMB); 156 (C_2-naphthalene); 170 (C_3-naphthaline); 178 (phenanthrene/anthracene); 184 (dibenzothiophene-DBT); 192 (C_1-phenanthrene); 198 (C_1-DBT); 206 (C_2-phenanthrene); 212 (C_2-DBT); 220 (C_3-phenanthrene); and 226 (C_3-DBT). The conditions for chromatography were: injector, 240°C; oven, 70 to 270°C at 4°C/min. The column was a grade AA, 30 m x 0.2 mm SE 54 glass capillary column with 25 cm/sec He flow. Injection size was 5 µl using the splitless injection mode.

Standards of known quantities of both alkanes and aromatic compounds were prepared for calibration and calculation of instrument response factors. Prior to analysis of each sample, hexamethyl benzene (HMB) was added as an internal

standard to achieve a final concentration of 12.6 ng/µl. The quantities of specific alkanes and aromatic compounds were calculated according to the following equations.

response factor

$$rf = (\text{area units of peak})/(\text{ng of compound injected})$$

internal standard factor

$$ISF = (\text{area}_{HMB} \times rf_{HMB})/(\text{ng/µl HMB injected})$$

Quantity of compounds

$$\text{ng/µl} = (\text{area of peak} \times \text{rf peak})/(ISF)$$

Quantity per gram dry sediment

$$\text{ng/g dry sed.} = (\text{ng/µl compound} \times \text{µl of sample})/(\text{g dry sediment extracted})$$

After calculation of quantities of specific compounds, the ratio of n-alkanes, C_{11}-C_{29} to pristane were calculated and the ratios of several aromatic compounds to dimethyl phenanthrene were calculated.

The dry weights of the sediment samples were determined by weighting 25 g wet sediment into aluminum pans and drying overnight at 100°C. After drying, the pans and sediment were placed into dessicators to cool. After cooling, the pans were reweighed to determine moisture content of the sediments.

VI Results

ENUMERATION OF MICROBIAL POPULATIONS

Table 2 shows the levels of bacteria, including both total direct and total viable counts.

Beaufort Sea

Enumeration of bacterial populations in the Beaufort Sea indicates that bacterial numbers decline somewhat during winter especially in surface waters (Table 3). As in other marine ecosystems, numbers of bacteria are highest in sediment, lower in water and lowest in ice. Also as occurs elsewhere, the numbers of viable bacteria are several orders of magnitude lower than the total numbers of bacteria observed using direct count procedures. Numbers of hydrocarbon utilizing bacteria represent only a small proportion of the total numbers of bacteria, less than a fraction of a percent of the total numbers of bacteria. Compared to other Alaskan Continental Shelf regions the numbers of viable bacteria in surface waters are significantly higher in the Beaufort Sea. For example, the average number of viable bacteria enumerated in the northern Bering Sea surface waters during several spring-summer sampling cruises was 6.9×10^2, which is over an order of magnitude lower than for comparable Beaufort Sea surface water samples. No such differences, however, occur in direct counts of water or sediment, nor in viable counts of sediment bacterial populations. The evidence suggests that the numbers of viable bacteria in the Beaufort Sea, at least those enumerated on marine agar, are an order of magnitude higher than the numbers of viable bacteria in nearby subpolar seas. Results using the INT method (Zimmermann et al., 1978) indicate that 1% or less of the bacteria enumerated by acridine orange direct microscopy, suggesting that most viable bacteria are enumerated on marine agar. In addition, counts of oligoheterotrophic bacteria were consistently several orders of magnitude lower

Table 2. Enumeration of total and viable microorganisms.

SAMPLE	DIRECT COUNT	PLATE COUNT MARINE AGAR 4°C
BBP1		$1.100X10^{03}$
BB0001		$1.000X10^{04}$
BB0002		$5.400X10^{04}$
BB0003		$5.300X10^{04}$
BB0004		$6.800X10^{05}$
BB0005		$4.500X10^{04}$
BB0006		$3.100X10^{04}$
BB0007		$1.900X10^{05}$
BB0008		$7.800X10^{05}$
BB0009		$8.700X10^{04}$
BB0010		$5.400X10^{04}$
BB0011	$5.700X10^{06}$	$6.200X10^{04}$
BB0012	$9.700X10^{06}$	$4.400X10^{04}$
BB0013	$1.400X10^{07}$	$3.400X10^{04}$
BB0014	$5.100X10^{06}$	$1.200X10^{05}$
BB0015	$5.500X10^{06}$	$2.200X10^{05}$
BB0016	$4.800X10^{08}$	$1.300X10^{05}$
BB0017	$5.900X10^{07}$	
BB0018	$3.000X10^{08}$	$2.400X10^{05}$
BB0019	$3.100X10^{07}$	$2.700X10^{05}$
BB0020	$7.300X10^{06}$	$2.300X10^{05}$
BB0021	$3.700X10^{07}$	$4.100X10^{04}$
BB0022	$9.200X10^{07}$	$3.200X10^{04}$
BB0023	$9.200X10^{07}$	$1.800X10^{06}$
BB0024	$5.200X10^{07}$	$6.800X10^{04}$
BB0025	$2.600X10^{07}$	$1.300X10^{05}$
BB0025A		
BB0026	$4.100X10^{07}$	$4.000X10^{05}$
BB0027	$1.700X10^{07}$	$6.800X10^{04}$
BB0027A		
BB0028	$2.300X10^{06}$	$9.700X10^{04}$
BB0029	$1.100X10^{07}$	$1.100X10^{05}$
BB0030	$1.700X10^{07}$	$2.900X10^{05}$
BB0031	$2.500X10^{07}$	$1.000X10^{05}$
BB0032	$8.300X10^{07}$	$1.100X10^{05}$
BB0033	$1.900X10^{07}$	$6.500X10^{03}$
BB101	$1.100X10^{10}$	$2.800X10^{05}$
BB102	$4.400X10^{09}$	$1.900X10^{06}$
BB103	$5.500X10^{09}$	$2.000X10^{05}$
BB104	$1.900X10^{09}$	$6.400X10^{05}$
BB105	$1.300X10^{09}$	$1.100X10^{05}$
BB106	$3.200X10^{07}$	$6.300X10^{04}$
BB107	$5.300X10^{07}$	$3.400X10^{04}$
BB108	$7.200X10^{08}$	$1.900X10^{05}$
BB109	$1.400X10^{10}$	$1.100X10^{05}$
BB110	$1.400X10^{08}$	$5.400X10^{04}$
BB111	$2.300X10^{08}$	$7.700X10^{04}$
BB112	$2.200X10^{08}$	$1.400X10^{04}$
BB113	$2.300X10^{08}$	$7.600X10^{03}$
BB114	$7.500X10^{07}$	$2.700X10^{04}$
BB115	$1.400X10^{08}$	$1.800X10^{03}$
BI101		
BI102		

BI103	1.000X10^{05}	3.700X10^{01}
BI104	1.900X10^{05}	7.300X10^{01}
BI105	1.600X10^{05}	1.600X10^{01}
BI106	8.500X10^{04}	1.100X10^{01}
BI107	7.800X10^{04}	3.000X10^{-01}
BI108	7.000X10^{04}	1.600X10^{01}
BI109	1.900X10^{05}	7.700X10^{01}
BI110	8.500X10^{04}	0.000X10^{00}
BI111	1.500X10^{05}	5.500X10^{02}
BI112	7.800X10^{04}	9.900X10^{00}
BI113	1.300X10^{05}	4.000X10^{01}
BI114	7.800X10^{04}	6.000X10^{01}
BI115	1.100X10^{05}	2.900X10^{02}
BI116	4.600X10^{04}	3.800X10^{01}
BI117	5.400X10^{04}	6.300X10^{01}
BI118	7.800X10^{04}	5.700X10^{00}
BI119	9.300X10^{04}	1.000X10^{01}
BI120	7.800X10^{04}	0.000X10^{00}
BI121	7.800X10^{04}	1.600X10^{00}
BI122	1.000X10^{05}	5.000X10^{00}
BI123	4.600X10^{04}	2.000X10^{01}
BW0001		1.900X10^{02}
BW0002		7.000X10^{01}
BW0003		4.800X10^{03}
BW0004		1.800X10^{04}
BW0005		6.000X10^{02}
BW0006		4.000X10^{03}
BW0006A		
BW0007		2.400X10^{03}
BW0007A		
BW0008		7.700X10^{02}
BW0009	5.400X10^{05}	8.400X10^{02}
BW0009A		
BW0010	1.400X10^{06}	1.500X10^{03}
BW0011	2.300X10^{06}	1.000X10^{04}
BW0011A		
BW0011B		
BW0012	1.400X10^{06}	6.700X10^{03}
BW0012A		
BW0013	5.600X10^{05}	1.000X10^{04}
BW0013A		
BW0013B		
BW0014	7.600X10^{05}	1.800X10^{04}
BW0014A		
BW0015	4.100X10^{06}	2.200X10^{04}
BW0016	8.700X10^{05}	1.600X10^{04}
BW0017	1.000X10^{06}	2.600X10^{04}
BW0018	7.100X10^{05}	1.800X10^{04}
BW0019	7.500X10^{05}	6.100X10^{03}
BW0019A		
BW0020	5.800X10^{05}	3.100X10^{03}
BW0021		5.100X10^{03}
BW0022	2.100X10^{05}	1.300X10^{03}
BW0023	6.800X10^{05}	2.700X10^{04}
BW0024	4.200X10^{05}	2.100X10^{04}
BW0024A		
BW0025	5.900X10^{05}	1.400X10^{04}
BW0026	4.100X10^{05}	9.600X10^{03}
BW0026A		
BW0027	3.800X10^{05}	9.700X10^{03}

BW0028	$4.900X10^{05}$	$2.600X10^{04}$
BW0028A		
BW0029	$2.300X10^{05}$	$2.900X10^{04}$
BW0030	$6.700X10^{05}$	$1.900X10^{04}$
BW0031	$2.400X10^{05}$	$9.200X10^{03}$
BW0032	$4.300X10^{05}$	$5.900X10^{03}$
BW0033	$7.400X10^{05}$	$1.800X10^{04}$
BW0034	$1.200X10^{06}$	$2.300X10^{04}$
BW0035	$3.100X10^{05}$	$3.500X10^{03}$
BW0036	$4.100X10^{05}$	$1.400X10^{03}$
BW0037	$3.400X10^{05}$	$7.100X10^{02}$
BW0038	$8.100X10^{05}$	$3.300X10^{03}$
BW0039	$1.300X10^{06}$	$2.300X10^{04}$
BW0040	$1.200X10^{06}$	$3.100X10^{02}$
BW0041	$1.100X10^{06}$	$2.300X10^{03}$
BW0042	$7.200X10^{05}$	$9.800X10^{02}$
BW0043		$1.700X10^{03}$
BW0079		
BW0080		
BW0081		
BW0082		
BW0083		
BW0084		
BW0091		
BW0092		
BW0093		
BW0094		
BW0095		
BW101		
BW102		
BW103	$6.400X10^{05}$	$1.300X10^{01}$
BW104	$6.200X10^{04}$	$2.000X10^{01}$
BW105	$7.000X10^{04}$	$4.700X10^{01}$
BW106	$1.600X10^{05}$	$5.000X10^{01}$
BW107	$1.000X10^{05}$	$4.000X10^{01}$
BW107A		
BW108	$2.800X10^{05}$	$1.400X10^{03}$
BW109	$3.600X10^{05}$	$6.100X10^{02}$
BW110	$1.700X10^{05}$	$5.700X10^{00}$
BW111	$1.500X10^{05}$	$6.200X10^{01}$
BW112	$2.800X10^{05}$	$3.000X10^{01}$
BW113	$1.100X10^{05}$	$3.300X10^{01}$
BW114	$7.000X10^{04}$	$2.000X10^{01}$
BW116	$2.300X10^{05}$	$1.000X10^{01}$
BW117	$1.200X10^{05}$	$9.100X10^{03}$
BW118	$7.800X10^{04}$	$8.300X10^{00}$
BW119	$1.600X10^{05}$	$7.100X10^{02}$
BW120	$9.300X10^{04}$	$1.300X10^{01}$
BW121	$9.300X10^{04}$	$3.300X10^{00}$
BW122	$1.600X10^{05}$	$1.200X10^{01}$
BW123	$1.600X10^{05}$	$1.300X10^{01}$
GB0101		$1.000X10^{04}$
GB0121		$1.100X10^{06}$
GB0134		$1.400X10^{06}$
GB0137		$6.200X10^{05}$
GB0146		
GB0148		$2.000X10^{04}$
GB201	$1.100X10^{06}$	$1.800X10^{05}$
GB202	$3.300X10^{09}$	$5.200X10^{05}$
GB203	$6.200X10^{09}$	$1.100X10^{05}$

GB204	$2.500X10^{09}$	$6.700X10^{04}$
GB206	$2.700X10^{09}$	$2.400X10^{04}$
GB207	$3.300X10^{09}$	$4.200X10^{05}$
GB210	$2.800X10^{09}$	$3.000X10^{06}$
GB211	$5.200X10^{09}$	$1.500X10^{05}$
GB212	$3.300X10^{09}$	$1.700X10^{05}$
GB213		$1.700X10^{05}$
GB214	$1.900X10^{09}$	$1.100X10^{06}$
GB216	$8.200X10^{08}$	$3.700X10^{03}$
GB217	$1.600X10^{09}$	$1.500X10^{05}$
GB218	$4.000X10^{08}$	$5.800X10^{03}$
GB219	$5.600X10^{09}$	$1.100X10^{05}$
GB220	$6.100X10^{07}$	$1.500X10^{03}$
GB223	$1.800X10^{09}$	$1.200X10^{05}$
GB224	$1.800X10^{09}$	$4.400X10^{05}$
GB225	$4.100X10^{09}$	$4.000X10^{06}$
GB226	$3.400X10^{09}$	$9.000X10^{06}$
GW0101	$1.200X10^{05}$	$5.500X10^{01}$
GW0102	$3.000X10^{05}$	$5.000X10^{01}$
GW0103	$1.400X10^{05}$	$2.000X10^{01}$
GW0104	$4.300X10^{05}$	$5.000X10^{01}$
GW0105	$3.800X10^{05}$	$6.000X10^{01}$
GW0106	$1.800X10^{05}$	$6.500X10^{01}$
GW0119	$3.600X10^{05}$	$3.500X10^{01}$
GW0124	$3.300X10^{05}$	$9.700X10^{01}$
GW0133	$3.800X10^{05}$	$9.200X10^{01}$
GW0137	$1.000X10^{05}$	$1.200X10^{02}$
GW0145	$2.800X10^{05}$	$1.300X10^{02}$
GW0148	$2.100X10^{05}$	$1.900X10^{02}$
GW0156	$5.000X10^{05}$	$2.500X10^{02}$
GW0159	$4.700X10^{05}$	$2.200X10^{02}$
GW201	$2.600X10^{05}$	$2.200X10^{04}$
GW202	$9.300X10^{04}$	$3.300X10^{01}$
GW203	$3.300X10^{05}$	$2.100X10^{01}$
GW204	$3.000X10^{05}$	$3.100X10^{01}$
GW205		
GW206	$9.300X10^{04}$	$1.400X10^{01}$
GW207	$1.000X10^{05}$	$9.700X10^{00}$
GW208		
GW209	$2.700X10^{05}$	$1.000X10^{03}$
GW210	$4.600X10^{04}$	$5.900X10^{01}$
GW211	$9.300X10^{04}$	$3.600X10^{01}$
GW212	$2.400X10^{05}$	$7.200X10^{01}$
GW213		$3.100X10^{01}$
GW214	$5.400X10^{04}$	$1.000X10^{02}$
GW215		
GW216	$6.200X10^{04}$	$3.200X10^{01}$
GW217	$1.600X10^{05}$	$2.300X10^{01}$
GW218	$1.500X10^{05}$	$3.300X10^{01}$
GW219	$1.200X10^{05}$	$1.200X10^{01}$
GW220	$4.600X10^{04}$	$3.800X10^{02}$
GW221	$1.200X10^{05}$	$3.000X10^{01}$
GW222	$1.000X10^{05}$	$1.500X10^{01}$
GW223	$7.000X10^{04}$	$6.000X10^{00}$
GW224	$3.100X10^{04}$	$3.000X10^{01}$
GW225	$1.000X10^{05}$	$7.700X10^{02}$
GW226	$2.200X10^{05}$	
GW227	$7.800X10^{04}$	
BB201	$1.600X10^{09}$	$1.600X10^{06}$
BB203	$2.600X10^{09}$	$3.100X10^{06}$

BB204	$1.900X10^{09}$	$3.200X10^{06}$
BB205	$1.100X10^{09}$	$2.100X10^{06}$
BB206	$1.900X10^{09}$	$1.500X10^{06}$
BB207	$1.600X10^{09}$	$6.200X10^{06}$
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BB212	$1.500X10^{09}$	$1.200X10^{07}$
BB214	$1.000X10^{09}$	$5.500X10^{06}$
BB215	$1.100X10^{09}$	$1.200X10^{07}$
BB216	$3.600X10^{09}$	$4.500X10^{07}$
BB217		
BB219	$6.900X10^{09}$	$2.500X10^{06}$
BB220	$7.600X10^{08}$	$1.700X10^{07}$
BW201	$9.300X10^{05}$	$3.700X10^{04}$
BW202	$6.000X10^{05}$	$3.200X10^{04}$
BW203	$4.500X10^{05}$	$2.400X10^{04}$
BW204	$2.600X10^{05}$	$1.800X10^{05}$
BW205	$3.400X10^{05}$	$2.600X10^{04}$
BW206	$4.000X10^{05}$	$7.200X10^{04}$
BW207	$2.100X10^{06}$	$2.100X10^{04}$
BW208	$3.700X10^{05}$	$3.600X10^{04}$
BW209	$4.500X10^{05}$	$3.700X10^{03}$
BW210	$4.200X10^{05}$	$1.200X10^{05}$
BW211	$2.600X10^{05}$	$1.900X10^{03}$
BW212	$6.500X10^{05}$	$1.000X10^{05}$
BW213	$5.700X10^{05}$	$9.200X10^{03}$
BW214	$5.300X10^{05}$	$7.700X10^{04}$
BW215	$3.100X10^{05}$	$2.200X10^{04}$
BW216	$3.200X10^{05}$	$8.700X10^{04}$
BW217	$2.800X10^{05}$	$1.200X10^{05}$
BW218	$6.000X10^{05}$	$8.700X10^{03}$
BW219	$2.900X10^{05}$	$1.700X10^{04}$
BW220	$3.100X10^{05}$	$1.000X10^{04}$
GB301	$5.000X10^{08}$	$8.900X10^{05}$
GB303	$5.400X10^{08}$	$8.700X10^{05}$
GB304	$1.100X10^{09}$	$2.600X10^{06}$
GB308	$6.100X10^{08}$	$1.700X10^{07}$
GB311	$2.600X10^{09}$	$2.300X10^{06}$
GB312		$2.900X10^{04}$
GB313	$2.900X10^{09}$	$6.000X10^{06}$
GB318	$8.400X10^{08}$	$6.900X10^{06}$
GB319	$3.700X10^{08}$	$1.000X10^{04}$
GB320		
GB324		
GB325	$2.700X10^{08}$	$8.400X10^{05}$
GB327	$5.900X10^{08}$	$6.500X10^{05}$
GB328		
GB329	$7.100X10^{09}$	$2.100X10^{07}$
GB333		
GB334		
GB335	$1.200X10^{09}$	$1.500X10^{05}$
GW301	$2.700X10^{05}$	$1.200X10^{01}$
GW302	$4.300X10^{05}$	$3.300X10^{01}$
GW303	$1.700X10^{05}$	$1.000X10^{01}$
GW304	$1.500X10^{05}$	$9.000X10^{01}$
GW305		
GW306		
GW307		
GW308	$1.700X10^{05}$	$1.000X10^{04}$
GW309	$1.900X10^{05}$	$4.200X10^{03}$
GW310	$2.100X10^{05}$	$1.100X10^{04}$

GW311	$3.800X10^{05}$	$1.600X10^{02}$
GW312	$2.500X10^{05}$	$5.000X10^{00}$
GW313	$2.200X10^{05}$	$2.000X10^{02}$
GW314		
GW315	$2.300X10^{05}$	$1.000X10^{03}$
GW316		
GW317	$4.400X10^{05}$	$1.100X10^{05}$
GW318	$2.600X10^{05}$	$7.700X10^{01}$
GW319	$6.200X10^{04}$	$9.300X10^{01}$
GW320	$2.200X10^{05}$	$1.100X10^{02}$
GW321		
GW322	$1.100X10^{06}$	$2.200X10^{02}$
GW323	$1.500X10^{05}$	$6.100X10^{02}$
GW324		
GW325	$1.100X10^{06}$	$2.900X10^{04}$
GW326		
GW327	$2.600X10^{05}$	$1.100X10^{02}$
GW328		
GW329	$1.200X10^{07}$	$4.700X10^{02}$
GW330		
GW331		
GW332		
GW333		
GW334		
GW335	$1.900X10^{05}$	$3.000X10^{01}$
GW336		
GW337		
NB002	$2.300X10^{08}$	$2.900X10^{06}$
NB003		$4.000X10^{06}$
NB004		
NB007	$2.300X10^{08}$	$6.100X10^{06}$
NB016	$1.100X10^{09}$	$3.000X10^{05}$
NB021	$1.500X10^{08}$	$2.900X10^{06}$
NB024		
NB027		
NB031		
NB036		
NB040	$5.000X10^{07}$	$3.800X10^{05}$
NB045		
NB047	$3.200X10^{07}$	$1.100X10^{06}$
NB049	$8.600X10^{07}$	$2.100X10^{07}$
NB101	$4.400X10^{07}$	$1.800X10^{05}$
NB103	$7.600X10^{07}$	$1.200X10^{06}$
NB107	$1.300X10^{08}$	$1.200X10^{06}$
NB111	$1.400X10^{08}$	$1.800X10^{05}$
NB113	$2.400X10^{08}$	$1.800X10^{06}$
NB115	$4.300X10^{08}$	$1.100X10^{07}$
NB117	$1.700X10^{08}$	$1.500X10^{06}$
NB119	$1.500X10^{08}$	$3.600X10^{06}$
NB124	$1.500X10^{08}$	$5.000X10^{05}$
NB126	$1.400X10^{08}$	$1.100X10^{06}$
NB127	$1.200X10^{08}$	$1.700X10^{06}$
NB128		$4.400X10^{06}$
NB129	$7.800X10^{07}$	$1.100X10^{06}$
NB130	$1.700X10^{08}$	$2.900X10^{06}$
NB131	$2.100X10^{08}$	$3.000X10^{06}$
NB132	$3.500X10^{08}$	$3.300X10^{06}$
NB133	$6.600X10^{08}$	$1.800X10^{06}$
NB134	$2.000X10^{08}$	$2.300X10^{06}$
NB135	$1.800X10^{08}$	$2.100X10^{06}$

NB137	$2.000X10^{08}$	$1.400X10^{06}$
NB138	$2.000X10^{08}$	$1.300X10^{06}$
NB139		
NB140	$2.700X10^{08}$	$2.600X10^{06}$
NB141	$1.900X10^{08}$	$1.100X10^{06}$
NB143		$2.100X10^{05}$
NB145	$3.500X10^{08}$	$1.700X10^{06}$
NB147	$9.000X10^{07}$	$4.600X10^{05}$
NB149	$2.300X10^{07}$	$1.300X10^{06}$
NB151	$1.600X10^{08}$	$2.900X10^{06}$
NB152		
NB153	$1.600X10^{08}$	$2.600X10^{06}$
NB154	$2.000X10^{08}$	$3.600X10^{06}$
NB156		
NB157	$8.300X10^{07}$	$4.700X10^{06}$
NB158	$1.800X10^{08}$	$3.800X10^{06}$
NB161	$3.600X10^{08}$	$3.500X10^{06}$
NB162	$9.400X10^{07}$	$2.700X10^{06}$
NB163	$1.100X10^{08}$	$2.900X10^{06}$
NWA29		
NWB29		
NW002	$3.700X10^{05}$	$4.600X10^{02}$
NW003	$4.000X10^{05}$	$5.300X10^{02}$
NW004	$4.800X10^{05}$	$3.600X10^{02}$
NW007	$2.800X10^{05}$	$1.400X10^{02}$
NW013	$2.400X10^{05}$	$1.100X10^{02}$
NW047	$1.100X10^{05}$	$9.500X10^{01}$
NW049	$2.700X10^{05}$	$1.700X10^{02}$
NW101	$1.700X10^{05}$	$8.000X10^{01}$
NW102		
NW103	$2.000X10^{05}$	$9.300X10^{01}$
NW104		
NW105	$1.300X10^{05}$	$4.500X10^{02}$
NW106	$2.000X10^{05}$	$1.200X10^{03}$
NW107	$1.800X10^{05}$	$6.000X10^{01}$
NW108		
NW109	$3.000X10^{05}$	$3.900X10^{02}$
NW110		
NW111	$2.100X10^{05}$	$1.100X10^{02}$
NW112		
NW113	$3.900X10^{05}$	$6.200X10^{01}$
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NW122	$2.300X10^{05}$	$9.200X10^{01}$
NW123		
NW124	$2.400X10^{05}$	$1.000X10^{02}$
NW125		
NW129		
NW130	$3.700X10^{05}$	$1.000X10^{03}$
NW131	$4.800X10^{05}$	$6.700X10^{01}$
NW132	$3.200X10^{05}$	$8.000X10^{01}$
NW133	$3.700X10^{05}$	$8.700X10^{01}$
NW134	$3.200X10^{05}$	$1.400X10^{03}$
NW135	$4.700X10^{05}$	$9.200X10^{02}$

NW136		
NW137	$2.100X10^{05}$	$7.800X10^{01}$
NW138	$3.200X10^{05}$	$6.800X10^{02}$
NW139		
NW140	$8.800X10^{05}$	$1.500X10^{03}$
NW141	$3.900X10^{05}$	$8.500X10^{01}$
NW142		
NW143	$4.100X10^{05}$	$1.300X10^{03}$
NW144	$3.500X10^{05}$	$7.700X10^{01}$
NW145	$1.800X10^{05}$	$1.000X10^{02}$
NW146	$2.500X10^{05}$	$4.500X10^{01}$
NW147	$6.100X10^{05}$	$1.600X10^{02}$
NW148		
NW149	$1.500X10^{05}$	$9.000X10^{01}$
NW150	$1.900X10^{05}$	$8.100X10^{02}$
NW151	$8.700X10^{04}$	$5.500X10^{02}$
NW152		
NW153		$1.600X10^{02}$
NW154	$2.100X10^{05}$	$1.600X10^{02}$
NW155	$9.600X10^{04}$	$5.000X10^{01}$
NW156		
NW157	$1.300X10^{05}$	$2.500X10^{02}$
NW158	$1.300X10^{05}$	$1.200X10^{02}$
NW159		
NW160	$1.300X10^{05}$	$4.100X10^{01}$
NW161	$1.900X10^{05}$	$2.100X10^{01}$
NW162	$2.900X10^{05}$	$1.500X10^{01}$
NW163	$2.200X10^{05}$	$4.300X10^{02}$
NW164		
NW165		
NZS13	$6.500X10^{05}$	$1.300X10^{02}$
NZ024	$3.300X10^{05}$	$1.400X10^{02}$
GB401		
GB410	$4.300X10^{07}$	$4.500X10^{05}$
GB411	$4.500X10^{07}$	$1.600X10^{07}$
GB412		
GB420	$5.200X10^{07}$	$1.100X10^{07}$
GB421	$7.200X10^{07}$	$1.100X10^{08}$
GB425	$4.700X10^{07}$	$1.300X10^{07}$
GB428	$1.400X10^{08}$	$5.100X10^{04}$
GB429	$5.000X10^{07}$	$3.800X10^{05}$
GB430		
GB431	$9.300X10^{07}$	$1.000X10^{06}$
GB432		
GB434	$1.600X10^{08}$	$3.900X10^{05}$
GB435	$5.000X10^{07}$	$1.400X10^{05}$
GB436		
GB437		
GB438	$8.300X10^{07}$	$1.600X10^{05}$
GB440	$1.000X10^{08}$	$6.000X10^{05}$
GB442	$6.800X10^{07}$	$5.900X10^{05}$
GB444		$2.500X10^{06}$
GB445		$2.100X10^{06}$
GW401		
GW402	$3.200X10^{04}$	$1.200X10^{03}$
GW403	$2.000X10^{04}$	$5.800X10^{02}$
GW404		
GW405	$2.100X10^{04}$	$4.700X10^{02}$
GW406	$3.100X10^{04}$	$8.800X10^{02}$
GW407	$2.800X10^{04}$	$5.600X10^{02}$

GW408		
GW409		
GW410		
GW411	$2.200X10^{04}$	$1.900X10^{03}$
GW412		
GW413		
GW414		
GW415	$2.000X10^{04}$	$1.500X10^{02}$
GW416		
GW417	$2.700X10^{04}$	$1.300X10^{03}$
GW418		
GW419		
GW420	$2.300X10^{04}$	$1.800X10^{02}$
GW421	$2.400X10^{04}$	$6.100X10^{02}$
GW422		
GW423		
GW424		
GW425	$5.600X10^{04}$	$1.100X10^{02}$
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GW429	$2.300X10^{04}$	$5.400X10^{01}$
GW430		
GW431	$2.500X10^{04}$	$7.400X10^{01}$
GW432	$1.400X10^{04}$	$6.400X10^{01}$
GW433	$2.000X10^{04}$	$2.800X10^{01}$
GW434	$4.000X10^{04}$	$1.900X10^{02}$
GW435	$1.900X10^{04}$	$3.200X10^{01}$
GW436		
GW437		
GW438	$1.500X10^{04}$	$1.200X10^{02}$
GW439		$1.200X10^{02}$
GW440	$2.400X10^{04}$	$4.800X10^{01}$
GW441	$1.900X10^{04}$	$9.300X10^{02}$
GW442	$2.500X10^{04}$	$2.400X10^{02}$
GW443		
GW444		$2.800X10^{01}$
GW445		$9.800X10^{01}$
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GB502	$7.500X10^{08}$	
GB503		
GB506	$1.100X10^{09}$	$4.600X10^{07}$
GB507		
GB508		
GB509		
GB510	$8.900X10^{08}$	$1.100X10^{06}$
GB511	$6.700X10^{08}$	$3.200X10^{05}$
GB512	$4.700X10^{08}$	$7.100X10^{05}$
GB513	$7.900X10^{08}$	$2.200X10^{06}$
GB514	$4.400X10^{08}$	$4.500X10^{05}$
GB515	$6.900X10^{08}$	$5.400X10^{05}$
GB516		
GB517		
GB518	$2.500X10^{08}$	$7.700X10^{04}$
GB519		
GB520	$5.000X10^{08}$	$5.400X10^{05}$
GB523	$3.900X10^{08}$	$6.400X10^{04}$
GB524	$4.700X10^{08}$	$2.300X10^{06}$
GB525		
GB526		

GB527		
GB528		
GB529	$4.500X10^{08}$	$7.300X10^{05}$
GB532		
GB536		
GB538	$5.400X10^{08}$	
GB545	$2.800X10^{08}$	$7.300X10^{04}$
GB549	$6.200X10^{08}$	$1.000X10^{07}$
GB550		
GB552		
GB554		
GB557	$6.400X10^{08}$	$4.400X10^{06}$
GB558	$8.300X10^{08}$	$4.700X10^{06}$
GW501	$3.000X10^{04}$	$6.100X10^{02}$
GW502	$4.100X10^{04}$	
GW503	$4.000X10^{04}$	
GW504	$2.500X10^{04}$	$4.300X10^{01}$
GW505	$4.100X10^{04}$	$3.400X10^{02}$
GW506	$3.500X10^{04}$	$1.400X10^{03}$
GW507		
GW508		
GW509	$3.700X10^{04}$	
GW510	$4.100X10^{04}$	$1.500X10^{02}$
GW511	$4.100X10^{04}$	$2.300X10^{02}$
GW512	$3.500X10^{04}$	$1.000X10^{01}$
GW513	$4.100X10^{04}$	$2.000X10^{01}$
GW514	$3.800X10^{04}$	$6.700X10^{00}$
GW515	$3.400X10^{04}$	$3.000X10^{00}$
GW516		
GW517		
GW518	$3.700X10^{04}$	$7.000X10^{01}$
GW519		
GW520	$3.300X10^{04}$	$2.000X10^{01}$
GW521	$5.300X10^{04}$	$5.300X10^{01}$
GW522	$9.300X10^{04}$	$1.700X10^{01}$
GW523	$8.300X10^{04}$	$1.300X10^{01}$
GW524	$5.200X10^{04}$	$3.700X10^{01}$
GW525		
GW526		
GW527		
GW528	$5.900X10^{04}$	$1.300X10^{02}$
GW529	$5.800X10^{04}$	$1.000X10^{02}$
GW530	$6.000X10^{04}$	$4.700X10^{01}$
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GW535	$3.700X10^{04}$	$6.000X10^{01}$
GW536	$7.500X10^{04}$	$4.100X10^{02}$
GW537		
GW538	$7.700X10^{04}$	$4.100X10^{02}$
GW539	$4.900X10^{04}$	$5.500X10^{02}$
GW540	$5.000X10^{04}$	
GW541	$1.100X10^{05}$	
GW542	$6.300X10^{04}$	$1.600X10^{02}$
GW543	$5.800X10^{04}$	
GW544		
GW545	$6.500X10^{04}$	$9.000X10^{01}$
GW545A	$6.200X10^{04}$	$2.400X10^{02}$
GW546		

GW547	9.000X10^{09}	4.600X10^{02}
GW548		
GW549	9.600X10^{09}	5.800X10^{02}
GW550	1.300X10^{05}	
GW551		
GW552	8.200X10^{09}	
GW553		
GW554		
GW555		
GW556	6.700X10^{09}	
GW557	8.700X10^{09}	2.000X10^{02}
GW558	4.800X10^{09}	3.000X10^{02}
GW559		3.700X10^{01}
GW560	1.000X10^{05}	1.000X10^{01}
GBS01	2.000X10^{09}	5.700X10^{09}
GBS02	6.500X10^{09}	4.800X10^{05}
GBS04	2.900X10^{09}	2.200X10^{05}
GBS05		
GBS08		
GBS09		
GBS10		
GBS11	2.500X10^{09}	7.300X10^{05}
GBS12	1.000X10^{09}	9.600X10^{09}
GBS17		
GBS18		
GBS19		
GBS22	2.300X10^{09}	2.600X10^{05}
GBS42	2.000X10^{09}	1.300X10^{05}
GBS43		
GBS44	1.800X10^{09}	5.500X10^{05}
GBS63		4.100X10^{05}
GWS00	4.000X10^{05}	1.100X10^{03}
GWS01	1.200X10^{05}	1.200X10^{02}
GWS02	3.500X10^{05}	2.800X10^{02}
GWS03	1.000X10^{06}	1.500X10^{03}
GWS04	5.400X10^{05}	3.700X10^{02}
GWS05		
GWS06		
GWS07		
GWS08		
GWS09		
GWS10		
GWS11	8.300X10^{05}	8.100X10^{02}
GWS12	2.000X10^{05}	1.400X10^{02}
GWS13		
GWS14		
GWS15		
GWS16		
GWS17		
GWS18		
GWS19		
GWS20		
GWS21		
GWS22	2.300X10^{05}	1.800X10^{02}
GWS23		
GWS24		
GWS25	2.500X10^{05}	2.600X10^{02}
GWS26		1.100X10^{02}
GWS27	3.200X10^{05}	3.300X10^{02}
GWS28	3.200X10^{05}	1.900X10^{02}

GW829		
GW830	$1.400X10^{05}$	$3.400X10^{02}$
GW831		
GW832	$2.600X10^{05}$	$3.200X10^{02}$
GW833	$1.400X10^{05}$	$3.100X10^{02}$
GW834		
GW835	$2.300X10^{05}$	$3.100X10^{02}$
GW836		
GW837	$5.400X10^{05}$	
GW838		
GW839	$4.600X10^{05}$	$3.500X10^{02}$
GW840		
GW841	$2.900X10^{05}$	$4.300X10^{02}$
GW842		
GW843	$4.200X10^{05}$	$9.500X10^{01}$
GW844		
GW845	$1.800X10^{05}$	$7.400X10^{01}$
GW846		
GW847	$4.800X10^{05}$	$2.600X10^{02}$
GW848		
GW849	$4.700X10^{05}$	$7.100X10^{02}$
GW850	$4.500X10^{05}$	$3.200X10^{02}$
GW851	$4.400X10^{05}$	$2.100X10^{02}$
GW852	$3.900X10^{05}$	$1.800X10^{02}$
GW853		
GW854	$6.500X10^{05}$	$3.000X10^{02}$
GW855		
GW856	$4.500X10^{05}$	$2.100X10^{02}$
GW857	$5.600X10^{05}$	$2.000X10^{02}$
GW858	$5.000X10^{05}$	$4.700X10^{02}$
GW859		
GW860	$5.200X10^{05}$	$4.600X10^{02}$
GW861	$3.400X10^{05}$	$6.000X10^{02}$
GW862		
GW863		$6.000X10^{02}$
GW865	$9.700X10^{05}$	$5.800X10^{01}$
GZ800	$3.400X10^{05}$	
GZ806	$1.200X10^{06}$	$8.600X10^{04}$
GZ825	$3.900X10^{05}$	$1.000X10^{02}$
GZ829	$2.200X10^{05}$	$1.400X10^{02}$
GZ831	$1.200X10^{05}$	$3.300X10^{02}$
GZ834	$3.900X10^{05}$	$3.500X10^{02}$
GZ836	$3.500X10^{05}$	$4.800X10^{02}$
GZ838	$3.400X10^{05}$	$6.900X10^{02}$
GZ839		
GZ840	$3.100X10^{05}$	$4.900X10^{02}$
GZ844	$9.300X10^{05}$	$1.100X10^{02}$
GZ848	$4.100X10^{05}$	$3.000X10^{02}$
GZ853	$4.100X10^{05}$	$2.900X10^{02}$
GZ855	$4.700X10^{05}$	$2.600X10^{02}$
GZ859	$5.200X10^{05}$	$4.600X10^{02}$
GZ862	$6.700X10^{05}$	$1.400X10^{03}$
GZ864	$2.400X10^{05}$	$7.100X10^{01}$
GB601		
GB602	$3.300X10^{08}$	$3.300X10^{06}$
GB603	$6.500X10^{08}$	$3.800X10^{06}$
GB604		$6.800X10^{05}$
GB605	$8.200X10^{08}$	$2.200X10^{06}$
GB606	$4.900X10^{08}$	$2.800X10^{05}$
GB607	$4.600X10^{08}$	$1.400X10^{05}$

GB608		
GB609	$6.400X10^{08}$	$1.200X10^{06}$
GB610	$5.000X10^{08}$	$1.200X10^{06}$
GB611	$4.000X10^{08}$	$4.700X10^{04}$
GB612		$1.200X10^{05}$
GB613	$5.800X10^{08}$	$1.100X10^{05}$
GB614	$3.700X10^{08}$	$1.800X10^{06}$
GB615		
GB616		$1.100X10^{06}$
GB617		
GB622		
GB624		
GB625	$4.800X10^{08}$	$7.000X10^{05}$
GB626		
GB627	$7.900X10^{08}$	$1.400X10^{06}$
GB628	$5.800X10^{08}$	$7.900X10^{06}$
GB629		
GB630		
GB631		
GB632		$4.600X10^{04}$
GB633	$4.400X10^{08}$	$6.800X10^{06}$
GB634	$4.800X10^{08}$	$8.500X10^{06}$
GB635		
GB636	$2.300X10^{08}$	
GB640		
GB644	$3.500X10^{08}$	$8.700X10^{05}$
GB649	$7.000X10^{08}$	
GB650	$3.100X10^{08}$	
GB652	$5.500X10^{08}$	
GB653	$4.300X10^{08}$	
GB654		
GB660	$3.400X10^{08}$	$6.900X10^{06}$
GB669		
GB677	$3.800X10^{08}$	
GW601	$1.100X10^{05}$	$1.200X10^{03}$
GW602	$9.300X10^{04}$	$3.100X10^{02}$
GW603	$8.200X10^{04}$	$3.200X10^{02}$
GW604	$8.300X10^{04}$	$1.400X10^{02}$
GW605	$6.000X10^{04}$	$2.500X10^{01}$
GW606	$8.200X10^{04}$	$6.000X10^{01}$
GW607	$8.500X10^{04}$	$1.800X10^{01}$
GW608	$1.100X10^{05}$	$2.400X10^{03}$
GW609	$5.200X10^{04}$	$2.400X10^{01}$
GW610	$5.600X10^{04}$	$6.900X10^{01}$
GW611	$9.500X10^{04}$	$7.300X10^{00}$
GW612	$6.200X10^{04}$	$1.400X10^{02}$
GW613	$3.900X10^{04}$	$8.800X10^{00}$
GW614	$1.200X10^{05}$	$5.400X10^{01}$
GW615	$8.500X10^{04}$	$4.400X10^{01}$
GW616	$7.800X10^{04}$	$7.500X10^{02}$
GW617	$6.600X10^{04}$	$1.100X10^{03}$
GW618	$4.900X10^{04}$	$8.200X10^{02}$
GW619	$1.100X10^{05}$	$2.200X10^{02}$
GW620	$1.100X10^{05}$	$4.200X10^{02}$
GW621	$1.600X10^{05}$	$3.200X10^{02}$
GW622	$8.200X10^{04}$	$2.100X10^{02}$
GW623	$6.400X10^{04}$	$1.700X10^{02}$
GW624	$4.900X10^{04}$	$3.500X10^{02}$
GW625	$9.300X10^{04}$	$7.700X10^{01}$
GW626	$6.800X10^{04}$	$8.000X10^{01}$

GW627	$9.900X10^{04}$	$6.200X10^{01}$
GW628	$1.200X10^{05}$	$9.800X10^{02}$
GW629		
GW630		
GW631	$8.200X10^{04}$	$9.500X10^{01}$
GW632	$6.200X10^{04}$	$7.500X10^{01}$
GW633	$1.100X10^{05}$	$5.900X10^{03}$
GW634	$1.200X10^{05}$	$4.500X10^{03}$
GW635	$9.700X10^{04}$	
GW636	$1.100X10^{05}$	
GW637	$6.600X10^{04}$	
GW638	$9.700X10^{04}$	$9.500X10^{02}$
GW639	$7.000X10^{04}$	
GW640	$8.900X10^{04}$	
GW641	$6.000X10^{04}$	
GW642	$1.100X10^{05}$	$1.500X10^{03}$
GW643	$9.300X10^{04}$	
GW644		
GW645	$5.000X10^{04}$	
GW646	$1.400X10^{05}$	$1.500X10^{03}$
GW647	$7.600X10^{04}$	$6.400X10^{03}$
GW648		
GW649	$6.000X10^{04}$	
GW650	$8.900X10^{04}$	
GW651	$6.400X10^{04}$	
GW652	$6.200X10^{04}$	
GW653	$7.800X10^{04}$	$2.800X10^{04}$
GW654	$7.400X10^{04}$	$2.000X10^{04}$
GW655	$6.400X10^{04}$	
GW656	$7.600X10^{04}$	
GW657	$6.600X10^{04}$	
GW658	$5.400X10^{04}$	
GW659	$1.800X10^{05}$	$1.200X10^{04}$
GW660	$7.400X10^{04}$	$6.000X10^{03}$
GW661	$7.200X10^{04}$	$4.500X10^{04}$
GW662	$6.800X10^{04}$	
GW663	$1.000X10^{05}$	
GW664	$1.400X10^{05}$	
GW665	$4.500X10^{04}$	
GW666	$7.200X10^{04}$	
GW667	$3.700X10^{04}$	
GW668	$4.900X10^{04}$	
GW669	$5.400X10^{04}$	
GW670	$8.200X10^{04}$	
GW671	$9.500X10^{04}$	
GW672	$8.900X10^{04}$	
GW673	$1.700X10^{05}$	
GW674	$4.300X10^{04}$	
GW675	$6.200X10^{04}$	
GW676	$1.000X10^{05}$	
GW677	$4.900X10^{04}$	
GW678	$7.600X10^{04}$	
GW679	$1.300X10^{05}$	
GW680	$6.800X10^{04}$	
GW681	$5.600X10^{04}$	
GW682	$6.400X10^{04}$	
GW683	$9.100X10^{04}$	
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BB602		
BB603		

BB604		
BB605		
BB606		
BB607		
BB608		
BB609	$1.600X10^{09}$	$5.700X10^{05}$
BB610	$1.300X10^{09}$	$2.600X10^{05}$
BB611	$5.400X10^{08}$	$1.200X10^{07}$
BB612	$1.900X10^{09}$	$2.500X10^{07}$
BB613	$1.300X10^{09}$	$1.700X10^{06}$
BB614	$2.000X10^{09}$	$9.400X10^{05}$
BB615	$1.000X10^{09}$	$6.800X10^{06}$
BB616	$1.700X10^{09}$	
BB617	$2.400X10^{09}$	$3.000X10^{07}$
BB618	$2.000X10^{09}$	
BB619	$2.200X10^{09}$	$1.400X10^{07}$
BB620	$1.100X10^{09}$	$1.300X10^{05}$
BB621		
BB622	$2.800X10^{09}$	$1.800X10^{07}$
BB623		
BB624	$3.100X10^{09}$	$1.800X10^{06}$
BB626	$9.900X10^{08}$	$3.400X10^{05}$
BB627	$9.700X10^{08}$	$1.900X10^{06}$
BB628		
BB629		
BB630	$1.600X10^{09}$	$6.400X10^{06}$
BB631	$2.200X10^{09}$	$2.200X10^{06}$
BB632	$1.200X10^{09}$	$7.800X10^{05}$
BB633	$2.000X10^{09}$	$3.300X10^{06}$
BB634	$1.400X10^{09}$	$1.500X10^{06}$
BB635	$1.600X10^{09}$	
BB636	$1.000X10^{09}$	$3.500X10^{05}$
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BB642	$1.900X10^{09}$	$2.800X10^{05}$
BB643	$1.000X10^{09}$	$2.300X10^{06}$
BB644	$1.400X10^{09}$	$3.700X10^{06}$
BB645	$1.600X10^{09}$	$2.900X10^{06}$
BB646		
BI009	$2.300X10^{05}$	
BW601		
BW604		
BW606		
BW608		
BW609	$2.000X10^{05}$	$6.200X10^{03}$
BW610	$3.900X10^{05}$	$5.800X10^{03}$
BW611		$1.300X10^{04}$
BW612	$4.500X10^{05}$	$9.700X10^{03}$
BW613	$2.200X10^{05}$	$1.800X10^{04}$
BW614	$5.800X10^{05}$	
BW615	$7.800X10^{05}$	$8.600X10^{03}$
BW616	$6.100X10^{05}$	
BW617	$8.700X10^{05}$	$1.800X10^{03}$
BW618	$7.500X10^{05}$	$1.300X10^{04}$
BW619	$4.800X10^{05}$	$4.000X10^{02}$
BW620	$4.600X10^{05}$	$4.000X10^{02}$
BW621	$9.200X10^{05}$	

BW622	$5.300X10^{05}$	$1.400X10^{03}$
BW623	$5.600X10^{05}$	$2.300X10^{03}$
BW624	$4.700X10^{05}$	$7.300X10^{01}$
BW625		
BW626	$5.500X10^{05}$	$8.600X10^{02}$
BW627	$1.000X10^{06}$	$3.500X10^{03}$
BW628	$6.800X10^{05}$	$9.000X10^{02}$
BW629	$5.900X10^{05}$	$8.300X10^{01}$
BW630	$6.600X10^{05}$	$5.400X10^{02}$
BW631	$7.600X10^{05}$	$1.400X10^{03}$
BW632	$8.000X10^{05}$	$1.900X10^{03}$
BW633	$6.600X10^{05}$	$1.600X10^{03}$
BW634	$1.000X10^{06}$	$2.100X10^{03}$
BW635	$5.500X10^{05}$	$1.200X10^{03}$
BW636	$7.300X10^{05}$	$3.900X10^{03}$
BW637	$8.000X10^{05}$	$8.600X10^{03}$
BW638	$1.100X10^{06}$	$3.000X10^{03}$
BW639	$5.400X10^{05}$	$9.700X10^{01}$
BW640	$4.900X10^{05}$	$6.000X10^{03}$
BW641	$5.900X10^{05}$	$1.000X10^{03}$
BW642	$5.700X10^{05}$	$1.900X10^{03}$
BW643	$9.700X10^{05}$	$1.300X10^{03}$
BW644	$1.100X10^{06}$	$3.700X10^{02}$
BW645	$1.200X10^{06}$	$1.000X10^{02}$
BW646	$8.500X10^{05}$	$2.800X10^{02}$
EB101	$1.300X10^{09}$	$1.400X10^{05}$
EB102	$9.000X10^{08}$	$8.400X10^{04}$
EB103	$2.000X10^{09}$	$6.200X10^{05}$
EB104	$2.200X10^{09}$	$2.300X10^{05}$
EB105	$7.900X10^{08}$	$1.100X10^{05}$
EB106	$2.800X10^{09}$	$5.700X10^{05}$
EB107	$2.400X10^{09}$	$4.500X10^{05}$
EB108	$6.900X10^{09}$	$6.700X10^{05}$
EB109	$1.900X10^{09}$	$4.300X10^{05}$
EB110	$2.100X10^{09}$	$5.400X10^{05}$
EB111	$1.500X10^{09}$	$3.500X10^{05}$
EB112	$1.400X10^{09}$	$3.000X10^{05}$
EB113	$1.500X10^{09}$	$2.600X10^{05}$
EB114	$2.000X10^{09}$	$2.900X10^{05}$
EB115	$1.500X10^{09}$	$2.400X10^{05}$
EB116	$2.800X10^{08}$	$2.100X10^{04}$
EB117	$1.500X10^{09}$	$1.600X10^{05}$
EB118	$1.500X10^{09}$	$2.600X10^{05}$
EB119	$1.000X10^{09}$	$4.300X10^{04}$
EB120	$9.000X10^{08}$	$1.600X10^{04}$
EB124	$3.700X10^{08}$	$4.000X10^{04}$
EB125		
EB126		
EB127	$2.600X10^{08}$	$1.100X10^{05}$
EB128	$1.400X10^{09}$	$5.400X10^{04}$
EB129	$1.800X10^{08}$	$3.400X10^{04}$
EW101	$1.100X10^{05}$	$1.100X10^{02}$
EW102	$2.900X10^{05}$	$2.100X10^{02}$
EW103	$1.100X10^{05}$	$1.600X10^{02}$
EW104	$1.300X10^{05}$	$1.500X10^{02}$
EW105	$2.400X10^{05}$	$1.100X10^{02}$
EW106	$7.800X10^{04}$	$2.400X10^{02}$
EW107		
EW108	$7.800X10^{04}$	$1.900X10^{02}$
EW109	$8.600X10^{04}$	$1.900X10^{02}$

EW110	$6.800X10^{04}$	$2.400X10^{02}$
EW111	$5.600X10^{04}$	$1.400X10^{02}$
EW112	$9.600X10^{04}$	$4.100X10^{02}$
EW113	$8.300X10^{04}$	$1.400X10^{02}$
EW114	$9.800X10^{04}$	$1.300X10^{02}$
EW115	$1.200X10^{04}$	$1.200X10^{02}$
EW116	$1.200X10^{05}$	$1.300X10^{02}$
EW117	$1.400X10^{05}$	$6.800X10^{01}$
EW118	$1.600X10^{05}$	$2.700X10^{02}$
EW119	$1.500X10^{05}$	$3.800X10^{02}$
EW120	$1.200X10^{05}$	$2.000X10^{01}$
EW121	$1.700X10^{05}$	$7.500X10^{01}$
EW122	$1.000X10^{05}$	$1.600X10^{01}$
EW123	$1.300X10^{05}$	$8.700X10^{01}$
EW124	$9.800X10^{04}$	$1.500X10^{02}$
EW125	$4.600X10^{05}$	$2.300X10^{02}$
EW126	$1.400X10^{05}$	$2.100X10^{02}$
EW127	$1.200X10^{05}$	$1.800X10^{02}$
EW128	$2.200X10^{05}$	$4.000X10^{02}$
EW129	$2.000X10^{05}$	$2.000X10^{03}$
EZ125	$1.000X10^{05}$	$2.000X10^{02}$
EZ126	$1.500X10^{05}$	$1.800X10^{02}$
AA206		
AA228	$7.000X10^{07}$	
AA301	$5.600X10^{05}$	
AA302	$1.600X10^{05}$	
AA303	$2.000X10^{05}$	
AA304		
AA305		
AA306		
AA307		
AA308		
AA309		
AA310	$2.400X10^{09}$	
AA311		
AB203	$5.300X10^{07}$	
AB211	$3.200X10^{06}$	
AB216	$9.400X10^{07}$	
AB222	$2.100X10^{07}$	
AB238	$1.800X10^{08}$	
AB247	$2.200X10^{07}$	
AB250	$1.400X10^{08}$	
AB253	$5.600X10^{08}$	
AB262	$1.800X10^{08}$	
AB270	$2.400X10^{09}$	
AB273	$4.700X10^{09}$	
AB276	$2.100X10^{09}$	
AB279	$7.400X10^{08}$	
AB285	$2.600X10^{09}$	
AB288	$4.200X10^{09}$	
AB291	$9.800X10^{09}$	
AB294	$3.400X10^{09}$	
AW202	$1.100X10^{06}$	
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AW211	$7.700X10^{05}$	
AW213	$2.900X10^{05}$	
AW214	$3.200X10^{05}$	
AW215	$1.600X10^{06}$	
AW216	$3.200X10^{05}$	
AW217	$4.000X10^{05}$	

AW219	$2.900X10^{05}$
AW220	$3.600X10^{05}$
AW221	$7.000X10^{06}$
AW222	$3.500X10^{05}$
AW223	$2.600X10^{05}$
AW225	$2.200X10^{05}$
AW226	$3.700X10^{05}$
AW227	$4.500X10^{05}$
AW228	$2.400X10^{05}$
AW229	$1.600X10^{05}$
AW232	$3.200X10^{05}$
AW233	$2.400X10^{05}$
AW234	$1.100X10^{06}$
AW235	$2.800X10^{05}$
AW236	$3.400X10^{05}$
AW237	$6.600X10^{05}$
AW238	$1.600X10^{05}$
AW239	$1.400X10^{05}$
AW241	$9.800X10^{04}$
AW242	$9.200X10^{04}$
AW243	$3.800X10^{05}$
AW244	$2.100X10^{05}$
AW245	$3.600X10^{05}$
AW246	$7.500X10^{05}$
AW247	$1.500X10^{05}$
AW248	$6.300X10^{05}$
AW250	$1.900X10^{05}$
AW251	$2.200X10^{05}$
AW253	$2.300X10^{05}$
AW254	$1.900X10^{05}$
AW256	$4.800X10^{05}$
AW257	$5.100X10^{05}$
AW258	$4.700X10^{05}$
AW259	$3.000X10^{05}$
AW260	$6.600X10^{05}$
AW261	$1.000X10^{06}$
AW262	$1.400X10^{05}$
AW263	$1.800X10^{05}$
AW265	$1.600X10^{05}$
AW266	$1.400X10^{05}$
AW267	$1.900X10^{05}$
AW270	$8.500X10^{04}$
AW271	$8.500X10^{04}$
AW273	$1.100X10^{05}$
AW274	$8.400X10^{05}$
AW276	$2.600X10^{05}$
AW277	$1.200X10^{05}$
AW279	$1.200X10^{05}$
AW280	$8.500X10^{04}$
AW282	$2.000X10^{05}$
AW283	$1.500X10^{05}$
AW284	$2.300X10^{05}$
AW285	
AW286	
AW288	
AW289	
AW291	
AW292	
AW294	
AW295	

Table 3. Counts of bacteria ± standard deviations (numbers g^{-1})

		Number of Samples	Direct Count	Viable Count	Hydrocarbon Utilizers
Ice	Winter 1976		$9.9\pm8.2\times10^{4}$	$6.6\pm1.8\times10^{1}$	-
Water	Summer 1975	40	$8.2\pm7.2\times10^{5}$	$9.6\pm4.8\times10^{3}$	-
	Winter 1976	20	$1.8\pm1.3\times10^{5}$	$6.1\pm7.0\times10^{2}$	-
	Summer 1976	20	$5.2\pm3.9\times10^{5}$	$5.0\pm3.2\times10^{4}$	-
	Summer 1978	50	$6.7\pm4.9\times10^{5}$	$3.5\pm2.9\times10^{4}$	$2.6\pm1.3\times10^{1}$
Sediment	Summer 1975	30	$6.2\pm1.1\times10^{8}$	$2.0\pm1.1\times10^{6}$	-
	Winter 1976	20	$3.7\pm1.0\times10^{8}$	$2.5\pm1.9\times10^{5}$	-
	Summer 1976	20	$2.1\pm0.9\times10^{9}$	$8.3\pm6.7\times10^{6}$	-
	Summer 1978	40	$1.6\pm0.8\times10^{9}$	$5.3\pm3.2\times10^{6}$	$2.5\pm2.2\times10^{4}$

than those for the copiotrophic bacteria enumerated on marine agar. The numbers of hydrocarbon utilizers were typical of pristane waters (Table 3).

