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Environmental Assessment of the Alaskan Continental Shelf

**Annual Reports of Principal Investigators
for the year ending March 1978**

Volume VI. Receptors — Microbiology



U.S. DEPARTMENT OF COMMERCE
National Oceanic and Atmospheric Administration



U.S. DEPARTMENT OF THE INTERIOR
Bureau of Land Management

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VOLUME II	RECEPTORS -- BIRDS
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Outer Continental Shelf Environmental Assessment Program
Boulder, Colorado

October 1978

U.S. DEPARTMENT OF COMMERCE
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RECEPTORS -- MICROBIOLOGY

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ANNUAL REPORT

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

RU #29

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I. Objectives - Summary

The main objectives of this study were to characterize microbial populations in Lower Cook Inlet, to determine microbial hydrocarbon biodegradation potentials in Lower Cook Inlet, to study degradation of petroleum under ice in the Beaufort Sea and to study degradation of petroleum in sediment in the Beaufort Sea. In situ experiments were begun and sampled in Elson Lagoon to determine the fate of oil trapped under ice or in sediment. A new Most Probable Number Procedure was developed for enumeration of hydrocarbon utilizing microorganisms. Hydrocarbon utilizers in Cook Inlet were found in higher numbers in Kachemak Bay, near Kennedy entrance and near Upper Cook Inlet than elsewhere in this area. Hydrocarbon biodegradation potentials in Cook Inlet were lower in November than in April. Hexadecane and naphthalene were utilized to a greater extent than pristane and benzanthracene, indicating the resistance of highly branched and condensed aromatic hydrocarbons to biodegradation. The numbers of viable heterotrophs enumerated in Cook Inlet were very low compared to Beaufort Sea and lower forty-eight coastal waters. The diversities of heterotrophic bacterial communities in Cook Inlet were high, indicative of a pristine area. The numerical taxonomic studies showed that exposure to oil enriched for hydrocarbon utilizers and that mixtures of hydrocarbons were more readily utilized than individual hydrocarbons. Our studies indicate that both simple aliphatic and aromatic compounds are degraded by microorganisms indigenous to areas of the Beaufort Sea, Gulf of Alaska and Cook Inlet.

II. Introduction - Scope of Work

This study is a continuation of an effort to characterize microbial populations and the ability of microorganisms to biodegrade petroleum hydrocarbons in proposed Alaskan OCS oil and gas lease areas. The approach has been to determine the distribution and population levels of several microbiological groups, *e.g.* hydrocarbon degraders within a geographic area, to extensively characterize selected microorganisms and using numerical taxonomy to determine the diversity of the microbial community and an inventory of the dominant microbial taxa within the geographic area. During this year microbial populations were characterized within Cook Inlet. We also have analysed further taxonomic data on microorganisms from the Beaufort Sea.

As part of this study intensive surveys have been conducted to determine the biodegradation potentials of indigenous microbial populations for petroleum hydrocarbons. During the past year hydrocarbon biodegradation potentials were estimated within Cook Inlet. Comparisons are contained in this report between hydrocarbon biodegradation potentials within Cook Inlet and previously studied Beaufort Sea and Gulf of Alaska regions. In addition to surveys to determine hydrocarbon biodegradation potentials, intensive studies have been initiated 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 and under ice.

III. Current State of Knowledge

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

IV. Study Area

The sampling sites used during this study are shown in Figure 1. Recent sampling has been restricted to Lower Cook Inlet (Figure 2) and Elson Lagoon in the Beaufort Sea region. Times of sample collection and mean temperatures and salinities of the samples are shown in Table 1.

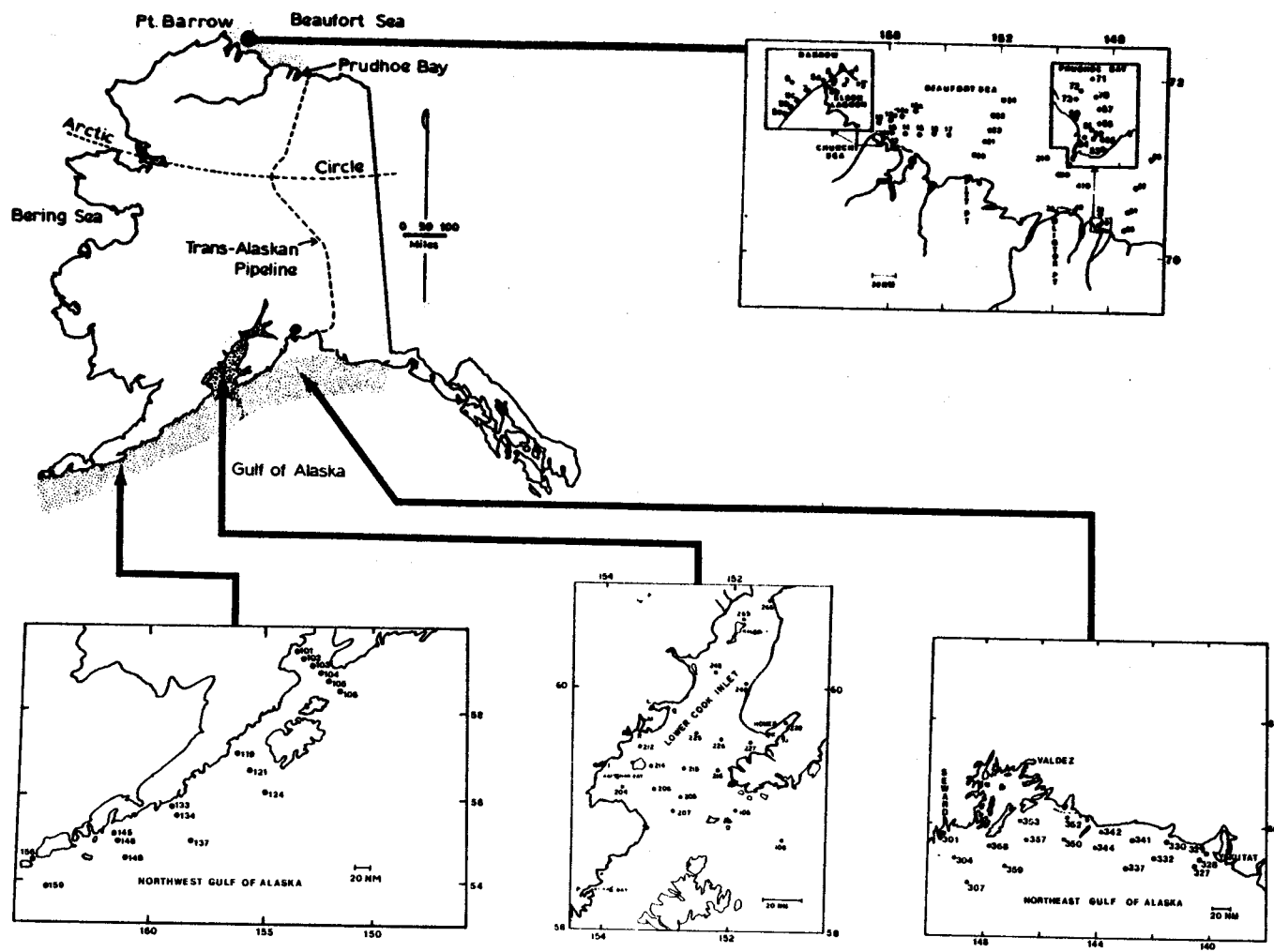
V. Methods and Rationale of Data Collection

Enumeration

The rationale of data collection for characterization of microbial populations was to sample surface water and sediment at a series of stations within a geographic area at 2 times of year. Microbial populations were enumerated from each sample using direct count and viable plate count procedures. These procedures have been described in previous reports.

Because of problems associated with viable plate counts for enumerating hydrocarbon utilizing microorganisms, a new Most Probable Number (MPN) method was developed and used in the recent Lower Cook Inlet

Figure 1. Sampling sites



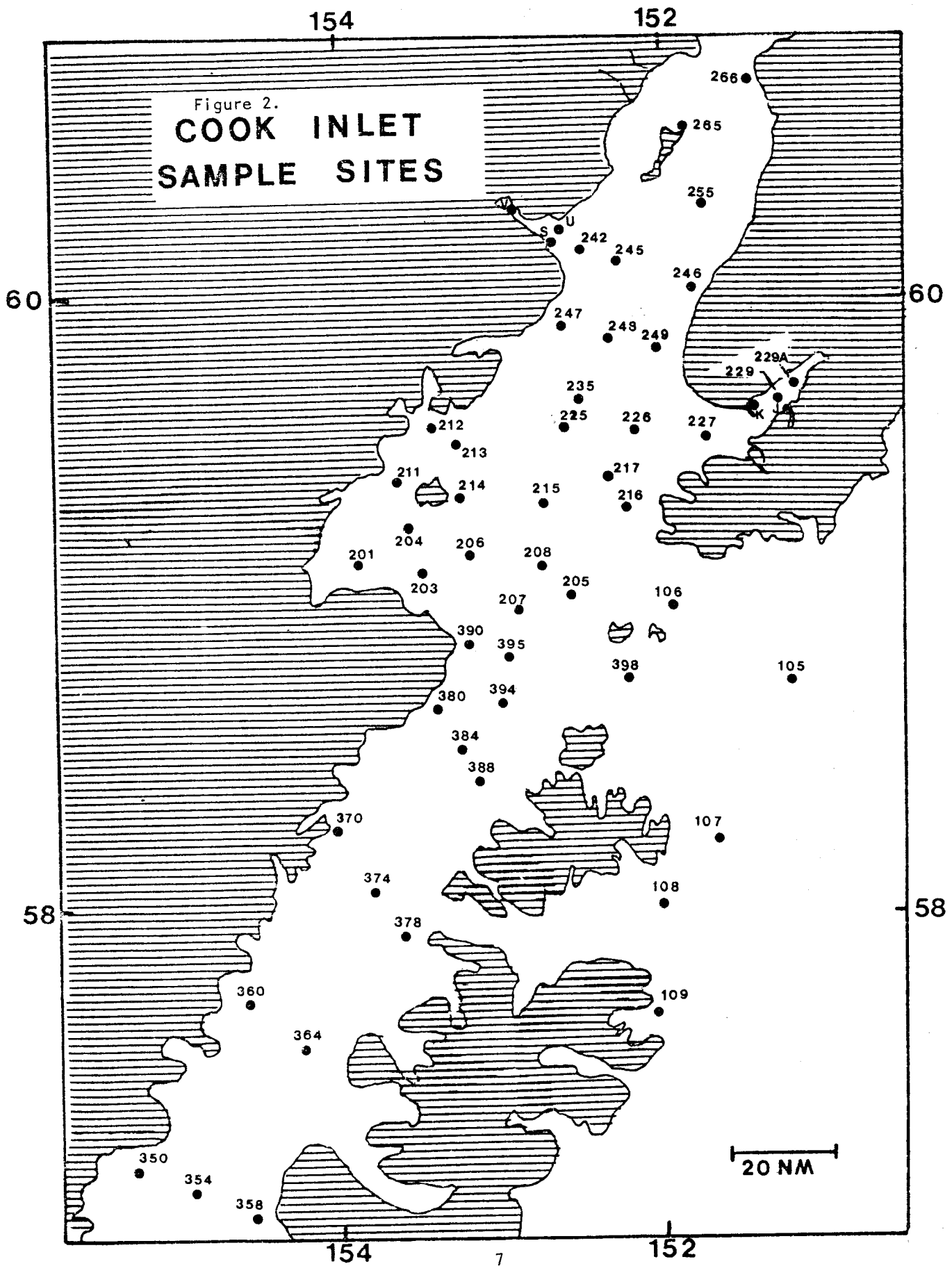


Table 1. Temperatures and salinities of samples

<u>Source</u>	Temperature (°C)		Salinity (o/oo)		
	<u>Mean</u>	<u>Range</u>	<u>Mean</u>	<u>Range</u>	
Beaufort Sea					
Aug.-Sept. 1975	water	+1.1	-1.2 - +3.2	17.4	11.1 - 27.0
Aug.-Sept. 1975	sediment	+1.0	-0.8 - +3.2	19.8	17.0 - 22.8
April 1976	ice	-2.0	-2.2 - -1.5	6.7	1.0 - 11.0
April 1976	water	-1.9	-2.0 - -1.5	24.3	17.0 - 31.0
April 1976	sediment	-1.8	-2.0 - -1.5	-	-
Aug. -Sept. 1976	water	+0.5	-1.3 - +2.7	16.5	5.1 - 29.5
Aug. -Sept. 1976	sediment	+0.1	-1.6 - +2.1	31.0	24.5 - 39.2
Northwest Gulf of Alaska					
Oct. 1975	water	+8.5	+7.3 - +9.4	31.0	29.5 - 32.0
Oct. 1975	sediment	+7.0	+4.7 - +9.3	32.1	30.8 - 32.5
Northeast Gulf of Alaska					
March 1976	water	+3.8	+2.0 - +5.0	31.7	30.7 - 32.3
March 1976	sediment	+4.7	+2.1 - +5.8	32.8	31.9 - 34.6
Cook Inlet					
Oct. 1976	water	+9.1	+6.0 - +12.0	24.2	17.0 - 27.5
Oct. 1976	sediment	+8.7	+6.0 - +10.0	26.5	23.2 - 28.0
April 1977	water	+3.8	+1.0 - +5.3	28.1	21.0 - 31.7
April 1977	sediment	+4.5	+2.3 - +7.5	31.5	30.2 - 32.6
Nov. 1977	water	+6.2	+2.2 - +7.8	30.5	23.0 - 32.2
Nov. 1977	sediment	+5.5	+2.0 - +7.3	31.2	29.0 - 33.4

studies. Most Probable Number estimates of hydrocarbon utiliziers were performed as follows: Dilutions of samples were added to 60 ml stoppered serum vials containing 10 ml autoclaved Bushnell Haas broth (Difco) with 3% added NaCl, and 50 μ l filter sterilized (0.2 μ m Millipore filter) Cook Inlet crude oil spiked with 1- 14 C n hexadecane (s.p. act. = 0.9 μ Ci/ml oil). Sterility of the oil was checked by plating portions of the oil onto marine agar 2216 (Difco) and observing for colony formation and by measuring 14 CO₂ production from uninoculated vials. Poisoned controls were prepared by adding 0.2 ml concentrated hydrochloric acid to the vials. A 3 tube MPN procedure was used. One set per sample was used. Following incubation at 5 C for 6 weeks, the solutions were acidified with 0.2 ml concentrated hydrochloric acid. After the solutions were allowed to cool, the 14 CO₂ was recovered by purging the vials with air and trapping the 14 CO₂ in 1 ml hyamine hydroxide in percolation tubes, 0.5 cm x 10 cm, containing glass beads. The hyamine hydroxide was washed from the tubes into scintillation vials with 3 one ml portions of methanol. The counting solution was 10 ml Omnifluor + toluene (New England Nuclear). Counting was with a Beckman liquid scintillation councer. Counts greater than or equal to 2 times control were considered as positive; counts less than 2 times control were considered as negative. The most probable number of hydrocarbon

degrading microorganisms was determined from the appropriate MPN Tables and recorded as most probable number per ml for water samples or most probable number per g dry wt. for sediment samples.

Taxonomic Characterization

Microorganisms selected at random from marine agar 2216 enumeration plates were considered as representative heterotrophic microorganisms. These organisms were extensively characterized. Approximately 300 phenotypic characteristics were determined for each strain. Characterization included morphological, physiological, biochemical and nutritional testing. A comprehensive list of tests was supplied in a previous annual report.

In addition to characterizing heterotrophic microorganisms, presumed hydrocarbon utilizing microorganisms were selected at random from oil-agar enumeration plates and MPN enumeration tubes. Approximately 100 phenotypic characteristics were determined for each strain of presumed hydrocarbon utilizing microorganism. These tests included extensive testing for the ability to utilize paraffinic and aromatic hydrocarbons. Table 2 shows a list of tests used for presumed hydrocarbon utilizers.

Following testing of heterotrophic and presumed hydrocarbon utilizing microorganisms, the data was analysed by cluster analysis.

Table 2.
FEATURES UTILIZED IN HYDROCARBON DATASETS

Gram positive.
Gram negative.
Cells are acid fast by Ziehl-Neelsen method.
Cells motile.
Cells are rod-shaped.
Cells are spherical.
Pleomorphic cells are characteristic.
Cells occur singly.
Cells occur in pairs.
Cells arranged in angular fashion after division (snapping).
Cells occur in chains.
Cells arranged in irregular aggregates.
Cells arranged in two-dimensional tetrads.
Organisms filamentous, greater than 10 micrometers, if multicellular the organism has little or no indentation at each point (For branched filaments also see Section 8).
Colonies are pure (paper) white on solid medium.
Colonies are gray on solid medium.
Diffusible (water-soluble) pigments are produced.
Non-diffusible red pigments are produced.
Non-diffusible brown pigments are produced.
Non-diffusible violet (purple) pigments are produced.
Non-diffusible golden (yellow) pigments are produced.
Non-diffusible orange pigments are produced.
Non-diffusible black pigments are produced.
Fluorescent pigment observable with short wavelength ultraviolet light (ca. 260 nm.).
Non-diffusible pink pigments are produced.
Agar macro-colonies are translucent.
Agar macro-colonies are transparent.
Agar macro-colonies are opaque.
Agar macro-colony margin is entire.
Colony surface is glistening.
Colony surface is dull (matte).
Sensitive to nitrofurantoin concentration (disc) 100 ug.
Sensitive to penicillin G concentration (disc) 2 units.
Sensitive to streptomycin concentration (disc) 2.0 ug.
Sensitive to novobiocin (albamycin) concentration (disc) 5 ug.
Sensitive to sulfisoxazole (gantrisin) concentration (disc) 5 ug.
Growth takes place at an initial pH of 4.0.
Growth takes place at an initial pH of 5.0.
Growth takes place at an initial pH of 6.0.
Growth takes place at an initial pH of 7.0.
Growth takes place at an initial pH of 8.0.
Growth takes place at an initial pH of 9.0.
Growth takes place at an initial pH of 10.0.
Growth at 5 C.
Growth at 10 C.
Growth at 15 C.
Growth at 20 C.
Growth at 25 C.
Growth at 37 C.
Growth at 43 C.
Added NaCl is required for growth.
Growth in the presence of 0.5% NaCl.
Growth in the presence of 3% NaCl.
Growth in the presence of 7.5% NaCl.

Table 2. con't

Growth in the presence of 10% NaCl.
Gelatin is hydrolyzed (liquefied).
Starch is hydrolyzed.
Tween 20 is hydrolyzed.
Tween 80 is hydrolyzed.
Hydrogen peroxide is decomposed.
Kovacs' oxidase test positive (smear from colony turns dark rple with tetramethylparaphenylenediamine dihydrochloride).
Methyl red test is positive.
Nitrate is reduced.
Nitrite is reduced.
D-Glucose catabolized aerobically.
D-Glucose catabolized anaerobically.
Acid produced from D-Fructose.
Acid produced from Lactose.
Acid produced from Maltose.
Acid produced from Sucrose.
Acid is produced from 1,2,3-Propanetriol (Glycerol).
Acid is produced from D-Mannitol.
Acid produced from D-Galactose.
D-Galactose is utilized.
D-Glucose is utilized (also see Section 24).
Lactose is utilized.
Sucrose is utilized.
Ethanol is utilized.
1,2,3-Propanetriol (Glycerol) is utilized.
D-Mannitol is utilized.
Cyclohexanol is utilized.
Meso-Inositol is utilized.
Phenol is utilized.
Acetic acid is utilized.
Palmitic acid is utilized.
Succinic acid is utilized.
10-Octadecynoic acid is utilized.
Oleic acid is utilized.
Citric acid is utilized.
Benzoic acid is utilized.
Cyclohexane carboxylic acid is utilized.
L-Asparagine is utilized.
L-Aspartic Acid is utilized.
L-Glutamic Acid is utilized.
L-Methionine is utilized.
L-Tryptophan is utilized.
L-Valine is utilized.
Ethanalamine can be used as the sole source of nitrogen.
Cyclohexanone can be used as the sole source of carbon.
N-Decane is utilized.
N-Hexadecane is utilized.
N-Nonane is utilized.
N-Octadecane is utilized.
N-Pentadecane is utilized.
1-Methylnaphthalene is utilized.
Omega-Phenyldecane is utilized.
Toluene is utilized.
Pristane (2,6,10,14-Tetra-methylpentadecane) is utilized.
Pentadecylcyclohexane is utilized.
2,2,4,4,6,8,8-Heptamethylnonane is utilized.
Ethylcyclohexane is utilized.
Dicyclohexyl is utilized.
Diphenylmethane is utilized.

Table 2. con't

Acenaphthalene is utilized.
9-Methylanthracene is utilized.
Naphthanol is utilized.
Prudhoe crude oil is utilized.
JP5 is utilized.
Gasoline (unleaded) is utilized.
Mineral oil is utilized.
API Reference Oil #1 IS utilized.
API Reference Oil #2 IS utilized.
API Reference Oil #3 IS utilized.
API Reference Oil #4 IS utilized.
Tolerant to mercury.
Tolerant to lead.
Urease (3.5.1.5) is produced.

Cluster analyses were performed using Jaccard coefficients and un-weighted average linkage sorting. Taxonomic groupings or clusters were recognized at greater than 70% similarity. The diversity of bacterial populations was estimated using the Shannon diversity index. The formula $\bar{H} = C/N (N \log_{10} N - \sum n_i \log_{10} n_i)$ was used where $C = 3.3219$, $N =$ total number of individuals and $n_i =$ total number of individuals in the i^{th} taxonomic grouping.

Hydrocarbon Biodegradation Activity

Natural Biodegradation Potential

Ten ml of water samples or 10 ml of a 1:100 dilution of sediment samples were added to 60 ml stoppered serum vials containing 10 ml autoclaved Rila marine salts solution and 50 μ l filter sterilized Cook Inlet crude oil spiked with ^{14}C radiolabelled hydrocarbon. Poisoned controls were prepared by adding 0.2 ml concentrated hydrochloric acid. The hydrocarbons used in these studies were: $1\text{-}^{14}\text{C}_{16}$ -hexadecane (Amersham Corp.), $1\text{-}^{14}\text{C}$ pristane (Cal Atomics), $1\text{-}^{14}\text{C}$ naphthalene (Amersham Corp.) and $1\text{-}^{14}\text{C}$ benzantracene (Amersham Corp.). The compounds were all 99+% purity analyzed hydrocarbons. The concentrations were adjusted to 0.9 $\mu\text{Ci } ^{14}\text{C}$ hydrocarbon/ml crude oil. After incubation at 5 C for 6 weeks, the $^{14}\text{CO}_2$ produced was recovered and counted as described above for the MPN procedure. Duplicate determinations were made for each. Counts from the controls were subtracted

from the non-poisoned counts and recorded as arbitrary units (CPM $^{14}\text{CO}_2$ produced) of hydrocarbon biodegradation potential. Since there were approximately 100,000 CPM in the spiked oil, every 1,000 units of $^{14}\text{CO}_2$ produced is equivalent to 1% conversion of hydrocarbon to CO_2 .

There was sufficient oxygen in the head space of the vials to support complete oxidation of the added hydrocarbon to CO_2 . We also have compared the $^{14}\text{CO}_2$ produced from vials flushed weekly with $^{14}\text{CO}_2$ produced during 6 weeks incubation and found no significant difference. We interpret this as indicating no oxygen limitation and no appreciable loss of $^{14}\text{CO}_2$ through the stoppers during our studies. The time course studies also showed that long incubation times were needed for assessing biodegradation potentials.

Non-nutrient Limited Biodegradation Potential

Non-nutrient limited hydrocarbon biodegradation potentials were determined in an identical manner to the natural hydrocarbon biodegradation potentials, except that 10 ml Bushnell Haas broth with 3% NaCl was added to each vial to remove inorganic nutrient limitations, replacing the 10 ml Rila marine salts solution.

In situ Biodegradation

Intensive *in situ* hydrocarbon biodegradation studies were initiated in the Beaufort Sea. A site was selected in Elson Lagoon. The site was

chosen because of its accessibility for diving operations, and because it is representative of the nearshore ecosystems likely to be the initial sites for oil development in the Beaufort Sea.

During January 1978, 7.5 cm diameter x 3 m length stainless steel cylinders were implanted into the ice. The ice depth at that time was approximately 1 meter. One ml Prudhoe Bay crude oil was injected into each cylinder by scuba divers. The oil appears to cover about 50% of the available ice surface. The cylinder does not cause the oil to pile up. Replicate cylinders were recovered after a few hours, and after 10 days' exposure to measure the initial weathering losses from the oil. Other cylinders were left in the ice and an attempt to recover them will be made in April to measure degradation of the oil exposed under ice for 4 months. The ice in the recovered cylinders was thawed and the oil recovered by repeated solvent extraction. The recovered oil will be analysed by gas liquid chromatography and mass spectrometry. The recovered oils have not yet been analysed and are being maintained at -20° C.

Oil was also exposed *in situ* in sediment. Two hundred ml Prudhoe Bay crude oil was placed in 0.25 m² Plexiglas trays. Freshly collected sediment was added to the trays to a depth of 5 cm. The trays were replaced *in situ*. Replicate trays were collected after 2 days' exposure. Additional trays will be collected in April after 4 months' exposure. An earlier *in situ* oil in sediment experiment had been

begun in May 1977, supported by the Office of Naval Research. Samples were collected for up to 8 months. This long term experiment was unfortunately disrupted when a NOAA-OCSEAP sampling vessel used the buoy marking the site of the experiment for navigational purposes. The propeller thrust overturned the trays, terminating the experiment.

Oil has been recovered from the sediment using sequential solvent extraction with diethyl ether, benzene and methylene chloride. The oil residues will be analysed by gas liquid chromatography and mass spectrometry.

VI. Results

Enumeration

A summary of all total direct count and viable heterotroph count data, accumulated for all areas that we have so far studied, is shown in Table 3. Enumerations of total and viable heterotrophic microorganisms at specific stations have been reported in previous quarterly reports. The data clearly shows that numbers of microorganisms in surface water are very low in Cook Inlet. Especially low are populations enumerated as viable heterotrophs.

The Most Probable Number enumeration method appears to be superior to previous methods for estimating numbers of hydrocarbon utilizing microorganisms. Figures 3 and 4 show the Most Probable Numbers of hydrocarbon utilizers enumerated in Cook Inlet during April 1977 and November 1977 respectively. The distribution pattern shows that hydrocarbon utilizers are in higher numbers in Kachemak Bay, near the forelands (Upper Cook Inlet) and at Kennedy entrance to the Inlet than elsewhere within the Inlet. The distribution within Cook Inlet is consistent with areas of reported occurrence of hydrocarbons in the sediment (R. Feely, Lower Cook Inlet Synthesis Meeting). The results are also consistent with a distribution pattern showing water flow up the eastern part of the Inlet, without extensive mixing with water from Kachemak Bay, and down the Inlet close to the western shore. We have no direct information on whether high concentrations of hydrocarbons

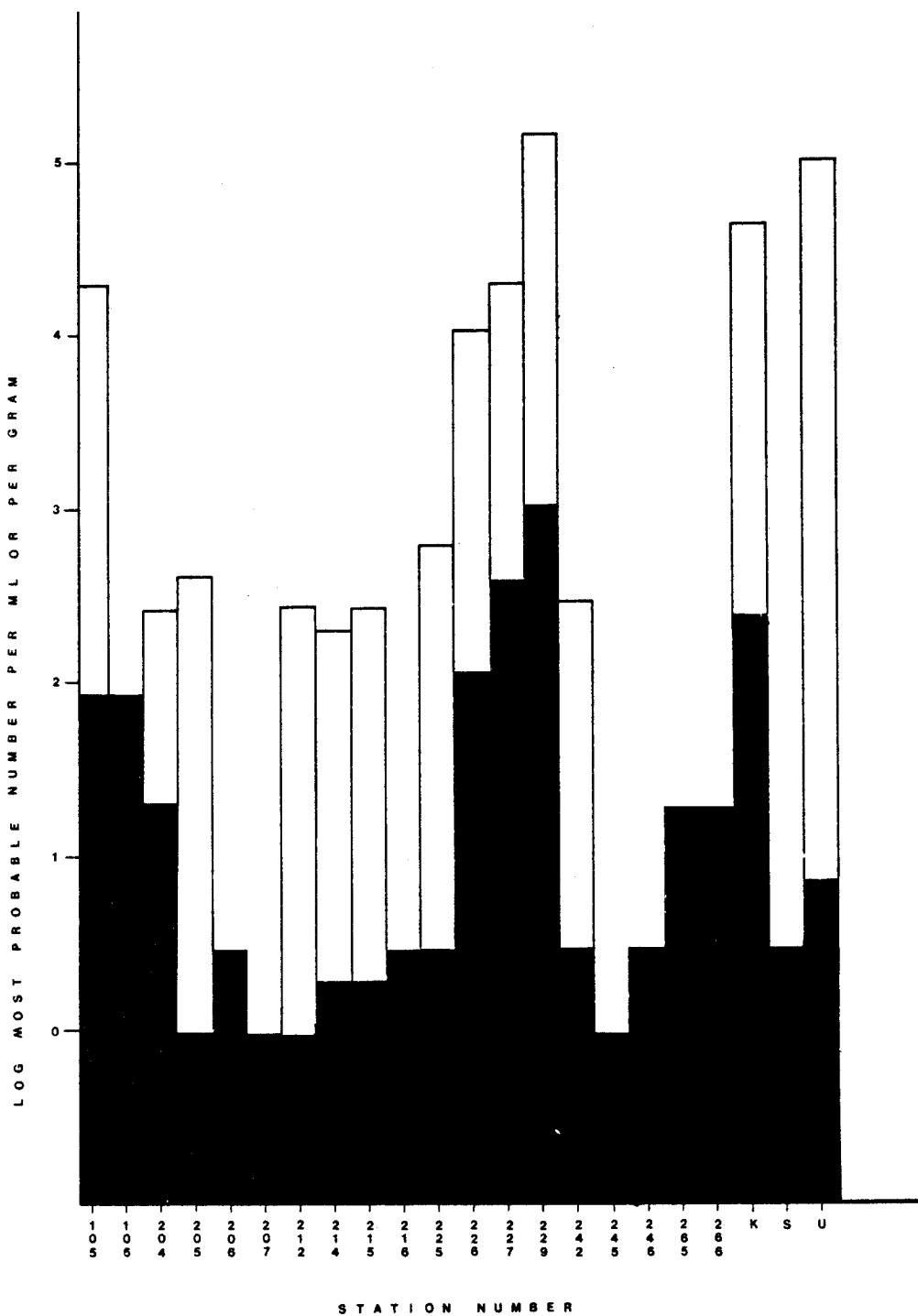
Table 3. Summary of enumeration data.

Sample	Number of Observations	Mean	Standard Deviation	Range
<u>Direct Count</u>				
Water (#/ml)				
N.W. Gulf of Alaska, October 1975	14	3.0×10^5	1.3×10^5	$1.0 \times 10^5 - 5.0 \times 10^5$
N.E. Gulf of Alaska, March 1976	23	1.4×10^5	8.7×10^4	$3.1 \times 10^4 - 3.3 \times 10^5$
Cook Inlet, October 1976	21	8.8×10^5	2.5×10^6	$6.6 \times 10^4 - 1.2 \times 10^7$
Cook Inlet, April 1977	23	2.5×10^4	8.8×10^3	$1.4 \times 10^4 - 5.6 \times 10^4$
Cook Inlet, November 1977	44	5.8×10^4	2.4×10^4	$2.5 \times 10^4 - 1.3 \times 10^5$
Beaufort Sea, August 1975	33	8.4×10^5	7.2×10^5	$2.1 \times 10^5 - 4.1 \times 10^6$
Beaufort Sea, April 1976	20	1.8×10^5	1.3×10^5	$6.2 \times 10^4 - 6.4 \times 10^5$
Beaufort Sea, August 1976	20	5.2×10^5	3.9×10^5	$2.6 \times 10^5 - 2.1 \times 10^6$
Sediment (#/g dry wt.)				
N.W. Gulf of Alaska, October 1975	0	-	-	-
N.E. Gulf of Alaska, March 1976	19	2.7×10^9	1.7×10^9	$1.1 \times 10^6 - 6.2 \times 10^9$
Cook Inlet, October 1976	12	1.6×10^9	1.9×10^9	$2.7 \times 10^8 - 7.1 \times 10^9$
Cook Inlet, April 1977	13	7.7×10^7	3.6×10^7	$4.3 \times 10^7 - 1.6 \times 10^8$
Cook Inlet, November 1977	19	5.9×10^8	2.1×10^8	$2.5 \times 10^8 - 1.1 \times 10^9$
Beaufort Sea, August 1975	23	6.2×10^7	1.1×10^8	$2.3 \times 10^6 - 4.8 \times 10^8$
Beaufort Sea, April 1976	15	2.7×10^9	4.2×10^9	$3.2 \times 10^7 - 1.4 \times 10^{10}$
Beaufort Sea, August 1976	13	2.1×10^9	1.6×10^9	$7.6 \times 10^8 - 6.9 \times 10^9$
<u>Marine Agar 40C</u>				
Water (#/ml)				
N.W. Gulf of Alaska, October 1976	14	1.0×10^2	6.9×10^1	$2.0 \times 10^1 - 2.5 \times 10^2$
N.E. Gulf of Alaska, March 1976	22	1.1×10^3	4.6×10^3	$6.0 \times 10^0 - 2.2 \times 10^4$
Cook Inlet, October 1976	21	8.0×10^3	2.4×10^4	$5.0 \times 10^0 - 1.1 \times 10^5$
Cook Inlet, April 1977	26	3.9×10^2	4.8×10^2	$2.8 \times 10^1 - 1.9 \times 10^3$
Cook Inlet, November 1977	35	5.2×10^2	9.9×10^2	$1.0 \times 10^1 - 5.1 \times 10^3$
Beaufort Sea, August 1975	43	7.3×10^3	7.3×10^3	$2.0 \times 10^1 - 2.8 \times 10^4$
Beaufort Sea, April 1976	20	2.7×10^3	7.6×10^3	$1.0 \times 10^1 - 3.2 \times 10^4$
Beaufort Sea, August 1976	20	4.4×10^6	1.9×10^7	$4.4 \times 10^2 - 8.8 \times 10^7$
Sediment (#/g dry wt.)				
N.W. Gulf of Alaska, October 1975	5	6.3×10^5	5.6×10^5	$1.0 \times 10^4 - 1.4 \times 10^6$
N.E. Gulf of Alaska, March 1976	20	9.9×10^5	2.1×10^6	$1.5 \times 10^3 - 9.0 \times 10^6$
Cook Inlet, October 1976	13	4.6×10^6	6.6×10^6	$1.0 \times 10^4 - 2.1 \times 10^7$
Cook Inlet, April 1977	15	1.1×10^7	2.7×10^7	$5.1 \times 10^4 - 1.1 \times 10^8$
Cook Inlet, November 1977	17	1.4×10^4	2.0×10^7	$9.1 \times 10^4 - 6.7 \times 10^7$

Table 3. Continued.

Sample		Number of Observations	Mean	Standard Deviation	Range
<u>Marine Agar 4°C</u>					
Sediment (#/g dry wt.)					
Beaufort Sea,	August 1975	33	3.1×10^5	4.0×10^5	$2.9 \times 10^3 - 1.5 \times 10^6$
Beaufort Sea,	April 1976	15	6.9×10^4	9.6×10^4	$2.4 \times 10^2 - 3.7 \times 10^5$
Beaufort Sea,	August 1976	14	1.1×10^7	2.8×10^7	$1.5 \times 10^5 - 1.1 \times 10^8$
<u>Marine Agar 20°C</u>					
Water (#/ml)					
N.W. Gulf of Alaska,	October 1975	7	5.3×10^2	6.4×10^2	$4.3 \times 10^1 - 2.0 \times 10^3$
N.E. Gulf of Alaska,	March 1976	22	1.1×10^4	4.2×10^3	$6.7 \times 10^0 - 2.0 \times 10^4$
Cook Inlet,	October 1976	21	1.0×10^4	2.4×10^4	$3.3 \times 10^1 - 9.7 \times 10^4$
Cook Inlet,	April 1977	12	1.6×10^3	1.7×10^3	$2.3 \times 10^1 - 5.1 \times 10^3$
Cook inlet,	November 1977	36	2.5×10^2	4.2×10^2	$3.0 \times 10^0 - 2.2 \times 10^3$
Beaufort Sea,	August 1975	43	9.9×10^3	9.0×10^3	$7.0 \times 10^1 - 2.9 \times 10^4$
Beaufort Sea,	April 1976	20	1.8×10^5	1.3×10^5	$6.2 \times 10^4 - 6.4 \times 10^4$
Beaufort Sea,	August 1976	20	5.0×10^4	4.8×10^4	$1.9 \times 10^3 - 1.8 \times 10^5$
Sediment (#/g dry wt.)					
N.W. Gulf of Alaska,	October 1975	3	8.0×10^5	3.5×10^5	$3.5 \times 10^5 - 1.2 \times 10^6$
N.E. Gulf of Alaska,	March 1976	20	2.9×10^6	7.7×10^6	$3.3 \times 10^3 - 3.2 \times 10^7$
Cook Inlet,	October 1976	13	7.8×10^6	1.0×10^7	$4.0 \times 10^3 - 3.2 \times 10^7$
Cook Inlet,	April 1977	6	3.2×10^7	4.5×10^7	$2.2 \times 10^5 - 1.3 \times 10^8$
Cook Inlet,	November 1977	36	2.6×10^2	4.2×10^2	$3.0 \times 10^0 - 2.2 \times 10^3$
Beaufort Sea,	August 1975	32	2.1×10^5	3.2×10^5	$6.5 \times 10^3 - 1.8 \times 10^6$
Beaufort Sea,	April 1976	15	2.5×10^5	4.7×10^5	$1.8 \times 10^3 - 1.9 \times 10^6$
Beaufort Sea,	August 1976	13	8.6×10^6	1.2×10^7	$3.5 \times 10^4 - 4.5 \times 10^7$

Figure 3. Most Probable Numbers of hydrocarbon utilizers in Cook Inlet - April 1977 samples.
 Solid bars= water Open bars=sediment



occur near the Kennedy entrance to the Inlet where high MPN estimates of hydrocarbon utilizers were found. It appears that the distribution of hydrocarbon utilizing microorganisms in Cook Inlet can be used as an indicator of areas of occurrence of hydrocarbons. We therefore predict that a source of hydrocarbons in the environment will be found near the Kennedy entrance to Cook Inlet.

Taxonomic Characterization

The results of cluster and feature frequency analyses on viable heterotrophic bacterial populations in the Beaufort Sea that were sampled during summer 1975 have been condensed to show the major characteristics of the dominant phenotypic clusters (Table 4). We have concluded that the bacterial populations in the Beaufort Sea are taxonomically distinct from bacterial populations in temperate marine ecosystems. Flavobacterium, Vibrio and Microcycclus have been tentatively identified as the dominant genera in Beaufort Sea water and sediment samples.

Cluster analyses have been performed for viable heterotrophic microbial populations in Cook Inlet isolated from November 1976 and April 1977. The results of the cluster analyses are shown as dendrograms (Figs. 5 and 6). Typically, clusters contained few organisms.

The Shannon diversity indices were calculated for the bacterial populations at each sampling station (Table 5). Diversity of populations

Table 4. Prominent features of major clusters of bacteria from the Beaufort Sea

Cluster L-1: 21 strains- Estimated 7.5% of population

Morphological characteristics

Gram negative, non-motile, highly pleomorphic rods. Some cells were horseshoe shaped, length 3-4 microns. Other cells were very large, often 10 microns length. Colonies small, non-pigmented, glistening, translucent, convex, with entire edge.

Physiological characteristics

Growth range 5-25°C, pH 6-8, 0.5-5% NaCl. Optimal pH 7-8, optimal NaCl concentration 3%.

Biochemical Characteristics

Generally no acid from carbohydrates. Starch, gelatin, Tween 20 and Tween 80 not hydrolyzed. Alkaline phosphatase positive. Catalase positive.

Antibiotic sensitivity

Sensitive to 11 of 12 antibiotics.

Nutritional characteristics

Require vitamins as growth factors. Utilize glucose, acetate, propionate, caprylate, fumarate, beta-hydroxybutyrate, citrate, alpha-ketoglutarate, pyruvate, glutarate, glycerol, p-hydroxybenzoate, alanine, serine, threonine, glutamate, gamma-aminobutyrate.

Distribution

Wide geographic distribution in both water and sediment.

Identification

Unidentified.

Cluster L-2: 9 strains- Estimated 3.2% of population

Morphological characteristics

Gram negative, pleomorphic, non-motile rods occurring singly, or in chains. Length varies but typically about 2 microns. Colonies small, non-pigmented, glistening, smooth, convex, with entire edge.

Physiological characteristics

Growth range 5-25°C, pH 6-9, 0.5-5% NaCl.

Biochemical characteristics

No acid produced from carbohydrates. No hydrolysis of polymers. Alkaline phosphatase positive, catalase weakly positive, oxidase negative.

Antibiotic sensitivity

Sensitive to 9 of 12 antibiotics.

Nutritional characteristics

Require vitamins as growth factors. Utilize xylose, glucose, cellobiose, acetate, caprylate, malonate, beta-hydroxybutyrate, alpha-ketoglutarate, pyruvate, glutarate, glycerol, p-hydroxybutyrate, glutamate, aminobutyrate.

Table 43. (continued)

Cluster L-2: 9 strains

Distribution

Water samples from stations 55 and 70.

Identification

Unidentified.

Cluster L-4: 60 strains- Estimated 21.6% of population

Morphological characteristics

Gram negative non-motile highly pleomorphic rods, 1.2-1.5 microns length, some elongated, greater than 20 microns. Cells occur singly and in chains. Round body (or spherical body) formation very common, especially in old cultures. Colony orange pigmented, translucent, glistening, smooth, convex.

Physiological characteristics

Growth range 5-20°C, pH 5-9, 0.5-5% NaCl.

Biochemical characteristics

Some produced acid from sucrose. Gelatin hydrolyzed by some. No amylase nor lipase produced. Alkaline phosphatase positive, catalase positive, oxidase negative, typically arginine decarboxylase positive. No nitrate reduction.

Antibiotic sensitivity

Sensitive to 6 of 12 antibiotics.

Nutritional characteristics

Require vitamins as growth factors. Utilize galactose, glucose, mannose, maltose, glutamate.

Distribution

Found at all stations. Incidence much higher in water than sediments.

Identification

Tentatively identified as Flavobacterium spp.

Cluster L-5: 4 strains- Estimated 1.4% of population

Morphological characteristics

Gram negative, non-motile, horseshoe shaped, curved rods, ca 3-4 microns length. Round bodies common. Cells occur singly. Colonies small (0.1 mm in a diameter), yellow pigmented, translucent, convex, entire, glistening, smooth.

Physiological characteristics

Growth range: 5-15°C, pH 6-9, 3-5% NaCl.

Biochemical characteristics

Acids not produced from carbohydrates. No hydrolysis of polymers. Alkaline phosphatase positive, catalase weakly positive, oxidase negative.

Antibiotic sensitivity

Sensitive to 6 of 12 antibiotics.

Nutritional characteristics

Fastidious. Require complex growth factors. Substrates not utilized without added amino acids and vitamins.

Table 4 . (continued)

Cluster L-5: 4 strains

Distribution

Mainly sediment at Station 2.

Identification

Tentatively identified as Microcycclus spp.

28 strains

Morphological characteristics

Morphologically similar to Cluster L-4, but smaller rods, length 0.8 micron. Orange pigments produced. Non-motile.

Physiological characteristics

Growth range 5-20°C, pH 6-8, 0.5-3% NaCl.

Biochemical characteristics

Some produced acid from D-glucose aerobically. No hydrolysis of polymers. Catalase positive, oxidase negative.

Antibiotic sensitivity

Sensitive to 8 of 13 antibiotics.

Nutritional characteristics

Require complex growth factors. Substrates not utilized without added amino acids and vitamins.

Distribution

Widely distributed. High incidence in water at Prudhoe Bay.

Identification

Tentatively identified as Flavobacterium spp.

Cluster L-7: 7 strains- Estimated 2.5% of population

Morphological characteristics

Gram negative, non-motile, straight or curved rods, two types were seen, the first type was broader, size ca 2-2.5 micron long and 1.0 micron wide, the second type was more slender, 0.6-0.8 micron wide and 2.5-3.0 micron long. Irregular pleomorphic shapes often developed. Cells occur singly. Colonies white-gray, opaque, entire, glistening, smooth and convex.

Physiological characteristics

Growth range 5-10°C, pH 5-9, 3-5% NaCl.

Biochemical characteristics

Acid from D-glucose anaerobically. Starch, gelatin, Tween 20 and Tween 80 hydrolyzed, alkaline phosphatase positive, NO₃ to NO₂

positive, oxidase variable, catalase variable.

Antibiotic sensitivity

Sensitive to 8 of 12 antibiotics.

Nutritional characteristics

Require amino acids as growth factors.

Distribution

Sediment from Sts. 10 and 71.

Identification

Tentatively identified Vibrio spp.

Table 4. (continued)

Cluster L-9: 4 strains - Estimated 1.4% of population

Morphological characteristics

Gram negative, typically large 4-5 micron length, straight rods. Cells occur singly and in chains. Motility positive in three of four strains. Colonies non-pigmented, translucent, convex, entire glistening and smooth.

Physiological characteristics

Growth range 5-15°C, pH 5-9, 3-5% NaCl.

Biochemical characteristics

Acid from D-glucose, both aerobically and anaerobically. Gelatin, Tween 20 and Tween 80 hydrolyzed, alkaline phosphatase positive. Catalase positive. Oxidase negative. Ammonia produced from peptones NO₃ reduced to NO₂.

Antibiotic sensitivity

Sensitive to 5 of 12 antibiotics.

Nutritional characteristics

Require vitamins for growth. Only pyruvate utilized on media B. Fructose, glucose, succinate, fumarate, lactate utilized on media E.

Distribution

Only found in Station 10 sediment.

Identification

Tentatively identified as Vibrio or Beneckeia spp.

Cluster L-10: 10 strains - Estimated 3.6% of population

Morphological characteristics

Gram negative, non-motile rods, 1-2.5 micron length, often slightly curved. Generally capsulated. Colonies small non-pigmented translucent, convex, entire, glistening and smooth.

Physiological characteristics

Growth range 5-25°C, pH 5-9, 0-5% NaCl.

Biochemical characteristics

No acid from carbohydrates. Starch and gelatin not hydrolyzed. Tween 20 hydrolyzed, Catalase positive. NO₃ reduced to NO₂.

Antibiotic sensitivity

Sensitive to 10 of 12 antibiotics.

Nutritional characteristics

Do not require growth factors. Utilize caprylate, pyruvate, alanine, aspartate, gamma-aminobutyrate. Carbohydrates not utilized.

Distribution

Found in sediment, at several stations.

Identification

Unidentified.

Table 4. (continued)

Cluster L-11: 6 strains - Estimated 2.1% of population

Morphological characteristics

Gram negative motile rods, curved axis, 2-3 micron length. Cells occur singly. Colonies white translucent, convex glistening, smooth and entire.

Physiological characteristics

Growth range 5-15°C, pH 5-10, 3-5% NaCl.

Biochemical characteristics

Acid from D-fructose and D-glucose aerobically and anaerobically. No hydrolysis of starch, gelatin, Tween 20 and Tween 80. Alkaline phosphatase positive. Catalase positive. Oxidase negative. Ammonia produced from peptone. NO₃ reduced to NO₂. Arginine decarboxylase positive.

Antibiotic sensitivity

Sensitive to 5 of 12 antibiotics.

Nutritional characteristics

Do not require growth factors. Utilized ribose, fructose, glucose, maltose, fumarate, citrate, alpha-ketoglutarate, pyruvate, glutarate, glycerol, L-arginine, N-acetylglucosamine.

Distribution

Sediment at stations 10 and 71.

Identification

Tentatively identified as Vibrio spp.

Cluster H-2: 13 strains.- Estimated 4.7% of population

Morphological characteristics

Gram negative, motile, straight and curved, rods, 2-4 micron length. Cells occur singly. Colonies gray, opaque, convex, entire, glistening and smooth.

Physiological characteristics

Growth range 5-15°C, pH 5-10, 3-5% NaCl.

Biochemical characteristics

Acids from fructose and glucose both aerobically and anaerobically. Catalase positive. Oxidase negative. NO₃ reduced to NO₂.

Antibiotic sensitivity

Sensitive to 5 of 12 antibiotics.

Nutritional characteristics

Require vitamins as growth factors. Utilized ribose, fructose, galactose, glucose, mannose, salicin, maltose, fumarate, glycerate, citrate, glutarate, glycerol, aspartate and N-acetylglucosamine.

Distribution

Sediment at stations 2 and 10.

Identification

Tentatively identified as Vibrio spp.

Table 4. (continued)

Cluster H-3: 7 strains - Estimated 2.5% of population

Morphological characteristics

Gram negative non-motile rods, 0.6-1.0 microns length. Cells occur singly. Colonies yellow, opaque, convex, entire, glistening, smooth.

Physiological characteristics

Growth range 5-20°C, pH 6-8, 3.0% NaCl.

Biochemical characteristics

Acid not produced. Starch hydrolyzed. Gelatin and Tween not hydrolyzed. Catalase positive, alkaline phosphatase negative, oxidase negative, NO₃ reduced to NO₂.

Antibiotic sensitivity

Sensitive to 8 of 11 antibiotics.

Nutritional characteristics

No substrates utilized on defined media. Require unknown growth factors.

Distribution

Sediment from stations 2 and 10.

Identification

Tentatively identified as Flavobacterium spp.

Cluster H-4: 36 strains Estimated 12.9% of population

Morphological characteristics

Gram negative rods, straight or curved, often comma-shaped. Highly pleomorphic with very large cells as well as round bodies often seen. Typical cells 2.5-3.5 microns long x 0.8-1.0 microns wide. Cells occur singly. Colonies orange, translucent or transparent, convex, entire, glistening and smooth.

Physiological characteristics

Growth range 5-25°C, pH 5-8, 0.5-3% NaCl.

Biochemical characteristics

Some produced acid from glucose and sucrose both aerobically and anaerobically. Starch and Tween 80 hydrolyzed. Most hydrolyzed gelatin. Alkaline phosphatase positive, most catalase positive. Ammonia produced from peptone.

Antibiotic sensitivity

Sensitive to 9 of 11 antibiotics.

Nutritional characteristics

Require amino acids or more complex growth factors.

Distribution

Distributed at all stations except station 10.

Identification

Tentatively identified as Flavobacterium spp.

Table 4. (continued)

Cluster H-5: 9 strains - Estimated 3.2% of population

Morphological characteristics

Gram negative, non-motile rods, ca 0.8-1.2 micron length. Colonies small, 0.5-0.8 mm in diameter, yellow, translucent, convex, entire glistening and smooth.

Physiological characteristics

Growth range 5-25°C, pH 5-8, 0-10% NaCl.

Biochemical characteristics

No acid from carbohydrates. Lipase positive. Alkaline phosphatase positive. Catalase negative. Oxidase negative. Ammonia produced from peptones.

Antibiotic sensitivity

Sensitive to 7 of 14 antibiotics.

Nutritional characteristics

Do not require growth factors. Utilized glucose, propionate, butyrate, valerate, isovalerate, caprylate, fumarate, beta-hydroxybutyrate, itaconate, 1-butanol, isoleucine.

Distribution

Stations 10, 30, 55 and 71.

Identification

Tentatively identified as Flavobacterium spp.

Cluster H-6: 7 strains - Estimated 7.5% of population

Morphological characteristics

Gram negative, non-motile, curved rods, 2-5 micron length. Cells occur singly. Some cells horseshoe shaped, some form rings. Colonies gray, entire, convex, glistening and smooth.

Physiological characteristics

Growth range 5-25°C, pH 5-9, 0-3% NaCl.

Biochemical characteristics

No acid from carbohydrates. Tween 20 hydrolyzed. No hydrolysis of starch, gelatin or Tween 80. Alkaline phosphatase positive, catalase positive. Ammonia produced from peptones. NO₃ reduced to NO₂.

Antibiotic sensitivity

Sensitive to 10 of 11 antibiotics.

Nutritional characteristics

Require vitamins as growth factors. Utilize fumarate, pyruvate, alanine, aspartate, glutarate, gamma-aminobutyrate.

Distribution

Sediment samples collected from Stations 30, 70 and 71.