Gulf of Alaska

There was no significant differences in viable counts between March and October samples in the Gulf of Alaska despite warmer fall temperatures (Table 4). In offshore surface waters viable counts ranged from 1.0×10^1 to 6.1×10^2/ml; intertidal water samples had significantly higher viable counts, 3.8×10^2 to 1.1×10^5/ml, viable counts in sediments ranged from 3.7×10^3 to 1.7×10^7/g dry wt. In most regions numbers of hydrocarbon degraders were low (Table 5).

Cook Inlet

Total numbers of microorganisms were about an order of magnitude lower in the northern and central portions of lower Cook Inlet than elsewhere in the lower Cook Inlet region (Fig. 2). The greatest microbial biomass was found just southeast of the entrance to Cook Inlet. In both lower Cook Inlet and Shelikof Strait the range of total numbers of microorganisms was similar, generally with only one order of magnitude variation.

Numbers of hydrocarbon utilizers within Cook Inlet were much more variable than numbers of total microorganisms (Fig. 3). Relatively higher concentrations of hydrocarbon utilizers were found within nearshore regions than within the central portions of the Inlet. High concentrations of hydrocarbon utilizers also were found at the upper end of Shelikof Strait and just southeast of the entrance to Cook Inlet. The largest determined number of hydrocarbon utilizers occurred in the northern portion of Kamishak Bay in a region known as Oil Bay.

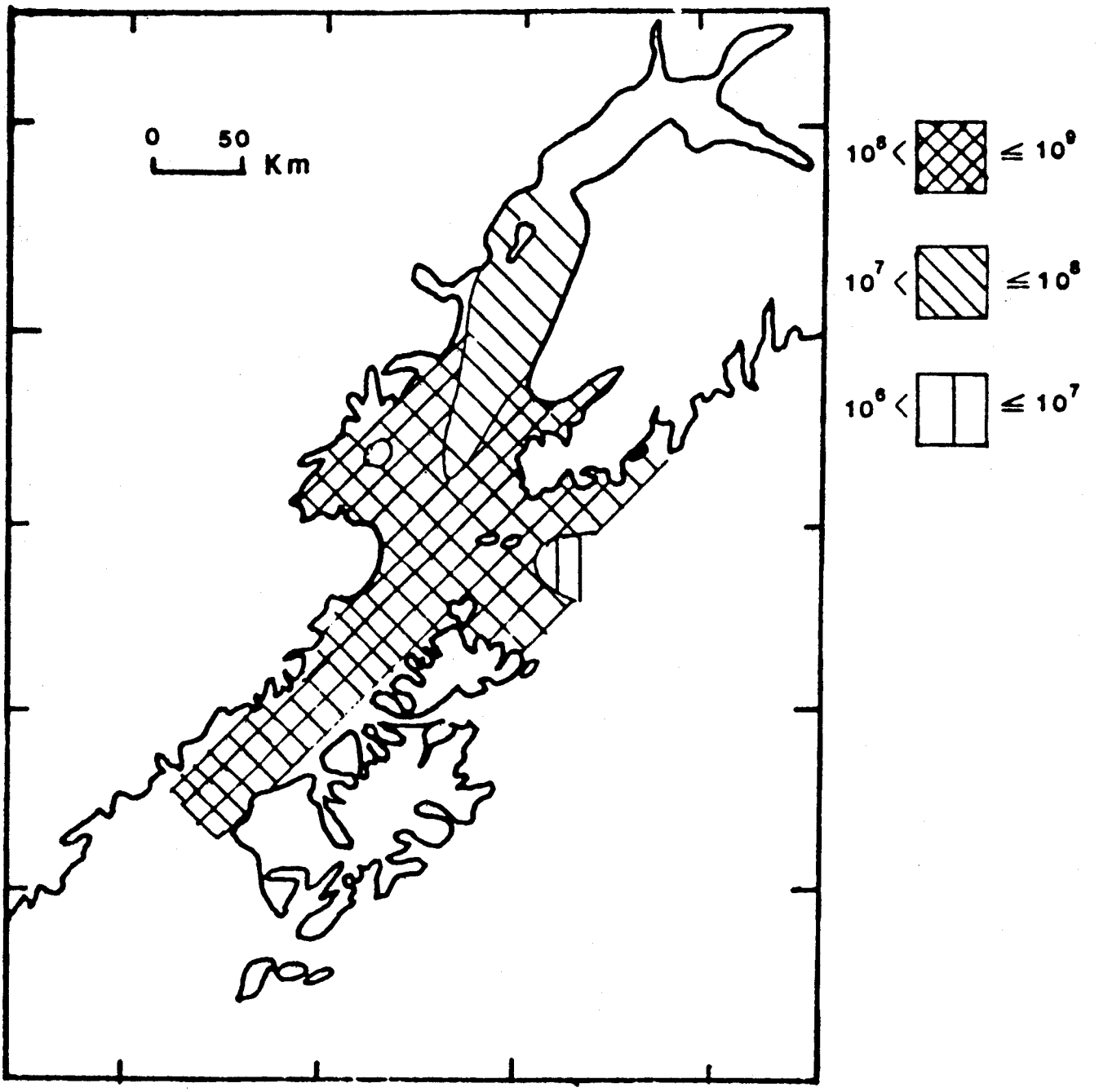

Figure 2. Direct counts of total microorganisms in Cook Inlet (g^{-1}).

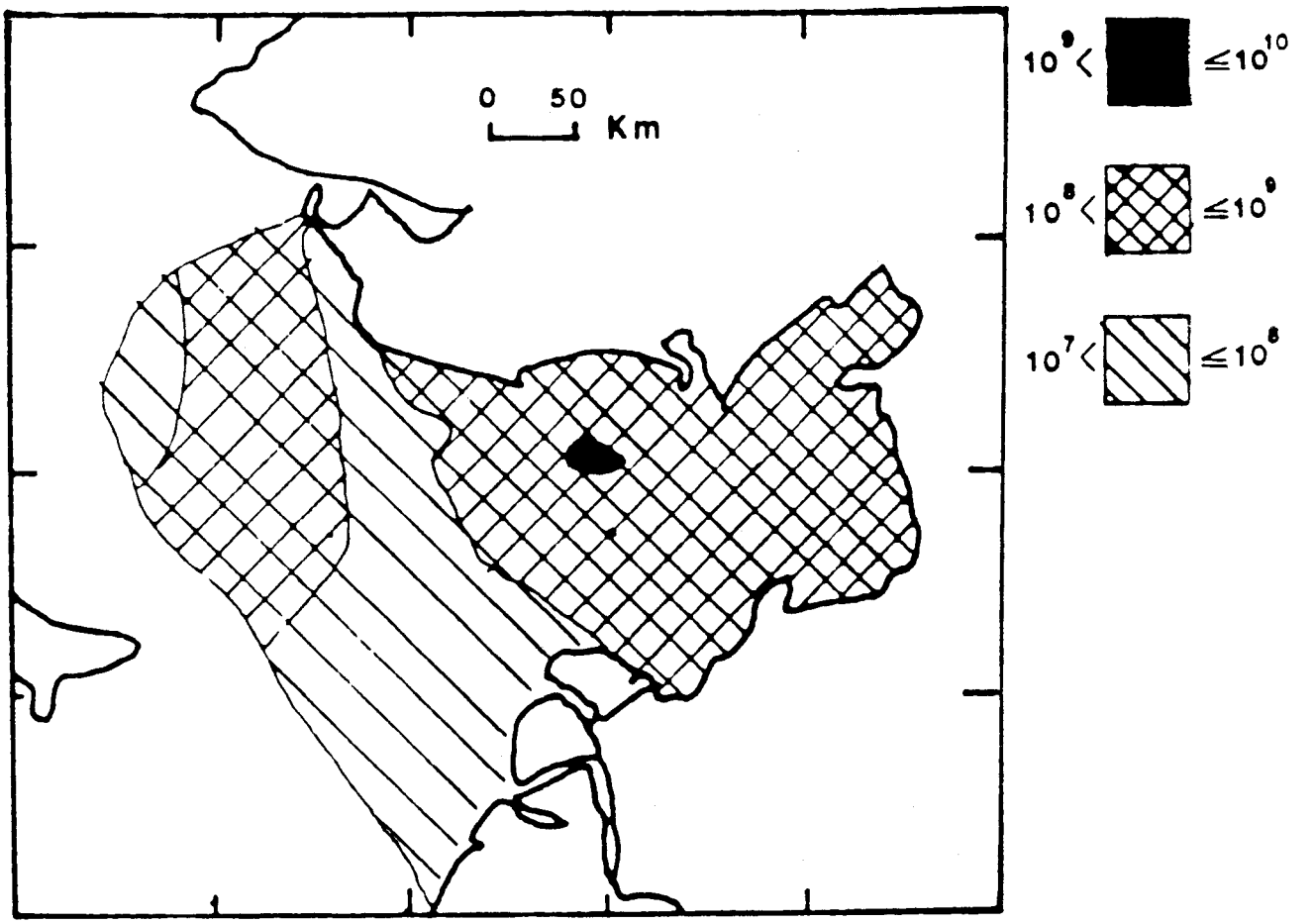

Figure 3. Direct counts of total microorganisms in Norton Sound (g^{-1}).

Table 4. Viable counts in Gulf of Alaska

Station	Viable count at 5°C (ml^{-1} or g^{-1})	Viable count at 20°C (ml^{-1} or g^{-1})
	Northwest Gulf of Alaska	
Water Samples		
101	$5{\cdot}5 \times 10^1$	-
106	$6{\cdot}5 \times 10^1$	-
119	$3{\cdot}5 \times 10^1$	$4{\cdot}3 \times 10^1$
124	$9{\cdot}7 \times 10^1$	$8{\cdot}0 \times 10^1$
133	$9{\cdot}2 \times 10^1$	$1{\cdot}1 \times 10^2$
137	$1{\cdot}2 \times 10^2$	-
145	$1{\cdot}3 \times 10^2$	$3{\cdot}1 \times 10^2$
148	$1{\cdot}9 \times 10^2$	$5{\cdot}7 \times 10^2$
156	$2{\cdot}5 \times 10^2$	$2{\cdot}0 \times 10^3$
159	$2{\cdot}2 \times 10^2$	$5{\cdot}6 \times 10^2$
Sediment samples		
101	$1{\cdot}0 \times 10^4$	-
121	$1{\cdot}1 \times 10^6$	$8{\cdot}4 \times 10^5$
134	$1{\cdot}4 \times 10^6$	$1{\cdot}2 \times 10^6$
137	$6{\cdot}2 \times 10^5$	$3{\cdot}5 \times 10^5$
	Northeast Gulf of Alaska	
Water samples		
1	$3{\cdot}3 \times 10^1$	$3{\cdot}9 \times 10^1$
4	$2{\cdot}1 \times 10^1$	$2{\cdot}0 \times 10^1$
7	$3{\cdot}1 \times 10^1$	$2{\cdot}2 \times 10^1$
29	$1{\cdot}5 \times 10^1$	$1{\cdot}8 \times 10^1$
30	$3{\cdot}3 \times 10^1$	$3{\cdot}2 \times 10^1$
32	$1{\cdot}2 \times 10^1$	$5{\cdot}8 \times 10^1$
52	$7{\cdot}2 \times 10^1$	$1{\cdot}0 \times 10^2$
53	$5{\cdot}9 \times 10^1$	$2{\cdot}6 \times 10^1$
57	$1{\cdot}0 \times 10^1$	$2{\cdot}7 \times 10^1$
59	$1{\cdot}4 \times 10^1$	$1{\cdot}7 \times 10^1$
A	$7{\cdot}7 \times 10^2$	$2{\cdot}9 \times 10^3$
D	$2{\cdot}2 \times 10^4$	$2{\cdot}0 \times 10^4$
E	$1{\cdot}1 \times 10^3$	$9{\cdot}9 \times 10^2$
F	$3{\cdot}8 \times 10^2$	$3{\cdot}8 \times 10^2$
Sediment samples		
1	$5{\cdot}2 \times 10^5$	$2{\cdot}1 \times 10^5$
4	$1{\cdot}1 \times 10^5$	$5{\cdot}5 \times 10^4$
30	$5{\cdot}8 \times 10^3$	$1{\cdot}2 \times 10^5$
32	$1{\cdot}1 \times 10^5$	$6{\cdot}0 \times 10^4$
37	$3{\cdot}7 \times 10^3$	$3{\cdot}3 \times 10^3$
52	$1{\cdot}7 \times 10^5$	$4{\cdot}7 \times 10^5$
53	$3{\cdot}0 \times 10^6$	$6{\cdot}1 \times 10^5$
57	$4{\cdot}2 \times 10^5$	$3{\cdot}1 \times 10^7$
A	$4{\cdot}0 \times 10^6$	$1{\cdot}7 \times 10^5$
D	$1{\cdot}8 \times 10^5$	$2{\cdot}4 \times 10^3$
F	$1{\cdot}5 \times 10^3$	$4{\cdot}4 \times 10^3$

Table 5. Enumeration of hydrocarbon utilizers.

Source	Sample	#/ml or #/g (dry wt) Mean	Standard deviation	Range	No. of samples
Northwest Gulf of Alaska					
Oct. 1975	Water	1.8	4.7	0.01-15	10
Oct. 1975	Sediment	8.9	3.1	0.1-12	8
Northeast Gulf of Alaska					
March 1976	Water	1.3	1.7	0.2-6	10
March 1976	Sediment	3.5	3.6	0.6-12	9
Cook Inlet					
Oct. 1976	Water	64	153	0.1-680	15
Oct. 1976	Sediment	2,980	7,020	66-26,000	11
April 1977	Water	37	130	0.01-680	20
April 1977	Sediment	6,670	9,110	1-33,000	11
Nov. 1977	Water	37	58	0.02-210	16
Nov. 1977	Sediment	8,420	8,370	23-20,000	8

Bering Sea

The data in Table 6 show population levels of microorganisms in water and sediment in several regions of the Bering Sea, including total counts and numbers of hydrocarbon degraders. Within Norton Sound, along a northwesterly path from the mouth of the Yukon River, concentrations of microbial biomass were found to be lower than elsewhere in the Sound (Fig. 4). The highest numbers of microorganisms were found near the reported Norton Sound gas seepage.

Northwest from the mouth of the Yukon River the numbers of measured hydrocarbon utilizers are low (Fig. 5). There is a localized area of high numbers of hydrocarbon utilizers near the southwest outlet of the Yukon River and a more extensive area of relatively high numbers of hydrocarbon utilizers at the southeast inner end of the Sound. No elevated numbers of hydrocarbon utilizers, above the background numbers characteristic of the region, were found near the site of the Norton Sound gas seepage.

The numbers of hydrocarbon degraders and total microbial counts are shown in Table 6. The lowest total numbers of microorganisms were found in the open waters of the northern Bering Sea whereas the most viable populations in water were found in the near shore waters of the Aleutian Shelf. Populations of hydrocarbon degrading microorganisms in the water column were very low in all areas of the Bering. Only the northern Bering and the north Aleutian Shelf had significant populations of hydrocarbon degrading organisms in water in ice covered areas. Very few of the sediments examined had large populations of hydrocarbon degraders, the majority of the stations sampled had less than 1000 hydrocarbon degraders per g of sediment.

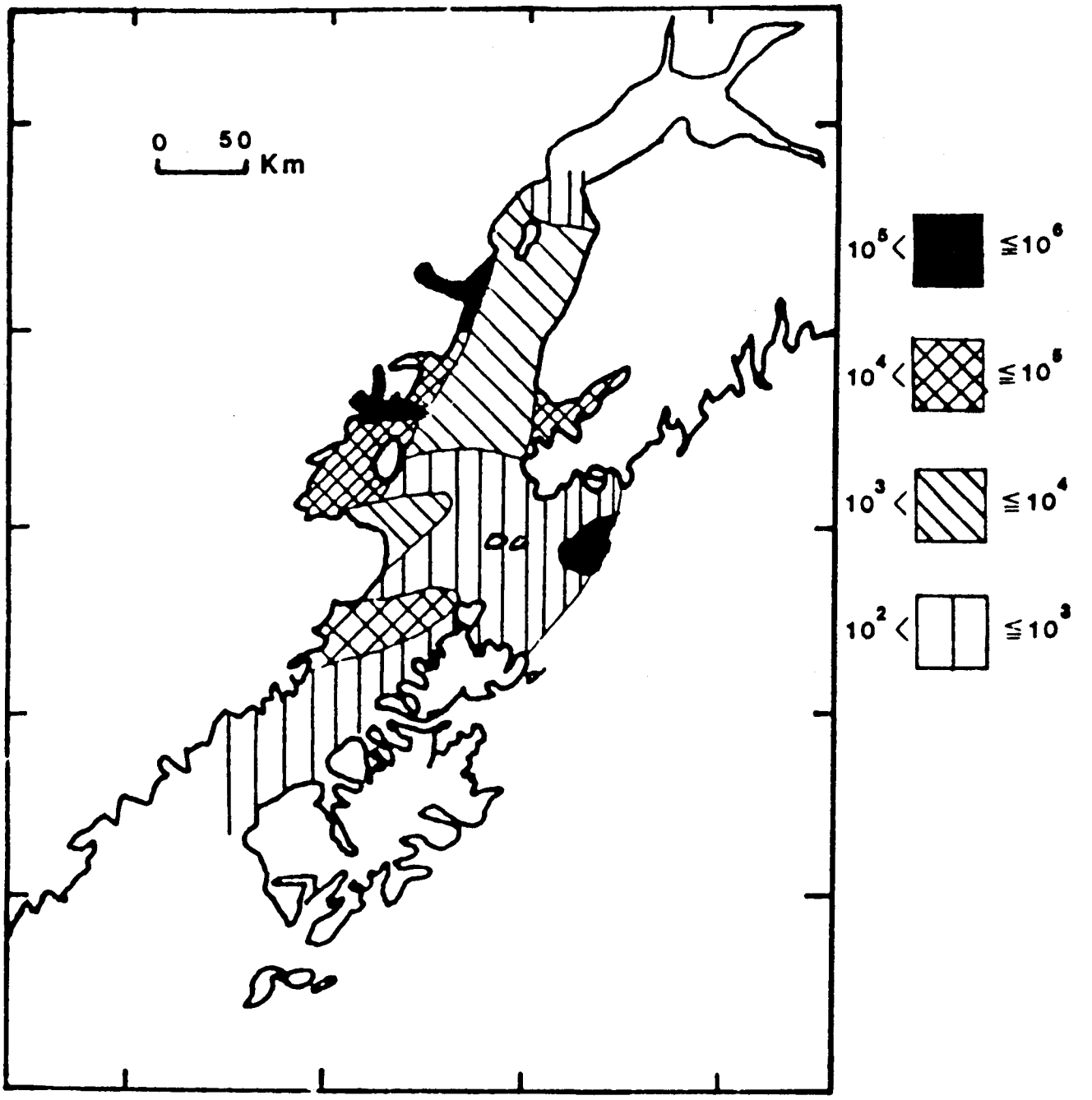

Fig. 4 Most Probable Numbers of hydrocarbon utilizers in Cook Inlet (g^{-1}).

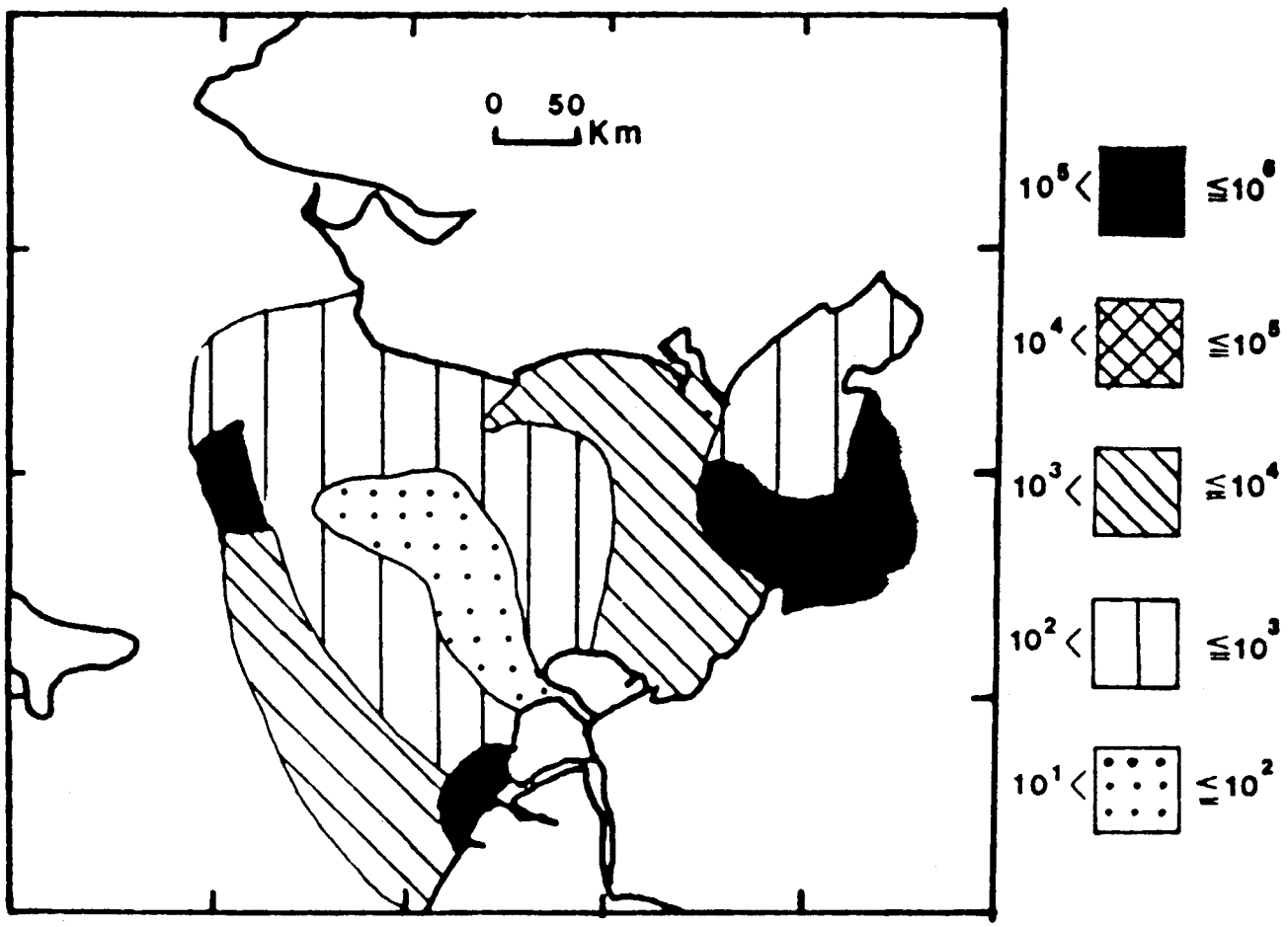

Figure 5. Most probable numbers of hydrocarbon utilizers in Norton Sound (g^{-1}).

Table 6. Enumeration of microbial populations including hydrocarbon utilizers.

	North Bering Sea April 1979	Norton Sound Aug. 1979	Mid-North Bering Sea May-June 1980	South Bering Sea Aug. 1980	South Bering Sea Jan. 1981
Direct Counts Water	1.4×10^5 ± 0.87	2.8×10^5 ± 1.47	1.98×10^5 ± 1.74	2.6×10^5 ± 1.5	9.3×10^5 ± 7.2
Direct Counts Sediment	1.7×10^9 ± 1.4	2.1×10^8 ± 1.88	2.3×10^9 ± 1.4	1.9×10^9 ± 2.5	2.95×10^9 ± 2.26
MPN Hydrocarbon Utilizers Water	11.3 ± 12	-	1.1 ± 5.7	0.1	2.2 ± 6.2
MPN Hydrocarbon Utilizers Sediment	2.6×10^3 ± 3.3	92×10^2 ± 163	$<3 \times 10^2$	30×10^2 ± 0	24.7×10^2 ± 17

TAXONOMY OF INDIGENOUS MICROBIAL POPULATIONS

General Characteristics of Beaufort Sea Isolates

Table 7 summarizes the occurrence of selected features in the bacterial populations. Gram-negative rods predominated in water and sediment. The majority of 4°C isolates from water were orange. Of the 20°C isolates, more from sediment than from water were pigmented. The 20°C isolates grew at higher temperatures and salinities than did the 4°C isolates. More sediment than water isolates tolerated low pH. Most isolates grew at 5 to 15°C, pH 6 to 8 and salinities of 3% (w/v) NaCl.

More sediment than water isolates hydrolysed starch and gelatin. The 20°C isolates from water and sediment were equally lipolytic, but more 4°C sediment isolates than water isolates were lipolytic. Nitrate reduction was common in 4°C sediment isolates but rare in 4°C water isolates. Almost equal numbers of 20°C isolates reduced nitrate. Few strains were oxidase-positive. Isolates

Table 7. Occurrence of selected features in water and sediment bacterial populations isolated at 4 and 20°C (expressed as a percentage of the total scored in each group for the given test)

	4°C isolates		20°C isolates	
Feature	Water	Sediment	Water	Sediment
Rod-shaped	100	100	98	99
Curved axis	29	56	13	34
Gram-negative	99	99	94	97
Motile	4	48	5	5
Capsule	19	26	8	18
Length				
1.1-2.0 µm	29	10	33	19
2.1-3.0 µm	40	30	26	27
3.1-4.0 µm	18	40	17	27
Non-pigmented	23	56	65	32
Orange	67	23	11	30
Yellow	5	10	21	33
Growth at:				
5°C	100	100	98	97
10°C	100	99	98	99
15°C	98	99	99	99
20°C	82	56	98	99
25°C	18	20	89	77
37°C	1	0	7	4
pH 5	52	82	53	74
pH 6	90	96	98	100
pH 7	98	97	98	99
pH 8	89	96	98	99
pH 9	73	84	87	46
0% (w/v) NaCl	8	16	37	32
0.5% (w/v) NaCl	8	52	04	92
3% (w/v) NaCl	92	93	97	99
5% (w/v) NaCl	72	69	92	63
7.5% (w/v) NaCl	27	20	63	48
10% (w/v) NaCl	17	17	53	46
Starch hydrolysis	9	36	17	55
Gelatin hydrolysis	41	50	18	43
Tween 20 hydrolysis	14	62	51	48
Tween 80 hydrolysis	20	62	79	74
Phosphatase	88	86	69	79
Catalase	90	72	70	77
Oxidase	0	16	4	18
Arginine decarboxylase	63	42	16	16
Lysine decarboxylase	18	19	5	6
Ornithine decarboxylase	5	10	9	19
Ammonia from pepton	9	37	36	69
Nitrate to nitrite	3	64	27	22

Acid from:				
Glucose (oxid.)	24	62	13	38
Glucose (ferm.)	30	60	49	50
Lactose	5	5	8	12
Sucrose	44	18	43	36
Utilization of:				
Carbohydrates	55	67	90	44
Glucose	58	50	85	31
Organic acids	37	69	88	50
Acetate	20	17	78	27
Pyruvate	30	50	76	38
Alcohols	29	56	79	31
Glycerol	28	35	66	25
Amino acids	32	40	83	37
Hydrocarbons	0	0	6	6
Growth factor:				
Type 1	4	24	17	14
Type 2	66	53	73	38
Type 3	25	18	6	37
Type 4	5	5	4	10

utilizing at least one substrate were counted for each class of substrates. The frequencies of utilization were in the order: carbohydrates > carboxylic acids > amino acids > alcohols >> hydrocarbons. The 4°C sediment isolates utilized more substrates than did the 4°C water isolates; the reverse was true for 20°C isolates. Since substrate utilization was determined with only vitamins as growth factors, fastidious organisms (growth factor requirement type 3 or 4) were not tested. Thus, only 70% of 4°C water isolates, 77% of 4°C sediment isolates, 90% of 20°C water isolates and 52% of 20°C sediment isolates could be tested. Obviously, growth factors are extremely important nutritional requirements for the Beaufort Sea bacteria.

Cluster analyses

Of the 4°C isolates (L series), 62% fell into 14 clusters at the 75% similarity level. Four of these clusters had only two members. The remaining 38% of the 4°C isolates did not cluster at this similarity level. The largest cluster (L4), tentatively identified as Flavobacterium, had 60 strains

(accounting for 20% of the total 4°C isolates) of which 67% were isolated from water (Fig. 6). The second largest cluster (L6), also tentatively identified as Flavobacterium, contained 28 strains, 80% of which were isolated from water.

The 20°C isolates (H series) fell into 13 clusters, which contained 60% of the isolates. Six clusters had only two members. The overall populations were very diverse (Kaneko et al., 1977b). In the largest cluster (H7), which has not been identified, 85% of the strains were isolated from water and accounted for 52% of all 20°C water isolates (Fig. 7). The second largest cluster (H4), tentatively identified as Flavobacterium, contained 13% of all 20°C isolates.

From the 27 original clusters, 47 representative strains were chosen (3 from each of the 10 largest clusters and 1 from each of the others). Cluster analyses failed to show similarity (75%) between the 4°C and 20°C clusters. Thus, the populations isolated at 4°C appear to be distinct from those isolated at 20°C. Tests on the 47 representative strains were repeated at 5°C since even identical organisms can show different test results at 5°C and 20°C. However, less than 3% of the repeated test results differed from the original results for both the 4°C and 20°C isolates.

Distribution of clusters

The distribution of the clusters is shown in Figs 6 and 7 for the 4°C and 20°C isolates, respectively. Some of the clusters (L1, L4, L6, H4, H7) were widely distributed geographically inwater and sediment, but most clusters showed restricted distribution, e.g. cluster L12 contained only strains from sediment near Point Barrow.

Classification of clusters

The features of the major clusters are show in Table 8. The growth factor requirements for all clusters are shown in Table 9. None of the isolates clustered with the reference strains and none was identified by the NIH/ATCC

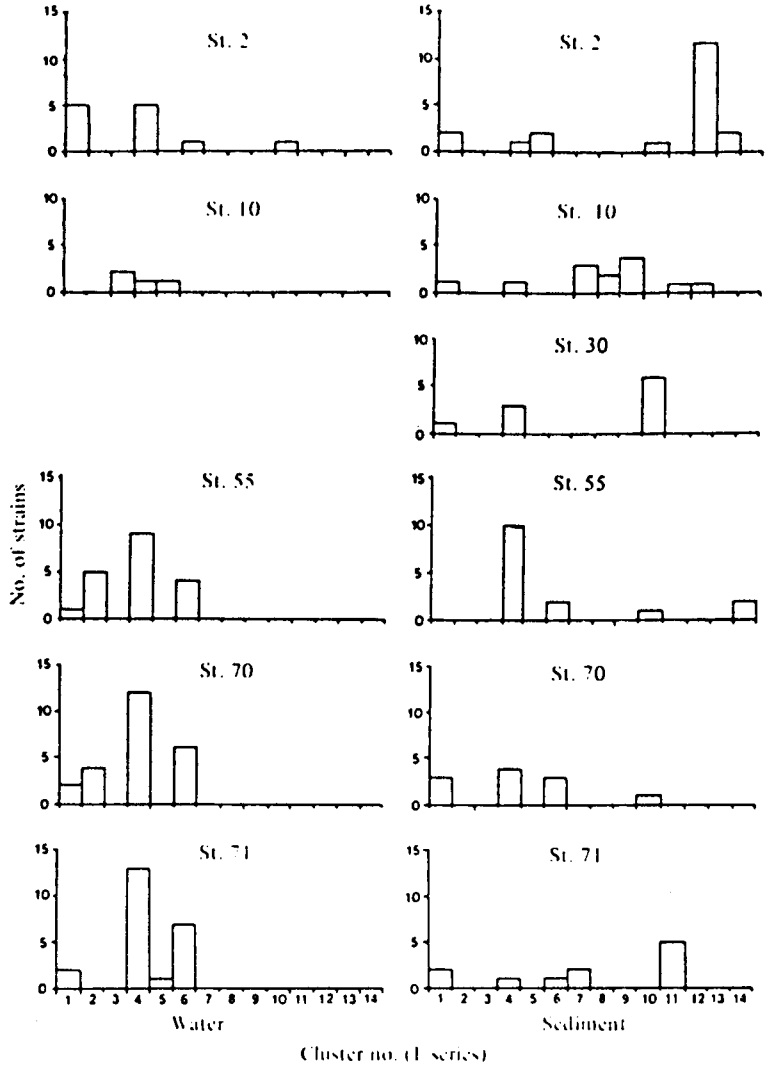

Fig. 6. Distribution of phenotypic clusters of 4 °C isolates.

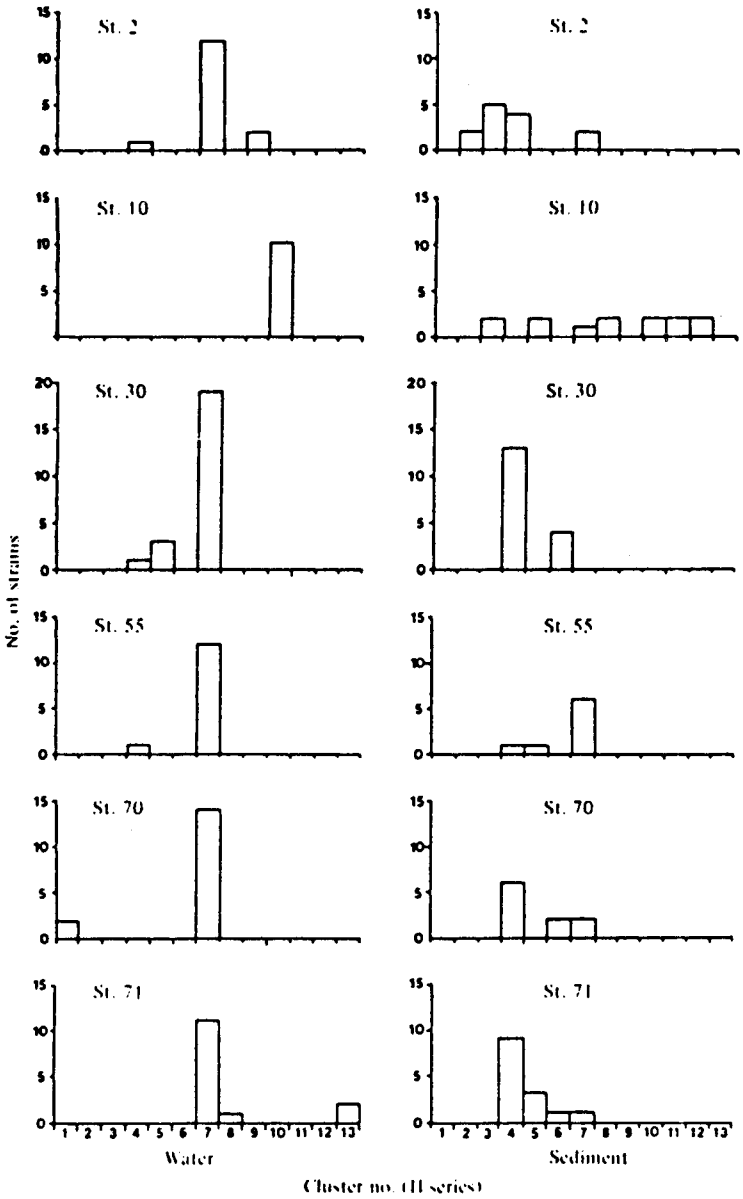

Fig. 7. Distribution of phenotypic clusters of 20 °C isolates.

Table 8. Feature frequencies* of selected characteristics of major clusters of bacterial populations isolated at 4 and 20°C

Cluster	L1	L2	L4	L5	L6	L7	L9	L10	L11	L12	H3	H4	H5	H6	H7	H8	H10
No. of strains	21	9	60	4	28	7	4	10	6	13	7	36	9	7	80	3	12
Cell morphology																	
Rod shaped	100	100	100	100	100	100	100	100	100	100	100	100	88	100	100	100	100
Curved-axis	28	44	11	100	32	71	25	70	100	84	0	88	33	57	6	0	25
Coccobacillary	0	0	3	0	0	0	0	0	0	0	16	0	0	0	2	0	0
Pear-shaped	14	88	15	25	21	0	0	20	25	15	0	2	0	28	38	33	0
Pleomorphic	95	77	96	100	96	85	100	88	100	92	33	85	55	42	38	66	83
Tapered ends	81	22	8	0	3	0	0	10	0	0	0	0	0	14	17	100	0
Rounded ends	100	100	100	100	100	100	100	100	100	100	100	100	100	100	97	100	91
Cell length																	
0.5-1.0µm	0	0	1	0	3	0	0	0	0	0	66	0	0	14	7	0	0
1.1-2.0µm	0	33	42	0	29	16	0	20	0	0	0	2	55	28	42	0	8
2.1-3.0µm	4	44	44	25	42	50	0	50	25	15	0	26	33	14	26	0	0
3.1-4.0µm	4	22	10	50	17	33	25	30	75	69	16	45	11	0	10	100	50
4.1-5.0µm	28	0	0	0	3	20	75	0	0	16	16	25	0	0	6	0	25
5.1-10.0µm	65	0	1	25	0	0	0	0	0	0	0	0	0	42	6	0	16
Cells width																	
<0.5µm	0	0	10	50	39	0	0	0	0	0	66	8	44	14	6	0	0
0·5-1·0µm	81	100	89	50	60	100	100	100	100	84	33	91	66	85	93	100	100
1·1-2·0µm	19	0	0	0	0	0	0	0	0	15	0	0	0	0	0	0	0
Cell arrangement																	
single cells	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
chain formation	5	33	63	0	67	0	25	50	0	0	0	0	33	14	8	33	41
irregular aggregates	0	0	3	0	3	0	25	0	0	0	0	2	11	0	21	0	41
Miscellaneous cell features																	
PHB inclusions	81	55	0	0	0	0	0	0	0	0	0	0	0	14	12	100	8
Capsule	23	0	22	0	32	28	0	60	25	0	0	8	11	0	7	0	8
Acid fast	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sudan black	95	77	3	0	3	0	0	0	0	0	0	2	0	28	30	100	0
Gram-negative	100	100	98	100	100	100	100	100	100	100	100	100	100	100	100	100	91
Motile	0	0	0	0	0	100	75	0	100	100	0	0	0	0	0	0	0
Colony pigmentation																	
Non-diffusible yellow	0	0	3	66	3	0	0	0	25	0	83	11	88	0	0	0	100
Non-diffusible orange	4	22	95	33	92	0	0	10	0	0	16	68	11	0	0	0	0
White	0	0	0	0	0	0	0	0	0	7	0	0	0	14	3	0	0
Grey	95	77	0	0	3	100	100	80	75	92	0	22	0	85	97	100	0
Colony size																	
<1mm	100	88	100	100	85	0	25	100	0	15	83	45	100	85	43	66	100
1-2mm	0	0	0	0	0	14	75	0	25	61	16	54	0	14	48	33	0
2-6mm	0	0	0	0	0	85	0	0	75	23	0	0	0	0	7	0	0

Colony morphology																	
Translucent	66	77	96	66	89	0	100	90	25	7	33	74	100	85	47	0	16
Transparent	23	22	3	33	10	14	0	10	0	0	0	22	0	14	2	33	0
Opaque	9	0	0	0	0	85	0	0	75	92	66	5	0	0	50	100	83
Entire	95	100	100	100	89	100	100	100	100	100	100	100	100	100	98	66	100
Convex	100	88	96	100	100	100	50	100	75	92	100	85	100	85	79	100	100
Mucoid	0	0	0	0	0	42	25	20	0	0	16	2	0	28	1	0	0
Glistening	100	100	100	100	100	100	100	100	100	100	83	100	100	100	86	33	75
Smooth	100	100	100	100	100	100	100	100		100	100	100	100	100	86	100	100
Growth in liquid media																	
No growth in liquid	4	33	5	33	7	0	0	0	0	0	0	0	0	0	0	0	0
Floccular	10	0	0	0	4	16	100	0	50	0	75	5	33	0	19	33	27
Ring	0	0	3	0	0	0	0	0	25	30	0	37	77	42	0	0	0
Pellicle	5	0	1	0	0	83	100	0	50	0	0	2	0	14	3	0	0
Even	47	16	35	0	28	33	100	100	75	100	25	94	88	100	27	66	36
Slight of no turbidity	68	100	75	100	92	33	0	20	0	0	75	31	0	42	73	100	90
Moderate turbidity	26	0	24	0	12	50	100	80	100	100	25	68	66	57	26	0	9
Heavy turbidity	5	0	0	0	0	16	0	0	0	0	0	0	33	0	0	0	0
Acid from																	
D-Ribose	0	0	2	0	0	0	0	0	100	81	0	9	0	14	35	0	0
D-Fructose	0	16	32	0	13	0	0	0	100	84	0	9	0	14	27	0	0
Celloboise	0	0	0	0	0	14	0	0	0	7	0	2	0	14	2	0	8
Lactose	0	0	0	0	0	0	0	0	0	0	0	5	0	0	3	0	0
Sucrose	0	14	57	0	50	0	0	11	0	0	0	68	0	0	48	0	8
D-Mannitol	0	0	0	0	0	0	0	0	25	0	0	20	0	14	6	0	0
D-Glucose (aerobic)	26	0	15	0	60	50	100	0	100	100	0	52	11	16	9	0	9
D-Glucose (fermentative)	26	12	20	0	27	75	100	0	100	100	16	69	33	0	59	33	0
Miscellaneous features																	
Indole produced	0	0	0	0	0	0	0	0	0	7	0	0	0	0	0	0	0
Ammonia produced	6	0	3	0	3	14	100	0	75	100	0	85	88	85	12	33	0
Nitrate reduced	0	0	0	0	0	100	100	100	100	100	83	11	0	85	3	33	100
Nitrite reduced	0	0	0	0	0	42	0	37	0	0	0	5	0	0	1	0	0
Arginine decarboxylase	56	71	70	66	65	14	0	40	50	23	50	29	22	33	15	0	0
Lysine decarboxylase	28	0	16	0	16	0	0	44	0	53	0	0	12	0	3	50	0
Ornithine decarboxylase	12	0	2	50	0	28	50	12	0	0	0	21	25	15	9	50	0
Gelatin hydrolysis	14	22	55	0	42	85	100	20	50	38	0	64	0	14	3	0	0
Starch hydrolysis	0	0	5	0	0	85	0	0	75	100	100	83	100	0	0	0	100

Tween 20 hydrolysis	12	33	1	0	21	100	100	100	100	100	28	18	100	100	45	66	50	
Tween 80 hydrolysis	40	75	11	25	11	100	100	50	100	100	0	91	88	42	82	100	90	
Catalase	93	66	94	66	92	28	100	100	100	100	75	75	50	100	54	66	100	
Oxidase	0	0	0	0	0	28	0	20	0	9	0	0	50	0	0	0	0	
Alkaline phosphatase	100	100	100	100	100	100	100	55	100	76	83	85	66	71	93	66	16	
Urease	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	
pH:growth at																		
pH4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	83	
pH5	18	11	94	25	14	80	100	90	100	100	28	86	100	85	25	100	100	
pH6	56	100	100	100	71	100	100	100	100	100	100	100	100	100	100	100	100	
pH7	100	100	100	100	82	100	100	100	100	100	85	100	100	100	98	100	100	
pH8	100	100	100	100	78	100	100	100	100	100	100	100	100	100	100	100	100	
pH9	57	100	100	100	17	85	100	100	100	100	0	2	77	100	97	100	100	
pH10	4	0	0	0	0	71	0	0	100	100	0	0	0	0	1	0	0	
Temperature:growth at																		
5°C	100	100	100	100	100	100	100	100	100	100	100	97	100	100	100	100	100	
10°C	100	100	100	100	96	100	100	100	100	100	100	100	100	100	100	100	100	
15°C	100	100	100	100	96	71	100	100	100	100	100	100	100	100	100	100	100	
20°C	95	100	96	0	89	0	0	100	0	0	100	100	100	100	100	100	100	
25°C	57	88	1	0	3	0	0	100	0	0	28	88	100	100	96	66	100	
37°C	0	0	0	0	0	0	0	0	0	0	0	2	0	0	1	0	25	
43°C	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	
NaCl:growth at																		
0.0%(w/v)	5	29	6	0	4	0	0	89	0	0	0	9	89	86	20	34	100	
0.5%(w/v)	71	100	98	0	92	0	0	100	0	0	16	82	100	100	97	66	100	
3.0%(w/v)	95	100	100	100	42	100	100	90	100	100	100	100	100	100	98	66	100	
5.0%(w/v)	66	100	100	100	17	85	100	10	100	46	28	77	100	0	98	100	100	
7.5%(w/v)	23	33	53	0	3	14	0	0	25	7	0	25	77	0	73	66	50	
10%(w/v)	23	14	18	0	12	0	0	0	0	0	0	42	100	14	62	33	8	
15%(w/v)	0	0	6	0	4	0	0	0	0	0	0	0	0	0	0	0	0	
Carbohydrates utilized																		
L-Arabinose	4	0	8	0	0	0	0	0	0	15	0	0	0	0	5	0	83	
D-Ribose	9	0	1	0	0	14	0	0	100	84	0	0	0	0	3	0	50	
D-xylose	33	88	8	0	7	0	0	0	0	0	0	0	0	0	80	0	75	
D-Fructose	4	66	45	0	0	0	0	0	100	100	0	0	0	0	58	33	100	
D-Galactose	4	66	78	0	3	0	0	0	0	100	0	0	0	0	59	0	33	
D-Glucose	81	88	68	25	14	0	50	0	100	100	0	2	55	0	94	66	100	
D-Mannose	0	0	58	0	3	0	0	0	50	100	0	0	0	0	1	0	66	
L-Sorbose	0	0	0	0	0	0	0	0	0	0	0	0	11	0	0	0	0	
Salicin	0	0	3	0	0	0	0	11	25	100	0	2	0	0	1	0	0	
Cellobiose	14	100	0	0	0	0	0	0	0	7	0	11	22	0	84	33	100	
Lactose	0	0	0	0	0	0	0	0	0	0	0	2	0	0	1	33	0	
Maltose	23	0	76	0	21	0	0	0	100	100	0	0	11	0	2	0	100	
Sucrose	9	11	45	0	3	0	0	0	0	0	0	0	0	0	0	0	100	
Trehalose	0	0	0	0	0	0	0	0	75	0	0	2	0	0	1	0	100	
Raffinose	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	91	

Alcohols utilized																	
1-Butanol	0	0	0	0	0	0	0	11	0	0	0	0	77	0	0	0	33
Ethanol	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1-Propanol	4	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	25
1,2 Propanediol	0	0	0	0	0	0	0	0	0	0	0	0	33	0	2	66	83
Glycerol	95	100	5	0	0	0	25	0	100	100	0	8	11	0	88	33	16
D-Arabitol	66	44	0	0	0	0	0	0	0	0	0	0	0	0	37	0	0
D-Mannitol	66	66	0	0	0	0	0	0	25	7	0	0	0	0	68	100	0
D-Sorbitol	76	22	0	0	0	0	0	0	0	0	0	0	0	0	28	0	0
meso-Inositol	4	77	0	0	0	0	0	0	0	0	0	0	0	0	67	0	0
Carboxylic acids utilized																	
Acetic acid	85	88	0	0	0	0	0	11	25	0	0	0	11	42	100	100	100
Butyric acid	76	77	0	0	0	0	0	0	0	0	14	0	100	28	64	100	83
Caproic acid	76	44	0	0	0	0	0	0	0	0	0	0	100	28	80	100	83
Caprylic acid	100	100	0	0	0	0	0	88	0	0	0	0	100	100	100	100	100
Lauric acid	14	22	0	0	0	0	0	0	0	0	0	0	33	14	22	0	41
Propionic acid	81	33	1	0	0	0	0	11	0	0	0	0	44	28	67	100	100
Valeric acid	0	33	0	0	0	0	0	11	0	0	0	0	100	14	36	33	91
Glutaric acid	95	100	13	0	0	0	0	33	100	100	0	0	44	42	100	100	33
Malonic acid	71	100	0	0	0	0	0	0	0	0	0	0	11	0	77	33	41
Succinic acid0	0	0	0	0	0	0	0	0	0	0	0	0	11	0	0	33	33
Fumaric acid	95	55	16	0	3	28	25	77	100	100	0	0	55	85	98	100	58
Itaconic acid	9	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	33
DL-Glyceric acid	28	88	0	0	0	0	0	0	100	100	0	0	11	0	84	0	66
β-Hydroxybutyric acid	95	88	0	0	0	0	0	0	0	0	0	0	100	42	100	100	83
DL-Lactic acid	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	33	0
L(+)-Tartaric acid	0	0	0	0	0	14	0	0	0	0	0	0	0	0	0	0	16
Citric acid	85	44	2	0	0	0	0	0	50	100	0	0	0	0	42	66	16
2-Ketogluconic acid	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	16
Pyruvic acid	100	100	6	0	0	28	100	100	100	15	0	0	88	100	84	100	100
2-Ketoglutaric acid	100	100	0	0	0	0	0	62	25	0	0	0	44	85	96	100	83
m-hydroxybenzoic acid	76	0	0	0	0	0	0	0	0	0	0	0	0	0	15	100	100
p-hydroxybenzoic acid	90	100	0	0	0	0	0	11	0	0	0	2	11	0	100	100	100
Galacturonic acid	4	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	8
D-Gluconic acid	0	0	0	0	0	0	0	0	0	69	0	2	11	0	2	0	16
Amino acids utilized																	
L-Alanine	85	66	0	0	0	0	0	100	25	0	0	0	0	100	92	100	0
Aminobutyric acid	81	100	0	0	0	0	0	90	0	0	0	0	0	100	95	50	8
L-Arginine	28	55	31	0	0	0	0	0	0	0	0	0	0	0	50	0	33
L-Asparagine	28	0	0	0	0	0	0	40	0	0	0	0	0	14	12	50	50
L-Aspartic acid	33	88	30	0	17	0	0	90	25	92	0	0	0	100	39	100	50
L-Cysteine	42	44	0	0	0	0	0	10	0	0	0	0	22	0	45	100	16
L-Cystine	28	55	0	0	0	0	0	0	0	0	0	0	11	0	6	0	33
Glycine	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0

L-Leucine	0	0	0	0	0	0	0	0	0	0	0	0	22	0	1	0	50
L-Isoleucine	0	0	0	0	0	0	0	0	0	0	0	0	55	0	1	0	66
L-Methionine	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0
L-Ornithine	57	55	00	3	0	0	0	0	0	0	0	0	0	28	74	50	58
L-Phenylalanine	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8	0	0
L-Proline	42	55	0	0	0	0	0	10	50	0	0	0	22	28	70	0	16
L-Serine	93	22	0	0	0	0	0	50	0	0	0	0	0	50	30	0	91
L-Threonine	81	0	0	0	0	0	0	0	0	0	0	0	0	0	10	100	8
L-Tryptophan	71	0	0	0	0	0	0	0	0	0	0	0	0	0	12	100	0
L-Tyrosine	66	0	0	0	3	0	0	0	0	0	0	0	11	0	10	100	0
L-ValineO	0	0	0	0	0	0	0	0	0	0	0	0	14	0	0	0	0
Amines utilized																	
Histamine	0	0	0	0	0	0	0	10	0	0	0	0	0	0	0	0	0
N-Acetylglucos-amine	42	0	1	0	0	0	0	11	100	100	0	0	14	0	1	0	0

The following substrates were not utilized by any cluster: L-rhamnose, 2-propanol, dulcitol, phenol, 2-phenylethanol, benzoic acid, ascorbic acid, o-hydroxybenzoic acid, stearic acid, L-lysine, α-amylamine, ethanolamine, putrescine, guanine, thymine, n-hexadecane, n-pentadecane, n-phenyldecane, pristane, pentadecycloheXane.

All isolates were facultative anaerobes.

*The "percentage positive" calculations do not include missing data, so for some tests the product of the feature frequency and the total number of organisms in the cluster is not an integral number.

Table 9. Growth factor requirements of dominant clusters of bacterial populations isolated at 4 and 20°C

4°C isolates: L series

Cluster	1	2	3	4	5	6	7	8	9	10	11	12	13	14
No. of strains	21	9	2	60	4	28	7	2	4	10	6	13	2	2
Growth factor:														
Type 1	-	-	-	-	-	-	-	-	-	+	+	-	-	+
Type 2	+	+	+	+	-	-	-	+	+	-	-	+	+	-
Type 3	-	-	-	-	±	±	+	-	-	-	-	-	-	-
Type 4	-	-	-	-	w	w	-	-	-	-	-	-	-	-

20°C isolates: H series

Cluster	1	2	3	4	5	6	7	8	9	10	11	12	13
No. of strains	2	2	7	36	9	7	80	3	2	12	2	2	2
Growth factor:													
Type 1	-	-	-	-	-	-	-	-	-	-	-	+	+
Type 2	-	-	-	-	+	+	+	+	+	+	+	-	-
Type 3	-	+	-	+	-	-	-	-	-	-	-	-	-
Type 4	+	-	+	-	-	-	-	-	-	-	-	-	-

+, > 80% positive; ±, 51-79% positive; w, 21-50% positive; -, <20% positive.

probabalistic identification matrices. The Beaufort Sea isolates are not identical with the common Gram-negative rods (some of which are from marine sources) contained in the matrices.

Of the 27 phenotypic clusters, 22 could be placed into seven categories:

1. Gram-negative rods producing yellow or orange pigments (clusters L3, L4, L6, H1, H2, H3, H4, H5, H13). According to the 8th edition of Bergey's Manual (Buchanan and Gibbons, 1974), Gram-negative, facultatively anaerobic rods, motile or non-motile, producing yellow, orange, red or brown pigments are classified in the genus Flavobacterium. This definition is vague and encompasses a heterogeneous collection of bacteria, e.g. it could include yellow-pigmented members of the Enterobacteriaceae. There were morphological, physiological, biochemical and nutritional differences between the Flavobacterium type strains in Bergey's Manual and our isolates. Many of our strains showed pleomorphism characteristic of Cytophaga species; however, no swarming was observed. The distinction between Flavobacterium and Cytophaga may be difficult (Hayes, 1963; Weeks, 1969; Hendrie et al., 1968). Several studies have attempted to clarify the taxonomy of yellow-pigmented Gram-negative bacteria (Goodfellow et al., 1976; Floodgate and Hayes, 1963). Clearly this group of organisms is quite heterogeneous.

2. Gram-negative bacteria forming full or partial rings (clusters L5, H6, H9). These organisms are morphologically similar to members of the genus Microcyclus, which is heterogeneous (Claus et al., 1968; Raj, 1977) and of uncertain affiliation (Buchanan and Gibbons, 1974). Our strains have different physiological and nutritional characteristics from previsouly describes species. Unlike Microcyclus marinus and several non-marine Microcyclus species

(Raj, 1977), our strains grew at 5°C and did not produce acids from carbohydrates.

3. Gram-negative, non-pigmented, fermentative, facultatively anaerobic, curved or straight rods (clusters L7, L8). The morphological and biochemical characteristics of these organisms closely resembled those of Vibrio species (Davis and Park, 1962) or Beneckea species (Baumann et al., 1971). All of the clusted organisms in this category were obligate psychrophiles. They had more complex growth requirements than the psychrophilic Vibrio marinus (Colwell and Morita, 1964) isolated from the North Pacific Ocean (Morita and Haight, 1964).

4. Gram-negative, non-pigmented, weakly fermentative, facultatively anaerobic, highly pleomorphic rods (clusters L1, L2, H7, H8). This category of clusters has not been identified with any genus described in Bergey's Manual. These non-motile organisms could be associated with several different genera.

5. Gram-negative, non-pigmented, non-fermentative, aerobic rods (cluster L10). This category has not been identified. These organisms reduce nitrate and are probably capable of anaerobic respiration. Phenotypically, they could belong to the genera Pseudomonas, Alcaligenes or Alteromonas (Baumann et al., 1972). With no observed motility and without flagella determination, the distinction is not clear.

6. Non-acid fast, coryneform bacteria showing rudimentary branching and snapping division (cluster H10). The coryneform bacteria may be Arthrobacter species even though they appeared to be Gram-negative. Indeed, many Arthrobacter species fail to appear Gram-positive (Mulder, 1964). Coccoid forms of these organisms and morphogenesis typcial of Arthrobacter were observed.

7. Gram-negative coccobacilli (clusters H11, H12). By the criteria of Baumann et al. (1968), the strains in cluster H12 appear to be Acinetobacter

species. Cluster H11 is not closely related phenotypically and remains unidentified. Organisms in cluster H12 were oxidase-negative, did not require growth factors and utilized many substrates including hydrocarbons. Hydrocarbon utilization by Acinetobacter has been reported by Finnerty et al. (1973).

Characteristics and identification of Northeast Gulf of Alaska isolates

Twenty-four clusters containing three or more strains were found at the 70% similarity level in the combined cluster analysis (performed after elimination of organisms that formed single-membered clusters in the four individual subset analyses) of isolates from the Northeast Gulf of Alaska. Seventeen clusters containing four or more isolates were considered to be major clusters. The largest cluster (E21) contained nearly equal numbers of isolates from water and sediment, all of which were isolated at 20°C. Nine of the clusters contained strains from multiple sampling sites (Table 10), but generally the clusters did not contain strains isolated at different temperatures nor from both water and sediment samples. Indeed, only 12% of the clusters contained strains isolated both from water and from sediment. Clusters E4, E5, E6, E7, E8, E10, E11, E15, E16, E18, E19, E20, E22, E23 and E24 only contained isolates from plates incubated at 5°C, and clusters E1, E3, E9, E12, E14, E17 and E21 only contained isolates from plates incubated at 20°C.

The feature frequencies of the 17 major clusters are shown in Table 11. Most of the organisms grew within the range of temperatures and salt concentrations normally found in the sampling region. Strains in clusters E8, E9, E10, E11, E12, E13, E14, E15, E16, E17, E18, E19, E20, E21, E22 and E23 did not require growth factors; those in E6, E7 and E24 required vitamins; those

Table 10. Sources of isolates in each cluster shown as the percentage isolated from each of the 15 sampling stations in the Northeast Gulf of Alaska.

Cluster	E1	E2	E3	E4	E5	E6	E7	E8	E9	E10	E11	E12	E13	E14	E15	E16	E17	E18	E19	E20	E21	E22	E23	E24
No. of strains	3	18	9	3	3	11	4	3	10	29	3	3	37	11	6	3	5	4	6	5	91	6	9	4
Station																								
1	-	11	-	67	-	-	-	-	-	-	100	-	8	27	-	-	-	-	-	-	21	-	-	-
4	-	11	-	-	-	9	-	-	-	-	-	-	-	-	100	-	-	-	33	-	-	-	-	-
7	-	-	-	-	-	-	-	-	100	-	-	-	-	-	-	-	-	-	-	-	15	-	-	-
29	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4	-	-	-
30	-	22	-	-	-	55	-	-	-	14	-	-	14	-	-	-	-	100	-	-	4	-	-	-
32	-	11	-	-	-	-	-	-	-	-	-	-	19	-	-	-	-	-	-	100	9	-	-	-
37	-	-	-	-	100	-	-	-	-	-	-	-	5	-	-	-	-	-	-	-	-	-	-	-
52	-	6	-	-	-	18	-	-	-	-	-	-	-	-	-	-	100	-	67	-	23	100	100	-
53	-	6	-	-	-	-	-	-	-	-	-	-	5	-	-	-	-	-	-	-	1	-	-	-
57	-	6	-	-	-	18	-	-	-	38	-	-	30	-	-	-	-	-	-	-	1	-	-	-
59	-	-	-	-	-	-	-	-	-	-	-	-	-	73	-	-	-	-	-	-	-	-	-	-
A	-	17	100	33	-	-	-	-	-	-	-	67	3	-	-	100	-	-	-	-	21	-	-	-
D	-	-	-	-	-	-	100	-	-	7	-	33	-	-	-	-	-	-	-	-	-	-	-	-
E	-	11	-	-	-	-	-	-	-	41	-	-	-	-	-	-	-	-	-	-	-	-	-	100
F	100	-	-	-	-	-	-	100	-	-	-	-	16	-	-	-	-	-	-	-	-	-	-	-

recovered in E3, E4 and E5 required yeast extract plus casamino acids; while those in E1 and E2 grew only on complex media.

None of the isolates formed defined clusters with the reference strains nor were any identified using the NIH/ATCC probabilistic identification matrices.

Based on the features shown in Table 11, six categories of the major clusters can be described:

1. Gram-negative, non-motile rods-coccobacilli often occurring as pairs (clusters E18 to E24). The morphological and metabolic features of the organisms closely resembled those of the Acinetobacter-Moraxella group as described in Bergey's Manual of Determinative Bacteriology (Lautrop, 1974). The genera Acinetobacter and Moraxella are distinguished by the oxidase test, strains of the former being negative while those of the latter are positive (Baumann et al., 1968; Lautrop, 1974). Additionally, strains of the genus Acinetobacter utilize carbohydrates, may form acid from sugar and are sensitive to penicillin (Lautrop, 1974; Thornley, 1967; Shewan, 1971). In the present study, strains in cluster E23 meet all the above characteristics of the genus Acinetobacter and strains in cluster E20 all those of Moraxella. The other clusters (E18, E19, E21, E22 and E24) represent intermediate cases which could be classified as either Acinetobacter or Moraxella depending on which of the above criteria were used.

2. Gram-negative, motile, oxidase-positive, fermentative, curved or straight rods (clusters E10, E13, E14 and E15). Members of these clusters resembled strains classified in the genera Aeromonas, Beneckea and Vibrio; the distinction between these taxa is not clear (Baumann et al., 1971).

3. Gram-negative, non-motile rods producing yellow, orange or brown pigments (clusters E2, E7 and E17). According to the taxonomic keys in

Table 11. Feature frequencies of selected characteristics of major clusters of bacterial populations isolated from the Northeast Gulf of Alaska*

Cluster	E2	E3	E6	E7	E9	E10	E13	E14	E15	E17	E18	E19	E20	E21	E22	E23	E24
No. of strains	18	9	11	4	10	29	37	11	6	5	4	6	5	91	6	9	4
Cell morphology																	
Sperical	0	77	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rod-shaped	100	100	100	100	100	100	97	100	100	100	0	0	0	0	0	0	0
Curved axis	0	0	0	0	0	0	8	0	100	0	0	0	0	0	0	0	0
Cocco-bacillary	0	0	0	0	0	0	2	0	0	0	100	66	100	100	66	77	100
Pleomorphic	0	77	0	0	0	0	51	0	0	0	0	0	0	0	0	0	0
Cell length																	
<2·0µm	99	0	100	100	0	100	82	100	100	100	100	100	100	100	100	100	100
>2·0µm	0	100	0	0	100	0	18	0	0	0	0	0	0	0	0	0	0
Cells width																	
0·5-1·0µm	100	100	100	100	100	100	89	100	100	100	0	16	0	5	16	33	0
1·1-2·0µm	0	0	0	0	0	0	10	0	0	0	100	83	100	94	83	66	100
Cell arrangement																	
Single cells	100	100	100	100	100	100	100	100	100	100	0	0	0	0	33	11	0
Pairs	0	0	0	0	0	0	0	0	0	0	100	100	100	100	66	88	100
Miscellaneous cell features																	
Endospores produced	0	0	0	0	100	0	0	0	0	0	0	0	0	0	0	0	0
PHB inclusions	0	0	0	0	0	100	2	18	0	0	0	0	0	0	0	0	0
Capsule	94	0	-	-	0	-	0	0	-	-	-	-	-	91	-	-	-
Sudan black	5	0	0	0	10	13	27	0	0	0	0	0	0	0	0	0	0
Gram-positive	0	100	0	0	100	0	0	0	0	0	0	0	0	0	0	0	0
Gram-negative	100	0	100	100	0	100	100	100	100	100	100	100	100	100	100	100	100
Motile	0	100	0	0	100	100	100	100	100	0	0	0	0	0	0	0	0
Colony pigmentation																	
Non-diffusible pink	0	100	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Non-diffusible brown	0	0	0	0	0	0	5	0	0	20	0	0	0	0	0	0	0
Non-diffusible yellow	94	0	0	100	0	0	0	0	0	20	0	0	0	0	0	0	0
Non-diffusible orange	5	0	0	0	0	0	0	0	0	60	0	0	0	0	0	0	0
White	0	0	0	0	0	0	0	0	100	0	0	100	40	0	100	100	100
Grey (no pigment)	0	0	100	0	100	100	91	100	0	0	100	0	60	100	0	0	0
Colony size																	
<1mm	11	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
1-2mm	83	22	0	25	20	6	8	0	16	80	50	16	60	58	16	0	0
2-6mm	5	77	100	75	80	93	91	100	83	20	50	83	40	40	83	100	100

Colony morphology																	
Translucent	0	0	27	25	0	58	67	54	0	20	0	0	0	1	0	0	25
Transparent	0	0	0	0	10	41	18	45	0	0	0	0	0	0	0	0	0
Opaque	100	100	72	75	90	0	13	0	100	80	100	100	100	98	100	100	75
Flat	0	0	0	0	0	0	8	0	0	0	0	0	0	0	0	0	0
Raised	22	100	100	100	100	100	91	100	100	80	100	100	100	100	100	100	100
Umbonate	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Convex	72	0	0	0	0	0	0	0	0	20	0	0	0	0	0	0	0
Mucoid	88	0	0	0	0	0	0	0	0	40	0	0	0	1	0	0	0
Acid from																	
D-Ribose	17	100	18	25	100	0	79	40	100	20	50	100	0	73	100	100	100
D-Fructose	6	50	0	50	90	0	94	63	83	0	50	0	0	39	80	100	100
Celloboise	5	66	0	50	80	83	72	80	16	0	100	50	20	67	100	100	100
Lactose	7	44	0	25	60	76	33	100	16	0	75	100	0	51	100	88	100
Sucrose	6	0	0	50	70	41	72	37	33	0	50	0	40	12	33	100	0
D-Mannitol	0	0	9	0	50	84	85	100	83	0	50	16	20	35	66	100	100
D-Glucose (aerobic)	0	44	0	100	100	96	100	100	66	0	50	100	0	45	50	88	100
D-Glucose (fermentative)	0	100	0	50	100	41	100	100	100	80	0	33	0	73	33	22	100
Miscellaneous features																	
Indole	0	0	0	0	30	0	69	9	33	0	0	0	0	24	40	55	0
Ammonia produced	0	25	0	0	100	47	95	63	25	20	25	0	0	68	-	-	0
Nitrate reduced	62	56	0	0	90	8	100	0	33	20	100	100	100	99	100	100	25
Nitrite reduced	14	55	0	0	60	6	2	0	0	0	0	0	0	13	0	0	75
Gelatin hydrolysis	44	75	0	100	100	100	86	100	100	0	0	0	20	9	0	0	0
Starch hydrolysis	66	0	0	25	100	3	100	9	0	0	0	16	0	2	0	0	25
Tween 20 hydrolysis	29	11	100	100	60	82	100	100	100	100	0	100	100	97	100	100	100
Tween 80 hydrolysis	33	0	100	25	0	79	97	81	33	80	0	100	100	56	83	88	100
β-Haemolysis sheep blood	0	100	-	-	100	-	0	72	-	0	-	-	-	0	-	-	-
L-Arginine decarboxylase	62	100	27	50	90	8	69	0	100	80	50	-	100	52	100	33	0
L-Lysine decarboxylase	20	33	27	0	66	0	13	0	0	20	25	0	0	8	0	0	0
L-Ornithine decarboxylase	17	22	0	0	40	0	5	0	0	50	25	16	0	9	0	0	0
Alkaline phosphatase	92	88	100	100	100	82	97	100	100	-	0	100	100	100	100	100	100
Urease	0	0	0	0	0	0	0	0	0	0	0	16	0	0	33	22	0
Catalase	83	100	0	100	100	100	97	90	100	100	100	100	100	100	100	100	100
Oxidase	44	100	100	0	40	100	100	100	100	60	100	83	100	98	66	0	75
pH:growth at pH5	0	0	0	0	0	0	0	0	0	0	0	0	0	7	0	0	0

pH6	0	100	0	0	100	48	64	100	100	40	75	80	0	97	100	100	100
pH8	88	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
pH9	33	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
pH10	0	0	36	50	70	100	83	36	100	100	100	100	100	65	100	100	100
Temperature:growth at																		
5°C	94	100	100	100	100	100	97	100	100	100	100	100	100	100	100	100	100
20°C	83	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
25°C	61	100	0	100	100	100	100	100	100	100	100	100	100	100	100	100	100
37°C	0	100	0	0	3	0	0	0	60	100	100	100	100	100	100	100	100
NaCl:growth at																		
0.5%(w/v)	0	100	0	100	100	100	48	100	100	100	100	100	100	100	100	100	100
5.0%(w/v)	0	100	54	100	100	100	89	100	100	100	100	100	100	100	100	100	100
7.5%(w/v)	0	100	0	100	100	100	5	100	100	100	100	100	100	100	100	100	100
10.0%(w/v)	0	88	0	0	100	100	0	100	0	80	100	100	100	100	100	100	100
15.0%(w/v)	0	0	0	0	100	0	0	0	0	0	0	0	0	46	0	0	0
Carbohydrates utilized																		
L-Arabinose	0	0	0	0	0	6	0	9	100	100	0	0	0	24	0	0	0
D-Ribose	0	0	0	0	100	3	97	27	33	100	50	0	0	24	100	0	0
D-Xylose	0	0	0	100	100	31	16	72	100	100	0	0	0	14	100	0	0
L-Rhamnose	0	0	0	0	0	0	0	0	0	100	0	0	0	15	0	0	0
D-Fructose	0	0	0	50	100	96	100	63	100	100	100	66	0	19	100	0	0
D-Galactose	0	0	45	50	0	79	100	100	100	100	50	16	0	29	50	0	0
D-Glucose	5	0	72	100	100	48	97	100	100	100	100	100	0	19	33	100	0
D-Mannose	0	0	0	100	100	0	100	72	100	100	0	0	0	23	33	0	0
L-Sorbose	0	0	0	0	0	100	2	36	0	40	100	100	60	14	83	0	0
Salicin	0	0	0	0	0	10	45	18	0	100	0	16	0	7	83	0	25
Cellobiose	0	0	0	0	0	41	62	90	16	100	0	0	0	17	50	0	0
Lactose	0	0	18	50	0	10	5	100	66	100	0	0	0	14	0	0	0
Maltose	0	-	100	100	100	44	90	0	50	100	0	0	0	5	16	0	0
Sucrose	0	0	0	50	100	13	75	18	16	100	0	0	0	23	16	66	0
Trehalose	0	0	0	0	100	0	88	9	100	-	0	0	0	15	16	0	0
Raffinose	0	0	0	0	0	0	2	36	0	100	0	0	0	19	0	0	0
Alcohols utilized																		
1-Butanol	0	0	0	0	30	0	0	0	0	100	0	0	0	27	0	0	0
Ethanol	0	0	0	0	0	0	5	63	0	80	0	0	0	14	0	0	0
1-Propanol	0	0	0	0	0	0	16	81	16	60	0	0	0	33	0	0	0
2-Propanol	0	0	0	0	0	0	2	36	0	100	0	0	0	1	0	0	0
D(-)-1,2-Propanediol	0	0	0	0	0	0	0	9	0	100	0	0	0	18	0	0	0
Glycerol	0	0	0	100	100	0	100	0	100	100	0	0	0	8	33	0	0
D-Arabitol	0	0	0	0	0	0	8	9	100	100	0	0	0	3	0	0	0
Dulcitol	0	0	0	0	0	0	5	0	0	100	0	0	0	0	0	55	0
D-Mannitol	5	0	0	100	100	96	97	100	100	100	0	0	0	5	33	0	0
D-Sorbitol	0	0	0	100	10	0	18	0	0	100	0	0	0	3	0	0	0
meso-Inositol	0	0	0	0	0	0	78	0	0	100	0	0	0	2	33	0	0
Phenol	0	0	0	0	0	0	0	9	0	100	0	0	0	2	0	0	0
2-Phenyl ethanol	0	0	0	0	0	0	0	0	0	100	0	0	0	0	0	0	0

Carboxylic acids utilized																	
Acetic acid	0	0	0	0	100	0	54	100	66	100	0	16	40	81	0	100	0
Butyric acid	0	0	0	0	100	0	21	45	100	100	0	50	0	97	50	77	0
Caproic acid	0	0	0	0	100	13	86	72	100	100	0	0	0	100	0	11	0
Caprylic acid	0	0	0	0	100	0	73	100	100	100	0	0	0	92	0	0	0
Lauric acid	0	0	0	0	0	0	13	0	0	100	0	0	0	7	16	66	0
Propionic acid	0	-	0	0	-	17	0	-	100	-	0	0	0	-	0	0	0
Valeric acid	0	33	0	0	100	0	64	72	100	100	0	0	100	64	66	55	0
Glutaric acid	0	0	0	0	0	0	18	0	0	100	0	0	0	83	0	0	0
Malonic acid	0	0	0	0	0	0	0	63	0	100	0	0	0	4	0	0	0
Succinic acid	0	-	0	0	-	51	85	-	100	-	0	0	0	-	83	0	0
Oleic acid	0	0	0	0	0	0	0	18	0	100	0	0	0	0	0	0	0
Fumaric acid	0	0	9	0	100	10	97	100	100	100	0	66	40	64	33	77	0
Itaconic acid	0	0	0	0	0	0	5	0	0	100	0	0	0	3	0	0	0
DL-Glyceric acid	0	0	-	-	100	-	100	36	-	100	-	-	-	18	-	-	-
β-Hydroxybutyric acid	0	0	0	0	0	0	13	27	100	100	0	0	0	60	0	0	0
DL-Lactic acid	0	0	0	0	0	0	100	27	100	100	75	83	100	82	100	55	0
L(+)-Tartaric acid	0	0	0	0	0	34	0	9	0	100	0	33	0	34	50	77	100
Citric acid	0	0	0	0	100	13	91	18	100	100	50	100	0	35	50	0	0
2-Ketogluconic acid	0	0	0	0	100	82	5	0	100	100	0	100	0	24	33	0	0
Pyruvic acid	0	0	9	0	100	100	83	100	100	100	50	83	60	100	100	44	0
2-Ketoglutaric acid	0	0	0	0	100	75	56	72	100	100	100	100	60	80	33	100	0
Benzoic acid	0	0	0	0	0	0	13	0	0	0	0	0	0	65	33	100	0
m-Hydroxybenzoic acid	0	0	0	0	0	0	0	0	0	100	0	0	0	3	0	0	0
p-Hydroxybenzoic acid	0	0	0	0	0	0	2	0	83	100	0	0	0	4	50	0	0
Ascorbic acid	0	0	0	0	0	58	16	36	0	0	0	0	0	6	0	11	0
Galacturonic acid	0	0	0	50	0	0	5	0	100	100	0	0	0	9	0	0	0
D-Gluconic acid	0	0	0	0	100	41	2	18	100	40	50	66	0	25	16	0	0
o-Hydroxybenzoic acid	0	0	0	0	10	0	2	0	0	100	100	33	60	22	66	22	0
Stearic acid	0	0	0	0	0	0	0	0	0	100	0	0	0	3	0	0	0
Amino acids utilized																	
L-Alanine	0	0	0	0	0	24	67	100	100	100	0	0	0	78	0	0	0
γ-Aminobutyric acid	0	0	0	0	0	0	2	18	100	100	0	83	0	82	0	77	0
L-Arginine	0	0	0	0	70	0	64	36	100	60	0	0	60	53	33	0	0
L-Asparagine	0	0	0	0	0	3	64	90	100	40	100	100	60	72	100	100	0
L-Aspartic acid	0	11	0	100	100	0	83	100	100	100	0	0	0	35	50	11	0
L-Cysteine	0	0	-	-	0	-	37	81	-	100	-	-	-	68	-	-	-
L-Cystine	0	0	0	0	0	0	8	0	0	100	100	83	60	22	50	100	0
L-Glutamic acid	0	0	0	0	60	58	97	63	100	100	0	0	0	42	66	22	25
Glycine	0	0	0	0	0	0	72	63	0	0	0	0	0	6	33	0	0
L-Leucine	0	0	0	0	0	3	8	0	100	100	0	0	0	76	83	0	0
L-Isoleucine	0	0	27	0	0	0	2	54	100	80	0	66	60	14	50	22	0
L-Lysine	0	0	0	0	0	0	12	36	33	0	0	66	60	80	0	0	0
L-Methionine	0	0	0	0	0	0	5	27	0	20	0	0	0	27	0	0	0

L-Ornithine	0	0	0	0	100	0	78	9	66	100	0	100	40	53	33	0	0
L-Phenylalanine	0	0	0	0	0	0	10	72	16	60	0	0	0	82	50	33	0
L-Proline	0	0	0	0	100	0	100	81	100	100	0	0	40	93	83	11	0
L-Serine	0	0	0	0	20	10	83	81	50	100	0	0	0	60	0	0	0
L-Threonine	0	0	0	0	0	0	94	72	0	100	0	0	0	5	0	0	0
L-Tryptophan	0	0	0	0	0	0	2	54	0	40	0	0	0	30	0	11	0
L-Tyrosine	0	0	0	0	0	0	14	63	0	80	0	0	0	100	0	100	0
L-Valine	0	0	0	0	0	0	0	27	0	60	0	0	0	9	0	0	0
Amines utilized																	
Histamine	0	0	0	0	0	0	0	63	0	100	0	0	0	14	0	0	0
Putrescine	0	0	0	0	0	0	0	0	100	0	0	16	0	0	16	0	0
N-Acetylglucos-amine	0	0	54	0	100	0	94	36	100	80	0	0	0	17	50	0	25
Guanine	0	0	0	0	0	0	0	36	0	100	0	0	0	4	0	0	0
Thymine	0	0	0	0	0	0	2	18	0	100	0	0	0	8	0	0	0
Hydrocarbons utilized																	
n-Hexadecane	0	0	0	0	0	0	5	0	0	100	0	0	0	9	0	0	0
2-Methylnaph-thalene	0	0	0	0	0	0	0	0	0	100	0	0	0	0	0	0	0
ω-Phenyldecane	0	0	0	0	0	0	0	0	0	100	0	0	0	0	0	0	0
Pristane	0	0	0	0	0	0	0	0	0	100	0	0	0	2	0	6	0
Pentadecyl-cyclohexane	0	0	0	0	0	0	2	9	0	100	0	0	0	4	0	0	0

* The following features were 100% positive for all strains: rounded ends, entire colonies, glistening colonies, smooth colonies, smooth colonies, facultative anaerobes, growth at pH 7, growth at 10°C, growth at 15°C, and growth at 3% NaCl.

Bergey's Manual of Determinative Bacteriology (Weeks, 1974), these strains are included in the genus Flavobacterium, a heterogeneous genus defined primarily on pigment production and failure to show 'unusual' characteristics such as ring formation or plant pathogenicity which would place the organisms in genera such as Microcyclus and Xanthomonas.

4. Gram-negative, non-motile, non-fermentative, oxidase-positive, catalase-negative rods which are not actively proteolytic in gelatin media (E6). These strains resembled non-motile strains of Alcaligenes except for the catalase test results (Holding and Shewan, 1974).

5. Gram-positive, motile rods producing pink colonies and spherical bodies in older cultures (E3). The morphogenesis of these strains is representative of coryneform bacteria (e.g. Arthrobacter) which also exhibit a morphological cycle (Bousfield, 1978; Mulder, 1964; Keddie, 1974, 1978).

6. Gram-positive-Gram-variable, motile, large rods forming endospores and growing aerobically (E9). The strains in this cluster clearly belong in the genus Bacillus (Gordon et al., 1973). The endospores were oval, terminal or subterminal and did not distend the sporangium. According to the keys of Gordon et al. (1973), strains occurring in this cluster are assigned to the Group I Bacillus species. The strains exhibited denitrifying activities and showed eurytolerance to physiological growth conditions, growing over a wide range of pH values, salt concentrations and temperatures.

In addition to the organisms that formed defined major clusters, several minor clusters showed characteristics of the genera Flavobacterium (strains in E8 were Gram-negative, motile rods producing yellow pigments), Pseudomonas (strains in E5 were Gram-negative, motile rods that grew only oxidatively) and Vibrio (strains in E11, E12 and E16 were Gram-negative rods generally with a

curved axis). Other clusters (E1 and E4) could not be associated with defined genera.

Characteristics and identification of Northwest Gulf of Alaska isolates

The bacterial populations in this region were quite diverse; only 12 clusters containing three or more members were found at the 70% similarity level and of these only four contained five or more strains. The larger clusters generally accommodated isolates from several locations (Table 12); the two largest clusters contained a mixture of isolates from water and sediment and from 5 and 20°C isolation temperatures. Only two of the clusters were restricted to isolates from a single location.

The feature frequencies of the 12 clusters are shown in Table 13. Most isolates were psychrotrophs capable of growth at 5 and 20°C, the normal temperature range for the area sampled; all grew well at 3% NaCl concentrations and several would not grow in the absence of added NaCl. Four of the clusters (W6, W8, W9 and W10) contained strains which exhibited complex nutritional growth factor requirements; those of one cluster (W7) required vitamins; but those in the remainder showed no growth factor requirements (W1, W2, W3, W4, W5, W11 and W12).

None of the reference strains were recovered within the 12 clusters and none of the isolates were identified using the computer comparison with ATCC strains. Based on the features shown in Table 13, seven categories of clusters can be described, five of which show major characteristics of defined generic groups:

1. Gram-negative, oxidase-positive, non-pigmented, fermentative, motile rods (clusters W2 and W3). These organisms were similar to Beneckea species (Baumann et al., 1971).

Table 12. Sources of isolates in each cluster shown as the percentage isolated from each of the 12 sampling stations in the Northwest Gulf of Alaska.

Cluster	W1	W2	W3	W4	W5	W6	W7	W8	W9	W10	W11	W12
No. of strains	4	3	20	3	3	3	5	7	9	4	3	3
Station												
101	-	33	30	-	-	-	-	14	-	-	-	-
106	-	-	5	-	-	-	-	-	-	-	-	-
119	-	-	5	-	33	-	-	-	-	-	-	-
121	-	-	-	-	-	-	20	14	-	-	-	-
124	25	33	-	33	-	-	-	14	-	-	-	-
133	25	33	25	33	-	-	-	-	11	-	-	-
134	-	-	-	-	-	33	80	29	-	-	-	-
137	25	-	30	33	67	-	-	-	89	100	-	-
145	25	-	-	-	-	-	-	14	-	-	33	-
148	-	-	5	-	-	33	-	-	-	-	-	-
156	-	-	-	-	-	33	-	-	-	-	-	-
159	-	-	-	-	-	-	-	14	-	-	67	100

Table 13. Feature frequencies of selected characteristics of major clusters of bacterial populations isolated from the Northeast Gulf of Alaska*

Cluster	W1	W2	W3	W4	W5	W6	W7	W8	W9	W10	W11	W12
No. of strains	4	3	20	3	3	3	5	7	9	4	3	3
Cell morphology												
Curved-axis	50	0	0	0	33	0	60	57	0	0	66	100
Pleomorphic	0	0	0	0	0	0	20	14	0	0	0	0
Rounded ends	100	100	94	100	100	100	100	71	100	100	100	100
Square ends	0	0	5	0	0	0	0	28	0	0	0	0
Cell length												
0.5-1.0µm	0	0	10	0	0	0	20	0	100	0	0	100
1.1-2.0µm	75	33	78	100	66	0	60	85	0	100	66	0
2.1-3.0µm	25	66	10	0	33	100	20	14	0	0	33	0
Cells width												
<0.5µm	0	0	5	0	0	0	0	0	0	0	0	100
0·5-1·0µm	100	100	89	100	100	100	100	100	100	100	100	0
1·1-2·0µm	0	0	5	0	0	0	0	0	0	0	0	0
Miscellaneous cell features												
PHB inclusions	0	0	5	0	0	66	0	42	0	0	0	0
Capsule	25	33	15	33	33	33	40	57	100	100	0	0
Sudan black	0	0	26	0	0	33	0	14	0	0	0	0
Gram-negative	100	100	100	100	100	100	100	85	100	100	100	100
Motile	100	66	78	100	100	66	20	42	0	0	0	0
Colony pigmentation												
Diffusible yellow	0	0	0	0	0	0	0	14	0	0	0	0
Non-diffusible violet	0	0	0	66	0	0	0	0	0	0	0	0
Non-diffusible yellow	0	0	10	0	0	0	0	0	100	100	100	100
Grey (no pigment)	100	100	89	33	100	100	100	100	0	0	0	0
Colony size												
<1mm	0	0	0	0	0	0	0	0	22	100	0	0
1-2mm	0	0	5	0	0	66	80	85	77	0	66	100
2-6mm	100	100	94	100	100	33	20	14	0	0	33	0
Colony morphology												
Translucent	50	100	89	0	66	100	100	71	0	0	100	100
Transparent	50	0	0	0	0	0	0	0	0	0	0	0
Opaque	0	0	10	100	33	0	0	28	100	100	0	0
Entire	75	0	89	100	100	100	100	100	100	100	100	100
Undulate	25	100	10	0	0	0	0	0	0	0	0	0
Raised	100	100	63	33	33	66	100	100	0	0	100	100
Umbonate	0	0	31	66	66	0	0	0	0	0	0	0
Convex	0	0	5	0	0	33	0	0	100	100	0	0
Mucoid	0	0	5	0	0	33	0	0	100	100	0	0

Acid from												
D-Ribose	0	-	92	50	50	-	50	0	0	0	0	0
D-Fructose	-	100	94	50	50	-	0	0	0	0	-	-
Celloboise	0	0	22	0	0	-	60	0	0	0	-	-
Lactose	0	0	10	0	0	-	66	-	0	0	-	-
Sucrose	0	0	0	50	33	-	0	0	0	0	-	-
D-Mannitol	0	100	94	100	66	-	0	0	0	0	-	100
D-Glucose (aerobic)	0	100	94	50	33	-	100	0	0	0	0	0
D-Glucose (fermentative)	50	100	88	100	66	0	100	0	0	25	66	0
Miscellaneous features												
Obligate aerobe	100	0	5	0	66	50	0	0	0	0	0	33
Facultative anaerobe	0	100	95	100	33	50	100	100	100	100	100	66
Indole	0	33	94	0	33	-	0	0	0	0	0	-
Ammonia produced	0	100	94	100	66	-	0	0	16	0	0	-
Nitrate reduced	0	0	95	100	66	0	100	66	75	50	0	0
Nitrite reduced	0	0	0	50	0	0	0	16	0	0	0	0
Starch hydrolysis	0	100	90	100	0	66	0	0	75	75	-	100
Tween 20 hydrolysis	75	66	95	100	0	100	0	50	0	0	0	0
Tween 80 hydrolysis	100	66	95	100	-	100	0	0	0	0	-	0
L-Arginine decarboxylase	0	33	65	0	0	0	100	83	25	50	50	-
L-Lysine decarboxylase	0	0	0	0	0	0	66	33	0	0	0	-
L-Ornithine decarboxylase	0	0	0	0	0	0	66	25	0	25	0	-
Alkaline phosphatase	100	100	90	50	100	100	100	100	71	-	100	100
Catalase	0	100	100	50	100	0	80	85	33	75	100	100
Oxidase	50	100	100	100	100	100	100	100	11	0	0	33
pH:growth at												
pH6	0	0	78	0	0	0	0	0	11	0	0	0
pH8	100	100	85	100	100	100	100	100	66	100	66	100
pH9	100	100	90	100	100	100	100	71	11	25	0	0
pH10	100	100	95	100	0	0	0	28	0	0	0	0
Temperature:growth at												
5°C	100	100	100	100	-	100	100	100	77	100	100	100
20°C	100	100	100	50	100	100	80	42	100	75	100	100
25°C	0	100	100	0	100	0	40	0	0	0	0	0
37°C	0	0	10	0	0	0	20	0	0	-	-	-

NaCl:growth at												
0.5%(w/v)o	0	33	100	50	0	0	0	0	0	0	0	0
5.0%(w/v)	100	100	75	50	100	0	100	42	11	0	0	0
7.5%(w/v)	0	0	85	0	0	0	0	0	0	0	0	0
Carbohydrates utilized												
D-Ribose	0	0	100	0	0	0	0	0	0	0	0	0
D-Fructose	0	100	100	0	0	0	0	0	0	0	0	0
D-Galactose	0	100	42	0	0	0	0	0	0	0	0	0
D-Glucose	100	100	100	0	0	0	0	0	0	0	0	0
D-Mannose	0	100	95	0	0	0	0	0	0	0	0	0
L-Sorbose	0	66	0	0	0	0	0	0	0	0	0	0
Salicin	0	66	10	50	0	0	0	0	0	0	0	0
Cellobiose	25	33	20	0	33	0	0	0	0	0	0	0
Lactose	25	0	0	50	33	0	0	0	0	0	0	0
Maltose	0	0	5	0	0	0	0	0	0	25	0	0
Sucrose	0	66	5	100	33	0	0	0	0	0	0	0
Trehalose	100	100	100	0	0	0	0	0	0	0	-	0
Alcohols utilized												
1-Propanol	0	100	0	50	0	0	0	0	0	0	0	0
Glycerol	25	100	100	50	0	0	0	0	0	0	0	0
D-Mannitol	0	100	100	0	66	0	0	0	0	0	0	0
D-Sorbitol	0	100	0	0	0	0	0	0	0	0	0	0
meso-Inositol	0	33	5	0	0	0	0	0	0	0	0	0
2-Phenyl ethanol	0	66	0	50	0	0	0	0	0	0	0	0
Carboxylic acids utilized												
Acetic acid	0	100	95	50	100	0	0	0	0	0	0	100
Caproic acid	0	0	15	0	0	0	0	0	0	0	0	0
Lauric acid	100	100	10	100	0	0	0	0	22	0	0	0
Propionic acid	0	0	20	0	0	0	0	0	0	0	0	0
Glutaric acid	0	100	40	50	0	0	0	0	0	0	0	0
Malonic acid	25	100	95	0	0	0	0	0	0	0	0	0
Succinic acid	25	100	0	50	0	66	0	0	0	0	0	0
Oleic acid	0	0	0	50	0	0	0	0	0	0	0	0
Itaconic acid	0	0	5	0	0	0	20	14	0	0	0	0
DL-Glyceric acid	25	0	65	0	0	0	0	0	0	0	0	0
β-Hydroxybutyric acid	0	0	0	50	66	0	0	0	0	0	0	0
DL-Lactic acid	0	100	35	0	100	0	0	0	0	0	0	100
L(+)-Tartaric acid	0	0	0	0	0	0	0	0	0	0	100	33
Citric acid	0	100	30	50	66	0	0	0	0	0	0	0
Pyruvic acid	75	100	95	50	100	0	0	0	0	0	100	33
2-Ketoglutaric acid	0	100	90	50	100	0	40	0	0	0	66	100
Ascorbic acid	0	0	65	0	0	0	0	0	0	0	0	0
Galacturonic acid	0	0	0	50	0	0	0	0	0	0	0	0
D-Gluconic acid	0	100	15	0	0	0	0	0	0	0	0	0
Stearic acid	0	0	0	0	0	0	0	0	25	0	-	0

Amino acids utilized												
L-Alanine	75	66	100	50	100	0	0	0	0	0	0	0
L-Arginine	100	66	40	50	100	0	100	33	0	0	-	0
L-Asparagine	25	100	100	50	100	0	80	0	0	0	0	0
L-Aspartic acid	0	100	100	0	0	0	100	14	0	0	100	100
L-Cysteine	0	100	40	50	0	0	20	0	0	0	0	0
L-Cystine	0	100	15	100	0	0	0	0	0	0	0	0
L-Glutamic acid	100	100	100	100	100	0	80	14	0	0	33	66
Glycine	75	100	100	0	0	0	0	0	0	0	0	0
L-Leucine	100	0	68	50	0	0	0	0	0	0	-	0
L-Isoleucine	0	0	15	50	66	0	0	0	0	0	-	0
L-Ornithine	0	66	15	50	100	0	0	0	0	0	0	0
L-Phenylalanine	0	0	10	50	100	0	0	0	0	0	-	0
L-Proline	100	100	100	100	100	0	100	14	0	0	0	0
L-Serine	100	100	100	0	100	0	0	0	0	0	0	0
L-Threonine	0	100	95	0	0	0	20	0	0	0	0	0
L-Tryptophan	0	0	5	0	0	0	0	0	0	0	0	0
L-Tyrosine	100	33	90	100	100	0	0	0	0	0	0	0
L-Valine	0	-	25	100	0	-	0	0	0	0	0	0
Amines utilized												
N-Acetylglucosamine	100	100	100	0	100	0	40	0	0	0	0	0
Guanine	0	33	0	50	0	0	0	0	0	0	0	33
Thymine	0	100	0	50	0	0	0	0	0	0	0	0
Hydrocarbons utilized												
Pentadecane	0	0	25	0	0	0	0	0	0	0	0	0
1-Methylnaphthalene	0	0	25	0	0	0	0	0	0	0	0	0
ω-Phenyldecane	0	0	10	0	0	0	0	0	0	0	0	0

*The following substrates were not used by strains in any of the clusters: L-arabinose, D-xylose, raffinose, L-rhamnose, 1-butanol, ethanol, 2-propanol, D-arabitol, dulcitol, phenol, caprylic acid, fumaric acid, benzoic acid, α-aminobutyric acid, L-lysine, L-methionine, ethanolamine, histamine, putrescine, 2-methylnaphthalene. The following features were 100% postive for all strains: rod-shaped, predominance of single cells, glistening colonies, smooth colonies, growth at pH 7, growth at 10°C, growth at 15°C and growth at 3.0% NaCl.