Identification

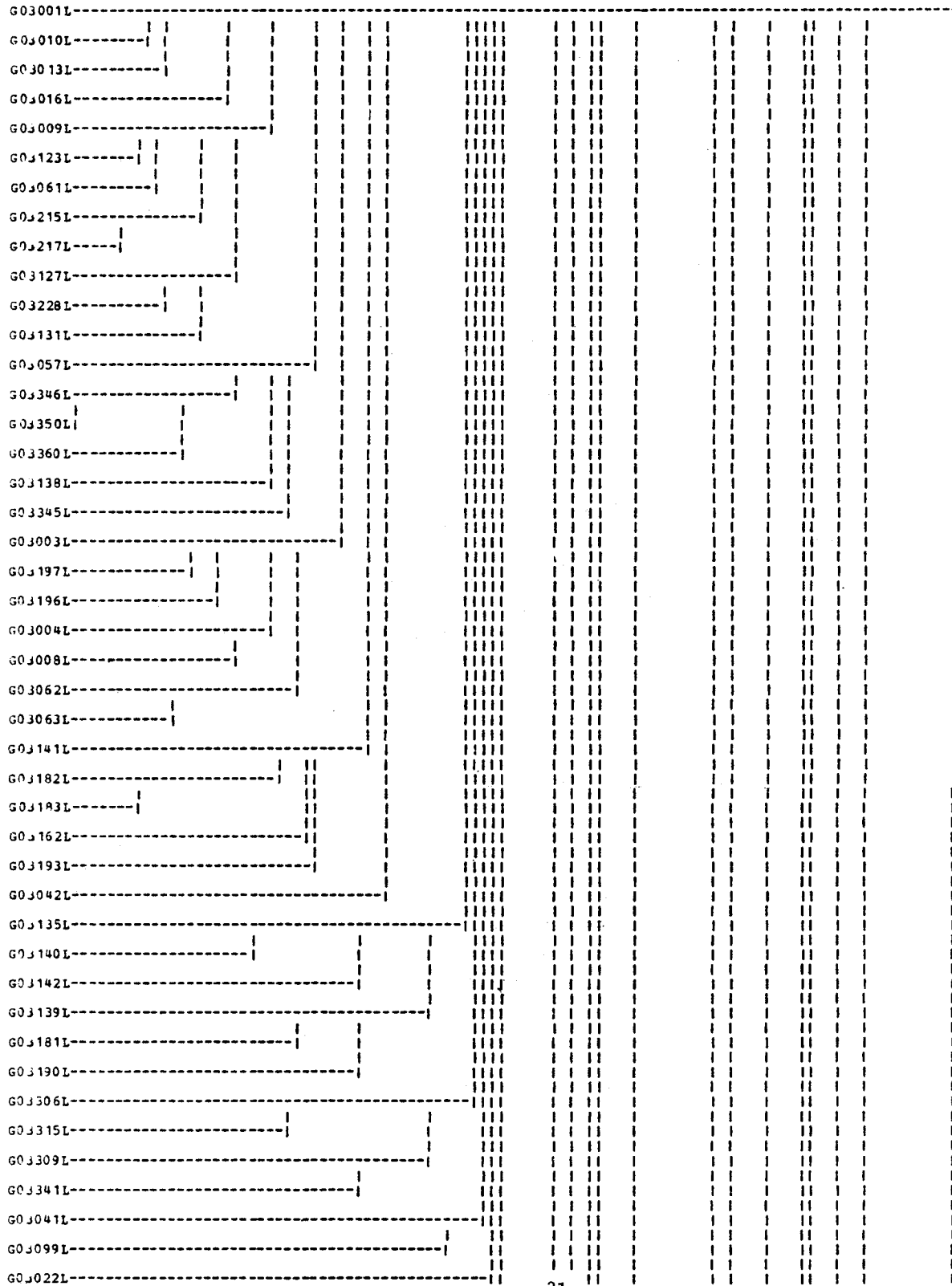
Tentatively identified as Microcycclus spp.

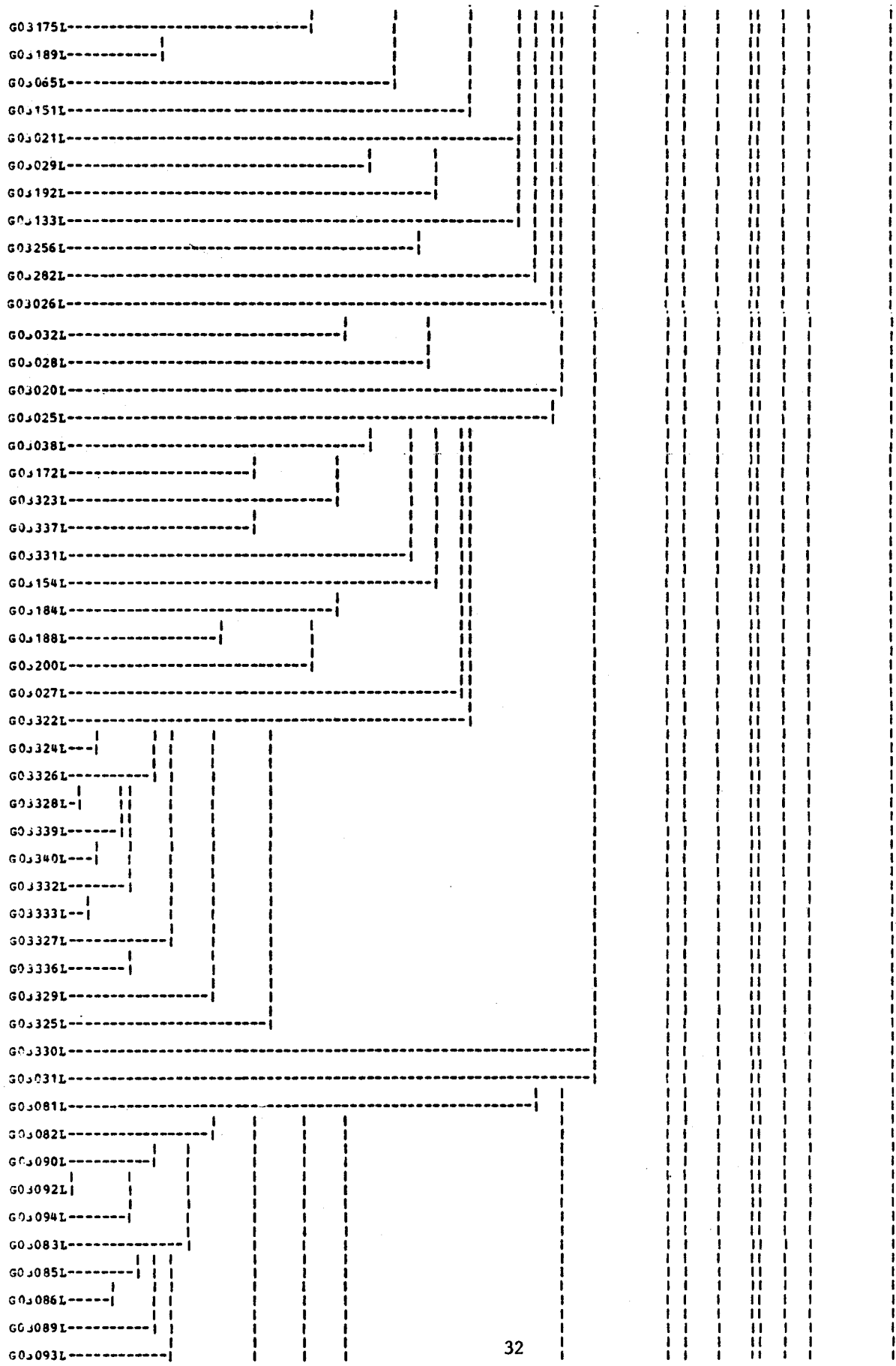
Figure 5. Dendrogram of Cook Inlet isolates from November 1976.

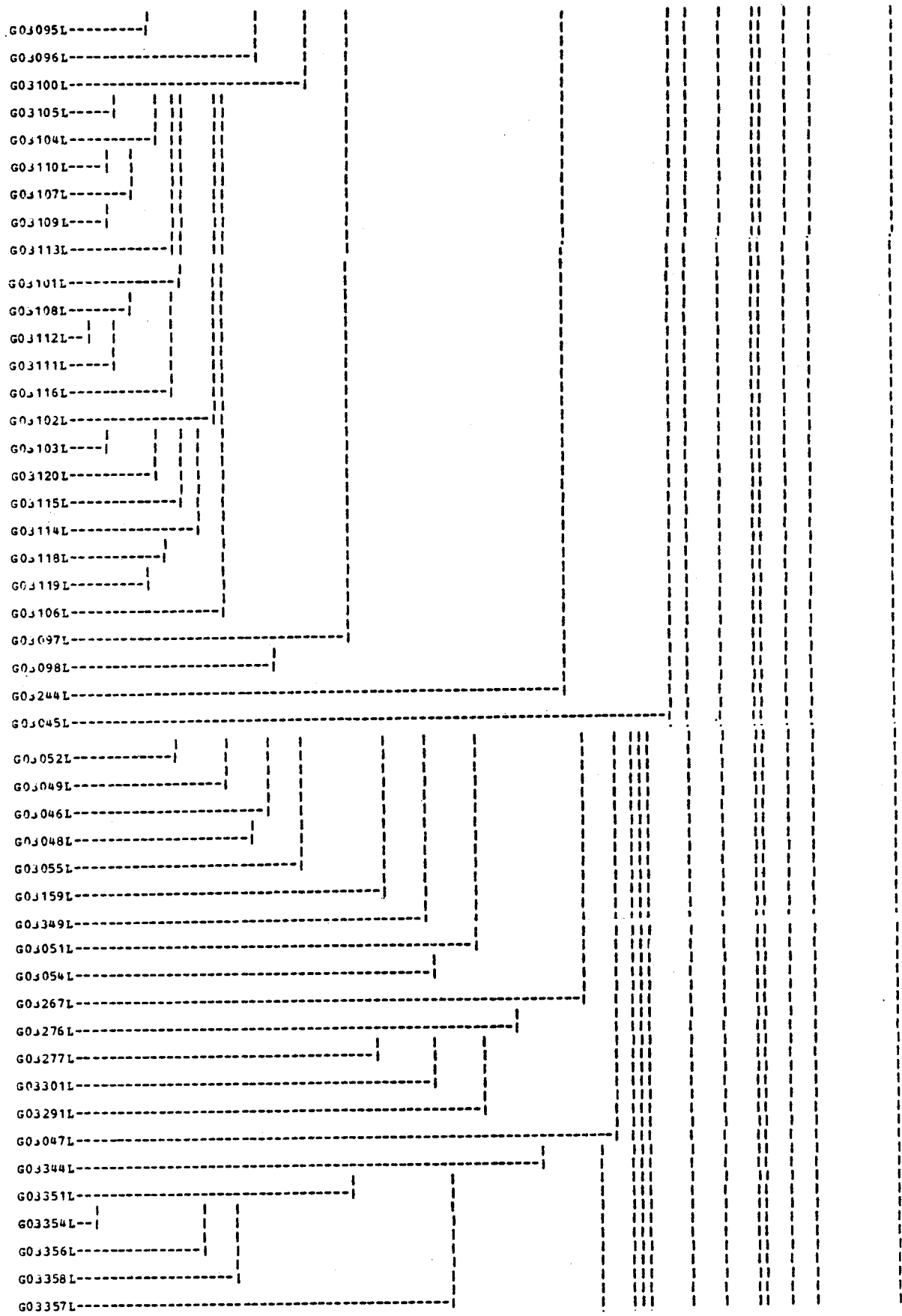
COEFFICIENT: S (J)

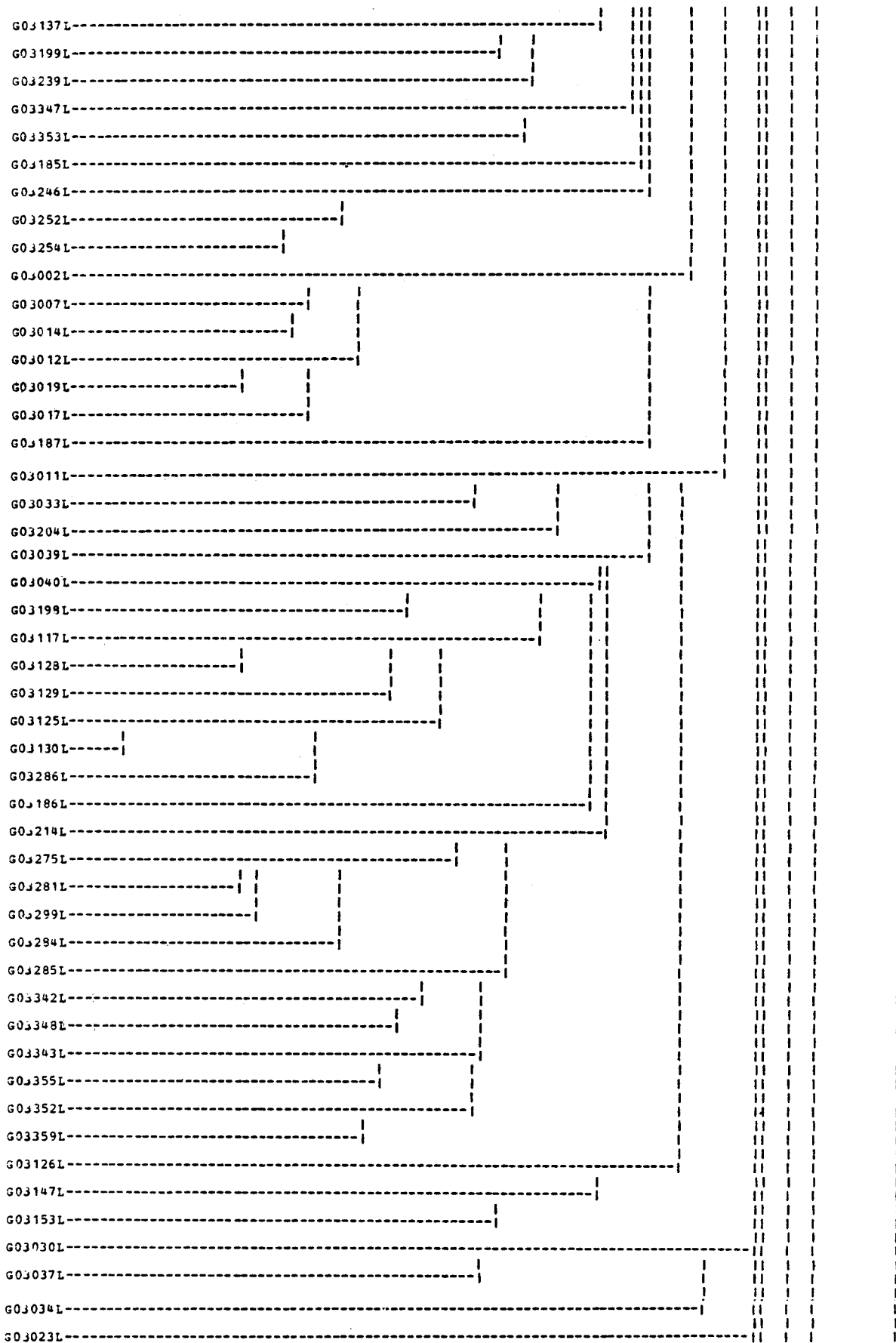
SIMILARITY AT TIP (LEFT) = .9545

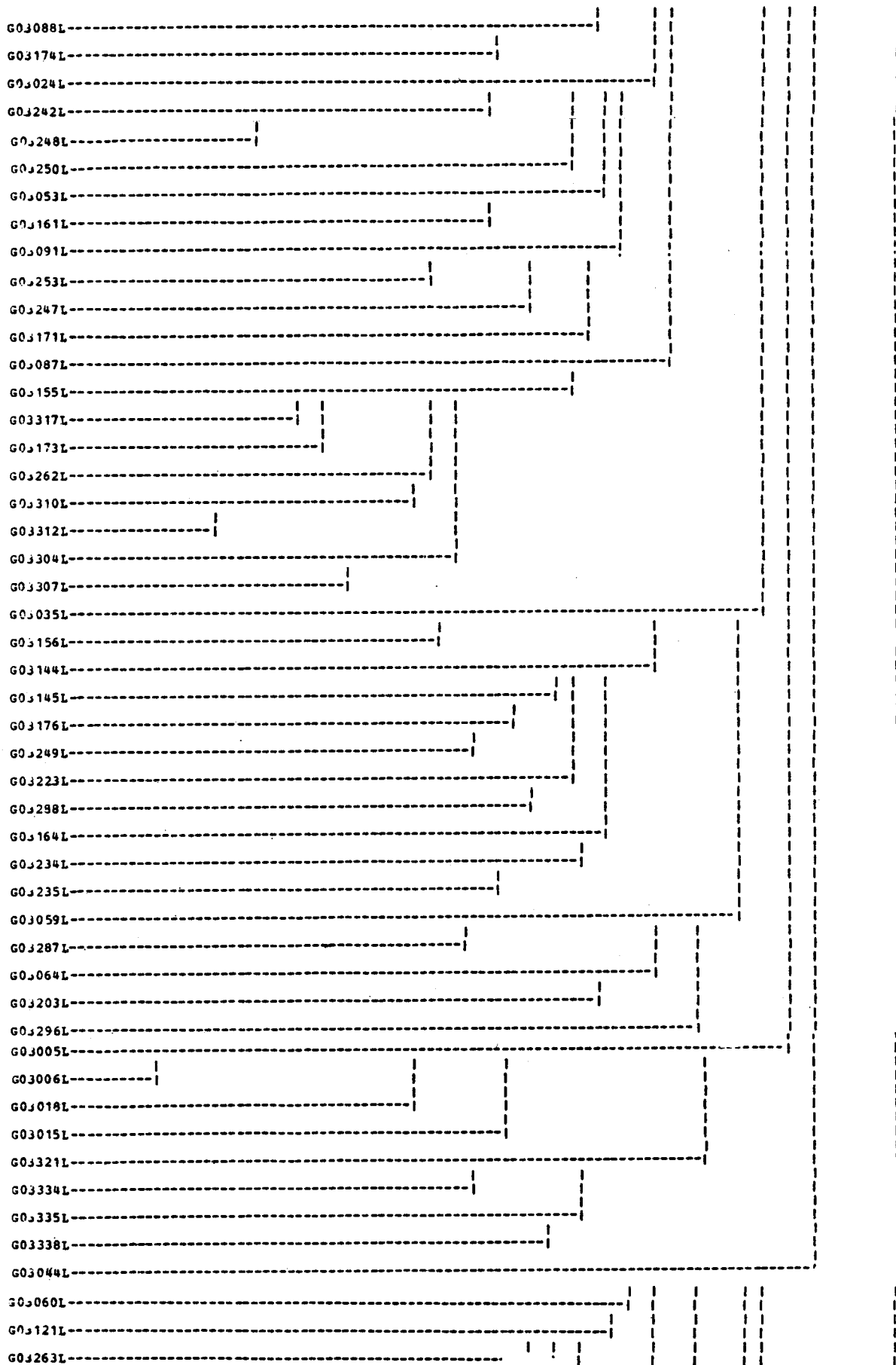
SIMILARITY AT BASE (RIGHT) = .2605

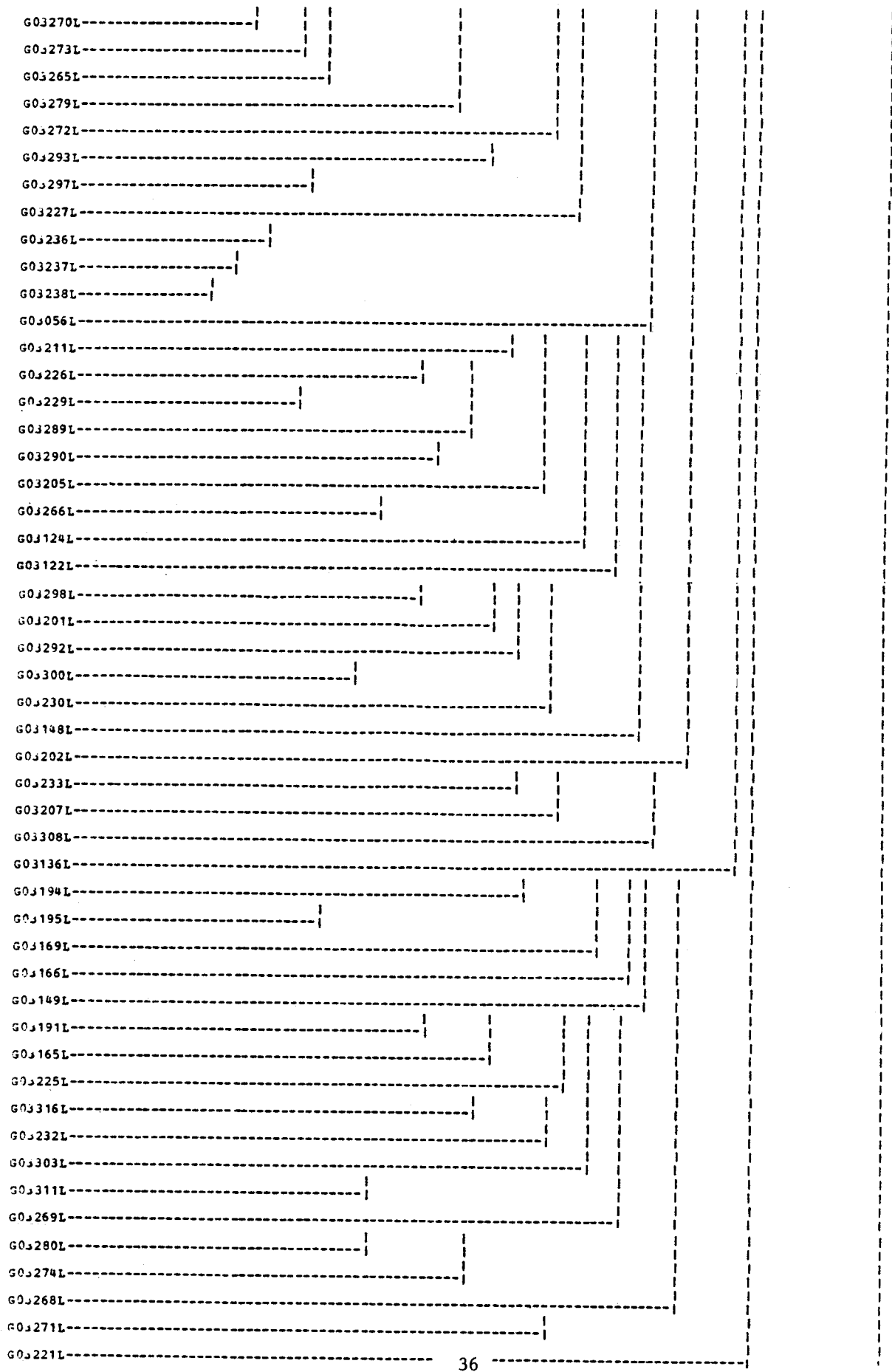












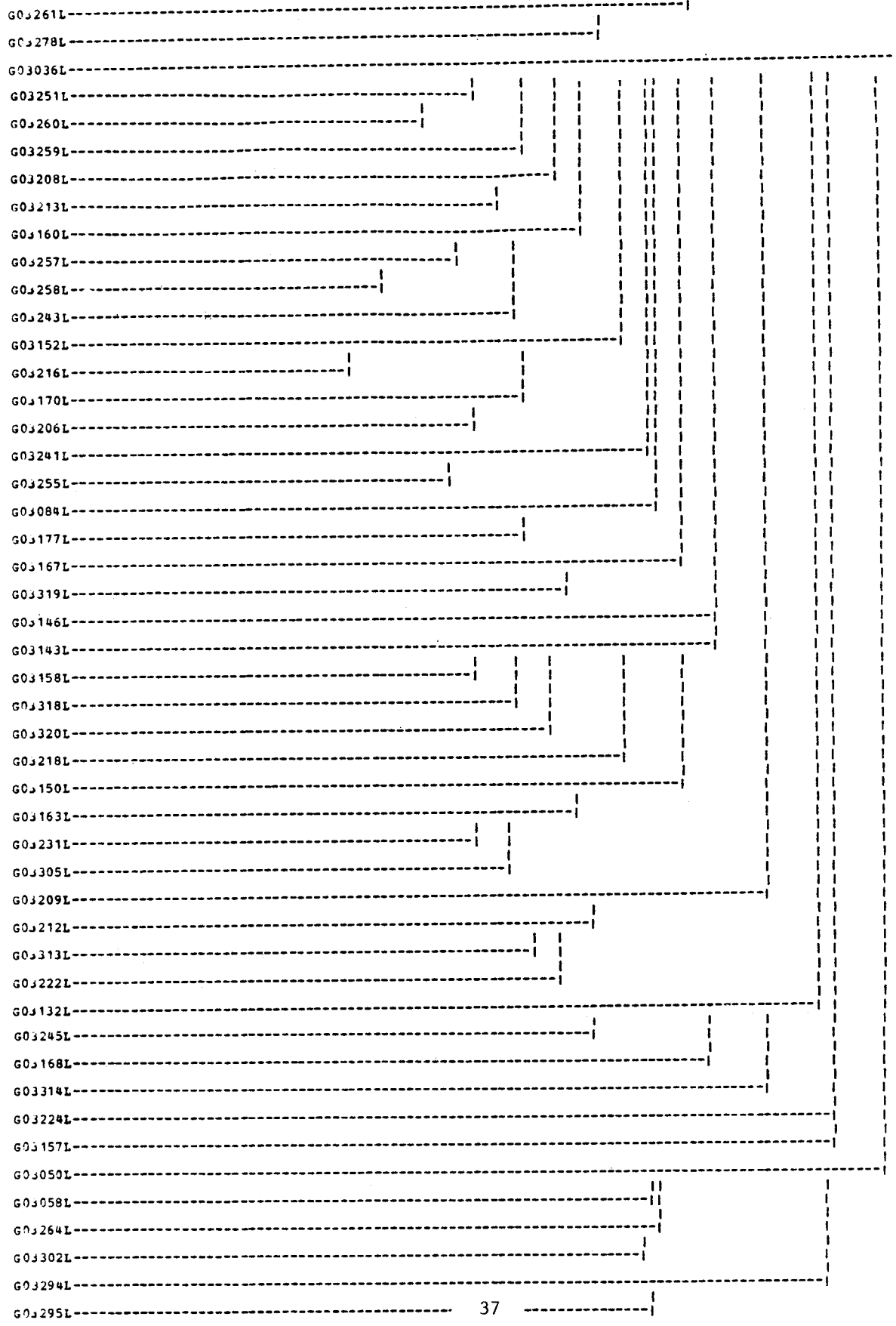
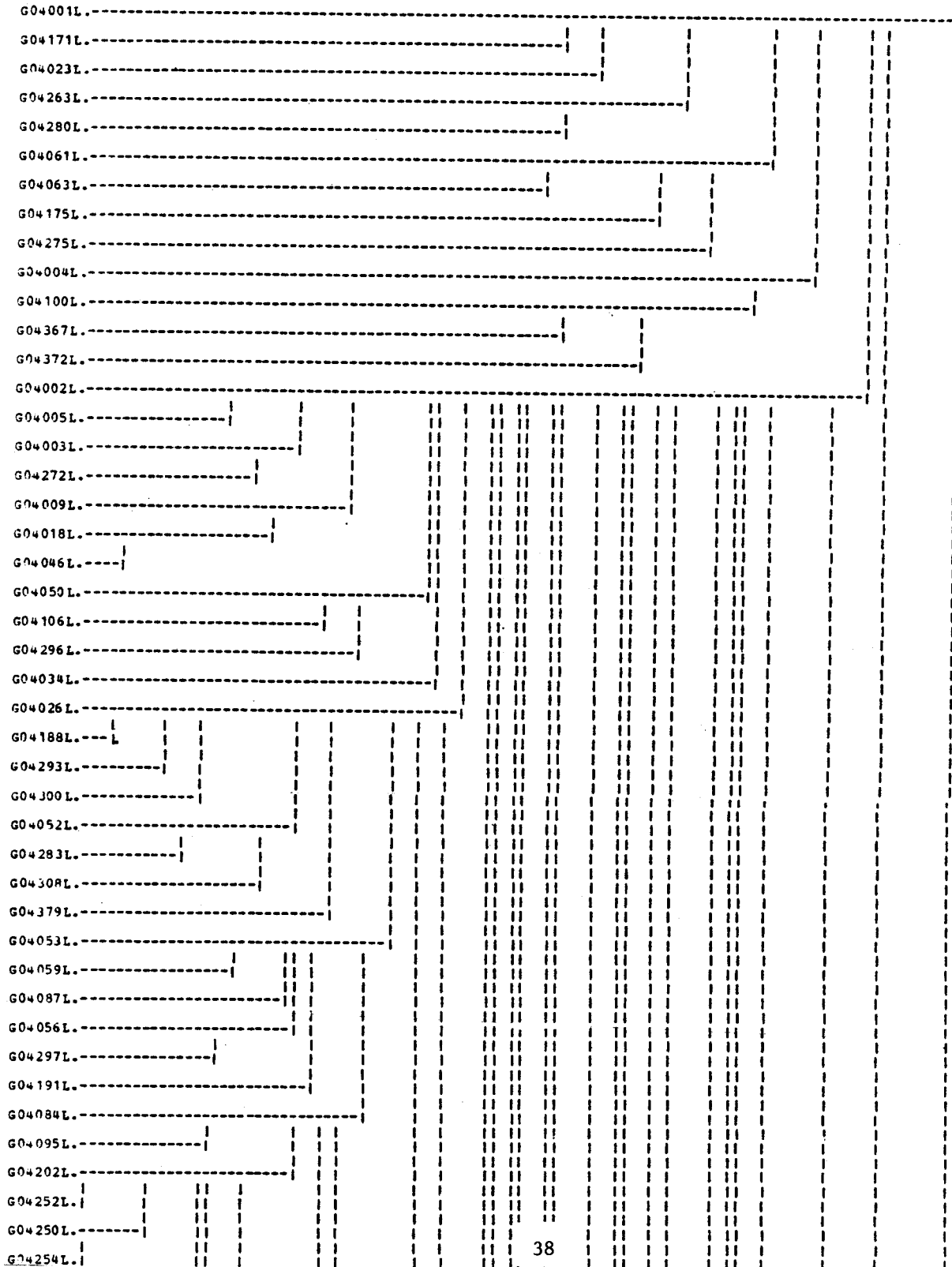


Figure 6. Dendrogram of Cook Inlet isolates from April 1977.

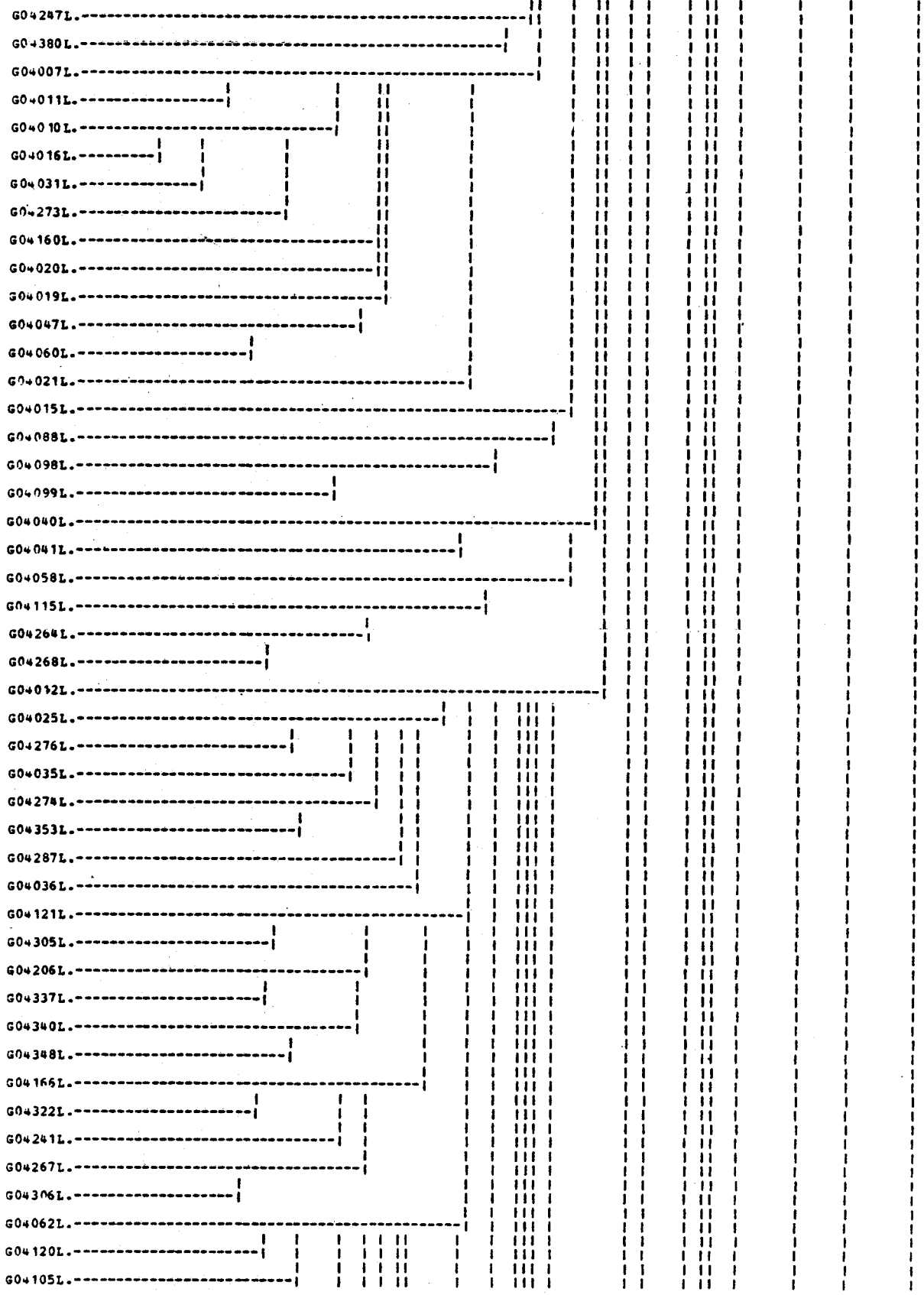
NT 100256

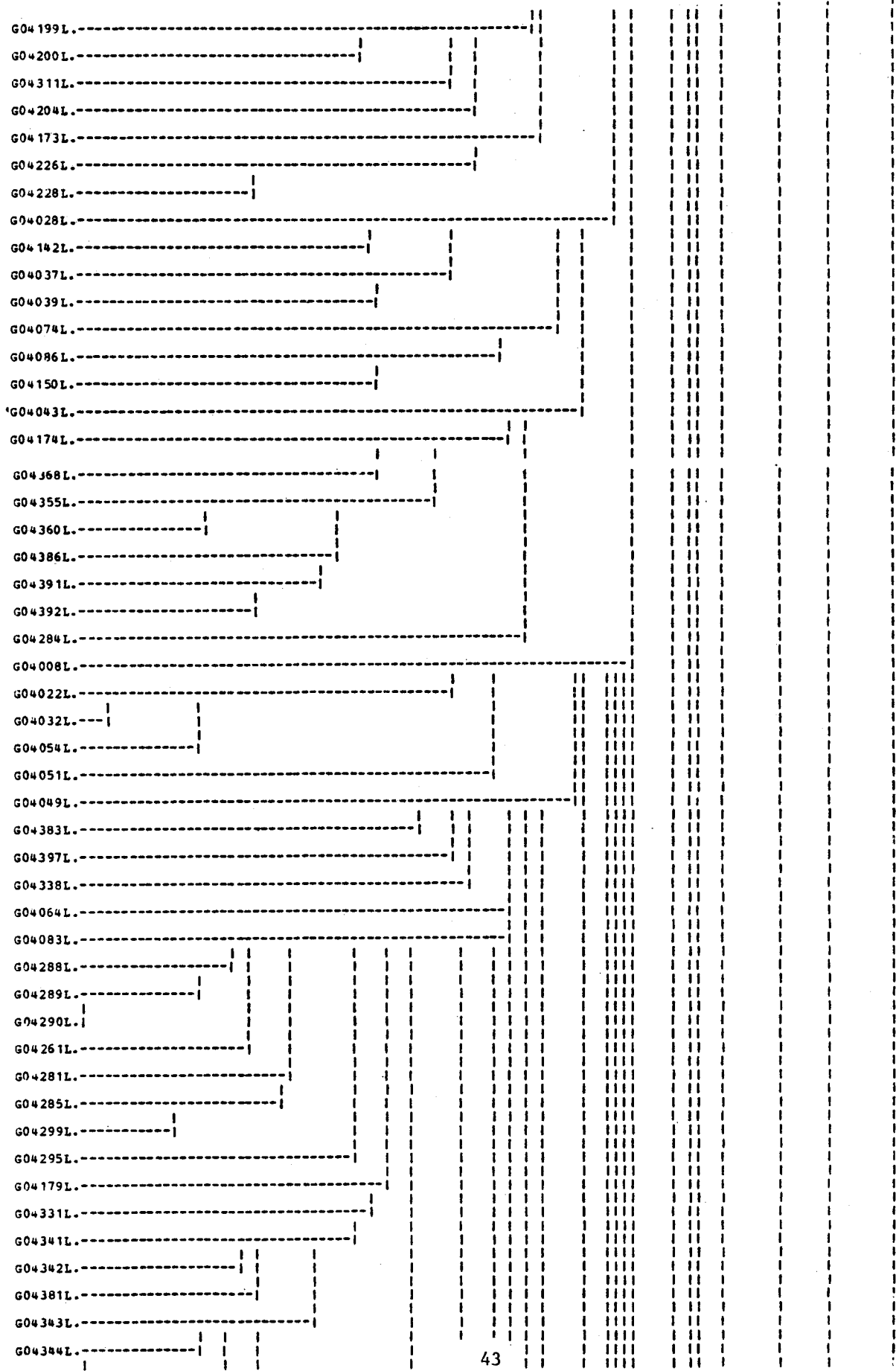
COEFFICIENT: S (J)
 SIMILARITY AT TIP (LEFT) = .9390
 SIMILARITY AT BASE (RIGHT) = .3569

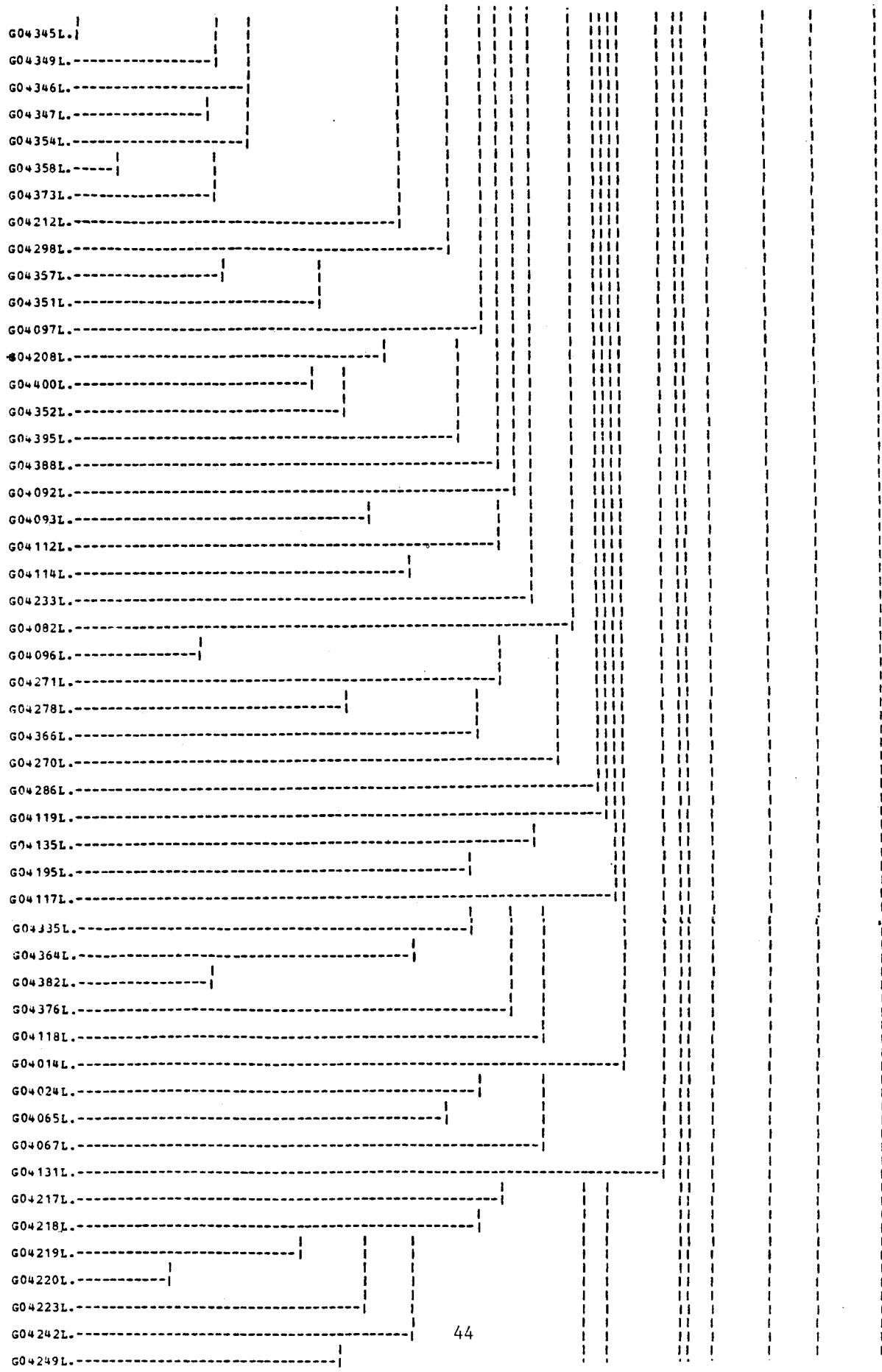


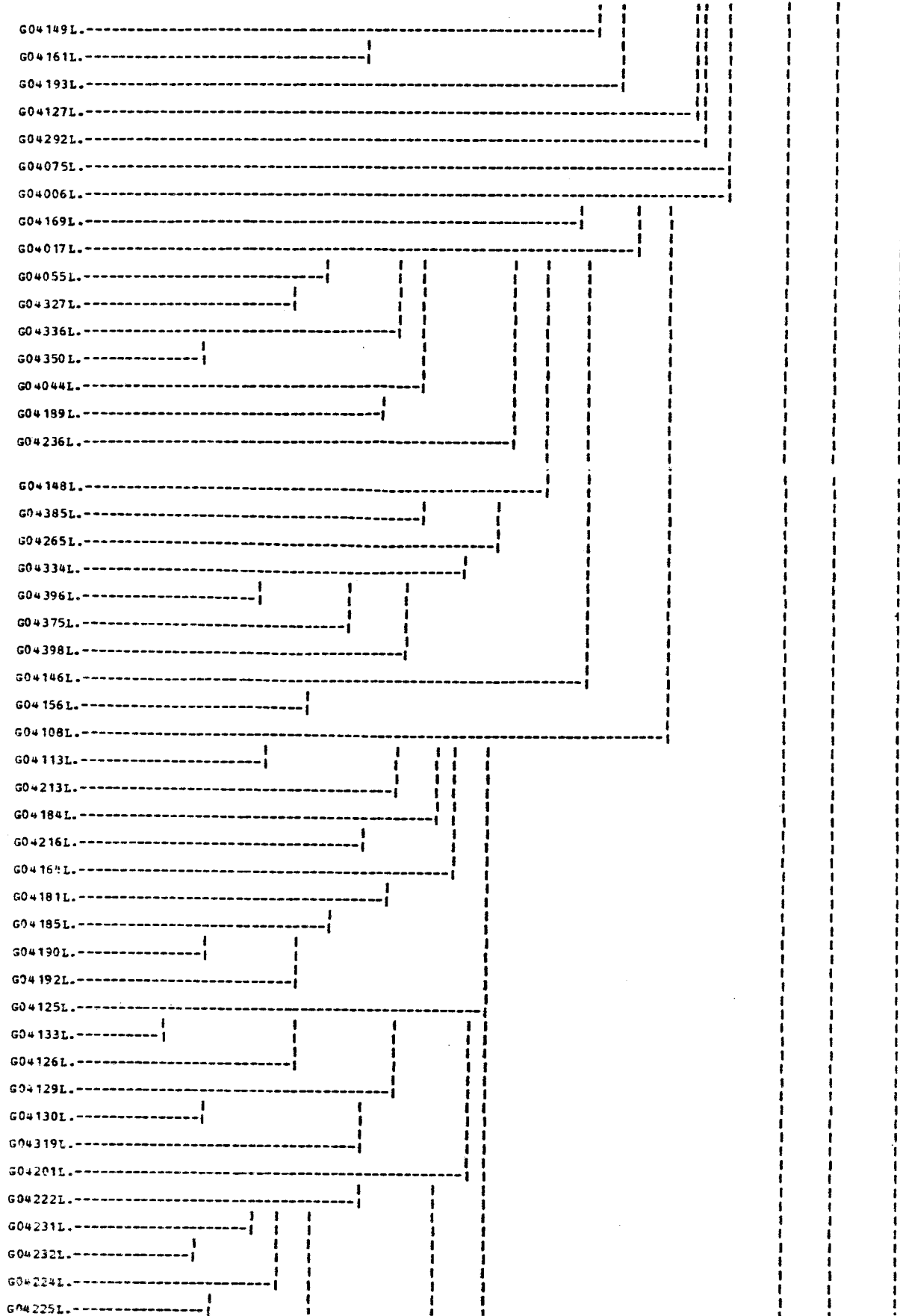
G04260L.-----
 G04205L.-----
 G04253L.-----
 G04248L.-----
 G04251L.-----
 G04245L.-----
 G04155L.-----
 G04203L.-----
 G04257L.-----
 G04258L.-----
 G04243L.-----
 G04282L.-----
 G04291L.-----
 G04144L.-----
 G04209L.-----
 G04214L.-----
 G04029L.-----
 G04172L.-----
 G04207L.-----
 G04042L.-----
 G04107L.-----
 G04089L.-----
 G04262L.-----
 G04377L.-----
 G04165L.-----
 G04180L.-----
 G04178L.-----
 G04247L.-----
 G04380L.-----

G04257L.-----
 G04258L.-----
 G04243L.-----
 G04282L.-----
 G04291L.-----
 G04144L.-----
 G04209L.-----
 G04214L.-----
 G04029L.-----
 G04172L.-----
 G04207L.-----
 G04042L.-----
 G04107L.-----
 G04089L.-----
 G04262L.-----
 G04377L.-----
 G04165L.-----
 G04180L.-----
 G04178L.-----









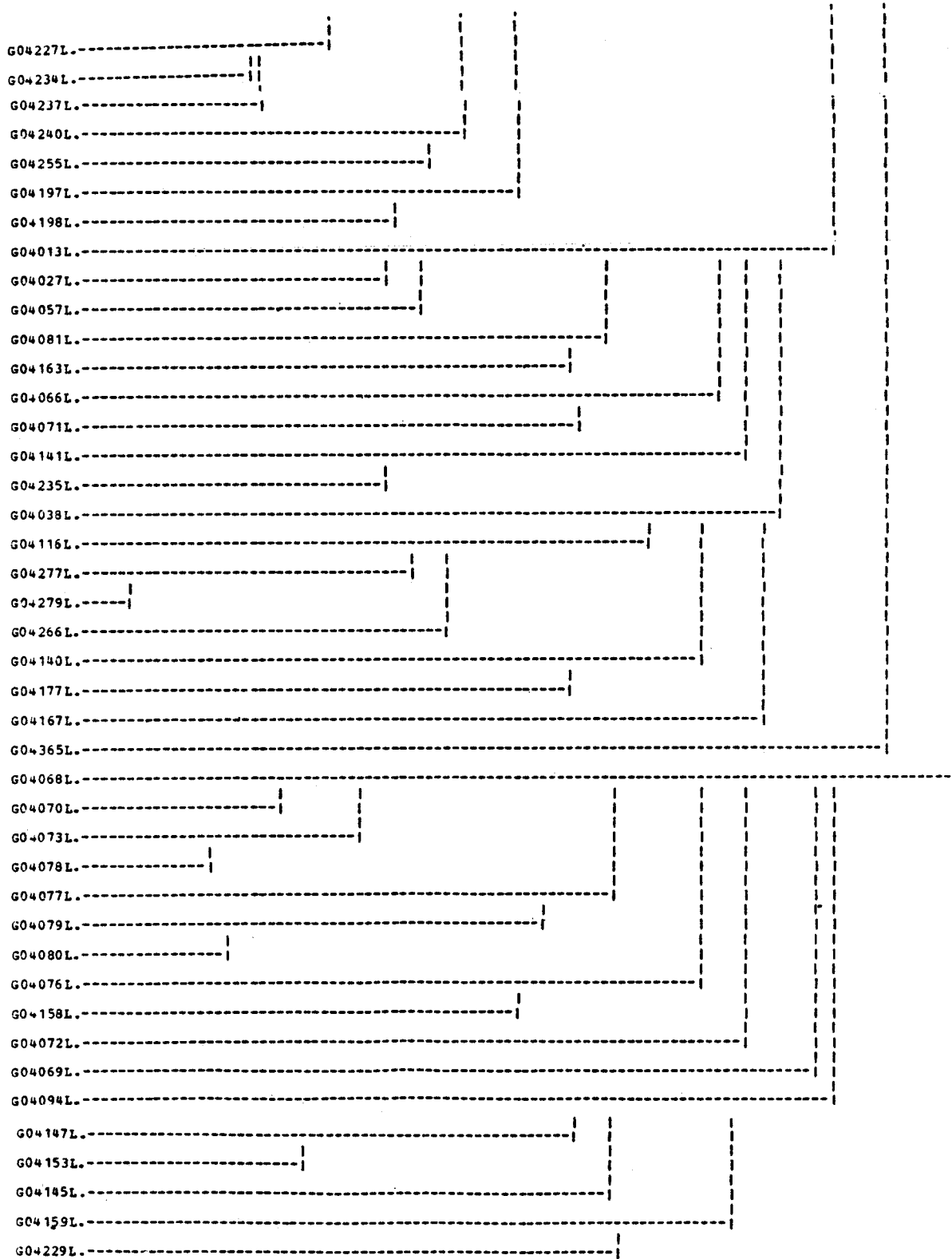


Table 5. Diversity indices (\bar{H}) for heterotrophic bacterial populations isolated at 4°C from Cook Inlet.

Station No.	November	April
	Water	
105	4.22	2.26
204	2.74	4.02
205	-	3.17
212	-	4.22
215	3.31	2.75
225	0.72	2.92
226	-	3.56
229	1.39	3.78
245	2.94	3.62
265	4.01	3.89
266	4.08	3.24
395	-	2.50
K	0.30	-
L	2.78	-
M	2.56	-
U	-	3.49
	Sediment	
105	2.85	-
204	3.72	-
205	-	3.69
212	-	3.32
215	3.72	3.92
225	3.78	3.38
226	-	3.49
229	3.40	3.34
L	1.39	-
M	3.88	-
U	-	4.12

from water were lower in November than April at a number of comparable stations. Notable exceptions were stations near Kennedy entrance and Upper Cook Inlet. No significant differences in diversity indices were seen between populations from November and April sediment samples.

The dendrograms from the cluster analyses of presumed hydrocarbon utilizing microorganisms are shown in Fig. 7 for organisms selected from enumeration plates and in Fig. 8 for organisms selected from Most Probable Number enrichments. The clustering patterns showed that closely related organisms had been isolated from Gulf of Alaska, Cook Inlet and Beaufort Sea water and sediment samples for presumed ability to utilize hydrocarbons. The cluster analyses also showed that many taxonomic groups were represented, *i.e.* many different microbial species and genera are able to grow on media with petroleum hydrocarbons added.

Examination of the feature frequency analyses showed that only 20% of the organisms used in the cluster analyses from the oil-agar enumeration plates had been scored positive for any of the hydrocarbons tested. The remaining 80% of the organisms were petroleum tolerant bacteria, able to grow in the presence of petroleum, and probably were low nutrient requiring bacteria able to grow on trace organic compounds in the control media. This fact emphasizes why we subtract control plate counts from oil-agar counts in the enumeration of hydrocarbon utilizing bacteria procedure and why plate count enumeration procedures

Figure 7. Simplified Dendrogram of presumed hydrocarbon utilizers from enumeration plates.