2. Gram-negative, oxidase-positive, non-pigmented, variably fermentative, motile, curved or straight rods (clusters W5 and W7). The morphological and biochemical characteristics of these organisms closely resembled those of Vibrio species (Davis and Park, 1962) or Beneckea species (Baumann et al., 1971). Members of these clusters were stenohaline growing only at 3% NaCl.

3. Gram-negative, facultatively anaerobic, straight rods producing non-diffusible yellow pigments (clusters W9 and W10). According to taxonomic keys in Bergey's Manual of Determinative Bacteriology (Weeks, 1974), strains in these clusters are classified in the genus Flavobacterium. Members of these clusters were fastidious; they were restricted to growth at a salt concentration of 3% NaCl and required complex media for growth.

4. Gram-negative, yellow-pigmented, non-motile rods forming partial rings (clusters W11 and W12). These organisms were morphologically similar to members of the genus Microcyclus (Claus et al., 1968; Raj, 1977); Staley, 1974). The strains were oxidase-negative and grew on a very limited number of substrates.

5. Gram-negative, oxidase-positive rods producing violet pigments (cluster W4). Isolates within this cluster produced violet pigments which were soluble in ethanol, but not water, and gave an absorption maximum at 579 nm characteristic of the pigment violacein produced by strains of the genus Chromobacterium (Sneath, 1956, 1974). The strains were motile and lost their pigment-producing ability on repeated subculturing.

6. Gram-negative, straight or curved rods that are catalase-negative (clusters W1 and W6). These strains have not been identified. Members of cluster W1 were non-fermentative; those of cluster W6 failed to grow in fermentation tests and thus could not be scored for these features.

7. Gram-negative, non-pigmented, pleomorphic rods exhibiting bipolar inclusions (cluster W8). These bacteria have not been identified. They showed a high degree of morphological variability and were nutritionally fastidious.

In addition to the organisms that were recovered in defined clusters, several individual organisms showed characteristics of coryneform bacteria (large rods forming spherical bodies in older culture) and several others were Gram-positive cocci which exhibited characteristics of the genus Micrococcus (Gram-positive cocci producing pigments and occurring singly or in pairs). Numerous other organisms were observed which were not readily associated with previously described taxa.

Probabilistic Identification Matrices

Three attributes (salt requirement, growth at 25°C and lack of pigment production), allow eight possible combinations of results (Table 14). However, because of the variability of groups 1 and 2, and 7 and 8 for requirements of salt, these group pairs were combined and a new feature frequency output was generated for distinguishing six groups; the resultant matrix was supplemented with two additional tests, growth in a medium with 0.5% NaCl and Tween 20 hydrolysis, which permitted further separation of additional subset pairs (Table 15). The super-matrix was designed to assign to strains to a proper sub-matrix for identification with a defined taxon. Taxa having variable results for one or more tests were placed in the group that most closely approximated its behavior for the variable test. For example, a taxon with scores of 96%, 88% and 14% for the tests of pigmentation, growth at 25°C and salt requirement, respectively, would be placed in group 2, while a taxon with scores of 96%, 88% and 53% would be placed in group 1. By design, the matrices contain at least two tests that completely separate all possible group pairs within the given subset. The number of groups and features contained in each

Table 14. Group Feature Frequencies for Three Selected Attributes

Feature	Group 1	2	3	4	5	6	7	8
Absence of Pigmentation	92%	95%	96%	95%	05%	01%	02%	02%
Growth at 25°C	94	98	33	01	87	81	10	11
NaCl Requirement	89	36	99	01	89	25	72	40

Table 15. Feature Frequency Table for Modified Groups with 5 Features.

Feature	Group 1,2	3	4	5	6	7,8
Absence of Pigmentation	97%	95%	95%	06%	01%	01%
Growth at 25°C	95	01	01	95	99	08
NaCl Requirement	73	99	01	95	04	60
Tween 20 Hydrolysis	75	92	72	39	27	08
Growth 0.5% NaCl	90	01	15	90	92	80

matrix are shown in Table 16. The inclusive matrix required 61 features to separate the 86 clusters. Nine features required for the complete separation of all group pairs in the inclusive matrix were not required in any of the super- sub-matrix combinations: cell length, 0.5-1.0 μm, ammonia production, celloboise utilization with production of acid, glycerol utilization, acetic acid utilization, alpha-keto-glutaric acid utilization, and L-methionine utilization.

Of the 1087 strains submitted to the super-matrix for assignment to appropriate sub-matrices, 17 were excluded from further testing as additional tests were suggested for these strains. Additionally, 36 of the test strains submitted to the battery of sub-matrices were found to have insufficient recorded test data for identification in the appropriate sub-matrix. Most of these strains (86%) were assigned to sub-matrices 2 and 6. The distribution of the 1034 remaining test strains, according to the identification score associated with the group with the highest normalized likelihood is shown in Table 17. Using the super- sub-matrix scheme, 949 (91.7%) had ID scores above 0.970. Using the inclusive matrix, 1058 (98.6%) resulted in ID scores above 0.970. Less than one percent (0.7%) of the strains entered in the inclusive scheme had ID scores below 0.900, and only half of these strains were assigned to incorrect groups. In the super- sub-matrix scheme, identification scores for 40 strains (3.9%) fell below 0.900, but only 6 strains were assigned to incorrect groups.

Using the R score to evaluate the efficiency of identification (27.6%) of strains tested in the super- sub-matrix scheme resulted in an R score of 1.0 compared to 7.4% in the inclusive scheme (Table 18). Employing ID and R score thresholds of 0.990 and 0.01, respectively, did not introduce a significantly higher rate of mis-identification when compared to the threshold identification

Table 16. Probability Matrix Specifications

Matrix	Name	Number of Features	Number of Group
1	Super-matrix	5	6
2	Sub-matrix 1	30	35
3	Sub-matrix 2	13	10
4	Sub-matrix 3	13	10
5	Sub-matrix 4	18	18
6	Sub-matrix 5	13	10
7	Sub-matrix 6	21	16
8	Inclusive Matrix	61	86

Table 17. Distribution of Test Strains According to Test Sub-Matrix and Highest ID Score

				Distribution of ID Scores								
Sub-Matrix	Total IN	Total OUT	Tests Suggested	≥.999	<.999 ≥.995	<.995 ≥.990	<.990 ≥.980	<.980 ≥.970	<.970 ≥.960	<.960 ≥.950	<.950 ≥.900	<.900
1	524	523	1	429(2)*	38	14	7	5	5(2)	2(1)	8	15(1)
2	58	51	7	35	6	0	1	4	0	1	1	3(1)
3	73	72	1	61	4	1	1	1	0	0	1	3(2)
4	133	131	2	100	14	7	2	1	0	0	0	7(1)
5	61	60	1	41	7	4(1)	1	1	1	0	1	4(1)
6	221	197	24	103	39	15(1)	19(1)	5(1)	1	2	5	8
Total	1070	1034	36	796(2)	108	41(2)	31(1)	17(1)	7(2)	5(1)	16	40(6)
Inclusive matrix	1087	1073	14	1015(2)	26	7	5	5(1)	3	2(1)	2	8(4)

*Numbers in parentheses indicate the absolute proportion of the cell frequency for which the highest normalized likelihood indicated an incorrect strain-group association.

Table 18. Distribution of Test Strains According to Sub-Matrix Trial and R Score

Sub-Matrix	Distribution of R Scores					
	10^0	$<10^0$ $\geq 10^{-1}$	$<10^{-1}$ $\geq 10^{-2}$	$<10^{-2}$ $\geq 10^{-3}$	$<10^{-3}$ $\geq 10^{-4}$	Total
1	100	227(4)	129(1)	49(1)	18	523(6)
2	37(1)*	12	2	0	0	51(1)
3	36	23	12	1	0	72
4	61	55(1)	11	3	1	131(1)
5	19	35(1)	6(1)	0	0	60(2)
6	32(1)	89(1)	60	11	5(1)	197(3)
Total	285(2)	441(7)	220(2)	64(1)	24(1)	1034(13)
Inclusive matrix	79	470(3)	286(3)	170(2)	68	1073(8)

*Numbers in parentheses indicate the absolute proportion of the cell frequency for which the highest normalized likelihood indicated an incorrect strain-group association.

score 0.999 set by the program IDDNEW (Table 19). Using the ID and R thresholds of 0.990 and 0.01 only 1 additional strain was identified incorrectly. The super- sub-matrices permitted identification of 92% of the strains, the inclusive matrix resulted in identification of 93% of the strains. Lowering the identification criteria to ID = 0.970 and R = 0.001 increased the identification rate in the super- sub-matrix scheme by 8%, with mis-identification of only one additional strain. Likewise, lowering the criteria for the inclusive matrix in the same manner increased the identification rate by 15% with mis-identification of only two additional strains. The most significant difference in identification rate achieved by the two sets of identification criteria was observed for strains associated with sub-matrix 5. When these strains were run in the inclusive matrix, 35% increase in identification was observed if the lower criteria (ID - 0.970 and R = 0.001) were used, as opposed to the criteria of ID = 0.990 and R = 0.01.

Values for total numbers of strains correctly assigned to a given sub-matrix, strains resulting in a correct group identification, strains with correct group identifications which exceeded the threshold criteria, strains resulting in incorrect group identifications which exceed threshold criteria using the different matrices (Table 20). The values for percent of error represent the probability of making an incorrect conclusion about an unknown strain's group association for each super- sub-matrix scheme and the inclusive scheme. Also included is a total error rate for the super sub-matrix scheme. The lowest error rate was observed for sub-matrix 3 using the lower set of criteria; the highest error rate was observed for sub-matrix 6 using the less lenient identification thresholds. The greatest difference in error rate across both identification threshold set was observed for the inclusive matrix, i.e., the inclusive matrix was 16% more efficient when using the lower rather

Table 19. Distribution of Test Strains According to Observed ID and R Scores as a Result of the Super-Sub-Matrix Scheme

Sub-Matrix	Total Strains Attempted	Distribution of ID and R Scores: ID<.990 R>.01	ID>.990 R<.01	ID<.990 R<.01	Strains Above Both	% ID	ID<.970 R>.001	ID>.970 R<.001	ID<.970 R<.001	Strains Above Both	% ID
1	517	15	53	13	436	84	23	15	3	476	92
2	50	8	0	0	42	84	3	0	0	47	94
3	70	4	0	0	66	94	2	0	0	68	97
4	130	9	4	0	117	90	6	0	0	124	95
5	58	7	0	0	51	88	5	0	0	53	91
6	194	34	11	4	145	75	16	3	1	174	90
Total	1019	77	68	17	857	84	55	18	4	942	92
Inclusive matrix	1065	15	228	10	812	76	13	57	2	993	93

NOTE: Table includes only those strains for which correct strain-group identifications were made and for which no additional tests were suggested.

TABLE 20. Error Rate Using Various Matrices.

ID CRITERIA	X 1	X 2	Y 1	Y 2	Z 1	Z 2	Q	R	% ERROR 1	% ERROR 2
Submatrix										
1	81	41	2	2	0	0	523	1	15.8	08.2
2	8	2	0	0	0	0	51	0	15.7	03.9
3	4	2	0	0	0	0	72	2	05.4	02.7
4	13	6	0	0	1	2	131	10	09.9	05.7
5	7	5	1	1	4	5	60	16	15.8	14.5
6	49	20	0	1	0	0	197	3	24.5	10.5
Total	162	76	3	4	5	7	1034	32	15.9	08.2
Inclusive Matrix	253	72	1	3	-	-	1073	-	23.7	06.9

X = total number of strains assigned to the correct group of origin for which ID and R scores were below threshold levels.

Y = total number of strains assinged to the correct sub-matrix but which identified as being members of the wrong group of origin above identification threshold levels.

Z = total number of strains assigned to the wrong sub-matrix identifying above identification thresholds.

Q = valid number of strains submitted to the correct sub-matrix.

R = valid number of strains submitted to the incorrect sub-matrix.

Threshold Criteria 1 = ID $\geq$ 0.990 and R $\geq$ 0.01

2 = ID $\geq$ 0.970 and R $\geq$ 0.001

than the higher identification threshold criteria. The error rate for the overall sub-matrix scheme was 7.6% less than that observed for the inclusive matrix using the higher threshold criteria; however, the sub-matrix scheme was slightly less efficient than in the inclusive scheme under the lower threshold (8.2% versus 6.9%).

Bacterial populations from Cook Inlet isolated on high and low nutrient media

The cluster analyses of bacteria isolated from high nutrient media showed that 64% of the isolates occurred in 37 phenotypic clusters of greater than 62% similarity (Figure 8). The average number of strains per phenotypic grouping was 5.2. Fourteen of the phenotypic clusters contained more than 4 strains. The two largest clusters each contained 21 isolates accounting for 14% of the total number of strains isolated on high nutrient media. The cluster analyses of the bacteria isolated on low nutrient media showed that 95% of the strains occurred in phenotypic clusters of greater than 62% similarity. The average number of strains per phenotypic cluster for bacteria isolated on low nutrient media was 11.6. A single phenotypic cluster contained 118 strains and a second cluster contained 46 strains; these two clusters accounted for almost 50% of the total number of strains isolated on low nutrient media.

An examination of the sources of the organisms occurring within each cluster showed that the larger clusters contained isolates from multiple stations that had been isolated from media with and without added oil. The minor clusters often contained isolates from only one source. In a number of cases the clusters contained equal numbers of strains from a given station that had been isolated on media with and without crude oil added. The plate counts of bacteria on low nutrient media were several orders of magnitude lower than those for high nutrient media indicating that the low nutrient bacterial populations could well be a subset of the populations obtained using high

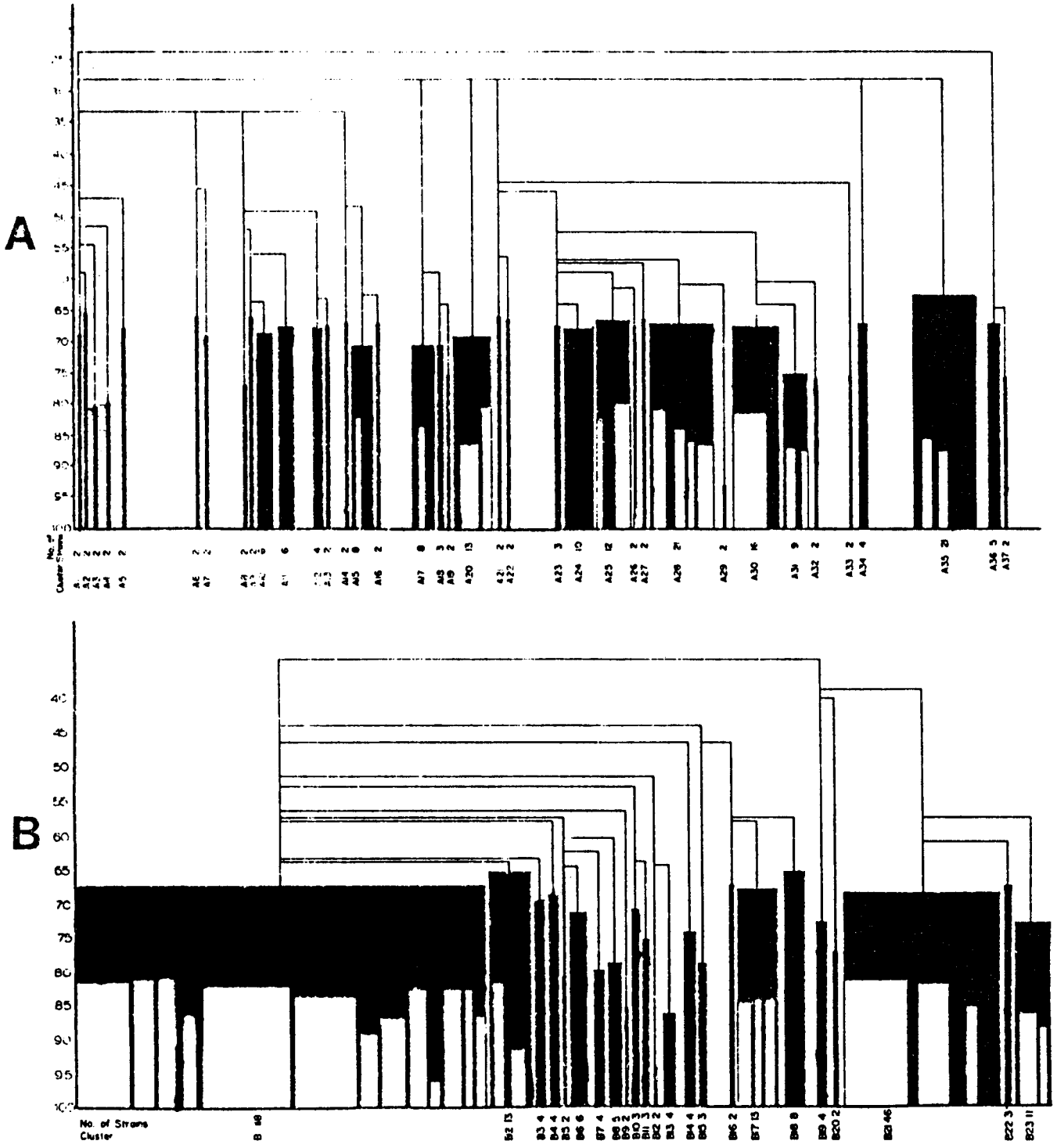

Fig. 8 Dendrograms showing clusters obtained for bacteria isolated from high (A) and low (B) nutrient media.

nutrient media; indeed the isolates from low nutrient media also could grow on marine agar.

Bacteria associated with edible crabs - potential human pathogens

The crabs obtained from near Kodiak Island contained bacteria that were identified as potential human pathogens. The isolates contained many taxa associated with domestic sewage, e.g. Klebsiella and Citrobacter. These isolates were most frequently found in association with gill tissues, but in some cases bacteria were isolated from muscle tissues as well. E. coli, which normally is used as an indicator of domestic sewage, was not found in any of the crab tissues. Yersinia enterocolytica, a human pathogen transmitted via the gastrointestinal tract and associated with several recent outbreaks of food poioning, was among the isolates obtained from the crabs collected near Kodiak Island. Tests with laboratory mice confirmed the pathogenicity of the Y. enterocolytica and also of the K. pneumoniae isolates. Crabs from the southern Bering Sea and those collected in the Gulf of Alaska away from Kodiak Island did not contain bacterial populations indicative of sewage contamination.

The scanning electron microscopic examination of Dungeness Crab tissue, showed that a diverse array of bacteria is assoicated with crab gill tissue, but there was no evidence of bacterial association with muscle tissue, in active and healthy looking crabs. Muscle tissue from dead and injured crabs obtained in the tank was found to have morphologically diverse type of bacteria. On the crab's shell, numerous morphologically different types of microorganisms exist, some of these organisms appear to be chitinoclastic, as shown by cracks in the shell surface and infiltration of bacteria into the shell. Plate counts of different tissues confirm our SEM observations, which indicated that tissues from Alaskan King Crabs showed higher numbers of viable

bacteria on gill tissue from crabs collected near Kodiak Island compared to those collected away from this populated area.

The results from the tank study indicated that all microorganisms added to the tank and to which the crabs were exposed were not uniformly concentrated in the tissues. Klebsiella sp., Bacillus sp., and Vibrio parahaemalyticus could be readily isolated from gill tissue. Beneckea harveyi (a bioluminescent organism) was readily isolated from both sediment and water column but not from tissues of crabs unless the crab had been exposed to this bacteria for a long period of time, in that case the B. harveyi did appear in gill but not muscle tissue. E. coli was not isolated from any of the crab tissues nor from water nor sediment samples.

DIVERSITY OF MICROBIAL COMMUNITIES

Beaufort Sea

Taxonomic diversity

A relatively high state of taxonomic diversity was characteristic of the marine bacterial communities of the Beaufort Sea (Table 21). Taxonomic diversity was significantly greater in sediment than in water communities and significantly greater in summer than during winter. Similar diversities have been found in subarctic marine ecosystems in sediment (Hauxhurst et. al, 1981), but in subarctic ecosystems somewhat higher diversities occur in surface waters than were found in the Beaufort Sea (Kaneko et. al, 1978). Also while there are seasonal shifts in bacterial diversity in Beaufort Sea surface water communities, in subarctic surface waters no comparable seasonal variations have been observed. The lower taxonomic diversity in Arctic waters during winter undoubtedly reflects the stress placed on such biological communities by harsh Arctic conditions including the limited substrates available from phytoplankton. In addition to seasonal shifts in diversity, definite

geographic trends in the diversity of bacterial communities were observed. During summer diversity is greatest in the western Beaufort Sea, whereas during winter the lowest diversities occur in the western Beaufort Sea. Community diversity was found to be identical at given Arctic habitats from one year to the next at a given time of year. However, the individual populations within the respective communities varied from one year to the next. It appears that there is a maximum taxonomic diversity for a bacterial community occupying a given habitat, but that different bacterial populations can occupy the niches of that ecosystem.

Physiological tolerance indices

As one would expect the Arctic populations are somewhat less tolerant to temperature fluctuations than subarctic populations, particularly with respect to tolerance of high temperatures. Most Beaufort Sea bacteria, however, are not true psychrophiles; psychrotrophs, capable of growth at temperatures of 25°C, comprise over 85% of the bacterial populations in these ecosystems. Relatively high physiological tolerance indices nevertheless are characteristic of Beaufort Sea bacterial communities (Table 22). High physiological tolerance indices are somewhat surprising considering the relatively low annual variations in temperature, salinity, and pH which occur in these marine ecosystems. The indigenous bacterial populations are quite tolerant of fluctuations in temperature, salinity, and pH, beyond the limits to which they ever are exposed naturally.

Nutritional utilization indices

The nutritional utilization indices indicate that the bacterial populations of the Beaufort Sea are relatively versatile (Table 23). However, hydrocarbons are not metabolized by the dominant populations of bacteria occurring either in water of sediment. With respect to other classes of

Table 21. Taxonomic Diversities of bacterial populations (H')

		H'
Ice	Winter 1976	3.0
Water	Summer 1975	2.6
	Winter 1976	2.1
	Summer 1976	2.6
	Summer 1978	2.6
Sediment	Summer 1975	3.5
	Winter 1976	3.6
	Summer 1976	4.0
	Summer 1978	4.1

H' = Shannon diversity index

Table 22. Physiological tolerance (P_x) indices of surface water and sediment bacterial communities in Beaufort Sea ecosystems sampled in 1976.

	Summer		Winter	
	Water	Sediment	Water	Sediment
P_T	0.60	0.55	0.56	0.50
P_T^H	0.62	0.72	0.65	0.70
P_S^H	0.26	0.35	0.30	0.35

P_T = physiological tolerance index for temperature
P_T^H = physiological tolerance index for pH
P_S^H = physiological tolerance index for salinity

Table 23. Nutritional utilization (N_x) indices of surface water and sediment bacterial communities in Beaufort Sea ecosystems sampled in 1976.

	Summer		Winter	
	Water	Sediment	Water	Sediment
N_c	0.55	0.67	0.40	0.56
N^c_a	0.29	0.56	0.31	0.50
N^a_{ca}	0.37	0.69	0.45	0.65
N^{ca}_{aa}	0.32	0.40	0.38	0.42
N^{aa}_h	0.00	0.00	0.00	0.00
N^h_T	0.31	0.46	0.31	0.45

N_c = nutritional utilization index for carbohydrates
N^c_a = nutritional utilization index for alcohols
N^a_{ca} = nutritional utilization index for carboxylic acids
N^{ca}_{aa} = nutritional utilization index for amino acids
N^{aa}_h = nutritional utilization index for hydrocarbons
N^h_T = nutritional utilization for all substrates

substrates, sediment populations are capable of growing on more organic substrates than water populations; sediment populations could utilize 50% more substrates than water populations. The major seasonal difference in the nutritional utilization indices occurred in the abilities of surface water populations to utilize carbohydrates. During summer carbohydrates were the most readily utilized substrates followed by carboxylic and amino acids. During winter carboxylic and amino acids were utilized by a greater proportion of the community than were carbohydrates. This shift in nutritional capabilities presumably reflects a shift in the food resources available as substrates to the bacterial populations of the Beaufort Sea.

Gulf of Alaska

Bacterial Population Characteristics

Selected morphological, physiological, and biochemical characteristics of representative dominant bacterial populations in each sample are shown in Table 24. Gram-negative rods predominated in all samples. Approximately one-half of the populations, represented by the isolates, were pigmented, predominantly with yellow, orange, and brown pigments. Slightly less than half of the isolates were motile. As expected, higher percentages of motile bacteria were found in water samples (49%) than in sediment samples (38%).

The majority of the bacterial populations grew at temperatures of 5-20°C, but true psychrophiles, incapable of growth at 20°C, only were found in 33 of the 45 samples. The majority of isolates at most stations required NaCl for growth. Three intertidal beach stations (D, M, and L) and one water station (152) showed anomalously low proportions of NaCl-requiring bacterial populations. This observation likely indicates the occurrence of bacterial populations of terrestrial origin, but which are capable of growth in the

Table 24. Selected features of populations in Gulf of Alaska and Cook Inlet water and sediment showing % positive.

Station	Gram neg.	Rods	Motile	Spores	Pig-mented	Psychro-phile	NaCl required	Nitrate reduced	Nitrate reduced	Gelatin hydrol.	Starch hydrol.
				WATER							
156	100	93	43	0	82	25	100	63	13	-	70
159	100	100	27	0	72	0	100	0	0	-	75
145	87	88	38	6	31	0	82	58	0	-	22
137	92	67	92	0	25	25	100	70	10	-	33
101	100	100	78	0	0	0	100	88	0	-	87
204	100	95	42	0	5	21	100	-	-	16	37
215	100	90	95	0	50	0	90	-	-	75	40
245	100	94	78	0	10	10	95	-	-	68	37
265	100	81	25	0	53	0	53	-	-	41	41
266	100	84	26	0	45	5	90	-	-	45	45
229	100	90	30	0	25	0	80	-	-	84	85
105	100	94	50	0	47	12	88	-	-	63	50
1	97	83	37	0	20	27	80	82	7	46	52
4	100	89	11	0	74	21	84	43	0	31	38
53	100	79	13	0	62	21	50	37	0	29	35
57	100	93	100	0	0	0	100	17	7	86	8
52	63	7	0	0	100	0	4	93	0	7	11
30	100	47	24	0	21	22	47	59	6	24	6
D	100	100	26	0	68	0	39	29	7	68	29
M	100	100	65	0	20	5	35	-	-	85	30
L	100	74	70	0	10	10	85	-	-	50	45
K	100	100	20	0	5	0	100	-	-	100	90
E	100	85	58	0	35	15	69	25	23	65	5
A	95	86	55	0	41	5	91	57	19	64	50
				SEDIMENT							
134	95	100	27	0	19	50	100	100	17	0	42
137	100	100	26	0	65	7	100	71	0	-	70
121	100	94	29	0	25	43	94	100	6	-	19
101	100	95	52	0	43	20	100	76	0	-	67
204	100	95	20	0	10	5	100	-	-	20	85
215	100	95	47	0	16	16	100	-	-	11	32
225	100	100	72	0	16	10	100	-	-	68	53
229	100	90	30	0	45	15	95	-	-	40	60
105	100	85	90	0	30	5	95	-	-	60	65
1	96	91	48	0	65	27	82	65	0	39	44
4	83	79	34	0	76	7	45	81	18	48	28
53	100	91	50	0	50	14	86	65	14	64	40
57	100	96	64	0	7	11	89	81	22	57	68
52	90	72	24	0	59	21	62	77	7	29	33
37	92	85	46	8	19	36	67	70	13	46	45
30	100	97	35	0	45	19	81	55	10	43	39
32	100	61	40	0	32	19	56	91	8	29	46
D	100	93	14	0	86	0	62	44	14	69	48
M	100	100	15	0	65	5	65	-	-	45	40
L	100	100	25	0	10	0	0	-	-	60	10
A	100	94	24	0	76	24	88	78	27	40	65

marine environment in these regions. At many other stations all of the dominant bacterial populations required NaCl.

The ability to reduce NO_3^- to NO_2^- was characteristic of many of the bacterial populations present at all but two stations. Nitrate reducers predominated in most sediment sampels and were only slightly less abundant in water samples. The ability to reduce nitrate can permit organisms to respire under anaerobic conditions, which are commonly found in marine sediments. Nitrite reduction was a common characteristic of bacterial populations at most stations, but the number of nitrite reducing populations was always far less than that of the corresponding nitrate reducers.

The ability to produce extracellular enzymes was tested using gelatin and starch, for protease and amylase enzymes, respectively. Isolates capable of hydrolyzing either gelatin (protein) or starch (polysaccharide) were found at all stations tested, in similar numbers. In this region saccharolytic bacterial populations appear to occur as frequently as proteolytic populations.

Physiological Tolerance Indices

The physiological tolerance indices for growth over ranges of temperature (P_T), pH (P_H), and salinity (P_S) are shown in Table 25. Considerable variations in P_T are apparent at different stations. There was no significant difference, though, in P_T values between communities samples in March and October. There was a significant difference ($\alpha<0.01$) in P_S values for communities in suface waters east and west of Kodiak Island: the P_S for western stations was 0.11; the mean P_S for eastern stations was 0.61. P_S values also were significantly higher in intertidal samples than in offshore samples. In most cases the temperature tolerance index (P_T) is distinctly larger than the indices for either pH range or NaCl concentration. This indicates that the majority of the communities samples are more tolerant to

Table 25. Physiological tolerance indices for temperature, pH, and salt for Gulf of Alaska and Cook Inlet bacterial communities.

	Water			Sediment		
Station	P_T	P_H	P_S	P_T	P_H	P_S
156	0.74	0.40	0.10	-	-	-
159	0.65	0.23	0.00	-	-	-
145	0.76	0.42	0.24	-	-	-
134	-	-	-	0.57	0.66	0.18
137	-	-	-	0.67	0.44	0.09
121	-	-	-	0.54	0.45	0.23
101	0.80	0.73	0.45	0.71	0.60	0.21
204	0.63	0.57	0.21	0.67	0.46	0.41
215	0.85	0.61	0.40	0.70	0.42	0.12
225	-	-	-	0.68	0.56	0.14
245	0.83	0.61	0.30	-	-	-
265	0.85	0.47	0.41	-	-	-
266	0.76	0.54	0.33	-	-	-
229	0.83	0.55	0.58	0.72	0.41	0.21
105	0.72	0.45	0.25	0.77	0.60	0.27
1	0.71	0.55	0.35	0.71	0.53	0.40
4	0.72	0.53	0.33	0.77	0.65	0.58
53	0.72	0.50	0.53	0.70	0.55	0.28
57	0.80	0.77	0.74	0.69	0.62	0.23
52	0.99	0.76	0.90	0.72	0.59	0.47
37	-	-	-	0.70	0.60	0.50
30	0.83	0.60	0.73	0.65	0.54	0.26
32	-	-	-	0.80	0.61	0.52
D	0.82	0.52	0.60	0.86	0.50	0.58
M	0.90	0.62	0.77	0.79	0.44	0.63
L	0.83	0.65	0.43	0.82	0.60	0.74
K	0.91	0.60	0.59	-	-	-
E	0.76	0.61	0.63	-	-	-
A	0.80	0.52	0.42	0.69	0.46	0.25
F	0.70	0.51	0.62	-	-	-

changes in temperature, over the experimental range, than to changes in either pH or salinity over the ranges tested. Direct comparison of tolerance indices for different environmental factors must be made with caution, however, as the calculated numerical values of the indices are dependent on the selected ranges of experimental values for each factor.

Nutrient Utilization Indices

The nutrient utilization indices are shown in Tables 26 and 28. Carbohydrates and amino acids generally had the highest utilization indices. All of the carbohydrates tested could be used by the bacterial communities in 11 different samples. Usually less than half of the alcohols and about half of the carboxylic acids tested could be used by the bacterial communities. Few individual hydrocarbon substrates could be utilized; in about 70% of the samples, the populations of the communities tested showed a complete lack of capability of utilizing any hydrocarbons. The total substrate utilization (N_T) values were somewhat lower in offshore waters (mean N_T = 0.51) than in offshore sediments (mean N_T = 0.61) or in intertidal samples (mean N_T = 0.61).

Taxonomic Diversity

The Shannon Weaver diversity indices (H') and equitability indices (J') are in Tables 27 and 28. There was no significant difference (α = 0.2) between the taxonomic diversity H' values for offshore water and offshore sediment communities, although the mean H' value of offshore water communities was 3.0, compared to an H' value of 3.8 for offshore sediment communities. Likewise, there was no significant difference in taxonomic diversities between intertidal water and intertidal sediment bacterial communities, although the mean H' for intertidal water (3.0) was lower than for intertidal sediment (3.4). The equitability values also were higher for offshore sediment (mean J' = 0.84) than for offshore waters (mean J' = 0.70), but this difference was significant

Table 26. Nutrient Utilization indices for Gulf of Alaska and Cook Inlet bacterial communities for various substrate classes[a].

	N_c	N_a	N_{ca}	N_{aa}	N_h	N_T	N_c	N_a	N_{ca}	N_{aa}	N_h	N_T
156	0.38	0.54	0.48	0.55	0.00	0.45	-	-	-	-	-	-
159	0.69	0.38	0.33	0.70	0.14	0.49	-	-	-	-	-	-
145	0.81	0.54	0.55	0.85	0.29	0.65	-	-	-	-	-	-
134	-	-	-	-	-	-	0.75	0.46	0.45	0.75	0.00	0.54
137	-	-	-	-	-	-	0.75	0.38	0.59	0.55	0.29	0.55
121	-	-	-	-	-	-	0.88	0.46	0.62	0.70	0.00	0.61
101	-	-	-	-	-	-	0.94	0.69	0.69	0.00	0.71	0.81
204	0.64	0.31	0.41	0.90	0.00	0.52	0.93	28	0.23	0.55	0.00	0.42
215	0.71	0.38	0.55	0.55	0.00	0.51	0.93	0.31	0.59	0.80	0.00	0.60
225	-	-	-	-	-	-	0.79	0.31	0.41	0.95	0.00	0.55
245	0.64	0.31	0.41	0.65	0.00	0.46	-	-	-	-	-	-
265	1.00	0.38	0.66	0.90	0.00	0.67	-	-	-	-	-	-
266	1.00	0.31	0.66	0.85	0.00	0.65	-	-	-	-	-	-
229	0.79	0.15	0.28	0.50	0.00	0.37	0.93	0.31	0.34	0.60	0.00	0.47
105	0.57	0.15	0.28	0.45	0.00	0.33	0.64	0.23	0.31	0.75	0.00	0.43
1	0.69	0.46	0.69	0.70	0.14	0.61	0.88	0.54	0.66	0.85	0.43	0.71
4	1.00	0.23	0.48	0.40	0.00	0.48	0.94	0.46	0.69	0.75	0.29	0.68
53	1.00	0.31	0.62	0.55	0.00	0.58	0.94	0.62	0.62	0.65	0.00	0.64
57	0.63	0.15	0.55	0.20	0.00	0.38	1.00	0.69	0.66	0.60	0.00	0.66
52	0.81	0.38	0.69	0.75	0.14	0.63	0.81	0.38	0.69	0.85	0.00	0.65
37	-	-	-	-	-	-	0.81	0.38	0.59	0.65	0.00	0.56
30	0.81	0.08	0.50	0.42	0.00	0.43	0.94	0.62	0.86	0.74	0.00	0.73
32	-	-	-	-	-	-	0.88	0.69	0.69	0.85	0.43	0.74
D	1.00	0.62	0.79	0.65	0.14	0.72	0.94	0.54	0.69	0.40	0.00	0.59
M	1.00	0.46	0.76	0.90	0.00	0.72	1.00	0.31	0.52	0.70	0.00	0.57
L	0.79	0.46	0.52	0.85	0.00	0.59	1.00	0.85	0.72	0.95	0.00	0.78
K	0.29	0.08	0.21	0.40	0.00	0.23	-	-	-	-	-	-
E	0.94	0.15	0.59	0.30	0.00	0.47	-	-	-	-	-	-
A	1.00	0.31	0.66	0.75	0.00	0.64	0.94	0.46	0.62	0.75	0.00	0.64
F	0.94	0.77	0.72	0.40	0.29	0.66	-	-	-	-	-	-

N_T all substrates tested

N_c carbohydrates; N_a alcohols; N_{ca} carboxylic acids; N_{aa} amino acids; N_h hydrocarbons

Table 27. Taxonomic diversity of Gulf of Alaska and Cook Inlet bacterial communities.

Station	Water H'	Water J'	Sediment H'	Sediment J'
159	3.3	0.87	-	-
159	2.2	0.56	-	-
145	3.3	0.83	-	-
134	-	-	3.6	0.81
137	-	-	3.4	0.80
121	-	-	3.7	0.90
101	0.0	0.00	4.1	0.93
204	3.0	0.70	3.7	0.86
215	3.3	0.76	3.7	0.87
225	-	-	3.8	0.89
245	2.9	0.68	-	-
265	4.0	0.98	-	-
266	4.1	0.95	-	-
229	1.4	0.32	3.4	0.79
105	4.1	0.99	2.9	0.67
1	4.4	0.90	3.9	0.86
4	4.0	0.94	3.9	0.80
53	4.3	0.94	4.2	0.94
57	1.1	0.29	3.5	0.72
52	2.3	0.48	5.6	0.95
37	-	-	4.2	0.89
30	2.9	0.71	4.1	0.83
32	-	-	3.8	0.79
D	4.1	0.83	4.5	0.91
M	2.6	0.60	3.9	0.90
L	2.8	0.65	1.4	0.32
K	0.3	0.70	-	-
E	2.6	0.56	-	-
A	4.2	0.94	3.6	0.81
F	4.1	0.85	-	-

Table 28. Summary of Physiological tolerance indices, nutrient utilization indices, and taxonomic diversities showing mean values.

	Intertidal	Offshore	West of Kodiak Island	Cook Inlet	East of Kodiak Island
			WATER		
P_T	0.82	0.78	0.72	0.78	0.80
P^H	0.58	0.55	0.35	0.57	0.62
P_S	0.58	0.41	0.11	0.57	0.61
N_c	0.85	0.76	0.63	0.76	0.82
N^c	0.41	0.32	0.49	0.28	0.27
N^a	0.61	0.51	0.45	0.46	0.59
N^{ca}	0.61	0.62	0.70	0.69	0.50
N^{aa}	0.06	0.04	0.14	0.00	0.05
N_T^n	0.58	0.51	0.53	0.50	0.52
H'	3.0	3.0	2.9	2.9	3.0
J'	0.64	0.70	0.75	0.67	0.71
			SEDIMENT		
P_T	0.79	0.69	0.59	0.71	0.72
P^H	0.50	0.55	0.52	0.51	0.59
P_S	0.55	0.28	0.17	0.18	0.41
N_c	0.97	0.87	0.79	0.86	0.90
N^c	0.54	0.46	0.43	0.35	0.55
N^a	0.64	0.57	0.55	0.44	0.68
N^{ca}	0.70	0.74	0.67	0.78	0.74
N^{aa}	0.00	0.13	0.10	0.12	0.14
N_T^n	0.65	0.61	0.57	0.55	0.67
H'	3.4	3.8	3.6	3.6	4.0
J'	0.74	0.84	0.84	0.84	0.85

only at the $\alpha = 0.1$ level. Particularly high H' and J' values were found in surface waters at the upper end of Cook Inlet (stations 265 and 266) and in a contiguous region southeast of the entrance to Cook Inlet (stations 1, 4, 53, and 105). Extremely low taxonomic diversities were found in water samples from stations 101 and K. There was no significant relationship between population size and taxonomic diversity in these communities.

Cook Inlet - copiotrophic and oligotrophic bacteria

The diversities of the heterotrophic (copiotrophic-high nutrient) bacterial communities at different seasons are shown in Table 29.

The diversity of bacteria isolated on high nutrient media clearly was higher than the diversity of bacteria isolated on low nutrient media (Table 30). The average Shannon diversity index for all isolates from high nutrient media with and without oil was 5.6 compared to 3.1 for isolates from low nutrient media. The Shannon diversity indices for isolates from sediment obtained on high nutrient media were higher than for comparable water isolates. There was no significant difference in diversity indices between sediment and water isolates obtained on low nutrient media.

Approximately 80% of the bacterial isolates required sodium chloride for growth. Only 15% of all isolates, though, were restricted to growth near 3% NaCl. Almost all such stenohaline bacteria were isolated on high nutrient media. Eighty-one percent of all isolates were able to grow at 7.5% NaCl and 20% could grow at 15% NaCl. Of the organisms capable of growth at 15% NaCl, nearly 75% were isolated from sediment. The temperature growth characteristics of the isolates showed that only 24 isolates were restricted to growth at temperatures below 25°C. All of these psychrophilic bacteria were isolated on high nutrient media. Ninety three percent of the isolates could grow at 25°C, but only 9% were capable of growth at 37°C.

Table 29. Diversity (H') of bacteria isolated on high nutrient media at different times in Cook Inlet

Sampling Time	Water	Sediment
Fall 1976	3.1	3.5
Spring 1977	3.6	3.6
Spring 1978	3.9	4.5
Spring 1979	4.1	5.6

Table 30. Diversity of bacteria isolated on different media.

Isolation Medium	Water		Sediment		Total	
	N*	H*	N	H	N	H
MA	94	4.1	69	5.4	163	5.6
MO	74	4.6	64	5.0	138	5.6
BA	80	2.8	40	2.6	120	3.1
BO	100	3.0	61	2.6	161	3.1

*N = number of isolates
H = Shannon diversity index

The physiological tolerance indices showed significant differences for temperature, pH and salinity between bacteria isolated on high and low nutrient media (Table 31). Isolates from low nutrient media could grow over a wider range of temperature, pH and salinity values than bacteria isolated on high nutrient media. The high physiological tolerance indices for the Alaskan isolates are in marked contrast to those calculated for salinity and temperature using the data of Mallory et al. which were P_T=0.44 and P_S=0.20 for Chesapeake Bay isolates. As a rule, there was greater variability in the physiological tolerance indices between bacteria isolated from Alaskan samples collected at different stations on high nutrient media than for bacteria isolated on low nutrient media. The physiological tolerance indices appear to reflect the diversity differences between the bacterial populations isolated on the high and low nutrient media.

Significant differences also were found for the nutritional utilization indices (Table 32), where the isolates from low nutrient media were nutritionally far more versatile than those isolated on high nutrient media. Isolates from low nutrient media could utilize 2-3 times the number of alcohol, carboxylic acid, amino acid and hydrocarbon substrates as could be used by the isolates obtained from high nutrient media. In the case of carbohydrates, though, both isolates from high and low nutrient media utilized similar numbers of carbohydrate substrates. Comparing our results to those obtained by Mallory et al. it appears that a higher proportion of the Alaskan isolates were euryheterotrophic, whereas the Chesapeake Bay isolates were more restricted in the substrates that could be utilized. For example, the nutritional utilization indices for the Chesapeake Bay isolates calculated based on the data of Mallory et al. are N_c=0.40, N_a=0.20, N_{aa}=0.10, and N_{ca}=0.10, which, with the exception of carbohydrates, are significantly lower than the

Table 31. Physiological tolerance indices for bacterial communities isolated on different media.

Sta.	P_H (pH)	P_T (temperature)	P_S (salinity)
		MEDIUM MA	
		Water	
265	0.58	0.79	0.43
235	0.78	0.80	0.80
394	0.64	0.83	0.79
354	0.40	0.79	0.38
		Sediment	
235	0.78	0.75	0.37
394	0.50	0.65	0.18
354	0.54	0.67	0.23
mean	0.60	0.75	0.45
		MEDIUM MO	
		Water	
265	0.71	0.77	0.42
235	0.76	0.82	0.49
394	0.72	0.80	0.80
354	0.30	0.76	0.18
		Sediment	
235	0.73	0.72	0.30
394	0.49	0.63	0.19
354	0.50	0.65	0.17
mean	0.60	0.73	0.36
		MEDIUM BA	
		Water	
265	0.80	0.80	0.85
394	0.80	0.80	0.80
354	0.80	0.82	0.79
		Sediment	
235	0.80	0.81	0.92
394	0.79	0.80	0.79
mean	0.80	0.81	0.83
		MEDIUM BO	
		Water	
265	0.80	0.80	0.91
235	0.67	0.85	0.79
394	0.80	0.79	0.76
354	0.80	0.80	0.80
		Sediment	
235	0.75	0.80	0.65
394	0.80	0.80	0.82
354	0.65	0.80	0.80
mean	0.75	0.81	0.79

Table 32. Nutritional utilization indices for bacterial communities isolated on different media.

Sta.	N_c (carbohydrates)	N_a (alcohols)	N_{ca} (carboxilic acids)	N_{aa} (amino acids)	N_h (hydrocarbons)
			MEDIUM MA		
			Water		
265	0.23	0.34	0.37	0.39	0.13
235	0.35	0.30	0.42	0.51	0.08
394	0.48	0.39	0.41	0.60	0.10
354	0.40	0.29	0.22	0.35	0.35
			Sediment		
235	0.28	0.23	0.31	0.42	0.15
394	0.14	0.23	0.16	0.31	0.00
354	0.15	0.13	0.18	0.22	0.08
mean	0.29	0.27	0.30	0.40	0.13
			MEDIUM MO		
			Water		
265	0.49	0.33	0.55	0.66	0.37
235	0.63	0.32	0.59	0.41	0.06
394	0.53	0.33	0.43	0.43	0.19
354	0.06	0.13	0.03	0.12	0.03
			Sediment		
235	0.33	0.26	0.39	0.52	0.35
394	0.11	0.09	0.08	0.28	0.09
354	0.06	0.08	0.07	0.12	0.03
mean	0.32	0.22	0.31	0.36	0.16
			MEDIUM BA		
			Water		
265	0.15	0.32	0.58	0.43	0.15
394	0.59	0.64	0.77	0.82	0.62
354	0.41	0.80	0.77	0.91	0.73
			Sediment		
235	0.11	0.20	0.50	0.31	0.04
394	0.37	0.65	0.78	0.87	0.66
mean	0.33	0.52	0.68	0.67	0.44
			MEDIUM BO		
			Water		
265	0.15	0.36	0.58	0.49	0.09
235	0.43	0.52	0.64	0.69	0.41
394	0.46	0.81	0.73	0.88	0.89
354	0.62	0.59	0.67	0.78	0.62
			Sediment		
235	0.30	0.28	0.56	0.59	0.16
394	0.49	0.68	0.75	0.83	0.70
354	0.63	0.83	0.78	0.82	0.71
mean	0.44	0.58	0.67	0.73	0.51

comparable utilization indices for the Alaskan isolates obtained on low nutrient media.

There was a higher incidence of pleomorphism among the isolates from low nutrient media (33%) than for the strains from high nutrient media (8%). Pleomorphism has been associated with oligotrophic bacteria; the increased surface area appears to be important for being able to utilize nutrients at very low concentrations (Moaledi, 1978; Poindexter, 1979, 1981a, 1981b). Many of the taxa from both high and low nutrient media in our study undoubtedly represent Vibrio species, which we have previously reported to be among the dominant bacterial populations of Alaskan Continental Shelf ecosystems (Kaneko et al., 1979; Hauxhurst et al., 1981). Moaledi (1981) also found that Vibrio species were among the dominant oligotrophic bacteria in the Plubsee, although various other pleomorphic genera of oligotrophs also occurred.

Bacterial communities associated with Arctic amphipods

To examine the bacterial communities associated with amphipod populations, populations of the amphipod Boeckosimus (= Onisimus) affinis were collected in Elson Lagoon, 50 m south of Plover Point near Pt. Barrow, Alaska. Amphipods were captured in wire mesh traps, baited with fish, which were suspended in the water column, beneath the ice. Animals used in this study were between 11 and 16 mm in length.

Scanning electron microscopic observation of the amphipod Boeckosimus affinis indicated a lack of associated surface microorganisms (Fig. 9a, b). Exhaustive viewing of all surface areas of multiple specimens did not reveal any associated microbial populations (Other species of amphipods collected from sediment had extensive microbial surface fouling indicating that the lack of microorganisms on the surface of B. affinis was probably not an artifact of specimen preparation).

Examination of the intestinal tract of B. affinis did not show any microorganisms associated with the lining of foregut, midgut and hindgut tissues (Fig. 10a). Microorganisms were observed, however, colonizing food particles within the midgut (Fig. 10b). Relatively low population densities were observed on the faecal matter in the hindgut, near the anal pore (Fig. 10c); the anal plates of the amphipod were colonized by a vibrio shaped bacterial population (Fig. 10d).

In the cluster analyses of viable microbial isolates 10 phena containing more than 2 strains were identified at the 75% similarity level. Eighty percent of all isolates examined occurred within these 10 clusters. The microorganisms associated with these amphipods are predominantly gram negative, motile, facultative anaerobic rods which appear to be in the Vibrio-Beneckea group as described by Shewan and Veron (1974).

The dominant microbial populations associated with B. affinis at the time of capture could metabolize proteinaceous material (e.g., gelatin, peptone, tryptone). The main amino acids utilized by these microorganisms were asparagine and proline; most other amino acids were utilized by none or only a very low proportion of the microbial strains. Urea could not be hydrolyzed by the associated microorganisms. A relatively low proportion of the bacterial isolates from freshly captured amphipods could utilize plant polymers (e.g., cellobiose, starch) although all could utilize simpler carbohydrates. Most could utilize lipoidal compounds (e.g., fatty acids, glycerol) but did not produce lipase enzymes. All isolates associated with the freshly caught B. affinis amphipods could hydrolyze chitin and starch. None of the isolates associated with the amphipods demonstrated the ability to metabolize hydrocarbons.