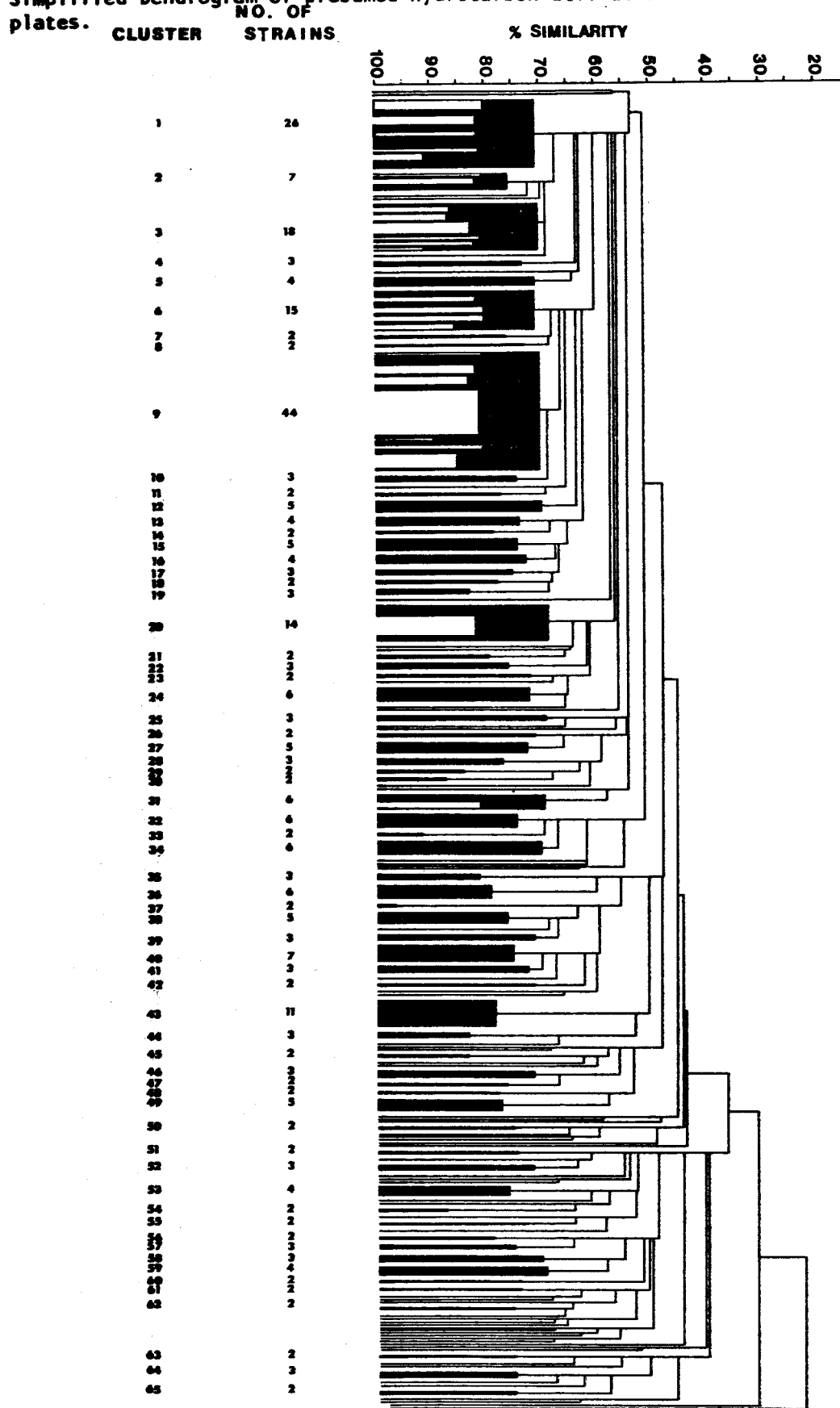
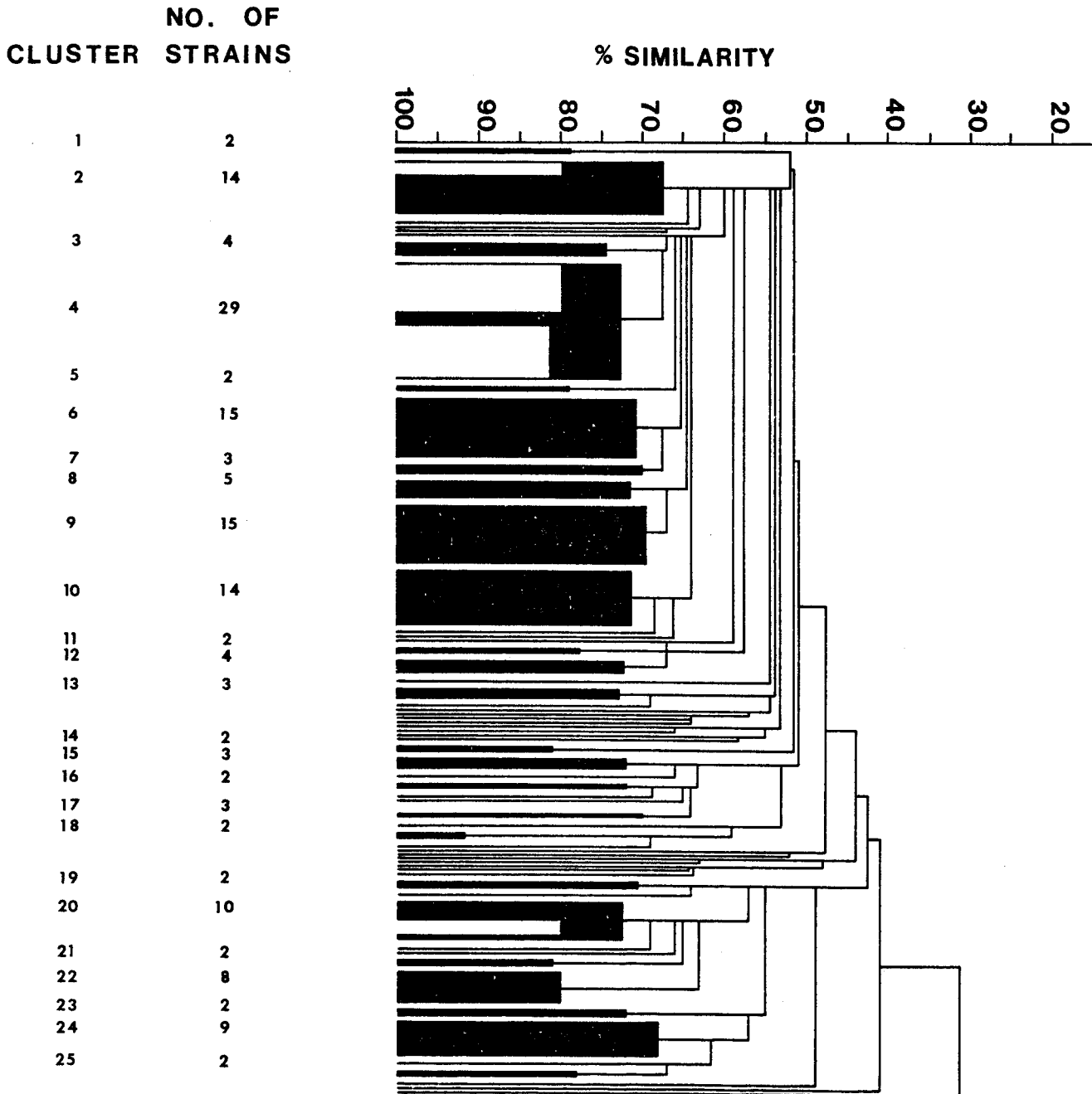


Figure 8. Simplified Dendrogram of presumed hydrocarbon utilizers from MPN enumerations.



should be replaced by Most Probable Number determinations for hydrocarbon utilizers. Several of the major clusters contained members that utilized hydrocarbons (Table 6). The organisms that utilized hydrocarbons typically formed subclusters within the major clusters representing 20-25% of the organisms in several major clusters. This indicates that some hydrocarbon utilizing bacterial species are very closely related to the non hydrocarbon utilizing species and may even be strains of the same species.

The feature frequencies showed that 75%, a much higher percentage, of the strains in the cluster analyses from the MPN enrichment were able to utilize hydrocarbons. In fact, all of the organisms in the major clusters were capable of hydrocarbon utilization (Table 6). Exposure to crude oil obviously selects and enriches for hydrocarbon utilizers. Further analyses of the hydrocarbon utilizers showed that a higher percentage of the strains could use mixtures of hydrocarbons, *e.g.* a crude oil, than could use pure hydrocarbons (Table 7). Both aliphatic and aromatic compounds could be utilized by some organisms.

Hydrocarbon Biodegradation Activity

In Cook Inlet natural hydrocarbon biodegradation potentials (no nutrients added) followed the order: naphthalene \geq hexadecane $>$ pristane \geq benzanthracene in both water and sediment samples

Table 6. Percent of strains in clusters scored positive for ability to utilize any hydrocarbon or hydrocarbon mixture.

From Enumeration Plates		From MPN Enrichments	
*Cluster No.	Percent Positive for hydrocarbon utilization	*Cluster No.	Percent Positive for hydrocarbon utilization
1	23	2	14
6	7	4	100
9	20	6	100
20	78	8	80
31	70	9	90
32	17	10	100
34	85	12	100
36	100	20	100
38	20	22	100
39	68	24	100
43	100		
49	100		
58	100		
59	100		

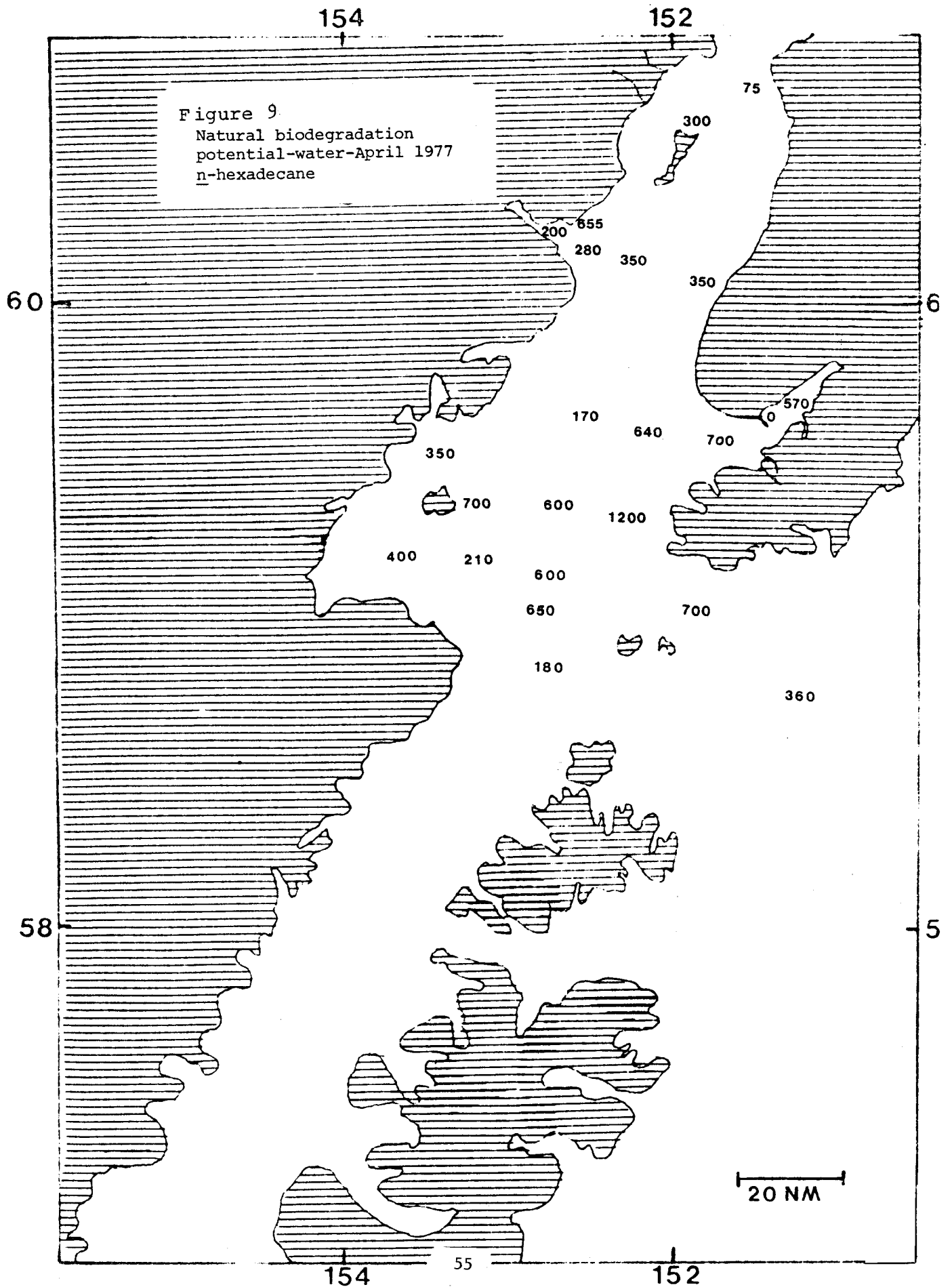
*Cluster No. refers to dendrogram

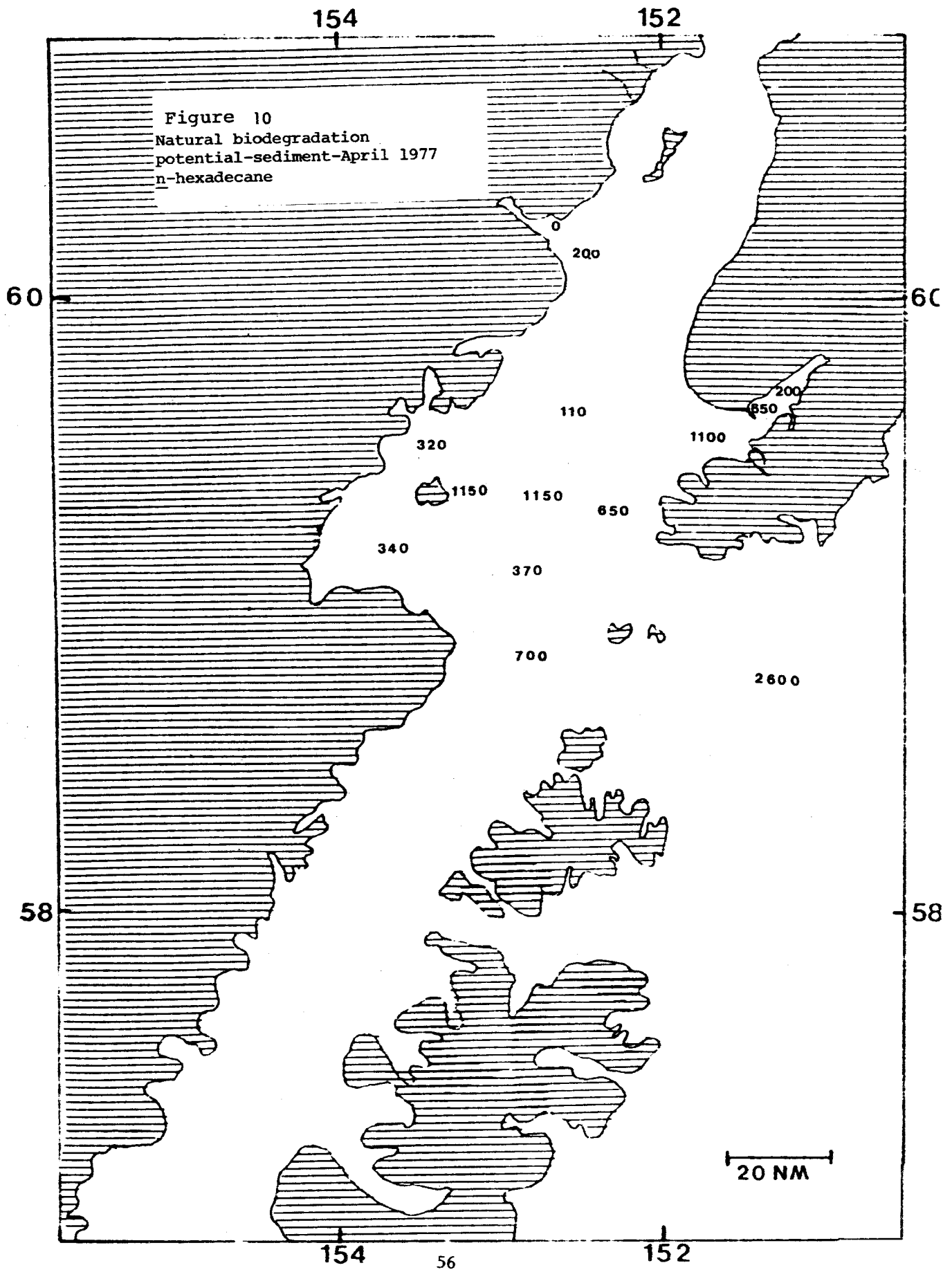
Table 7. Percent utilization of hydrocarbons by strains in major clusters from MPN enrichment cluster analyses.

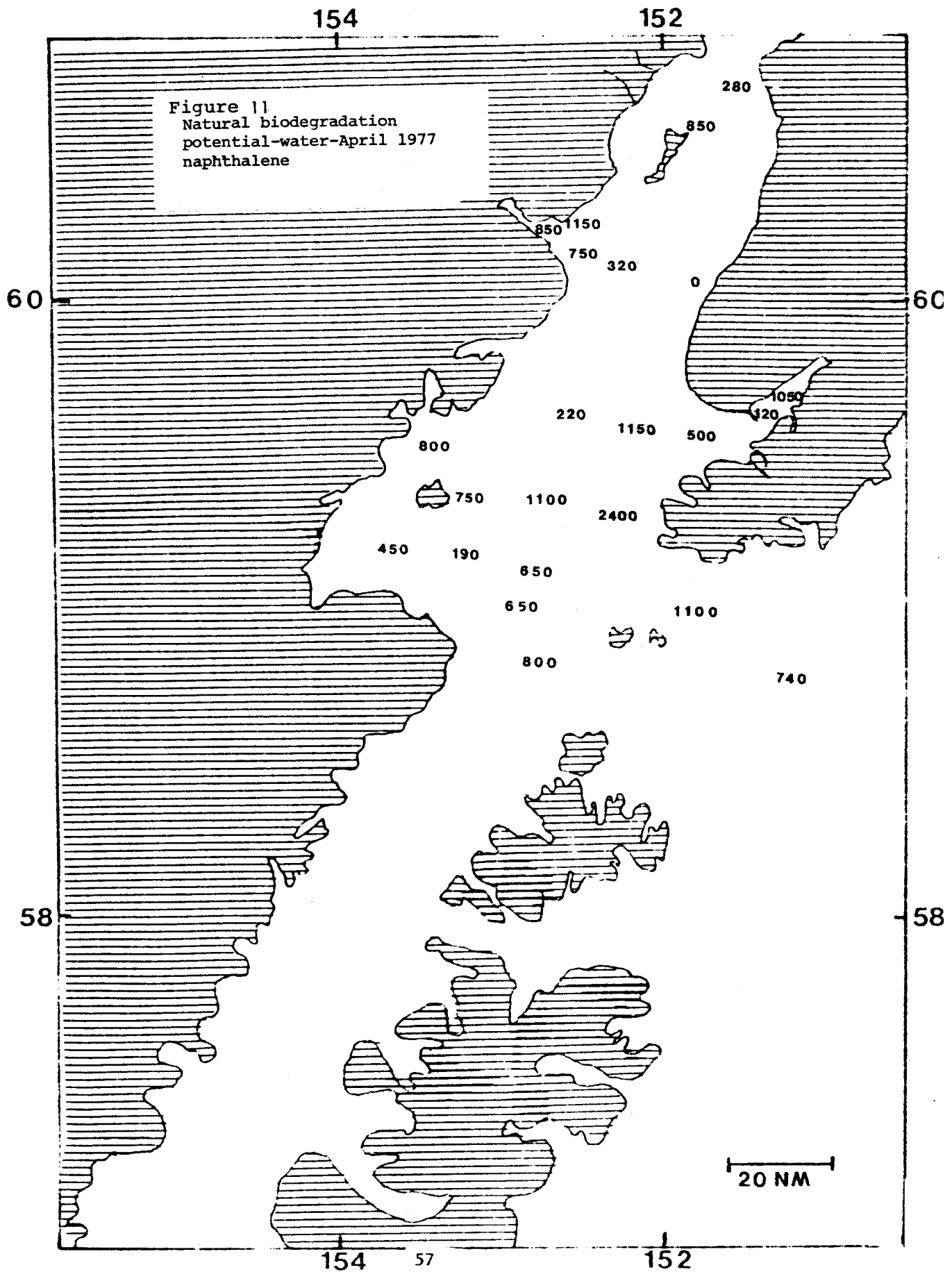
<u>Hydrocarbon Utilized</u>	<u>Cluster No.</u>									
	<u>2</u>	<u>4</u>	<u>6</u>	<u>8</u>	<u>9</u>	<u>10</u>	<u>12</u>	<u>20</u>	<u>22</u>	<u>24</u>
N-Decane	7	0	0	0	0	0	0	100	0	79
N-Hexadecane	0	14	0	0	20	57	0	90	100	44
N-Nonane	0	0	0	0	0	0	0	90	100	79
N-Octadecane	0	0	0	0	0	0	0	100	100	67
N-Pentadecane	0	0	0	0	0	0	0	90	100	89
1-Methylnaphthalene	14	0	0	0	0	0	0	100	100	100
Omega-Phenyldecane	7	0	0	40	0	0	0	100	100	67
Toluene	7	0	0	0	13	0	0	90	100	89
Pristane	0	0	0	0	0	0	0	100	100	0
Pentadecylcyclohexane	0	0	0	0	0	21	0	100	100	0
Ethylcyclohexane	0	0	0	0	13	0	0	100	100	100
Dicyclohexyl	0	0	0	0	0	0	0	100	100	0
Diphenylmethane	0	0	0	0	0	0	0	90	100	67
Acenaphthalene	0	0	0	0	0	7	0	100	100	100
9-Methylanthracene	0	0	0	0	0	0	0	100	100	56
Napthanol	0	0	0	0	0	0	0	0	0	0
Prudhoe crude oil	0	100	100	20	80	100	100	100	100	78
JP 5	0	0	0	0	0	0	0	100	100	100
Gasoline (unleaded)	0	0	0	0	0	0	0	100	100	56
Mineral oil	0	45	100	80	87	0	100	100	100	100
API reference oil #1	0	0	0	0	7	0	0	100	100	56
API reference oil #2	0	0	0	0	0	0	0	100	75	56
API reference oil #3	0	0	0	0	0	0	0	100	100	22
API reference oil #4	0	0	0	0	0	0	0	90	100	22

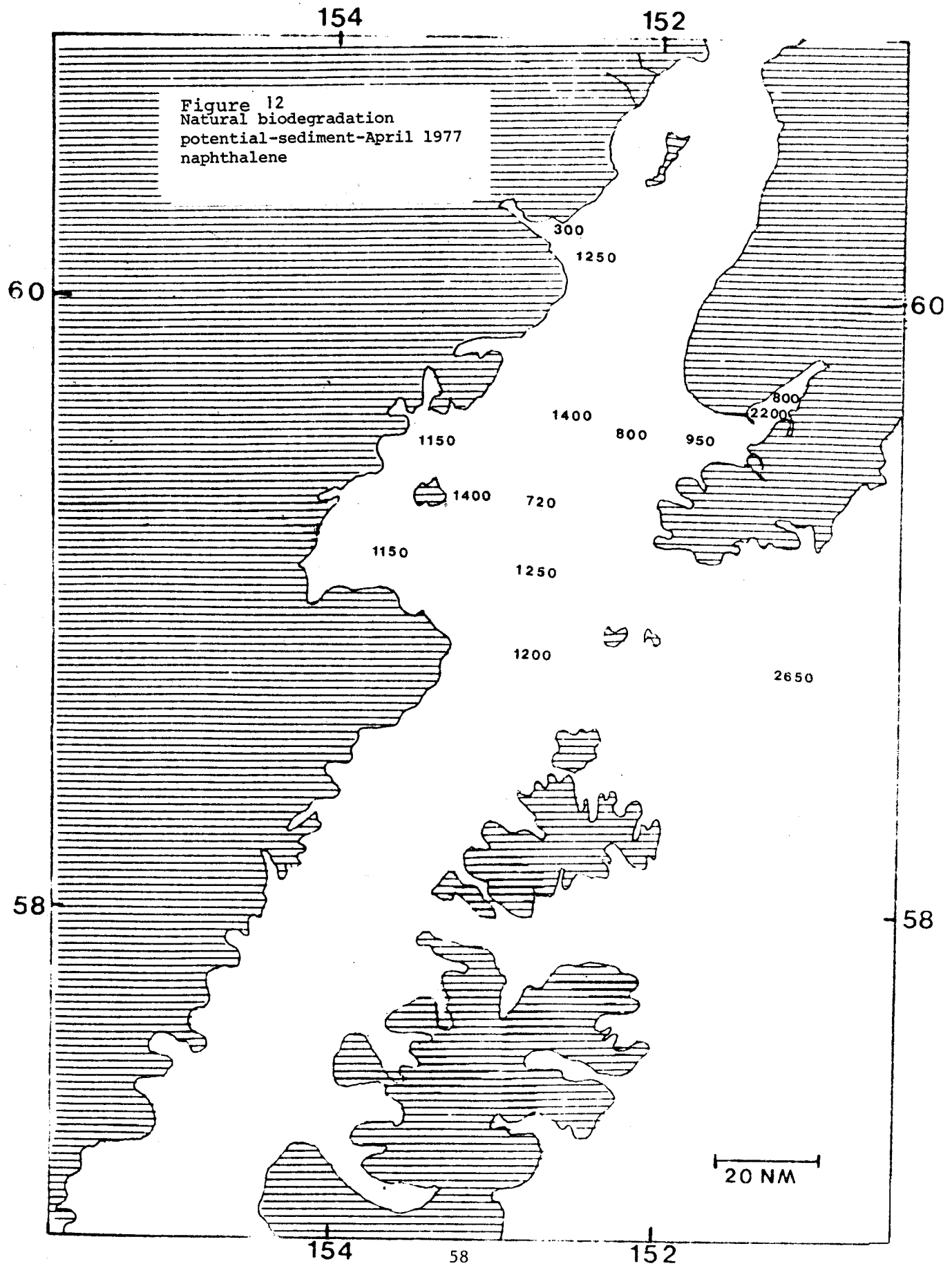
collected during both April and November 1977 (Figs. 9-17). Natural biodegradation potentials for pristane and benzantracene were often zero (not shown in Figures). The natural hydrocarbon biodegradation potentials were higher in April than in November for water samples. Natural hydrocarbon biodegradation potentials in sediment were similar for comparable station samples in April and November.

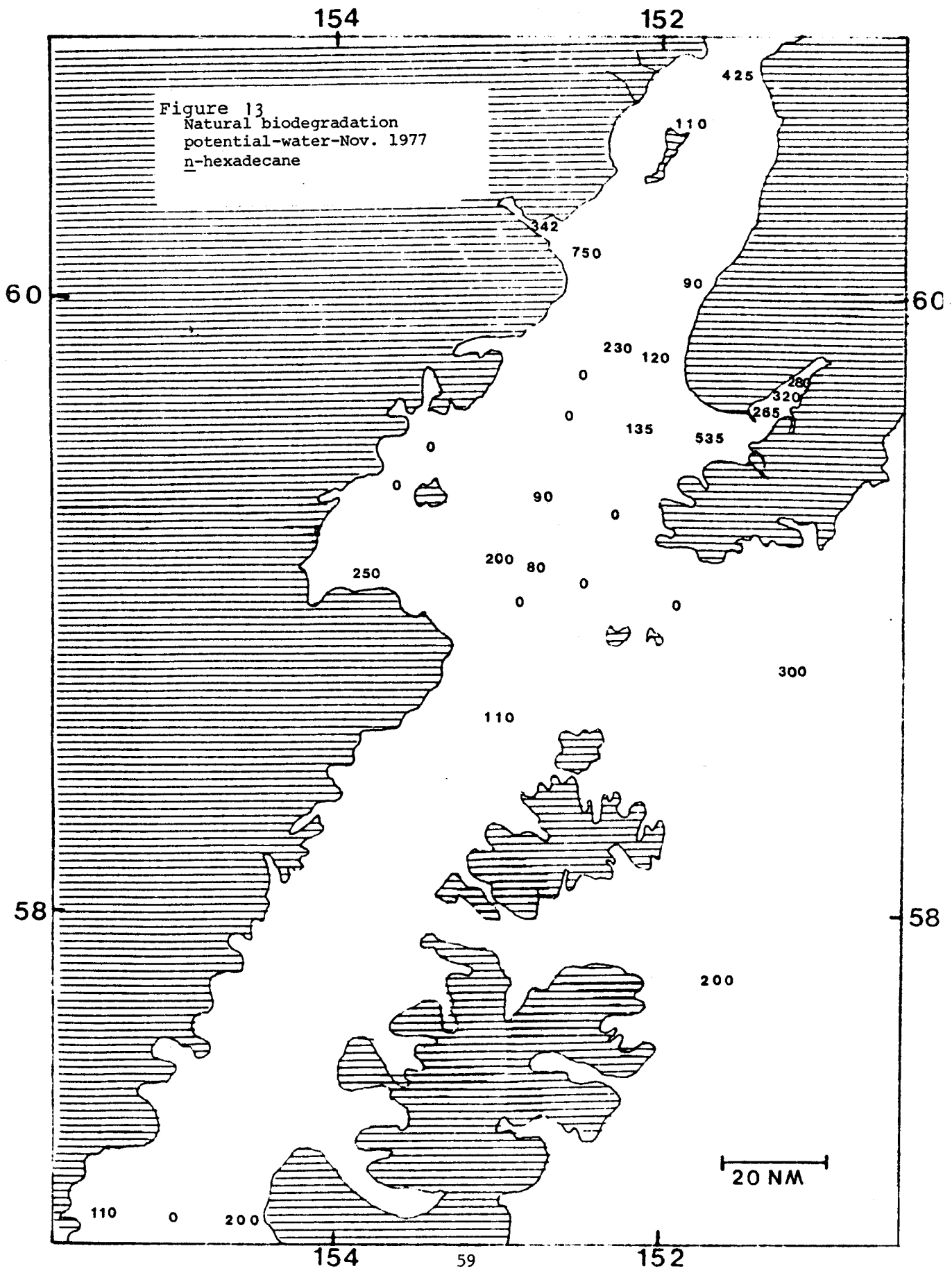
The non-nutrient limited biodegradation potentials followed the order: hexadecane > naphthalene >>> pristane > benzantracene (Figs. 17-32). In almost all cases the removal of nutrient limitation resulted in higher biodegradation potentials for hexadecane and naphthalene but not for pristane and benzantracene. The lack of stimulated degradation for pristane and benzantracene when nutrient limitations were removed probably indicates that these compounds are resistant to mineralization by the available enzymatic systems. Pristane and benzantracene could have been partially degraded without production of $^{14}\text{CO}_2$. The non-nutrient limited biodegradation potentials were much higher in April than November samples for all substrates. It is possible that some additional growth factor was required by microorganisms in the November samples. The large difference between the biodegradation potentials of November and April samples probably indicates that the fate of spilled oil in Cook Inlet will be highly dependent on the time of year that contamination occurs. The hydrocarbon











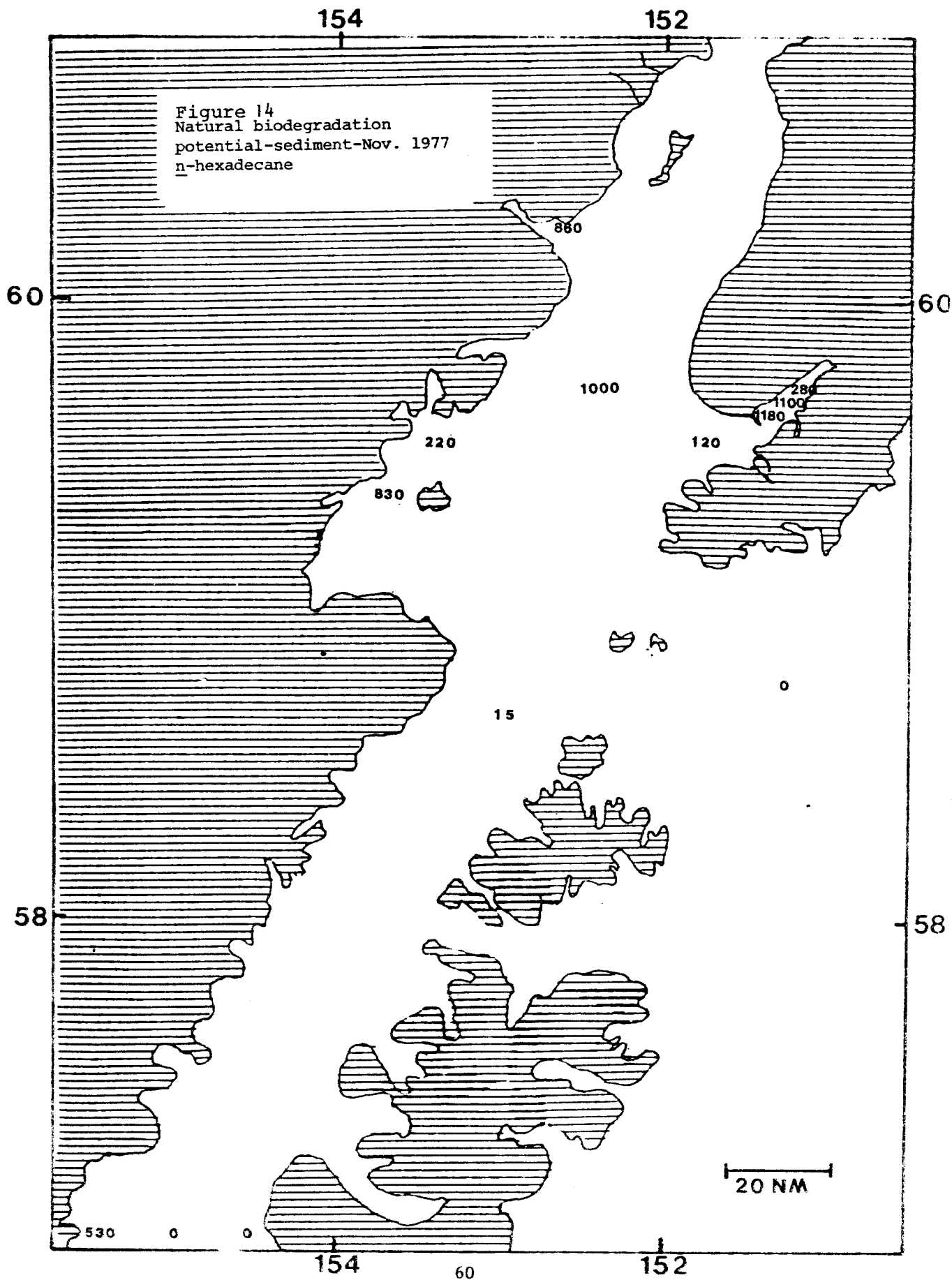
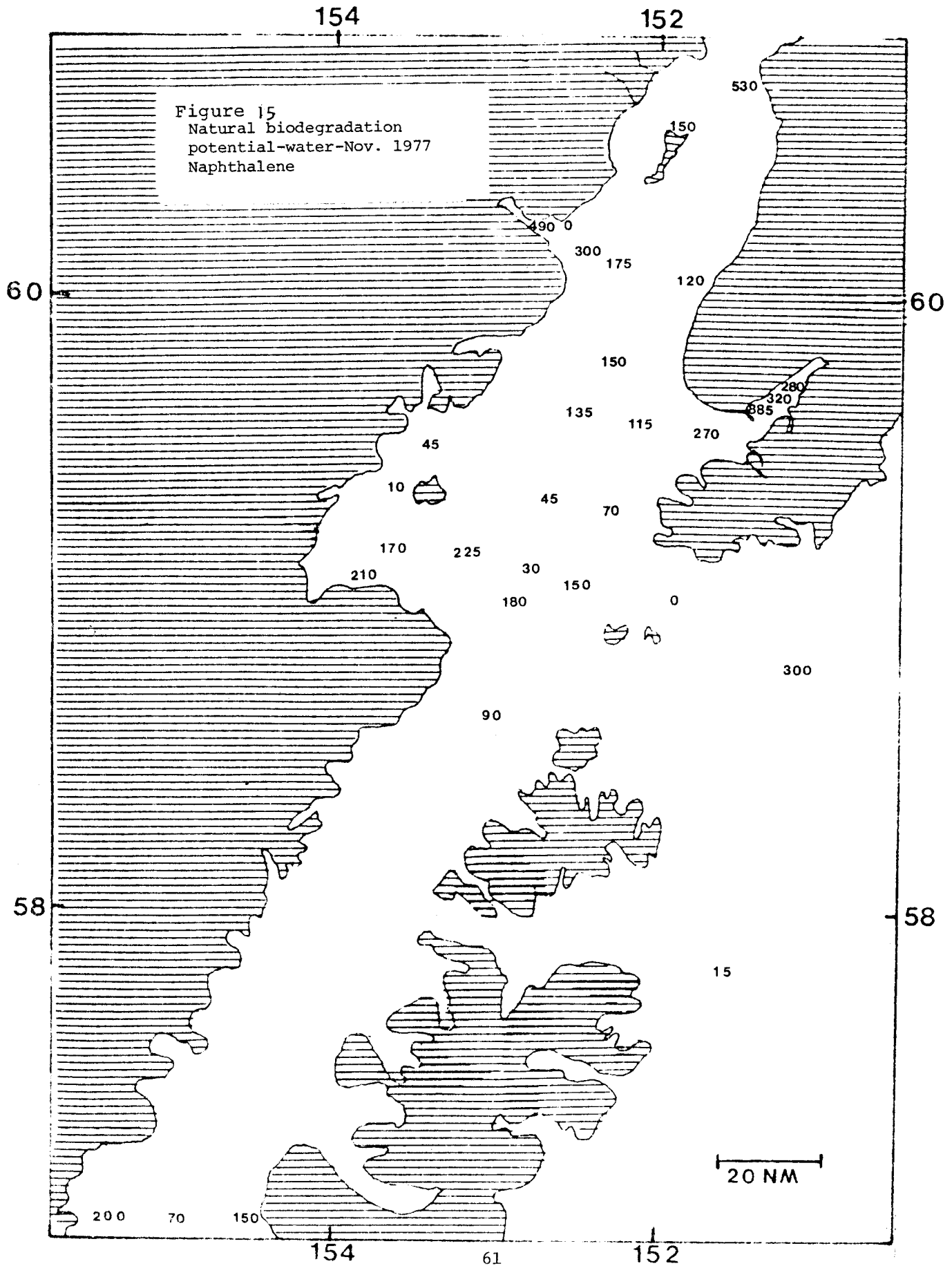
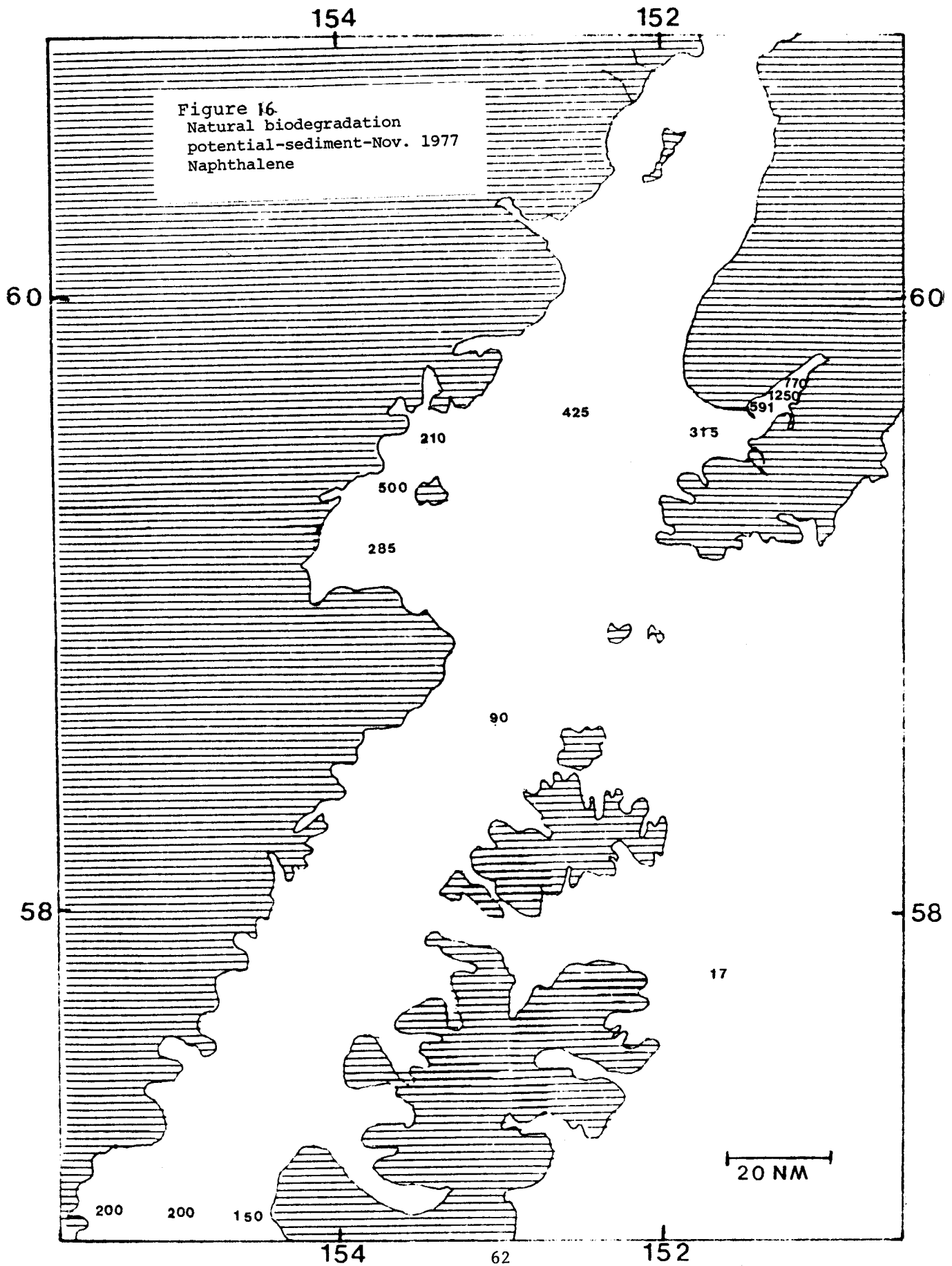
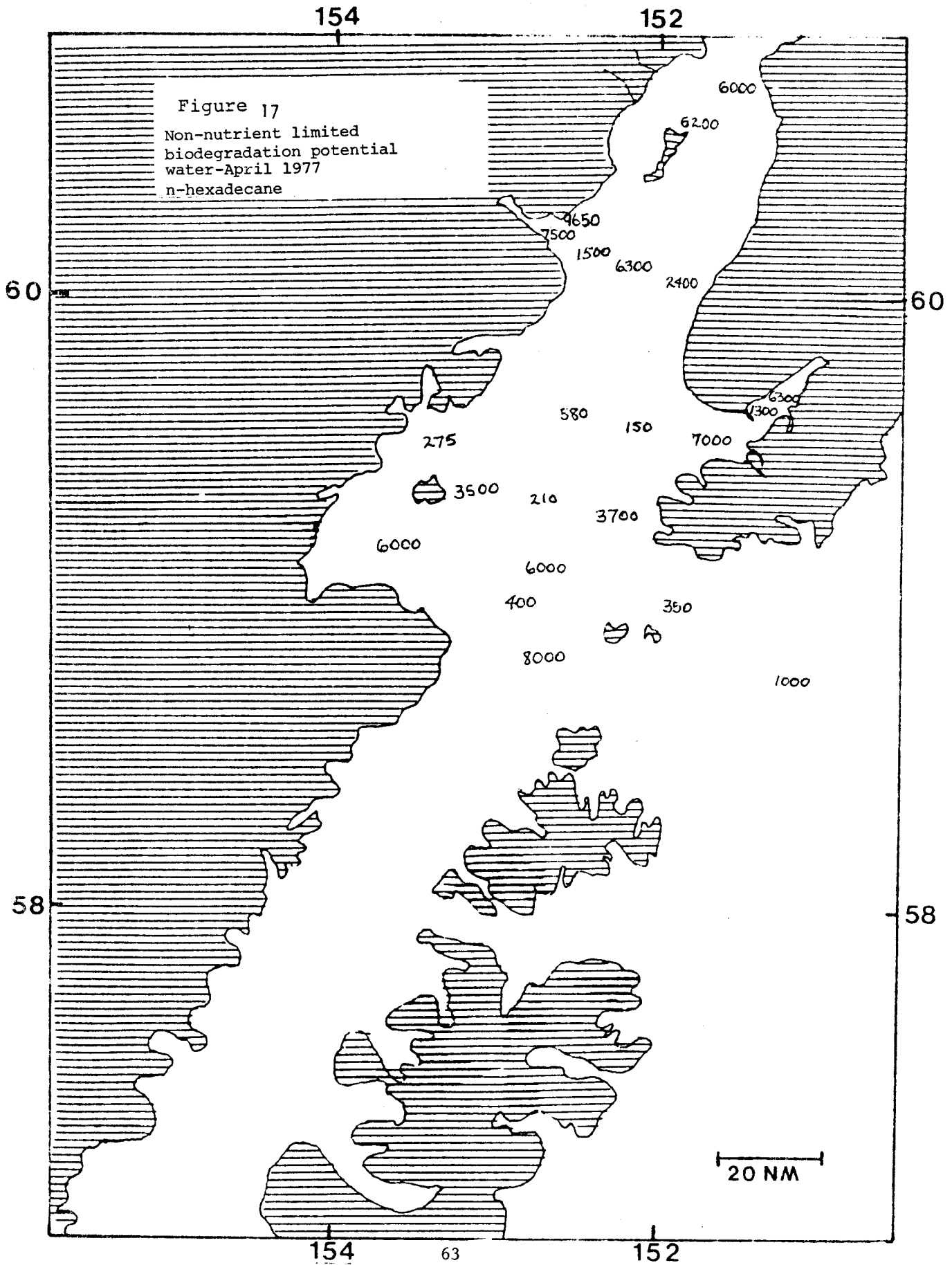
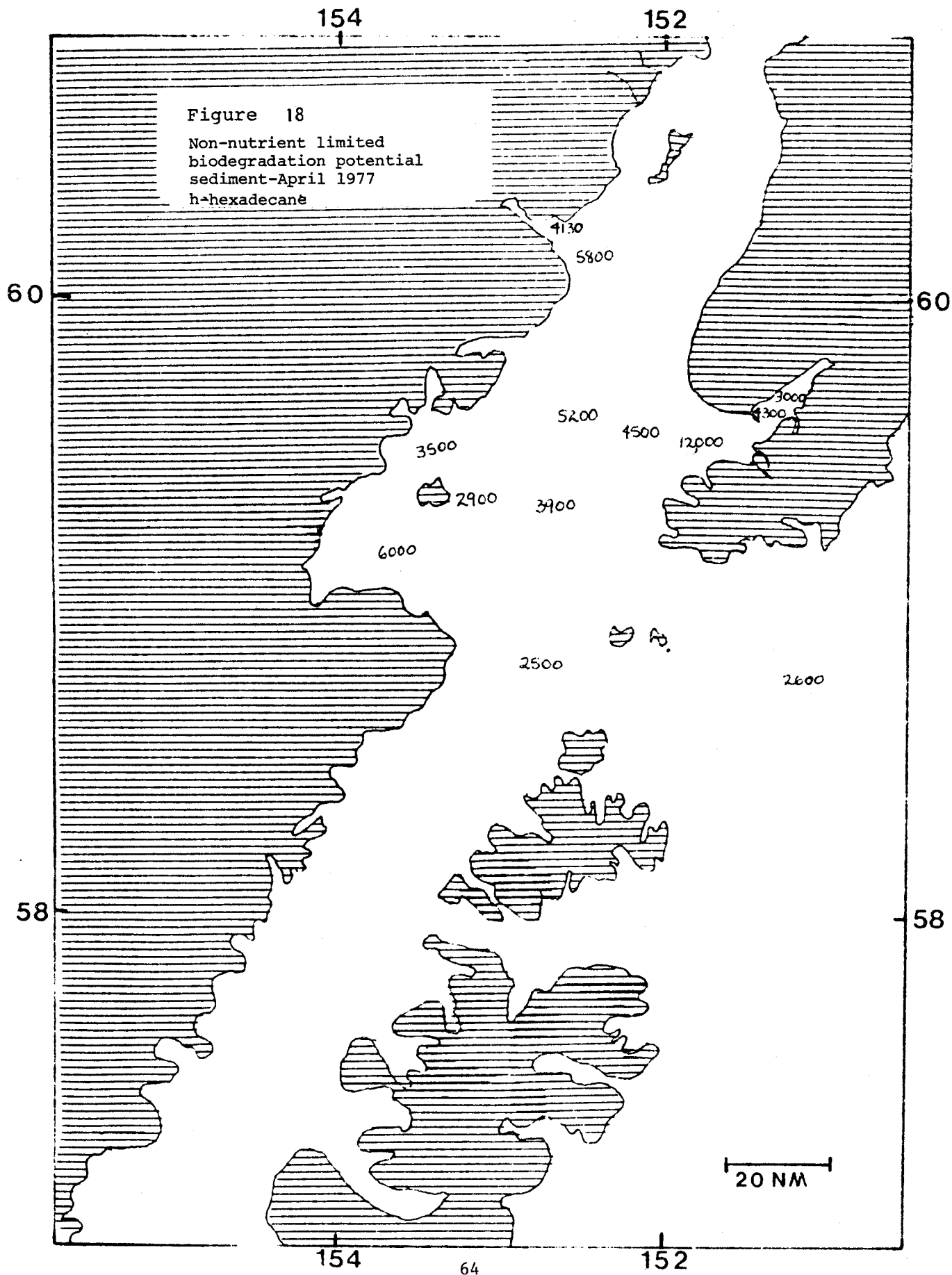


Figure 15
Natural biodegradation
potential-water-Nov. 1977
Naphthalene









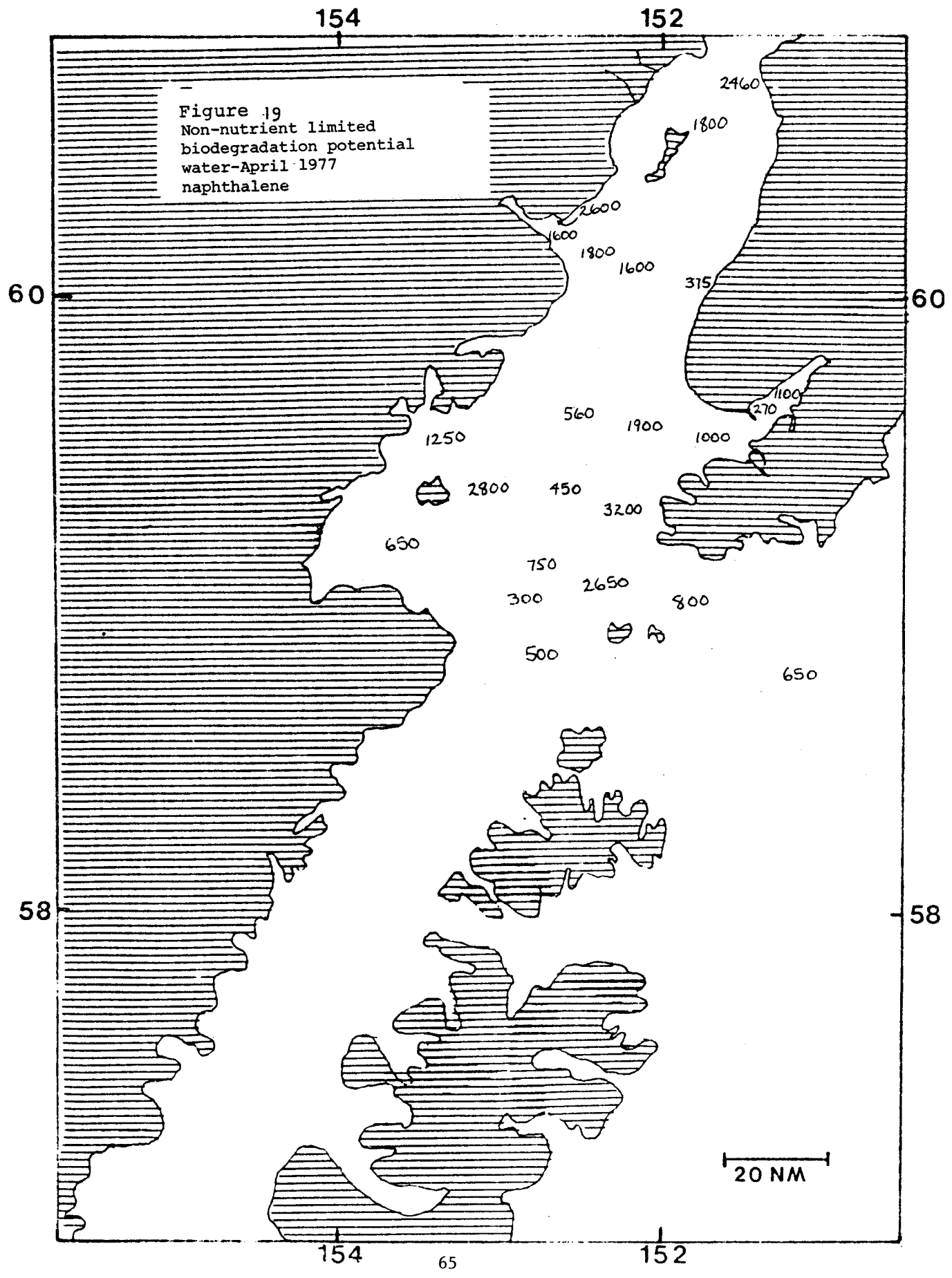
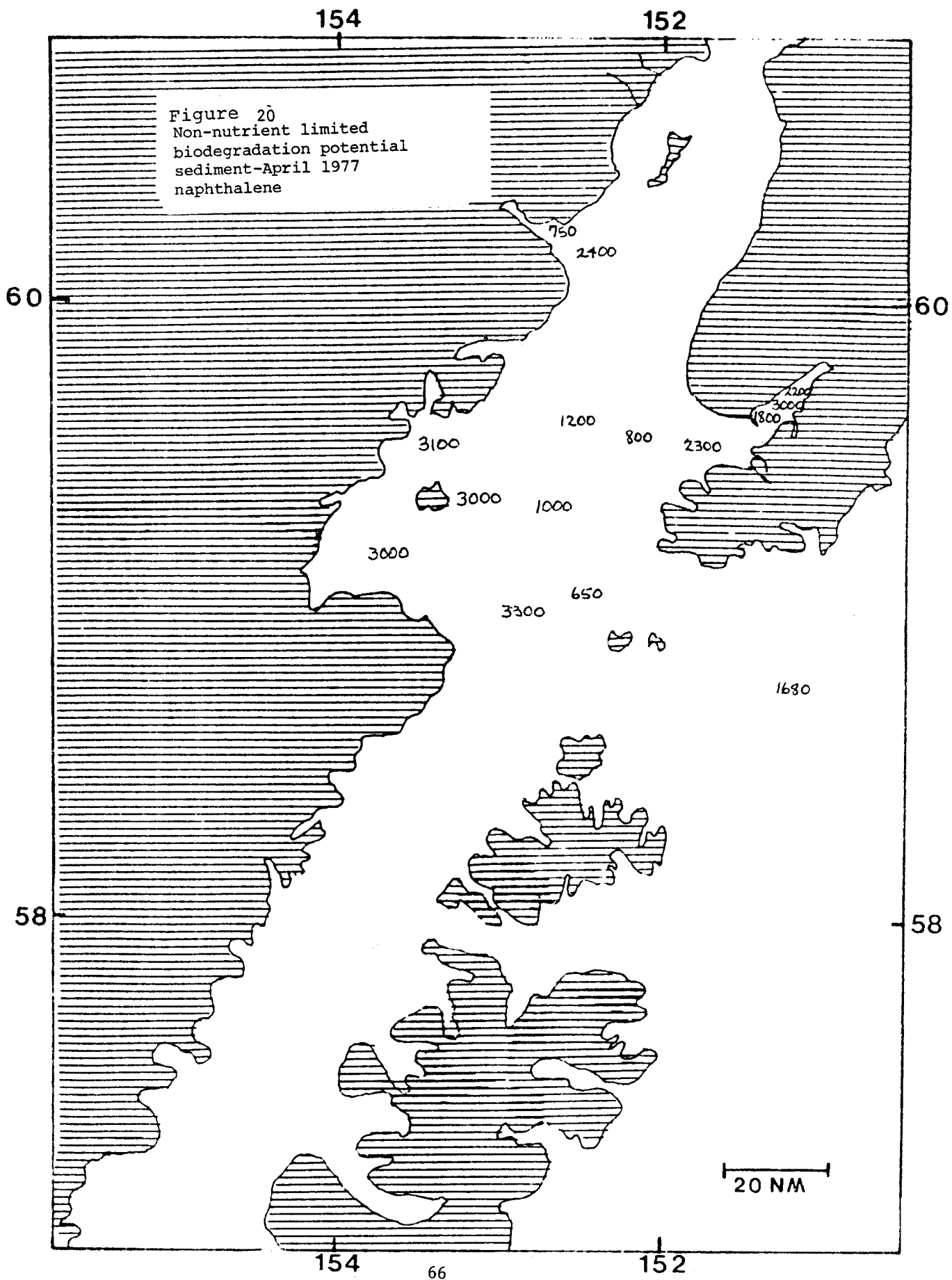
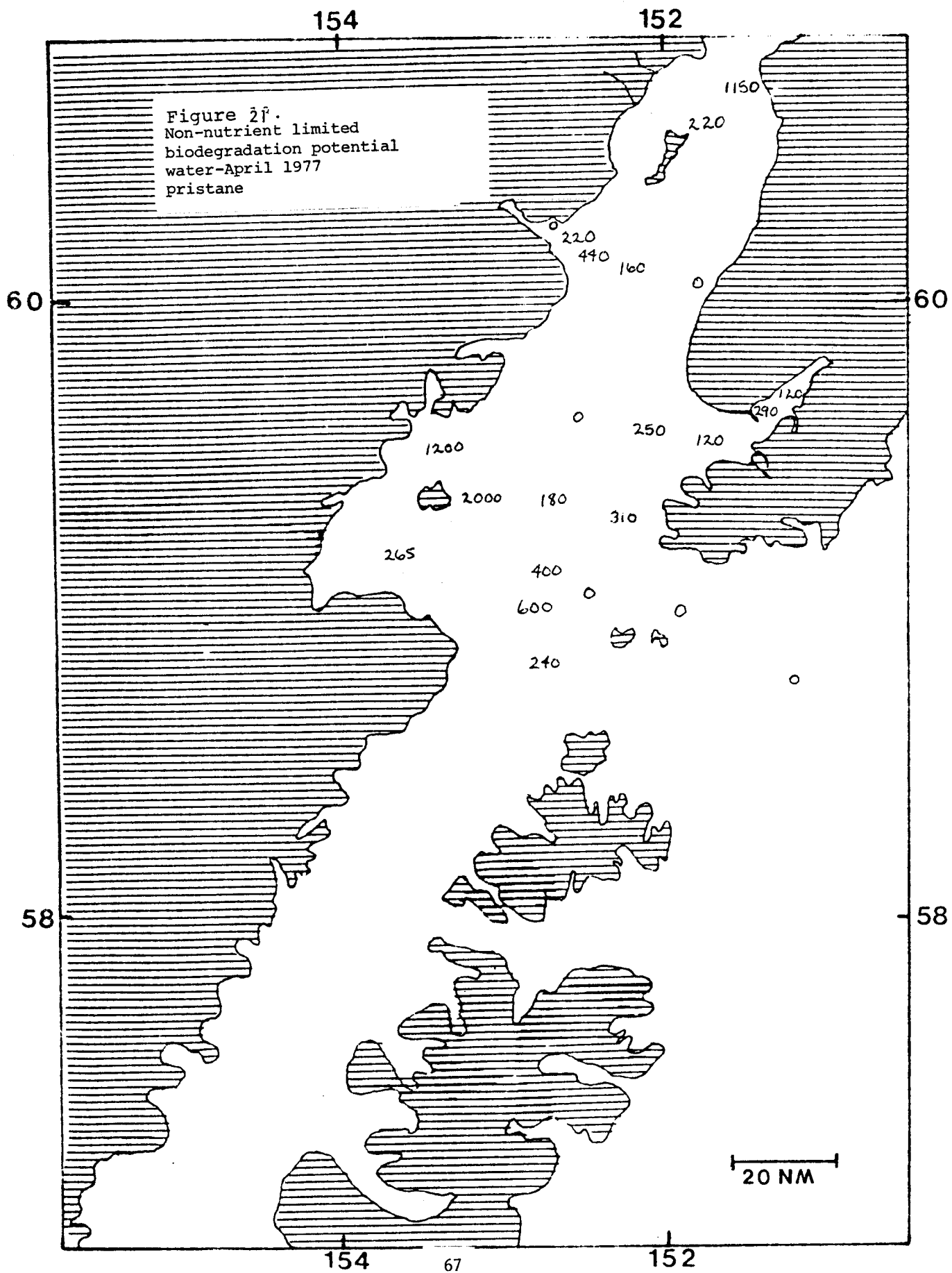
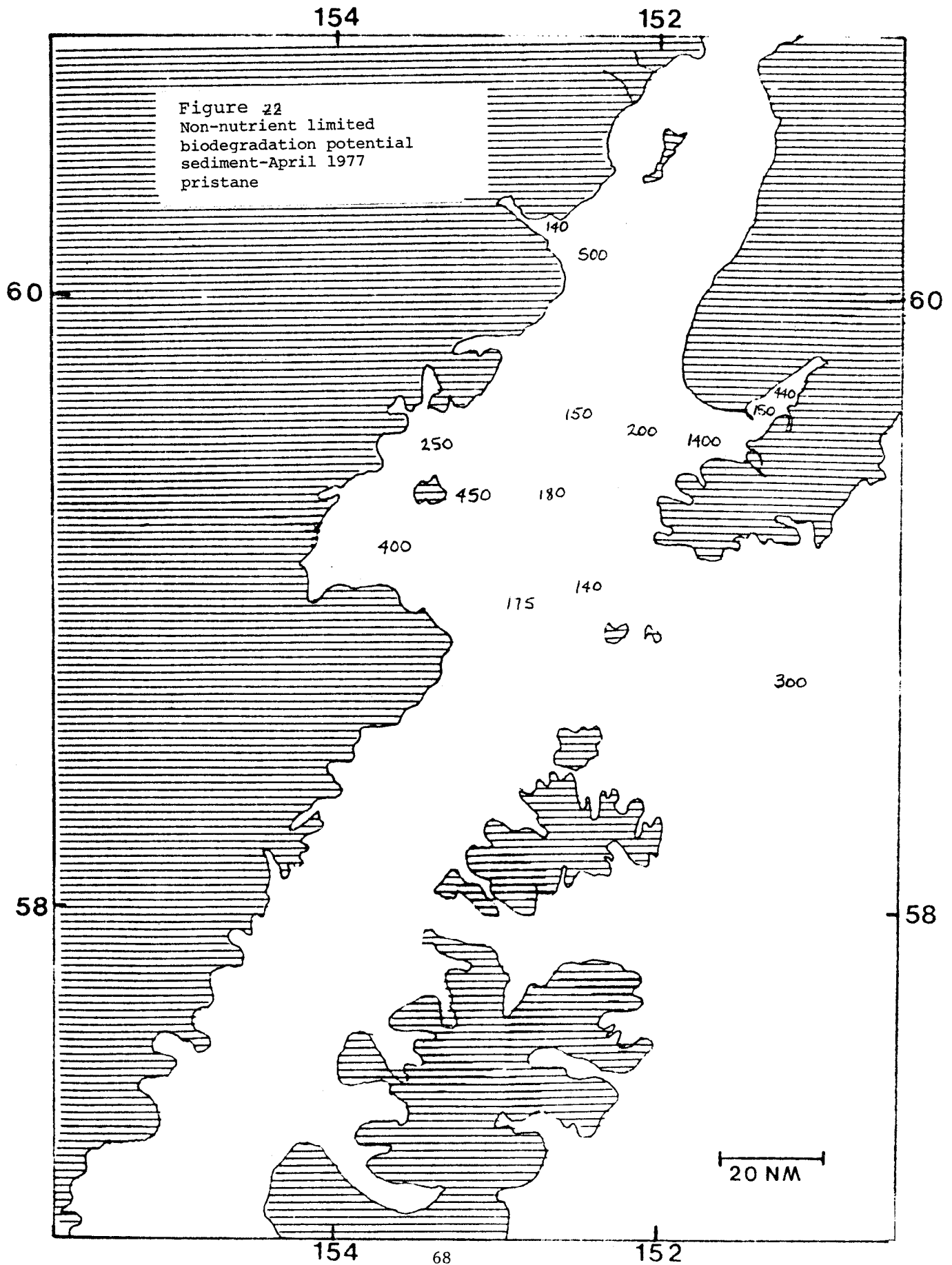
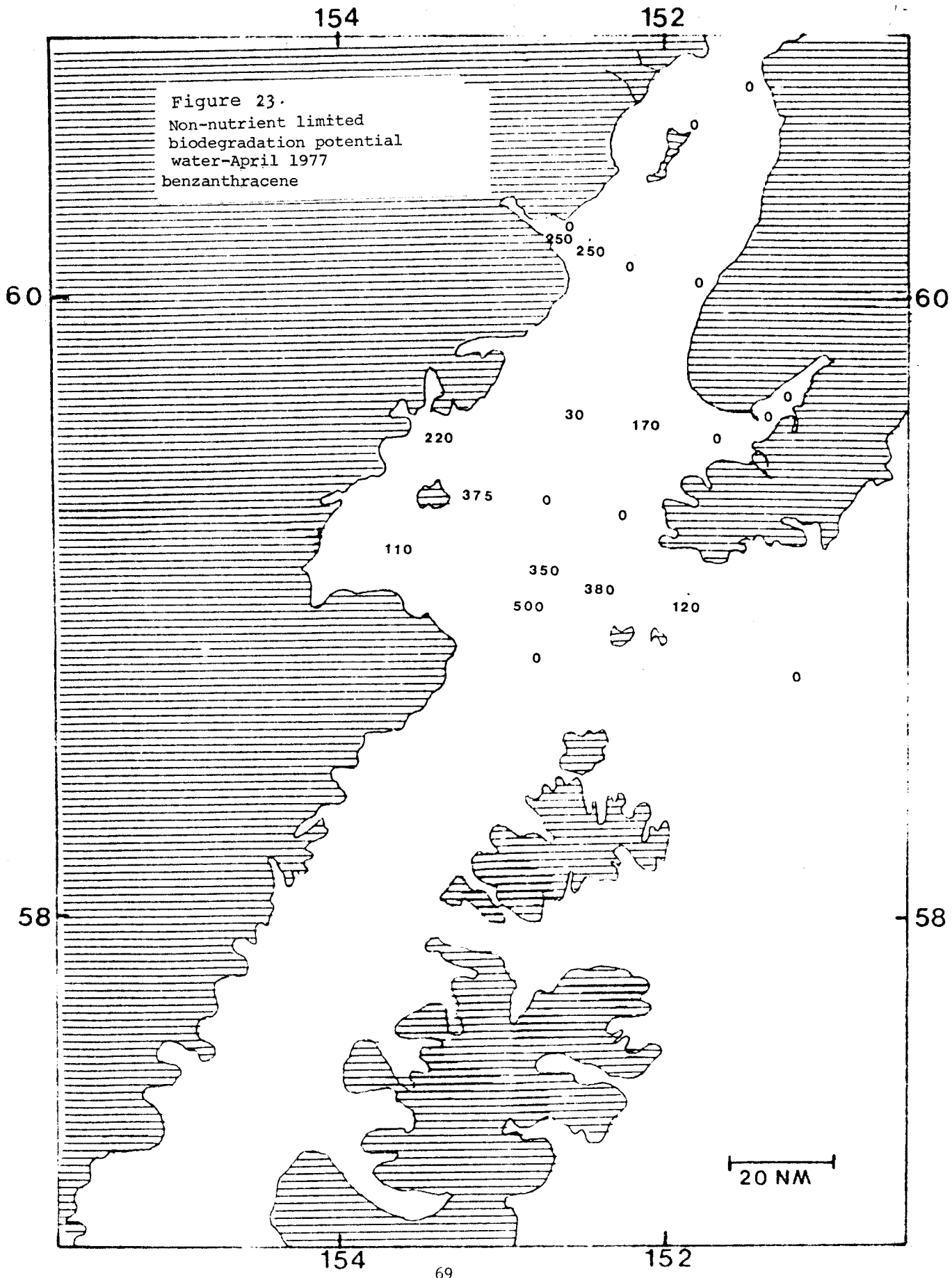


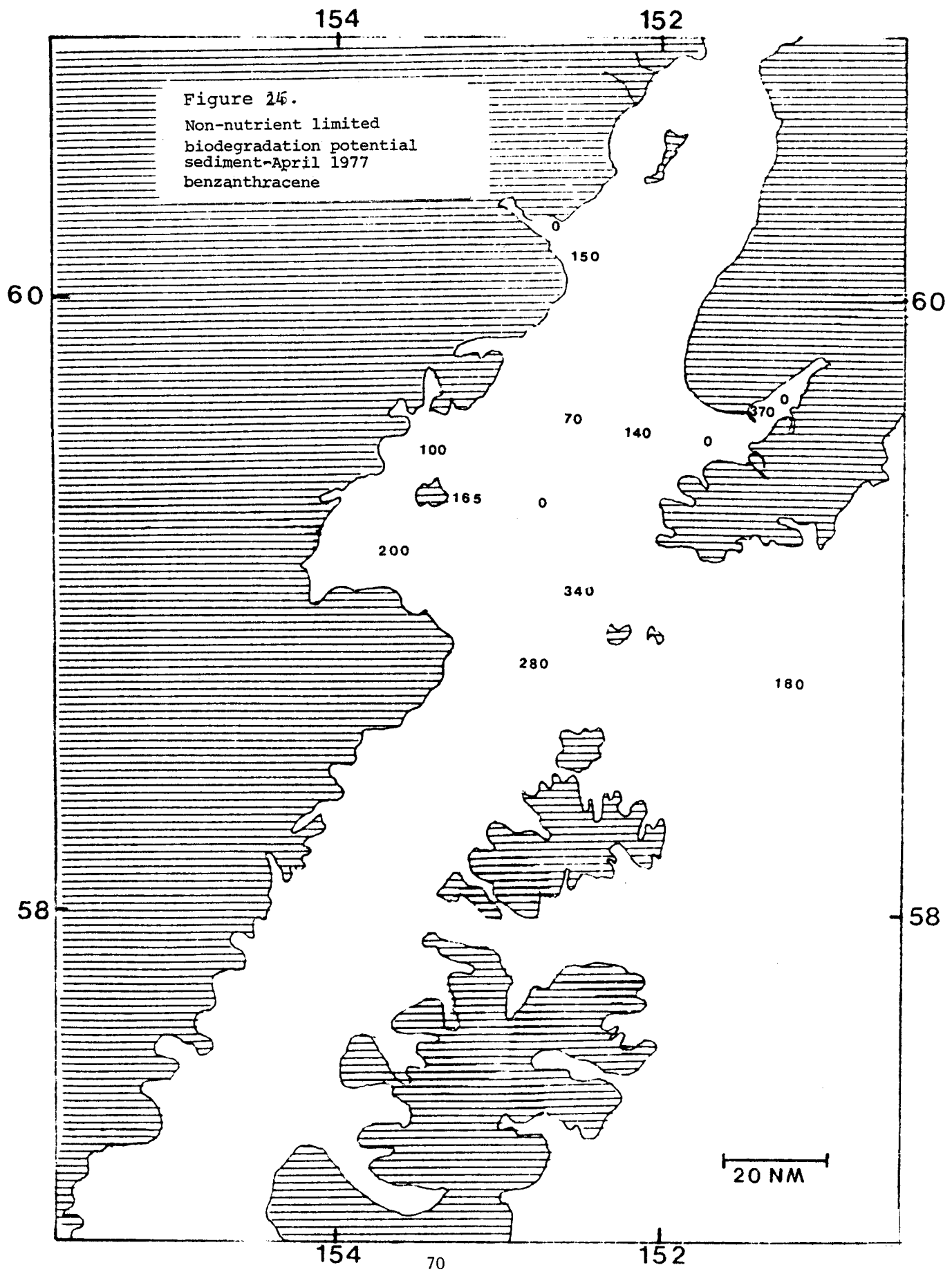
Figure 20
Non-nutrient limited
biodegradation potential
sediment-April 1977
naphthalene

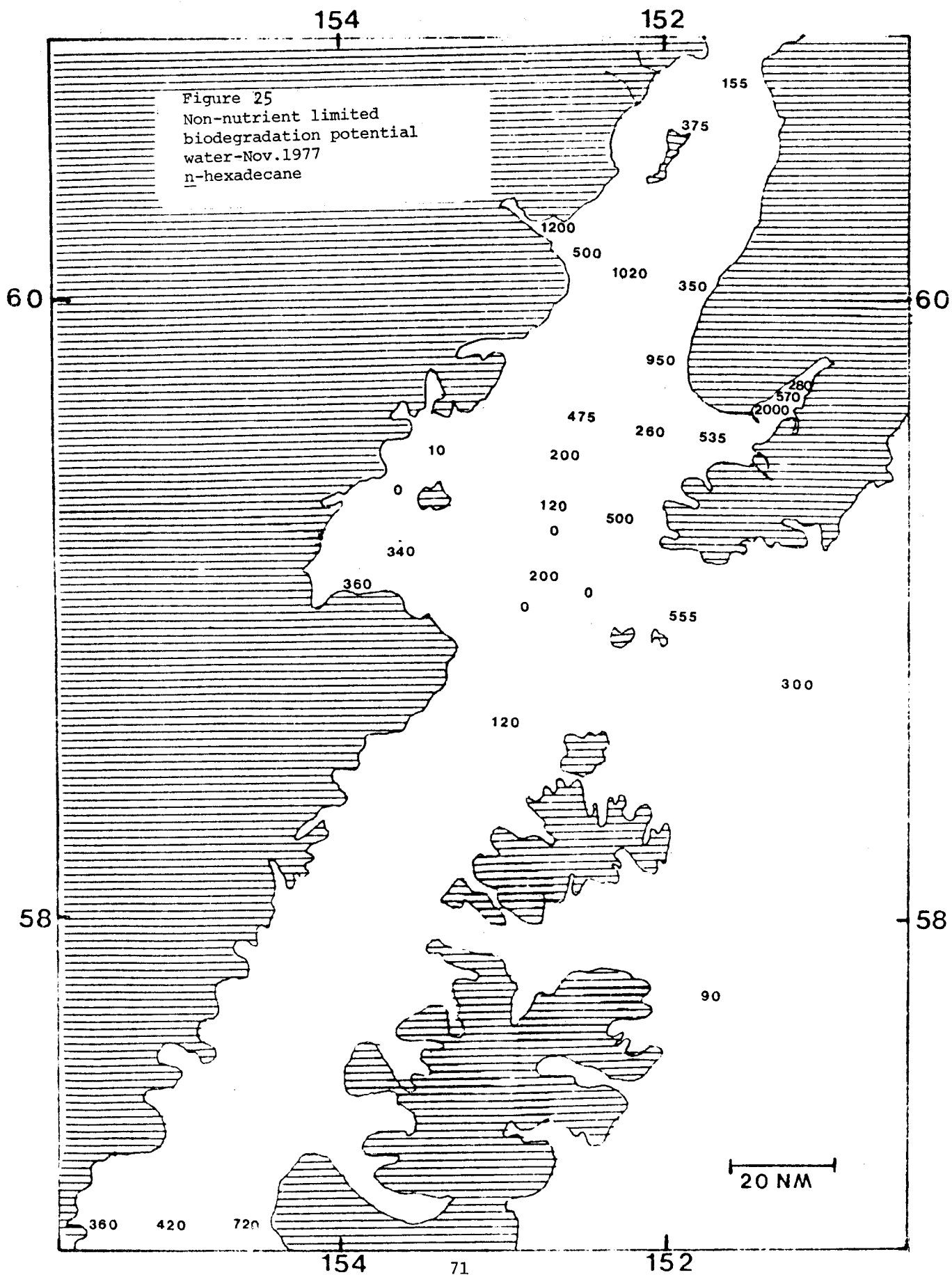


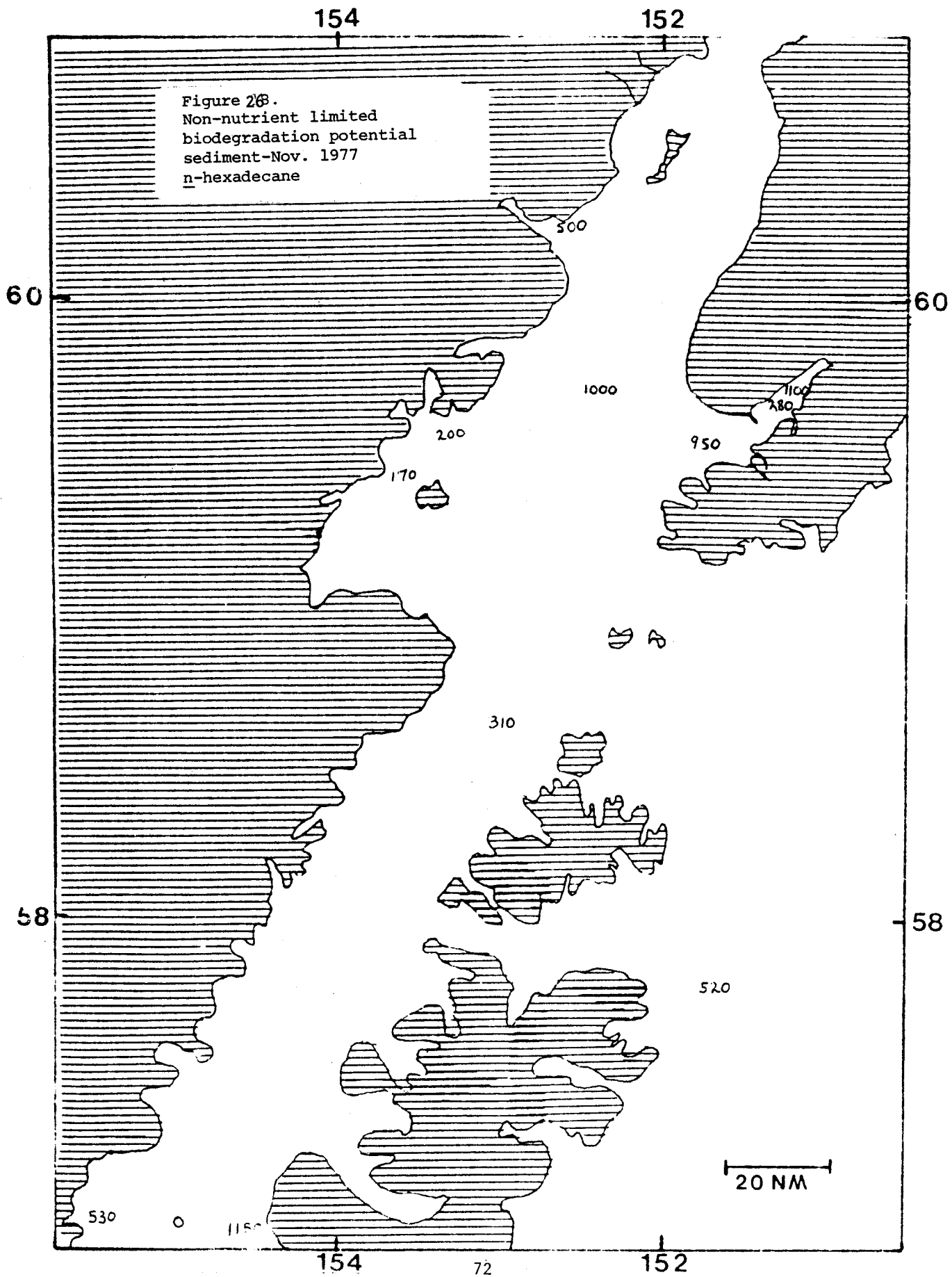


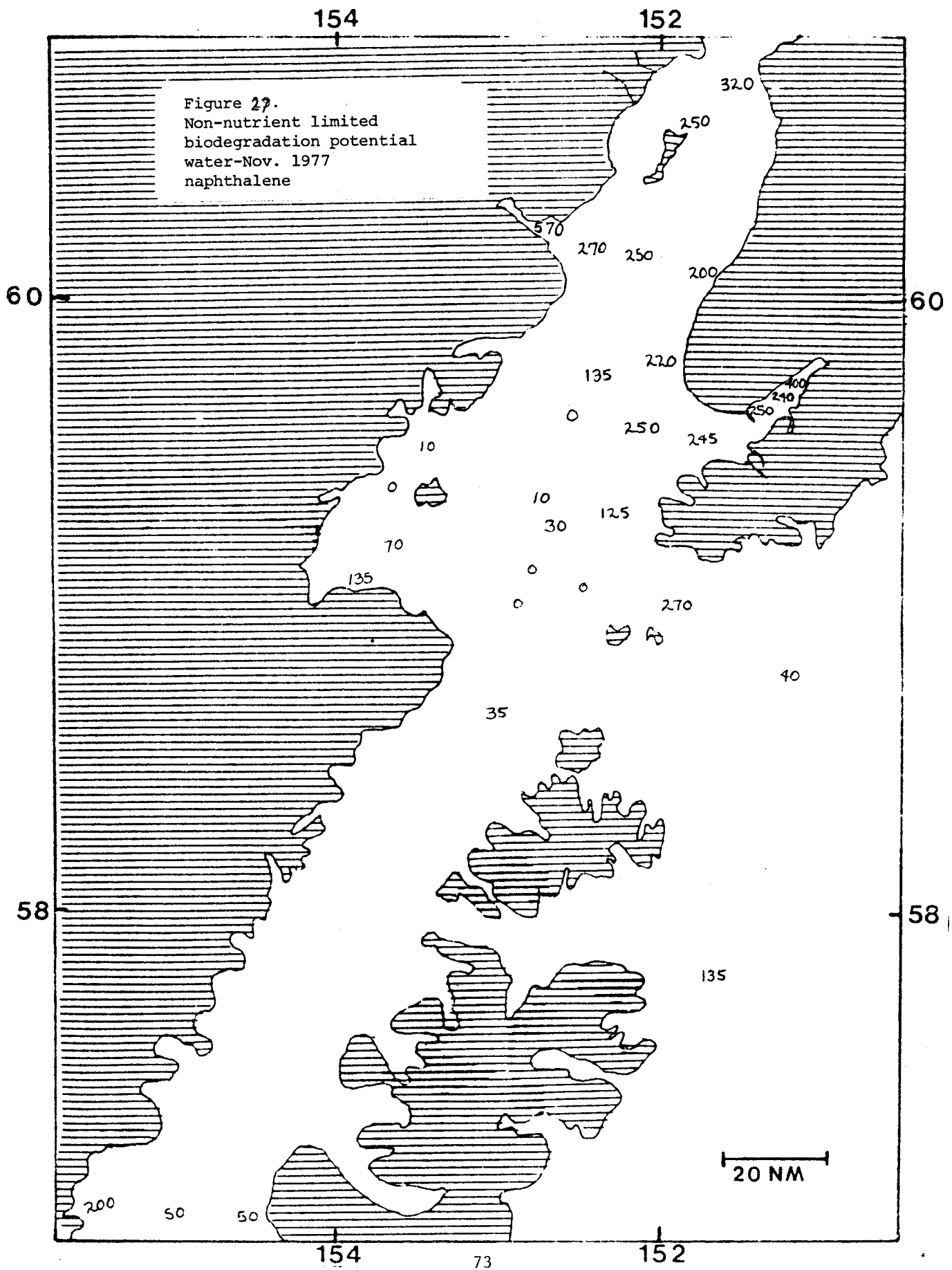


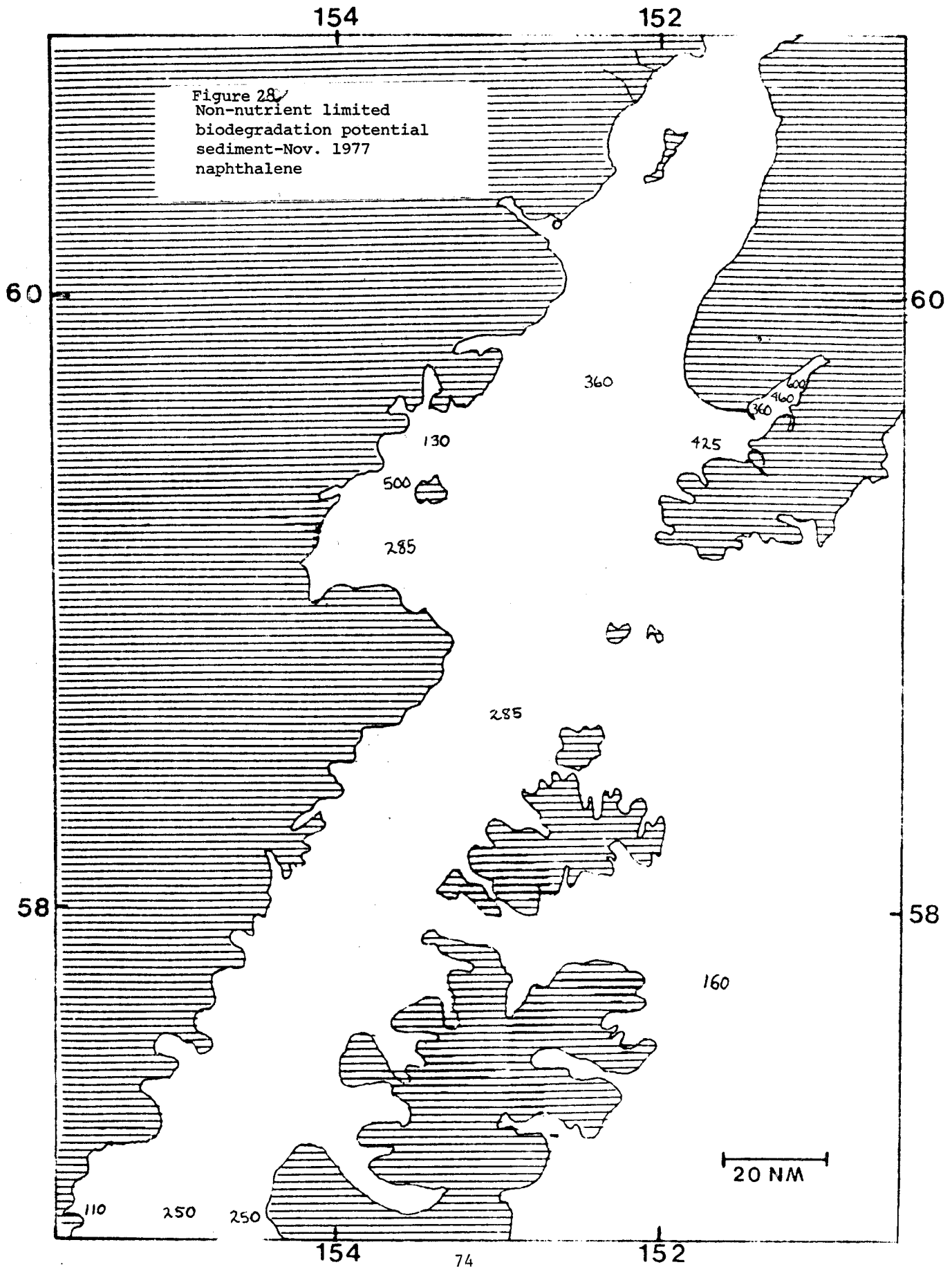


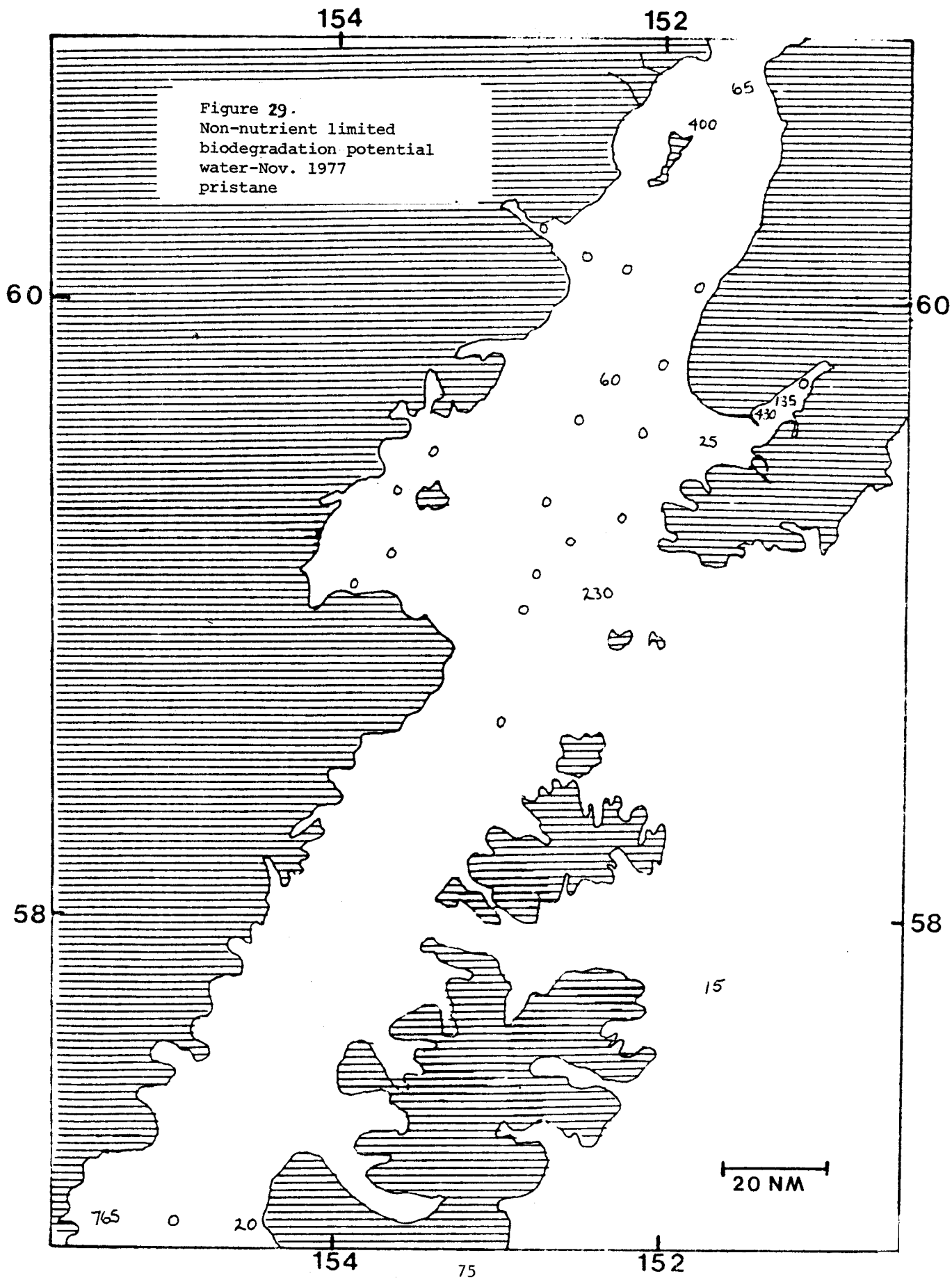


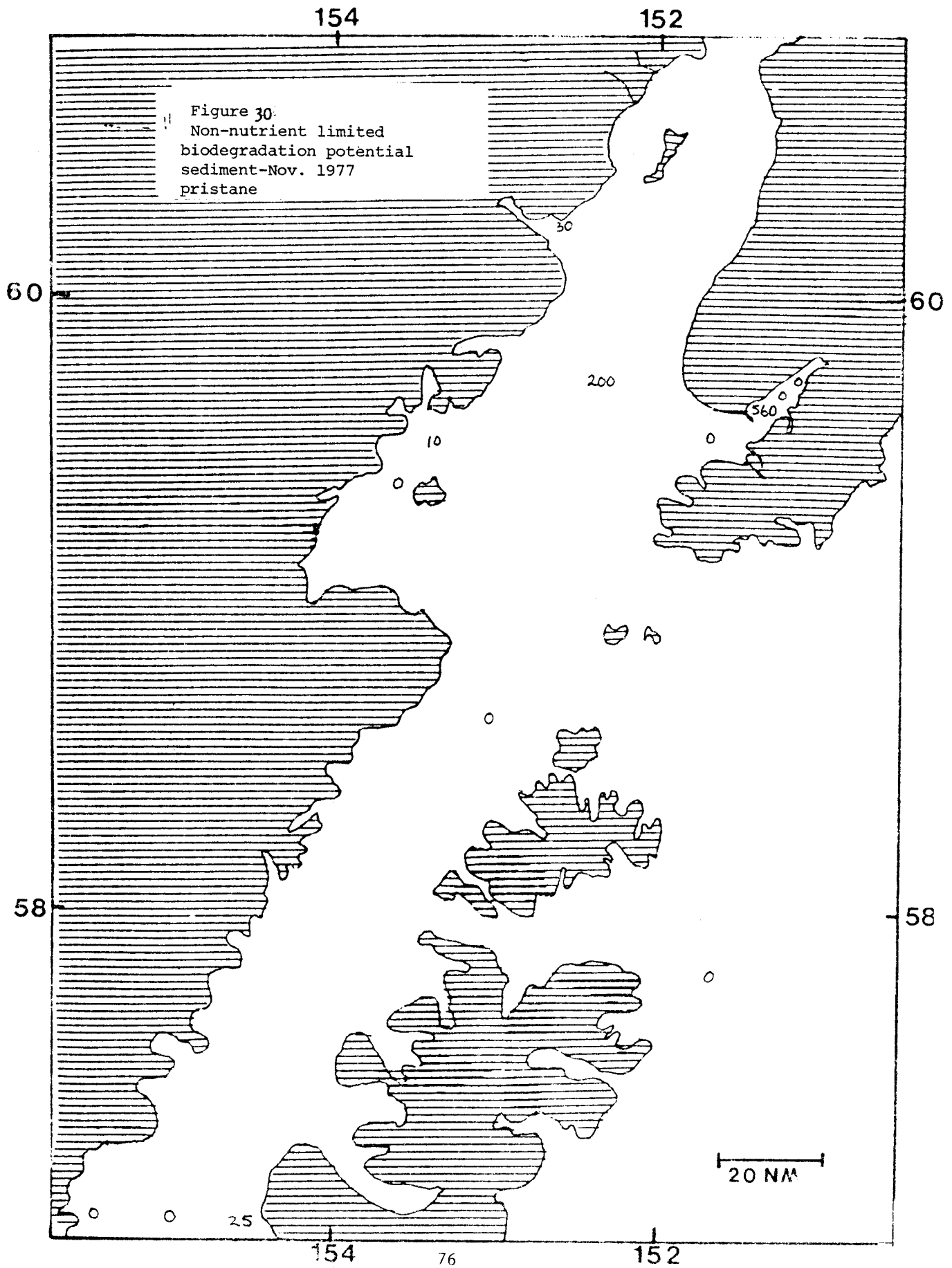












154

152

Figure 3p.
Non-nutrient limited
biodegradation potential
water-Nov. 1977
benzanthracene

60

60

130

300

5

50

110

58

58

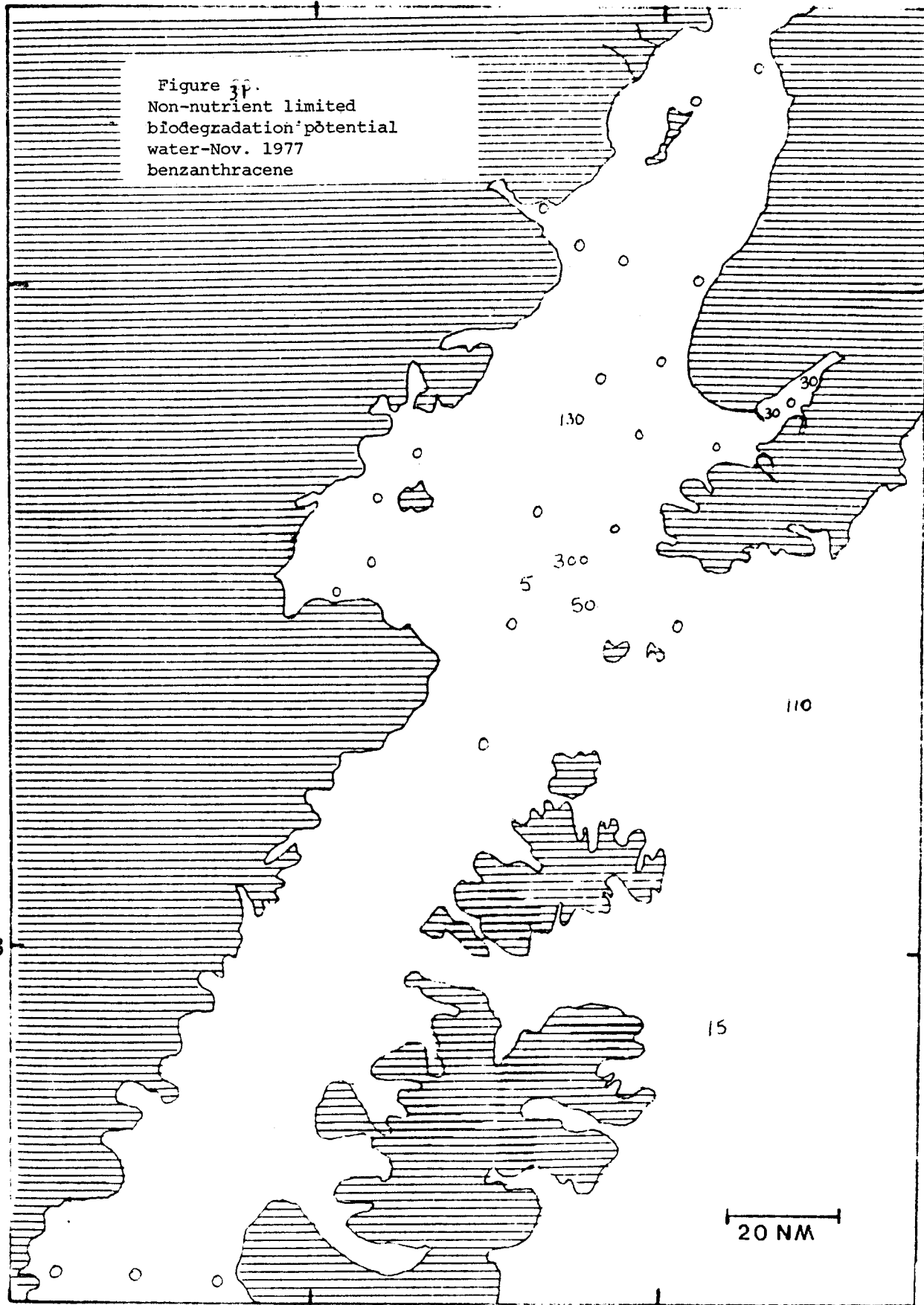
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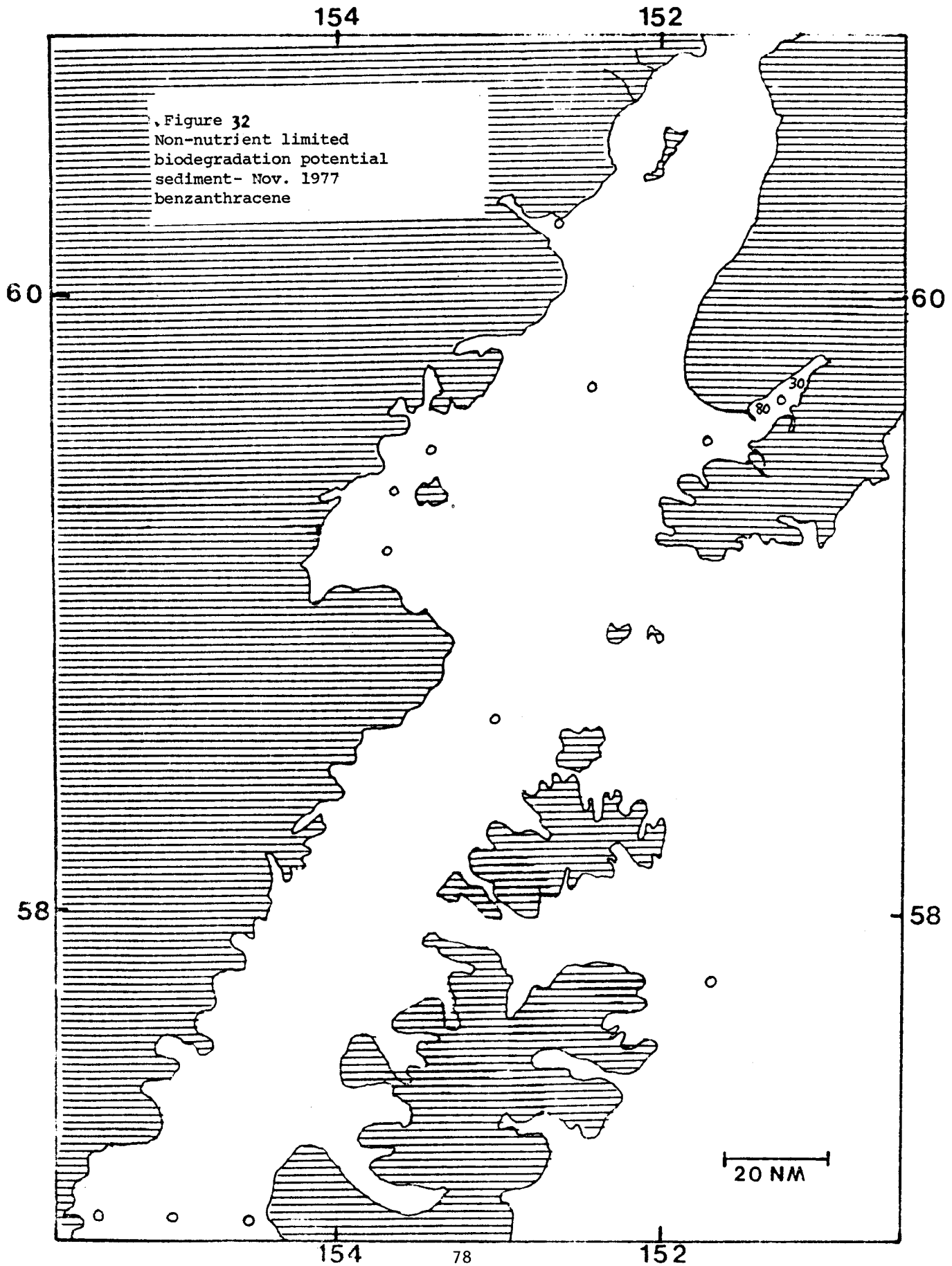
20 NM

154

77

152





biodegradation potentials were generally higher in Kachemak Bay and near Upper Cook Inlet than in the central areas of Lower Cook Inlet. This pattern is consistent with the distribution pattern of hydrocarbon utilizing microorganisms enumerated by the MPN method.

The use of crude oil as a carrier in the biodegradation activity measurements allows for sparing or cooxidation that would occur in actual oil contamination incidents. The four ^{14}C hydrocarbons used are representative of major classes of hydrocarbons found in petroleum, *i.e.* straight chain paraffins, branched paraffins, simple aromatics and polynuclear aromatics. Branching and multiple condensed aromatic rings appear to greatly reduce biodegradability.

The "real biodegradation rates" that would occur in oil contamination incidents, probably lie somewhere between the natural and non-nutrient limited biodegradation potentials. Whether the "real" rates approach the non-nutrient limited biodegradation potentials will depend on the degree of mixing in a given area allowing for nutrient replenishment. In areas of Cook Inlet, where there is high mixing energy, the real degradation rates should approach the non-nutrient limited biodegradation potential rates. The biodegradation potentials measured in our study are potentials for complete biodegradation to CO_2 and H_2O , *i.e.*, mineralization. Degradation of hydrocarbons to products such as alcohols or acids, which are intermediary degradation products, would

not be measured in our biodegradation potentials. The degradation rates that were measured in this study show total biodegradation of hydrocarbons are, thus, conservative in the estimate of hydrocarbons removed.

VII. Discussion and Conclusions

The numbers of viable microorganisms in Gulf of Alaska and Cook Inlet are clearly lower than in the Beaufort Sea. The reason for the low numbers of viable microorganisms in this area is still unknown. The taxonomic analyses show that Beaufort Sea microorganisms are distinctly adapted to the Arctic marine ecosystem. The taxonomic studies of hydrocarbon utilizers indicate that microorganisms respond to the presence of petroleum with an enrichment in the population of hydrocarbon utilizers. The diversities of heterotrophs in Cook Inlet were generally high, indicating that this area is generally not now under severe natural or man made stress.

The Most Probable Number enumerations of hydrocarbon utilizers and biodegradation potentials in Cook Inlet showed that Kachemak Bay, Kennedy entrance and Upper Cook Inlet had higher numbers of hydrocarbon utilizers, suggesting previous exposure to hydrocarbons, i.e. a source of hydrocarbons in these areas. The biodegradation potentials indicated that branched paraffins and condensed aromatic hydrocarbons would be degraded much more slowly in an oil than simple aromatics and unsubstituted paraffins. The biodegradation potentials also indicated that there are seasonal differences in rates of petroleum hydrocarbon biodegradation in Cook Inlet; rates in November being lower than rates in April.

VIII. Study Needs

There are several ongoing areas of research for this project which have not yet been completed. Our major ongoing research for

the Beaufort Sea area is the in situ ice and sediment studies of petroleum biodegradation described earlier in this report. We need to analyse the hydrocarbons in the residual petroleum already recovered in this study. We further need to continue recovering samples from this study site on a long term basis to examine the persistence of petroleum hydrocarbons in Arctic marine sediment. A similar in situ study is needed in a southern Alaskan area, eg. within Cook Inlet near Homer. Larger scale multidisciplinary oil spill experiments in nearshore Arctic and subarctic ecosystems are needed, but environmental protection requirements may preclude such experiments.

There remains a large portion of the Alaskan OCS area in which we know little or nothing about the microbial communities. If ice conditions permit, an area in the Beaufort Sea between Prudhoe Bay and the Canadian border will be examined this summer. This will still leave the Kodiak lease area, all of the Bering Sea and the Chukchi Sea, including Bristol Bay and Norton Sound areas without microbiological OCSEAP studies. It is necessary to examine microbial populations in these potential lease areas. Some of these areas are rich shellfishing areas which could be adversely affected by changes in microbial populations. Our studies have shown that there are several orders of magnitude difference in viable microorganisms between the Gulf of Alaska and the Beaufort Sea. We do not know if there is a gradual rise in population levels moving northward or an abrupt change somewhere north of the Aleutian Islands. Hydrocarbon biodegradation potentials should be known for these areas before petroleum

development. Also, there should be a taxonomic inventory or characterization of the microbial communities in these areas as will have been accomplished in Cook Inlet and the Beaufort Sea.

Studies are needed on the effects of petroleum hydrocarbons on microorganisms indigenous to OCS lease areas. We intend to examine some of the changes in microbial community structure in our in situ Beaufort Sea oil in sediment studies. We also intend to carry out some pilot studies to examine denitrification rates in sediment in Cook Inlet and the effects of oil on denitrification rates. We also plan to develop a new medium to determine what proportion of the indigenous heterotrophs in an area can tolerate the presence of petroleum hydrocarbons. These effects studies should be expanded to determine what levels of chronic hydrocarbon inputs can be tolerated by the microbial populations.

Many of these study needs have been outlined in previous reports and at meetings with NOAA OCSEAP staff. Adequate funding, program continuity and program coordination are essential for fulfilling microbiological information gaps.

IX. Summary of 4th Quarter Activities

The in situ oil under ice and oil in sediment experiments described earlier in this report, were established in Elson Lagoon in January. This was the only field work accomplished during this time period. Other activities included participation in the Lower Cook Inlet and Beaufort Sea synthesis meetings with subsequent report writing.

In the laboratory modifications were made in a gas chromatograph and a mass spectrometer installed to analyse the residual oil from the in situ oil experiments. Taxonomic testing was completed on microorganisms isolated from the November, 1977 sampling cruise in Cook Inlet. Determinations of hydrocarbon biodegradation potentials for these samples were also completed during the past three months.

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

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Date submitted

March 29, 1978

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

A. Objectives

In general terms, our main objectives during the last year were to obtain information about marine microbial function in the Beaufort Sea and Cook Inlet, Alaska and to obtain information about the effects of crude oil on specific processes. The microbial functions that we were primarily concerned with were, relative microbial activity and respiration in surface waters and sediments and rates of nitrogen fixation in the sediments. By both field and laboratory observations, we were to estimate the effects of crude oil on these processes.

B. Conclusions and Implications

1. One of the most important findings of the last year concerned the potential incorporation of crude oil into the sediments of Cook Inlet and the Shelikof Strait via surface oil spills in the Upper Cook Inlet. This conclusion can be reached by integrating the results of several studies; namely the results of Drs. Feely and Cline (RU#154), Miller and Allen (RU #436) and ourselves. It was previously assumed that since most of the suspended matter in these waters consists of glacial flour, there would be little absorption of crude oil onto these particles. It was also assumed, prior to our studies, that there was very little microbial activity to be found in these waters because of the shallow photic zone. Both assumptions appear to be incorrect.