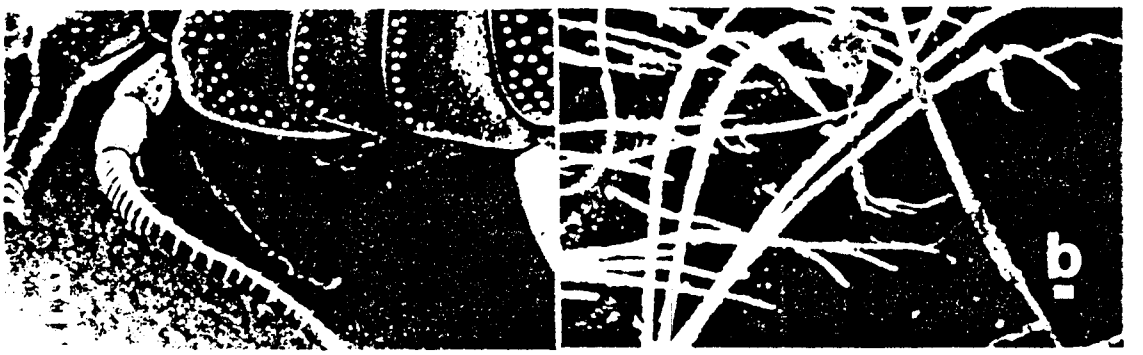

FIG. 9. Scanning electron micrographs of surface regions of *B. affinis*. (*a*) Low-magnification view. Note lack of visible surface fouling. Bar = 100 μm. (*b*) High-magnification view of pleopod surface showing total lack of associated bacterial populations. Bar = 1 μm.

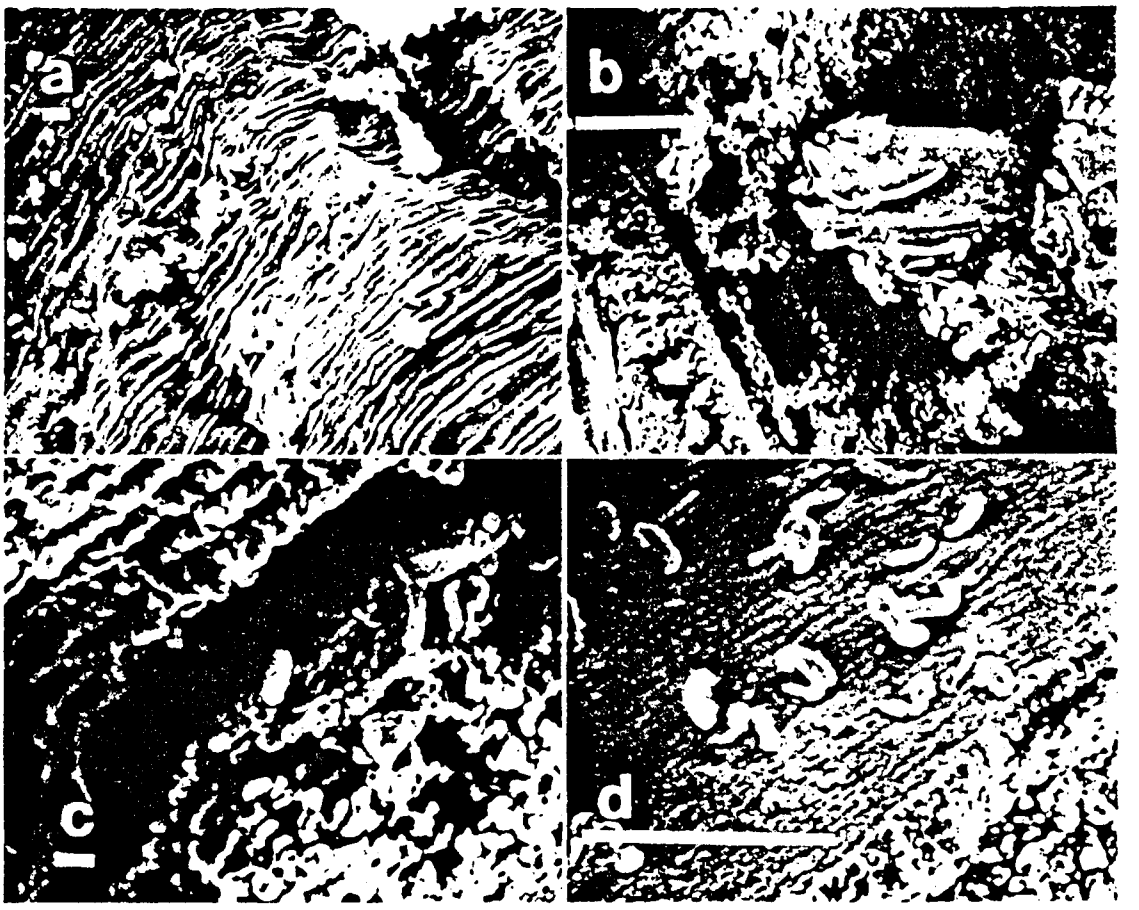

FIG. 10. Scanning electron micrographs of regions of the gut of the amphipod *B. affinis*. (*a*) Section of amphipod foregut showing absence of bacteria associated with the gut lining. Bar = 1 μm. (*b*) Cross section of the midgut showing food particles. Lining of gut is shown in the lower left of the micrograph. Note bacterial cells, including curved rods, on the surface of a food particle in the center of the micrograph. Bar = 10 μm. (*c*) Faecal matter at anal pore of amphipod showing a few bacterial cells on the surface. Bar = 1 μm. (*d*) Anal plates of the amphipod showing occurrence of vibrio-shaped bacteria. Bar = 10 μm.

During the experimental exposure period there was a shift in the metabolic capabilities of the microbial populations associated with B. affinis. This is shown by an increase in the nutrient utilization indices during the exposure period, indicating a diversification of the metabolic capabilities of the microbial isolates (Table 33). There also was a general shift in the physiological tolerance ranges of the bacterial isolates. Initially the dominant populations were psychrophilic and stenohaline; following captivity (without feeding) the dominant populations were psychrotrophic and euryhaline. This shift in physiological tolerance ranges in reflected in the increased physiological tolerance indices for the bacterial populations associated with amphipods held in captivity (Table 34).

Additionally, there was a decrease in the relative numbers of vibrio-like organisms (CFU on TCBS agar relative to CFU on marine agar 2216) when exposed to petroleum hydrocarbons. Populations of vibrio-like organisms shifted from being the major (dominant) portion of the population to being present in much lower proportions compared to the total viable population when oil was added. The presence of water soluble oil components may have stressed the association between amphipods and bacteria such that the initially dominant vibrio-like populations (Vibrio-Beneckea group) declined in numbers and importance.

The taxonomic diversity of the microbial community also changed during the experimental exposure (Table 35). There was a general increase in the diversity of the microbial community associated with the amphipods, shown by changes in both H and J, during the experimental period. An increase in microbial diversity occurred in both controls and oil exposed cases; the increase was greater for populations exposed to oil. No replicates were performed for each treatment and combination thus statistical analyses could not be performed to determine the significance of this observation. The

Table 33. Nutrient utilization indices for bacterial populations associated with the amphipod B. affinis.

Treatment	N_c	N_a	N_{ca}	N_{aa}	N_T
at time of capture	0.36	0.08	0.12	0.16	0.18
7 day control	0.60	0.09	0.37	0.47	0.38
7 day + oil	0.50	0.43	0.50	0.67	0.53
14 day control	0.75	0.31	0.59	0.75	0.60
14 day + oil	0.51	0.38	0.48	0.74	0.53

Table 34. Physiological tolerance limits for bacterial populations associated with the amphipod B. affinis.

Treatment	P_T	P_H	P_S	P_C
at time of capture	0.33	0.25	0.29	0.29
7 day control	0.62	0.21	0.53	0.45
7 day + oil	0.59	0.25	0.50	0.45
14 day control	0.68	0.52	0.47	0.44
14 day + oil	0.58	0.68	0.46	0.45

Table 35. Diversity ($\bar{H}$) and equitability (J) of bacterial populations associated with the amphipod B. affinis: changes during captivity (starvation) and exposure to oil.

Treatment	$\bar{H}$	J
at time of capture	1.00	0.22
0 time control	1.41	0.30
7 day control	1.69	0.37
14 day control	2.02	0.44
7 day + oil	2.79	0.71
14 day + oil	2.59	0.66

consistent increase during the exposure period, nevertheless, appears to clearly indicate a pattern of increased diversification in the microbial community.

Denitrification - potential activities

The rates of denitrification (N_2O production) from unamended sediments are shown in Fig. 11. There was a high degree of variance in the rates of denitrification between different sediment samples. Variability between replicates from the same sample was always less than 10%. No duplicate samples were analyzed from the same sampling site, but samples collected within 1 km of each other showed up to 50% variability.

No detectable N_2O was produced from approximately one-third of the unamended samples from Norton Sound and the northern Bering Sea. In the western Bering Sea (Navarin Basin), approximately 50% of the samples produced $\leq$ 1 pmol of N_2O/g per day. The rate of N_2O production from unamended Beaufort Sea sediment samples was below the level of detection with a TCD. All Cook Inlet and Gulf of Alaska unamended samples produced detectable levels of N_2O. The highest rate of N_2O production, 2.3 nmol/g per day, occurred on the western side of Cook Inlet (wthin Kamishak Bay). Relatively high rates of N_2O production, from unamended sediments, 1.2 to 1.7 nmol/g per day, were found on either side of the Aleutian Islands, near Unimak Pass. Addition of organic carbon and nitrogen produced variable results: in several cases, addition of peptone led to a 50% reduction of N_2O evolution; in others, rates of N_2O production were unaffected or slightly stimulated.

Significantly higher rates of N_2O production were found when the samples were amended with nitrate (Fig. 12) compared with unamended samples. All samples amended with nitrate produced detectable amounts of N_2O. In the upper Bering Sea, the rates of N_2O production typically were three to four orders of

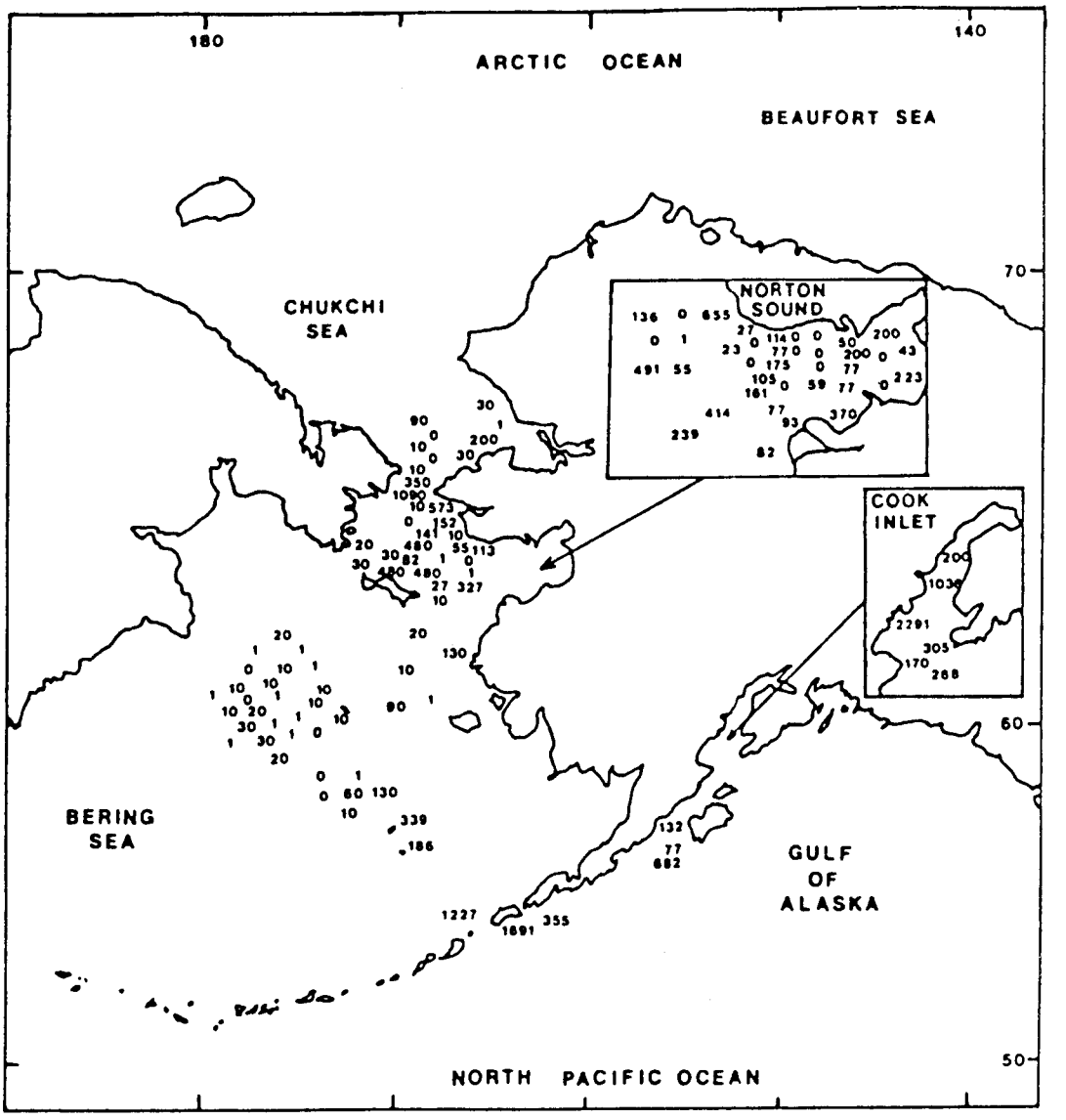

FIG. 11. *Rates of denitrification from unamended sediments, medium R (picomoles of N_2O per gram per day).*

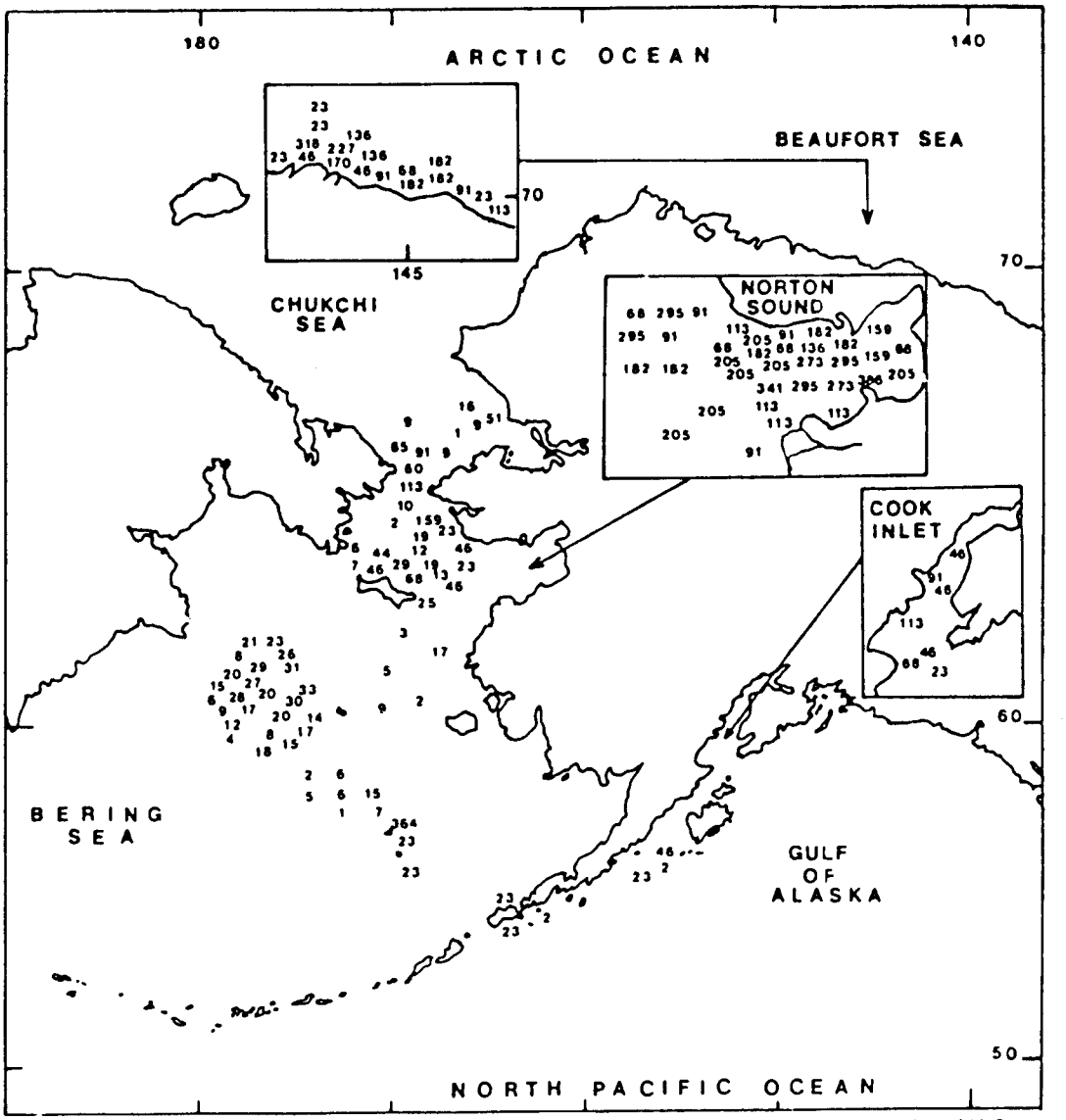

FIG. 12. *Rates of denitrification from sediments amended with nitrate, medium RN (nanomoles of N_2O per gram per day).*

magnitude above rates from unamended treatments at comparable sites. Data from the Norton Sound also showed that addition of NO_3^- generally stimulated N_2O production from three to four orders of magnitude compared with unamended samples. In the western Bering Sea (Navarin Basin), the degree of stimulation was about three orders of magnitude. In the Gulf of Alaska-Cook Inlet region, the degree of stimulation was generally only one to two orders of magnitude over unamended treatments.

Addition of an organic carbon source together with NO_3^- further stimulated N_2O production in almost all cases, generally by twice the values obtained when NO_3^- was the sole amendment. A comparison of the rates of denitrification from sediment collected in various Alaskan continental shelf regions is shown in Table 36. There was a sixfold difference in rates of denitrification for samples collected at the same geographic coordinates 14 months apart in the upper Bering Sea. The apparent regional differences in rates of denitrification were obscured by the high degree of variance within each of the broad sampling regions, as evidenced by the high standard deviations. The differences between regions were not statistically significant. It does appear, however, that potential rates of denitrification are as high in Arctic sediments as in subarctic Alaskan continental shelf sediments.

Effects of oil on denitrification

Without added nitrate there was a statistically significant difference in denitrification (N_2O production) between oiled and unoiled sediments (Table 37). This difference was found for both short-term (1 week) and long-term (up to 2 years) exposures. No N_2O production from unamended samples was observed during the incubation period from any of the oiled sediments. With added nitrate there was no statistically significant difference between oiled and

Table 36. Production of N_2O by sediment slurries from various regions of the Alaskan continental shelf

Region	Sampling date	Mean N_2O production (nmol/g per day) from slurries incubated in[a]: R	RN	RNB
Beaufort Sea	Aug. 1978		113 (82)	184 (129)
Upper Bering Sea	April 1979	0.2 (0.2)	71 (51)	106 (72)
Upper Bering-Chukchi Seas	June 1980	0.04 (0.06)	20 (20)	22 (23)
Norton Sound	Aug. 1979	0.1 (0.2)	181 (85)	197 (104)
Navarin Basin	May 1980	0.03 (0.07)	15 (9)	41 (32)
Cook Inlet	May 1979	0.7 (0.8)	62 (31)	163 (107)
Gulf of Alaska-lower Bering Sea	May 1979	0.6 (0.6)	21 (14)	84 (69)

[a] Standard deviation is given in parentheses.

Table 37. Effects of crude oil on nitrogen fixation and denitrification

Region	Sampling date	Exposure time	Control	Experimental
[a]Elson Lagoon	Jan. 1980	1 wk	200	<4
	Jan. 1980	24 mo	100	<4
	Aug. 1979	8 mo	5	<4
	Aug. 1979	16 mo	4	<4
	May 1980	5 mo	<4	<4
	May 1980	28 mo	<4	<4
[b]Elson Lagoon	Jan. 1980	1 wk	46.3	48.8
	Jan. 1980	24 mo	56.3	55.2
	Aug. 1979	8 mo	166.3	179.2
	Aug. 1979	16 mo	166.3	190.9
	May 1980	5 mo	58.6	80.1
	May 1980	28 mo	58.6	50.1

[a] Values for denitrification in unamended samples (picograms of N_2O produced per gram per hour).

[b] Values for denitrification in NO_3-amended samples (nanorgrams of N_2O produced per gram per hour).

unoiled sediments; i.e., denitrification potentials were not altered by exposure to oil.

Oil biodegradation - potential activities

Beaufort Sea

In the Beaufort Sea, natural biodegradation potentials measured with [^{14}C]hexadecane were significantly higher in winter-spring than in summer-fall samples (Table 38). The natural biodegradation potentials in the Beaufort Sea summer-fall samples were almost nil.

Cook Inlet - Gulf of Alaska

Water samples collected in Cook Inlet had low natural biodegradation potentials in both spring and fall (Tables 38 and 39). Somewhat higher natural biodegradation potentials were found in Cook Inlet sediment in summer-fall samples than in winter-spring samples. Natural biodegradation potentials were not higher in beach or nearshore samples than in offshore samples. The winter-spring northeast Gulf of Alaska natural biodegradation potentials were higher than in any of the other subarctic samples.

In Cook Inlet, natural biodegradation potentials followed the order naphthalene > hexadecane > pristane $\geq$ benzanthracene (Fig. 13). Natural biodegradation potentials for pristane and benzanthracene were often zero. The non-nutrient-limited biodegradation potentials followed the order hexadecane > naphthalene >> pristane > benzanthracene. In almost all cases, the removal of nutrient limitation resulted in higher biodegradation potentials for hexadecane and naphthalene, but not for pristane or benzanthracene.

The gas chromatogrophic analysis of residual oil from selected samples showed a significant correlation ($r = 0.83$) between the biodegradation potential based on hexadecane and the amount of oil remaining (Fig. 14). The biodegradation potentials for naphthalene, pristane, and benzanthracene did not

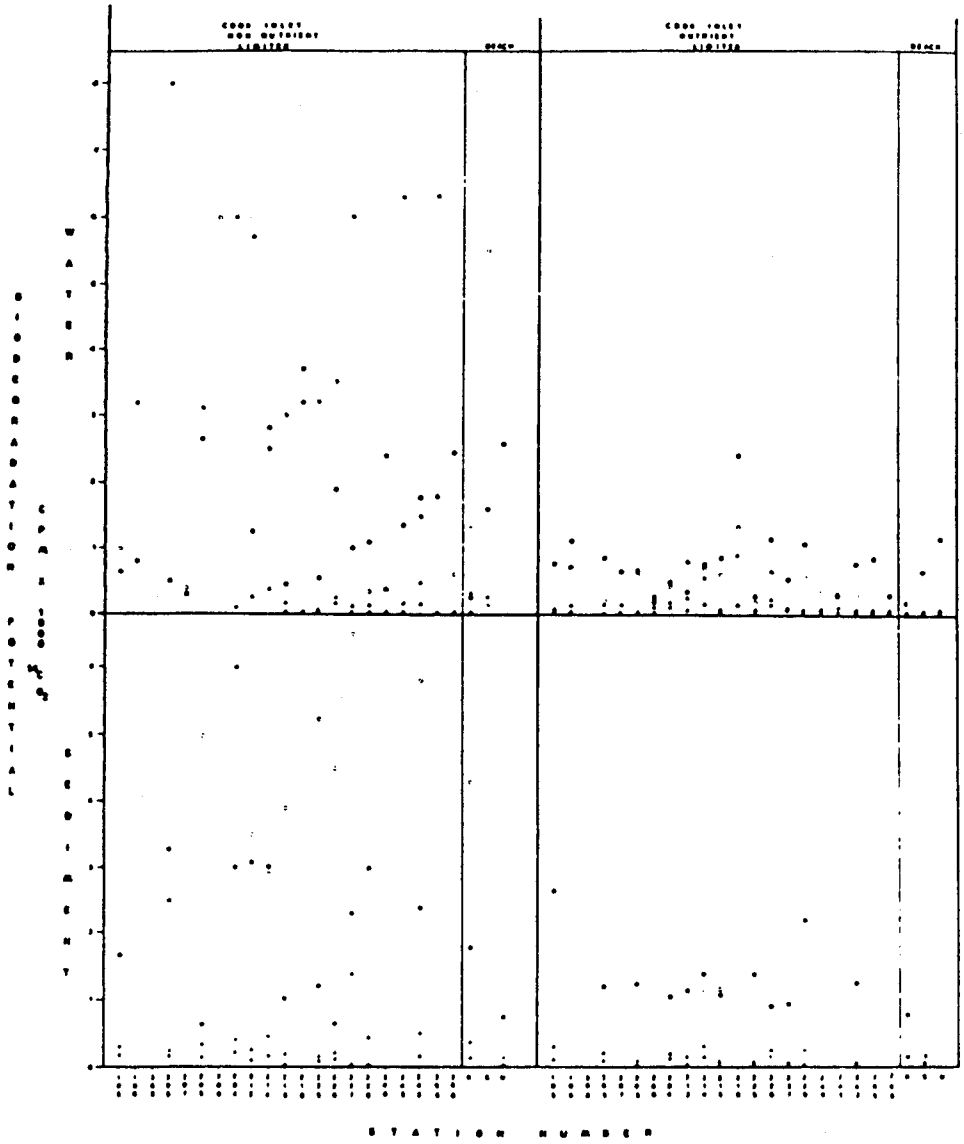

FIG. 3. *Natural and non-nutrient-limited biodegradation potentials in Cook Inlet for winter-spring 1977. Symbols:* (○) *hexadecane;* (●) *naphthalene;* (△) *pristane;* (▲) *benzanthracene.*

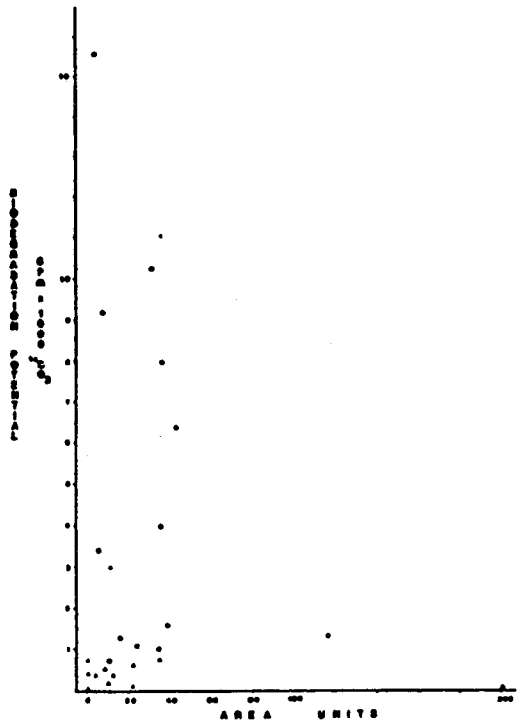

FIG. 14. *Comparison of biodegradation potentials and gas chromatographic analyses of remaining oil. Symbols are as in Fig. 13.*

Table 38. Natural biodegradation potentials based on [^{14}C]hexadecane

Source	Sample	cpm of $^{14}CO_2$ produced			No. of samples
		Mean	Standard deviation	Range	
Beaufort Sea					
April 1976	Ice	1,321	1,009	50-3,000	16
April 1976	Water	4,803	4,196	100-13,500	16
April 1976	Sediment	4,546	3,096	160-11,000	14
Aug.-Sept. 1976	Water	63	100	0-400	15
Aug.-Sept. 1976	Sediment	137	126	50-450	12
Northwest Gulf of Alaska					
Oct. 1975	Water	575	250	400-750	3
Oct. 1975	Sediment	2,000		2,000	1
Northeast Gulf of Alaska					
March 1976	Water	4,925	1,955	2,000-8,000	17
March 1976	Sediment	5,485	1,920	2,100-9,300	15
Cook Inlet					
Oct. 1976	Water	369	261	50-1,000	13
Oct. 1976	Sediment	1,850	1,560	100-4,750	10
April 1977	Water	468	300	50-1,200	20
April 1977	Sediment	563	394	100-1,100	12
Nov. 1977	Water	37	58	0.02-210	16
Nov. 1977	Sediment	8,420	8,370	23-20,000	8

Table 39. Mineralization of hydrocarbons from representative samples collected in Cook Inlet during 6 wk incubation.

	SPRING				FALL			
site	hexadecane	pristane	naphthalene	benz-anthracene	hexadecane	pristane	naphthalene	benz-anthracene
105	0.3-1.9%	<0.3%	0.3-1.9%	<0.3%	0.3-1.9%	<0.3%	<0.3%	<0.3%
106	0.3-1.9%	<0.3%	0.3-1.9%	<0.3%	0.3-1.9%	<0.3%	0.3-1.9%	<0.3%
204	5.0-10.0%	<0.3%	0.3-1.9%	<0.3%	0.3-1.9%	<0.3%		
205	5.0-10.0%	<0.3%	0.3-1.9%	0.3-1.9%	<0.3%	<0.3%	<0.3%	<0.3%
206	2.0-4.9%	<0.3%	<0.3%	<0.3%	<0.3%	<0.3%	<0.3%	<0.3%
212	0.3-1.9%	0.3-1.9%	0.3-1.9%	<0.3%	<0.3%	<0.3%	<0.3%	<0.3%
214	2.0-4.9%	2.0-4.9%	2.0-4.9%	0.3-1.9%	<0.3%	<0.3%	<0.3%	<0.3%
215	2.0-4.9%	<0.3%	0.3-1.9%	<0.3%	<0.3%	<0.3%	<0.3%	<0.3%
225	0.3-1.9%	<0.3%	0.3-1.9%	<0.3%	0.3-1.9%	<0.3%	<0.3%	<0.3%
226	<0.3%	0.3-1.9%	2.0-4.9%	<0.3%	0.3-1.9%	<0.3%	0.3-1.9%	<0.3%
227	5.0-10.0%	<0.3%	0.3-1.9%	<0.3%	0.3-1.9%	<0.3%	0.3-1.9%	<0.3%
229	5.0-10.0%	<0.3%	0.3-1.9%	<0.3%	2.0-4.9%	<0.3%	0.3-1.9%	<0.3%
295	5.0-10.0%	0.3-1.9%	0.3-1.9%	<0.3%	<0.3%	<0.3%	<0.3%	<0.3%
265	>10%	<0.3%	0.3-1.9%	<0.3%	0.3-1.9%	0.3-1.9%	<0.3%	<0.3%
266	5.0-10.0%	0.3-1.9%	2.0-4.9%	<0.3%	<0.3%	<0.3%	0.3-1.9%	<0.3%
105	2.0-4.9%	<0.3%	0.3-1.9%	<0.3%	<0.3%	<0.3%	<0.3%	<0.3%
106	<0.3%	<0.3%	0.3-1.9%	0.3-1.9%	<0.3%	<0.3%	<0.3%	<0.3%
204	5.0-10.0%	0.3-1.9%	2.0-4.9%	<0.3%	<0.3%	<0.3%	<0.3%	<0.3%
205	<0.3%	<0.3%	<0.3%	<0.3%	<0.3%	<0.3%	<0.3%	<0.3%
206	<0.3%	<0.3%	<0.3%	<0.3%	<0.3%	<0.3%	<0.3%	<0.3%
212	2.0-4.9%	<0.3%	2.0-4.9%	<0.3%	<0.3%	<0.3%	<0.3%	<0.3%
214	2.0-4.9%	0.3-1.9%	2.0-4.9%	<0.3%	<0.3%	<0.3%	<0.3%	<0.3%
215	2.0-4.9%	<0.3%	0.3-1.9%	<0.3%	<0.3%	<0.3%	<0.3%	<0.3%
225	5.0-10.0%	<0.3%	0.3-1.9%	<0.3%	0.3-1.9%	<0.3%	0.3-1.9%	<0.3%
227	>10%	0.3-1.9%	2.0-4.9%	<0.3%	0.3-1.9%	<0.3%	0.3-1.9%	<0.3%
229	2.0-4.9%	0.3-1.9%	2.0-4.9%	<0.3%	0.3-1.9%	<0.3%	0.3-1.9%	<0.3%
295	2.0-4.9%	<0.3%	2.0-4.9%	<0.3%	<0.3%	<0.3%	<0.3%	<0.3%

show significant correlation with the amount of oil remaining. Low biodegradation potentials were found for these three substrates even when gas chromatography showed extensive losses of resolvable hydrocarbons.

Bering Sea

The data in Table 40 shows the biodegradation potentials for hexadecane, pristane, and 9-methylanthracene for indigenous microbial populations in various regions of the Bering Sea, including within Norton Sound; variability of replicate determinations for any of the parameters on a given sample was less than 10% of the mean.

The biodegradation potentials for representative hydrocarbons reflect the low bacterial populations in regions of the Bering Sea. Results from over 60% of the samples collected showed no biodegradation of hexadecane, pristane, or 9-methylanthracene in water samples. In the remaining cases, often only $\underline{n}$-C_{16} was degraded under the conditions of the experiment. In the northern Bering mean biodegradation potentials were 1.7% for $\underline{n}$-C_{16} in water and 7.2% in water samples from the north Aleutian Shelf. Other hydrocarbons were degraded minimially or not at all. The most extensive biodegradation of hydrocarbons was found in the near shore sediments of the north Aleutian Shelf in winter. The second most extensive degradation was found in the northern Bering Sea in winter followed by the same area in spring.

Table 41 shows the summary results of detailed analysis of radiolabelled biodegradation experiments. Hydrocarbons from the biodegradation potential experiment vials were extracted after ^{14}C-CO_2 were collected and fractioned into undegraded and degraded components. The results indicate that only hexadecane was mineralized extensively within the three week incubation period of the experiment, with a maximum of 56% of added ^{14}C-hexadecane being

Table 40. Hydrocarbon biodegradation potentials.

	North Bering Sea April 1979	Norton Sound Aug. 1979	Mid-North Bering Sea May-June 1980	South Bering Sea Aug. 1980	South Bering Sea Jan. 1981
Biodeg. Potential C_{16} Water	1.7 ± .5	-	0.0	0	7.2 ± 7.3
Biodeg. Potential C_{16} Sediment	2.7 ± 3.8	-	1.2 ± 6.6	0	11.8 ± 6.4
Biodeg. Potential Pristane Water	0.1 ± 0.1	-	<0.1	0	0
Biodeg. Potential Pristane Sediment	0.2 ± 0.07	-	<0.1	0	1.3 ± 1.8
Biodeg. Potential 9-Methyl-anthracene Water	<0.1	-	0	0	0
Biodeg. Potential 9-Methyl-anthracene Sediment	<0.1	-	0	0	0.2 ± 0.6

mineralized. The portion of the hexadecane that was chemically modified ranged from 2 to 62%, indicating that microorganisms were either incorporating hexadecane into cellular components as a polar compound or were not degrading hexadecane as an energy source.

The other substrates examined for biodegradation; pristane, 9-methyl anthracene, and benzanthracene were mineralized to a maximum of 3%. However, even when extensive mineralization was not detected, these substrates sometimes were degraded to polar compounds to a significant extent. Pristane was degraded from 2 to about 20%, 9-methyl anthracene was degraded from 1 to 13% and benzanthracene was degraded from 0-4%. These results indiate that petroleum added to the Bering Sea can be degraded but complete mineralization to CO_2 and H_2O will be a slow process.

Oil weathering - microbial biodegradation of petroleum hydrocarbons

Beaufort Sea - in situ exposure

The total microbial biomass remained relatively constant, ca 5×10^8 bacterial cells/g dry wt sediment, during the 2 years of exposure to petroleum hydrocarbons (Table 42). During the exposure period there was a slow, but significant increase in the concentration of hydrocarbon utilizing microorganisms and the proportion of the total microbial community comprised of this specialized group (Table 42). Many months were required before a substantial increase in numbers of hydrocarbon utilizers was noted. Numbers of hydrocarbon utilizers in unoiled control sediments never exceeded 100 per gram dry wt at any of the collection times.

The nutrient concentrations over the experimental period were relatively low: ammonium ions, 85 µM ± 45 (mean ± standard deviation); nitrate-nitrite ions, 2.9 µM ± 0.7 phosphate ions, 6.6 µM ± 3.0. The C:N and C:P ratios at the beginning of the experimental period both were approximately 40000:1 and

Table 41. Analysis of hydrocarbons extracted from biodegradation potential vials.

	August 1980			January 1981		
	range	mean ± S. dev.		range	mean ± S. dev.	
Hexadecane						
mineralized	0-9%	2	2	0-56	14	14
degraded	2-22%	8	5	2-62	21	16
Pristane						
mineralized	0-1	0	-	0-3	1	1
degraded	2-23	7	4	2-18	9	4
9-methyl anthracene						
mineralized	0	0	-	0-2	1	1
degraded	2-13	6	3	1-13	5	4
Benzanthracene						
mineralized	-	-	-	0-1	<1	<1
degraded	-	-	-	0-4	1	1

Table 42. Enumeration of total and hydrocarbon utilizing microbial populations.

Exposure Time	Direct Count (# x 10^8/g)	MPN Hydrocarbon Utilizers (#/g)	% of Hydrocarbon utilizers in total population
0	4.9	30	6.1×10^{-6}
0.5 h	4.7	40	8.5×10^{-6}
72 h	4.5	40	8.9×10^{-6}
1 mo	5.0	210	4.2×10^{-5}
4 mo	6.2	420	6.8×10^{-5}
8 mo	4.8	2100	4.4×10^{-4}
1 y	5.3	2100	4.0×10^{-4}
1 1/2 y	5.1	2800	5.5×10^{-4}
2 y	5.9	24000	4.1×10^{-3}

20000:1 respectively. This ratio decreased only slightly as the hydrocarbon concentration declined, the N and P concentration remaining relatively constant during the experimental period. Oxygen concentrations were not measured. However, the coloration of the upper 1 cm was light brown and was colonized with polychaete worms, indicating that this upper layer was probably aerobic. Bioturbation by polychaete worms has been reported to be a mechanism through which oxygen is introduced into sediment to support hydrocarbon biodegradation (Gordon et al., 1978). Beneath this surface layer the sediment was uniformly gray (but not black), which is probably indicative of a reduced oxygen tension. It should be noted, however, that color and the presence of sediment fauna may be poor indicators of O_2 availability (Sorenson et al., 1979; Revsbeck et al., 1980). Temperature during the exposure period was between 4.0°C and -1.8°C.

The concentration of resolved aliphatic and aromatic hydrocarbons was highly variable (Table 43). The oil appeared (visually) to have been trapped in pockets within the sediment; the oil clearly ws not uniformly distributed, although it had been thoroughly mixed into the sediment at the start of the experiment. There was a relatively high degree of variability between replicate samples and between the various early sampling times (shown by the high standard deviation values), which probably reflects and confirms the uneven redistribution of hydrocarbons within the sediment.

Only after 1 1/2 years of exposure was there a significant decrease in the heptadecane:pristane ratio. This drop in the n-alkane/isoprenoid hydrocarbon ratio was reproducible. This ratio is an index of biodegradation since normal alkanes are usually degraded by microorganisms more rapidly than highly branched isoprenoid alkanes. A C_{17}:pristane ratio of <1 was considered as clear evidence for biological weathering of the Prudhoe Bay crude oil.

Table 43. Weight of resolved aliphatic and aromatic hydrocarbons and C_{17}:pristane ratio showing mean and standard deviation values in parentheses.

Exposure Time	Aliphatics µg/g dry wt	Aromatics µg/g dry wt	Heptadecane / Pristane
Prudhoe crude	-----	-----	1.16 (-)
0	400 (254)	180 (115)	1.15 (0.04)
0.5 h	280 (29)	87 (20)	1.14 (0.03)
24 h	307 (352)	174 (34)	1.20 (0.11)
48 h	217 (68)	105 (7)	1.16 (0.09)
72 h	630 (88)	132 (40)	1.15 (0.04)
7 d	178 (56)	73 (28)	1.25 (0.05)
14 d	236 (50)	101 (17)	1.19 (0.07)
21 d	337 (175)	143 (16)	1.23 (0.04)
28 d	120 (27)	45 (8)	1.37 (0.08)
3 mo	153 (46)	53 (12)	1.28 (0.29)
4 mo	314 (194)	115 (83)	1.09 (0.13)
8 mo	267 (105)	110 (60)	1.19 (0.11)
1 1/2 y	142 (69)	68 (15)	0.86 (0.08)
2 y	100 (8)	37 (9)	0.67 (0.15)

The detailed analyses of the aliphatic and aromatic fractions showed a lack of both abiotic and biodegradative weathering of the oil until after one year's exposure (Figs. 15 and 16). Low molecular weight alkanes (ca C_9-C_{10}) and aromatics (unsubstituted naphthalene) remained in relatively constant concentrations relative to higher molecular weight compounds during the first year of exposure. Following 1 year's exposure, alkanes of chain length $\leq C_{17}$ declined in concentration relative to both pristane and n-alkanes $\geq C_{18}$ (Fig. 15).

There was an enhancement of alkanes $\geq C_{18}$ relative to pristane in the samples collected following 1 year's exposure. Following several months of exposure there was a relative decline in the proportion of the naphthalene series in the aromatic fraction; higher molecular weight aromatic compounds of the phenanthrene and dibenzothiophene series did not decline in concentration as rapidly (Fig. 16).

Bering Sea - microcosm exposure

Slow rates of hydrocarbon biodegradation in the Bering Sea were indicated in the laboratory experiments as shown in Figures 17, 18, and 19 which illustrate the results of chemical analysis of a long term flow through oil biodegradation experiment. In no case was any significant change in the ratios of aliphatic compounds to pristane observed over the six week period of incubation. The total concentration of recoverable aliphatic compounds did decline by about 75% primarily due to abiotic factors such as evaporation and wash out from the incubation vessel. The nutrient solution was forced to flow through the sediments due to the design of the experimental apparatus.

Analysis of the aromatic fraction of the hydrocarbon extracts showed both a decline in concentration as well as a change in ratios of various aromatic hydrocarbons to C_2 phenanthrene. The concentration of C_2 phenanthrene declined

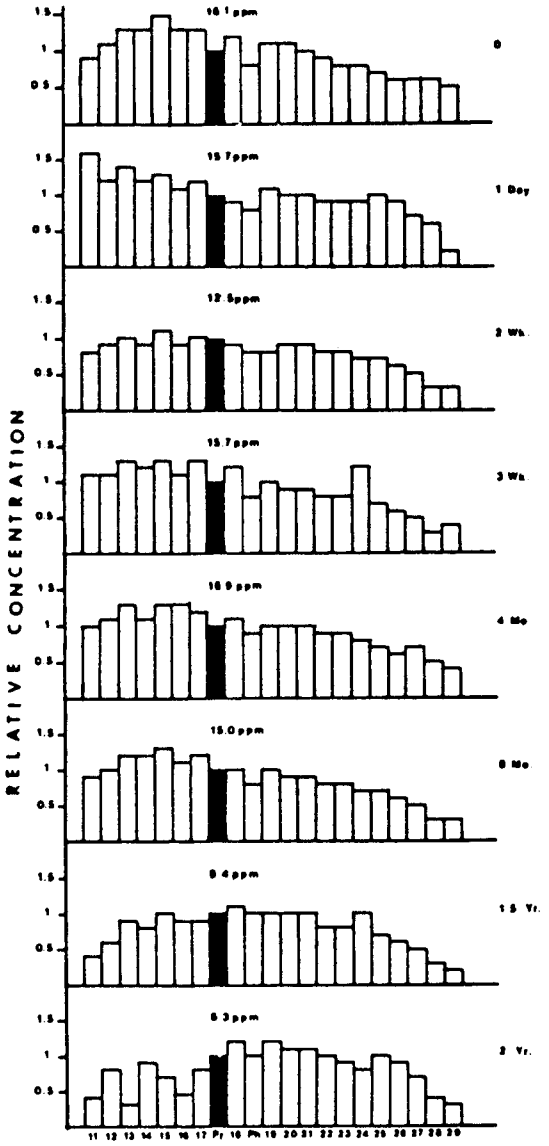

Fig. 15 Simplified histogram presentation of chromatographic analyses of the aliphatic (f_1) fraction. Concentrations of selected alkanes are shown relative to pristane (high relative concentrations are shown as a broken bar with the ratio shown numerically above the bar). The absolute concentration of pristane is given (as ppm shown above the pristane bar), permitting calculation of the actual concentrations of the other compounds. Pr = pristane; Ph = phytane. Numbers refer to chain lengths of normal alkanes.

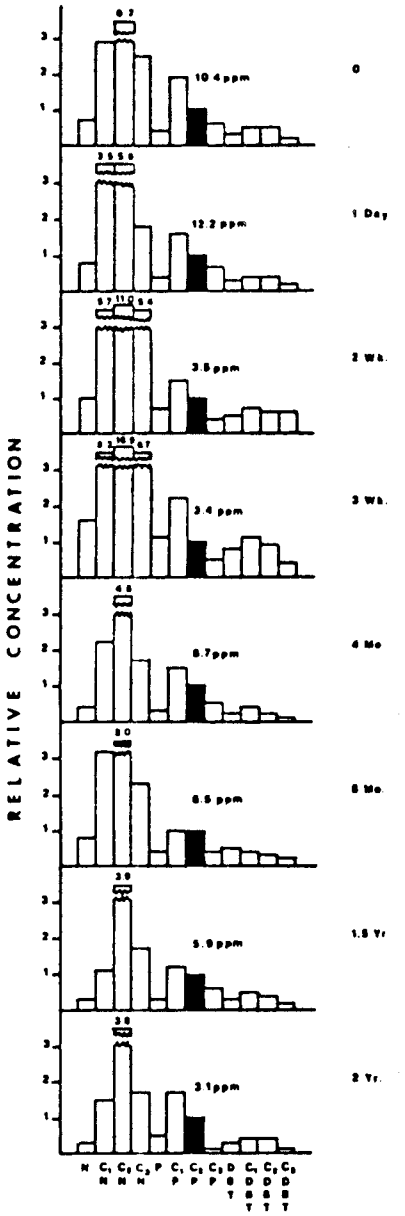

Fig. 16 Simplified histogram presentation of gas chromatographic–mass spectral analyses of the aromatic (f_2) fraction. Concentrations of selected aromatic compounds are shown relative to C_2 phenanthrenes (high relative concentrations are shown as a broken bar with the ratio shown numerically above the bar). The absolute concentration of the C_2 phenanthrenes is given (as ppm above the C_2 phenanthrenes bar) permitting calculation of the actual concentrations of the other compounds. N = naphthalene; P = phenanthrene; DBT = dibenzothiophene; C_1, C_2, C_3 = the degree of alkyl substitution.

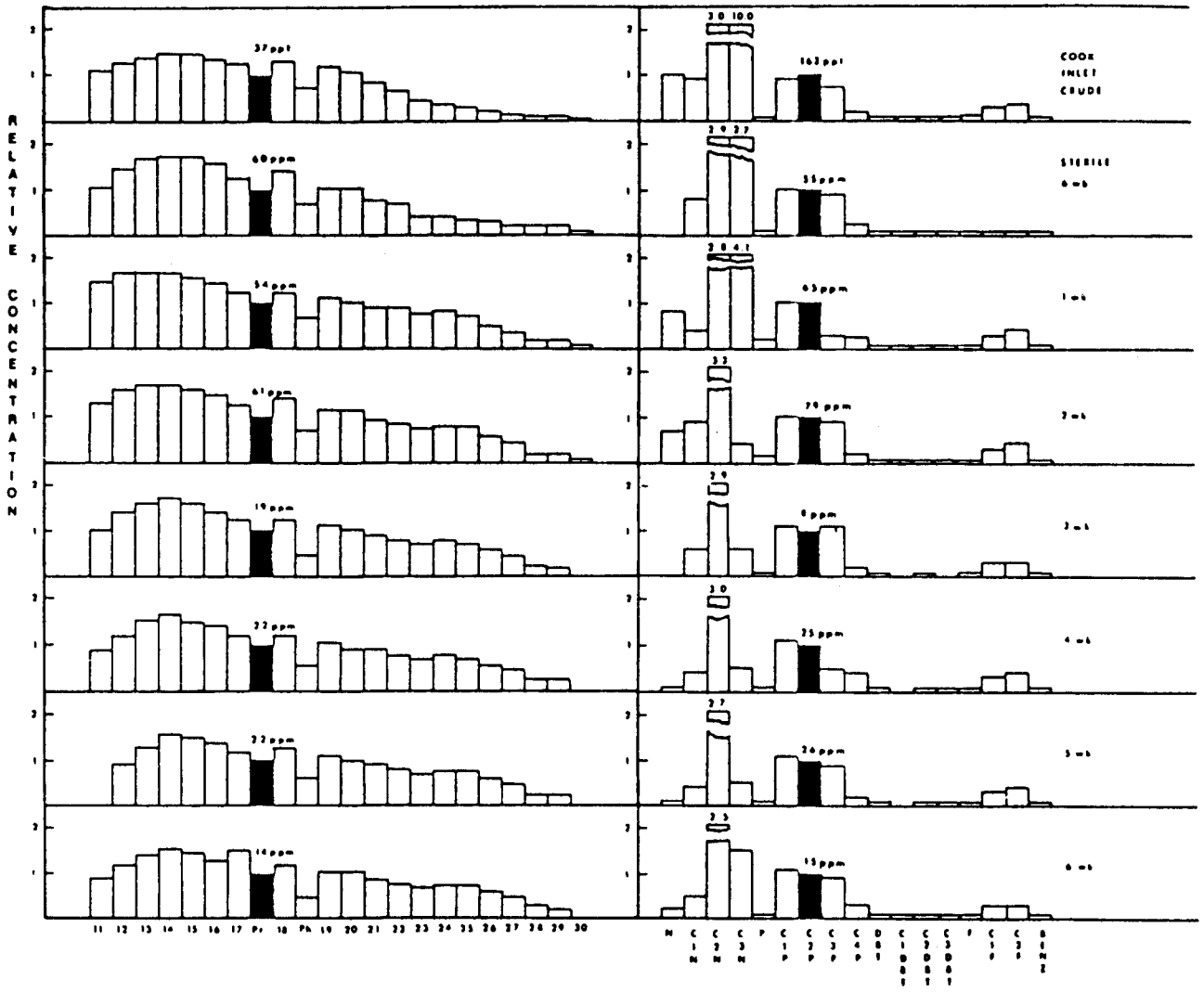

Fig. 17. Simplified histogram presentation of gas chromatographic- mass spectral analysis of f_1 and f_2 fractions extracted from a gravelly sediment. Concentrations of selected alkanes shown relative to pristane. Concentrations of selected aromatic compounds shown relative to C_2 phenanthrenes (high relative concentrations are shown as a broken bar wiht the ratio shown numerically above the bar. The absolute concentrations of pristane and C_2 phenanthrene are given as ppm (shown numerically above the respective bars) permitting the calculation of the absolute amounts of the other compounds. Pr = pristane; Ph = phytane; numbers on X axis refer to chain lengths of n-alkanes; N = naphthalene; P = phenanthrene; DBT = dibenzothiophene; F = fluorene; and BENZ = benzanthrene; C_1, C_2, and C_3 refer to the degree of alkyl substitution of the aromatic compounds.