Drs. Feely and Cline (1977) reported that preliminary data taken in the Upper Cook Inlet indicate that crude oil can be absorbed onto the suspended matter found in the waters of the Upper Cook Inlet. We found that the northern waters of the Cook Inlet showed very high levels of microbial activity (the highest values observed anywhere in the Cook Inlet and the Shelikof Strait) and that the level of activity was correlated with the turbidity of the water. Microscopic observation of the water samples revealed that most of the bacteria present were associated with these particles. The presence of these bacteria on these particles may explain why the glacial flour in the waters of Cook Inlet can absorb components of crude oil where as glacial flour itself might not have these properties. It is quite likely that the bacteria render the surface of these particles more hydrophobic, thus making it more likely that hydrocarbons will absorb onto the surface. If crude oil did become associated with the particles on the water column in this region, it would be important to determine where these particles would settle and what effect this might have. We have enough information at this time to partially answer these questions.

The study of surface water circulation patterns by Miller and Allen (1976) indicate that the net flow of this water mass is to the south

along the western edge of the inlet and then down through the Shelikof Strait. The studies of Feely and Cline (1977) have also shown that the suspended matter settles out of the water column as this water mass moves south. They felt that most of the particles settle out into the sediments of the Shelikof Strait. Figure 3 (page 795) of their report which illustrates the distribution of total suspended matter in surface waters bears a striking resemblance to the patterns of relative microbial activity and respiration percentages that we have observed in the surface waters of Cook Inlet and Shelikof Strait (Figs. 10,12,and 13). Our data as well as that reported by Feely and Cline indicate that as the northern water mass moves south, it is mixed with the open ocean water that is consistently moving to the northwest from the southeast entrances of the inlet. Our studies on relative microbial activity in the sediments indicate that these particles probably settle out of the water column in both the lower Kamishak Bay area as well as the Shelikof Strait. The information collected thus far indicates that there is a strong possibility that crude oil spilled on the surface in the upper Cook Inlet will end up in the sediments of Kamishak Bay and the Shelikof Strait.

Both our studies on the effects of crude oil on nutrient uptake and respiration in marine microbial populations and a recent study by Hodson et al. (1977) have shown that marine microbial populations are inhibited in the presence of crude oil. Dr. Atlas (RU #30) has observed that bacterial populations in Beaufort Sea water show an increase in numbers after extended exposure to crude oil; however, the species diversity markedly decreases. These data suggest that crude oil suppresses the growth of certain bacteria whereas it acts as an energy source for other bacteria thus altering the natural balance within the population. Data collected in our aquarium studies and Dr. Atlas's Beaufort Sea chemostat studies (not funded by OCSEAP), have shown that presence of crude oil does not adversely effect the ability of the microbial population to utilize certain soluble organic nutrients after extended exposure. This is only one of hundreds of roles bacteria perform in the marine environment.

Another function performed by marine bacteria is nitrogen fixation. We have studied the effects of crude oil on nitrogen fixation (acetylene reduction) in sediment samples taken from both the Cook Inlet and the Beaufort Sea. Studies made on samples in the field have shown little or no effect of crude oil on this process. In another series of experiments conducted on sediments transported to our laboratory at Oregon State have shown that crude oil might effect this process over extended exposure. In this series, the samples were amended with sucrose which usually enhances acetylene reduction. We felt that by speeding up the process, effects of crude oil on the growth of nitrogen fixing bacteria might be determined. The results of these experiments show that crude oil does not consistently inhibit acetylene reduction. This determination is relatively crude and extrapolations of these observations back to nature should be taken with caution. Further study should give a more precise picture of what crude oil effects might have in the environment. The

results of our sediment work in Cook Inlet have shown that nitrogen fixation is probably quite important to the detrital food chain in Kachemak Bay and in other areas in and around Cook Inlet.

Other possible effects of crude oil which have yet to be tested are the effect on bacterial colonization of detritus particles and the effect on the grazing of bacteria by protozoa and higher animals. Any detrimental effect in these functions would soon alter the biological productivity on all trophic levels (including all species of commercial importance).

In summary, it can no longer be assumed that crude oil spills in Cook Inlet would primarily impact only shoreline communities. There is a very real possibility that this oil could be absorbed onto the suspended particulate matter in the water column and carried to the sediments on the west side of the inlet and into the sediments of the Shelikof Strait. The possible long range effects of this on the biological productivity of Cook Inlet is yet to be determined.

2. The correlation between relative microbial activity and crude oil Biodegradation potentials.

Up to this time, we have assumed that areas which showed the highest relative microbial activity would be the ones which also would show the highest rates of crude oil degradation. The results of two studies conducted on marine sediments indicates that this might not be the case. We correlated the relative microbial activity with the release of $^{14}\text{CO}_2$ from sediments which were exposed to crude oil which contained labeled hexadecane and pristane under conditions where neither nitrogen nor phosphorous was limiting. A study on the Beaufort Sea sediments gave a correlation coefficient of -0.42 and a similar study in the Cook Inlet gave a correlation of 0.09. At least under these conditions, this assumption was not shown to be true. It must be kept in mind that the method used to determine crude oil biodegradation potentials was a highly artificial system and these measurements may not reflect relative degradation rates in situ. Dr. Atlas has observed that areas which show the highest concentrations of hydrocarbon utilizing bacteria in Cook Inlet are also the areas where we have observed the highest levels of relative microbial activity. Further study will be required before the best method of estimating in situ crude oil degradation potentials is identified.

3. Crude oil degradation potentials in various geographical locations

We measured the crude oil biodegradation potentials in the sediment samples that were sent to us by Dr. Carey (RU #6) from the summer, 1977 Beaufort Sea cruise. The highest rates were observed in the sediments taken to the east of Prudhoe Bay. Theoretically, the highest rates should be associated with sediments that have been chronically exposed

to hydrocarbons. If this is the case, these sediments have been exposed to elevated levels of either natural or polluting hydrocarbons.

14C Studies of crude oil degradation potential in Cook Inlet using the ¹⁴C labeled crude oil mentioned above, has not produced consistent geographical trends.

4. The presence of Desulfovibrio sp. in Cook Inlet sediments

Of potential interest to those planning to construct metal structures that come into contact with sediments is the presence of a group of anaerobic bacteria known as Desulfovibrio. The growth of these organisms may produce considerable corrosion of most metals. These organisms are also known to grow in drilling muds with the resulting release of H₂S. During the April, 1977 Cook Inlet cruise, the presence of these organisms was measured in a number of sediment samples. Except for the sediment analyzed from station 214, all samples tested showed significant concentrations of this organism in Cook Inlet sediments (10³ to 10⁶ bacteria per ml sediment). In most dilution tubes, growth was visible within four weeks incubation at 8 C.

II. Introduction

A. General nature and scope of the study

Our main objective has been to study the natural levels of relative microbial heterotrophic activity, respiration percentages and nitrogen fixation rates in natural microbial populations found in the Beaufort Sea and Cook Inlet under contrasting seasonal conditions. Our other objectives have been to evaluate the effects of crude oil on microbial activity and nitrogen fixation rates. These studies have been interpreted in light of other data that has also been collected on the same samples. These data include inorganic nutrient data, sample temperature, salinity and location; direct count, plate count and crude oil degradation potential data collected by both ourselves and Dr. Atlas, and microbial population characterizations made by Dr. Atlas.

B. Specific Objectives

1. Cook Inlet

a. To continue studies of relative microbial activity and respiration (mineralization) of natural microbial populations found in water and sediment samples. The samples were to be taken in such a way as to characterize these measurements both geographically and temporally. These studies were to fill some of the data gaps which still exist from past studies in this region. These data may also be used in the future to estimate the degree of perturbation caused by chronic crude oil input.

Characterization of water masses using microbial measurements which might be used to follow the movement of water masses within the Inlet.

b. To evaluate the extent of nitrogen fixation in the sediments and gut contents of animals found in this region and to determine what, if any, effect crude oil might have on this process. Significant impact on function of any process in the nitrogen cycle could have a profound effect on all trophic levels in the Cook Inlet.

c. To evaluate techniques which might be used to determine crude oil degradation in sediments.

d. To provide nutrient data on all water and sediment samples taken by both microbiological groups. These data are important in evaluating other data collected by us, especially data on N_2 fixation and denitrification.

2. Beaufort Sea

a. To obtain information concerning the effects of added crude oil on the natural microflora of the sediments. These studies were to include crude oil effects on microbial function as measured by uptake and respiration characteristics using several labeled compounds. These studies were also to include the study of nitrogen fixation and the effects of crude oil on this process. These studies are designed to simulate the introduction of crude oil into the sediments by buried pipeline breaks similar to those discussed during the February, 1977 synthesis meeting held in Barrow, AK.

b. To continue collecting data on relative microbial activity and respiration percentages in this region during the August-September, 1978 Glacier cruise in this region. Nitrogen fixation rates will also be estimated on sediment samples collected at the same time.

c. To provide nutrient data on all water and sediment samples collected by both Dr. Atlas and ourselves.

d. To estimate the effects of crude oil on natural microbial populations which undergo osmotic stress during freezing and thawing.

3. General

a. To coordinate our sampling efforts and experimentation with that of Dr. Atlas and his associates at the University of Louisville. This will minimize duplication of effort and maximize the usefulness of the resulting data.

b. To continue our laboratory studies at Oregon State University on the effects of crude oil on nitrogen fixation in marine sediments. We also plan to study crude oil degradation by bacteria isolated from the Beaufort Sea and the Cook Inlet.

II. Field and Laboratory Activities.

C. Relevance to problems of petroleum development

Our major area of concern is the interaction between the crude oil that might be accidentally spilled during the course of petroleum production in potential lease areas and the microorganisms present that might be perturbed by such a spill. Our studies will produce information about microbial function in these areas before extensive perturbation has occurred.

We are also determining what effect crude oil has on specific microbial function. These data will provide information about what types of microbial function are most likely to be impacted by a crude oil spill. In addition, field studies on the effects of crude oil on microbial function will provide information about what geographical areas are most likely to be impacted. These data along with data provided by other investigators can be used to locate areas which are particularly sensitive to crude oil perturbation. These data, in turn, can be used by planners and managers in both government and industry to better assess the potential risks involved and better estimate what measures must be taken to minimize risks to the environment.

Another study is being made on the degradation of crude oil by microorganisms. It is important to determine the rate of crude oil degradation under a variety of environmental conditions so that under a given set of circumstances, a prediction of crude oil residence time can be estimated. This is a very complex problem because so many variables effect this process. This information must be obtained if estimates of potential impact are to be made. We have conducted some studies in this area but the majority of this work is being conducted by Dr. Atlas and his associates.

III. Current state of knowledge

Most of the information that is known about the distribution and relative activity in the sediments and waters of the Beaufort Sea and the Cook Inlet has been generated by both Dr. Atlas (RU #29 and 30) and ourselves. This information is available in our past reports to NOAA under this project. Information about the effects of crude oil on microbial activity and nitrogen fixation in these two regions can also be found in our past reports. To our knowledge, there have been no other papers published on the effects of crude oil on nitrogen fixation in marine sediments; however, there has been a recent study published on

the effects of crude oil on the uptake and respiration of labeled glucose by microrganisms in seawater near Vancouver Island, B.C. (Hodson et al., 1977).

There is a great deal of information that has accumulated concerning crude oil biodegradation processes; however, there have been relatively few field studies on this process in Alaska waters. Besides Dr. Atlas's and our work, the other studies that have been published are Kinney et al. (1969); Arhelger et al. (1977); and Robertson et al. (1973).

IV. Study areas

During the past year we have conducted three field studies; one in the Cook Inlet in April, 1977, another in November 1977 and a third in the Beaufort Sea in January, 1978. The latter study was conducted as a part of the crude oil degradation study in Elson Lagoon near Barrow, AK. In September, 1977, we received a set of 20 sediment samples that were taken by Dr. Carey's group during the Glacier Beaufort Sea cruise. The stations that were sampled during these operations are illustrated in Figs. 1 to 4. The exact locations of the sampling sites are given in Tables 2 to 4.

V. Methods

A. Sampling procedures

1. Cook Inlet Cruises

The water samples were taken in sterile Niskin plastic water sample bags fitted in Niskin "butterfly" water samplers. With the exception of the depth profile studies made during the November cruise, all water samples were taken within two meters of the surface and were processed within two hours after they were collected.

All sediment samples except those taken at the beach stations were obtained by a Van Veen grab. Approximately 150 grams of surface sediment were taken from the top two to four cm. Portions of these samples were used to determine percent respiration, relative microbial activity and nitrogen fixation rates within four hours of their collection.

2. Beaufort Sea (Glacier cruise)

Sediment samples were taken using a Smith-McIntyre grab. The subsamples were taken as described above.

3. Beaufort Sea (Elson Lagoon, Jan., 1978).

Water samples were taken through hole drilled through the ice using a Niskin "butterfly" water sampler. The samples were kept at in situ

temperature while they were transported back to the Naval Arctic Research Laboratory (NARL). The samples were processed within 5 hours after they were collected. Sediment samples were taken directly from the bottom by divers.

B. Relative microbial activity and percent respiration determinations

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

After addition of subsample, the 50 ml serum bottles that were used for reaction vessels were sealed with rubber serum bottle caps fitted with plastic rod and cup assemblies (Kontes Glass Co., Vineland, N.J.:K-882320) containing 25 x 50 mm strips of fluted Whatman #1 chromatography paper. The samples were incubated in the dark within 0.5 C of the in situ temperature. After the incubation period, the bottles were injected through the septum with 0.2 ml of 5N H₂SO₄ in order to stop the reaction and release the ¹⁴CO₂. After the addition of the acid, 0.15 ml of the CO₂ absorbent, β-phenethylamine, 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 CO₂. The filter papers containing the ¹⁴CO₂ were removed from the cup assemblies and added to scintillation vials containing 10 ml of toluene based scintillation fluor (Omiflour, New England Nuclear).

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

In the sediment samples, a 1.0 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-¹⁴C L-glutamic acid with a specific activity of 237 mCi/mole (Amersham-Searle) was used in all water samples giving a final concentration of 5.4 ug/liter. Glutamic acid with a lower specific activity (10 mCi/mole) was used in all sediment samples except those collected at Elson Lagoon in January, 1978. In these sediments, the higher specific activity glutamic acid was used. U-¹⁴C D-glucose with a specific activity of 328 mCi/mole (Amersham-Searle) was used in all water and sediment samples. The final concentration used was 3.8 ug/liter. The ¹⁴C sodium acetate used in the April Cook Inlet sediment samples had a specific activity of 54 mCi/mole and a final concentration of 6.9 ug/liter.

Triplicate subsamples were analyzed for each sample and the results reported here 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 associated with the CO₂ fraction by the total amount of labeled carbon taken up by the cells (both cell and CO₂ radioactivity) and multiplying this ratio by 100. All samples were incubated in the dark at a temperature within 0.5 C of the in situ temperature.

C. Direct Cell Counts

Ten ml of seawater was fixed in the field laboratory by adding it to 0.6 ml of membrane (0.45 µm) filtered formaldehyde (37%). The vials containing the fixed water samples were sealed and stored until they could be counted in our laboratory at Oregon State University. In the sediment studies, the final dilution of the sediments in the heterotrophic potential studies was used and treated the same as the seawater samples.

From 5 to 17 ml of sample were filtered through a 0.2 µm Nuclepore filter. When a relatively high number of organisms was present, the samples were diluted with membrane filtered artificial seawater. The number of organisms per field was kept within acceptable limits and the volume filtered was kept above 5 ml. Controls were run using filtered artificial seawater and all of the reagents used in the staining and mounting procedure. These counts were no more than 5% of those found in the samples and were considered insignificant.

The staining procedure used was that of Zimmerman and Meyer-Reil (1974). This procedure involves staining the cells trapped on the membrane filter with acridine orange and then destaining with isopropyl alcohol. The membranes were dried and mounted on microscopic slides. Immersion oil was used as the mounting medium.

The bacterial cells were counted using a Zeiss IV F1 epi-fluorescence condenser microscope fitted with filters KP 500, IP 490, FT 510, and LP 520. The eyepiece used was Kpt W 12.5 x and the objective was plan 100 x. Approximately 50 restriction fields were counted per sample. Representative fields were counted from the center of the membrane filter to the outside edge of the filtration circle.

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

D. Desulfovibrio concentrations in sediments

Subsamples of a 10^{-3} dilution were added to screw top test tubes so that no air would be introduced to the semisolid anaerobic medium. Serial dilutions were made in the range of 10^{-5} to 10^{-6} in most cases. The medium used was a modification of marine desulfovibrio medium PM 10 E (ZoBell and Morita, Deep Sea Research 3:66-73, 1955). This medium contained the following ingredients: Na formaldehyde sulfoxylate, 0.1 g; K_2HPO_4 , 0.5 g; NH_4Cl , 1 g; Na_2SO_4 , 1 g; $CaCl_2 \cdot 2H_2O$, 0.1 g; $MgSO_4 \cdot 7H_2O$, 1 g; agar, 3 g; H_2O , 1 liter. The medium was adjusted to a pH of 7.5 and sterilized by autoclaving at 30 psi for 15 min. Prior to use, the medium was steamed for one hour to drive off gases within the medium.

The test tubes were kept at 4 C or below during transport to our laboratory at Oregon State University. These tubes were incubated at 8 C for four weeks before they were checked for growth. The incubation temperature was then increased to 25 C and checked again for growth after an additional two weeks incubation.

E. Crude oil degradation potential in sediments

These experiments were conducted on sediment samples that were brought back to the laboratory at Oregon State. The samples were kept at or below 4 C between the time they were collected and the start of the experiment. Subsamples of the sediment were diluted 1:10 in seawater salts supplemented with Nitrogen and Phosphorus. Ten ml of this dilution were placed into 50 ml serum bottles. To this was added 0.1 ml of Cogk Inlet Crude oil which contained 7.5×10^{-3} μCi pristane and 7.5×10^{-3} μCi hexadecane. The bottles were sealed with rubber stoppers fitted with a plastic bucket containing fluted filter paper. Duplicate subsamples and one acidified control were used to determine crude oil mineralization in each of the sediments analyzed. The samples were incubated at 8 C for 31 days. At the end of the incubation period, the evolved $^{14}CO_2$ was collected and assayed for radioactivity.

F. Nitrogen fixation and nitrogen fixing bacterial concentrations in sediments

Nitrogen fixation in the sediments was determined in the field by using the acetylene reduction method (Stewart *et al*, 1967, Proc. N.A.S.-U.S. 58:2071 - 2078). Ten ml subsamples of sediment were added to respective 50 ml serum bottles: one control and two duplicate samples were used for each analysis. After the bottles were sealed with a rubber stopper, the samples were gassed for one min with He at a flow rate of 10 cc/sec. Ten ml of acetylene was then added to each bottle and the bottles were allowed to incubate for 24 hr before incubation was terminated with one ml of 50% trichloroacetic acid (TCA). The controls were treated in the same way before incubation and were used to determine

the amount of ethylene that was released abiotically. After the incubation was terminated, the tops of the rubber stoppers were sealed with silicone cement. The bottles were kept at or below 4 C until they could be assayed for ethylene in our laboratory at Oregon State University. The analysis for ethylene was made on a Hewlett Packard model 5830A gas chromatograph. The column used was 1.9 meter of 1/8" stainless steel tubing packed with Porapak R 80-100 mesh and the oven temperature was 40 C. The carrier gas was nitrogen flowing at a rate of 29 cc/min. The resulting levels of ethylene were normalized using incubation times and gram dry weight conversions.

During the November, 1977 Cook Inlet cruise, we collected a number of benthic organisms to determine if any nitrogen fixation activity was associated with them. As soon as the animals were collected, they were placed on ice until they could be processed. Either whole animals or just the gut (in larger animals) were blended in a blender fitted with a microcup. Ten ml of the blended tissue was added to a 50 ml serum bottle and assayed for nitrogen fixation rates as described above. These samples were fixed with two ml of saturated HgCl₂ solution at the end of the 24 hr incubation period. The samples were incubated at temperature within 0.5C of that found in situ. After the samples were fixed, they were returned to the laboratory for gas analyses and dry weight determinations.

The studies on the effects of crude oil on nitrogen fixation in sediments which had been exposed to sucrose to stimulate this process were conducted in our laboratory at Oregon State. The sediment samples used in these experiments were kept at or below 4C during transport and storage. Most of these determinations were made within four weeks after the samples were collected. Sucrose at a final concentration of 10⁻³M was used to stimulate nitrogen fixation because it has been found to be one of the most effective energy sources for that purpose by both ourselves and other investigators (Keirn-Brezonik, 1971).

Unless otherwise indicated, the incubation temperature used was 5C. The incubation times varied from 4 to 7 days. All rates were calculated in terms of pmoles nitrogen fixed per gram dry weight of sediment per hour. A conversion factor of 0.33 was used to calculate the amount of nitrogen fixed from the amount of ethylene produced.

G. Crude oil effects on uptake and respiration of labeled organic compounds

These studies included both field and laboratory observations. Field observations of crude oil effects were made on sediment samples collected during the recent Elson Lagoon study and on water samples collected during the November Cook Inlet cruise. The observations made on sediments collected during the Glacier cruise in the Beaufort Sea and the sediments collected during the November Cook Inlet cruise were made

in our laboratory at Oregon State. The method used to handle these sediments has been described above.

During these studies, we analyzed the effects of crude oil (both Prudhoe Bay and Cook Inlet crude), "weathered" crude oil and aqueous extracts of crude oil. The "weathered" crude oil was prepared by evaporating off the lower boiling point hydrocarbons under a stream of air. This produced a crude oil that was reduced in weight by 27% and one that had lost virtually all of the lower boiling point hydrocarbons as determined by gas chromatography. The crude oil aqueous extract was prepared by adding 5 ml of crude oil to 1 liter of sterile artificial seawater solution and gently stirring this mixture at 5°C for 24 hours. The aqueous phase was decanted and dispensed into sterile dilution bottles.

H. Enumeration of bacteria using various agar media

During the course of these studies, a number of different media were used to determine the relative concentrations of various types of bacteria. Two media were used to determine "total" numbers of bacteria present in sediment samples. These were marine agar 2216 (Difco) and Lib X agar. Lib X agar is made up of 1.2 g Bacto-yeast extract, 2.3 g Trypticase (BBL), 0.3 g sodium citrate, 0.3 g L-glutamic acid, 0.05 g sodium nitrate, 0.005 g ferrous sulfate, 33 g Rila marine salts dissolved in 1 liter of distilled water, and 12 g agar. The pH is adjusted to 7.5 and autoclaved. In the studies designed to determine the effects of crude oil on bacterial growth, 5 ml of crude oil was added to autoclaved Lib X agar and then mixed in a blender. Uninoculated control plates were always used to insure that no contamination had occurred during this process.

The concentration of Vibrio sp. was determined on TCBS agar (Difco) plates. The concentration of anaerobic bacteria was determined on Glucose agar plates which were made up of a 1:10 dilution of Lib X medium with 0.5% glucose. The Tween 80 medium was Sierra Agar with marine salts and Tween 80. The concentration of lipase producing organisms was determined by counting only those colonies which showed a positive lipase reaction on these agar plates. The concentration of obligate anaerobic bacteria was calculated by subtracting the total Vibrio count from the Glucose agar count. The percent of obligate anaerobic bacteria, the percent lipolytic bacteria and the percent fermentative bacteria were all calculated relative to the Lib X counts.

I. Statistical analysis

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

$$r = \frac{NXY - (EX)(EY)}{[NEX^2 - (EX)^2] - [(NEY^2 - (EY)^2]}$$

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

VI. Results

A. Experiments designed to expose potential problems in methodology

Many of the studies reported here involve the use of relatively new techniques or new applications to standard techniques. As a result, a great deal of testing was required to insure that the observations that we were making were not prejudiced by faulty assumptions or techniques. The following is a description of these studies.

1. Problems in sampling error and sample handling.

a. It has been reported that sterile plastic sample bags that we employ in obtaining water samples may alter microbial function in the collected sample. We checked to see if these samplers affected glucose and glutamic acid uptake and respiration by comparison to similar samples taken by other means. We observed no alteration in function.

b. In some cases, we have had to store sediment samples so that further analyses could be conducted after the termination of the field period. We have attempted to keep these samples at or below the in situ temperature until they can be processed. Comparisons between field laboratory data have shown that in general, nitrogen fixation rates decrease and glucose and glutamic acid uptake rates increase with increased incubation time. These observations reconfirm the validity of our usual practice of conducting as many observations as possible in the field on samples as soon as they are collected.

c. Since we are not able to carry our gas chromatograph into the field, we had to devise some method of storing and transporting gas samples from our nitrogen fixation studies. After trying a number of different techniques we found that if the reaction was run in a 50 ml serum bottle, the reaction could be terminated by injecting 1 ml of saturated $HgCl_2$ solution through the septum. If the septum was then sealed with a silicone cement, the sample could be stored at normal refrigerator temperatures for several weeks

without significant gas loss. We found that trichloroacetic acid (TCA) could also be used to terminate the reaction successfully but in sediments with large amounts of carbonate, CO_2 was released causing a positive pressure within the reaction vessel.

2. Problems in assaying radioactivity

a. The beta rays which emit from ^{14}C labeled compounds are very easily blocked. For this reason, we wanted to make sure that radioactivity measurements we were making in the cell fraction within sediments were not significantly affected by the presence of the sediment particles themselves. This was done in two ways; by running a dilution series and looking for changes in radioactivity and by combusting all of the cellular material to CO_2 and assaying this fraction without interference from the sediment particles. In the first experiment, a sediment with a ^{14}C labeled natural microbial population was diluted by a factor of 100, 500, 1,000, 5,000, and 10,000. The DPM observed at each of these dilutions were corrected for the respective dilution factor and compared. The first two dilutions did show significant quenching but there was no significant quenching observed above the 500 : 1 dilution. Since a 1,000 dilution was routinely used in these studies, it was felt that this was not a serious problem.

It occurred to us that the above test would still not exclude the possibility that sediment particles might still be interfering with the method used to assay radioactivity. A better but much more difficult test would involve combusting the sediment samples containing labeled microorganisms. In this way all of the radioactivity associated with the cells would be oxidized to CO_2 which could be trapped and assayed separately from the sediment itself. Such a series of tests have been initiated on sediment samples we have collected from both the Beaufort Sea and the Cook Inlet. Although not all of the tests have been completed as of this date, preliminary results indicate that as much as 38% of the radioactivity in our sediment cell fractions might be absorbed by sediment particles so that they are not counted by liquid scintillation. Although the data we have reported on relative levels of microbial activity in the sediments are still valid, actual rates would undoubtedly be higher than those we have reported and the respiration percentages would be lower if further tests confirm our original observations. These changes would if anything further exaggerate comparisons we have made between levels of activity and respiration differences in water and sediment microbial populations and does not substantially effect the conclusions we have drawn from these data. We are currently exploring different methods to separate the cells from the sediment either by solubilizing the cellular components or oxidizing the samples to CO_2 prior to assay.

b. In the experiments concerning the effect of crude oil on glucose and glutamic acid respiration, we felt that there might be some problem recovering all of the CO₂ from the aqueous phase because of the film of crude oil on the water surface. A series of 12 identical subsamples containing labeled carbonate dissolved in artificial seawater was used. One tenth of a ml of crude oil was added to one half of the samples. The CO₂ was collected in the usual manner and the resulting radioactivity in the two sets was compared. No differences were observed therefore we concluded that the presence of crude oil in this system did not materially effect the recovery process.

c. It is known that crude oil can act as a significant quenching agent in the scintillation cocktails that we use to assay radioactivity. We originally thought that any crude oil that remained on the filters (used to retain the cell fraction) would dissolve into the toluene fluor. Once in the fluor, the quench caused by the crude oil should be reflected in a depressed channels ratio which would then be translated into a corrected count. The results of our earlier experiments indicated that this might not be the case. We checked this by adding crude oil to radioactive cells and assaying for the radioactivity on the membrane filters after filtration. The activity was lower in these samples than in the controls indicating that enough crude oil remained in contact with the cells to reduce counting efficiency without altering the channels ratio enough to account for all quenching. We have since modified our technique so that no crude oil is associated with the cell fraction when it is filtered and thus no crude oil is present in the scintillation fluor during the counting process.

The work that we conducted on the summer Beaufort Sea sediments was done using the original technique therefore, the effects of crude oil on the uptake of glucose and glutamic acid may be in error as they are reported in our last quarterly report. The effects that we observed in crude oil aqueous samples would not be effected by crude oil quenching; however, the depression in radioactivity observed in the cells that had been exposed to either raw or weathered crude oil are probably lower than the actual levels. All data reported in this communication have been interpreted in terms of these new findings.

3. Determination of the most effective method for determining relative microbial activity.

a. There are several different approaches one can use to obtain information about the relative levels of microbial activity in marine samples. When we initiated our studies in this program, we chose to assay both the uptake and respiration of glutamic acid at

several substrate concentrations. This technique enabled us to measure the kinetics of glutamic acid utilization. It is a rather cumbersome technique since it involves a large number of subsamples to make a single determination. Since time and manpower restraints are particularly acute in the field, more samples could be analyzed if only one substrate concentration were used in these studies. We made a comparison between these two methods and found that the correlation coefficients were generally above 0.90 (Table 1 and Griffiths et al., 1977). Since the correlations were so high, we started using the single concentration method exclusively for routine field microbial activity determinations. This has resulted in the much higher resolution found in our most recent field studies.

b. During the course of our studies, we have utilized a number of labeled compounds. These include ^{14}C labeled glucose, glutamic acid, acetate, and algal protein hydrolysate. Of these, we have found that the first two provided the most useful information therefore we use both of these compounds routinely to characterize various microbial populations.

B. Abiotic variables observed in field studies

1. Cook Inlet

During the last year, we participated in two Cook Inlet cruises; one on board the Discoverer in April, 1977 and the other on board the Surveyor in November, 1977. During the first cruise, 44 water and 15 sediment samples were collected from 7 beach and 29 offshore stations. During the second cruise, 60 water and 20 sediment samples were collected from 3 beach and 56 offshore stations. The location of these stations and their respective station numbers are illustrated in Figs. 1-3. The exact position of these stations and a summary of abiotic variables are listed in Tables 2 and 3. During the April cruise, the surface waters were generally colder and more saline than they were during the November cruise.

Distinct patterns of surface water salinity were observed during both of the Cook Inlet cruises (Figs. 5 and 6). During both the cruises, the lowest salinities were observed in the northern section of Cook Inlet and the highest values were observed to the southeast. The higher resolution study in November gives the most comprehensive picture of surface water salinity patterns. The salinities to the west are lower than those found in the eastern half of the inlet. Salinities in the region of Kalgin Island and Tuxedni Bay were below 30 o/oo. The area to the south and west including Kamishak Bay and the western half of the Shelikof Straight had surface water salinities below 31 o/oo. With the exception of Kachemak Bay, all other areas studied had salinities above 31 o/oo.

2. Beaufort Sea

During the Glacier cruise in August-September, 1977, 20 sediment samples were taken from 20 offshore stations. The locations and station numbers for these stations are illustrated in Fig. 4. The exact position and depth information for these stations are given in Table 4. These samples were collected for us by Dr. Carey's associates and were analyzed in our laboratory at Oregon State after the cruise. The salinity temperature data collected during this cruise will be reported by Drs. English and Horner.

During the winter Elson Lagoon study, 4 water, 1 ice, and 9 sediment samples were collected near our Beaufort Sea station #3. These samples were collected as a part of the in situ crude oil degradation studies which we are conducting with Dr. Atlas' group (RU #30).

C. Relative microbial activity and respiration percentages in water samples

1. Cook Inlet

Patterns of relative microbial activity in offshore surface waters have been found to be similar in all three Cook Inlet cruises in which we have participated (Figs. 7-10, Tables 5 and 6). This was true if microbial activity was measured using either glucose or glutamic acid as the labeled substrate (Figs. 7 and 8). In all cases, the relative microbial activity in the northern waters was the highest, the lowest values were observed to the south and east of the inlet and in open ocean waters. Intermediate values were observed in samples collected along the western side of the inlet. A comparison of microbial activity levels during all three sampling periods have shown differences in mean values when comparing one cruise to another. There have also been local differences observed between values; however, these differences are not statistically significant. As has been the case in the past, relative microbial activity in water samples taken near the beach are higher than those taken further offshore (Table 7).

Consistent respiration percentage patterns were also seen when the results of the data collected on these three cruises are compared (Figs. 11-13). The values in the area near Kalgin Island and Tuxedni Bay are very low ranging from 31 to 40%. Contours of increasing values run in lines which run diagonally from the northeast to the southwest. Intermediate values are found along these contours in the center of the inlet and the highest values are found in the southeastern portion of the inlet and in the open seawater. Again, differences were seen between values observed in one location from cruise to cruise but the differences between mean values are not statistically significant.

2. Beaufort Sea

During this period, the only observations that were made on seawater samples taken from the Beaufort Sea were conducted in Elson Lagoon. Four water samples and one ice sample were analyzed for glucose and glutamic acid uptake and respiration (Table 8). The levels of relative microbial activity were essentially the same as that observed in the same location in April, 1976 (almost two years earlier). These levels are approximately one order of magnitude lower than that normally found in the summer in Elson Lagoon. The percent respiration was also about the same as that observed during the April, 1976 study.

D. Relative microbial activity and respiration percentages in sediment samples

1. Cook Inlet

In general, the highest levels of microbial activity were observed in Tuxedni Bay, Kachemak Bay and in the southern portion of Kamishak Bay (Figs. 14 and 15, Tables 9 and 10). During the April cruise, extremely high values were observed in the two samples taken in the Shelikof Strait; however, these high values were not observed during the November cruise when a much more comprehensive study was conducted. It must be concluded at this time that there are very large seasonal changes in the microbial activity in the Shelikof Strait sediments or that the extremely high values observed during the April cruise was caused by some experimental error. During the November cruise, the levels of microbial activity were lower in the Shelikof Strait than they were in the Cook Inlet.

The levels of microbial activity were lower in sediments analyzed during the October, 1976 and November, 1977 cruises than they were in the April cruise but these differences are not statistically significant using Student's "t" test.

The percent respiration values observed in the sediment samples taken from the Shelikof Strait in November were higher than those observed in Cook Inlet sediments. As we have observed in the past, the percent respiration values in sediment samples were lower than those observed in water samples.

2. Beaufort Sea

The sediment samples analyzed from the summer Glacier cruise indicated that the highest levels of microbial activity were found in the area near Point Barrow and the lowest activities were seen near Prudhoe Bay at stations 74a and 80a and near the Canadian border at station 110 (Figs. 16 and 17 and Table 11). This was true when either glucose or glutamic acid was used in the determinations. Since the samples were stored for up to four weeks before they could be analyzed, it is impossible

to make a valid comparison between these data and that observed in the field during the 1976 Glacier cruise where only fresh samples were analyzed. The average percent respiration observed in sediments while using glutamic acid as the substrate was 38%. This is higher than the value of 28% observed during the previous cruise, however, this difference may be due to the condition of the samples at the time of assay.

During the January Elson Lagoon study, 9 sediment samples were analyzed for relative microbial activity and percent respiration (Table 8). The rate of glutamic acid uptake and percent respiration in the undisturbed samples (samples BB402-406) was essentially the same as that observed in the same location in April, 1976. There was no significant difference between the uptake and respiration found in the control tray sediment and sediment found in a sample taken a few meters from the crude oil degradation study site. There were also no differences seen between these controls and the sediments that had been taken from the trays and treated with crude oil at the beginning of the experiment (July, 1977). Differences were seen however, between these sediments and the sediment that was used to initiate a new series of experiments on crude oil degradation. The first sediments were representative of the top 4 cm of the sediment. The sediment used in the degradation study preparation was taken from deeper sediments and were not as active (sample BB407). After the sediment had set under the ice for 24 hours, the observed activity doubled (samples BB408 and BB409). After the sediments sat for another 24 hours, the activity levels were equal to that observed in the undisturbed sediment (sample BB410).

E. Nitrogen fixation rates observed in the Beaufort Sea and Cook Inlet

In the sediments analyzed from the April cruise, the highest rates of nitrogen fixation observed were seen in Kachemak Bay and in the Shelikof Strait (Fig. 18 and Table 12). The average rate of nitrogen fixation observed was 0.28 ng nitrogen fixed per gram dry weight of sediment per hour. During the November cruise a more comprehensive study was made of nitrogen fixation rates in sediments (Figs. 19 and 20). The average rate observed at that time in Cook Inlet was 0.82 ng per g dry wt. per h (1.16 ng per g per h if all stations from the Shelikof Strait are included). Although the average rate was higher in the samples analyzed from the November cruise, the difference was not significant at the 95% confidence level when compared to the April results. As was the case in the April cruise, sediments taken from Kachemak Bay and the Shelikof Strait showed the highest activities. There were 7 stations which were sampled during both cruises. If the rates observed at these two times are compared, the resulting correlation coefficient is 0.88 indicating that relative levels of nitrogen fixation in Cook Inlet are probably consistent geographically (Table 13). Further comparison of this kind will be made during the next scheduled Cook Inlet cruise. These data will be required to substantiate this conclusion.

Nitrogen fixation rates were also measured in the sediment samples that we received from the Beaufort Sea (Fig. 21 and Table 14). The highest levels were observed in the samples taken near Point Barrow. The average rate of nitrogen fixation observed in these sediments was 0.06 ng per g dry weight per hour. This is significantly lower than the average values observed in the Cook Inlet; however, this comparison should be taken with caution since it is quite likely that the actual rates were higher than those reported here due to the storage time involved.

Nitrogen fixation rates were observed in a number of water samples taken from the Cook Inlet during both cruises. In all cases, the fixation rates were too low to measure. The rates of nitrogen fixation observed in the benthic organism preparations were also very low. These observations will be repeated during the next Cook Inlet cruise using a refinement on the method employed in this study.

F. Crude oil biodegradation potential studies

Crude oil degradation potentials were measured in diluted sediment samples using crude oil containing ^{14}C labeled pristane and hexadecane. The levels of radioactivity observed in those samples collected during the April and October Cook Inlet cruises are illustrated in Figs. 22-24. During the April cruise, the highest rates of degradation were observed in samples collected in Tuxedni and Kachemak Bays. The more comprehensive study conducted in November; however, shows no distinct pattern of hydrocarbon biodegradation potential.

A similar study was conducted on the sediments collected during the summer, 1977 Glacier cruise. Most of the highest values measured were observed in sediments taken to the east of Prudhoe Bay (Fig. 25).

G. Enumeration of bacteria

During all of our past studies, we have made bacterial concentration measurements using epifluorescent microscopy. The bacterial concentrations observed in both water and sediment samples collected during the April cruise are given in Table 15. Average values that we observed in both types of samples were essentially the same as we observed in samples taken during the October, 1976 cruise in the same area. No direct count data were made on samples that had been collected since then because we have judged that this information was of only limited use and it was essentially a duplication of similar observations being made by Dr. Atlas's group on the same samples.

During the April cruise we measured the presence of Desulfovibrio sp. bacteria in the sediment of Cook Inlet. This study was conducted because under the right conditions the organisms can greatly accelerate metal corrosion; a possible concern to those planning the construction

of drilling platforms in Cook Inlet. The relative levels of these organisms in sediments are illustrated in Fig. 26.

Since Dr. Atlas and his associates did not have access to sediment samples that we received from the Beaufort Sea, we made our own measurements of bacterial concentrations using different agar media. (This is a type of measurement that they routinely make on the samples that we take together). The results of this study are given in Table 16. In this study we compared the total counts obtained on two general purpose marine agar media; Lib X and Marine agar 2216. The counts obtained on Lib X were on the average twice those obtained on 2216. The average concentration of organisms reported here are close to the values that Dr. Atlas obtained in the Glacier cruise the summer before.

We calculated a series of correlation coefficients on bacterial concentrations as determined by counts on various media and the rates of glucose and glutamic acid uptake in the same samples (Table 18). The correlation coefficients were higher between the uptake rates and Lib X medium counts than with 2216 counts. A similar comparison was made on sediment samples collected during the April, 1977 Cook Inlet cruise (Table 19). In both of these studies, the correlation between bacterial concentration (as determined by plate counts) and substrate uptake was higher when glucose was used than when glutamic acid was used to measure activity.

During the April cruise, we also estimated the concentration of presumptive nitrogen fixing bacteria found in the sediment samples collected (Table 20). These values did not correlate well with the rates of nitrogen fixation that were observed in the same samples.

H. Effects of crude oil on microbial function

We measured the effects of crude oil on the growth of bacteria plated on a agar medium. The inocula that we used were subsamples of the sediment samples that were collected during the November Cook Inlet cruise. Both Lib X plates with and without crude oil were used. There was an average reduction of 33% in the number of colonies that formed on the crude oil supplemented agar. The same inhibitory effect was not observed, however, in sediment samples collected in Elson Lagoon (Table 17).

We have also conducted studies on the effect of crude oil on nitrogen fixation rates using the acetylene reduction method. The effect of crude oil acetylene reduction was measured in 10 sediment samples during the November 1977 Cook Inlet cruise. No significant difference was seen between samples that had been treated with Cook Inlet crude oil and those that were not (Table 12). A series of related studies has been conducted on sediment samples collected from several sources (Table 21).

These included 12 sediment samples collected during the November, 1977 Cook Inlet cruise, 4 from the April cruise, 9 samples from the summer Beaufort Sea cruise, and 5 Yaquina Bay, Oregon samples. All of these studies were conducted on samples that had been returned to our laboratory at Oregon State. In these studies, the effect of crude oil on acetylene reduction was measured using both untreated samples and samples to which sucrose had been added to stimulate acetylene reduction. In the case where no sucrose had been added, we observed little or no effect. In the samples which were supplemented with sucrose, both decreases and increases were observed. If there is an effect, it is not consistently negative.

The effect of crude oil on the uptake and respiration of glucose and glutamic acid by microbial populations in sediment and water samples has been studied. Two sets of data were collected from samples in the field and one set was taken from samples processed in our laboratory at Oregon State. After conducting these studies, it was discovered that cell fractions which were coated with crude oil at the time of assay produced erroneously low counts because of reduction in the counting efficiency caused by the crude oil. We have since modified our techniques so that this will no longer be a problem. The data presented here has been interpreted in light of this finding.

The effects of crude oil on the rate of glucose oxidation was measured in 21 water samples collected during the November Cook Inlet cruise (Table 22). The average percent reduction in the samples exposed to crude oil was 41. The other field study was conducted on 8 Beaufort Sea samples during the January, 1978 field trip. The average reduction in glucose oxidation rates in the presence of crude oil was measured at 45% (Table 22).

The effects of crude oil on glucose respiration was measured in 20 summer Beaufort Sea sediment samples as soon as they were received. The average reduction in respiration rate was 35% (Table 23). When the same experiment was conducted two weeks later, a reduction of 20% was observed. During the same studies, crude oil effects on glutamic acid respiration were also measured. The average reduction in respiration observed in these studies was 33% and 15% respectively. These data suggest that the apparent effect of crude oil on the microbial populations in sediments decreases with increased storage time.

A comparison of the effects of crude oil, crude oil extract, and "weathered" crude oil on glucose and glutamic acid respiration by microbial populations was made on the 20 Beaufort Sea sediments. The average percent reduction was essentially the same in all of these preparations when glucose was used. When the effect on glutamate respiration was measured, both crude oil and weathered crude oil had the same effect, the effect of the aqueous extract was less pronounced.

A pilot study was conducted to determine what effect crude oil has on the kinetics of respiration (Table 24). This study was conducted on sediment samples from the Beaufort Sea that had been stored for 2 months before they were used in these experiments, therefore, these results should be interpreted with caution. The calculated values for V_{\max} were about the same in both sets of samples. In all cases, however, the T_t and the $K_t + S_n$ values increased in the samples that had been exposed to crude oil. This is the type of response one would expect if there was some component of the crude oil that the cells were utilizing as glucose. It is also the response one would expect from uncompetitive inhibition in classical enzyme kinetics. Further studies with fresh samples will be made during the next cruise to substantiate these observations.

VII. Discussion

A. Glucose and glutamic acid uptake and respiration in water and sediment samples

1. Cook Inlet

At this point, data have been collected during three cruises in the Cook Inlet; October, 1976, April, 1977 and November, 1977. Each successive study has produced higher resolution data which have shown consistent trends. The resulting patterns when interpreted in light of what is known about the hydrography and chemistry of the region, produces an overall picture of the dynamics of the system which will assist those in government and industry in making a more accurate assessment of the potential problems related to crude oil production in Cook Inlet.

A discussion of the conclusions drawn from these data as well as the facts and assumptions on which these were based have already been mentioned at length in section I.B.1. of this report. The following is an amplification of the data presented in that section.

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

As far as we know, this is the first study made in which patterns of microbial activity in marine waters have been used to characterize more than one distinct water mass and to indicate regions of interaction between those water masses. The two water masses in question are clearly shown by the surface water salinity data illustrated in Figs. 5 and 6. This is a similar pattern to that observed by Kinney et al. (1969). This is also the same type of pattern that one would expect from the current data presented by Miller and Allen. Since these observations were taken at various times, it would appear that this is a relatively consistent feature in Cook Inlet. These same patterns are clearly shown in the relative levels of microbial activity and respiration percentages observed in the same region (Figs. 7-13) during all three Cook Inlet cruises.

At this point it is important to reflect on what these observations mean in terms of what is occurring in these water masses. The water mass to the southeast is coastal water which is being pushed into the inlet by inshore currents moving to the west. These waters probably contain very low levels of available organic nutrients. As a result, the level of microbial activity is low and the percent respiration is high. It has already been established by a number of investigators (Wright and Hobbie, 1966; Vaccaro and Jannasch, 1966; Crawford et al., 1974; and Carney and Colwell, 1976) that the uptake rate of simple labeled amino acids and sugars by natural microbial populations usually reflect the levels of nutrients present in the surrounding water. The significance of the percent respiration data is less clear. A relatively high percent respiration value indicates that the population is using proportionately more of the nutrient as an energy source and less of it to produce cellular material. There are at least two conditions in which this might occur. If the cells are starved, the cells will utilize most of added nutrient for energy requirements before biosynthesis is initiated. A more likely explanation is that growth factors are not present in sufficient concentration to allow biosynthesis to occur even though nutrients are available to the cells during the course of the experiment.

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

Drs. Cline and Feely have shown in their studies that the water mass at any given point in Cook Inlet is well mixed vertically. We conducted relative microbial activity at three locations and at various depths during the November cruise. We found no significant microbial activity stratification with depth. It would thus appear that the observations made in the surface waters should hold true for the entire water column in a given location.

We have also observed that the relative levels of microbial activity are directly related to the levels of suspended matter in the surface waters. When relative microbial activity as measured using glutamic acid is compared with turbidity in the same samples, correlation coefficients of 0.87 and 0.89 were observed for all water samples collected during the April and November cruises respectively. This correlation is also substantiated by the suspended matter patterns reported by Feely and Cline (1977). There is a striking similarity between these patterns

and the patterns of microbial activity and respiration percentages reported here. During our determinations of bacterial concentrations using epifluorescent microscopy, we have observed that 70-80% of the bacteria present in water samples are associated with the particulate matter.

Feely and Cline also reported that much of the suspended matter found in the northern waters probably makes its way into the sediments of the Shelikof Strait. Our studies of relative microbial activity in the Cook Inlet and Shelikof Strait sediments during the April cruise tend to support this hypothesis; however, a somewhat different pattern was observed during the November cruise. During both cruises, relatively high rates of microbial activity were observed in the sediments of Tuxeni Bay, Kachemak Bay and the southern portion of Kamishak Bay. The high rates of activity seen in Kachemak Bay are probably due to the trapping of nutrients within the bay. It has been observed by other investigators that the net flow of water through this bay is very low. The extremely high rate observed in the sediments of the Homer boat basin is undoubtedly due to organic pollutants introduced from man's activities there. The extent to which this nutrient input affects the microbial activity in the rest of the bay is unknown.

The high levels of microbial activity observed in Tuxedni Bay sediments are probably due to the sedimentation of the microbiologically active suspended matter in the water column in this area. Assuming that the bacterial populations associated with these particles remain active even after they have settled into the sediments, then measurements of microbial activity in the sediments can be used as a tracer to determine the sedimentation patterns of the suspended matter found in the northern water mass. If we make this assumption, it would appear that at least some of this matter settles into the sediments in the southern Kamishak Bay area (Figs. 14 and 15). If the values observed for the two Shelikof Strait sediment samples analyzed during the April cruise are accurate, then it would appear that significant quantities of this material also settle out in the Shelikof Strait under certain conditions.

2. Beaufort Sea

During the Glacier cruise (summer, 1976), we did not see any significant trends in microbial activity in the sediment relative to geographical location. A similar study made on sediments collected during the summer, 1977 cruise did show a geographical trend. The levels of microbial activity were highest in the stations closest to Point Barrow. The difference in these two sets of observations could be due to yearly variations but is most likely related to the way in which the samples were taken. During the previous summer, the sediment samples were taken with a Van Veen grab. During the 1977 cruise, the sediment samples were taken with a Smith-McIntyre grab. The latter sampler, gives a much less disturbed sample thus the subsamples taken from it are more representative

of the original. For this reason, we are recommending that either a Smith-McIntyre grab or box corer be used in all future microbiological work. The importance of working with relatively undisturbed sediments was graphically shown during our more recent Beaufort Sea study. Samples taken from the surface of the sediment by divers were very similar in their microbial characteristics to sediment samples taken from control and oiled sediment trays in the oil degradation study in Elson Lagoon. Another sample taken by divers a few days later which included sediments from a much greater depth had very low activity. When the same sample had been placed in a tray and left for 24 hours, the activity was the same as that found in undisturbed surface sediments. The observed increase was undoubtedly caused by a silting in by surrounding sediments.

Both water and sediment samples taken during the same time, gave relative microbial activity levels and respiration percentages which were essentially the same as those observed at the same location in April, 1976. These observations further support our earlier observation that the levels of microbial activity are approximately one order of magnitude lower in the winter than they are in the summer. The respiration percentages are, on the other hand, higher in the winter than they are in the summer (see our last annual report for details).

B. Nitrogen fixation studies

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

Although nitrogen fixation rates in the seawater samples that we have analyzed have been too low to measure, most of the sediments analyzed showed detectable levels of nitrogen fixation. The sediments in some areas, notably Kachemak Bay and the Shelikof Straight, showed relatively high rates. During both cruises, we have measured levels of nitrogen fixation in Kachemak Bay sediments at about 1 ng nitrogen fixed per g dry wt. per h. If this is indeed representative of average nitrogen fixation rates over the normal year, and if nitrogen is limiting to bacteria growth in these sediments, then this level of nitrogen fixation could account for a yearly production of bacterial biomass in Kachemak Bay of 400 tons.

The studies of Dr. Feder and his associates (1977) have shown that the food chain can be very short from bacterial biomass to commercially valuable species. In the case of the King crab, one of the major pathways runs from bacteria in detritus to detrital feeding clam to crab. Anything that might affect bacterial production will be directly reflected in changes at higher trophic levels. We have conducted a series of experiments designed to measure the effects of crude oil on rates of nitrogen fixation. In untreated sediment samples, we found that crude oil did not significantly alter nitrogen fixation rates. These data suggested that the immediate effects of crude oil on this process is probably not an important factor; however, it is still not known what the longer term effects might be. It is quite likely that crude oil might not affect existing enzyme systems but it might affect enzyme induction, enzyme synthesis or cell growth. One approach that we have taken to evaluate the possible long term effects was to stimulate nitrogen fixation rates in sediment samples by adding sucrose. When this was done, the samples containing crude oil sometimes inhibited and sometimes stimulated rates of nitrogen fixation in the presence of sucrose. We are planning a series of experiments with pure cultures of nitrogen fixing bacteria to obtain more specific information on what effect crude oil has on this process.

Another approach to this problem is also being conducted in the Elson Lagoon studies that have already been mentioned. During this series of experiments, we will be comparing nitrogen fixation rates in sediment samples that have been exposed to crude oil over an extended period of time. This type of study should produce the most definitive answers to the long term effects problem.

There have been very few reports in the literature concerning the rates of nitrogen fixation in marine sediments. Of these, the study that most closely approximates ours was that of Herbert (1975). This was an in situ study of nitrogen fixation in sediment cores taken at a location on the northeast coast of Scotland. Herbert observed a maximum nitrogen fixation rate of 1.84 ng nitrogen fixed per g dry wt. per h. This rate is the average rate of nitrogen fixation observed by us in all sediment samples analyzed during the November Cook Inlet cruise. The maximum rate that we observed was 6.3 ng/g/h. In another study, Brooks et al. (1971) reported a range of nitrogen fixation in 8 sediments taken from a Florida estuary of from 0.64 to 6.0 ng N/g/h. The highest value that they observed was very close to the highest value that we observed in November (Homer boat basin). Marsho et al. (1975) reported an average annual nitrogen fixation rate of 2.9 ng N/g/h in sediments taken from 7 stations in the Rhode River close to Chesapeake Bay. These data suggest that the rates of nitrogen fixation that we observed in Cook Inlet and the Shelikof Strait are close to that observed in other marine sediments and relatively high when compared to sediments that were most similar (the Herbert study). Again this substantiates the potential importance of nitrogen fixation in Cook Inlet.

C. Effects of crude oil on nutrient uptake and mineralization rates by microbial populations

Our effects of crude oil studies on nutrient uptake and mineralization have shown that both of these processes are inhibited by the presence of crude oil. This was true when either glucose or glutamic acid was the nutrient source. This effect was seen in sediment samples when glucose was the substrate and when the microorganisms were exposed to either crude oil, an aqueous extract of crude oil or "weathered" crude oil.

In a recent report by Hodson et al. (1977), the effects of four oils on glucose uptake and mineralization were observed in seawater samples collected from the CEPEX seawater enclosure bags located in Saanich Inlet, British Columbia. They observed an inhibition effect similar to that observed by us in Cook Inlet waters. Our aquarium studies and the results of studies conducted by Atlas et al. (1976) on the long term effects of crude oil on microbial populations in Prudhoe Bay waters have shown that the presence of crude oil does not inhibit heterotrophic activity in marine microbial populations after extended exposure. In fact, heterotrophic activity may actually increase as the crude oil is degraded. What our short term exposure experiments do suggest is that components of crude oil may act as an environmental stress which effectively eliminates certain forms and encourages the growth of hydrocarbon utilizing bacteria. Dr. Atlas and his associates have been conducting chemostat studies at Barrow, Alaska under another program. These studies were designated to measure the fate and effects of oil on marine microorganisms in seawater under simulated in situ conditions. They observed that the species diversity index dropped with exposure time to crude oil. (R. M. Atlas, personnel communication). They have thus observed what we would have predicted from our acute effects studies.

If the presence of crude oil decreases the diversity of the species composition of a natural microbial population, it must affect the number of functions that that population is capable of performing. We are in the process of determining the microbial functions that are critical to the Arctic and Subarctic marine ecosystems and to assess what effect crude oil has on these processes

VIII. Conclusions

1. Evidence is accumulating which suggests that crude oil which is spilled in the turbid waters of the Upper Cook Inlet may become associated with the suspended matter found in these waters. If this occurs, then crude oil components would become associated with the sediment when these particles settle out of the water column. Our studies and the studies of Drs. Feely and Cline suggest that these particles probably settle out into the sediments of the southern Kamishak Bay and/or the sediments of the Shelikof Strait.

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

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

4. Our studies on the effects of crude oil on nitrogen fixation in natural sediment samples showed that the presence of crude oil had little or no short term adverse effect on this process. The method used to determine longer term effects produced inconclusive evidence as to what effect may be produced in actual sediment samples in nature.

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

6. Observations made in Beaufort Sea sediment samples show that the highest rates of nitrogen fixation and relative levels of microbial activity were highest in the area near Point Barrow with the highest rates of crude oil biodegradation found to the east of Prudhoe Bay.

7. Other observations in the Beaufort Sea confirm earlier findings which indicated that the relative levels of microbial activity in the winter are about one order of magnitude lower than that found in the summer. Both the level of microbial activity and respiration in the water and sediment at one station in Elson Lagoon was the same in January, 1978 as it was in April, 1976.

8. The patterns of microbial activity and respiration percentages in the Cook Inlet surface waters are relatively constant. High levels of microbial activity and low respiration percentages were found in the northern water mass and the reverse pattern was seen in the waters to the south and southeast. These differences probably reflect qualitative and/or quantitative differences in organic nutrients present in these two water masses.

IX. Needs for future research

A. Detrital food web studies

With the increasing emphasis on food web studies in the Cook Inlet and the area around Kodiak Island, it is imperative that an analysis of microbiological variables be included in these studies. As more is known about the dynamics of the detrital food web, it is becoming increasingly obvious that bacteria play a critical role in the energy transfer within this system (Fenchel and Jorgensen, 1977; Mann, 1972). It has been estimated that the majority of primary productivity in inshore communities is carried out by macrophytes rather than by phytoplankton (Mann, 1972). It has also been estimated that only about 10% of macrophytic biomass is utilized directly by grazers. Since this material has such high carbon to nitrogen ratios, it by itself is a very poor food source for marine animals. Observations to date suggest that this material must be colonized by bacteria before it can become a valuable food source for other organisms. The bacteria utilize the carbon from the plant material and absorb nitrogen and phosphate from the water to form a food source with relatively low carbon to nitrogen and carbon to phosphate ratios. When the plant material is ingested by marine animals, the bacteria are digested and the rest of the material is defecated. The resulting fecal pellets are recolonized by bacteria and reinjected etc., etc. The role of protozoa and meiofauna in the efficiency of energy transfer mediated by the bacterial population is poorly understood but it is known that both of these groups graze on bacteria and are thus increasing the efficiency of the bacterial degradation of detritus particles by some unknown mechanism. Of the inorganic nutrients required for this bacterial process, fixed nitrogen is most likely to be limiting, thus the importance of nitrogen fixation in sediments. Of the above mentioned processes, only one, to our knowledge, has been studied in terms of crude oil effects. This is our study of the effects of crude oil on nitrogen fixation in sediments which has been described above. It seems quite likely that there might be some critical step in energy transfer system that is susceptible to the presence of petroleum hydrocarbons.

Since relatively little is known about this critical process in the inshore food web, it is imperative that a study be initiated which would include microbiologists and benthic ecologists with specific areas of expertise. Before the effects of crude oil on this process can be initiated, basic observations must be made on the rate of energy flow through the major elements of the benthic community. As far as the microbiological studies are concerned, these would include measurements of bacterial biomass, growth rates, metabolic activity rates, as well as grazing rates by protozoa, protozoa growth rates, and biomass determinations. Once the methods have been established to make these observations, and some basic information has been established, then the effects of crude oil on these processes can be determined.

B. In situ studies of the fate and effects of crude oil in marine sediments

We recommend that the fate and effects studies which have been initiated in Elson Lagoon near Barrow, AK be continued. Both Dr. Atlas and ourselves have been involved in this study using oiled sediment samples in plastic trays. Dr. Atlas has been primarily studying the fate aspects of the project and we have concentrated on the effects aspects. This is a project which should be continued for an extended period of time so that the long range effects of crude oil as well as the long range fate can be determined under actual field conditions.

We also recommend that a similar study be initiated in the Cook Inlet since the conditions found in these two regions are so radically different. A similar approach should also be used to study the fate and effects of crude oil in the water column - a more challenging technological problem.

C. Background information on the microbiology of new lease areas

Our study of microbiological activity and respiration in the Cook Inlet and Shelikof Strait have given us a great deal of information about the dynamics of the system and have helped pinpoint potential problem areas within Cook Inlet. There is still a need to collect similar data in such areas as Bristol Bay and Norton Sound where currently none of this information is available.

X. Summary of last quarter operations

A. Field studies

During this quarter we conducted a field trip in January, 1978 to the Naval Arctic Research Laboratory in Barrow. We worked for about 10 days collecting sediment trays inoculated with crude oil which had been placed there last summer. The divers that had collected the first set of trays also established a new experiment by placing a new set of trays at the same location. These trays will be sampled again in April and September of this year. We measured relative rates of microbial activity and respiration percentages in sediment, ice and water samples. We also conducted crude oil effects studies on the same samples. Subsamples of sediments were returned to our laboratory at Oregon State for further analysis.

B. Laboratory studies

Much of our effort during this time has been devoted to analyzing samples that were returned from both the November Cook Inlet cruise and the January NARL field trip. We have also been analyzing the data that was collected during these two field trips. In addition, we have continued our crude oil degradation studies and have started analyzing degraded

crude oil samples using our new GC - calculator interface. Studies have also been initiated to improve our current methodology and to gain expertise in conducting energy charge measurements in sediment microbial populations.

XI. Projected activities for next quarter

A. Field studies

In April we will be participating in the Cook Inlet chemistry cruise. During this cruise, we will be collecting sediment and water samples from both the Shelikof Strait and the Cook Inlet. The effects of crude oil on microbial activity and respiration and on nitrogen fixation in sediments will be studied. We will be coordinating our studies with those conducted by both the chemists and the benthic ecologist involved in the cruise.

We will also participate in a field trip to NARL to continue our study of the long term effects of crude oil on the microbial community in the Elson Lagoon sediments.

B. Laboratory studies

We will be continuing the studies that we have outlined under the previous section. In addition, we will be analyzing the nutrient chemistry information generated from the last few field trips as well as the data generated by the field trips being conducted during this quarter.

C. Problems encountered

An analysis of our sediment data indicates that the Van Veen grab samplers that have been available for our use in the past are not adequate for our work. In future field work we will require a sediment sampler that will produce a more representative sample.

We have also encountered problems with methodology that have been mentioned in this report. Studies are being conducted to eliminate problem areas.

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Table 1. Correlation coefficients calculated from the comparison between the maximum potential uptake rate of glutamic acid (V_{\max}) and the uptake rate of glutamic acid at the concentration listed in the table.

Location of the study	WATER SAMPLES		
	Number of samples analyzed	Substrate concentration $\mu\text{g/liter}$	Correlation coefficient
Antarctic, February, 1972	83	7.9	0.92
Beaufort Sea, August, 1975	47	2.3	0.88
Beaufort Sea, April, 1976	25	3.5	0.90
Beaufort Sea, August, 1976	16	2.7	0.77
Gulf of Alaska, March, 1976	26	3.5	0.95
Lower Cook Inlet, October, 1976	35	2.7	0.95
Total samples	232		
SEDIMENT SAMPLES			
Beaufort Sea, August, 1975	23	13.0	0.90
Beaufort Sea, April, 1976	13	3.5	0.94
Beaufort Sea, August, 1976	11	72.7	0.98
Gulf of Alaska, March, 1976	20	80.9	0.98
Lower Cook Inlet, October, 1976	12	72.7	0.97
Total Samples	79		

Table 2. Summary of water depth, temperature, and salinity data and positions of stations sampled during the April, 1977 Cook Inlet cruise. Sediment samples having the same number as the listed water samples were taken at the same locations. (*) those stations at which temperature and salinity were determined using a portable field salinometer of relatively low accuracy. (a) those beach samples taken 10 meters offshore. (b) those beach samples taken in the surf line.

Station number	Water Sample number	Depth of water column (meters)	Temperature		Salinity 0/00		Lat. North	Long. West
			Surface	Bottom	Surface	Bottom		
D *	GW401 (b)	shore	6.5	--	16.5	--	57 39.0	152 31.0
266 *	GW402	36	1.0	--	21.0	--	60 41.2	151 25.0
265 *	GW403	39	0.44	0.38	27.38	27.41	60 34.3	151 51.4
244	GW404	56	2.21	2.36	30.08	30.21	60 09.6	152 15.0
245 *	GW405	46	2.9	--	26.0	--	60 06.8	152 14.0
S *	GW406 (a)	shore	2.8	--	24.5	--	60 10.7	152 36.0
S *	GW407 (b)	shore	4.5	--	24.0	--	"	"
T *	GW408 (a)	shore	2.8	--	25.0	--	60 09.3	152 38.0
T *	GW409 (b)	shore	4.0	--	25.0	--	"	"
242	GW410	39	2.35	2.33	30.19	30.19	60 09.5	152 25.0
U *	GW411 (a)	shore	6.0	--	15.0	--	60 12.7	152 36.5
V *	GW412 (a)	shore	4.0	--	22.0	--	60 13.7	152 46.8
V *	GW413 (b)	shore	2.0	--	23.0	--	"	"
242	GW415	33	2.29	2.31	30.11	30.10	60 09.5	152 25.0
242	GW416	33	2.28	2.29	30.08	30.08	"	"
246 *	GW417	15	2.5	--	25.5	--	60 03.0	151 46.2
235	GW418	40	2.81	2.83	30.60	30.64	59 40.8	152 38.6
236	GW419	48	4.06	3.96	31.41	31.37	59 40.9	151 14.1
227	GW420	90	4.46	4.36	31.41	31.49	59 33.5	151 36.4
K *	GW421 (a)	shore	2.5	--	25.5	--	59 36.1	151 25.0
K *	GW422 (b)	shore	5.0	--	26.0	--	"	"
J *	GW423 (a)	shore	4.6	--	26.8	--	59 35.3	151 10.7
J *	GW424 (b)	shore	5.0	--	25.0	--	"	"
229	GW425	66	4.16	4.11	31.25	31.33	59 37.6	151 18.0
216	GW426	86	4.95	4.97	31.46	31.46	59 18.2	152 14.1
217	GW427	74	4.64	4.47	31.47	31.51	59 27.0	152 23.2
226	GW428	60	4.66	4.66	31.46	31.47	59 33.5	152 18.7
225	GW429	39	3.68	4.44	31.25	31.48	59 31.4	152 41.5
213	GW430	33	3.13	3.13	30.98	30.98	59 30.0	153 13.2
214	GW431	49	3.28	3.27	31.10	31.09	59 18.2	153 14.3
204	GW432	36	2.72	2.95	30.79	30.91	59 14.2	153 39.7
206	GW433	39	4.89	4.95	31.51	31.53	59 09.8	153 08.2
212	GW434	27	2.30	2.48	30.59	30.66	59 32.4	153 21.8
215	GW435	76	3.41	4.62	31.16	31.51	59 21.9	152 48.7
212	GW436	22	2.38	2.39	30.68	30.70	59 33.4	153 24.5
208	GW437	104	4.92	5.39	31.62	31.92	59 14.7	152 45.5
205	GW438	148	5.00	5.42	31.64	32.14	59 06.3	152 43.1
207	GW439	148	4.82	5.28	31.67	31.92	58 59.9	152 52.0
395	GW440	170	--	--	--	--	58 53.0	152 54.0
106	GW441	202	4.75	5.56	31.49	32.08	59 00.4	152 00.6
105	GW442	118	4.74	7.49	31.29	32.63	58 50.0	151 20.7
398	GW443	122	4.69	5.32	31.74	32.11	58 48.8	152 11.9
388	GW444	169	--	--	--	--	58 28.6	153 10.9
378	GW445	95	4.22	4.98	31.72	31.95	58 02.0	153 29.5

Table 3. Summary of water depth, temperature, and salinity data and positions of stations sampled during the November, 1977 Cook Inlet cruise.

Station number	Sample number	Depth in meters	Temperature		Salinity 0/00		Lat. North	Long. West
			Surface	Bottom	Surface	Bottom		
429	GW501	88	7.11	7.14	31.668	32.218	57 58.0	151 58.8
418	GW502	168	6.43	5.96	32.020	32.872	58 04.8	151 42.0
407	GW503	75	6.16	5.50	32.224	33.162	58 19.0	151 25.7
398	GW504	124	6.96	5.64	31.996	33.084	58 48.9	152 11.6
J*	GW505	surf.	7.5		29		59 35.5	151 10.5
K*	GW506	surf.	3.5		29		59 36.5	151 25.5
380	GW507	75	4.78	7.39	29.856	31.380	58 39.5	153 23.5
370	GW508	125	5.20	7.27	30.207	32.272	58 17.2	154 02.3
360	GW509	225	6.99	5.44	30.658	33.139	57 57.0	154 41.3
350	GW510	265	6.68	5.85	30.538	32.909	57 31.4	155 32.8
354	GW511	251	6.09	4.96	31.836	33.502	57 27.5	155 14.5
358	GW512	186	6.25	5.38	31.750	33.068	57 18.4	154 57.0
364	GW513	214	6.06	5.04	31.900	33.423	57 50.1	154 25.0
378	GW514	91	6.38	6.91	31.242	32.278	58 01.6	153 29.0
374	GW515	193	6.98	5.46	31.342	33.097	58 10.8	153 45.0
388	GW516	214	7.34	5.81	31.249	32.939	58 27.0	152 57.5
384	GW517	178	7.31	6.40	30.884	32.684	58 33.4	153 14.3
394	GW518	126	7.43	6.62	31.035	32.486	58 42.4	152 59.7
390	GW519	163	5.08	6.68	30.202	32.600	58 53.3	153 11.5
395	GW520	168	7.11	6.11	31.903	32.784	58 53.3	152 54.0
205	GW521	141	7.82	6.50	31.040	32.652	59 06.2	152 41.3
206	GW522	87	6.61	8.04	30.309	31.984	59 09.4	153 07.1
212	GW523	27	5.71	5.71	30.096	30.099	59 32.5	153 21.2
211	GW524	19	5.60	5.63	30.252	30.265	59 26.1	153 37.3
213	GW525	37	5.97	5.99	29.895	29.902	59 29.4	153 12.7
214	GW526	50	6.24	6.59	29.977	30.483	59 17.8	153 14.0
203	GW527	44	5.05	5.47	30.302	30.469	59 06.2	153 29.1
201	GW528	18					59 12.8	153 52.4
204	GW529	33					59 14.3	153 38.5
215	GW530	81	6.47	8.14	30.632	31.931	59 21.1	152 48.8
208	GW531	106	7.00	7.89	31.032	32.062	59 15.0	152 44.9
207	GW532	167	7.03	6.36	31.402	32.703	58 59.8	152 52.9
216	GW533	75	7.81	7.92	31.413	31.544	59 18.0	152 15.0
217	GW534	71	7.56	7.59	31.494	31.531	59 27.7	152 22.9
225	GW535	64	7.13	7.12	31.306	31.318	59 31.5	152 41.9
226	GW536	49	7.18	7.21	31.336	31.268	59 33.3	152 18.6
228	GW537	46					59 32.9	151 53.4
227	GW538	88	7.23	7.38	30.991	31.144	59 33.5	151 36.1
249	GW539	44	6.29	6.29	30.802	30.811	59 51.3	152 02.1
246	GW540	20			30.714		60 02.5	151 47.5
266	GW541	40	4.4		23.031		60 41.2	151 25.6
265	GW542	23					60 33.6	151 51.6
255	GW543	50	5.35	5.35	28.810	28.991	60 19.9	151 45.9
248	GW544	51	5.87	6.05	29.760	29.962	59 50.5	152 21.4
235	GW545	36	6.08	6.09	30.046	30.067	59 42.1	152 38.0
234	GW546	43	6.33	6.35	30.356	30.360	59 37.6	152 55.8
245	GW547	44	5.29	5.70	29.358	29.616	60 06.7	152 14.5
241	GW548	31					60 06.8	152 36.0
U*	GW549	surf.	2.0		27		60 12.8	152 36.1
V*	GW550	surf.	2.0		28		60 13.7	152 45.7
241	GW551	31					60 06.8	152 36.0
242	GW552	35	5.05	5.39	29.343	29.442	60 09.0	152 25.5
247	GW553	34					59 56.0	152 37.1
233	GW554	26					59 50.4	152 56.5
213	GW555	35	5.56	5.56	30.280	30.287	59 29.4	153 12.7
236	GW556	47	6.95	6.95	31.390	31.383	59 41.3	152 14.1
229A	GW557	24	5.48	5.58	30.729	30.811	59 40.4	151 14.3
229	GW558	75	6.15	6.98	30.902	31.111	59 37.5	151 17.8
106	GW559	209	6.90	6.62	31.540	32.386	59 00.6	152 01.0
105	GW560	126	6.45	6.77	31.174	32.605	58 49.8	151 19.2

(*) Shore stations. Temperature and salinity measurements were made using a portable salinometer.

Table 4. Description of sample position, water column depth, grab numbers (OSU), sample numbers, station numbers and sampling date for all sediment samples collected during the summer, 1977 Glacier Cruise.

Sample number	Grab number	Station number	Sample depth (meters)	Date	Lat	Position	Long.
BB301	1523	14b	123	8/7	71° 46'		155° 35'
" 302	1529	18	384	8/9	71 57.5		154 34
" 303	1536	19	51	8/10	71 34.2		153 39.5
" 304	1544	24b	55	8/11	71 19.2		152 54
" 305	1555	25a	40	8/11	71 13.8		152 57.9
" 306	1568	25	24	8/11	71 0.6		153 01
" 307	1569	24	79	8/12	71 21.2		152 35
" 308	1580	24a	102	8/12	71 23.0		152 41
" 309	1586	31a	42	8/15	71 07.2		149 56.7
" 310	1588	74a	21	8/16	70 39.0		148 28.5
" 311	1595	80a	30	8/18	70 31.4		147 30.5
" 312	1598	91	31	8/18	70 23.2		146 33.3
" 313	1600	99	3841	8/20	72 53.8		146 27
" 314	1621	115	659	8/25	70 42.8		141 39.5
" 315	1628	110	26	8/26	69 49.5		141 28.5
" 316	1642	113	50	8/26	70 10.0		141 17.7
" 317	1648	114	106	8/27	70 33		142 27.5
" 318	1654	103	146	8/27	70 37.5		143 57
" 319	1660	95	521	8/29	71 01.5		145 26
" 320	1662	93	42	8/30	70 40.5		146 31

For tempt salinity data see Dr. Horner's annual report.

Table 5. Relative levels of microbial activity and percent respiration of glutamic acid and glucose observed in all water samples collected during the April cruise.

Station number	Sample number	* Substrate Uptake		Percent Respiration	
		Glucose	Glutamic Acid	Glucose	Glutamic Acid
D	GW401	6.7	38.8	51	76
266	GW402	8.5	52.4	15	36
265	GW403	2.7	24.7	17	34
244	GW404	1.6	14.6	15	31
245	GW405	1.6	11.7	27	35
S	GW406	2.6	15.2	19	38
S	GW407	3.3	25.7	20	44
T	GW408	2.4	13.5	15	34
T	GW409	1.1	8.3	17	34
242	GW410	1.9	15.5	16	32
U	GW411	7.9	67.7	23	58
V	GW412	39.7	129	15	50
V	GW413	18.0	78.9	16	39
242	GW415	1.8	9.3	5	27
242	GW416	--	17.9	--	33
246	GW417	11.5	66.5	14	34
235	GW418	2.0	6.8	16	34
236	GW419	0.3	0.9	34	52
227	GW420	0.4	0.9	26	61
K	GW421	3.7	8.0	47	53
K	GW422	6.7	28.1	22	54
J	GW423	0.5	1.8	41	49
J	GW424	1.0	4.8	25	58
229	GW425	0.9	0.9	36	67
216	GW426	0.3	2.0	47	61
217	GW427	0.3	1.0	39	64
226	GW428	0.3	0.7	42	61
225	GW429	0.6	1.5	36	55
213	GW430	0.3	1.8	40	41
214	GW431	0.3	0.5	35	42
204	GW432	0.8	3.0	34	48
206	GW433	0.4	0.6	31	56
212	GW434	1.1	3.8	32	54
215	GW435	0.5	1.5	37	62
212	GW436	0.6	2.0	26	40
208	GW437	0.2	0.8	36	61
205	GW438	1.0	2.7	36	64

Table 5. (con't)

Station number	Sample number	* Substrate Uptake		Percent Respiration	
		Glucose	Glutamic Acid	Glucose	Glutamic Acid
207	GW439	1.0	2.0	39	67
395	GW440	0.2	0.7	45	68
106	GW441	0.2	0.5	39	45
105	GW442	0.4	2.0	45	72
398	GW443	0.7	2.9	49	70
388	GW444	2.0	2.6	16	70
378	GW445	0.8	2.2	30	62

(*) The uptake rates are reported as ng substrate/liter/h.

Table 6. Relative levels of microbial activity and percent respiration of glutamic acid and glucose observed in all water samples collected during the November cruise.

Station number	Sample number	* Substrate Uptake		Percent Respiration	
		Glucose	Glutamic Acid	Glucose	Glutamic Acid
429	GW501	<0.2	1.1	L	60
418	GW502	0.4	0.9	67	59
407	GW503	0.3	1.4	41	68
398	GW504	<0.2	0.4	L	71
J	GW505	<0.2	1.1	L	63
K	GW506	11.3	18.0	25	61
380	GW507	<0.2	1.0	L	61
370	GW508	<0.2	1.0	L	63
360	GW509	<0.2	0.6	L	59
350	GW510	<0.2	1.0	L	67
354	GW511	<0.2	1.1	L	62
358	GW512	<0.2	0.9	L	56
364	GW513	<0.2	0.8	L	61
378	GW514	<0.2	1.2	L	68
374	GW515	<0.2	<0.4	L	L
388	GW516	<0.2	<0.4	L	L
384	GW517	<0.2	0.8	L	76
394	GW518	<0.2	<0.4	L	L
390	GW519	<0.2	0.4	L	79
395	GW520	<0.2	0.7	L	76
205	GW521	<0.2	<0.4	L	L
206	GW522	<0.2	0.9	L	62
212	GW523	<0.2	0.5	L	67
211	GW524	<0.2	1.3	L	67
213	GW525	0.4	2.0	39	57
214	GW526	<0.2	0.8	L	69
203	GW527	0.4	2.8	35	63
201	GW528	0.9	3.8	44	65
204	GW529	0.8	3.2	21	61
215	GW530	0.2	0.5	47	64
208	GW531	0.2	1.3	31	79
207	GW532	<0.2	0.4	L	90
216	GW533	<0.2	0.6	L	82
217	GW534	<0.2	0.5	L	81
225	GW535	0.3	2.0	60	60

Table 6 (con't)

Station number	Sample number	* Substrate Uptake		Percent Respiration	
		Glucose	Glutamic Acid	Glucose	Glutamic Acid
226	GW536	<0.2	1.1	L	63
228	GW537	0.2	0.5	89	64
227	GW538	<0.2	2.0	L	76
249	GW539	1.9	7.5	36	51
246	GW540	2.0	8.5	19	44
266	GW541	6.6	61.0	12	32
265	GW542	1.8	16.5	14	35
255	GW543	2.0	4.1	16	47
248	GW544	<0.2	1.3	L	31
235	GW545	<0.2	2.8	L	45
234	GW546	0.3	3.3	15	36
245	GW547	0.3	4.3	29	48
241	GW548	4.4	30.3	13	35
U	GW549	4.3	19.0	14	31
V	GW550	6.1	44.4	13	39
241	GW551	2.4	11.9	17	37
242	GW552	0.4	2.5	22	39
247	GW553	3.3	21.0	14	34
233	GW554	0.6	2.6	27	56
213	GW555	0.5	3.3	33	58
236	GW556	0.2	1.6	36	66
229A	GW557	0.4	2.5	32	54
229	GW558	0.4	2.6	43	65
106	GW559	<0.2	1.0	L	65
105	GW560	<0.2	0.6	L	70

(*) The uptake rates are reported as ng substrate/liter/h.

(L) Those values which were too low to obtain an accurate rate.

Table 7. A comparison of glutamic acid uptake in water samples taken in the surf zone and 10 meters offshore during the April Cook Inlet cruise.

Glutamic acid uptake (ng/l/h)	
<u>Surfline</u>	<u>Offshore</u>
14	4.9
26	5.8
39	21
8.3	0.9
256	236
51	50
3.5	2.1
7.3	0.4
3.3	2.6
2.4	1.1
40	18
6.7	3.7
1.0	0.5

Table 8. Relative levels of microbial activity and percent respiration of glucose and glutamic acid observed in samples collected at Elson Lagoon, Barrow, AK in January, 1978

WATER SAMPLES				
Sample number	<u>*Substrate Uptake</u>		<u>Percent Respiration</u>	
	<u>Glucose</u>	<u>Glutamic acid</u>	<u>Glucose</u>	<u>Glutamic Acid</u>
BW401	0.4	4.8	51	69
♠BI401	0.5	4.5	37	69
BW402	0.3	3.8	46	64
BW403	0.3	3.4	34	68
BW408	0.3	2.4	32	61
SEDIMENT SAMPLES				
BB402	3.4	22.6	25	44
BB403	7.8	27.6	20	41
BB404	5.7	44.3	20	45
BB405	2.9	14.5	27	49
BB406	2.0	14.9	27	48
BB407	0.2	2.8	42	55
BB408	1.2	6.0	23	51
BB409	1.1	6.1	24	50
BB410	3.4	19.9	23	48

♠ Melted ice water

* Substrate uptake reported as ng substrate taken up/unit/h. The unit for water samples was liters and the unit for sediment samples was g. dry wt.

Table 9. Relative levels of microbial activity and respiration percentages observed using glucose, glutamic acid and acetate in all sediment samples collected during the April cruise.

Station number	Samples number	* <u>Substrate Uptake</u>			<u>Percent Respiration</u>		
		Glucose	Acetate	Glutamic acid	Glucose	Acetate	Glutamic acid
D	GB401	0.6	1.8	0.01	36	73	48
U	" 411	24.0	19.2	0.89	16	75	45
V	" 412	18.0	7.5	0.66	14	70	37
227	" 420	18.4	5.7	0.36	16	54	49
K	" 421	44.8	13.3	1.57	22	32	54
229	" 425	12.8	6.0	0.30	19	60	45
213	" 430	3.7	6.7	0.14	36	49	41
214	" 431	2.0	4.9	0.08	19	63	40
204	" 432	13.2	7.3	0.37	22	55	46
212	" 434	6.4	4.6	0.16	32	57	41
212	" 436	5.1	13.3	0.17	26	62	45
395	" 440	11.6	9.3	0.27	16	62	45
105	" 442	1.4	6.3	0.11	23	61	42
388	" 444	618	72.4	9.22	65	84	57
378	" 445	211	22.1	10.50	35	44	60

* Uptake rates reported as ng/g dry wt/h.

Table 10. Relative levels of microbial activity and percent respiration of glutamic acid and glucose observed in all sediment samples collected during the November cruise.

Station number	Sample number	* Substrate Uptake		Percent Respiration	
		Glucose	Glutamic Acid	Glucose	Glutamic Acid
429	GB501	0.9	75	27	58
418	GB502	3.2	61	24	55
K	GB506	32.6	787	19	47
380	GB507	1.5	20	16	L
370	GB508	1.9	20	33	L
360	GB509	2.6	22	22	46
350	GB510	0.7	20	29	L
354	GB511	1.5	20	39	53
358	GB512	<0.4	20	L	L
364	GB513	1.2	33	35	42
378	GB514	0.9	31	40	61
374	GB515	1.4	20	51	L
388	GB516	<0.4	20	L	L
384	GB517	<0.4	16	L	75
394	GB518	<0.4	20	L	L
390	GB519	2.2	20	36	70
395	GB520	0.7	20	40	L
212	GB523	2.6	41	26	57
211	GB524	5.4	73	23	51
213	GB525	3.1	38	34	43
214	GB526	3.4	61	13	48
203	GB527	14.5	204	17	47
204	GB529	7.1	106	21	45
207	GB532	1.4	76	32	55
227	GB538	6.7	129	19	55
U	GB549	23.3	572	13	45
233	GB554	3.9	87	20	46
229A	GB557	21.5	252	18	48
229	GB558	6.4	231	19	47

* Uptake rates reported on ng/g dry wt/hr.

Table 11. Relative rates of microbial activity and respiration percentages observed in sediment samples collected during the summer, 1977 Glacier cruise in the Beaufort Sea.

Sample number	Substrate Uptake		Percent Respiration	
	* Glucose	** Glutamic acid	Glucose	Glutamic acid
BB301	56.5	0.95	22	53
BB302	25.6	0.27	24	49
BB303	15.3	0.62	15	28
BB304	4.8	0.49	22	38
BB305	6.0	0.46	18	35
BB306	26.4	1.06	34	40
BB307	7.3	0.34	16	37
BB308	20.3	0.43	17	38
BB309	7.2	0.49	24	38
BB310	0.8	0.03	28	50
BB311	1.1	0.04	25	39
BB312	6.3	0.50	23	37
BB313	0.9	0.06	42	38
BB314	2.0	0.41	38	40
BB315	1.1	0.04	20	35
BB316	5.5	0.43	28	38
BB317	6.6	0.38	18	33
BB318	4.1	0.49	24	27
BB319	2.3	0.42	29	32
BB320	3.8	0.42	24	44
Average	10.2	0.42	25	38

* Substrate uptake reported as ng/g dry wt/h.

** Substrate uptake reported as μ g/g dry wt/h.

Table 12. Rates of nitrogen fixation observed in sediments collected during the April cruise.

<u>Station number</u>	<u>Sample number</u>	<u>Nitrogen fixation rates</u> <u>ng/g dry wt./hr</u>	
		<u>Field</u>	<u>Laboratory</u>
D	GB401	0.06	
U	GB411	0.09	0.16
V	GB412	0.02	
227	GB420	0.52	0.65
K	GB421	1.36	
J	GB424	0.11	
229	GB425	0.90	0.90
204	GB432	0.05	
212	GB434	0.02	
215	GB435	0.00	
212	GB436	0.03	
208	GB437	0.01	
395	GB440	0.43	0.39
105	GB442	0.00	
388	GB444	0.33	0.50
Average		0.28 ng/g hr	
Correlation coefficient lab vs. field on four samples		0.96	
Average rate observed in five sediments taken from Yaquina Bay, Oregon		1.07 ng/g/hr	

Table 13. Rates of nitrogen fixation in sediments collected during the November cruise.

Station number	Sample number	*Nitrogen Fixation Rates		Laboratory Observations No Oil	April rates
		Field Observations No oil	Oil added		
429	GB501	0.3			
418	GB502	0.9			
K	GB506	6.3	3.6	1.4	1.4
380	GB507	0.3	0.7	0.1	
370	GB508	0.5			
360	GB509	1.3		0.1	
350	GB510	1.2		0.2	
354	GB511	4.4	4.1	0.4	
358	GB512	2.5		0.1	
364	GB513	2.3		0.4	
378	GB514	0.8		0.1	
374	GB515	2.5	2.2	0.5	
388	GB516	0.8			
384	GB517	0.5	0.3		
394	GB518	0.3	0.2		
390	GB519	0.3			
395	GB520	0.5			0.4
212	GB523	0.05			0.03
211	GB524	0.15			
213	GB525	0.08			
214	GB526	0.07			
203	GB527	0.3			
204	GB529	0.15	0.2		0.05
207	GB532	0.8		0.1	
227	GB538	1.7	1.4	0.3	0.5
U	GB549	0.2			0.09
233	GB554	0.2			
229A	GB557	0.5	0.6		
229	GB558	1.0	1.0	0.5	0.9
Average		1.1	1.4	0.4	
		@1.8			
		@@2.1			

* Rates of nitrogen fixation reported as ng nitrogen fixed per g dry wt. per h.

April Nitrogen fixation rates observed in sediments collected at the same stations during the April, 1977 cruise.

@ Mean value for sediments which had been analyzed for crude oil effects.

@@ Mean value for sediments which had also been analyzed in our laboratory several weeks after the field observations.

Table 14. Nitrogen fixation rates observed in Beaufort Sea sediment samples as calculated using the acetylene reduction method.

Sample number	* Nitrogen fixation rate
301	0.21
302	0.20
303	0.18
304	0
305	0
306	0.12
307	0
308	0.09
309	0.03
311	0.05
312	0
313	0
314	0.02
315	0.01
316	0.07
317	0.003
318	0
319	0.09
320	0
Average	0.06

* Values reported as ng nitrogen fixed per gram dry weight of sediment per h.

Table 15. Concentrations of bacteria in water and sediment samples collected during the April cruise as determined by epifluorescent microscopy.

WATER			
Sample number	10^5 Cells/ml	Sample number	10^5 Cells/ml
GW401	2.4	GW423	1.1
GW402	17.4	GW424	1.0
GW403	23.3	GW425	1.4
GW404	10.9	GW426	2.2
GW405	17.4	GW427	1.4
GW406	19.6	GW428	1.3
GW407	32.7	GW429	0.9
GW408	14.1	GW430	1.0
GW409	12.4	GW431	1.7
GW410	12.4	GW432	1.8
GW411	17.0	GW433	1.7
GW412	96.4	GW434	1.1
GW413	138	GW435	0.8
GW415	1.0	GW436	5.7
GW416	1.1	GW437	0.9
GW417	121	GW438	0.8
GW418	6.5	GW439	1.0
GW419	1.2	GW440	0.8
GW420	1.0	GW441	5.3
GW421	1.2	GW442	2.0
GW422	1.3	GW443	0.7
Average	3.0×10^6	GW444	1.4
		GW445	2.0

SEDIMENTS	
	10^9 Cells/ml
GB401	0.4
GB411	4.8
GB412	7.1
GB420	3.6
GB421	3.1
GB425	4.8
GB430	5.9
GB431	7.3
GB432	9.2
GB434	7.2
GB436	8.9
GB440	5.8
GB442	3.3
GB444	2.1
GB445	3.1
Average	2.5×10^9

Table 16. Concentrations of various types of bacteria found in the Beaufort Sea sediment samples. All numbers are in number of cells per g dry wt. sediment.

Sample Number	Total Counts		Anaerobic count	Total vibrios	Percent fermentative	Percent lipase+
	Lib-X	2216				
BB301	3.0E7		7.7E6	7.7E5	26	6
BB302	1.2E7	5.4E6	3.5E6	7.9E4	29	5
BB303	1.0E7	7.0E6	1.4E6	8.7E5	14	10
BB304	8.8E6		1.3E6	9.5E5	15	12
BB305	4.5E6	2.4E6	7.5E5	4.0E5	17	2
BB306	1.6E7	4.3E6	2.6E6	7.4E5	16	9
BB307	1.9E7	1.0E7	4.5E6	2.0E6	24	10
BB308	1.9E7	8.6E6	4.8E6	1.1E6	25	10
BB309	7.1E6		2.0E6	8.0E6	29	20
BB310	1.5E6	4.5E5	3.1E5	1.3E5	20	9
BB311	8.4E5	5.1E5	4.5E4	2.6E4	5	23
BB312	5.8E6		4.3E5	2.3E5	7	11
BB313	1.5E6	5.0E5	2.3E4	1.1E4	1.6	18
BB314	9.4E5		2.1E4	2.2E4	2	19
BB315	9.4E5		9.8E4	3.2E4	10	12
BB316	9.3E6	3.6E6	8.1E5	4.3E5	9	15
BB317	2.7E7	3.8E6	9.8E6	1.5E5	36	8
BB318	3.9E6	1.1E6	2.7E5	1.8E5	7	5
BB319	3.5E6	6.9E5	3.0E5	7.9E4	8.5	17
BB320	6.8E6		8.2E5	1.4E5	12	12
Average	9.4E6	3.7E6	2.1E6	4.6E5	16	12

Percent fermentative and percent lipase positive based on Lib-X total counts as 100%. All count data based on plates that had been incubated at 0.5C for 14 days.

Table 17. Concentrations of various types of bacteria found in Beaufort Sea Sediment samples collected during the January Elson Lagoon study.

Sample number	Total # Bacteria			Percent Lipolytic Bacteria
	Lib X	Marine Agar 2216	Lib X + 1% Crude oil	
BB402	1.9E6	1.6E6	1.2E6	15
BB403	2.3E6	2.1E6	8.2E5	15
BB404	3.6E6	2.7E6	2.0E6	3
BB405	2.9E6	1.4E6	1.0E6	10
BB406	3.2E6	7.8E6	3.9E6	8
BB407	2.9E5	2.2E5	1.0E6	12
BB408	2.4E5	3.1E5	3.7E5	6
BB409	7.6E6	9.3E6	6.5E6	88
BB410	8.8E5	8.5E5	1.4E6	11

Table 18. Correlation coefficients calculated from various measurements made on Beaufort Sea sediments.

	Glutamic acid									
Glucose uptake	0.68	Glucose uptake								
Lib "X" plate counts	0.57	0.73	Lib "X" plate counts							
2216 plate counts	0.35	0.60	0.73	2216 plate counts						
Tween 80 plate counts	0.49	0.79	0.90	0.92	Tween 80 plate counts					
Anaerobes	0.37	0.61	0.95	0.55	0.84	Anaerobes				
Total vibrios	0.39	0.32	0.53	0.87	0.60	0.38	Total vibrios			
Total sucrose utilizers	0.52	0.67	0.62	0.60	0.70	0.47	0.49	Total sucrose utilizers		
% fermentative bacteria	0.12	0.12	0.25	-0.01	0.22	0.30	0.07	0.03	% fermentative bacteria	
% lipase producers	-0.35	-0.44	-0.45	-0.31	-0.43	-0.40	-0.19	-0.29	-0.03	% lipase producers

Table 19. Correlation coefficients observed between measurements made in 16 sediment samples collected during the April cruise.

		<i>* Total plate counts</i>	<i>Total fermentative organisms</i>	<i>Total Vibrio counts</i>	<i>Total sucrose + Vibrio counts</i>	<i>Glutamic acid uptake rates</i>	<i>Glucose uptake rates</i>
Total fermentative organisms	0.98						
Total <u>vibrio</u> counts	0.96	0.99					
Total sucrose + <u>Vibrio</u> counts	0.97	0.97	0.97				
Glutamic acid uptake rates	0.61	0.65	0.61	0.59			
Glucose uptake rates	0.92	0.92	0.87	0.86	0.84		
Acetate uptake rates	0.94	0.92	0.87	0.86	0.77	0.97	

* Plate count determinations made on Lib X agar plates.

Table 20. Concentration of presumptive nitrogen fixing bacteria in sediments collected during the April, 1977 Lower Cook Inlet cruise.

<u>Sample number</u>	<u>Station number</u>	<u>Counts x 10³ per g dry wt.</u>
GB401	D	4.0
GB410	242	<0.1
GB411	U	2.4
GB412	V	1.2
GB420	227	340
GB421	K	22
GB424	J	1.3
GB425	229	800
GB430	213	0.02
GB431	214	<0.01
GB432	204	40
GB434	212	--
GB435	215	0.8
GB436	212	0.2
GB437	208	14
GB440	395	56
GB442	105	0.7
GB444	388	<0.01
GB445	378	0.2

Table 21. The effects of crude oil on nitrogen fixation in sediment samples which had not been treated and those which had been treated with sucrose.

All values reported as ng nitrogen fixed per g. dry wt./h.

Sample number	No Sucrose		Sucrose	
	No oil	Oil	No Oil	Oil
A. Yaquina Bay, OR				
A.	0.28	0.22	16.1	17.2
B.	0.5	0.5	0.6	0.6
C.	0.86	0.75	8.5	3.8
D.	0.36	0.28	3.3	
E.	1.55	0.22	9.7	7.1
B. Cook Inlet, April, 1977				
411	0.08	0.11	9.0	3.4
420	0.42	0.33		0.6
425	0.48	0.44	4.5	8.5
440	0.22	0.14	0.67	0.67
444	0.26	0.26	0.57	0.49
C. Beaufort Sea, September, 1977				
301	0.21	0.23	0.32	0.23
302	0.20	0.20	0.31	0.31
303	0.19	0.16	0.25	0.21
306	0.04	0.05	1.25	0.51
308	0.13	0.12	0.15	0.17
314	0.02	0.02	0.67	0.84
315	0.02	0.02	0.07	0.04
317	0.01	0.01	0.29	0.12
319	0.09	0.10	1.57	1.24
D. Cook Inlet, November, 1977				
506	1.43	1.38	1.77	1.43
507	0.07	0.09	0.13	0.18
509	0.07	0.17	0.17	0.26
510	0.18	0.18	0.22	0.26
511	0.43	0.35	1.06	0.79
512	0.10	0.08	0.40	0.33
513	0.36	0.33	0.55	0.44
514	0.09	0.05	0.30	0.28
515	0.48	0.35	0.61	0.52

Table 21.

Sample number	No Sucrose		Sucrose	
	No oil	Oil	No Oil	Oil
D. Cook Inlet, November, 1977 (con't)				
532	0.11	0.09	0.21	0.17
538	0.33	0.23	0.36	0.33
558	0.51	0.45	0.57	0.54
E. Beaufort Sea, January, 1978				
402	0.15	0.16	0.50	0.32
403	0.15	0.15	0.51	0.25
404	0.09	0.10	0.63	0.41
405	0.08	0.08	0.22	0.20
406	0.05	0.04	0.18	0.18
407	0.10	0.08	0.13	0.12
408	0.11	0.10	0.14	0.14
409	0.11	0.10	0.27	0.23
410	0.08	0.07	0.15	0.16

Table 22. Effects of crude oil on glucose respiration rates observed in the field.

A. Water samples collected during the November Cook Inlet cruise.

Sample number	*DPM		Percent Reduction
	No oil	Oil	
505	246	156	37
506	3866	1553	60
511	226	201	11
513	171	L	
514	260	L	
517	204	93	54
528	722	199	72
530	155	123	21
533	165	78	53
534	122	105	14
535	404	189	53
536	221	87	61
538	516	133	74
539	1265	388	69
540	1235	411	67
541	6491	5806	11
542	2054	844	54
543	704	951	0
546	396	345	13
551	1651	1584	4
552	314	194	38
557	457	185	60
558	570	404	29
Average			41%

B. Sediment samples collected during the January Beaufort Sea field trip.

Sample number	No oil	Oil	Oil Extract	Percent Reduction
402	259		108	58
403	447		50	89
404	435	345		21
405	307	202		34
406	236	152		36
408	110	47		58
409	124	98		21
410	347	205		41
Average				45%

(*) Disintegrations per min. - a measurement of radioactivity which is directly proportional to the amount of glucose respired.

(L) Too low to be detected.

Table 23. Percent reduction in respiration rates observed in sediment samples exposed to crude oil, crude oil aqueous extract or weathered crude oil.

A. First study on summer Beaufort Sea samples exposed to crude oil.

Sample #	Percent Reduction	
	Glucose	Glutamic acid
301	30	55
302	49	17
303	40	37
304	25	45
305	46	56
306	58	37
307	42	19
308	41	0
309	46	38
310	0	50
311	22	23
312	39	32
313	14	24
314	42	48
315	8	19
316	65	48
317	55	50
318	42	5
319	12	0
320	19	50
Average	35	33

Table 23. (con't)

B. Second study on the above samples conducted two weeks later.

Sample #	Glucose			Glutamic Acid		
	Oil	Extract	Weathered	Oil	Extract	Weathered
301	16	39	21	6	3	0
302	59	9	49	36	0	35
303	8	22	0	9	0	7
304	30	52	23	16	12	13
305	26	60	0	6	0	13
306	21	60	12	0	0	0
307	65	20	63	43	0	40
308	7	40	16	0	0	0
309	59	21	66	46	23	67
310	0	0	0	13	0	6
311	0	0	0	0	0	0
312	23	43	27	13	2	9
313	0	0	0	0	0	0
314	32	3	15	29	0	44
315	2	15	12	8	12	21
316	21	0	30	8	12	21
317	21	66	39	0	0	0
318	9	16	0	29	0	24
319	40	38	7	10	0	0
320	12	0	22	7	26	7
Average	20	23	21	15	8	19

Table 24. Kinetic experiments on the effects of crude oil on glucose uptake in sediment samples collected in Elson Lagoon in January, 1978.

Sample number	No Oil			Crude Oil Extract		
	V_{\max}	T_t	$K_t + S_n$	V_{\max}	T_t	$K_t + S_n$
BB402	0.14	188	26	0.15	469	73
BB403	0.25	178	45	0.25	205	50
BB405	0.10	368	35	0.08	928	75
BB408	0.09	395	37	0.06	1438	80
BB410	0.09	401	35	0.12	350	41

V_{\max} is the maximum potential rate of glucose uptake.

T_t is the time in hours required for the natural microbial population to utilize all of the naturally occurring glucose in the sample.

$K_t + S_n$ is the transport constant plus the natural substrate concentration.

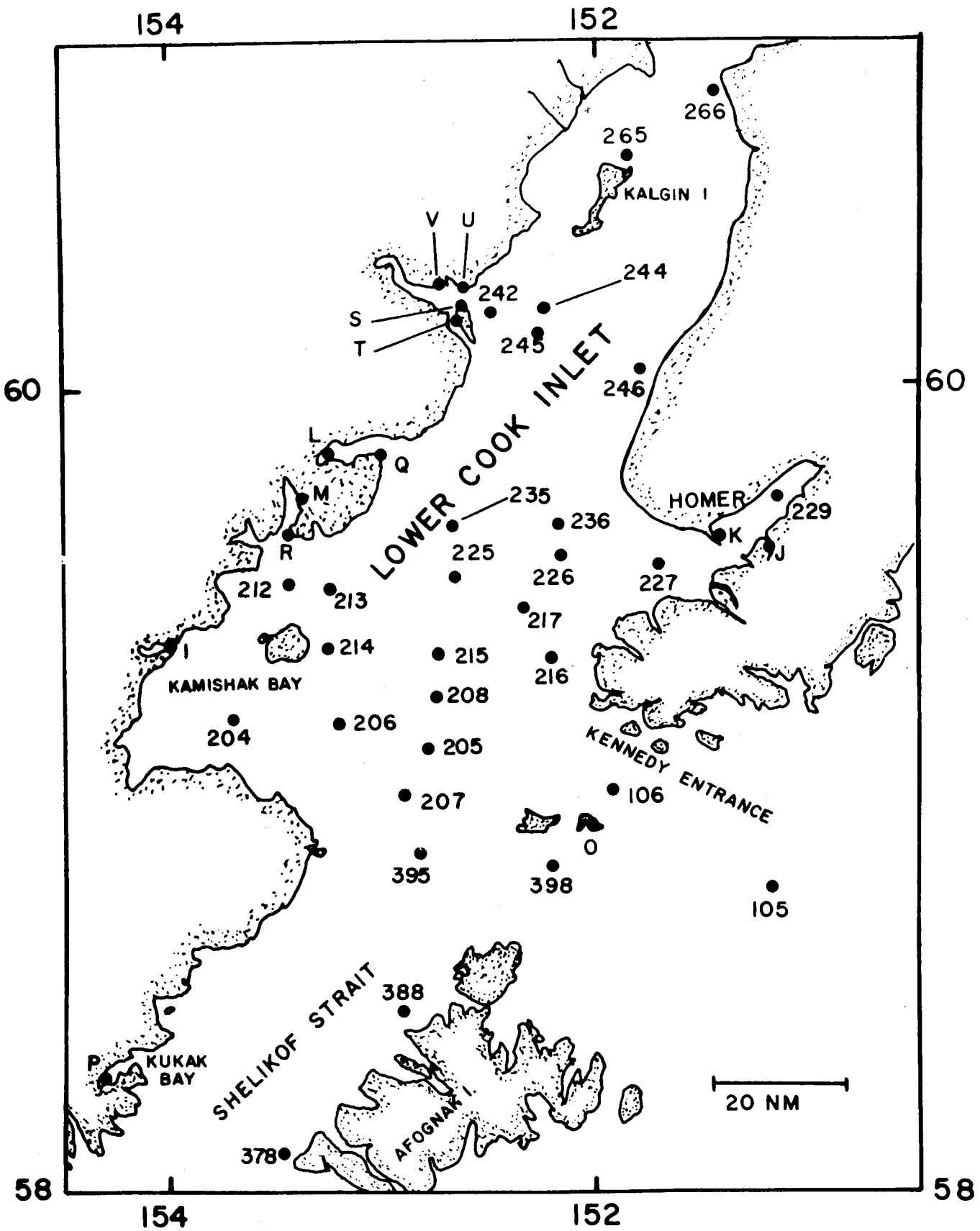


Figure 1. Station locations and numbers for stations occupied in the Cook Inlet area during the April cruise.

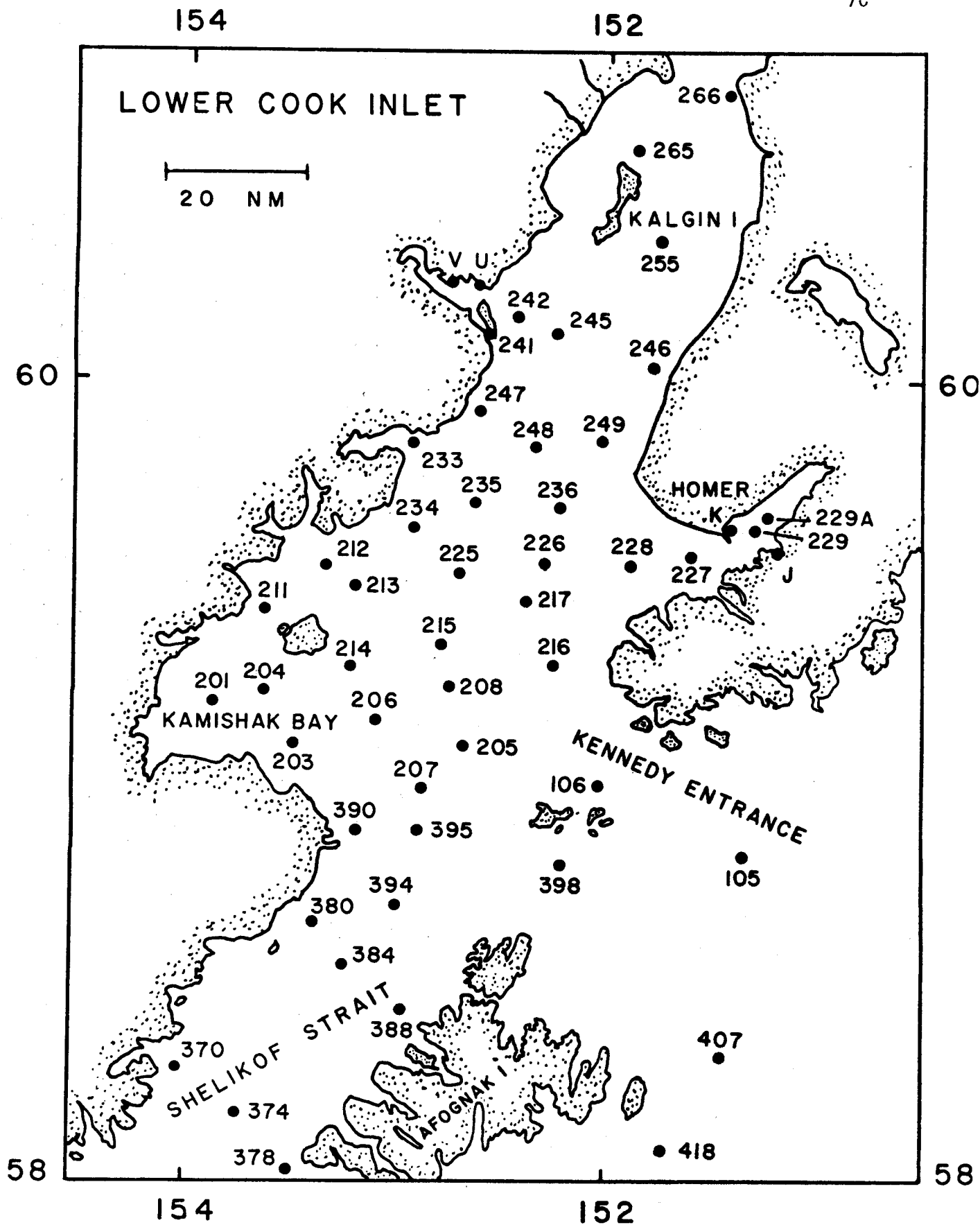


Figure 2. Station locations and numbers for stations occupied in the Cook Inlet during the November cruise.