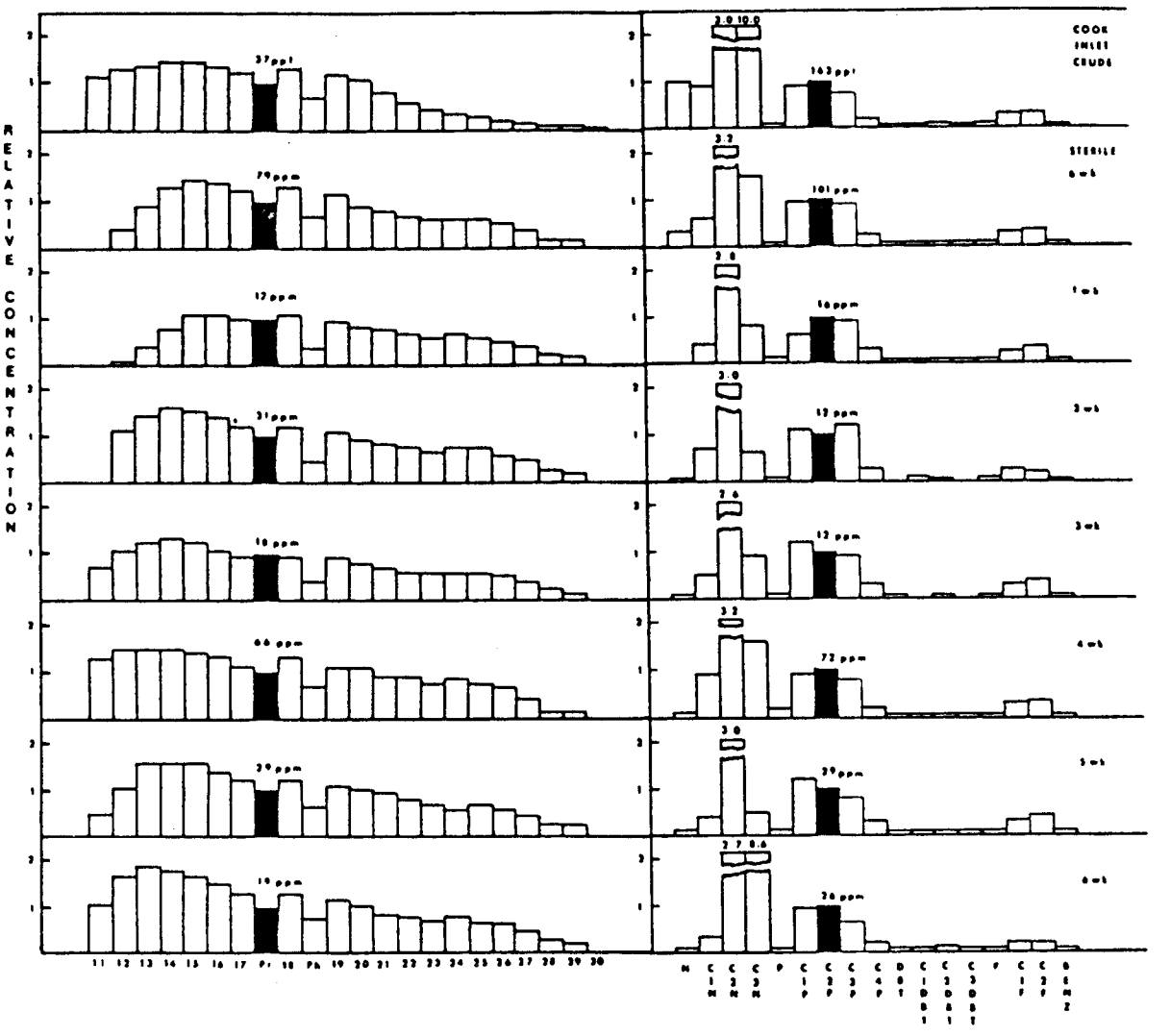

Fig. 18. Simplified histogram presentation of gas chromatographic- mass spectral analysis of f_1 and f_2 fractions extracted from a sandy sediment. Concentrations of selected alkanes shown relative to pristane. Concentrations of selected aromatic compounds shown relative to C_2 phenanthrenes (high relative concentrations are shown as a broken bar wiht the ratio shown numerically above the bar. The absolute concentrations of pristane and C_2 phenanthrene are given as ppm (shown numerically above the respective bars) permitting the calculation of the absolute amounts of the other compounds. Pr = pristane; Ph = phytane; numbers on X axis refer to chain lengths of n-alkanes; N = naphthalene; P = phenanthrene; DBT = dibenzothiophene; F = fluorene; and BENZ = benzanthrene; C_1, C_2, and C_3 refer to the degree of alkyl substitution of the aromatic compounds.

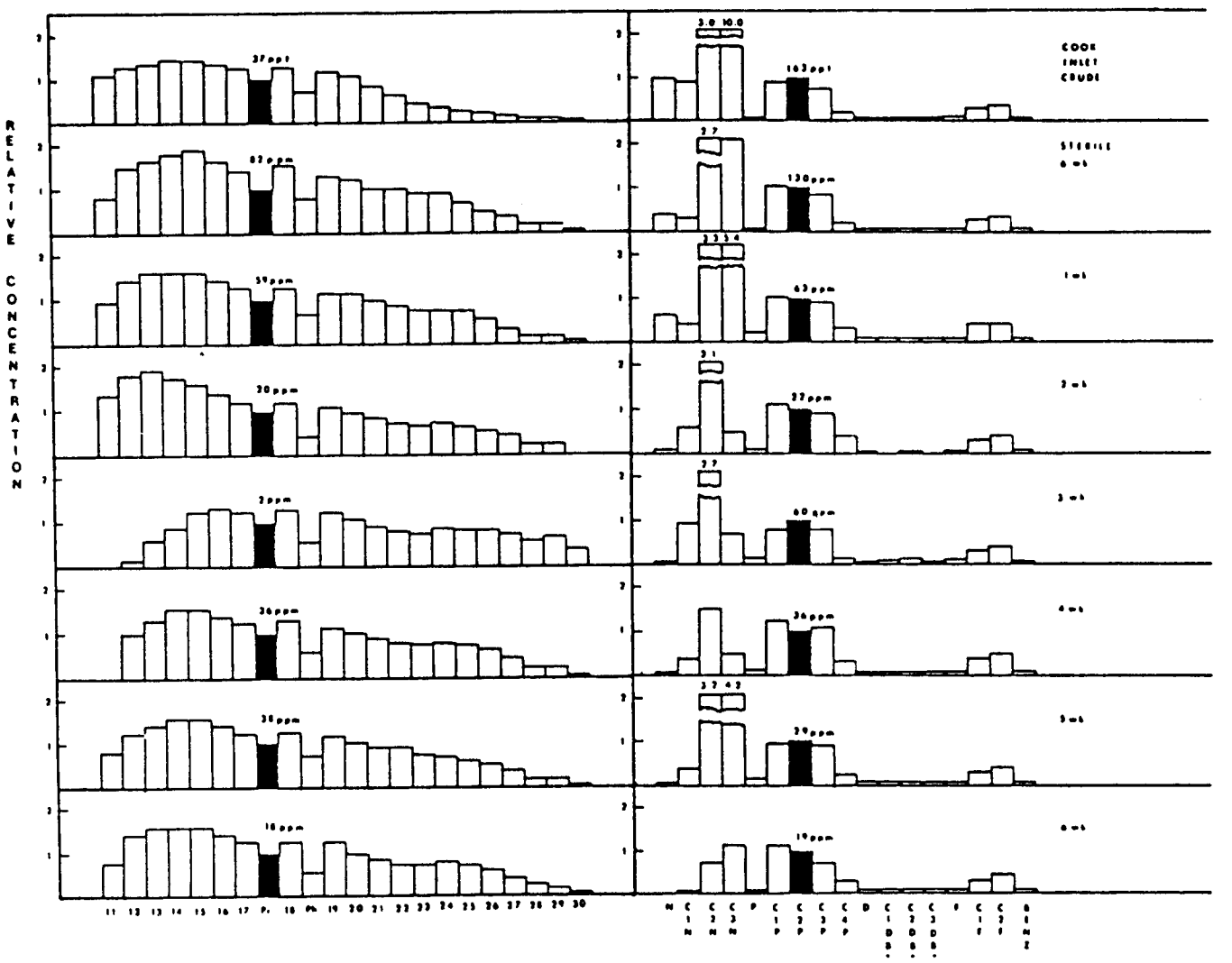

Fig. 9. Simplified histogram presentation of gas chromatographic- mass spectral analysis of f_1 and f_2 fractions extracted from a silty sediment. Concentrations of selected alkanes shown relative to pristane. Concentrations of selected aromatic compounds shown relative to C_2 phenanthrenes (high relative concentrations are shown as a broken bar wiht the ratio shown numerically above the bar. The absolute concentrations of pristane and C_2 phenanthrene are given as ppm (shown numerically above the respective bars) permitting the calculation of the absolute amounts of the other compounds. Pr = pristane; Ph = phytane; numbers on X axis refer to chain lengths of n-alkanes; N = naphthalene; P = phenanthrene; DBT = dibenzothiophene; F = fluorene; and BENZ = benzanthrene; C_1, C_2, and C_3 refer to the degree of alkyl substitution of the aromatic compounds.

over the period of the experiment about 75 to 85%. The compounds showing the greatest change in concentrations relative to C_2 phenanthrene were naphthalene, C_1, C_2, and C_3 substituted naphthalene. Very little change was observed in the ratios of phenanthrene, substituted phenanthrenes, dibenzothiophenes, fluorenes, or benzanthracene to C_2 phenanthrene. The decline of the naphthalene series relative to C_2 phenanthrene is probably due to dissolution in water as well as biologic degradation. Naphthalene and substituted naphthalenes are more soluble in water than phenanthrene or substituted phenanthrenes and would more easily wash out of the incubation vessels than higher molecular weight aromatic compounds.

VII Discussion

ENUMERATION OF MICROBIAL POPULATIONS

The enumeration data present a paradox. Why are numbers of viable bacteria higher in the Beaufort Sea than in subarctic seas overlying the Alaskan Continental Shelf? While the current studies do not provide any definitive answers it may well be that at the low temperatures of the surface waters of the Beaufort Sea bacteria survive for prolonged periods; accordingly the difference between the direct and viable counts may be interpreted as representing dead and dying bacteria.

TAXONOMY OF INDIGENOUS MICROBIAL POPULATIONS

Bacteria in the Beaufort Sea

The Arctic Beaufort Sea, like other polar seas, is characterized by low temperatures, extensive ice cover, constant sunlight during summer and constant darkness during winter. Bacteria in the Beaufort Sea are adapted to these extreme environmental conditions. Our isolates clearly showed adaptation to growth at low temperatures. Of the 4°C isolates, 25% were obligate psychrophiles unable to grow above 15°C. The remainder of the 4°C isolates and all of the 20°C isolates were psychrotrophs; 95% of these could not grow at 37°C. In comparison to our findings, McDonald et al. (1963) found 31% of their isolates from Canadian Arctic sediments could grow at 0°C but not at 25°C. In studies in the Antarctic Ocean, Wiebe and Hendricks (1974) found 37% of their 4°C isolates could not grow above 15°C and Morita (1975) reported that 35% of his isolates could not grow at 20°C. Thus, the Arctic and Antarctic seas are dominated by psychrotrophs and have a high incidence of obligate psychrophiles.

Nearshore areas of the Beaufort Sea show large seasonal fluctuations in salinity. Summer freshwater input and ice melt result in low salinities. Freezing out of salts during winter ice formation results in hypersaline layers which move to the bottom. Most of our isolates could not grow with added NaCl.

The optimum salt concentration was 3% (w/v) NaCl. Most of the 20°C isolates tolerated a wide range of salt concentrations, but many of the 4°C water isolates were restricted to near 3% (w/v) NaCl for growth.

We are not certain why so many strains were oxidase-negative. It is possible that test conditions, e.g. age of cultures, affected the results. Using identical methods in a number of other studies, we have observed much higher proportions of oxidase-positive organisms.

All isolates were facultative anaerobes by the agar-butt stab method. The oxidative/fermentative metabolism tests on MOF medium were used to distinguish aerobes from facultative anaerobes.

Pigmentation can protect bacteria against the lethal effects of intense solar radiation (Mathews and Sistrom, 1959). A very high proportion of the bacterial isolates obtained from surface waters at 4°C were pigmented; it is possible that the orange or yellow pigmentation is an adaptive protective mechanism for Beaufort Sea bacteria exposed to intense sunlight during the Arctic summer.

An unexpected finding was that most bacteria required vitamins or more complex growth factors. The probable source of nutrients for Beaufort Sea bacteria is primary producing phytoplankton. The annual spring bloom of under-ice algae is probably the prime source of nutrients for bacterial growth (Horner and Alexander, 1972). Phytoplankton are known producers of vitamins and amino acids which can supply bacterial growth factor requirements (Carlucci and Bowes, 1970; MacLeod et al., 1954; Burkholder and Burkholder, 1956). Some flavobacteria have complex requirments for vitamins and amino acids (Prince et al., 1954; Prince and Cleverdon, 1955; Weeks and Beck, 1960).

The cluster analyses and comparison with previously described taxa indicate that the dominant bacteria of the Beaufort Sea are quite different

from those found in temperate marine environments. Most of the phenotypic clusters could be tentatively classified as members of genera which are listed as of uncertain affiliation in Bergey's Manual (Buchanan and Gibbons, 1974). Pseudomonas species, which are often found to be a dominant genus in marine environments (Simidu et al., 1977; Pfister and Burkholder, 1965; Murchelano and Brown, 1970), did not comprise a large proportion of the Beaufort Sea bacteria. We did find several clusters of presumed Vibrio species; such species have been found in high proportions in temperate marine environments (Kaneko and Colwell, 1973, 1974; Lovelace et al., 1967; Cook and Goldman, 1976). Flavobacteria are usually not the dominant taxa in temperate marine ecosystems. In Chesapeake Bay, Lovelace et al. (1967) found 56% Vibrio, 18% Pseudomonas and 6% Flavobacterium species. In Antarctic marine waters, Pfister and Burkholder (1965) found Pseudomonas species to be dominant and pigmented bacteria to comprise a low proportion of the bacterial population. The latter study, in another polar marine environment, contrasts with our findings.

With respect to the genera represented in our cluster analyses, the species were psychrophilic or psychrotrophic. In many cases these may represent new species. The environmental conditions of the Beaufort Sea have apparently resulted in selection of heterogeneous bacterial taxa that are uniquely adapted as members of the bacterial community capable of survival and proliferation in these ecosystems.

Gulf of Alaska Isolates

The dominant Moraxella-Acinetobacter populations found in the Northeast Gulf of Alaska were absent from the Northwest Gulf of Alaska. Indeed, while Acinetobacter and Moraxella strains are readily isolated from marine habitats, they have not previously been found as dominant marine bacterial populations. Some caution must be used in comparing and drawing firm conclusions about

regional differences in species compositions in Alaskan Outer Continental Shelf areas since samples were collected at different times in each area; caution should also be used when comparing the sizes of populations in these different studies since different numbers of isolates were used in each study.

As in the Beaufort Sea (Kaneko et al., 1979), there were several clusters that were equated with Vibrio-Beneckea species and there was a notable lack of Pseudomonas strains in both the Northeast and Northwest Gulf regions. Vibrio-Beneckea species, which are commonly found in marine ecosystems (Kaneko and Colwell, 1973, 1974), were frequently identified in this study. The repeated failure to find dominant populations of Pseudomonas is not likely to be an artefact of isolation procedures since, using the same basic medium marine agar 2216, ZoBell and colleagues (ZoBell and Upham, 1944; ZoBell, 1946) isolated numerous Pseudomonas species in the Pacific between Hawaii and California. Simidu et al. (1977) also isolated Pseudomonas species from the Pacific Ocean.

A general feature of the isolates from the Northeast Gulf of Alaska is their eurytolerance to temperature, salt and pH; most isolates grew at 5 to 37°C, 0.5 to 10% NaCl and pH values of 6 to 10. There was a high incidence of diverse pigmented bacteria (44% of total isolates) in this region, many of which formed single-membered clusters; this finding is similar to our observations in other Alaskan Continental Shelf regions. Some are undoubtedly flavobacteria, but some of these pigmented bacteria may be coryneforms (Keddie, 1978); difficulties in identifying marine members of the coryneform group have been discussed by Bousfield (1978). There appears to be a spatial discontinuity in the distribution of Microcyclus species; this genus is common to the contiguous region of the Northwest Gulf of Alaska, Bering Sea and Arctic Ocean, but not the Northeast Gulf of Alaska.

A surprisingly high proportion of the isolates from the Northwest Gulf of Alaska appear to be 'true marine bacteria' as shown by their salt requirements. Most of the organisms isolated from this region possess the features of autochthonous marine microorganisms. Most isolates are psychrotrophs capable of growth over the full range of temperatures that occur in surface and bottom water layers of these habitats. No obligate psychrophiles were found. There is no universal definition for a true marine bacterium. ZoBell (1961, 1963) considers a marine bacterium to be one which, on initial isolation, grows in full-strength seawater (3% NaCl), but not at one-quarter to one-third the osmotic pressure of seawater. Bacteria which, on initial isolation, are restricted to growth at or near 3% NaCl and which grow in the range of 0 to 20°C were considered to meet the requirements of true marine bacteria. Other eurytolerant bacteria exist quite well in marine ecosystems as evidenced by the large number of such organisms isolated, but it is virtually impossible to classify such bacteria as truly marine since they could also exist in terrestrial and freshwater ecosystems. It is best to consider such organisms, which are able to grow in seawater under ambient conditions and which are isolated from marine ecosystems, as indigenous marine bacteria; they are functionally marine bacteria if not indeed true marine bacteria.

There was a lack of dominant population in the Northwest Gulf of Alaska. Rather, the populations in this region were very diverse and most organisms formed single-membered clusters. There was a relatively high mortality rate among these original isolates as evidenced by the reduction from the original 25 isolates from each station to those used in the analyses. We attribute this loss of viability in part to the complex nutritional requirements shown by many of the isolates which may not have been met during subculturing. The more stringent nutritional requirements of the Northwest Gulf isolates, compared

with the Northeast Gulf isolates, are interesting to note; they probably reflect regional differences in nutrient availability.

Many of the pigmented isolates, which comprised 37% of the strains included in the cluster analysis, formed single-membered clusters. The incidence of pigmented bacteria in the Northwest Gulf of Alaska was somewhat lower than in the Northeast Gulf and higher than has been reported for some temperate marine waters (Lovelace et al., 1967). Microcyclus species were tentatively identified, based on morphology, near the Aleutian Islands; they have also been found in the Beaufort Sea (Kaneko et al., 1979) and Bering Sea (unpublished data) but not in the Northeast Gulf of Alaska.

The strains examined in this study show a heterogeneity of features that does not permit a clear separation of genera based on the use of individual 'key' phenotypic features; this is evidenced by the spectrum of features found for isolates that appear to be Moraxella-Acinetobacter species. Problems with relying on a single feature for determining taxonomic status are illustrated in this study, e.g. the reliance on yellow-orange pigmentation for classification of Flavobacterium species and on a negative oxidase test for classification of Acinetobacter.

The results of the catalase tests present a particular problem in classifying several phenetic groups in this study. The only Gram-negative, catalase-negative strains described in Bergey's Manual of Determinative Bacteriology are included in the genus Derxia (Becking, 1974). While the relationship of strains isolated from the Gulf of Alaska to the genus Derxia cannot be positively excluded, the features of the catalase-negative strains from the Gulf of Alaska do not resemble those in the description of Derxia gummosa. The catalase-negative isolates could represent a new group of generic

rank, but it is not possible to make this conclusion based largely on this phenotypic feature which may be of dubious taxonomic value.

This study highlights the difficulties in classifying bacteria from diverse habitats, which have been poorly studied, even when a large number of phenotypic features are considered. Many of the isolates examined in this study could not be identified.

The failure to recover reference strains within phenetic clusters makes identification of the Gulf of Alaska isolates difficult. The reference strains were selected because they represent genera normally reported for marine ecosystems. Most of the ATCC strains selected as reference strains were originally isolated from marine ecosystems, many from the Pacific Ocean near the Gulf of Alaska regions sampled in this study. In retrospect, it is not surprising that ATCC reference strains from temperate regions of the Pacific Ocean would not be included in phenetic clusters containing strains isolated from sub-Arctic Gulf of Alaska ecosystems. Most of the ATCC strains included in constructing the probabilistic identification matrices are not marine isolates and would not be expected to show a high similarity with marine isolates from the Gulf of Alaska. Species distribution appears to be quite different between the Northeast and Northwest Gulf of Alaska regions as well as between these regions and elsewhere in the Pacific Ocean and in other Alaskan Continental Shelf regions. Many new 'species' or genera probably exist within the Gulf of Alaska and other northern marine ecosystems.

Probabilistic Identification of Bacteria from Alaskan OCS regions

The work on the numerical taxonomy of bacteria from the Gulf of Alaska dealt with the difficulty in identifying a clear separation of genera based on the use of arithmetically defined key features. An identical problem was encountered in the attempts to select key features for use in the development

of the primary identification matrix (super-matrix). The results of several attempts to separate the represented groups based on several different combinations of group agglomerates, consistently failed to yield clear separations of the groups represented. Growth at various concentrations of NaCl, at various temperatures, at differeing pH values, and the distribution of pigmented bacterial strains were cited as important discriminants for the differentiation of Gulf of Alaska isolates by Hauxhurst et al. (1980); similarly the work by Kaneko, et al. (1977, 1979) support the use of the above parameters for grouping marine bacteria from the Beaufort Sea. The requirement for NaCl separates marine from non-marine bacteria and growth at 25°C separates psychrophilic-psychrotrophic from mesophilic populations. One might argue that the subjective selection of these characters violates the assumption of lack of bias in qualifying groups on the basis of arithmetically determined separators. However, these features were used to divide the spectrum of taxonomically defined groups into subsets of workable sizes. Subsequent choices of highly discriminant characters within subgroups were based on the arithmetically determined measures of separation values, character correlation and redundancy.

Since success of identification procedures depends on minimizing errors we should consider the sources of potential error in the procedures used in this study. Error may result from: data acquisition, poor sampling of the phenetic hyperspace, shortcomings of the clustering algorithm, and inappropriate threshold levels for probabilistic identification (Sneath and Sokal, 1973). Hauxhurst et al. (1980) found a testing error rate of 3% for Gulf of Alaska isolates at isolation temperatures of 5°C and 20°C. The total error rates for the super- and sub-matrix and inclusive schema described in this study were 8% and 7%, respectively, for lenient identification thresholds of ID score equal to 0.970 and R score equal to 0.001. If the inherent testing error rate

suggested by Hauxhurst, et al. (1980) holds for the testing of Alaskan outer-continental shelf marine isolates in general, the proportion of unexplained error is only 5% for the super- sub-matrix procedure and 4% for the inclusive matrix, the residual 3% error being due to errors in the test procedures employed to acquire the data.

In considering the source of this residual error we must note that individual group feature probabilities contained in the computer matrix represent the expected probability that a member of that group will give a positive reaction for the particular feature. Modal strains used in the construction of the matrices do not consider the biological outliers. Although an individual strain may be shown to be most similar to group "X", for example, the strain may be a peripheral member of the other strains contained in group "X". Thus, the peripheral strain is likely to exhibit low likelihood scores with group "X" during identification attempts, especially since the character set on which similarity was originally estimated had been significantly reduced. Of the four strains assigned to wrong groups and falling above the identification threshold criteria after the super-matrix, two strains may be labeled as peripheral strains of large OTU's; similarly, two of the three strains mis-identified by the inclusive scheme may be labeled as peripheral members of large OTU's. The remaining mis-identified strains contained large amounts of missing data.

We also note that the choice of the particular ID and R thresholds to be used as conclusive identification criteria is dependent on the purpose for which the identification matrices are employed. Wayne, et al. (1980) used an ID score threshold of 0.990 and an R score threshold of 0.01 for medically important mycobacteria. However, the use of lower threshold criteria in this study permitted a significantly higher identification rate for

outer-continental shelf isolates with a concurrent increase in the mis-identification rate of only two additional strains over the mis-identification rate observed under the criteria set forth by Wayne. Under strict identification criteria, identification and mis-identification rates are lowered. This may be the desirable situation in a medical diagnostic laboratory as erroneous conclusions about the nature of a particular microorganism may have serious consequences. In instances where human health is not a consideration, however, lowering the identification criteria for probabilistic identification methods may increase the efficiency of performing taxonomic studies with only a slight increase in identification error rate, i.e., lenient identification threshold criteria sometimes allows one to sacrifice a slightly higher mis-identification rate for correct identification of a greater proportion of test strains.

Prior to this study, there has been no documented use of super- and sub-matrix arrangements for probabilistic identification methods. The results herein bear out the conclusion that this type of arrangement permits a considerable increase in experimental economy. The inclusive matrix alone allows an 81% decrease in binary bits of information required for the proper description of unknown bacterial strains. Use of the super- and sub-matrix scheme for identification purposes requires a maximum 16% of the original set of binary information. In a more practical sense, the super- and sub-matrix scheme may require that as few as 18 tests be performed on a set of unknown organisms for conclusive identification as compared to the 61 tests required by the inclusive matrix and the 320 test originally conducted for each isolate. If strains are isolated from diverse ecological habitats such that all six combinations of super- sub-matrices become necessary for complete identification, a maximum of 52 tests need to be performed for each strain; the

practical difference between the 52 tests of the super- sub-matrix scheme and the 61 tests of the inclusve scheme represents at least a nine-test economy.

Bacteria associated with edible crabs - potential human pathogens

The evidence indicates that certain bacteria from foreign contamination sources, e.g. sewage, can become associated with crab tissues and survive in the marine environment for prolonged periods. E. coli, however, does not survive in marine ecosystems, even when associated with crab tissues and thus is a poor indicator organism for detectecting fecal contamination in these ecosystems. Other bacteria such as Klebsiella and Vibrio species can survive in association with crabs. It appears that as long as the crab is healthy bacteria are restricted to the shell and gill tissues. However, if the crab is injured, becomes weakened, and/or dies the surface bacterial contaminants rapidly penetrate throught the hemolymph to the muscle tissues. In such crabs human pathogens can enter the muscle tissues. Several human pathogens were isolated from crabs collected during 1975 in the vicinity of Kodiak Island. Clearly at that time the crabs near Kodiak were contaminated with a source of inadequately treated sewage. Our studies indicate that once crabs become contaminated with human pathogens, the pathogenic bacteria remain viable in association with surface tissues of the crabs; the pathogenic bacteria are retained by the crabs as they migrate and when the crabs become ill or die the bacteria migrate into the edible muscle tissues posing a potential human health hazard.

DIVERSITY OF BACTERIAL COMMUNITIES

Beaufort Sea

The taxonomic diversity studies are interesting in that they support general ecological theory as evidenced, for example, by the lower diversities in surface waters during winter when the communities are particularly stressed.

They are also interesting in that, despite the high physiological versatilies of the populations, the taxonomic diversity reflects population shifts due to environmental variations. Benthic communities which are exposed to less variability in terms of abiotic parameters, i.e., are exposed to less ecological stress, have higher diversities than surface water communities and do not exhibit seasonal variability. Our studies indicate that bacterial communities maintain a high state of diversity unless severely stressed; this appears to be true of most ecosystems including Arctic marine ecosystems where one might have predicted a greater degree of specialization, i.e., less versatility. Of particular note is that Beaufort Sea ecosystems appear to have a specified number of niches that vary seasonally in surface waters. The formation of coastal ice effectively removes bacterial populations from surface waters and following the spring melt there is an annual successional process that must reestablish the surface water bacterial community. Different populations are included in the community in different years, but the same level of community diversity is achieved each summer. It is likely that random recruitment determines which populations successfully occupy the niches of this ecosystem and that parameters such as phytoplankton productivity (substrate availability) and temperature (abiotic factors) determine the structure of the stable climax community that develops.

The bacterial populations occurring in the surface waters exhibit definite adaptations that enhance their ability to survive (Kaneko et. al, 1979), including a predominance of pigmented bacteria in surface waters during summer when the bacteria are exposed to continuous sunlight; pigmentation protects bacteria against the potential lethal effects of ionizing radiation. As with taxonomic diversity, the physiological tolerance and indices attest to the diversity and versatility of the community. It is interesting that the

bacterial populations of the Beaufort Sea maintain the ability to tolerate conditions to which they are not exposed. Psychrophiles do occur in the Beaufort Sea in higher proportions than in subarctic seas, but psychrotrophs dominate even in this polar sea. The salinity tolerance indices suggest that surface water populations are better adapted to low salinities and that benthic bacterial populations are adapted to higher salinities. Benthic communities are exposed to hypersaline waters produced when ice forms; the dense saline waters sink to the benthos. In contrast surface communities are exposed to low salinity surface waters in the spring from ice melt and river runoff.

The nutritional utilization indices suggest that these ecosystems are phytoplankton driven, particularly during summer. This is evidenced by the large number of carbohydrates that can be utilized by the bacterial populations. During winter there is a shift toward utilization of non-carbohydrate substrates, including carboxylic and amino acids, suggestive of a shift to a detrital food web. The potential for utilization of particular classes of substrates, as expressed by the nutritional utilization indices, presumably reflects the natural patterns of substrate availability and utilization in Beaufort Sea ecosystems. This hypothesis is substantiated by a work of Griffiths and Morita (1981a, b) which shows that the ratio of glucose to glutamate uptake rates changes during a marine phytoplankton bloom. During periods of little primary productivity, this ratio is close to 0.1; however, during the height of the bloom, this ratio increases to 1.0; this shift appears to reflect a change in the type of organic nutrients available to the microbial community. During a bloom, the phytoplankton released carbohydrates such as glucose which in turn can be utilized by the bacteria present.

The inability of the dominant populations to metabolize hydrocarbons indicates that these are not major natural substrates and that a relatively

long period of adaptation may be needed for the indigenous bacterial communities of the Beaufort Sea to respond to inputs of hydrocarbons resulting from offshore oil and gas development in this region. The relative lack of exposure of most of the bacterial communities of the Beaufort Sea to petroleum hydrocarbons also is shown by the low numbers of hydrocarbon utilizers. Indeed studies have shown that petroleum biodegradation in Beaufort Sea ecosytems will be slow and that petroleum pollutants will persist (Atlas, 1978; Atlas et. al, 1978; Horowitz and Atlas, 1978; Haines and Atlas, 1982).

Gulf of Alaska

A high state of diversity was found to be a characteristic of bacterial communities in the Gulf of Alaska; the maintenance of high diversity appears to be an adaptive feature of subarctic marine bacterial communities. The measured taxonomic diversity indices for Gulf of Alaska bacterial communities were similar to those previously found for Arctic marine bacterial communities during summer (Kaneko et al., 1977). Although seasonal differences in taxonomic diversity were found in arctic waters, no significant seasonal differences in taxonomic diversity indices were found for bacterial communities in subarctic Gulf of Alaska waters sampled in March and October. The calculated Shannon diversity indices for Gulf of Alaska bacterial communities were comparable to those reported by Martin and Bianchi (1980) ($H' \simeq 4$) for oligotrophic marine waters of the French Mediterranean region (Martin and Bianchi, 1980).

We have hypothesized that taxonomic diversity of the heterotrophic bacterial community would be lower in surface waters than in sediments due to "stress" from irregular fluctuations of temperature and salinity, high light intensities, and low concentrations of available nutrients. This study may support this hypothesis since taxonomic diversity in offshore waters was lower

than in offshore sediments, but the difference was not statistically significant indicating further studies conducted with larger numbers of isolates will be needed to establish the validity of this hypothesis. In Arctic marine ecosystems taxonomic diversity was significantly lower in surface water than in sediment (Kaneko et al., 1977).

We also have proposed that taxonomic diversity would be lower for bacterial communities within intertidal habitats than for offshore communities; this was not found to be the case. The regular tidal fluxes do not appear to severely stress intertidal bacterial communities. Additionally, we have postulated that there would be an inverse relationship between population size and taxonomic diversity; high population sizes should reflect competitive success of a limited number of populations. No significant correlation, however, was found between population size and diversity for Gulf of Alaska bacterial communities.

The question of interpretation of diversity indices must be raised. What does a diversity index say about the community? Communities with low taxonomic diversities are relatively homogeneous; they are specialized and generally have low genetic heterogeneity. Communities with high diversities are heterogeneous and have high informational content within the gene pool of the community. Although there is no simple relationship between community stability and diversity, overly specialized communities are particularly susceptible to disruption by environmental perturbations, whereas diverse communities are better adapted for self-maintenance in fluctuating environments. Communities existing under severe environmental stress generally are quite specialized and thus have low diversities. From our experience, an H' value of <3.0 appears to represent relatively "low" diversity for bacterial communites, indicative of

some form of environmental "stress", which exerts selective pressure on the bacterial community and results in the predominance of specialized populations.

Diversity indices have been used for assessing environmental stress caused by pollution (Cairns, 1979; Patrick, 1963; Patrick et al., 1954; Pielou, 1975). Communities in ecosystems characterized by a lack of environmental variability, e.g., in benthic deep ocean trenches, and those under natural stress, e.g., in polar ice caps, similarly, may have low diversities. High diversities are expected for communities under biological accommodation. The diversity index reflects the informational content within the community and the "status" of the community, but does not define the specific causal factors responsible for establishing a particular level of informational heterogeneity.

The Shannon Weaver index used in this study is a general diversity index, i.e., it measures both the species richness (number of different "species") and the evenness (distribution of individuals within "species") components of diversity. H' is theoretically sensitive to the sampled population size, expecially when fewer than 100 representatives of the community are sampled. Bianchi (paper presented 2nd Internation Microbial Ecology Symposium, Sept. 1980, Warwich, England) has found, though, only relatively small differences (<0.4) in H' values for marine bacterial communities when the actual sample size used in the calculation was varied between 20 and 150 strains. Because H' is sensitive to changes in rare species, its use has been criticized (Peet, 1974; Pielou, 1975). In our study the selection of representatives of the community (isolates) follows screening (plating), which eliminates rare species; we thus are really calculating diversity of the major (dominant) populations within the community. The random selection of isolates from dilution plates also means that each individual in this study represents between 10^2 and 10^5 organisms in the original sample, depending on the

concentration of bacteria in the sample. This fact can be used for justifying the validity of the results of this study even though only a limited number of strains were examined for each sample.

The selection procedures, however, raise an additional problem for diversity measurements; all plating procedures are selective and thus exclude a protion of the community from the study. We found that marine agar 2216 gave higher counts than other media, including MSWYE and low nutrient media, for these samples. We thus considered marine agar 2216 to be the "least selective," since it permitted growth of the highest proportion of populations in the community of any of the media tested.

The equitability index (J') estimates the evenness with which importance is apportioned between species. Questions have been raised about the appropriateness of using J' unless the entire community is censused (Peet, 1974), an impossibility for bacterial communities. It has been pointed out that J' is sensitive to changes in the number of speccies, especially if one utilizes the actual number od species observed for calculating J'; in our study the number of possible taxa in the community exceeds the sizes of the sampled populations, and therefore the maximal diversity used in calculating the denominator for J' was based on the assumption that each bacterial isolate could represent a different taxon; this assumption decreases the sensitivity of J' to small changes in the number of taxa observed.

The physiological tolerance indices devleoped in this study assess the abilities of the members of the bacterial community to grow under extreme conditions and not just simply to survive. The high physiological tolerance indices for Gulf of Alaska bacterial communities are somewhat surprising considering the relatively low annual variations in temperature, salinity, and pH which occur in these subarctic marine ecosystems. Most populations were

quite tolerant of fluctuations in temperature, salinity, and pH, beyond the limits to which they ever are exposed naturally. The lower salinity tolerance indices in the western Gulf of Alaska compared to those in Cook Inlet and the eastern Gulf correlate with expected areas of freshwater input; little runoff should occur from the Aleutian Islands, while east of Kodiak Island there are major river sources of freshwater. The salinity tolerance indices also indicate that intertidal communities are more tolerant than offshore communities to variations in salinity; this is adaptive since nearshore communities are subjected to greater fluctuations in salinity than offshore communities. The lack of statistically singificant differences in physiological tolerance indices between water and sediment communities may reflect extensive turnover in the water column, which was suggested by the temperature and salinity (density) measurements.

The nutritional versality index (N_T) developed in this study is virtually synonymous with the average carbonaceous compound index (UAI) developed by Martin and Bianchi (1980), although different substrates were used in determination of the two indices. Average UAI values for oligotrophic Mediterranean waters were found to be approximately 40% (equivalent to an N_T value of approximately 0.40). Martin and Bianchi (1980) reported increases of UAI values to 52-57% during peak phytoplankton bloom, i.e., higher UAI values occurred during a period of organic enrichment than under oligotrophic conditions. The mean N_T value of 0.53 for offshore Gulf of Alaska waters is somewhat higher than the UAI of 0.40 reported for oligotrophic Mediterranean waters; direct comparison, however, is not possible in an absolute sense since different substrates were used in the calculations. The higher N_T values found for Gulf of Alaska offshore sediment and intertidal communities than for offshore waters support the hypothesis that low nutrient ("oligotrophic")

conditions support low nutritional versatility, whereas environments with higher nutrient availabilities support bacterial communities with higher nutritional versatilities.

Employing a large number of biochemically diverse substrates permits factoring the nutritional versatility into utilization indices for individual classes of compounds. The similar utilization indices for amino acids and carbohydrates are noteworthy, as are the similar proportions of bacterial populations exhibiting extracellular proteolytic and saccharolytic activities. In other regions of the Pacific Ocean, proteolytic capacities have been found to far exceed saccharolytic activities for bacterial populations (ZoBell, 1946). The nutritional utilization indices presumably reflect substrate utilization patterns within the natural habitats of these communities. This suggests that the bacterial communities may be deriving their energy from phytoplankton-produced nutrients, which are rich in carbohydrates.

The maintenance of a high degree of informational heterogeneity is characteristic of bacterial communities, even those occurring in relatively stable environments such as marine ecosystems. It appears to be of adaptive advantage to maintain the capabilities to tolerate physiological stress beyond the range to which the habitat is ever subjected and to maintain a nutritionally versatile community in marine ecosystems.

Cook Inlet Bacterial Communities Isolated on High and Low Nutrient Media

The concentration of nutrients in the isolation media had a significant effect on the diversity and characteristics of the bacterial populations isolated in this study. The use of a high nutrient media has been extensively used in marine microbiology (ZoBell, 1946b). ZoBell and others have found that a nutrient rich medium supports the growth of higher numbers of marine microorganisms than media of other composition (Carlucci, 1974; ZoBell, 1946b).

The recognition that most true marine bacteria in the water column grow in a nutrient deprived environment has raised questions as to whether low nutrient media would be more appropriate for the isolation and study of marine bacteria (Carlucci, 1974; Carlucci and Shimp, 1974; Jannasch, 1967). It has been recognized that high nutrient concentrations are inhibitory to the growth of some marine bacteria and that such bacteria grow best at low nutrient concentrations. Although many bacteria occurring in Cook Inlet grow on low nutrient media, such media are more selective than high nutrient media, as evidenced by the lower diversities of bacterial populations isolated compared to nutrient rich media. The characteristics of bacteria isolated on low and high nutrient media were significantly different, suggesting that the bacteria isolated on the different media represent bacterial populations that occupy different ecological niches within their environment. It is possible that in natural marine ecosystems some bacteria are associated with nutrient rich particles (e.g., detritus including dead organisms and excretions) while others grow under conditions of near starvation (e.g., on the minimal concentrations of dissolved organic carbon). The question of whether copiotrophic and oligotrophic bacteria are differentially distributed in the marine ecosystem requires further study centering on whether the isolates obtained from marine agar 2216 are particle associated and whether suspended particles constitute a less variable habitat than the aqueous environment

While one might postulate that bacteria isolated on low nutrient media would be able to grow only under greatly restricted conditions, this was not found to be the case. The bacteria isolated on low nutrient media were extremely versatile, being more tolerant to variations in salinity and pH and nutritionally less fastidious than bacterial populations isolated on rich media. The bacteria isolated on low nutrient media were eurytolerant for

various physiological parameters and were capable of metabolizing a large number of organic substrates. The indigenous microorganisms of Cook Inlet appear to be quite heterogeneous and adapted to growth over a wide range of conditions that may naturally occur in both water and sediment in this region. The lack of significant proportions of psychrophilic and stenohaline bacteria from any of the isolation media supports the contention that it is advantageous for the indigenous bacteria to be versatile rather than specialized. A high state of taxonomic diversity within the bacterial community also appears to be of adaptive advantage.

Interestingly, the inclusion of crude oil into both high and low nutrient media did not alter the general properties of the isolates. A significantly higher proportion, though, of bacteria isolated on low nutrient media could metabolize hydrocarbons compared to those isolated on high nutrient media. Exposure to hydrocarbons may lead to changes in community composition in the environment (A. Horowitz, M. I. Krichevsky and R. M. Atlas. 1979. Abstracts of Annual Meeting of American Society for Microbiology, Q84) although Cook Inlet crude oil did not exhibit selective toxicity when included in isolation media. It has been reported previously that the overall nutrient balance rather than the concentrations of specific organic compounds has the greatest effect on the growth of some low nutrient bacteria (Poindexter, 1981). The inclusion of hydrocarbons was not toxic to "low nutrient" bacteria even though the inclusion of crude oil in the medium greatly elevated the concentration of organic compounds.

Bacterial communities associated with Arctic amphipods

The lack of commensal microorganisms attached to the gut lining has been previously reported for some marine invertebrates (Boyle and Mitchell 1978). The gut of the isopod Limnoria lacks a resident microflora, but the surface of

the animal has been found to be heavily fouled with microorganisms (Boyle and Mitchell 1980). In contrast the copepod Acartia tonsa has both surface and specific associated gut microflora (Sochard et al. 1979). Boeckosimus affinis lacked microbial populations associated both with the surface of the animal and the lining of the gut. The only microbial populations associated with B. affinis were observed on the food particles in the midgut, on the anal plates and on the faecal matter. It is not clear whether the amphipods were deriving nutrition from the microorganisms on the food particles. There was no evidence that the amphipods examined in this study possess a true normal gut microflora.

The lack of a surface microflora associated with B. affinis is interesting. The animals do burrow in sediment and should become fouled with sediment particles and microorganisms. However, the animals used in these experiments were captured on bait suspended in the water column and it is unknown how long these animals had been swimming in search of food. Microorganisms and sediment particles may have been removed during swimming or during processing of the amphipods. Also, it is not known how long before capture the animals had last molted which could have removed surface fouling.

The bacterial populations associated with the amphipods were not identical to those previously reported for water and sediment in this region (Kaneko et al. 1979). Schwartz et al. (1976) similarly reported that the intestinal microflora of deep sea amphipods were not identical with water and sediment isolates. Taxonomically, the dominant bacterial populations associated with the amphipods B. affinis appear to be in the Vibrio-Beneckea group (Bauman and Bauman 1977; Leifson et al. 1964; Shewan and Veron 1974). Marine vibrios have been shown to be the dominant microorganisms in the gut of the copepod Acartia (Sochard et al. 1980).

The changes in diversity and equitability support the concept that during captivity a successional process occurs in which the initially dominant bacterial populations decline in importance and a less specialized and more diverse bacterial community becomes associated with the amphipods. This also is supported by the increases in the physiological tolerance ranges and nutritional versatility indices of the bacterial populations. Our results support a hypothesis that the microbial populations associated with Arctic amphipods are in a continuous state of flux. The successional changes in the dominant microbial populations associated with the amphipods may relate to the feeding habits of the amphipods. It is likely that the microbial populations will vary depending on what food sources the amphipods ingest.

Exposure to petroleum resulted in a significant change in the microbial populations associated with the amphipods. It appears that hydrocarbons greatly accelerated successional changes within the microbial community associated with these Arctic amphipods. When exposed to hydrocarbons the dominance of vibrio-like organisms clearly diminished. This could have occurred due to toxicity of the hydrocarbons to the vibrio-like organisms or due to an alteration in the successional process.

Denitrification - Potential Activities

Several recent studies have examined denitrification in coastal marine sediments. Koike and Hattori (1978a, 1978b) examined denitrification and ammonia formation in coastal sediments. They reported rates of nitrogen gas production in sediments surface layers of about 10^{-2} g-atom of N_2/g per h irrespective of location (three sediments from Japanese bays were used inthese studies). Oren and Blackburn (1979) reported denitrification rates of sediment cores from Danish fjords of 12.5 nmol of N_2-N/ml per day for 1 to 3 cm. Sørensen (1978), using the acetylene inhibition technique, reported a maximum

rate of denitrification in coastal marine sediment of 35 nmol of N/ml of sediment per day at 2.5°C with peak denitrification rates occurring at 2 to 3 cm. Sørensen (1978) found that the greatest rates of denitrification occurred near the surface sediment in the transitional zone from aerobic to anaerobic conditions. Grundmanis and Murray (1977) also found nitrification and denitrification to be important in surface sediments and implicated bioturbation as a major factor affecing the rates of these processes. Knowles and Wishart (1977) found very low natural rates of nitrogen fixation in sediments from Beaufort Sea (about 25 mg of N/m^2 per yr). At 5°C they calculated rates of nitrogen fixation in unamended marine Arctic sediments as 0.1 to 0.3 nmol of C_2H_4/g per day. Similar rates, 0.1 to 1.1 nmol of C_2H_4/g per day, were reported by Maruyama et al. (1974) for sediments from Tokyo Bay that were incubated at 3°C.

Koike and Hattori (1978a) reported that the rate of denitrification is proportional to nitrate concentrations in the range of 0 to 30 µg-atoms of N per liter. They concluded that denitrification in Bering Sea sediments was apparently controlled by the supply of nitrate and nitrite to the sediments (Koike and Hattori, 1978a). In the present study there was a significant correlation ($\alpha < 0.001$) between rates of denitrification in unamended sediments and nitrate-nitrite concentrations, although the correlation coefficient r) was only 0.6. In many cases the available nitrate in the interstitial water should have been consumed within approximately 48 h. In some cases more N_2O was produced than the available NO_3^- measured. The reasons for this phenomenon are unknown; it is possible, but unlikely, that in some of these cases nitrification was not totally blocked. 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. The acetylene blockage of N_2O reduction has been reported also to block nitrification (Bremner and Blackmer, 1979); no tests of the efficiency of nitrification blockage, though, were made in the present study.

Some interesting comparisons of the rates of nitrogen fixation and denitrification within localized areas are shown in Table 44. In upper Cook Inlet, which has very coarse sediments, unamended 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 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 which enter from terrestrial and river runoff. Significant seasonal differences were found in the relative rates of denitrification and nitrogen fixation in Elson Lagoon. During winter, measured rates of nitrogen fixation were equal to rates of denitrification, but during spring and summer rates of nitrogen fixation were significantly higher than rates of denitrification.

The present study adds a significant amount of data on the potential rates of nitrogen fixation and denitrification in Alaskan continental shelf sediments. Koike and Hattori (1978c) previously measured a rate of denitrification of 1.2 ng-atoms of N/g of dry sediment per h in surface sediments of the southern Bering Sea. They estimated the loss of combined nitrogen by denitrification to be 5×10^{11} g of N/yr over the entire shelf region, assuming a uniform rate of denitrification over the Bering Sea shelf and that denitrification occurred only to a depth of 2 cm an donly on one-half of the shelf. In the current study, the rate of denitrification at sites

Table 44. Comparison of mean rates of nitrogen fixation and denitrification in sediments from different regions of the Alaskan continental shelf

Region	N_2 fixation (µg-atoms N_2-N/m^2 per h)	Denitrification (µg-atoms N_2O-N/m^2 per h)
Upper Cook Inlet	0.3	0.1
Kamishak Bay	1.0	25.6
Shelikof Strait	2.4	2.1
Norton Sound	0.8	4.3
Elson Lagoon		
Winter	0.4	0.3
Spring	4.6	<0.1
Summer	2.1	<0.1

*Nitrogen fixtation data courtesy R. Griffiths and R. Morita

comparable to those examined by Koike and Hattori (1978c) averaged 0.9 ng-atoms of N/g of dry sediment per h. Our data were within the range reported in the study by Koike and Hattori (1978c). Assuming that denitrification occurs in the entire Bering Sea shelf, we would estimate, based on unamended denitrification activity measurements, gross losses of 2.2×10^{12} g of N/yr from the Bering Sea. Similarly, we calculate gross rates of nitrogen fixation of 0.4×10^{12} g of N/yr per Bering Sea shelf. The net loss of fixed forms of nitrogen from the Bering Sea sediments, thus, is estimated as 1.8×10^{12} g/yr. In these calculations we assume that the conversion factor of 0.33 from ethylene to nitrogen is correct for this region, that rates of N_2O production in unamended sediment slurries determined by the acetylene blockage assay approximate "natural" rates of denitrification, and that there is a 5-cm activie depth (10^5 g [dry weight] of sediment/m^2). No measurements were made in situ or with ^{15}N to confirm the validity of these assumptions. In situ variations in temperature and oxygen concentration will affect rates of nitrogen fixation and denitrification, and therefore caution should be used in extrapolating annual global scale fluxes from these measurements.