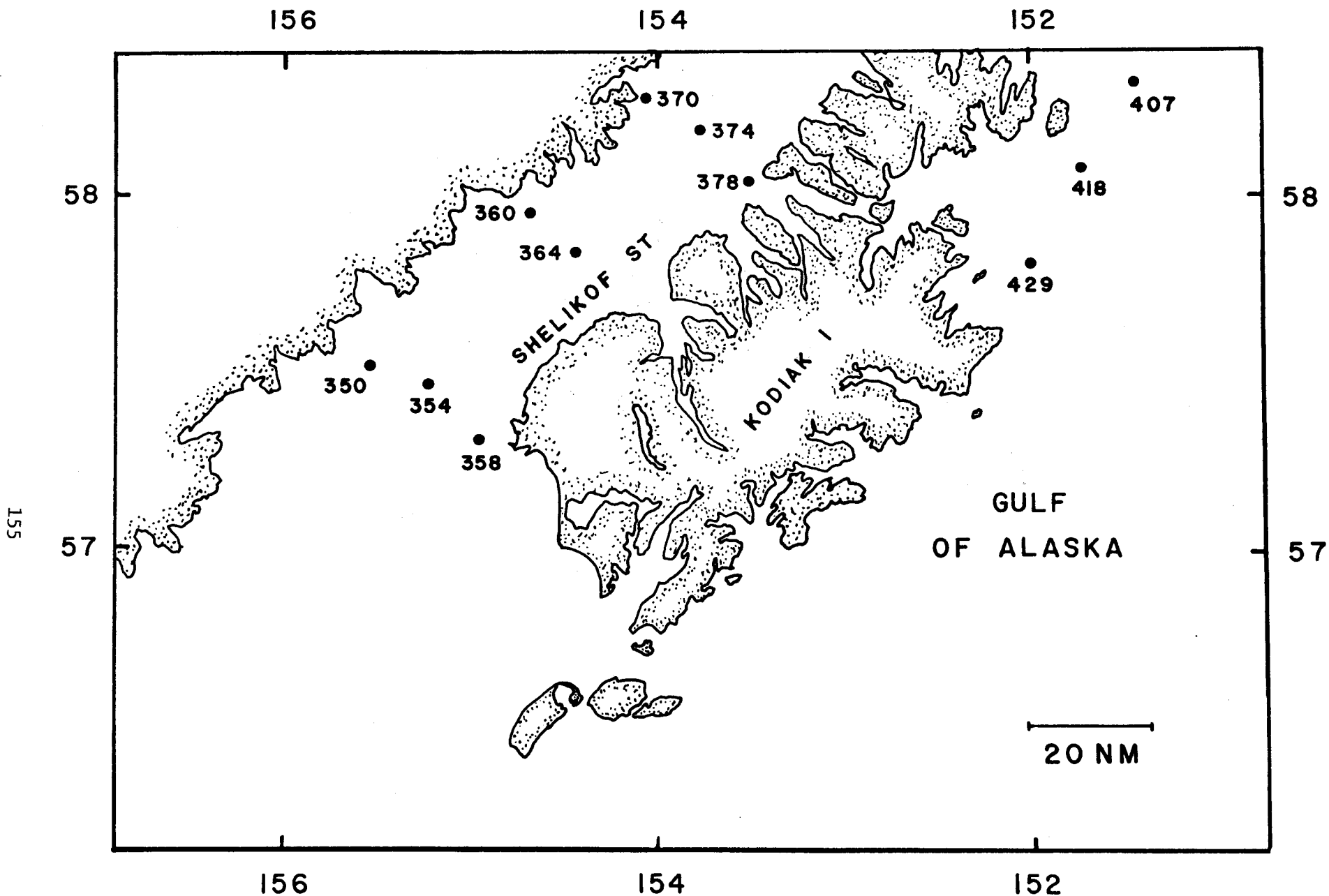


Figure 3. Station locations and numbers for stations occupied in the Cook Inlet during the November cruise.

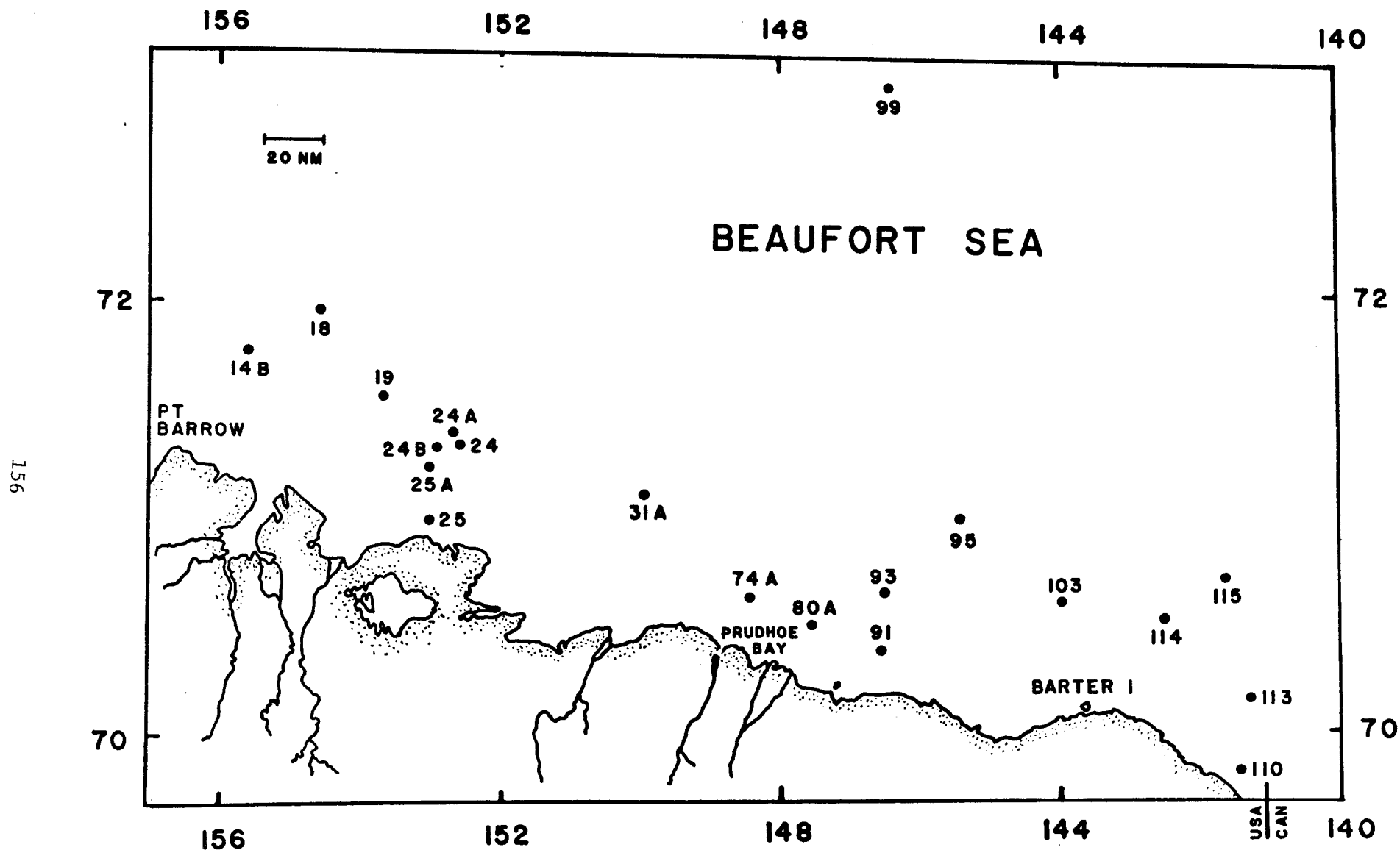


Figure 4. Station locations and numbers for stations occupied in the Beaufort Sea during the September cruise.

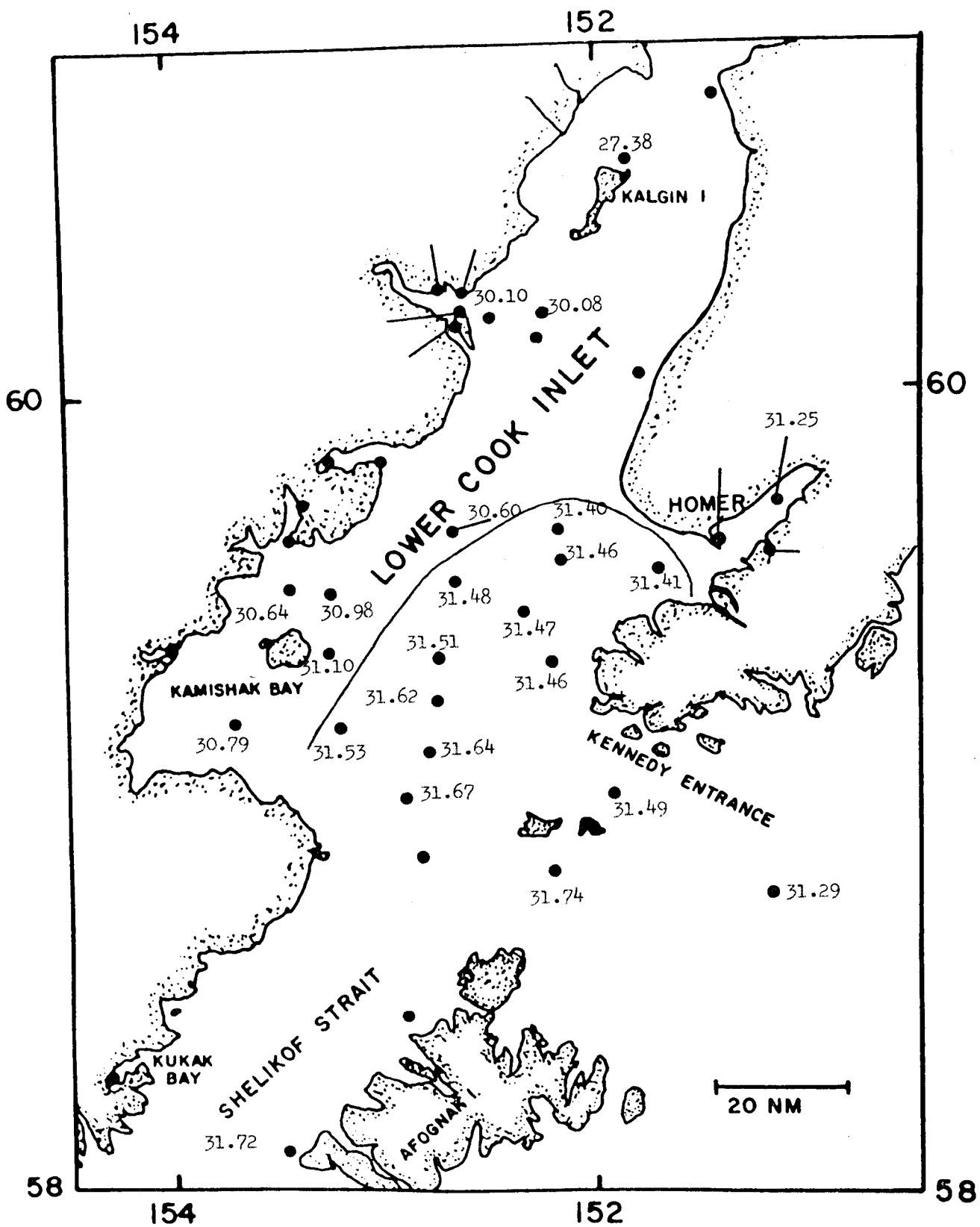


Figure 5 . Salinity measurements made in surface water samples analyzed during the April cruise. The unit used is parts per thousand.

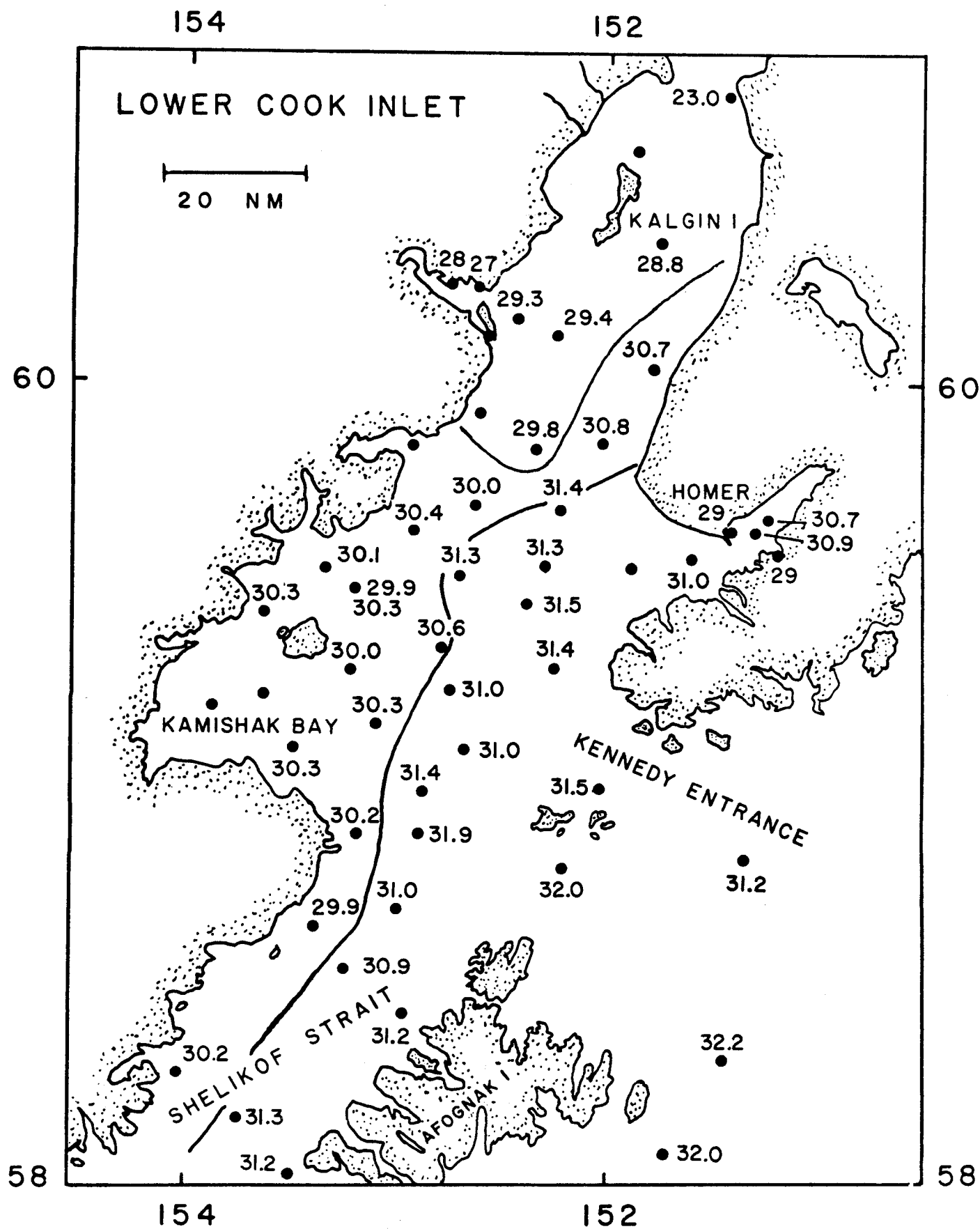


Figure 6. Salinity measurements made in surface water samples analyzed during the November cruise. The unit used is parts per thousand.

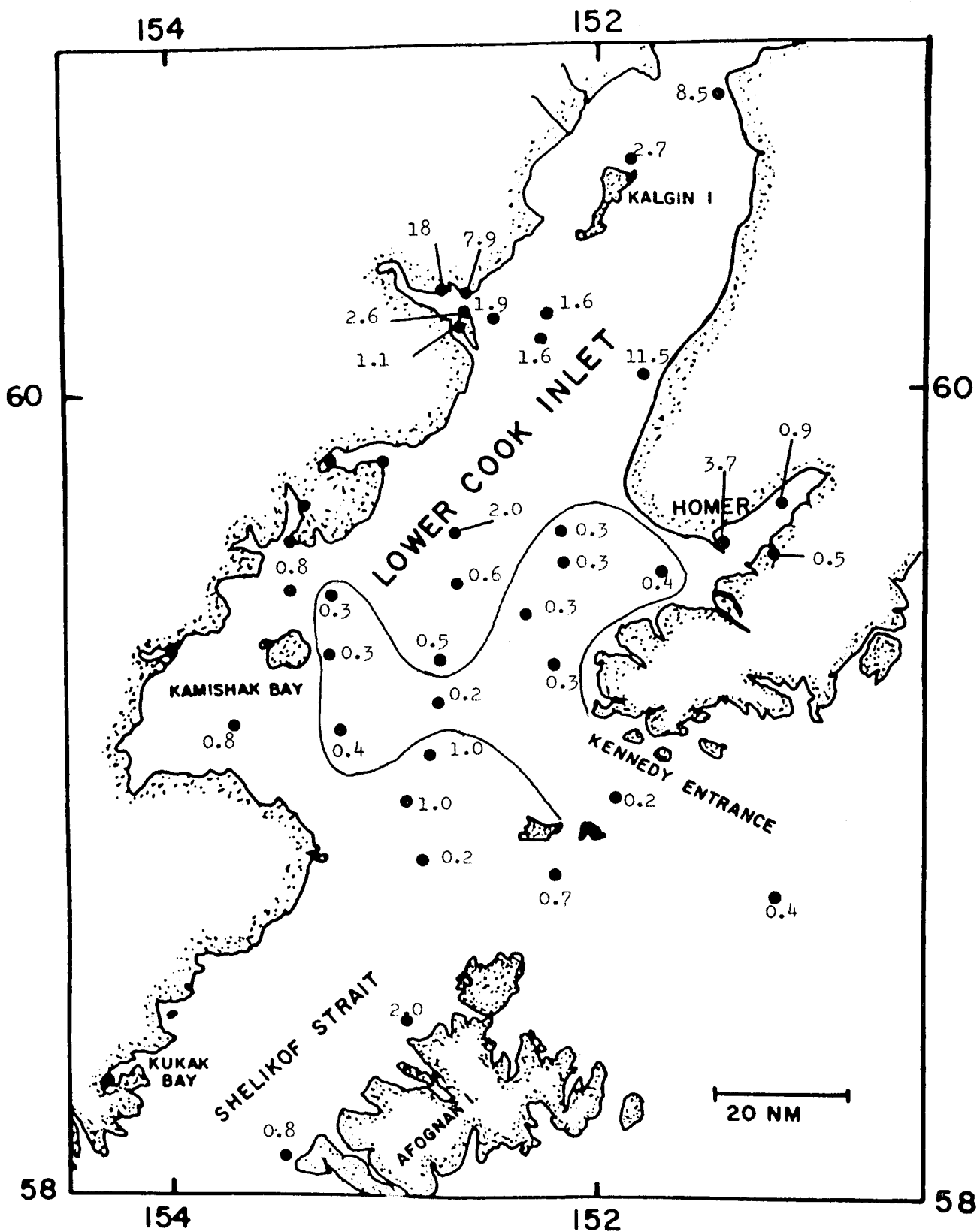


Figure 8. Relative microbial activity in water samples as measured using glucose during the April cruise. The units are reported as ng/liter/h.

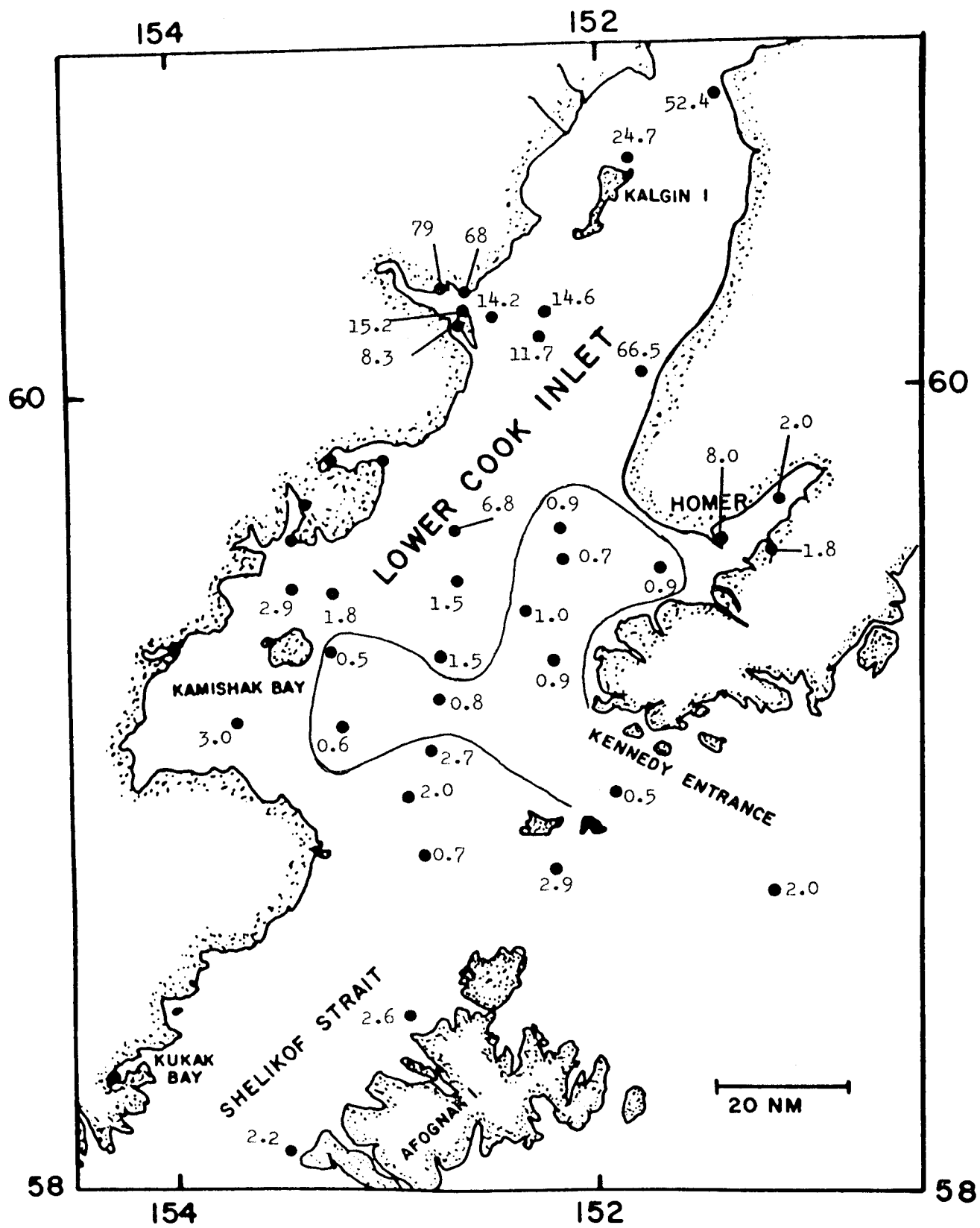


Figure 9. Relative microbial activity in water samples as measured using glutamic acid during the April cruise. The units are reported as ng/liter/h.

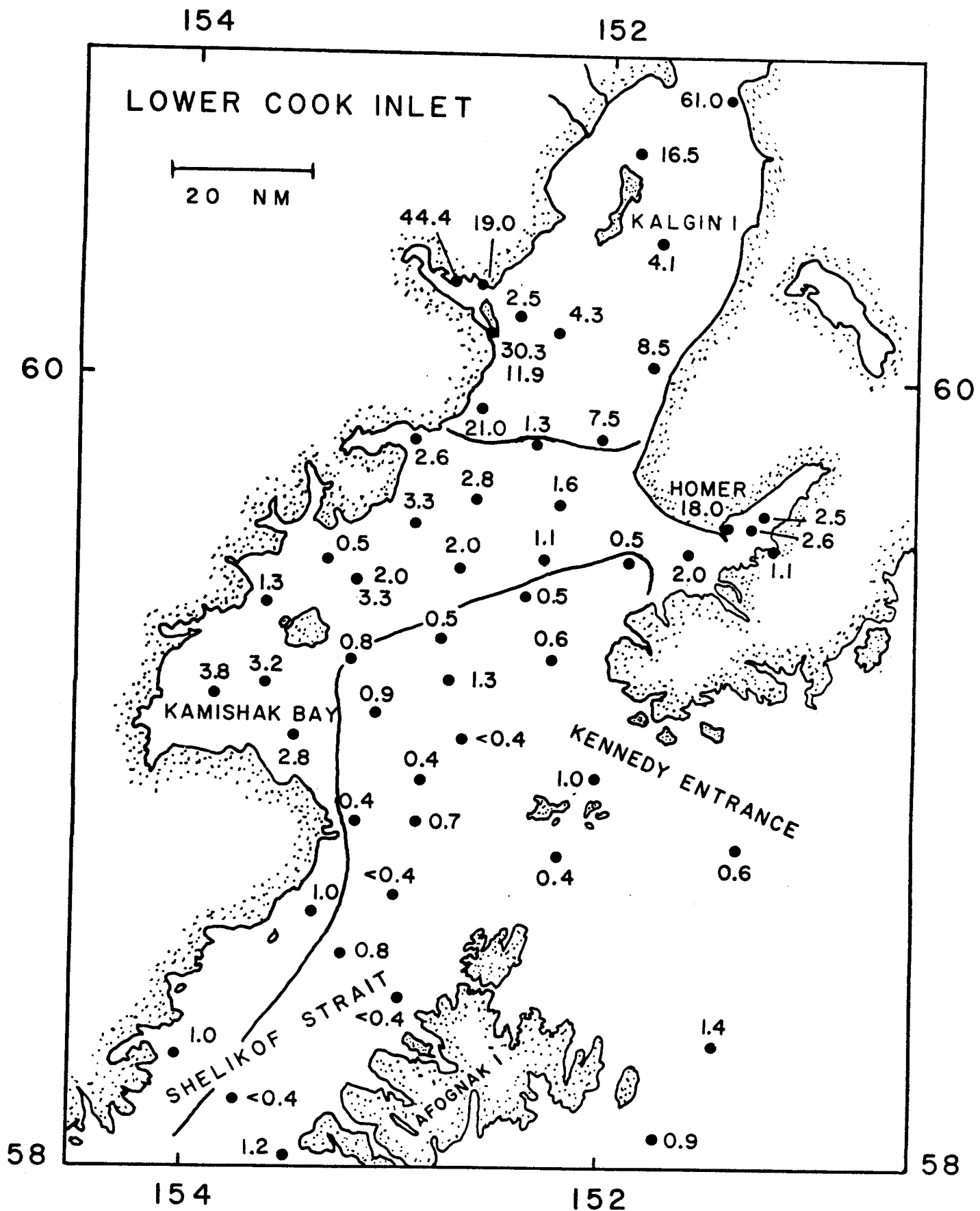


Figure 10. Relative microbial activity in water samples as measured using glutamic acid during the November cruise. The units are reported as ng/liter/h.

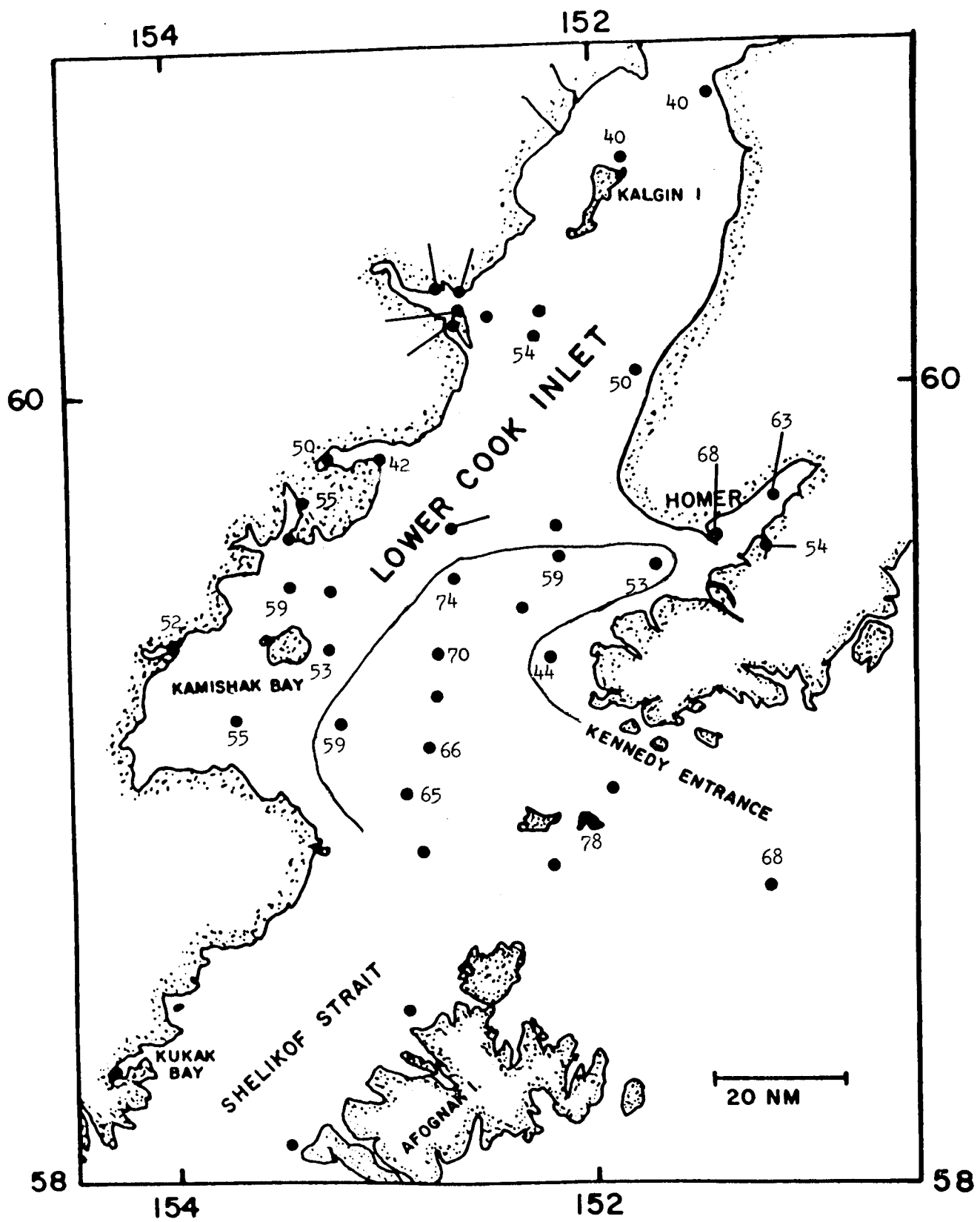


Figure 11. Respiration percentages observed in water samples as measured using glutamic acid during the October cruise,

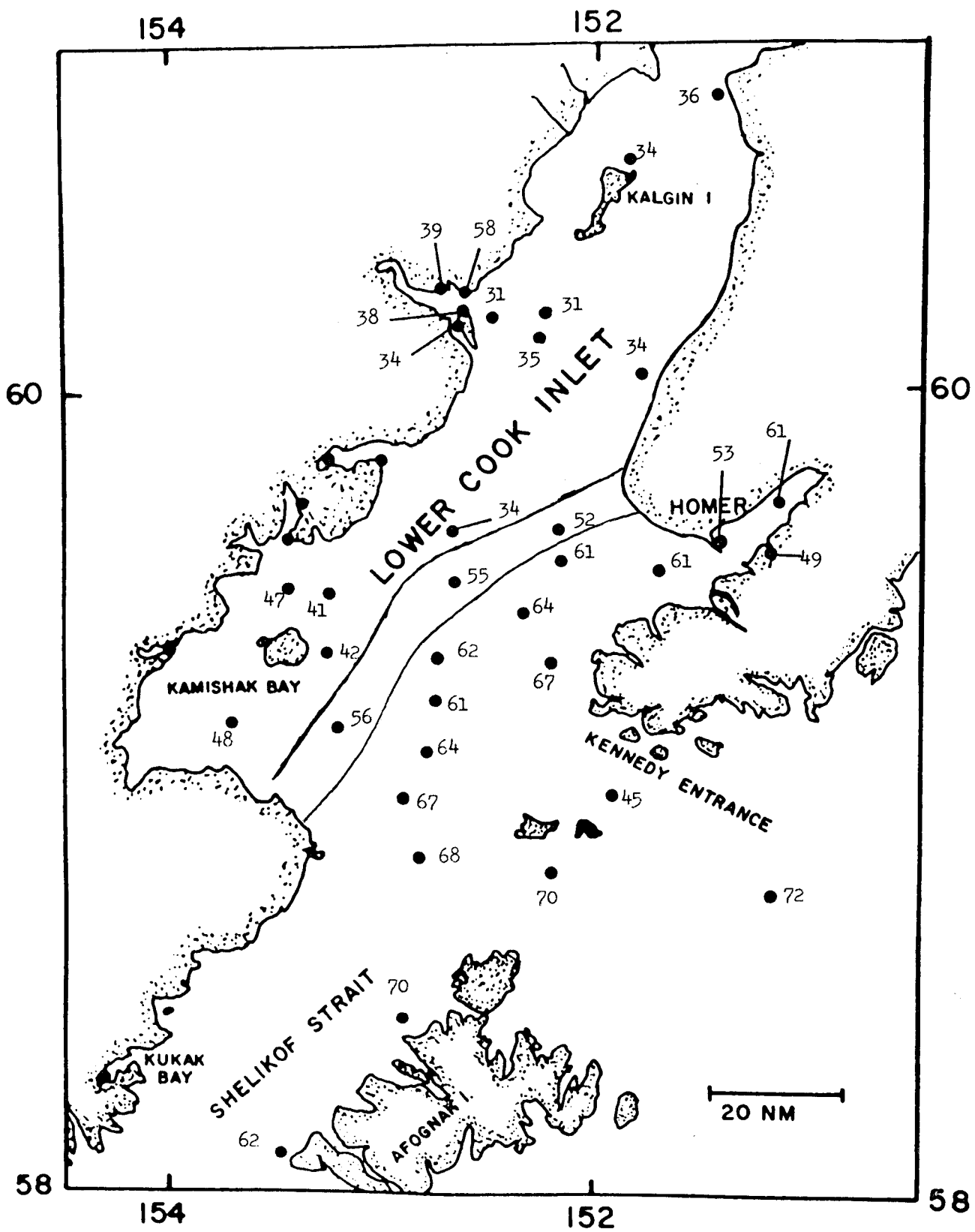


Figure 12. Respiration percentages observed in water samples as measured using glutamic acid during the April cruise.

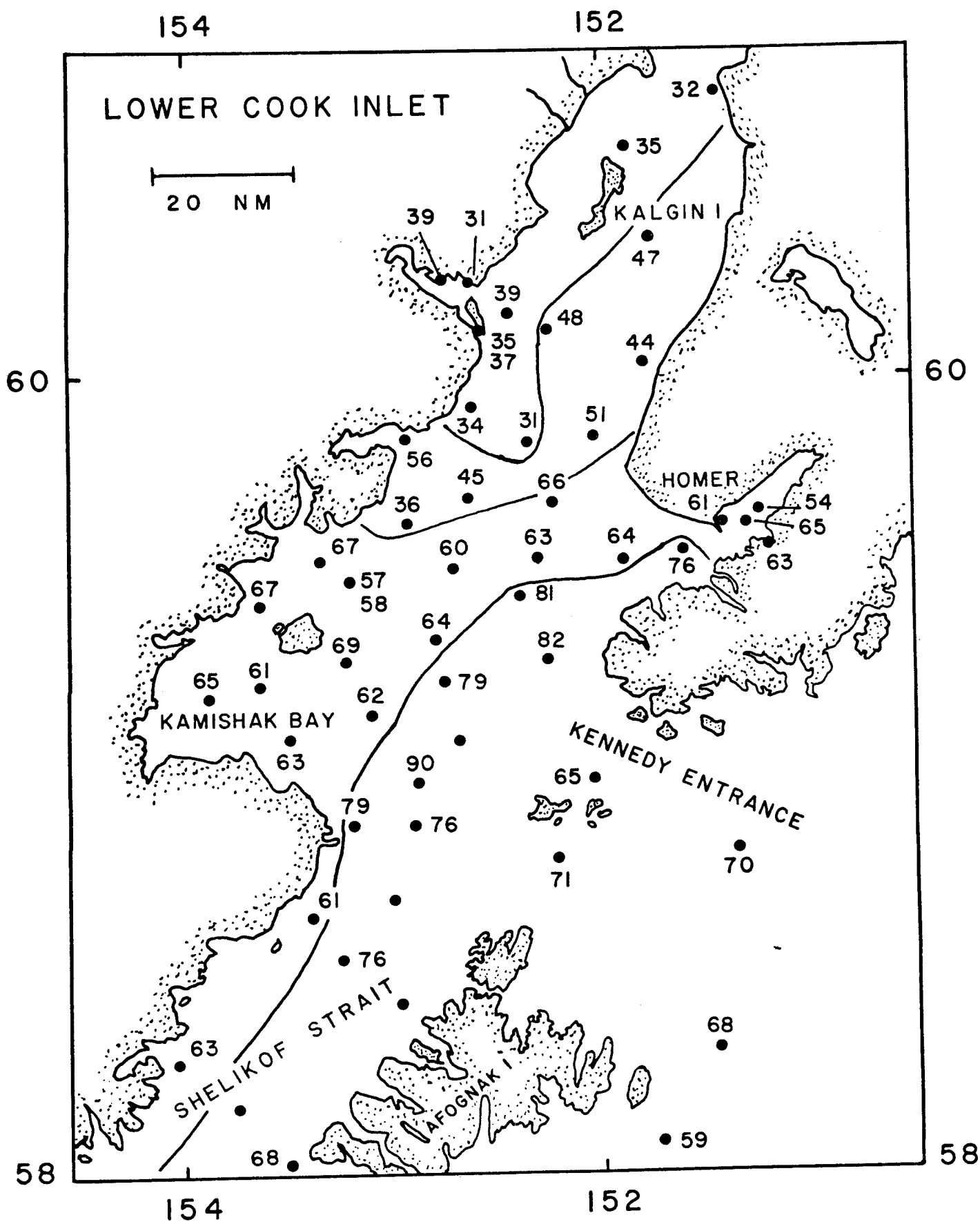


Figure 13. Respiration percentages observed in water samples as measured using glutamic acid during the November cruise.

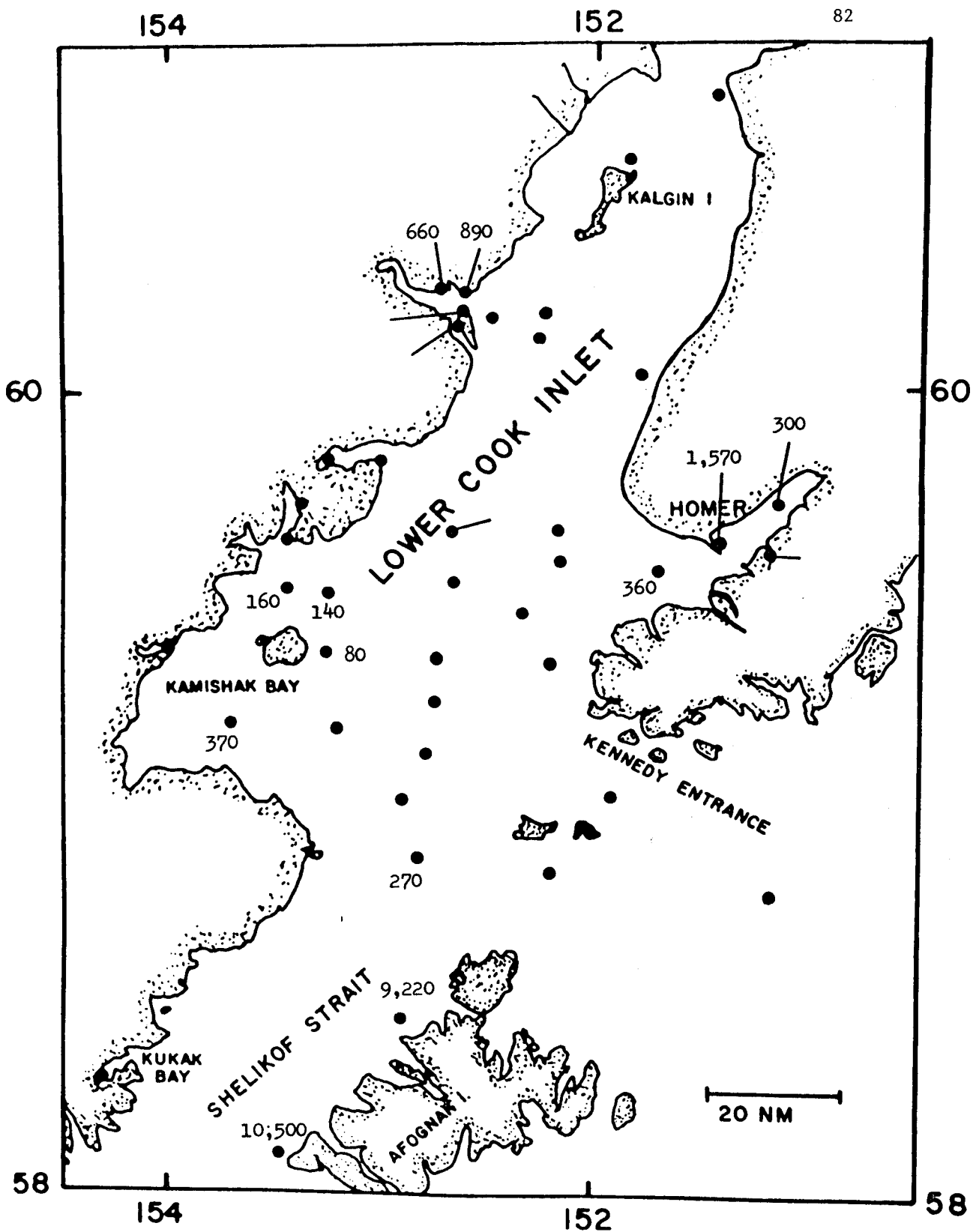


Figure 14. Relative microbial activity in sediment samples as measured using glutamic acid during the April cruise. The units are reported as ng/g. dry wt./h.

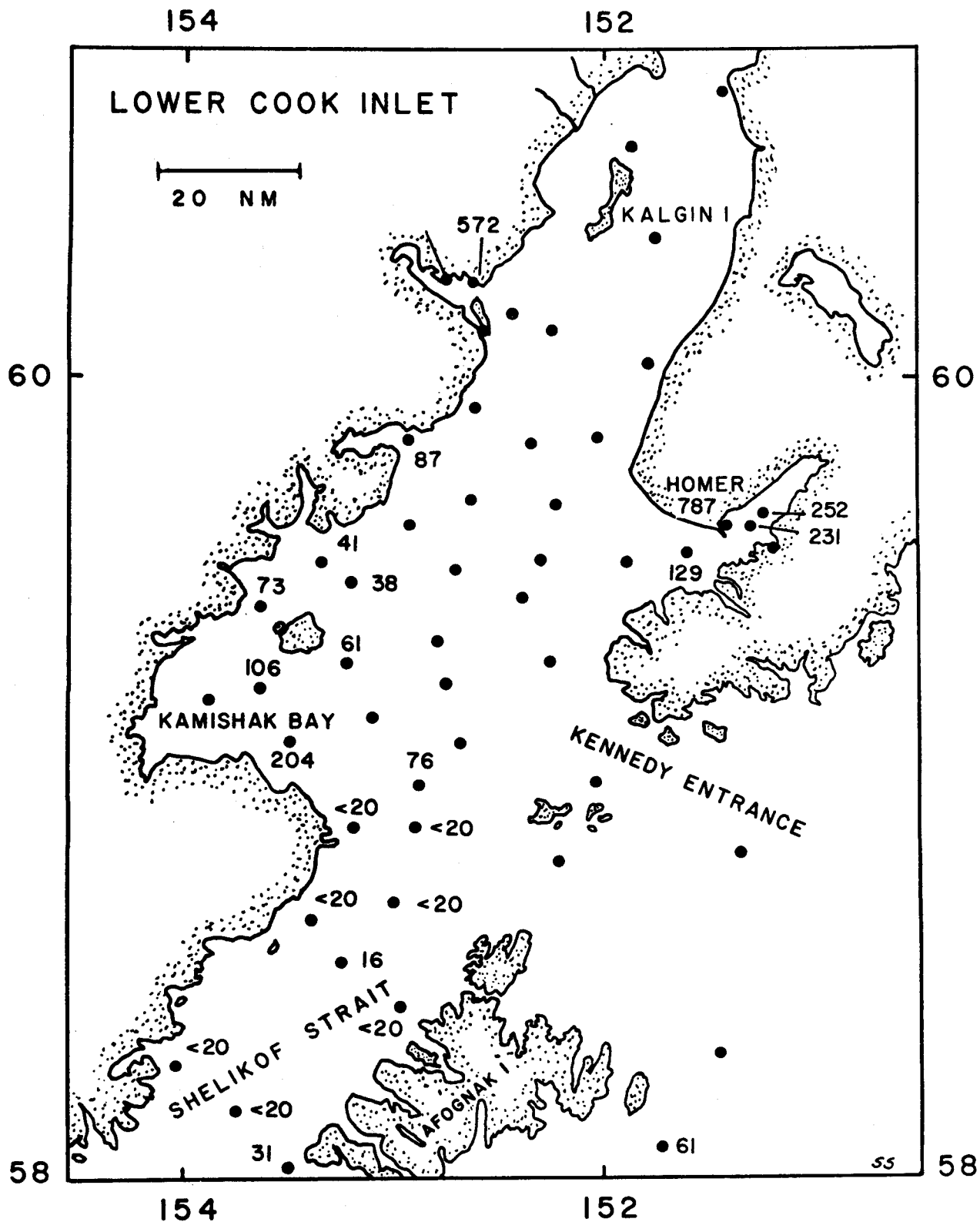


Figure 15. Relative microbial activity in sediment samples as measured using glutamic acid during the November cruise. The units are reported as ng/g. dry wt./h.

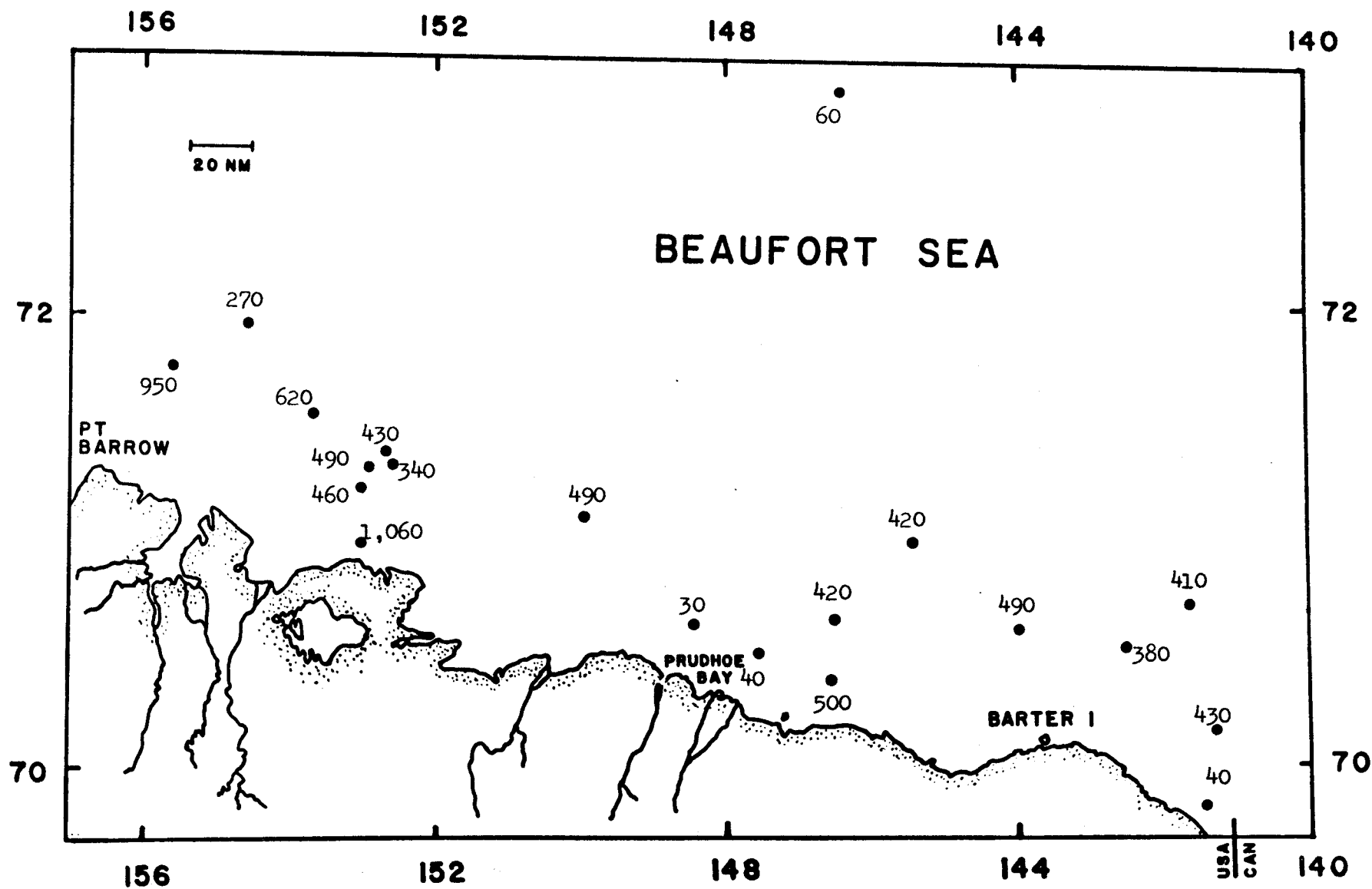


Figure 16. Relative microbial activity in sediment samples as measured using glutamic acid during the September Beaufort Sea cruise. The units are reported as ng/g. dry wt./h.

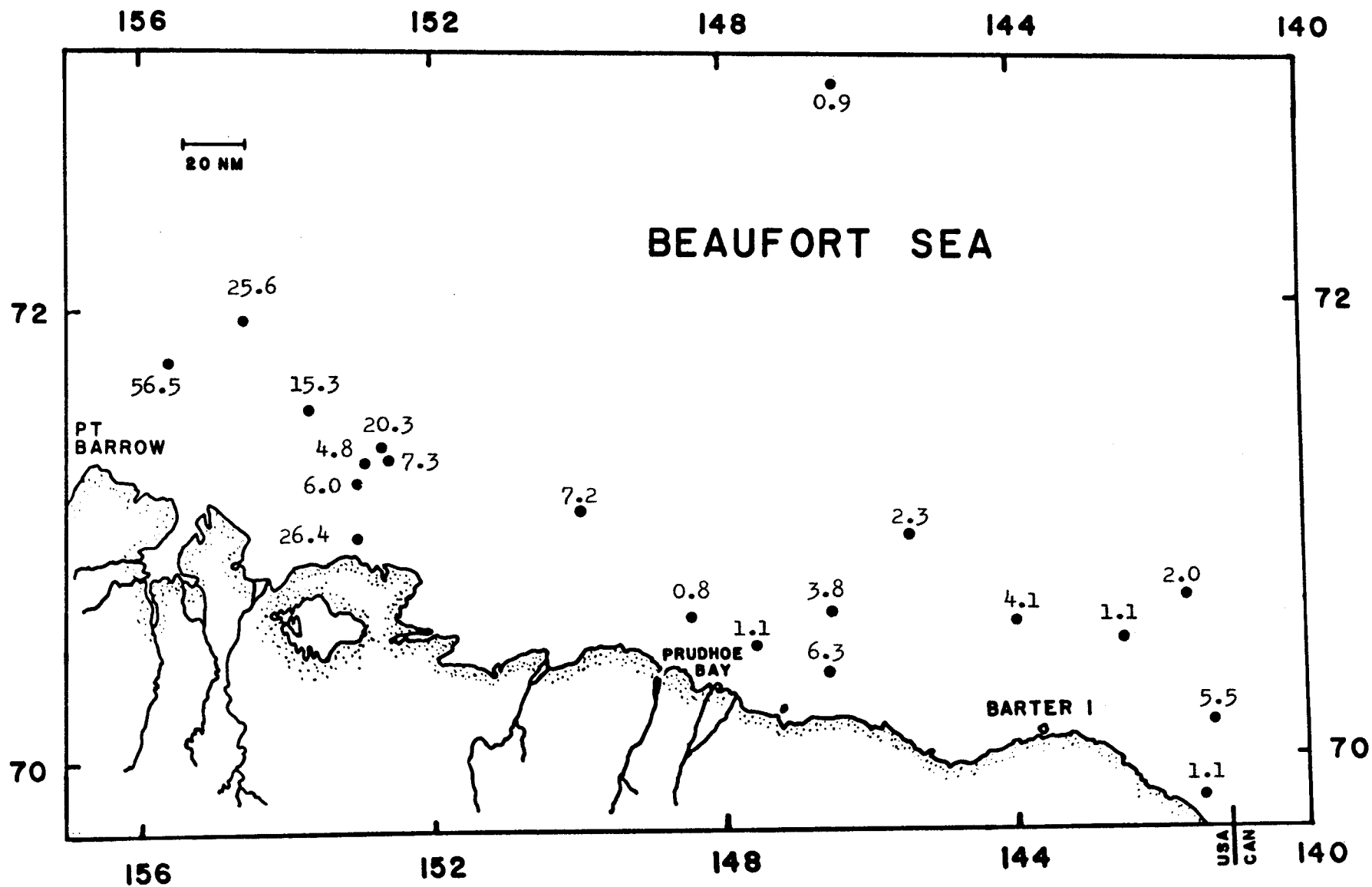


Figure 17. Relative microbial activity in sediment samples as measured using glucose during the September Beaufort Sea cruise. The units are reported as ng/g.dry wt./h.