OIL BIODEGRADATION

Beaufort Sea

This study clearly indicates that weathering of oil in Beaufort Sea sediments will be a slow process. Microbial degradation of petroleum hydrocarbons occurred in contaminated Arctic sediments but only after significant exposure periods; evidence for biological modification of petroleum in experimentally oil contaminated Beaufort Sea sediment was not observed until after a year.

Several factors probably contributed to the slow rate of microbial weathering. The hydrocarbon-degrading microbial population did not increase

rapidly following addition of oil to the sediments. Only after 8 months of exposure were there sufficient numbers of hydrocarbon utilizers to establish a significant response within the microbial community to the presence of the oil. The initial lack of response of the microbial community could not have been entirely due to the low temperature. The hydrocarbon utilizing microorganisms were psychrotrophic or psychrophilic; such microorganisms are capable of active growth and metabolism at temperatures below 0°C (Traxler, 1973; ZoBell, 1973; Robertson et al., 1973; Morita, 1975). Also Prudhoe Bay crude oil has not been shown to contain a toxic fraction which is inhibitory to microbial hydrocarbon biodegradation at low temperatures (Atlas, 1975). The nitrogen and phosphorus concnetrations were adequate to support only limited hydrocarbon biodegradation; optimal rates of hydrocarbon biodegradation typically occur at C:N and C:P ratios of 10:1 and 30:1 (Atlas and Bartha, 1972) which would have required several orders of magnitude higher concentrations of N and P; concentrations of available N and P which were actually measured, were below those needed to support maximal rates of biodegradation of the hydrocarbons added to the sediments. Oxygen availability also may have been a rate limiting factor. Gibbs and Davis (1976) have shown that O_2 and N are limiting factors in fine grained sediment columns. The sediments in Elson Lagoon are very fine grained (silty-clay). Rates of exchange of nutrients and oxygen between the interstitial water of the sediment and the overlying water column are likely to be quite low in such sediments. It is likely that all of the above factors contributed to the limited rates of oil biodegradation. The fact that the inorganic N and P concentrations remained relatively constant in the interstitial water during the experimental period indicates that these inorganic nutrients were not being rapidly utilized during oil degradation; otherwise the concentration of N and P in the interstitial water would have

declined with time. We speculate that the concentrations of inorganic N and P in the interstitial water were below the threshold concentrations needed to support rapid microbial utilization of hydrocarbons or that hydrocarbon biodegradation was blocked by some other factor. Alternatively, hydrocarbon degradation could have been proceeding slowly throughout the experimental period, but the population of hydrocarbon degraders was low enough that chemical changes in the oil were not observed; the delay of observable changes in C_{17}/pristane ratios would be due to the length of time needed to develop a relatively large degrading population and for that population to utilize enough oil to be detectable by current analytical techniques.

Not only was biodegradation limited but abiotic weathering also was restricted. Low molecular weight aliphatic and aromatic compounds, which normally are rapidly lost from surface oil slicks by evaporation and dissolution, remained as a significant feature of the residual hydrocarbon mixture for over one year. Oil which becomes entrained in Arctic sediments without signficant surface weathering, thus, would retain toxic low molecular weight aromatic hydrocarbons for prolonged periods of time. This would retard ecological recovery of benthic Arctic sediments which become oiled by ruptures of buried oil transfer pipelines. Weathering of the oil was atypical in several ways. Loss of low molecular weight compounds (aromatics such as naphthalene and aliphatics through $\underline{n}$-C_{14}) was not the first event resulting inmajor modification of the hydrocarbon mixture. Aliphatic hydrocarbons were not preferentially degraded over aromatic hydrocarbons; aromatic compounds were lost at rates comparable to those for aliphatic compounds. There was preferential biodegradation of $\underline{n}$-alkanes $\leq nC_{17}$ over alkanes $\geq nC_{18}$. These were significant deviations from the normal oil weathering process.

An important feature of the oil weathering process was the patchy oil distribution. The appearance of isolated pockets of crude oil within the sediment would reduce the surface area/volume ratio of the oil exposed to microbiological weathering. Our original sediment-oil mixture had a uniform distribution of oil throughout the sediment. Accumulation of the oil in pockets reduced the interfacial surface area of the oil available for microbial degradation of the petroleum hydrocarbons. The mechanisms by which oil in sediments would gather into small pools may be due to differential solubility. The implication of this observation is that accidentally spilled oil would tend to gather into small pools and prolong the time for recovery of the ecosystem from oil contamination. A patchy distribution of hydrocarbons was a characteristic of Chedabucto Bay sediments following weathering of oil spilled by the tanker Arrow; degradation of oil in Chedabucto Bay was particularly slow in low wave energy environments (Rashid, 1974). This may have been due to a lack of resupply of oxygen and nutrients needed to support microbial hydrocarbon biodegradation. In polar seas, such as the Beaufort Sea, ice dampens wave action contributing to the limitation of nutrient and oxygen resupply to the sediments.

The losses of hydrocarbons from Beaufort Sea sediments, from both abiotic and biotic weathering, (based on our in situ exposure experiments) are estimated at a rate of about 0.2 mg oil degrading/g dry wt sediment/y. This value is calculated as the difference in the mean values of the resolvable hydrocarbons at the start and end of the experiment divided by the total time of the experimental exposure. At the observed rate of degradation it will take many years to remove hydrocarbons from heavily oiled Arctic sediments by natural wethering processes. This prediction is supported by actual observations of oil, from several accidental spills, weathering in cold

sediments. Colwell et al. (1978) found that biodegradation of oil spilled near the Straits of Magellan by the tanker Metula was slow with marked persistence 2 years after the spill. Mayo et al. (1978) found that petroleum residues, from a pipeline spill as Searsport, Maine, weathered particularly slowly in the cold anoxic fine grained sediments of the contaminated cove. It is likely that only prevention of Arctic oil spills can preclude long term impact and persistence of hydrocarbons in benthic sediments of the Beaufort Sea.

Bering Sea

The extremely low rates of petroleum biodegradation observed in the Bering Sea are somewhat surprising. Previous studies have indicated slow, but significantly higher rates of hydrocarbon degradation in other regions of the Alaskan Continental Shelf including in more northerly regions of the Beaufort Sea as well as in the more southerly Cook Inlet (Haines and Atlas, 1982; Roubal and Atlas, 1978; Atlas, 1975; Horowitz and Atlas, 1978; Bunch and Harland, 1976). Although studies were not performed to determine if degradation rates would have been higher at warmer temperatures (the temperatures used approximated those found during warm periods in the Bering Sea), temperature alone cannot account for the low rates of hydrocarbon biodegradation. Studies conducted in the English Channel and North Sea, at temperatures comparable to those of the Bering Sea, have demonstrated rapid increases in oil degrading microbial populations in reponse to the introduction of petroleum hydrocarbons into the environment and rather rapid rates of hydrocarbon biodegradation at temperatures below 10°C (Aminot, 1981; Atlas et al., 1981; Colwell et al., 1978; Oppenheimer et al., 1977). For example, following the Amoco Cadiz spill extensive biodegradation occurred in the water column, as evidenced by a depletion of nitrate and dissolved oxygen (Aminot 1981). Hydrocarbon degrading populations in intertidal sediments impacted by the Amoco Cadiz spill were

several orders of magnitude higher than at comparable unoiled control sites (Atlas et al., 1981). As with temperature, concentrations of available nutrients alone cannot explain the lack of biodegradaton in the Bering Sea samples. Mean available inorganic nitrogen concentrations in Bering Sea sediments in the study area were 0.01 mM nitrate and 0.5 mM ammonium; in surface water samples concentrations of inorganic nirtrogen concentrations were 0.2 μM ammonium and 4 μM nitrate. Low nutrient concentrations have been found to limit petroleum biodegradation in marine environments (Gibbs and Davis, 1976), but in the present study the addition of nutrients to replicate samples did not enhance the rate of petroleum biodegradation; the model flow-through experiment had added nitrate and phosphate, but there no increase in the rate of hydrocarbon biodegradation was observed over several weeks compared to unsupplemented sediments.

The most likely explanation for the overall lack of observed petroleum biodegradation in the Bering Sea is the lack of previous exposure to petroleum pollutants, and thus, the limited numbers of indigenous microrganisms that are adapted to hydrocarbon utilization. In areas of the world's oceans where there has been extensive prior exposure to petroleum hydrocarbons, such as along the shipping lanes of the English Channel, sizeable competent microbial populations have developed that are capable of rapidly responding to the introduction of petroleum pollutants. In contrast, the Bering Sea is relatively pristine and a much longer period of time is required following an oil spillage to establish significant populations of hydrocarbon degraders and rapid rates of hydrocarbon biodegradation. Extensive studies in the Bering Sea, especially in the Norton Sound region around a reported gas seepage, failed to show any detectable petroleum hydrocarbons (Atlas et al., 1983).

As a consequence of the slow rates of hydrocarbon biodegradation it is likely that hydrocarbons introduced into the Bering Sea will have sufficient residence times to be transported to intertidal or benthic sediments. For modelling and trajectory purposes the rates of hydrocarbon biodegradation for petroleum hydrcarbons in most regions of the Bering Sea must be considered zero. Consequently, the potential for long distance transport of petroleum pollutants in this region is serious and even offshore major oil spillages could easily lead to the disruption of the delicate ecological balance of many of the coastal communities in this region. The introduction and probable long term persistence of oil into the Bering Sea as a result of offshore oil and gas development also is of potential serious concern because of the extensive commercial fish and shellfish industries in the southern Bering Sea. Long term distruption of coastal ecosystems has occurred at some oil spill sites, particularly when the oil is entrapped within embayments, such as in the extensively studied spillages at West Falmouth (Sanders, et al., 1980). The lack of rapid biodegradative processes in the Bering Sea increases the probability of hydrocarbon tainting of fish and shellfish following accidental oil spillages into these northerly marine ecosystems. Clearly great care must be taken in the development of offhore oil resources in this region to prevent serious environmental perturbation.

VIII Literature cited

Abbott, B. J., and W. G. Gledhill. 1971. The extracellular accumulation of metabolic productus by hydrocarbon-degrading microorganisms. Adv. Appl. Microbiol. 14:249-388.

Ahearn, D. G., S. P. Meyers, and P. G. Standard. 1971. The role of yeasts in the decomposition of oils in marine environments. Dev. Ind. Microbiol. 12:126-134.

Akagi, Y., N. Taga, and U. Simidu. 1977. Isolation and distribution of oligotrophic marine bacteria. Can. J. Microbiol. 23: 981-987.

Aminot, A. 1980. Mise en e'vidence et e'stimation quantitative de la biode'gradation in situ des hydrocarbons de L'Amoco Cadiz. In Proceedings of the International Symposium on the Amoco Cadiz: Fates and Effects of the Oil Spill. Centre Oceanologique de Bretagne, Brest, France, in press.

Aminot, A. 1981. Ananomalies du systeme hydrobiologique cotier apres L'ecouage de l'Amoco Cadiz. Consderations qualatatives et quantatatives sur la biodegradation in situ des hydrocarbures. p223-242. In Fates and Effects of the Oil Spill Amoco Cadiz, Proc. Intl. Symp. Centre Oceanologique de Bretagne, Brest, France.

Arhelger, S. D., B. R. Robertson, and D. K. Button. 1977. Arctic hydrocarbon biodegradation, p. 270-275. In D. Wolfe ed., Fate and effects of petroleum hydrocarbons in marine ecosystems and organisms. Pergamon Press, Inc., Elmsford, N.Y.

Atlas, R. M. 1975. Effects of temperature and crude oil composition on petroleum biodegradation. Appl. Microbiol. 30:396-403.

Atlas, R. M. 1977a. Stimulated petroleum biodegradation. Crit. Rev. Microbiol. 5:371-386.

Atlas, R. M. 1977b. Studies on petroleum biodegradation in the Arctic, p. 261-269. In D. Wolfe ed., Fate and effects of petroleum hydrocarbons in marine ecosystems and organisms. Pergamon Press, Inc., Elmsford, N.Y.

Atlas, R. M. 1978a. An assessment of the biodegradation of petroleum in the Arctic, p. 86-90. In M. W. Loutit and J. A. R. Miles ed., Microbial ecology. Springer-Verlag, Berlin.

Atlas, R. M. 1978b. Measurement of hydrocarbon biodegradation potentials and enumeration of hydrocarbon utilizing microorganisms using ^{14}C radiolabeled spiked crude oil, p. 196-204. In J. W. Costerton and R. R. Colwell ed., Native aquatic bacteria: enumeration, activity and ecology. ASTM-STP 695. American Society for Testing and Materials, Philadelphia.

Atlas, R. M. 1981. Microbial degradation of petroleum hydrocarbons: an environmental perspective. Microbiol. Rev., 45, 180-209.

Atlas, R. M., and R. Bartha. 1972a. Biodegradation of petroleum in seawater at low temperatures. Can. J. Microbiol. 18:1851-1855.

Atlas, R. M., and R. Bartha. 1972b. Degradation and mineralization of petroleum by two bacteria isolated from coastal water. Biotechnol. Bioeng. 14:297-308.

Atlas, R. M., and R. Bartha. 1972c. Degradation and minerlization of petroleum in seawater: limitation by nitrogen and phosphorus. Biotechnol. Bioeng. 14:309-317.

Atlas, R. M., and R. Bartha. 1973a. Abundance, distribution and oil biodegradation potential of microorganisms in Raritan Bay. Environ. Pollut. 4:291-300.

Atlas, R. M., and R. Bartha. 1973b. Effects of some commercial oil herders, dispersants and bacterial inocula on biodegradation of oil in seawater, p. 283-289. In D. G. Ahearn and S. P. Meyers ed., The microbial degradation of oil pollutants. Publication no. LSU-SG-73-01. Center for Wetland Resources, Louisiana State University, Baton Rouge.

Atlas, R. M., and R. Bartha. 1973c. Fate and effects of oil pollution in the marine environment, Residue Rev. 49:49-85.

Atlas, R. M., and R. Bartha. 1973d. Inhibition by fatty acids of the biodegradation of petroleum. Antonie van Leeuwenhoek. J. Microbiol. Serol. 39:257-271.

Atlas, R. M., and R. Bartha. 1973e. Stimulated biodegradation of oil slicks using oleophilic fertilizers. Environ. Sci. Technol. 7:538-541.

Atlas, R. M., and P. D. Boehm, and J. A. Calder. 1981. Chemical and biological weathering of oil from the Amoco Cadiz oil spillage in the littoral zone. Estuarine Coastal Mar. Sci., in press.

Atlas, R. M., and A. Bronner. 1980. Microbial hydrocarbon degradation within intertidal zones impacted by the Amoco Cadiz oil spillage. In Proceedings of the International Symposium on the Amoco Cadiz: Fates and Effects of the Oil Spill. Centre Oceanologique de Bretagne, Brest, France, in press.

Atlas, R. M., and M. Busdosh. 1976. Microbial degradation of petroleum in the Arctic, p. 79-86. In J. M. Sharpley and A. M. Kaplan ed., Proceedings of the Third International Biodegradation Symposium. Applied Science Publishers, Ltd., London.

Atlas, R. M., A. Horowitz, and M. Busdosh. 1978. Prudhoe crude oil in arctic marine ice, water, and sediment ecosystems: degradation and interactions with microbial and bentic communities. J. Fish. Res. Board Can. 35:585-590.

Atlas, R. M., G. Roubal, A. Bronner, and J. Haines. 1980a. Microbial degradation of hydrocarbons in mousse from IXTOC-I. In Proceedings of Conference on Researcher/Pierce IXTOC-I Cruises. National Oceanographic and Atmospheric Administration, AOML, Miami, in press.

Atlas, R. M., G. Roubal, and J. Haines. 1980b. Biodegradation of hydrocarbons in mousse from the IXTOC-I well blowout. In Proceedings of Symposium on Assessment of the Environmental Impact of Accidental Oil Spills in the Oceans. Presented before the Division of Environmental Chemistry of the American Chemical Society, 24-29 August 1980, San Francisco. Ann Arbor Science Publications, Inc., Ann Arbor, Mich.

Atlas, R. M., E. A. Schofield. 1975. Petroleum biodegradation in the Arctic, p. 183-198. In A. W. Bourquin, D. G. Ahearn, and S. P. Meyers ed., Impact of the use of microorganisms on the aquatic environment. U.S. Environmental Protection Agency, Corvallis, Ore.

Atlas, R. M., E. A. Schofield, F. A. Morelli, and R. E. Cameron. 1976. Interactions of microorganisms and petroleum in the Arctic. Environ. Microbiol. 34:60-68.

Atlas, R.M., M.I. Venkatesan, I.R. Kaplan, R.A. Feely, R.P Griffiths, and R.Y. Morita. 1983. Distribution of hydrocarbons and microbial populations related to sedimentation pressures in lower Cook Inlet and Norton Sound Alaska. Arctic: in press.

Austin, B., J. J. Calomiris, J. D. Walker, and R. R. Colwell. 1977a. Numerical taxonomy and ecology of petroleum degrading bacteria. Appl. Environ. Microbiol. 34:60-68.

Austin, B., R. R. Colwell, J. D. Walker, and J. J. Calomiris. 1977b. The application of numerical taxonomy to the study of petroleum degrading bacteria isolated from the aquatic environment. Dev. Ind. Microbiol. 18:685-696.

Bailey, N. J. L., A. M. Jobson, and M. A. Rogers. 1973. Bacterial degradation of crude oil: comparison of field and experimental data. Chem. Geol. 11:203-221.

Bailey, C. A., and M. E. May. 1979. Evaluation of microbiological test kits for hydrocarbon fuel systems. Appl. Environ. Microbiol. 37:871-877.

Barber, M. and Kuper, S. W. A. 1951. Indentification of Staphylococcus pyogenes by the phosphatase reaction. Journal of Pathology and Bacteriology 63, 65-68.

Barnsley, E. A. 1975. The bacterial degradation of fluoranthene and benzoapyrene. Can. J. Microbiol. 21:1004-1008.

Barsdate, R. M. 1973. Ecologic changes in an Arctic tundra pond following exposure to crude oil, p. 52. In Impact of oil resource development on northern plant communities. Occasional Publications of Northern Life no. 1. Institute of Arctic Biology, University of Alaska, Fairbanks.

Bartha, R., and R. M. Atlas. 1973. Biodegradation of oil in seawater: limiting factors and artificial stimulation, p. 147-152. In D. G. Ahearn and S. P. Meyers ed., The microbial degradation of oil pollutants. Publication no. LSU-SG-73-01. Center for Wetland Resources, Louisiana State University, Baton Rouge.

Bartha, R., and R. M. Atlas. 1976. Biodegradation of oil on water surfaces. U.S. patent 3,939,127, May 25, 1976.

Bartha, R., and R. M. Atlas. 1977. The microbiology of aquatic oil spills. Adv. Appl. Microbiol. 22:225-266.

Baumann, P., Doudoroff, M. and Stanier, R. Y. 1968. A study of the Moraxella group. II. Oxidase-negative species genus Acinetobacter. Journal of Bacteriology 95, 1520-1541.

Baumann, P., Baumann, L. and Mandel, M. 1971. Taxonomy of marine bacteria: the genus Beneckea. Journal of Bacteriology 107, 268-294.

Baumann, L., Baumann, P., Mandel, M. and Allen, R. D. 1972. Taxonomy of aerobic eubacteria. Journal of Bacteriology 110, 402-429.

Bauman, P., and L. Bauman. 1977. Biology of the marine enterobacteria: Genera Beneckea and Photobacterium. Ann. Rev. Microbiol. 31: 39-61.

Beam, H. W., and J. J. Perry. 1973. Co-metabolism as a factor in microbial degradation of cycloparaffinic hydrocarbons. Arch. Mikrobiol. 91:87-90.

Beam, H. W., and J. J. Perry. 1974a. Microbial degradation and assimilation of n-alkyl-substituted cycloparaffins. J. Bacteriol. 118:394-399.

Beam, H. W., and J. J. Perry. 1974b. Microbial degradation of cycloparaffinic hydrocarbons via co-metabolism and commensalism. J. Gen. Microbiol. 82:163-169.

Becking, J. H. 1974. Genus Derxia. In Bergey's Manual of Determinative Bacteriology, 8th edn, pp. 260-261. Edited by R. E. Buchanan R. and N. E. Gibbons. Baltimore: Williams and Wilkins.

Bergstein, P. E., and J. R. Vestal. 1978. Crude oil biodegradation in Arctic tundra ponds. Arctic 31:158-169.

Berridge, S. A., R. A. Dean, R. G. Fallows, and A. Fish. 1968a. The properties of persistent oils at sea, p. 2-11. In P. Hepple ed., Scientific aspects of pollution of the sea by oil. Institute of Petroleum, London.

Berridge, S. A., M. T. Thew, and A. G. Loriston-Clarke. 1968b. The formation and stability of emulsions of water in crude petroleum and similar stocks, p. 35-59. In P. Hepple ed., Scientific aspects of pollution of the sea by oil. Institute of Petroleum, London.

Blumer, M., M. Ehrhardt, and J. H. Jones. 1972. The environmental fate of stranded crude oil. Deep Sea Res. 20:239-259.

Blumer, M., and J. Sass. 1972a. Indigenous and petroleum-derived hydrocarbons in a polluted sediment. Mar. Pollut. Bull. 3:92-94.

Blumer, M., and J. Sass. 1972b. Oil pollution: persistence and degradation of spilled fuel oil. Science 176:1120-1122.

Boehm, P. D., and D. L. Fiest. 1980a. Comparative weathering patterns of hydrocarbons from the Amoco Cadiz oil spill observed at a variety of coastal environments. In Proceedings of the International Symposium on the Amoco Cadiz: Fates and Effects of the Oil Spill. Centre Oceanologique de Bretagne, Brest, France, in press.

Boehm, P. D., and D. L. Fiest. 1980b. Aspects of the transport of petroleum hydrocarbons to the offshore benthos during the IXTOC-I blowout in the Bay of Campeche. In Proceedings of Conference on Researcher/Pierce IXTOC-I Cruises. National Oceanographic and Atmospheric Administration, AOML, Miami, in press.

Bousfield, I. J. 1978. The taxonomy of coryneform bacteria from the marine environment. In Coryneform Bacteria, pp. 217-234. Edited by I. J. Bousfield and A. G. Callely. London: Academic Press.

Boylan, D. B., and B. W. Tripp. 1971. Determination of hydrocarbons in seawater extracts of crude oil and crude oil fractions. Nature London 230:44-47.

Boyle, P. J., and R. Mitchell. 1978. Absence of microorganisms in crustacean digestive tracts. Science 200: 1157-1159.

Boyle, P. J., and R. Mitchell. 1980, p. 197-186. Interactions between microorganisms and wood-boring crutaceans. In Biodeterioration Proc. 4th Int. Biodeter. Symp. Berlin. T. A. Oxley, D. Allsop, and G. Becker, eds. Pitman Pub. Lt.

Bridie, A. L., and J. Box. 1971. Biological degradation of mineral oil in seawater. J. Inst. Pet. London 57:270-277.

Brown, D. W., L. S. Ramos, A. J. Fiedman, and W. D. Macleod. 1969a. Analysis of trace levels of petroleum hydrocarbons in marine sediments using a solvent-slurry extraction procedure, p. 161-167. In Trace organic analysis: a new frontier in analytical chemistry. Special publication no. 519. National Bureau of Standards, Washington, D.C.

Brown, L. R., W. E. Phillips, G. S. Pabst, and C. M. Ladner. 1969b. Physical, chemical and microbiological changes occurring during degradation of oil in aquatic and brackish water environments. Presented at the American Society for Mechanical Engineers Annual Winter Meeting, 16-20 November, Los Angeles, Calif.

Brown, L. R., and R. G. Tischer. 1969. The decomposition of petroleum products in our natural waters. Water Resources Research Institute, Mississippi State University, State College.

Buchanan, R. E. and Gibbons, N. E. editors 1974. Bergey's Manual of Determinative Bacteriology, 8th edn. Baltimore: Williams and Wilkins.

Buckley, E. N., R. B. Jonas, and F. K. Pfaender. 1976. Characterization of microbial isolates from an estuarine ecosystem: relationship of hydrocarbon utilization to ambient hydrocarbon concentrations. Appl. Environ. Microbiol. 32:232-237.

Buckley, E. N., and F. K. Pfaender. 1980. Response of the pelagic microbial community to oil from the IXTOC-I blowout: II. Model ecosystem studies. In Proceedings of Conference on Researcher/Pierce IXTOC-I Cruises. National Oceanographic and Atmospheric Administration, AOML, Miami, in press.

Bunch, J. N., and R. C. Harland. 1976. Biodegradation of crude petroleum by the indigenous microbial flora of the Beaufort Sea. Beaufort Sea Technical Report 10. Environment Canada, Victoria, B.C.

Burkholder, P. R. and Burkholder, L. M. 1956. Vitamin B_{12} in suspended solids and marsh muds collected along the coast of Georgia. Limnology and Oceanography 1, 202-208.

Burwood, R., and G. C. Speers. 1974. Photo-oxidation as a factor in the environmental dispersal of crude oil. Estuarine Coastal Mar. Sci. 2:117-135.

Busdosh, M., K. W. Dobra, A. Horowitz, S. E. Neff, and R. M. Atlas. 1978. Potential long term effects on Prudhoe Bay crude oil in Arctic sediments on indigenous benthic invertebrate communities, p. 857-869. In Proc. conf. on assessment of ecological impacts of oil spills. Meeting held 14-17 June, 1978 at Keystone Colorado. American Institute of Biological Sciences.

Calder, J. A., and P. D. Boehm. 1980. Year study of weathering processes acting on the Amoco Cadiz oil spill. In Proceedings of the International Symposium on the Amoco Cadiz: Fates and Effects of the Oil Spill. Centre Oceanologique de Bretagne, Brest, France, in press.

Callaway, J. C., Tomalinson, R. D., Gordon, L. I., Barsrow, L. and Parla, P. K. 1972. An instruction manual for use of the Technicon Autoanalyser II in precision seawater analysis, Tech. Rep. I. Corvallis, OR, Oregon State University.

Calomiris, J. J., B. Austin, J. D. Walker, and R. R. Colwell. 1976. Enrichment for estuarine petroleum-degrading bacteria using liquid and solid media. J. Appl. Bacteriol. 42:135-144.

Cantwell, S. G., E. P. Lau, D. S. Watt, and R. R. Fall. 1978. Biodegradation of acyclic isoprenoids by Pseudomonas species. J. Bacteriol. 135:324-333.

Caparello, D. M., and P. A. Larock. 1975. A radioisotope assay for the quantification of hydrocarbon biodegradation potential in environmental samples. Microb. Ecol. 2:28-42.

Carlucci, A. F. and Bowes, P. M. 1970. Vitamin production and utilization by phytoplankton in mixed culture. Journal of Phycology, 6, 393-400.

Carlucci, A.F. 1974. Nutrients and microbial response to nutrients in seawater, p. 245-248. In R.R. Colwell and R.Y. Morita eds., Effect of the Ocean Environment on Microbial Acitivities. University Park Press, Baltimore, Maryland.

Carlucci, A.F. and S.L. Shimp. 1974. Isolation and growth of a marine bacterium in low concentrations of substrate, p. 363-367. In R.R. Colwell and R.Y. Morita eds., Effect of the Ocean Environment on Microbial Activities. University Park Press, Baltimore, Maryland.

Cerniglia, C. E., and D. T. Gibson. 1977. Metabolism of naphthalene by Cunninghamella elegans. Appl. Environ. Microbiol. 34:363-370.

Cerniglia, C. E., and D. T. Gibson. 1978. Metabolism of naphthalene by cell extracts of Cunninghamella elegans. Arch. Biochem. Biophys. 186:121-127.

Cerniglia, C. E., and D. T. Gibson. 1979. Algal oxidation of aromatic hydrocarbons: formation of 1-naphthol from naphthalene by Agmenellum quadruplication, strain PR-6. Biochem. Biophs. Res. Commun. 88:50-58.

Cerniglia, C. E., and D. T. Gibson. 1980a. Fungal oxidation of benzoapyrene and ±-trans-7,8-dihydroxy-7,8-dihydrobenzoapyrene: evidence for the formation of a benzoapyrene 7,8-diol-9,10-epoxide. J. Biol. Chem. 225:5159-5163.

Cerniglia, C. E., and D. T. Gibson. 1980b. Fungal oxidation of ±-9,10 dihydroxy-9,10-dihydrobenzoapyrene: formation of diastereometic benzoapyrene 9,10-diol 7,8-epoxides. Proc. Natl. Acad. Sci. U.S.A. 77:4554-4558.

Cerniglia, C. E., D. T. Gibson, and C. van Baalen. 1980. Oxidation of naphthalene by cyanobacteria and microalgae. J. Gen. Microbiol. 116:495-500.

Cerniglia, C. E., R. L. Hebert, R. H. Dodge, P. J. Szaniszlo, and D. T. Gibson. 1978a. Some approaches to studies on the degradation of aromatic hydrocarbons by fungi, p. 360-369. In A. L. Bourquin and P. H. Pritchard ed., Microbial degradation of pollutants in marine environments. EPA-600/9-79-012. Environmental Research Laboratory, Gulf Breeze, Fla.

Cerniglia, C. E., R. L. Hebert, P. J. Szaniszlo, and D. T. Gibson. 1978b. Fungal transformation of naphthalene. Arch Microbiol. 117:135-143.

Cerniglia, C. E., J. J. Perry. 1973. Crude oil degradation by microorganisms isolated from the marine environment. Z. Allg. Mikrobiol. 13:299-306.

Cerniglia, C. E., C. van Baalen, and D. T. Gibson. 1980a. Metabolism of naphthalene by the cyanobacterium Oscillatoria sp., strain JCM. J. Gen. Microbiol. 116:485-494.

Cerniglia, C. E., C. van Baalen, and D. T. Gibson. 1980b. Oxidation of bi-phenyl by the cyanobacterium Oscillatoria sp., strain JCM. Arch. Microbiol., in press.

Chakrabarty, A. M. 1972. Genetic basis of the biodegradation of salicylate in Pseudomonas. J. Bacteriol. 112:815-823.

Chakrabarty, A. M., G. Chou, and I. C. Gunsalus. 1973. Genetic regulation of octane disimilation plasmid in Pseudomonas. Proc. Natl. Acad. Sci. U.S.A. 70:1137-1140.

Chouteau, J., E. Azoulay, and J. C. Senez. 1962. Anaerobic formation of n hept-1-ene from n heptane by resting cells of Pseudomonas aeruginosa. Nature London 194:576-578.

Claus, D., Bergendahl, J. E. and Mandel, M. 1968. DNA base composition of Microcyclus species and organisms of similar morphology. Archiv fu;r Mikrobiologie 63, 26-68.

Cobet, A. B., and H. E. Guard. 1973. Effect of a bunker fuel on the beach bacterial flora, p. 815-819. In Proceedings of Joint Conference on Prevention and Control of Oil Spills. American Petroleum Institute, Washington, D.C.

Colwell, R. R. 1978. Enumeration of specific populations by the most-probable-number MPN method, p. 56-61. In J. W. Costerton and R. R. Colwell ed., Native aquatic bacteria: enumeration, activity and ecology. ASTM-STP 695. American Society for Testing and Materials, Philadelphia.

Colwell, R. R., A. L. Mills, J. D. Walker, P. Garcia-Tello., and V. Campos-P. 1978. Microbial ecology studies of the Metula spill in the Straits of Magellan. J. Fish. Res. Board Can. 35:573-580.

Colwell, R. R. and Mortia, R. Y. 1964. Reisolation and emendatin of description of Vibrio marinus Russell Ford. Journal of Bacteriology 88, 831-837.

Colwell, R. R., and J. D. Walker. 1977. Ecological aspects of microbial degradation of petroleum in the marine environment. Crit. Rev. Microbiol. 5:423-445.

Colwell, R. R., J. D. Walker, and J. D. Nelson, Jr. 1973. Microbial ecology and the problem of petroleum degradation in Chesapeake Bay, p. 185-197. In D. G. Ahearn and S. P. Meyers ed., The microbial degradation of oil pollutants. Publication no. LSU-SG-73-01. Center for Wetland Resources, Louisiana State University, Baton Rouge.

Colwell, R.R., A.L. Mills, and J.D. Walker. 1978. Microbial Ecology studies of the Metula spill in the Straits of Magellan. J. Fish. Res. BD. Can. 35:573-580.

Cook, F. D., and D. W. S. Westlake. 1974. Microbiological degradation of northern crude oils. Environmental-Social Committee; Northern Pipelines,

Task Force on Northern Oil Development, report no. 74-1. Catalog no. R72-12774. Information Canada, Ottawa.

Cook, T. M. and Goldman, C. K. 1976. Bacteriology of Chesapeake Bay surface waters. Chesapeake Science 17, 40-49.

Cook, W. L., J. K. Massey, and D. G. Ahearn. 1973. The degradation of crude oil by yeasts and its effects on Lesbistes reticulatus, p. 279-282. In D. G. Ahearn and S. P. Meyers ed., The microbial degradation of oil pollutants. Publication no. LSU-SG-73-01. Center for Wetland Resources, Louisiana State University, Baton Rouge.

Cooney, J. J., P. Edmonds, and Q. M. Brenner. 1968. Growth and survival of fuel isolates in hydrocarbon-fuel emulsions. Appl. Microbiol. 16:569-571.

Cooney, J. J., and R. J. Summers. 1976. Hydrocarbon-using microorganisms in three freshwater ecosystems, p. 141-156. In J. M. Sharpley and A. M. Kaplan ed., Proceedings of the Third International Biodegradation Symposium. Applied Science Publishers, Ltd., London.

Cooney, J. J., and J. D. Walker. 1973. Hydrocarbon utilization by Cladosporium resinae, p. 25-32. In D. G. Ahearn and S. P. Meyers ed., The microbial degradation of oil pollutants. Publication no. LSU-SG-73-01. Center for Wetland Resources, Louisiana State University, Baton Rouge.

Cowell, E. B. ed.. 1971. The ecological effects of oil pollution on littoral communities. Applied Science Publishers, Ltd., London.

Cretney, W.I., C. S. Wong, D. R. Green, and C. A. Bawden. 1978. Long-term fate of a heavy fuel oil in a spill-contaminated coastal bay. J. Fish. Res. Board Can. 35:521-527.

Cripps, R. E., and R. J. Watkinson. 1978. Polycyclic aromatic hydrocarbons: metabolism and environmental aspects, p. 113-134. In J. R. Watkinson ed., Developments in biodegradation of hydrocarbons-1. Applied Science Publishers, Ltd., London.

Crow, S. A., W. L. Cook, D. G. Ahearn, and A. W. Bourquin. 1976. Microbial populations in coastal surface slicks, p. 93-98. In J. M. Sharpley and A. M. Kaplan ed., Proceedings of the Third International Biodegradation Symposium. Applied Science Publishers, Ltd., London.

Crow, S. A., S. P. Meyers, and D. G. Ahearn. 1974. Microbiological aspects of petroleum degradation in the aquatic environment. Mer 12:37-54.

Cundell, A. M., and R. W. Traxler. 1973. The isolation and characterization of hydrocarbon-utilizing bacteria from Chedabucto Bay, Nova Scotia, p. 421-426. In Proceedings of Joint Conference on Prevention and Control of Oil Spills. American Petroleum Institute, Washington, D.C.

Cundell, A. M., and R. W. Traxler. 1974. Hydrocarbon degrading bacteria associated with Arctic oil seeps. Dev. Ind. Microbiol. 15:250-255.

Cundell, A. M., and R. W. Traxler. 1976. Psychrophilic hydrocarbon-degrading bacteria from Narragansett Bay, Rhode Island, U.S.A. Mater. Org. 11:1-17.

Daley, R. J. and Hobbie, J. E. 1975. Direct counts of aquatic bacteria by a modified epifluorescence technique. Limnol. Oceanogr., 20, 875-882.

Davies, J. A., and D. E. Hughes. 1968. The biochemistry and microbiology of crude oil degradation, p. 139-144. In J. D. Carthy and D. R. Arthur ed., The biological effects of oil pollution on littoral communities supplement to Field Stud., vol. 2. E. W. Classey, Ltd., Hampton, Middesex, England.

Davies, J. S., and D. W. S. Westlake. 1979. Crude oil utilization by fungi. Can. J. Microbiol. 25:146-156.

Davis, J. B. 1956. Microbial decomposition of hydrocarbons. Ind. Eng. Chem. 48:1444-1448.

Davis, H. G. and Park, R. W. A. 1962. A taxonomic study of certain bacteria currently classified as Vibio species. Journal of General Microbiology 27, 101-119.

Davis, S. J., and C. F. Gibbs. 1975. The effect of weathering on a crude oil residue exposed at sea. Water Res. 9:275-285.

Dean, R. A. 1968. The chemistry of crude oils in relation to their spillage on the sea, p. 1-6. In J. D. Carthy and D. R. Arthur ed., The biological effects of oil pollution on littoral communities supplement to Field Stud., vol. 2. E. W. Classey, Ltd., Hampton, Middlesex, England.

Dean-Raymond, D., and R. Bartha. 1975. Biodegradation of some polynuclear aromatic petroleum components by marine bacteria. Dev. Ind. Microbiol. 16:97-110.

Delaune, R. D., G. A. Hambrick III, and W. H. Patrick, Jr. 1980. Degradation of hydrocarbons in oxidized and reduced sediments. Mar. Pollut. Bull. 11:103-106.

Diamond, v. Chakrabarty. 1980. 48 U.S.L.W. 4714 u.S. June 16, 1980.

Dibble, J. T., and R. Bartha. 1976. The effect of iron on the biodegradation of petroleum in seawater. Appl. Environ. Microbiol. 31:544-550.

Dibble, J. T., and R. Bartha. 1979a. Effect of environmental parameters on the biodegradation of oil sludge. Appl. Environ. Microbiol. 37:729-739.

Dibble, J. T., and R. Bartha. 1979b. Leaching aspects of oil sludge biodegradation in soil. Soil Sci. 127:365-370.

Dibble, J. T., and R. Bartha. 1979c. Rehabilitation of oil-inundated agricultural land: a case history. Soil Sci. 128:56-60.

Donoghue, N. A., M. Griffin, D. G. Norris, and P. W. Trudgill. 1976. The microbial metabolism of cyclohexane and related compounds, p. 43-56. In J. M. Sharpley and A. M. Kaplan ed., Proceedings of the Third

International Biodegradation Symposium. Applied Science Publishers, Ltd., London.

Dunn, N. W., and I. C. Gunsalus. 1973. Transmissible plasmid coding early enzymes of naphthalene oxidation in Pseudomonas putida. J. Bacteriol. 114:974-979.

Fall, R. R., J. L. Brown, and T. L. Schaeffer. 1979. Enzyme recruitment allows the biodegradation of recalcitrant branched hydocarbons by Psuedomonas citronellolis. Appl. Environ. Microbiol. 38:715-722.

Ferris, J. P., L. H. MacDonald, M. A. Patrie, and M. A. Martin. 1976. Aryl hydrocarbon hydroxylase activity in the fungus Cunninghamella bainierii. Evidence of the presence of cytochrome P450. Arch. Biochem. Biophys. 175:443-452.

Finnerty, W. R., Kennedy, R. S., Spurlock, B. O. and Young, R. A. 1973. Microbes and petroleum: perspectives and implications. In The Microbial Degradation of Oil Pollutants, pp. 105-125. Edited by D. G. Ahearn and S. P. Meyers. Baton Rouge: Center for Wetland Resources. Louisiana State University Publication No. LSU-SG-73-01.

Floodgate, G. D. 1972a. Biodegradation of hydrocarbons in the sea, p. 153-171. In R. Mitchell ed., Water pollution microbiology. John Wiley and Sons, Inc., New York.

Floodgate, G. D. 1972b. Microbial degradation of oil. Mar. Pollut. Bull. 3:41-43.

Floodgate, G. D. 1973. A threnody concerning the biodegradation of oil in natural waters, p. 17-24. In D. G. Ahearn and S. P. Meyers ed., The microbial degradation of oil pollutants. Publication no. LSU-SG-73-01. Center for Wetland Resources, Louisiana State University, Baton Rouge.

Floodgate, G. D. 1976. Oil biodegradation in the oceans, p. 87-92. In J. M. Sharpley and A. M. Kaplan ed., Proceedings of the Third International Biodegradation Symposium. Applied Science Publishers, Ltd., London.

Floodgate, G. D. 1979. Nutrient limitation, p. 107-118. In A. W. Bourquin and P. H. Pritchard ed., Proceedings of workshop, Microbial Degradation of Pollutants in Marine Environments. EPA-66019-79-012. Environmental Research Laboratory, Gulf Breeze, Fla.

Floodgate, G. D. and Hayes, P. R. 1963. The Adansonian taxonomy of some yellow pigmented marine bacteria. Journal of General Microbiology 30, 237-244.

Foster, J. W. 1962a. Bacterial oxidation of hydrocarbons, p. 241-271. In O. Hiyashi ed., Oxygenases. Academic Press, Inc., New York.

Foster, J. W. 1962b. Hydrocarbons as substrates for microorganisms. Antonie van Leeuwenhoek J. Miocrobiol. Serol. 28:241-274.

Francke, H. C., and F. E. Clark. 1974. Disposal of oil wastes by microbial assimilation. Report Y-1934. U.S. Atomic Energy Commission, Washington, D.C.

Frankenfeld, J. W. 1973. Factors governing the fate of oil at sea; variations in the amounts and types of dissolved or dispersed materilas during the weathering process, p. 485-495. In Proceedings of Joint Conference on Prevention and Control of Oil Spills. American Petroleum Institute, Washington, D.C.

Friello, D. A., J. R. Mylroie, and A. M. Chakrabarty. 1976. Use of genetically engineered multi-plasmid microorganisms for rapid degradtion of fuel hydrocarbons, p. 205=214. In J. M. Sharpley and A. M. Kaplan ed., Proceedings of the Third International Biodegradation Symposium. Applied Science Publishers, Ltd., London.

Gatellier, C. R. 1971. Les facteurs limitant la biode'gradation des hydrocarbons dans l'epuration des eaux. Chim. Ind. Paris 104:2283-2289.

Gatellier, C. R., J. L. Oudin, P. Fusey, J. C. Lacase, and M. L. Priou. 1973. Experimental ecosystems to measure fate of oil spills dispersed by surface active products, p. 497-507. In Proceedings of Joint Conference on Prevention and Control of Oil Spills. American Petroleum Institute, Washington, D.C.

Gibbs, C. F. 1975. Quantitative studies in marine biodegradaton of oil. I. Nutrient limitation at 14°C. Proc. R. Soc. Lonon Ser. B 188:61-82.

Gibbs, C. F., and S. J. Davis. 1976. The rate of microbial degradation of oil in a beach gravel column. Microb. Ecol. 3:55-64.

Gibbs, C. F., K. B. Pugh, and A. R. Andrews. 1975. Quantitative studies in marine biodegradation of oil. II. Effects of temperature. Proc. R. Soc. London Ser. B 188:83-94.

Gibson, D. T. 1968. Microbial degradation of aromatic compounds. Science 161:1092-1097.

Gibson, D. T. 1971. The microbial oxidation of aromatic hydrocarbons. Crit. Rev. Microbiol. 1:199-223.

Gibson, D. T. 1975. Oxidation of the carcinogens benzoapyrene and benzoaanthracene to dihydrodiols by a bacterium. Science 189:295-297.

Gibson, D. T. 1976. Microbial degradation of polycyclic aromatic hydrocarbons, p. 57-66. In J. M. Sharpley and A. M. Kaplan ed., Proceedings of the Third International Biodegradation Symposium. Applied Science Publishers, Ltd., London.

Gibson, D. T. 1977. Biodegradation of aromatic petroleum hydrocarbons, p. 36-46. In D. Wolfe ed., Fate and effects of petroleum hydrocarbons in marine ecosystems and organisms. Pergamon Press, In., New York.

Gibson, D. T., and W. K. Yeh. 1973. Microbial degradation of aromatic hydrocarbons, p. 33-38. In D. G. Ahearn and S. P. Meyers ed., The microbial degradation of oil pollutants. Publication no. LSU-SG-73-01. Cneter for Wetland Resources, Louisiana State University, Baton Rouge.

Goodfellow, M., Austin, B. and Dickinson, C. H. 1976. Numerical taxonomy of some yellow-pigmented bacteria isolated from plants. Journal of General Microbiology 97, 219-233.

Gordon, D. C., P. D. Keizer, and J. Dale. 1974. Estimates using fluorescence spectroscopy of the present state of petroleum hydrocarbon contamination in the water column of the northwest Atlantic Ocean. Marine Chemistry 2: 251-261.

Gordon, D. C., J. Dale, and P. D. Keizer. 1978. Importance of sediment working by the deposit-feeding polychaete Arenicola marina on the weathering rate of sediment-bound oil. J. Fish. Res. Board Can. 35:591-603.

Gordon, R. E., Haynes, W. C. and Pang, C. H.-N. 1973. The genus Bacillus. U. S. Department of Agriculture Handbook no. 427.

Gordon, D. C., Dale, J. and Keizer, P. D. 1978. Importance of sediment working by the deposit-feeding polychaete Arenicola marina on the weathering rate of sediment bound oil. J. Fish. Res. Bd. Can., 35, 591-603.

Griffiths, R.P., and Morita, R.Y. 1981a. Study of microbial activity and crude oil-microbial interactions in the waters and sediments of Cook Inlet and the Beaufort Sea. Final report RU190. NOAA/OMPA Alaskan Project Office, Juneau.

Griffiths, R.P., and Morita, R.Y. 1981b. Microbial processes as related to transport in the North Aleutian Shelf and St. George Lease areas. Final report RU395. NOAA/OMPA Alaskan Project Office, Juneau.

Grouse, P. L., J. S. Mattson, and H. Petersen ed.. 1979. USNS Potomac oil spill Melville Bay, Greenland, 5 August 1977. NOAA-S/T 79-202. U.S. Department of Commerce, Wahsington, D.C.

Gudin, C., and W. J. Syratt. 1975. Biological aspects of land rehabilitation following hydrocarbon contamination. Environ. Pollut. 8:107-112.

Guire, P. E., J. D. Friede, and R. K. Gholson. 1973. Production and characterization of emulsifying factors from hydrocarbonoclastic yeast and bacteria, p. 229-231. In D. G. Ahearn and S. P. Meyers ed., The microbial degradation of oil pollutants. Publication no. LSU-SG-73-01. Center for Wetland Resources, Louisiana State University, Baton Rouge.

Gunkel, W. 1967. Experimentell-okologische Untersuchungen uber die limitierenden Faktoren des mickrobiellen Olabbaues in marinen Milieu. Helgo. Wiss. Meeresunters. 15:210-224.

Gunkel, W. 1968a. Bacteriological investigations of oil-polluted sediments fromt he Cornish coast following the "Torrey Canyon" disaster, p. 151-158. In J. D. Carthy and D. R. Arthur ed., The biological effects of oil pollution on littoral communities supplement to Field Stud., vol. 2. E. W. Classey, Ltd., Hampton, Middlesex, England.

Gunkel, W. 1968b. Bacteriological investigations of oil-polluted sediments from the Cornish coast following the Torrey Canyon disaster. Helgol. Wiss. Meeresunters. 17:151-158.

Gunkel, W., G. Gassmann, C. H. Oppenheimer, and I. Dundas. 1980. Preliminary results of baseline studies of hydrocarbons and bacteria in the North Sea: 1975, 1976 and 1977, p. 223-247. In Ponencias del Simposio International en: Resisistencia a los Antibiosis y Microbiologia marina. Santiago de Compestela, Spain.

Gutnick, D. L., and E. Rosenberg. 1977. Oil tankers and pollution: a microbiological approach. Annu. Rev. Microbiol. 31:379-396.

Haines, J. R., and M. Alexander. 1974. Microbial degradation of high-molecular weight alkanes. Appl. Microbiol. 28:1084-1085.

Haines, J.R. and R.M. Atlas. 1982. In situ microbial degradation of Prudhoe Bay crude oil in Beaufort Sea sediment. Mar. Environ. Res. 7:91-102.

Hambrick, G. A., III, R. DeLaune, and W. H. Patrick, Jr. 1980. Effect of estuarine sediment pH and oxidation-reduction potential on microbial hydrocarbon degradation. Appl. Environ. Microbiol. 40:365-369.

Harrison, W., M. A. Winnik, P. T. Y. Kwong, and D. Mackay. 1975. Crude oil spills. Disappearance of aromatic and aliphatic components from small sea-surface slicks. Environ. Sci. Technol. 9:231-234.

Hauxhurst, J.D., T. Kaneko, and R.M. Atlas. 1981. Characteristics of Bacterial Communities in the Gulf of Alaska. Microb. Ecol. 7:167-182.

Hauxhurst, J. D., Krichevsky, M. I., and Atlas, R. M. 1980. Numerical taxonomy of bacteria from the Gulf of Alaska. J. Gen. Microbiol. 120:131-148.

Hayes, P. R. 1963. Studies on marine flavobacteria. Journal of General Microbiology 30, 1-19.

Head, P. C. 1971. An automated phenolhypochlorite method for the determination of ammonia in seawater. Deep Sea Res., 8, 531-532.

Hendrie, M. S., Mitchell, T. G. and Shewan, J. M. 1968. In Identification Methods for Microbiologists, Part B, pp. 67-78. Edited by B. M. Gibbs and D. A. Shapton. London: Academic Press.

Herbes, S. E., and L. R. Schwall. 1978. Microbial transformation of polycyclic aromatic hydrocarbons in pristane and petroleum-contaminated sediments. Appl. Environ. Microbiol. 35:306-316.

Higashihara, T., A. Sato, and U. Simidu. 1978. An MPN method for the enumeration of marine hydrocarbon degrading bacteria. Bull. Jpn. Soc. Sci. Fish. 44:1127-1134.

Hill, E. C. 1978. Biodegradation of hydrocarbon-based products in industrial use, p. 201-226. In J. R. Watkinson ed., Developments in biodegradation of hydrocarbons-1. Applied Science Publishers, Ltd., London.

Hill, E. C., and A. R. Thomas. 1976. Microbiological aspects of supersonic aircraft fuel, p. 157-174. In J. M. Sharpley and A. M. Kaplan ed., Proceedings of the Third International Biodegradation Symposium. Applied Science Publishers, Ltd., London.

Holding, A. J. and Shewan, J. M. 1974. Genera of uncertain affiliation; genus Alcaligenes. In Bergey's Manual of Determinative Bacteriology, 8th edn, pp. 273-277. Edited by R. E. Buchanan and N. E. Gibbons. Baltimore: Williams and Wilkins.

Hopper, D. J. 1978. Microbial degradation of aromatic hydrocarbons, p. 85-112. In J. R. Watkinson ed., Developments in biodegradation of hydrocarbons-1. Applied Science Publishers, Ltd., London.

Horner, R. and Alexander, V. 1972. Algal populations in Arctic sea ice: an investigation of heterotrophy. Limnology and Oceanography 17, 454-458.

Horowitz, A., and R. M. Atlas. 1977a. Continuous open flow-through system as a model for oil degradation in the Arctic Ocean. Appl. Environ. Microbiol. 33:647-653.

Horowitz, A., and R. M. Atlas. 1977b. Response of microorganisms to an accidental gasoline spillage in an Arctic freshwater ecosystem. Appl. Environ. Microbiol. 33:1252-1258.

Horowitz, A., and R. M. Atlas. 1978. Crude oil degradation in the Arctic: changes in bacterial populations and oil composition during one year exposure in a model system. Dev. Ind. Microbiol. 19:517-522.

Horowitz, A., D. Gutnick, and E. Rosenberg. 1975. Sequential growth of bacteria on crude oil. Appl. Microbiol. 30:10-19.

Horowitz, A., A. Sexstone, and R. M. Atlas. 1978a. Hydrocarbons and microbial activities in sediment of an Arctic lake one year after contamination with leaded gasoline. Arctic 31:180-191.

Horowitz, A. amd R.M. Atlas. 1978. Crude oil degradation in the Arctic: changes in bacterial populations and oil composition during one year exposure in a model ecosystem, Dev. Ind. Microbiol. 19:517-522.

Horvath, R. S. 1972. Microbial co-metabolism and the degradation of organic compounds in nature. Bacteriol. Rev. 36:146-155.

Hou, C. T., and A. I. Laskin. 1976. Microbial conversion of dibenzothiophene. Dev. Ind. Microbiol. 17:351-362.

Hunt, P. G., W. E. Rickard, F. J. Deneke, F. R. Koutz, and R. P. Murrmann. 1973. Terrestrial oil spills in Alaska: environmental effects and recovery, p. 733-740. In Proceedings of the Joint Conference on Prevention and Control of Oil Spills. American petroleum Institute, Washington, D.C.

Iizuka, H., M. Ilida, and S. Fujita. 1969. Formation of n-decene-1 from n-decane by resting cells of C. rugosa. Z. Allg. Mikrobiol. 9:223-226.

Jamison, V. M., R. L. Raymond, and J. O. Hudson, Jr. 1975. Biodegradation of high-octane gasoline in groundwater. Dev. Ind. Microbiol. 16:305-312.

Jamison, V. M., R. L. Raymond, and J. O. Hudson. 1976. Biodegradation of high-octane gasoline, p. 187-196. In J. M. Sharpley and A. M. Kaplan ed., Proceedings of the Third International Biodegradation Symposium. Applied Science Publishers, Ltd., London.

Jannasch, H.W. 1967. Growth of marine bacteria at limiting concentrations of organic carbon in seawater. Limnol. Oceanogr. 12:264-271.

Jannasch, H,W. and G.E. Jones. 1959. Bacterial populations in sea water as determined by different methods of enumeration. Limno. Oceanog. 4: 128-139.

Jannasch, H. W., K. Eimhjellen, C. O. Wirsen, and A. Farmaian. 1971. Microbial degradation of organic matter in the deep sea. Science 171:672-675.

Jensen, V. 1975. Bacterial flora of soil after application of oily waste. Oikos 26:152-158.

Jobson, A., F. D. Cook, and D. W. S. Westlake. 1972. Microbial utilization of crude oil. Appl. Microbiol. 23:1082-1089.

Jobson, A., M. McLaughlin, F. D. Cook, and D. W. S. Westlake. 1974. Effect of amendments on the microbial utilization of oil applied to soil. Appl. Microbiol. 27:166-171.

Johnson, R. 1979. Computer-aided identification. FDA By-Lines 9, 235-250.

Johnston, R. 1970. The decompostion of crude oil residues in sand columns. J. Mar. Biol. Assoc. U.K. 50:925-937.

Jones, J. G., and M. A. Eddington. 1968. An ecological survey of hydrocarbon-oxidizing microorganisms. J. Gen. Microbiol. 52:381-390.

Jordan, R. E., and J. R. Payne. 1980. Fate and weathering of petroleum spills in the marine environment. Ann Arbor Science Publications, Inc., Ann Arbor, Mich.

Jurtshuk, P., and G. E. Cardini. 1971. The mechanism of hydrocarbon oxidation by a Corynebacterium species. Crit. Rev. Microbiol. 1:239-289.

Kaneko, T. and Colwell, R. R. 1973. Ecology of Vibrio parahaemolyticus in Chesapeake Bay. Journal of Bacteriology 113, 24-32.

Kaneko, T. and Colwell, R. R. 1974. Distribution of Vibrio parahaemolyticus and related organisms in the Atlantic Ocean off South Carolina and Georgia. Applied Microbiology 28, 1009-1017.

Kaneko, T., Holder-Franklin, M. and Franklin, M. 1977a. Multiple syringe inoculator for agar plates. Applied and Environmental Microbiology 33, 982-985.

Kaneko, T., R.M. Atlas, and M. Krichevsky. 1977b. Diversity of bacterial populations in the Beaufort Sea. Nature 270: 596-599.

Kaneko, T., M.I. Krichevsky, and R.M. Atlas. 1979. Numerical taxonomy of bacteria from the Beaufort Sea. J. Gen. Microbiol. 110:111-125.

Kaneko, T., Hauxhurst, J., Krichevsky, M. I., and Atlas, R. M. 1978. Numerical taxonomic studies of bacteria isolated from arctic and subarctic marine environments, pp. 26-30. In M. W. Loutit and J.A.R. Miles eds., Microbial Ecology. Berlin and Heidelberg: Springer-Verlag.

Kappeler, Th., and K. Wuhrmann. 1978a. Microbial degradation of the water-soluble fraction of gas oil-I. Water Res. 12:327-334.

Kappeler, Th., and K. Wuhrmann. 1978b. Microbial degradation of the water-soluble fraction of gas oil-II. Bioassays with pure strains. Water Res. 12:335-342.

Karrick, N. 1977. Alterations in petroleum resulting from physicochemical and microbiological factors, p. 225-299. In D. C. Malins ed., Effects of petroleum on Arctic and subarctic marine environments and organisms, vol. 1. Nature and fate of petroleum. Academic Press, Inc., New York.

Kator, H. 1973. Utilization of crude oil hydrocarbons by mixed cultures of marine bacteria, p. 47-66. In D. G. Ahearn and S. P. Meyers ed., The microbial degradation of oil pollutants. Publication no. LSU-SG-73-01. Center for Wetland Resources, Louisiana State University, Baton Rouge.

Kator, H., and R. Herwig. 1977. Microbial responses after two experimental oil spills in an eastern coastal plain estuarine ecosystem, p. 517-522. In Proceedings of the 1977 Oil Spill Conference. American Institute of Petroleum, Washington, D. C.

Kator, H., R. Miget, and C. H. Oppenheimer. 1972. Utilization of paraffin hydrocarbons in crude oil by mixed cultures of marine bacteria. Paper no. SPE 4206. Symposium on Environmental Conservation. Society of Petroleum Engineers, Dallas, Tex.

Kator, H. C. H. Oppenheimer, and R. J. Miget. 1971. Microbial degradation of a Louisiana crude oil in closed flasks and under simulated field conditions, p. 287-296. In Joint Conference on Prevention and Control of Oil Spills. American Petroleum Institute, Washington, D.C.

Keizer, P. D., and D. C. Gordon, Jr. 1972. Detection of trace amounts of oil in seawater by fluorescence spectroscopy. J. Fish. Res. Bd. Can. 30: 1029-1046.

Keizer, P. D., T. P. Ahern, J. Dale, and J. H. Vandermeulen. 1978. Residues of Bunker C oil in Chedabucto Bay, Nova Scotia, 6 years after the Arrow spill. J. Fish. Res. Board Can. 35:528-535.

Kincannon, C. B. 1972. Oily waste disposal by soil cultivation process. EPA-R2-72-100. U.S. Environmental Protection Agency, Washington, D.C.

King, D. H., and J. J. Perry. 1975. The origin of fatty acids in the hydrocarbon-utilizing microorganism, Mycobacterium vaccae. Can. J. Microbiol. 21:85-89.

Kinney, P. J., D. K. Button, and D. M. Schell. 1969. Kinetics of dissipation and biodegradation of crude oil in Alaska's Cook Inlet, p. 333-340. In Proceedings of 1969 Joint Conference on Prevention and Control of Oil Spills. American Petroleum Institute, Washington, D.C.

Klug, M. J., and A. J. Markovetz. 1967a. Degradation of hydrocarbons by members of the genus Candida. J. Bacteriol. 93:1847-1852.

Klug, M. J., and A. J. Markovetz. 1967b. Thermophilic bacteria isolated on n-tetradecane. Nature London 215:1082-1083.

Kodama, K., S. Nakatani, K. Umehara, K. Shimizu, Y. Minoda, and K. Yamada. 1970. Microbial conversion of petro-sulfur compounds. III. Isolation and identification of products from dibenzothiophene. Agric. Biol. Chem. 34:1320-1324.

Komagata, K., T. Nakase, and N. Katsuya. 1964. Assimilation of hydrocarbons by yeasts. I. Preliminary screening. J. Gen. Appl. Microbiol. 10:313-321.

Krichevsky, M. 1977. Coding and management of microbiological data. Developments in Industrial Microbiology 18, 309-318.

Krichevsky, M.I. 1979. The microbial information system MICRO-IS: A system overview. In U.S. Department of Health, Education, and Welfare, Food and Drug Administration, Washington, D.C. FDA By-Line 9:217-222.

Kuznetzov, S.I., G.A. Dubinina, and N.A. Lapteva. 1979. Biology of Oligotrophic Bacteria. Ann. Rev. Microbiol. 33: 377-387.

Lee, R. F. 1977a. Accumulation and turnover of petroleum hydrocarbons in marine organisms, p. 60-70. In. D. A. Wolfe ed., Fate and effects of petroleum hydrocarbons in marine organisms and ecosystems. Pergomon Press, Inc., New York.

Lee, R. F. 1977b. Fate of petroleum components in estuarine waters of the Southeastern United States, p. 611-616. In Proceedings of the 1977 Oil Spill Conference. American Petroleum Institute, Washington, D.C.

Lee, R. F., and C. Ryan. 1976. Biodegradation of petroleum hydrocarbons by marine microbes, p. 119-126. In J. M. Sharpley and A. M. Kaplan ed., Proceedings of the Third International Biodegradation Symposium. Applied Sciences Publishers, Ltd., London.

Lehmicke, L. G., R. J. Williams, and R. L. Crawford. 1979. ^{14}C-most-probable-number method for enumeration of active heterotrophic microorganisms in natural waters. Appl. Environ. Microbiol. 38:644-649.

Lehtomake, M., and S. Niemela. 1975. Improving microbial degradation of oil in soil. Ambio 4:126-129.

Leifson, E. 1963. Determination of carbohydrate metabolism of marine bacteria. Journal of Bacteriology 85, 1183-1184.

Leifson, E., B. J. Cosenza, R. Murchelano, and R. C. Cleverdon. 1964. Motile marine bacteria. I. Techniques, ecology and general characteristics. J. Bacteriol. 87:652-666.

LePetit, J., and M. H. Barthelemy. 1968. Les hydrocarbures en mer: le probleme de l'epuration des zones littorales par les microorganismes. Ann. Inst. Pasteur Paris 114:149-158.

LePetit, J., and M.-H. N'Guyen. 1976a. Besoins en phosphore des bacteries metabolisant les hydrocarbons en mer. Can. J. Microbiol. 22:1364-1373.

LePetit, J., and S. Tagger. 1976b. De'gradation des hydrocarbures en pre'sence d'autres substances organiques par des bacte'ries isole'es de l'eau de mer. Can. J. Microbiol. 22:1654-1657.

LePetit, J., M.-H. N'Guyen, and S. Tagger. 1977. Aquelques donnees sur l'ecologie d'une zone marine littorales recevant les rejets d'une raffinerie de petrole. Environ. Pollut. 13:41-56.

Liston, J., Wiebe, W. and Colwell, R. R. 1963. Quantitative approach to the study of bacterial species. Journal of Bacteriology 85, 1061-1070.

Llanos, C., and A. Kjoller. 1976. Changes in the flora of soil fungi following oil waste application. Oikos 27:337-382.

Lloyd, M., Zar, J.H., and J.R. Karr. 1968. On the calculation of informational measures of diversity. Am. Mid. Nat. 79: 257-272.

Lough, A. K. 1973. The chemistry and biochemistry of phylenic, pristanic and related acids. Prog. Chem. Fats Other Lipids 14:1-48.

Lovelace, T. E., Tubiash, H. and Colwell, R. R. 1967. Quantitative and qualitative commensal bacterial flora of Crassostrea virginica in Chesapeake Bay. Proceedings of the National Shellfishereis Association 58, 82-87.

Ludzack, F. L., and D. Kinkead. 1956. Persistence of oily wastes in polluted water under aerobic conditions. Ind. Eng. Chem. 48:263-267.

Macleod, R. A., Onofrey, E. and Norris, M. E. 1954. Nutrition and metabolism of marine bacteria. I. Survey of nutritional requirements. Journal of Bacteriology 68, 680-686.

Malins, D. C. 1977. Effects of petroleum on Arctic and subarctic marine environments and organisms. New York, Academic Press.

Mallory, L.M., Austin, B., and R.R. Colwell. 1977. Numerical taxonomy of oligotrophic bacteria isolated from the estuarine environment. Can. J. Microbiol. 23: 733-750.

Markovetz, A. J. 1971. Subterminal oxidation of aliphatic hydrocarbons by microorganisms. Crit. Rev. Microbiol. 1:225-237.

Masters, M. J., and J. E. Zajic. 1971. Myxotrophic growth of algae on hydrocarbon substrates. Dev. Ind. Microbiol. 12:77-86.

Mateles, R. I., J. N. Baruah, and S. R. Tannenbaum. 1967. Growth of a thermophilic bacteria on hydrocarbons: a new source of single-cell protein. Science 157:1322-1323.

Mathews, M. M. and Sistrom, W. R. 1959. Function of carotenoid pigments in non-photosynthetic bacteria. Nature, London 184, 1892-1893.

Maunder, B. R., and J. S. Waid. 1973. Disposal of waste oil by land spreading, p. 142-160. In Proceedings of the Pollution Research Conference, 20-21 June 1973, Wairakei, New Zealand. Information series no. 97. New Zealand Department of Sceintific and Industrial Research, Wellirgton.

Mayo, D. W., D. S. Page, J. Cooley, E. Solenson, F. Bradley, E. S. Gilfillan, and S. A. Hanson. 1978. Weathering characteristics of petroleum hydrocarbons deposited in fine clay marine sediments, Searsport, Maine. J. Fish Res. Board Can. 35:552-562.

Mayo, D. W., Page, D. S., Cooley, J., Solenson, E., Bradley, F., Gilfillan, E. S. and Hanson, S. A. 1978. Weathering characteristics of petroleum hydrocarbons deposited in fine clay marine sediments, Searsport, Maine. J. Fish. Res. Bd. Can., 35, 552-562.

McAuliffe, C. 1966. Solubility in water of paraffin, cycloparaffin, olefin, acetylene, cycloolefin, and aromatic hydrocarbons. J. Phys. Chem. 70:1267-1275.

McAuliffe, C. D., A. E. Smalley, R. D. Groover, W. M. Welsh, W. S. Pickle, and G. E. Jones. 1975. The Chevron Main Pass Block 41 oil spill: chemical and biological investigations, p. 555-566. In Proceedings 1975 Conference on Prevention and Control of Oil Pollution. American Petroleum Institute, Washington, D.C.

McDonald, I. J., Quadling, C. and Chambers, A. K. 1963. Proteolytic activity of some cold-tolerant bacteria from Arctic sediments. Canadian Journal of Microbiology 9, 303-315.

McKenna, E. J., and R. E. Kallio. 1964. Hydrocarbon structure: its effect on bacterial utilization of alkanes, p. 1-14. In H. Heukelian and N. C. Dondero ed., Principles and applications in aquatic microbiology. John Wiley and Sons, Inc., New York.

McKenna, E. J., and R. E. Kallio. 1965. The biology of hydrocarbons. Annu. Rev. Microbiol. 19:183-208.

McKenna, E. J., and R. E. Kallio. 1971. Microbial metabolism of the isoprenoid alkane, pristane. Proc. Natl. Acad. Sci. U.S.A. 68:1552-1554.

Merkel, G. J., S. S. Stapleton, and J. J. Perry. 1978. Isolation and peptidoglycan of gram-negative hydrocarbon-utilizing thermophilic bacteria. J. Gen. Microbiol. 109:141-148.

Miller, T. L., and M. J. Johnson. 1966. Utilization of normal alkanes by yeasts. Biotechnol. Bioeng. 8:549-565.

Mills, A. L., C. Breuil, and R. R. Colwell. 1978. Enumeration of petroleum-degrading marine and estuarine microorganisms by the most-probable-number method. Can. J. Microbiol. 24:552-557.

Mironov, O. G. 1970. Role of microorganisms growing on oil in the self-purification and indication of oil pollution in the sea. Oceanology 10:650-656.

Mironov, O. G., and A. A. Lebed. 1972. Hydrocarbon-oxidizing microorganisms in the North Atlantic. Hydrobiol. J. 8:71-74.

Mitchell, E. 1975. Trophic relationships and competition for food, p. 123-133. In Proc. Canadian Soc. Zoologists Ann. Meet., 1974.

Moaledi, von K. 1978. Qualatative analysis of an oligocarbophilic aquatic microflora in the Plubsee. Arch. Hydrobiol. 82: 98-113.

Morita, R. Y. and Haight, R. D. 1964. Temperature effects on the growth of an obligate psychrophilic marine bacterium. Limnology and Oceanography 9, 103-106.

Morita, R. Y. 1975. Psychrophilic bacteria. Bact. Rev., 39, 144-167.

Morrison, S. I., and D. C. White. 1980. Effects of grazing by estuarine gammaridean amphipods on the microbiota of allocthonous detritus. Appl. Environ. Microbiol. 40:659-671.

Mulder, E. G. 1964. Arthrobacter. In Principle and Application in Aquatic Microbiology, Proceedings of Rudolfs Research Conference, pp. 254-276. Edited by H. Heukelekian and N. C. Dondero. New York: John Wiley.

Mulkins-Phillips, G. J., and J. E. Stewart. 1974a. Distribution of hydrocarbon-utilizing bacteria in northwestern Atlantic waters and coastal sediments. Can. J. Microbiol. 20:955-962.

Mulkins-Phillips, G. J., and J. E. Stewart. 1974b. Effect of environmental parameters on bacterial degradation of Bunker C oil, crude oils, and hydrocarbons. Appl. Microbiol. 28:915-922.

Mulkins-Phillips, G. J., and J. E. Stewart. 1974c. Effect of four dispersants on biodegradation and growth of bacteria on crude oil. Appl. Microbiol. 28:547-552.

Murchelano, R. A. and Brown, C. 1970. Heterotrophic bacteria in Long Island Sound. Marine Biology 7, 1-6.

Nakatani, S., T. Akasaki, K. Kodama, Y. Minoda, and K. Yamada. 1968. Microbial conversion of petro-sulfur compounds. II. Culture conditions of dibenzothiophene-utilizing bacteria. Agric. Biol. Chem. 32:1205-1211.

National, Academy of Sciences. 1975. Petroleum in the marine environment. National Academy of Sciences, Washington, D.C.

Nie, N.H., Hull, C.H., Jenkins, J.G., Steinbrenner, K. and D.H. Bent. 1975. Statistical Package of the Social Sciences. McGraw Hill, New York.

Nyns, E. J., I. P. Auquiere, and A. L. Wiaux. 1968. Taxonomic value of property of fungi to assimilate hydrocarbons. Antonie van Leeuwenhoek J. Microbiol. Serol. 34:441-457.

Odu, C. T. I. 1972. Microbiology of soils contaminated with petroleum hydrocarbons. I. Extent of contamination and some soil and microbial properties after contamination. J. Inst. Pet. London 58:201-208.

Olivieri, R., P. Bacchin, A. Robertiello, N. Oddo, L. Degen, and A. Tonolo. 1976. Microbioal degradation of oil spills enhanced by a slow-release fertilizer. Appl. Environ. Microbiol. 31:629-634.

Ooyama, J., and J. W. Foster. 1965. Bacterial oxidation of cycloparaffinic hydrocarbons. Antonie van Leeuwenhoek J. Microbiol. Serol. 31:45-65.

Oppenheimer, C.H., W. Gunkel, and G. Gassman. 1977. Microorganisms and hydrocarbons in the North Sea during July-August, 1975. p 593-610. In Proc. 1977 Oil Spill Conf. Prevention, Behavior, Control and Cleanup of Oill Spills. Amer. Petrol. Inst., Washington, D.C.

Parekh, V. R., R. W. Traxler, and J. M. Sobek. 1977. n-Alkane oxidation enzymes of a Pseudomonad. Appl. Environ. Microbiol. 33:881-884.

Percy, J. A. 1976. Response of arctic marine crustaceans to crude oil and oil-tainted food. Environ. Pollut. 10: 155-162.

Percy, J. A. 1977a. Effects of dispersed oil upon the respiratory metabolism of an arctic marine amphipod Onisimus (= Boeckosimus) affinis, p. 191-200. In D. A. Wolfe (ed.), Fate and effects of petroleum hydrocarbons in marine ecosystems and organisms. Pergamon Press, New York.

Percy, J. A. 1977b. Responses of Arctic marine benthic crustaceans to sediments contaminated with crude oil. Environ. Pollut. 13: 155-162.

Percy, J. A., and T. C. Mullin. 1977. Effects of crude oil on the locomotion activity of Arctic marine invertebrates. Mar. Poll. Bull. 8: 35-46.

Perry, J. J. 1977. Microbial metabolism of cyclic hydrocarbons and related compounds. Crit. Rev. Microbiol. 5:387-412.

Perry, J. J. 1979. Microbioal cooxidations involving hydrocarbons. Microbiol. Rev. 43:59-72.

Perry, J. J., and C. E. Cerniglia. 1973. Studies on the degradation of petroleum by filamentous fungi, p. 89-94. In D. G. Ahearn and S. P. Meyers ed., The microbial degradation of oil pollutants. Publication no. LSU-SG-73-01. Center for Wetland Resources, Louisiana State University, Baton Rouge.

Pfaender, F. K., E. N. Buckley, and R. Ferguson. 1980. Response of the pelagic microbial community to oil from the IXTOC-I blowout: I. In situ studies. Proceedings of the Conference on Researcher/Pierce IXTOC-I Cruises. National Oceanographic and Atmospheric Administration, AOML, Miami, in press.

Pfister, R. M. and Burkholder, P. R. 1965. Numerical taxonomy of some bacteria isolated from antarctic and tropical seawaters. Journal of Bacteriology 90, 863-872.

Pierce, R. H., A. M. Cundell, and R. W. Traxler. 1975. Persistence and biodegradation of spilled residual fuel oil on an estuarine beach. Appl. Microbiol. 29:646-652.

Pinholt, Y., S. Struwe, and A. Kjoller. 1979. Microbial changes during oil decomposition in soil. Holarctic Ecol. 2:195-200.

Pirnik, M. P. 1977. Microbial oxidation of methyl branched alkanes. Crit. Rev. Microbiol. 5:413-422.

Pirnik, M. P., R. M. Atlas, and R. Bartha. 1974. Hydrocarbon metabolism by Brevibacterium erythrogenes: normal and branched alkanes. J. Bacteriol. 119:868-878.

Poindexter, J.S. 1979. Morphological adaptation to low nutrient concentrations, p. 341-356. In M. Shilo ed., Strategies of microbial life in extreme environments. Dahlem Konferenzen Life Sciences Research Report 13. Verlag Chemie, Weinheim, West Germany.

Poindexter, J.S. 1981a. The caulobacters: ubiquitous unusual bacteria. Microbiol Rev. 45:123-179.

Poindexter, J.S. 1981b. Oligotrophy: fast and famine existence. Adv. Microbial. Ecol. 5:63-90.

Polyakova, I. N. 1962. Distribution of hydrocarbons in water of Neva Bay. Mikrobiologiya 31:1076-1081.

Prince, H. N. and Cleverdon, R. C 1955. The flavobacteria. II. Utilization of nitrogen compounds. Journal of Bacteriology 69, 307-309.

Prince, H. N., Beck, E. S., Cleverdon, R. C. and Kulp, W. L. 1954. The flavobacteria. I. Nutritional requirements. Journal of Bacteriology 68, 326-328.

Pritchard, P. H., and T. J. Starr. 1973. Microbial degradation of oil and hydrocarbons in continuous culture, p. 39-46. In D. G. Ahearn and S. P. Meyers ed., The microbial degradation of oil pollutants. Publication no. LSU-SG-73-01. Center for Wetland Resources, Louisiana State University, Baton Rouge.

Pritchard, P. H., R. M. Ventullo, and J. M. Suflita. 1976. The microbial degradation of diesel oil in multistage continuous culture systems, p. 67-78. In J. M. Sharpley and A. M. Kaplan ed., Proceedings of the Third International Biodegradation Symposium. Applied Science Publishers, Ltd., London.

Raj, H. D. 1977. Microcyclus and related ring-forming bacteria. CRC Critical Reviews in Microbiology 5, 243-269.

Rashid, M. A. 1974. Degradation of Bunker C. oil under different coastal environments of Chedabucto Bay, Nova Scotia. Estuarine Coastal Mar. Sci. 2:137-144.

Ratledge, C. 1978. Degradation of aliphatic hydrocarbons, p. 1-46. In J. R. Watkinson ed., Developments in biodegradation of hydrocarbons-1. Applied Science Publishers, Ltd., London.

Raymond, R. L., J. O. Hudson, and V. W. Jamison. 1976. Oil degradation in soil. Appl. Environ. Microbiol. 31:522-535.

Raymond, R. L., and V. W. Jamison. 1971. Biochemical activities of Nocardia. Adv. Appl. Microbiol. 14:93-122.

Raymond, R. L., V. M. Jamison, and J. O. Hudson. 1967. Microbial hydrocarbon co-oxidation. I. Oxidation of mono- and dicyclic hydrocarbons by soil isolates of the genus Nocardia. Appl. Microbiol. 15:857-865.

Raymond, R. L., V. W. Jamison, and J. O. Hudson. 1976. Beneficial stimulation of bacterial activity in ground waters containing petroleum products, p. 319-327. In Water 1976. American Institute of Chemical Engineers, New York.

Reisfeld, A., E. Rosenberg, and D. Gutnick. 1972. Microbial degradation of oil: factors affecting oil dispersion in seawater by mixed and pure cultures. Appl. Microbiol. 24:363-368.

Revsbeck, N. P., Sorensen, J., Blackburn, H. and Lomholt, J. P. 1980. Distribution of oxygen in marine sediments measured with microelectrodes. Limnol. Oceanog., 25, 403-411.

Rheinheimer, G. 1980. Aquatic Microbiology. John Wiley and Sons, New York.

Robertson, B., S. Arhelger, P. J. Kinney, and D. K. Button. 1973a. Hydrocarbon biodegradation in Alaskan waters, p. 171-184. In D. G. Ahearn and S. P. Meyers ed., The microbial degradation of oil pollutants. Publication no. LSU-SG-73-01. Center for Wetland Resources, Louisiana State University, Baton Rouge.

Robertson, B. R., S. D. Arhelger, R. A. T. Law, and D. K. Button. 1973b. Hydrocarbon biodegradation, p. 449-479. In D. W. Hood, W. E. Shiels, and E. J. Kelley ed., Environmental studies of Port Valdez. Occasional publication no. 3. University of Alaska, Institute of Marine Science, Fairbanks.

Robichaux, T. J., and H. N. Myrick. 1972. Chemical enhancement of the biodegradation of crude oil pollutants. J. Petrol. Technol 24:16-20.

Rogoff, M. H. 1961. Oxidation of aromatic compounds by bacteria. Adv. Appl. Microbiol. 3:193-221.

Rogosa, M., Krichevsky, M. I. and Colwell, R. R. 1971. Method of coding data on microbial strains for computer manipulation. International Journal of Systematic Bacteriology, Supplement 21, 1A-184A.

Roubal, G., and R. M. Atlas. 1978. Distribution of hydrocarbon-utilizing microorganisms and hydrocarbon biodegradation potentials in Alaska continental shelf areas. Appl. Environ. Microbiol. 35:897-905

Roubal, G., and R. M. Atlas. 1979. Hydrocarbon biodegradation in Cook Inlet, Alaska. Dev. Ind. Microbiol. 20:498-502.

Roubal, G., A. Horowitz, and R. M. Atlas. 1979. Disappearance of hydrocarbons following a major gasoline spill in the Ohio River. Dev. Ind. Microbiol. 20:503-507.

Sanders, H.L., J.F. Grassle, G.R. Hampson, L.S. Moore, S. Guner-Price, and C.C. Jones. 1980. Anatomy of an oil psill: long term effects from the grounding of the barge Florida off West Falmouth, Massachusetts. J. Mar. Res. 38:265-280.

Schaeffer, T. L., S. G. Cantwell, J. L. Brown, D. S. Watt, and R. R. Fall. 1979. Microbial growth on hydrocarbons: terminal branching inhibits biodegradation. Appl. Environ. Microbiol. 38:742-746.

Schwartz, J. B., A. A. Yayanos, and R. R. Colwell. 1976. Metabolic activities of the intestinal microflora of a deep-sea invertebrate. Appl. Environ. Microbiol. 31:46-48.

Schwarz, J. R., J. D. Walker, and R. R. Colwell. 1974a. Growth of deep-sea bacteria on hydrocarbons at ambient and in situ pressure. Dev. Ind. Microbiol. 15:239-249.

Schwarz, J. R., J. D. Walker, and R. R. Colwell. 1974b. Hydrocarbon degradation at ambient and in situ pressure. Appl. Microbiol. 28:982-986.

Schwarz, J. R., J. D. Walker, and R. R. Colwell. 1975. Deep-sea bacteria: growth and utilization of n-hexadecane at in situ temperature and pressure. Can. J. Microbiol. 21:682-687.

Seesman, P. A., J. D. Walker, and R. R. Colwell. 1976. Biodegradation of oil by marine microorganisms at potential off-shore drilling sites. Dev. Ind. Microbiol. 17:293-297.

Seki, H. 1976. Method for estimating the decomposition of hexadecane in the marine environment. Appl. Environ. Microbiol. 31:439-441.

Senez, J. C., and E. Azoulay. 1961. Dehydrogenation of paraffinic hydrocarbons by resting cells and cell free extracts of Pseudomonas aeruginosa. Biochim. Biophys. Acta 47:307-316.

Seubert, W., and E. Fass. 1964. Untersuchungen uber den bakteriellen Abbau von Isoprenoiden. V. Der Mechanisms des Isoprenoid-Abbaues. Biochem. Z. 341:35-44.

Sexstone, A. J., and R. M. Atlas. 1977a. Mobility and biodegradability of crude oil in Arctic tundra soils. Dev. Ind. Microbiol. 18:673-684.

Sexstone, A. J., and R. M. Atlas. 1977b. Response of populations in arctic tundra soils to crude oil. Can. J. Microbiol. 23:1327-1333.

Sexstone, A. J., and R. M. Atlas. 1978. Persistence of oil in trundra soils. Dev. Ind. Microbiol. 19:507-515.

Sexstone, A. J., K. Everett, T. Jenkins, and R. M. Atlas. 1978a. Fate of crude and refined oils in north slope soils. Arctic 31:339-347.

Sexstone, A. J., P. Gustin, and R. M. Atlas. 1978b. Long-term interactions of microorganisms and Prudhoe Bay crude oil in tundra soils at Barrow, Alaska. Arctic 31:348-354.

Shannon, C.E. 1948. A mathematical theory of communication. Bell. Syst. Technol. J. 27:379-423.

Shelton, T. B., and J. V. Hunter. 1975. Anaerobic decomposition of oil in bottom sediments. J. Water Pollut. Control Fe. 47:2256-2270.

Sieburth, J. McN. 1976. Bacterial substrates and productivity in marine ecosystems. Ann. Rev. Ecol. Syst. 1: 259-285.

Sieburth, J. McN. 1979. Sea microbes. Oxford University Press, New York.

Sierra, G. 1957. A simple method for the detection of lipolytic activity of microorganisms and some observations on the influence of contact between cells and fatty substrates. Antonie van Leeuwenhoek 23, 15-22.

Simidu, U., Kaneko, E. and Taga, N. 1977. Microbiological studies of Tokyo Bay. Microbial Ecology 3, 173-191.

Skerman, V. B. D. 1969. Abstracts of Microbiological Methods. New York: Wiley-Interscience.

Smith, J. E. 1968. "Torrey Canyon" pollution and marine life. Cambridge University Press, England.

Sneath, P.H.A., and Sokal, R.R. 1973. Numerical Taxonomy. W.H. Freeman and Co., San Francisco.

Sochard, M. R., D. F. Wilson, B. Austin and R. R. Colwell. 1979. Bacteria associated with the surface and gut of marine capepods. Appl. Environ. Microbiol. 37: 750-759.

Society of American Bacteriologists 1957. Manual of Microbiological Methods. New York: McGraw-Hill.

Sokal, R. R. and Sneath, P. H. A. 1963. Principles of Numerical Taxonomy. San Francisco: W. H. Freeman.

Soli, G. 1973. Marine hydrocarbonoclastic bacteria: types and range of oil degradation, p. 141-146. In D. G. Ahearn and S. P. Meyers ed., The microbial degradation of oil pollutants. Publication no. LSU-SG-73-01. Center for Wetland Resources, Louisiana State University, Baton Rouge.

Sorensen, J., Jorgensen, B. B. and Revsbeck, N. P. 1979. A comparison of oxygen, nitrate, and sulfate respiration in coastal marine sediments. Microbial Ecology, 5, 105-115.

Sparrow, E. B., C. V. Davenport, and R. C. Gordon. 1978. Response of microorganisms to hot crude oil spills on a subarctic taiga soil. Arctic 31:324-338.

Stanier, R. Y., Palleroni, N. J. and Doudoroff, M. 1966. The aerobic pseudomonads: a taxonomic study. Journal of General Microbiology 43, 159-271.

Stewart, J. E., and L. J. Marks. 1978. Distribution and abundance of hydrocarbon-utilizing bacteria in sediments of Chedabucto Bay, Nova Scotia, in 1976. J. Fish. Res. Board Can. 35:581-584.

Stirling, L. A., R. J. Watkinson, and I. J. Higgins. 1977. Microbial metabolism of alicyclic hydrocarbons: isolation and properties of a cylcohexane-degrading bacterium. J. Gen. Microbiol. 99:119-125.

Strickland, J. D. H. and Parsons, T. R. 1972. A practical handbook of seawater analysis, Bull. 167. Ottawa, Fisheries Research Board of Canada.

Tagger, S., L. Deveze, and J. LePetit. 1976. The conditions for biodegradation of petroleum hydrocarbons at sea. Mar. Pollut. Bull. 7:172-174.

Tagger, S., L. Deveze, and J. LePetit. 1979. Sur L'epuration biologique d'une zone littorale marine affectee par des rejets d'hydrocarbures. Environ. Pollut. 18:275-288.

Teal, J. M., K. Burns, and J. Farrington. 1978. Analyses of aromatic hydrocarbons in intertidal sediments resulting from two spills of No. 2 fuel oil in Buzzards Bay, Massachusetts. J. Fish Res. Board Can. 35:510-520.

Traxler, R. W. 1973. Bacterial degradation of petroleum materials in low temperature manine environments, p. 163-170. In D. G. Ahearn and S. P. Meyers ed., The microbial degradation of oil pollutants. Publication no. LSU-SG-73-01. Center for Wetland Resources, Louisiana State University. Baton Rouge.

Traxler, R. W., and J. M. Bernard. 1969. The utilization of n-alkanes by Pseudomonas aeruginosa under conditions of anaerobiosis. Int. Biodeterior. Bull. 5:21-25.

Trecani, V. 1964. Microbial degradation of hydrocarbons. Prog. Ind. Microbiol. 4:3-33.

Trudgill, P. W. 1978. Microbial degradation of alicyclic hydorcarbons, p. 47-84. In J. R. Watkinson ed., Developments in biodegradation of hydrocarbons-1. Applied Science Publishers, Ltd., London.

Van der Linden, A. C. 1978. Degradation of oil in the marine environment, p. 165-200. In J. R. Watkinson ed., Developments in biodegradation of hydrocarbons-1. Applied Science Publishers, Ltd., London.

Van der Linden, A. C., and G. J. E. Thijsse. 1965. The mechanisms of of microbial oxidations of petroleum hydrocarbons. Adv. Enzymol. 27:469-546.

Van Eyk, J., and T. J. Bartels. 1968. Paraffin oxidation in Pseudomonas aeruginosa. I. Induction of paraffin oxidation. J. Bacteriol. 96:706-712.

Vandermeulen, J. H., and R. W. Traxler. 1980. Hydrocarbon-utilizing microbial activity in marsh, mudflat and sandy sediments from north Brittany. In Proceedings of The International Symposium on the Amoco Cadiz: Fates and Effects of the Oil Spill. Centre Oceanologique de Bretagne, Brest, France, in press.

Verstraete, W., R. Vanlooke, R. deBorger, and A. Verlinde. 1975. Modelling of the breakdown and the mobilization of hydrocarbons in unsaturated soil layers, p. 98-112. In J. M. Sharpley and A. M. Kaplan ed., Proceedings of the Third International Biodegradation Symposium. Applied Science Publishers, Ltd., London.

Walczak, C. A. and Krichevsky, M. I. 1977. Interactive computer editing of phenetic data for numerical taxonomy. American Society for Microbiology Annual Meeting, abstract no. 138.

Walczak, C. A., Johnson, R. and Krichevsky, M. I. 1978. A flexible approach to phenotypic feature frequency calculations. American Society for Microbiology Annual Meeting, abstract no. 156.

Walczak, C.A. 1979. Complex data analysis. FDA By Lines 9: 251-253.

Walczak, C.A., and Krichevsky, M.I. 1980. Computer methods for describing groups from binary phenetic data: Modification of numeraicl taxonomy programs to increase flexibility. Int. J. Syst. Bacteriol. 30:622-626.

Walczak, C. A. 1982. Computer methods for describing groups from binary phenetic data: Calculation of group descriptors based on feature frequencies. Int. J. Syst. Bacteriol. In press.

Walker, J. D., H. F. Austin, and R. R. Colwell. 1975. Utilization of mixed hydrocarbon substrate by petroleum-degrading microorganisms. J. Gen. Appl. Microbiol. 21:27-39.

Walker, J. D., L. Cofone, Jr., and J. J. Cooney. 1973. Microbial petroleum degradation: the role of Cladosporium resinae, p. 821-825. In Proceedings of Joint Conference on Prevention and Control of Oil Spills. American Petroleum Institute, Washington, D. C.

Walker, J. D., and R. R. Colwell. 1974a. Microbial degradation of model petroleum at low temperatures. Microb. Ecol. 1:63-95.

Walker, J. D., and R. R. Colwell. 1974b. Microbial petroleum degradation: use of mixed hydrocarbon substrates. Appl. Microbiol. 27:1053-1060.

Walker, J. D., and R. R. Colwell. 1975. Some effects of petroleum on estuarine and marine microorganisms. Can. J. Microbiol. 21:305-313.

Walker, J. D., and R. R. Colwell. 1976a. Enumeration of petroleum-degrading microorganisms. Appl. Environ. Microbiol. 31:198-207.

Walker, J. D., and R. R. Colwell. 1976b. Long-chain n-alkanes occurring during microbial degradation of petroleum. Can. J. Microbiol. 22:886-891.

Walker, J. D., and R. R. Colwell. 1976c. Measuring the potential activity of hydrocarbon-degrading bacteria. Appl. Environ. Microbiol. 31:189-197.

Walker, J. D., and R. R. Colwell. 1976d. Petroleum: degradation by estuarine microorganisms, p. 197-204. In J. M. Sharpley and A. M. Kaplan ed., Proceedings of the Third International Biodegradation Symposium. Applied Science Publishers, Ltd., London.

Walker, J. D., R. R. Colwell, and L. Petrakis. 1975a. Bacterial degradation of motor oil. J. Water Pollut. Control Fed. 47:2058-2066.

Walker, J. D., R. R. Colwell, and L. Petrakis. 1975b. Degradation of petroleum by an alga, Prototheca zopfii. Appl. Microbiol. 30:79-81.

Walker, J. D., R. R. Colwell, and L. Petrakis. 1975c. Evaluation of petroleum-degrading potential of bacteria from water and sediment. Appl. Microbiol. 30:1036-1039.

Walker, J. D., R. R. Colwell, and L. Petrakis. 1975d. Microbial petroleum degradation: application of computerized mass spectrometry. Can. J. Microbiol. 21:1760-1767.

Walker, J. D., R. R. Colwell, and L. Petrakis. 1976a. Biodegradation of petroleum by Chesapeake Bay sediment bacteria. Can. J. Microbiol. 22:423-428.

Walker, J. D., R. R. Colwell, and L. Petrakis. 1976b. Biodegradation rates of components of petroleum. Can. J. Microbiol. 22:1209-1213.

Walker, J. D., R. R. Colwell, and L. Petrakis. 1976c. Comparison of the biodegradability of crude and fuel oils. Can. J. Microbiol. 22:598-602.

Ward, D., R. M. Atlas, P. D. Boehm, and J. A. Calder. 1980. Microbial biodegradation and the chemical evolution of Amoco Cadiz oil pollutants. Ambio 9:277-283.

Ward, D. M., and T. D. Brock. 1976. Environmental factors influencing the rate of hydrocarbon oxidation in temperate lakes. Appl. Environ. Microbiol. 31:764-772.

Ward, D. M., and T. D. Brock. 1978a. Anaerobic metabolism of hexadecane in marine sediments, Geomicrobiol. J. 1:1-9.

Ward, D. M., and T. D. Brock. 1978b. Hydrocarbon biodegradation in hypersaline environments. Appl. Environ. Microbiol. 35:353-359.

Ward, D., Atlas, R. M., Boehm, P. D. and Calder, J. A. 1980. Microbial biodegradation and the chemical evolution of Amoco Cadiz oil pollutants. Ambio, 9, 277-283.

Wayne, L. G., Krichevsky, E. J., Love, L. L., Johnson, R., and Krichevsky, M. I. 1980. Taxonomic probability matrix for use with slowly growing mycobacteria. Int. J. Syst. Bacteriol. 30:528:538.

Weeks, O. B. 1969. Problems concerning the relationships of cytophaga and flavobacteria. Journal of Applied Bacteriology 32, 13-18.

Westlake, D. W. S., A. M. Jobson, and F. D. Cook. 1978. In situ degradation of oil in a soil of the boreal region of the Northwest Territories. Can. J. Microbiol. 24:254-260.

Westlake, D. W. S., A. Jobson, R. Phillippe, and F. D. Cook. 1974. Biodegradability and crude oil composition. Can. J. Microbiol. 20:915-928.

Wiebe, W. J. and Hendricks, C. W. 1974. Distribution of heterotrophic bacteria in a transit of the Antarctic Ocean. In Effect of the Ocean Environment on Microbial Activties, pp. 524-535. Edited by R. R. Colwell and R. Y. Morita. Baltimore: University Park Press.

Williams, P. A. 1978. Microbial genetics relating to hydrocarbon degradation, p. 135-164. In J. R. Watkinson ed., Developments in bodegradation of hydrocarbons-1. Applied Science Publishers, Ltd., London.

Wodzinsky, R. S., and D. LaRocca. 1977. Bacterial growth kinetics on diphenylmethane and naphthalene-heptamethylnonane. Appl. Environ. Microbiol. 33:660-665.

Yamada, K., Y. Monoda, K. Komada, S. Nakatani, and T. Akasaki. 1968. Microbial conversion of petro-sulfur compounds. I. Isolation and identification of dibenzothiphene-utilizing bacteria. Agric. Biol. Chem. 32:840-845.

Yamagita, T. Ichikawa, T., Tsui, T., Kamata,Y., Ito, K., and Sasaki, M. 1978. Two trophic groups of bacteria, oligotrophs and eutrophs: their distributions in fresh and sea water areas in the central northern Japan. J. Gen. Appl. Microbiol. 24:59-88.

Zajic, J. E., B. Supplisson, and B. Volesky. 1974. Bacterial degradation and emulsification of No. 6 fuel oil. Environ. Sci. Technol. 8:664-668.

Zimmerman, R., R. Gibson, and J. Harrington. 1979. Herbivory and detritivory among gammaridean amphipods from a Florida seagrass community. Mar. Biol. 54: 41-47.

ZoBell, C. E. 1946. Action of microorganisms on hydrocarbons. Bacteriol. Rev. 10:1-49.

ZoBell, C. E. 1950. Assimilation of microorganisms on hydrocarbons. Adv. Enzymol. 10:443-486.

ZoBell, C. E. 1964. The occurrence, effects and fate of oil polluting the sea. Adv. Water Pollut. Res. 3:85-118.

ZoBell, C.E. 1946a. Marine Microbiology. Chronica Botanica Co., Watham, Mass.

ZoBell, C. E. 1969. Microbial modification of crude oil in the sea, p. 317-326. In Proceedings of Joint Conference on Prevention and Control of Oil Spills. American Petroleum Institute, Washington, D. C.

ZoBell, C. E. 1971. Sources and biodegradation of carcinogenic hydrocarbons, p. 441-451. In Proceedings of Joint Conference on Prevention and Control of Oil Spills. American Petroleum Institute, Washington, D.C.

ZoBell, C. E. 1973a. Bacterial degradation of mineral oils at low temperatures, p. 153-161. In. D. G. Ahearn and S. P. Meyers ed., The microbial degradation of oil pollutants. Publication no. LSU-SG-73-01, Center for Wetland Resources, Louisiana State University, Baton Rouge.

ZoBell, C. E. 1973b. Microbial degradation of oil: present status, problems and perspectives, p. 3-16. In D. G. Ahearn and S. P. Meyers ed., The microbial degradation of oil pollutants. Publication no. LSU-SG-73-01. Center fore Wetland Resources, Louisiana State University, Baton Rouge.

ZoBell, C. E., and J. F. Prokop. 1966. Microbial oxidation of mineral oils in Barataria Bay bottom deposits. Z. Allg. Mikrobiol. 6:143-162.

ZoBell, C. E. 1973. Bacterial degradation of mineral oils at low temperatures. In The microbial degradation of oil pollutants, Publ. No. LSU-SG-73-01, ed. by D. G. Ahearn and S. P. Meyers. Baton Rouge, LA, Center for Wetland Resources, Louisiana State University, 153-161.

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Collaborative projects
Samples were collected jointly with Robert Griffiths and Richard Morita of Oregon State University. They helped to develope sampling strategies and to interpret data; their staff memebers helped with the field collections.
Data was stored and analyzed at the Microbial Systematics Unit of the National Institutes of Health under the direction of Micah Krichevsky. Both he and his staff helped with analysis of the data.

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XX Conclusions

Bacterial communities in proposed Alaskan OCS regions are taxonomically and physiologically diverse. The bacterial populations are not dominated by pseudomonads as in some subpolar regions of the Pacific ocean; rather Vibrio and various other genera of gram negative bacteria are most common in Alaskan OCS regions. Many of the bacteria isolated in this study do not appear to be identical with previously described species. The bacterial population levels are typical of unpolluted pristine continental shelf regions.

The bacterial populations in these regions perform biochemical transformations that are important to the overall ecological functioning of the system. Seasonal changes in the composition of the bacterial community reflect changes in the flow of carbon through the food web, e.g., whether the system is phytoplankton driven or supported by a detrital food web. The denitrifying and nitrogen fixing activities of sediment bacteria regulate the availability of fixed forms of nitrogen, thereby controlling productivity.

The composition of the community changes in response to ecological stress. The introduction of petroleum hydrocarbons or other organic pollutants into these systems will alter the composition and functioning of the bacterial community. For example, the introduction of hydrocarbons into sediment alters the denitrification potential and thus the productivity capacity of the ecosystem. Of serious concern is the introduction of pathogenic bacteria in sewage associated with increased human activities resulting from oil development. This study showed that pathogenic bacteria associated with sewage can become associated with edible crabs; proper sewage treatment and preventing contamination of valuable marine food resources is a necessary part of Alaskan OCS oil and gas development.

Should petroleum hydrocarbons accidentally contaminate Alaskan OCS regions, there are indigenous populations of hydrocarbon degrading bacteria. These hydrocarbon degrading bacteria are present in low numbers, especially in water. The oil-degrading capacity of these bacterial populations is not sufficient to extensively degrade spilt oil before it impacts sediments and coastlines. The rate of petroleum hydrocarbon degradation in Alaskan OCS regions would be slow, and hydrocarbon pollutants resulting from OCS development would persist for long periods. Eventually, though, most hydrocarbon contaminants would be degraded by the hydrocarbon degrading bacteria. During the recovery period the numbers of hydrocarbon degraders would be elevated in the contaminated region, providing an indicator that can be used in ecological monitoring.

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