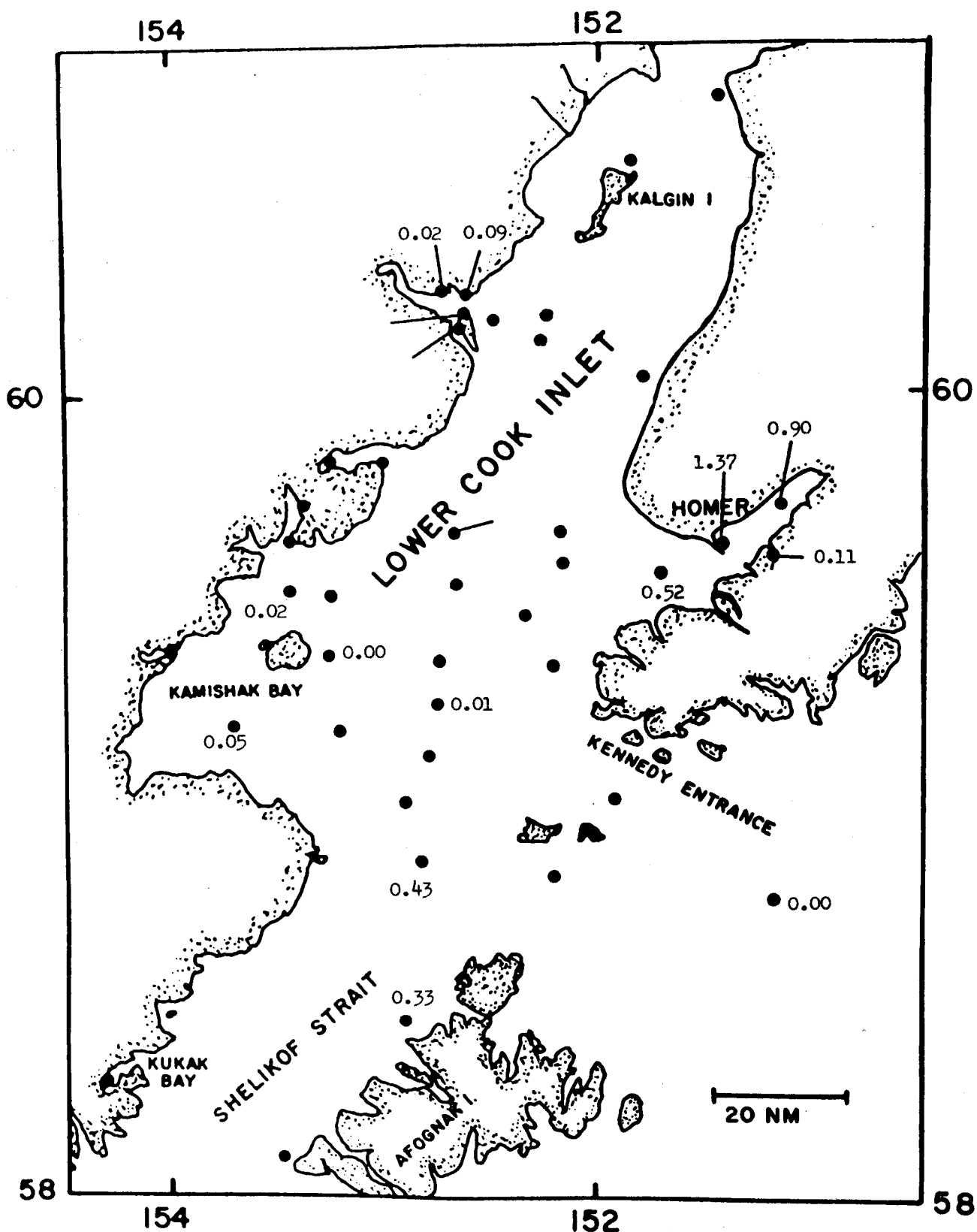


Figure 18. Observed rates of nitrogen fixation in sediment samples collected during the April cruise. The unit of measurement used was ng nitrogen fixed/gram dry weight of sediment/h.

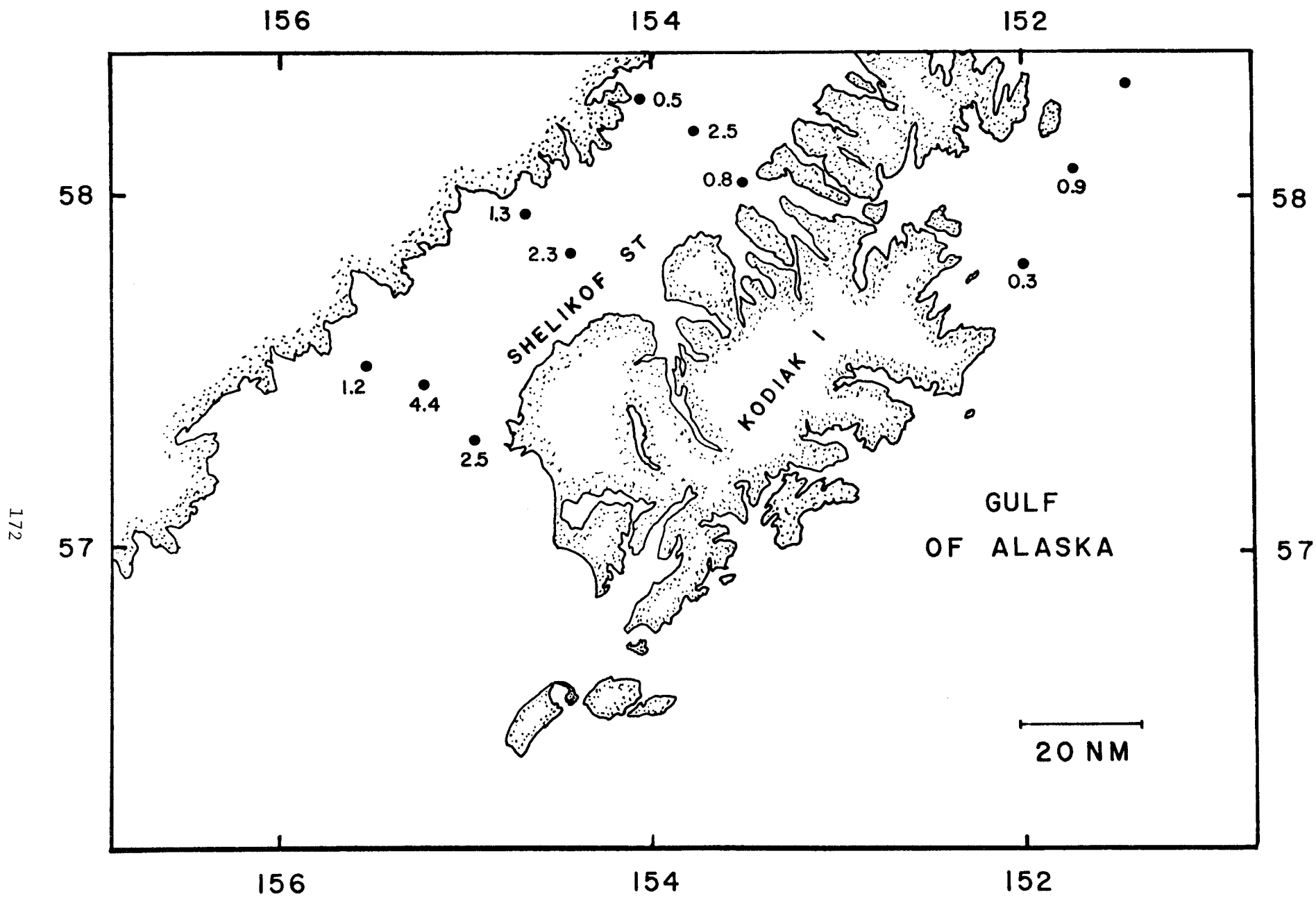


Figure 20. Nitrogen fixation rates in sediment samples collected during the November cruise. The unit of measurement used was ng nitrogen fixed/g. dry wt./h.

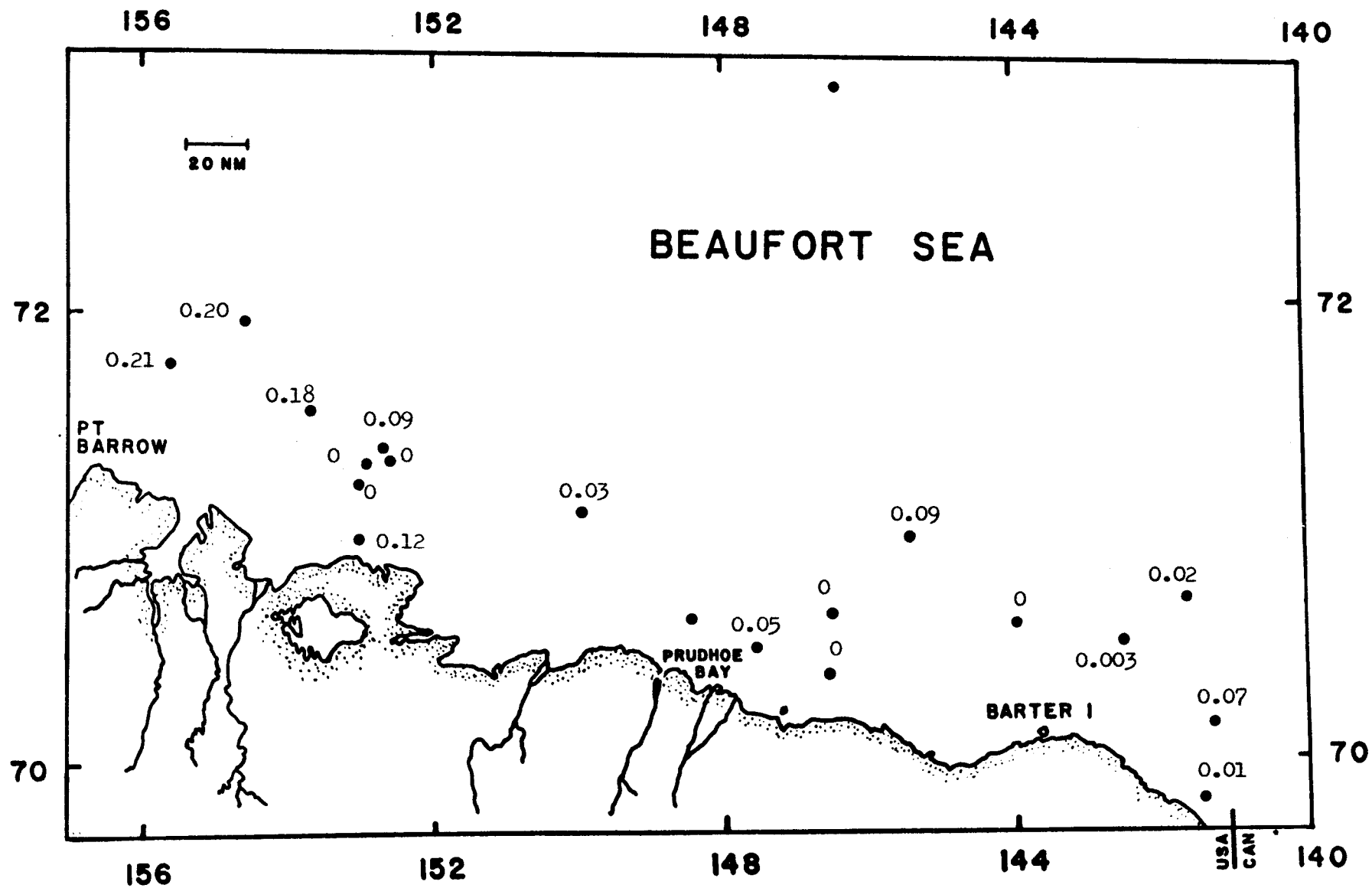


Figure 21. Nitrogen fixation rates in sediment samples collected during the September Beaufort Sea cruise. The unit of measurement used was ng nitrogen fixed /g. dry wt./h.

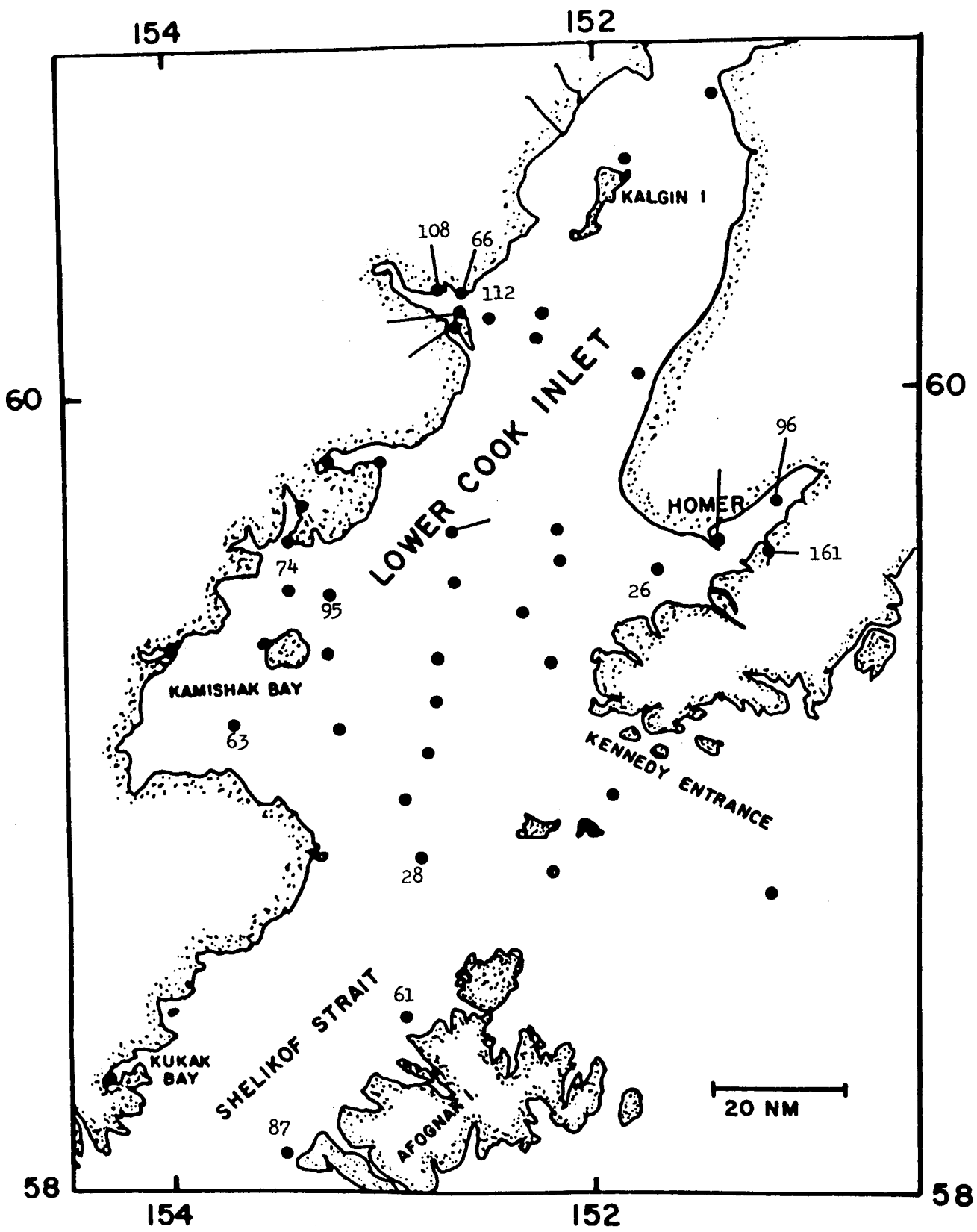


Figure 22. Crude oil degradation potential measurements in sediments collected during the April cruise. Units reported as DPM/ gr. dry weight.

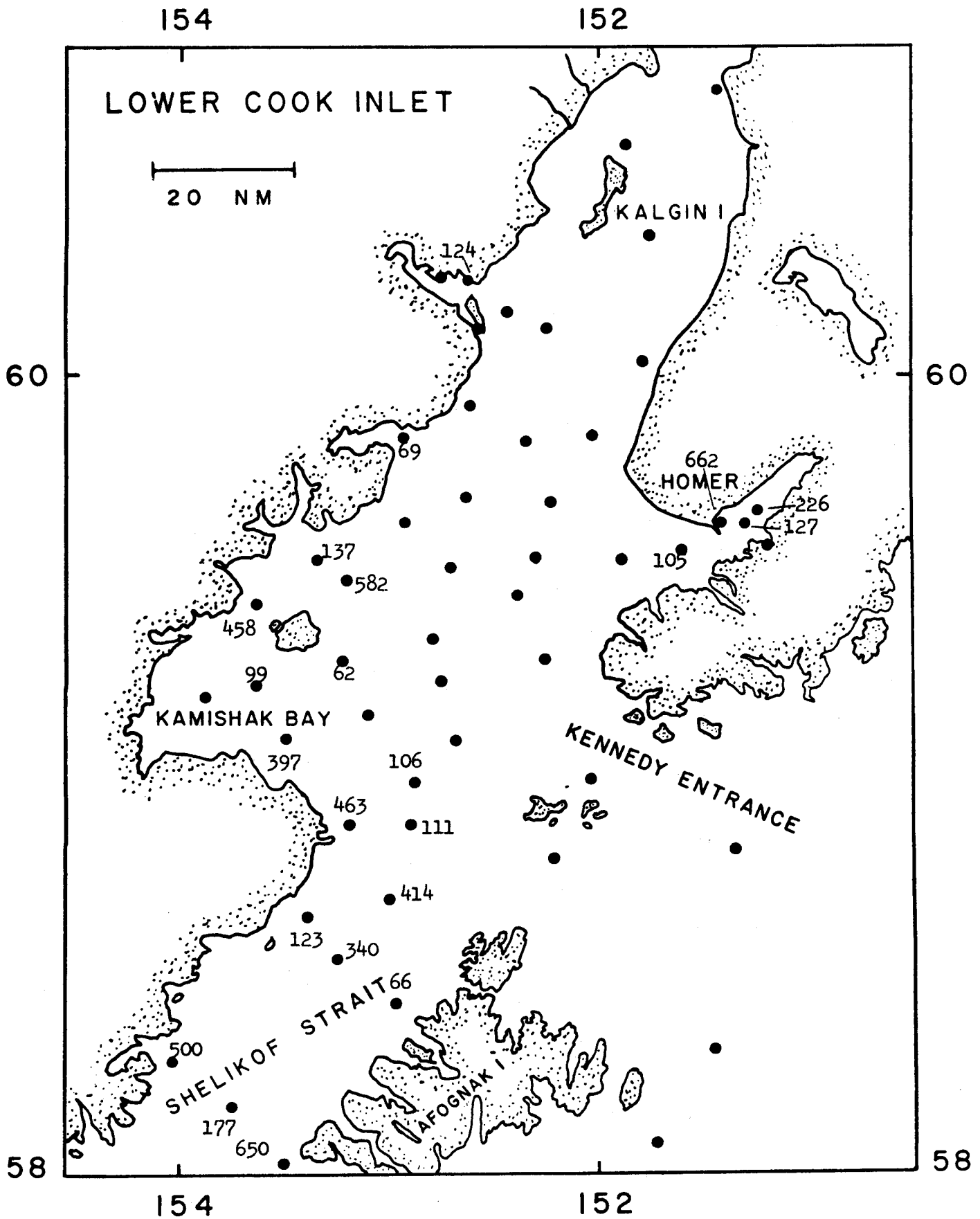


Figure 23. Crude oil degradation potentials observed in Cook Inlet sediment samples collected during the November cruise. The unit of measurement used was DPM/g. dry wt.

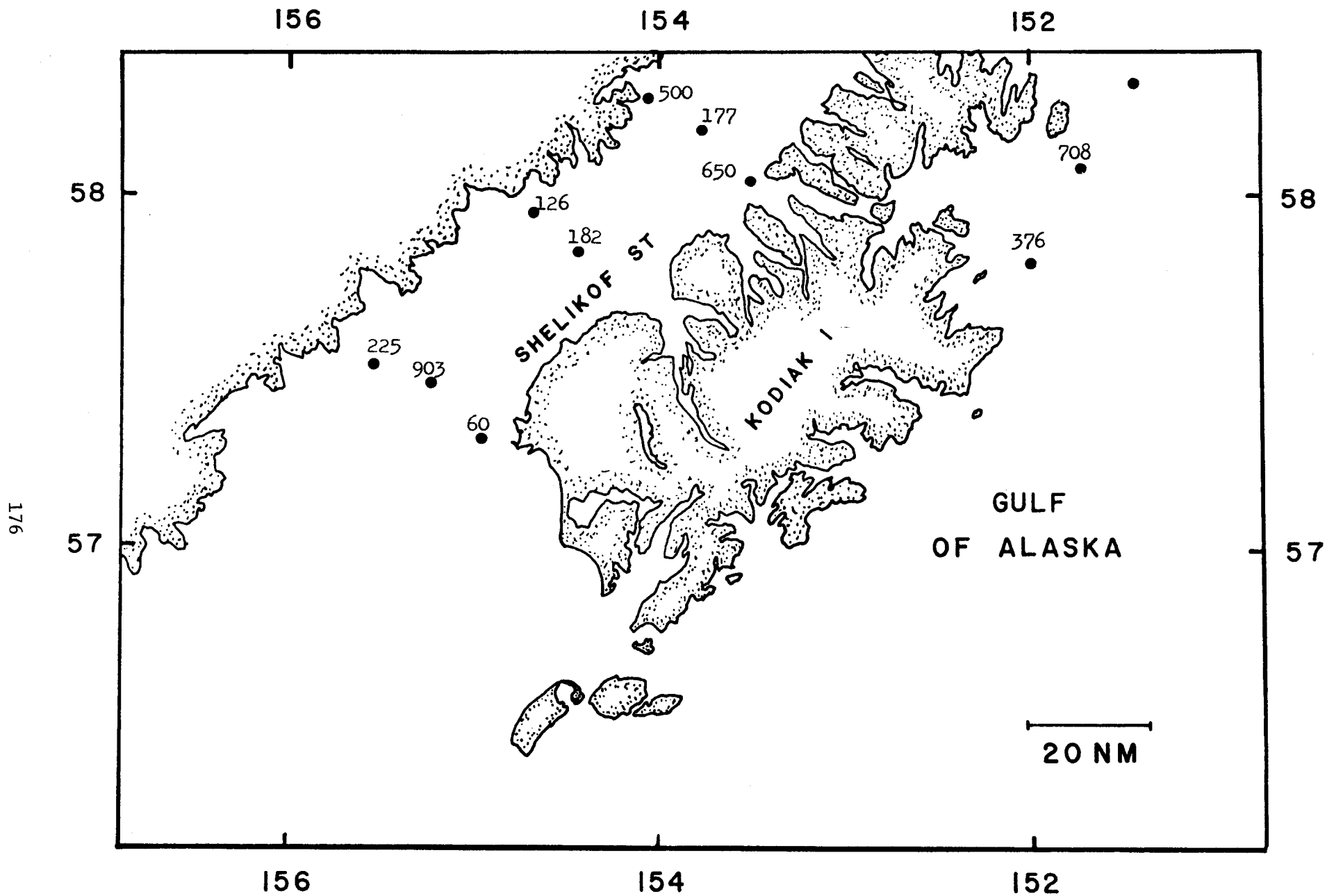


Figure 24. Crude oil degradation potentials observed in Cook Inlet sediment samples collected during the November cruise. The unit of measurement used was DPM/g. dry wt.

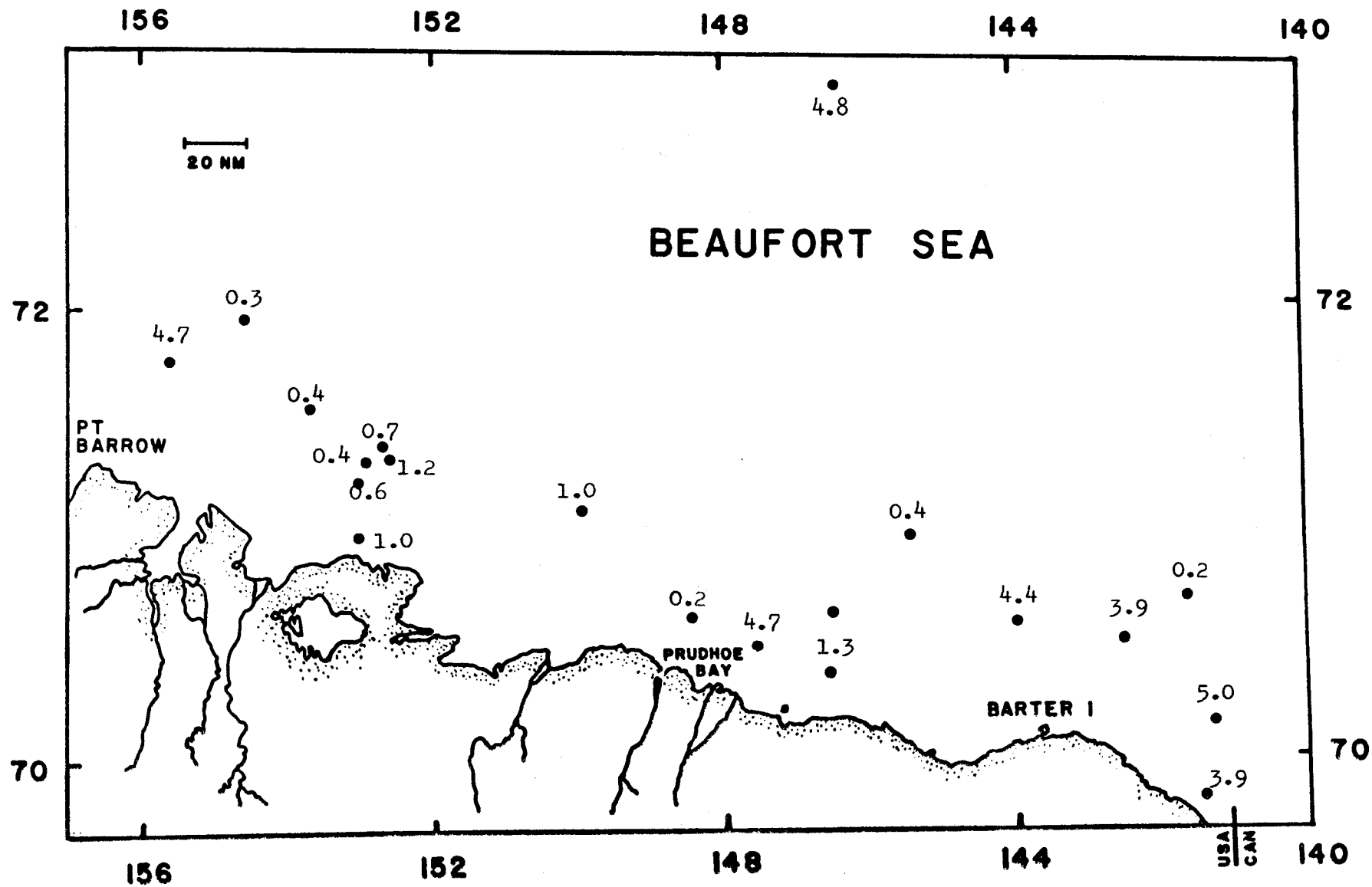


Figure 25. Crude oil biodegradation potentials observed in the Beaufort Sea sediments collected during the September cruise. The unit of measurement used was DPM in thousands/g. dry wt.

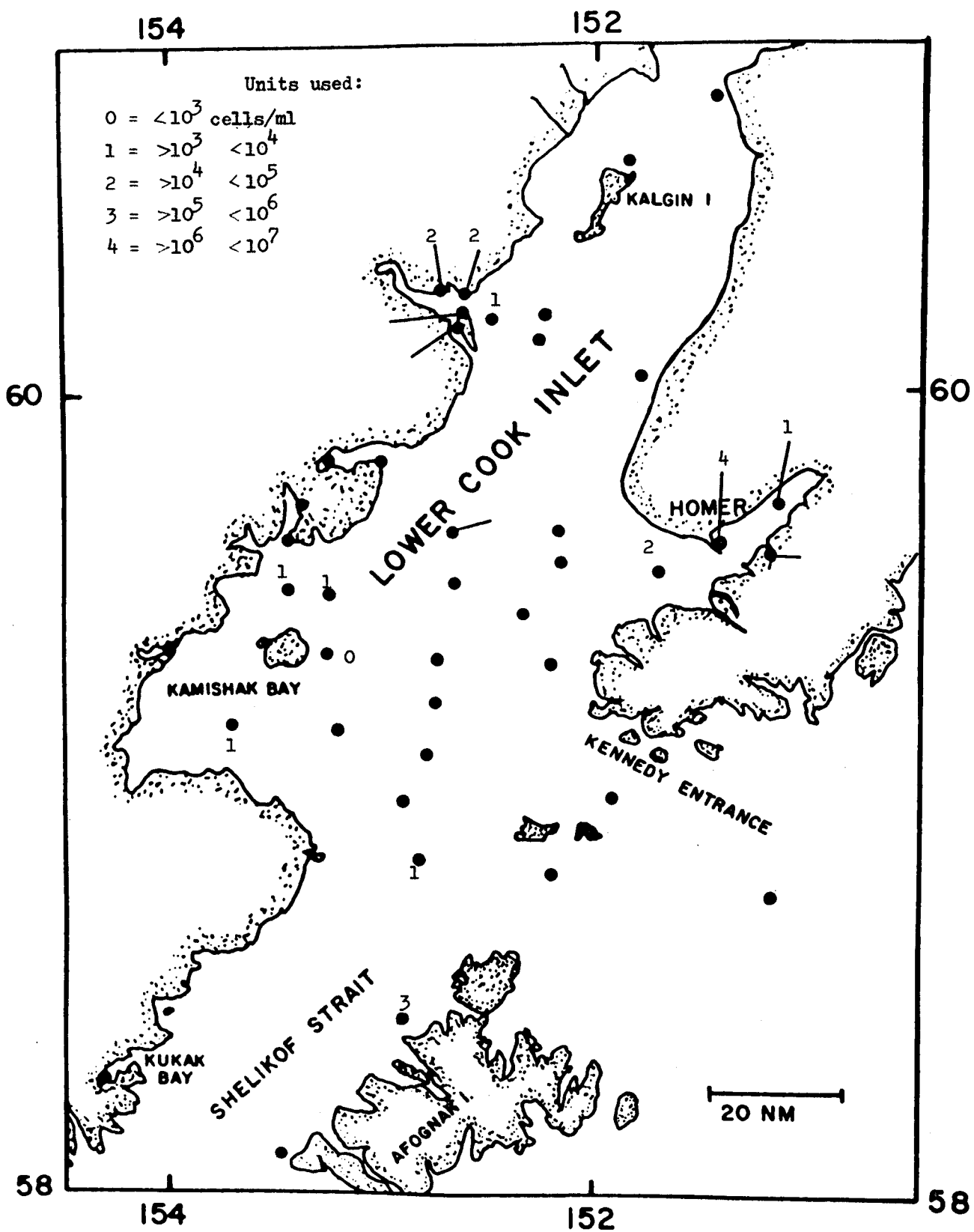


Figure 26. Relative concentrations of *Desulfovibrio* sp. present in sediments analyzed during the April cruise.

DETERMINE THE FREQUENCY AND PATHOLOGY OF MARINE FISH
DISEASES IN THE BERING SEA, GULF OF ALASKA, AND BEAUFORT SEA

by

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OUTER CONTINENTAL SHELF ENERGY ASSESSMENT PROGRAM
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I. Summary of objectives, conclusions, implications with respect to Outer Continental Shelf oil and gas development.

A. Summary of Objectives

The overall objectives are to determine the frequency and pathology of marine animal diseases in the Bering Sea and Gulf of Alaska (GOA). Special emphasis was placed on the northern GOA during the present reporting period.

B. Summary of Conclusions

Four major pathological conditions were found in 6 species of marine fish from the GOA in 1977. The species involved, the type of condition, and the average prevalence and the range of frequencies at the sampling stations of each condition were as follows: Pacific cod (Gadus macrocephalus), pseudo-branchial tumors, 2.5% (1.0 to 50%); pollock (Theragra chalcogramma), pseudo-branchial tumors, 0.7% (0.3 to 14.3%); rock sole (Lepidopsetta bilineata), epidermal papillomas, 0.2% (0.0 to 0.5%); flathead sole (Hippoglossoides elassodon), epidermal papillomas, 0.4% (1.7 to 16.7%); Pacific ocean perch (Sebastes alutus), epithelioid tumors, 0.6% (0.2 to 21.0%); and Pacific cod, skin ulcers, 0.9% (0.6 to 46.2%). The geographical distributions of all these conditions, except the tumors of Pacific ocean perch, were concentrated in the northwestern GOA, east and northeast of Kodiak Island. All of the tumors had in common the presence of tumor-specific cells known as X-cells which suggests a common etiology. Possible causes of these tumors include a virus(es), natural or man-made toxic chemical(s), or a single-celled parasite.

C. Implications with Respect to Outer Continental Shelf Oil and Gas Development

These field studies have increased our knowledge of the health status of demersal fishes in the GOA. Although the overall prevalence of the observed pathological conditions were relatively low, sampling stations with frequencies

of over 14% were found for all the conditions except one. Therefore, in the aftermath of possible incidents of crude oil contamination of the GOA, the finding of high frequencies of diseased fish alone will not be sufficient to show the harmful effects of oil.

In addition, information has been gained concerning which species are particularly susceptible to diseases and which diseases seem closely correlated or not correlated with man's activities.

II. Introduction

A. General nature and scope of study.

The purpose of this investigation is to obtain baseline data on the prevalence, distribution, and pathology of diseases presently existing in fish and invertebrates in the Bering, Beaufort, and Chukchi Seas, Norton Sound, and Gulf of Alaska (GOA). This effort requires both field and laboratory activities. Field activities are performed in cooperation with the Resource Assessment and Conservation Engineering Division (RACE), NWAFC, Seattle, WA., (OCSEAP R.U. #175). Animals captured by R.U. #175 are divided into subsamples, and most or all of the individuals in each are examined for externally visible pathological conditions. The biological and pathological characteristics of each affected animal are determined.

B. Specific objectives.

The specific objectives of this investigation include the following: (1) determine the frequency of each major type of pathological condition in demersal fishes and invertebrates in the northern GOA; (2) establish the geographical distribution of each disease; (3) define the histopathological features of each disease by examining tissues from lesions and associated major internal organs and blood, using light and/or electron microscopy; (4) isolate disease-associated microorganisms from lesions and internal tissues, use taxonomic tests to identify them, and determine if any microorganisms is disease specific; and (5) compare the size, weight, age, and sex frequencies of diseased animals with those of normal-appearing animals of the same species.

C. Relevance to problems of petroleum development.

The principal contributions of these studies are the demonstration that significant frequencies of disease exist in the demersal fish populations associated with Alaska's Outer Continental Shelf. In addition, these studies provide information concerning the species free of detectable diseases and those

species affected by specific diseases, the geographical distribution of affected fish, and the possible causes of some of the diseases. Therefore, in the aftermath of possible incidents of crude oil contamination of Alaskan marine waters, the simple existence of diseased fish will not be sufficient to show the harmful effects of oil. If, however, certain aspects of pathological conditions deviate significantly from the parameters established during these baseline studies, then oil contamination could be a possible cause.

III. Current state of knowledge.

Six major pathological conditions have been reported by us in fish from Alaskan marine waters. They include epidermal papillomas of rock sole (Lepidopsetta bilineata) and flathead sole (Hippoglossoides elassodon), pseudobranchial tumors of cod (Gadus macrocephalus) and pollock (Theragra chalcogramma), gill-associated tumors of Pacific ocean perch (Sebastes alutus), lymphocystis of yellowfin sole (Limanda aspera), skin lesions in cod, and larval trematode infestations characterized by black spots in the skin of Pacific herring (Clupea harengus pallasi), toothed smelt (Osmerus mordax dentex), and saffron cod (Eleginus gracilis) (McCain et al 1978, Wellings et al 1977, Alpers et al 1977a and 1977b, Myers et al, manuscript in preparation). Two important pathological conditions found in epibenthic invertebrates were infestation of sea stars (Leptasterias sp.) by parasitic gastropods, and extensive attachment of leech eggs to the appendages of shrimp (Sclerocrangon boreas) (Katherine King, manuscript in preparation). Tumor bearing cod, pollock, and rock sole, and cod with skin lesions were found in both the Bering Sea and GOA. Lymphocystis of yellowfin sole was observed only in the Bering Sea; tumor-bearing Pacific ocean perch and flathead sole were found only in the GOA; and the trematode infestations of toothed smelt, Pacific herring, and saffron cod were detected only in the Norton Sound/Chukchi Sea.

The causes of all but possibly two of their pathological conditions are not known. The exceptions are lymphocystis of yellowfin sole, which is caused by a virus, and the apparently bacterially-caused skin ulcers of cod. Cod and pollock pseudobranchial tumors (probably carcinomas), gill-associated tumors of Pacific ocean perch and epidermal papillomas of sole, are neoplasms of unknown cause(s).

IV. Study Area

During the last year, the area of the northern GOA investigated had the approximate boundaries of:

54.5° to 58° N. Lat.

133° to 152° W. Long.

V. Sources, Methods, and Rationale of Data Collection

Research efforts were of two general types, field and laboratory activities. Field activities were performed aboard the NOAA Ship Miller Freeman and the Polish research ship Professor Siedlecki. Gear characteristics, trawling methods, and sampling procedures were similar to those used by Kaimmer et al. (1976). Each catch was sorted according to species by our personnel and by members of OCSEAP R.U. #175, and a randomly-selected subsample of each species (200 to 400 fish) was used to estimate size, age composition, and sex ratios. The samples were examined for externally-visible pathological conditions and, when feasible, for readily recognizable internal disorders.

The types and frequencies of each abnormality found during these examinations were recorded on an individual data sheet. The species, sex, length, weight, and age of each affected fish were determined; and the type, location, and size of the pathological condition(s) was recorded. Either the diseased tissue alone, or the affected tissue in combination with the major internal

organs, was preserved in 10% formalin with phosphate-buffered saline and returned to the laboratory and examined histologically. Isolates of bacteria, fungi, and virus were obtained by standard microbiological procedures.

Field data were recorded on 4 different types of data sheets corresponding to 4 data management record types. These record types included, File Header (describing the cruise and investigators), Station Header (describing the haul related data), Species Catch (describing the fish caught and the frequency of pathological conditions), and the above mentioned Individual Data.

The information on the Data Sheets was key-punched onto computer cards by the Fisheries Analysis Center, University of Washington, Seattle. Duplicate computer cards are made out and sent to OCSEAP, and the original cards will be used for transferring the data to magnetic tape.

Laboratory activities involved processing tissue specimens and data obtained in the field. Specimens to be examined by light microscopy were embedded in paraffin, sectioned, and stained with hemotoxylin and eosin, May-Grunwald-Giemsa, or Massons trichrome (Preece 1972).

Specimens of tissue for examination by electron microscopy were fixed in a solution containing glutaraldehyde, formalin, and acrolein (Hawkes 1974) in the field, returned to the laboratory, and post-fixed in osmium tetroxide, dehydrated, embedded in plastic, and sectioned for both light and electron microscopy. Richardson's stain was used for sections cut for light microscopy. Thin sections were triple stained with lead citrate, uranyl acetate, and again with lead citrate, and examined with a transmission electron microscope.

VI. Results

During 1977, research efforts emphasized the health status of demersal fishes in the GOA. During 2 separate cruises, one on the NOAA Ship Miller Freeman and

the other on the Polish R/V Professor Siedlecki, 5 major pathological conditions were found in 4 species of fish (Table 1). Thirty-nine other species of demersal fish were examined and found to be free of detectable diseases (Table 2). The remainder of this section will be concerned with the distribution, frequency, pathology, and biology of fish with these conditions.

A. Pseudobranchial Tumors of Pollock

The geographical distribution of this condition was generally confined to the western portions of the GOA near Kodiak and the Kenai Peninsula (Figure 1). Only one trawl in the central region of the Gulf produced tumor-bearing pollock, and none were found in the eastern region. The prevalence of this condition in individual hauls ranged from 0.3% to 14.3% with an average frequency of 0.7% (Table 1). The disease was not depth related. The sex ratio was 2.8:1, males to females. The significance of this ratio is not yet known, since data for the normal pollock population has not yet been completed by the RACE division of NWAFC (R.U. #175). The sex data may simply reflect unequal proportions of male and female pollock captured. Of those tumor-bearing pollock aged, most were age 2 (34 out of 38), with the ages ranging up to 10 years (Figure 2). The relationship of this condition to age, growth rate, and population density is unknown, pending completion of compilation of normal pollock data.

The appearance and size of the pollock tumors were very similar to that previously reported for pseudobranchial tumors of pollock in the Bering Sea (Annual Report, April, 1977, OCSEAP R.U. 332). Four of the 38 tumor-bearing pollock had unilateral tumors.

The microscopic anatomy of these tumors was very much like that described for the same condition in Pacific cod, (Alpers et al 1977a) with the following exceptions: (1) granulomas common to Pacific cod tumors were not seen in pollock

tumors; (2) the fibrous stroma of the pollock tumors was populated with numerous melanophores, while melanophores were seldom observed in the stroma of Pacific cod tumors; and (3) the pollock tumors had a marked infiltration of macrophages and lymphocytes, this was not the case in the Pacific cod tumors.

B. Pseudobranchial Tumors of Pacific Cod

The overall prevalence of pseudobranchial tumors of Pacific cod in the GOA was 2.5% (range 1.0 to 50.0%)(Table 1). The condition did not appear to be related to sex, as was shown by the male: female ratio of 1.3:1. The age of tumor-bearing fish was from 1 to 4 years, and 69% of them were 2 or 3 years (Figure 2). Pacific cod with tumors were distributed in a broad geographical area of the western GOA near Kodiak Island the Kenai Peninsula (Figure 3). An index of this broad distribution is that 41% of the hauls in which Pacific cod were captured had tumor-bearing cod. The geographical distribution of this condition does not seem to be depth related.

The gross appearance and histopathology of the pseudobranchial tumors have been previously reported (McCain et al 1978, Alpers et al 1977a).

C. Skin Ulcers of Pacific Cod

Pacific cod with skin ulcers were found in only 5 of 61 (8%) hauls in which cod were captured, and these hauls tended to be in the north-central portion of the GOA (Figure 4). The overall prevalence was 0.9% (Table 1). The male to female ratio of 0.8:1 suggests that this condition is not sex-related. The age of diseased Pacific cod ranged from 1 to 5, with 61% being 2 or 3 years of age (Figure 2).

Although two types of lesions were observed on Pacific cod in the Bering Sea, a "ring-shaped" lesion and an ulcer, only the skin ulcer was found in the GOA. The ulcers ranged in diameter from 5 to 20 mm, and distributed over the exposed body surface in a random manner, with several fish having more than 40 ulcers.

The histopathological properties of the ulcers and the etiology of the ulcers have been previously reported (Annual Report, April, 1977, OCSEAP, R. U. #332) and will be described only briefly here. A typical ulcer showed either a focally or completely exfoliated epidermis, exposing the underlying dermal components. The dermis exhibited a chronic inflammatory response consisting of increased vascularization, perivascular hemorrhage, hyperemia, and infiltration with a mixed population of lymphocytes, macrophages, and melanophore-containing cells. The underlying musculature is usually not involved, but occasionally well encapsulated fibrogranulomatous centers are observed beneath the hypodermis.

Preliminary evidence suggests that these ulcers are caused by a pseudomonas-like bacteria. In previous work in the Bering Sea, taxonomically identical bacteria were isolated, sometimes in pure culture from the ulcers of 5 different Pacific cod.

D. Epidermal Papillomas of Rock Sole

Only 1 haul yielded rock sole with epidermal papillomas. This haul was taken near Afognak Island (Figure 5) at a depth of 63 m. Since the mean depth of all the hauls in which rock sole were captured was 138.5 m, the depth-related nature of this condition was again demonstrated. Of the 3 tumor-bearing fish captured in that haul, representing an individual haul frequency of 0.5% (3/615) and an overall prevalence of 0.2% (Table 1), 2 were age 8 and 1 was 7 years.

The gross appearance and histopathology of the rock sole tumors are very similar to epidermal papillomas reported for other flatfish (Wellings et al 1976) and have been previously described (McCain et al 1978, Wellings et al 1977).

E. Epidermal Papillomas of Flathead Sole

Although hauls containing tumor-bearing flathead sole were limited to a small geographical area east of Kodiak Island (Figure 6), this condition does

appear to be more widespread in the GOA than the similar condition in rock sole; 6 of 49 hauls in which flathead sole were captured had tumor-bearing flathead sole. The overall prevalence was 0.4% (10/2439) with a frequency range for individual hauls of 1.7 to 16.7% (Table 1). The ages of tumor-bearing fish were evenly distributed between 4 and 11 years (Figure 2).

The gross appearance of the flathead tumors differed slightly from that of the rock sole tumors. Three basic types of flathead sole tumors were observed: (a) the typical epidermal papilloma, 7 fish had this type; (b) a tumor type identified as an angioepithelial nodule (AEN) (Wellings et al 1976) which is known to be the progenitor of the epidermal papilloma, these tumors were on 3 fish; and (c) an unclassified form of an epidermal papilloma that has not been previously described. This latter tumor was similar in texture and appearance to an epidermal papilloma, except that this tumor was black rather than brown and more loosely attached to the underlying dermis. The appearance and histopathological characteristics (see above) of this tumor suggest that it is an epidermal papilloma in the process of regression.

The microscopic anatomy of the epidermal papillomas and AENs was similar to that previously reported by Wellings et al (1976) for similar tumors found in other pleuronectids. The unclassified tumor differed from the above tumors by having signs of a pronounced inflammatory host response. The stroma of the tumor contained numerous pleomorphic melanophores, macrophages, lymphocytes, and eosinophilic granular cells. A high percentage of tumor-specific cells, known as X-cells (Wellings et al 1976), near the surface of the tumors were degenerated; the X-cells in typical epidermal papillomas are very seldom necrotic.

F. Epithelioid Tumors of Pacific Ocean Perch

Epithelioid tumors of Pacific ocean perch (POP) were located on membranes associated with gills, and on body surfaces. Tumor-bearing POP were in

7 of 36 POP-containing hauls which were taken along the northwestern to northeastern periphery of the GOA (Figure 7). The prevalence ranged from 0.2 to 21.0%, with an overall average of 0.6% (Table 1). No relationship between the depth of a haul and the frequency of tumor-bearing POP was observed. Also, males and females had similar tumor frequencies, as was suggested by the ratio of tumor-bearing males to females of 0.9:1.0. Only POP 8 years or older had detectable tumors, the maximum age was 17 years (Figure 2).

The epithelial tumors appeared as multiple raised nodules and/or flat, spreading growths of dull red to pink. A variety of anatomical structures were found with these tumors, including: (1) the translucent membrane on the body surface between the cleithrum and the posterior holobranch of the gills (Figure 8); (2) the membrane on the underside of the opercula (Figure 9); (3) the gill rakers, rays, and filaments (Figure 8); (4) the body surface sometimes associated with the pectoral, pelvic, caudal and anal fins, and (5) several structures (anterior isophagus, various epithelial membranes) of the buccal cavity as a result of spreading from the above-mentioned primary tumor sites (Figure 10a and 10b). Fish with the most severe and widespread tumors were also the oldest (ages 14 to 17 years).

Although the histological characteristic of the POP tumors have many similarities to the previously mentioned X-cell tumors (pseudobranchial tumors of Pacific cod and pollock, and skin tumors of rock sole and flathead sole), there are several important differences; the most dramatic of which was the commonly observed invasiveness of the POP tumors. Several tumors contained areas on their periphery in which X-cells had spread into connective and/or epithelial tissues (Figure 11). In addition secondary tumors or metastases with no connection with primary tumors were found.

Another characteristic of POP tumors seldom observed in the other X-cell tumors was a mononuclear infiltrate composed mainly of lymphocytes which was present to a variable degree in most tumors. Normally the infiltrate was diffuse and mild, but dense foci were occasionally noted.

POP tumors were generally more vascular. The stroma surrounding the nests of tumor cells was collagenous and contained numerous capillaries, venules, and arterioles (Figure 12).

Electron microscopic examination of several different types of POP epithelioid tumors has demonstrated that these tumors are composed of X-cells morphologically very similar to X-cells found in the epidermal papillomas of pleuronectids (Brooks et al. 1969) and the pseudobranchial tumors of Pacific cod (Alpers et al. 1977a) (Figures 13 and 14).

VII. Discussion

Several aspects of the geographical distribution, prevalence, age, sex, and pathology of the diseased fish from the GOA warrant further discussion. However, such discussion at this time will be somewhat restricted because data on the above mentioned subjects for normal fish captured in the same hauls containing diseased fish are not yet available to us.

Fish with 5 of the 6 pathological conditions (POP tumors were the exception) were largely captured in the northwestern periphery of the GOA. The following reasons may independently or collectively account for this observation: (1) disease frequency may be related to fish density, and more areas with high fish densities may be in the northwestern GOA; (2) since 4 of the 5 conditions were found in even higher prevalences in the Bering Sea, some diseased fish may have migrated into the GOA from the north; (3) the bottom types in the northwestern GOA may contribute to disease induction, and (4) infectious agents and/or disease-related chemicals may be in higher concentrations in the northwestern GOA.

The overall average frequency of diseases in the GOA was relatively low, with only pseudobranchial tumors of Pacific cod having a frequency of over 1.0%. In the Bering Sea, for example, average disease frequencies ranged from 1.3 to 8.7%, as compared to 0.2 to 2.5% in the GOA. Nevertheless, in the GOA, each of the fish diseases, with the exception of the rock sole skin tumors, had frequencies of between 14 and 50% in certain hauls. Thus, unexplained disease "hot spots" do exist in the GOA.

Three diseases, pseudobranchial tumors of Pacific cod and pollock and the skin ulcers of Pacific cod, appear to largely affect fish less than 5 years of age. On the other hand, mainly older (over 8 years) POP had tumors. The reasons for this apparent age specificity are not clear. One likely explanation is that sampling bias caused by sampling techniques and/or the life histories of the affected fish permit only certain age groups to be captured. For example, POP can be captured with an age range of 8 to 21 years or 2 to 15 years depending upon the mesh size of the trawls and the sampling location (Major and Shippen 1970).

Epidermal or epithelioid tumors represent 5 of the 6 diseases found in the GOA. All of these tumors have in common the presence of the tumor-specific X-cell. The existing histochemical and ultrastructural evidence strongly suggests the X-cells from each tumor type are very similar. Therefore, it is possible that the epidermal papillomas, the pseudobranchial tumors, and the epithelioid tumors have a common etiology. The nature of this etiology is not known. Possible causes include a tumorigenic virus, a single cell parasite, or chemical carcinogens.

With the possible exception of the epidermal papillomas of pleuronectids, the X-cell-containing tumors are invasive. The POP tumors appear to be the most invasive.

VIII. Conclusions

Three of the 4 major pathological conditions involving 6 species of fish were tumors with a possibly common etiology. The 3 types of tumors, epidermal papillomas of pleuronectids, pseudobranchial tumors of gadids, and epithelioid tumors of Sebastes sp., all contained morphologically identical, tumor-specific cells known as X-cells. The origin of X-cells is not known; although they could be virally or chemically transformed host cells, or single-cell parasites.

For reasons not yet understood, all but one of the pathological conditions occurred most often and in highest frequencies in the northwestern GOA, east and northeast of Kodiak Island. The one exception, epithelioid tumors of Sebastes sp., was geographically distributed along the northeastern and eastern periphery of the GOA.

Thus, the GOA contains at least four demersal fish diseases which are endemic in certain areas. The overall prevalence of the conditions was relatively low, ranging from 0.2 to 2.5%, although some sampling stations had epizootic levels of disease frequency ranging from 14.3 to 50% for 5 of the 6 fish species affected.

IX. Needs for Further Study

Investigations of the health status of demersal fishes in Alaskan marine waters are very complementary to resource assessment studies. Marine animals captured and examined for population studies can also be examined for pathological conditions with only a small increase in time and personnel. Thus, as long as OCSEAP-supported resource assessment programs are carried out in Alaskan waters, it would seem to be in OCSEAP's best interest to continue marine animal disease studies.

X. Summary of Fourth Quarter Activities

A. Ship and Laboratory Activities

1. Ship or Field Trip Activities

None

2. Scientific Party

Bruce B. McCain, Ph.D.
NMFS, NOAA, NWAFC

Role: Principal investigator, coordinates field and laboratory activities, participates in histopathological and microbiological analyses, and writes progress reports and manuscripts.

Harold O. Hodgins, Ph.D.
NMFS, NOAA, NWAFC

Role: Principal investigator, supervises NMFS investigations and reviews all reports and manuscripts.

Albert K. Sparks, Ph.D.
NMFS, NOAA, NWAFC

Role: Principal investigator, supervises the collection and histological analyses of invertebrates.

William D. Gronlund, M.S.
NMFS, NOAA, NWAFC

Role: Principal investigator, participates in field activities, data processing, and analyses of biological data.

Mark S. Myers
NMFS, NOAA, NWAFC

Role: Performs histopathological analyses of tissue specimens and participates in field activities and data processing.

Kenneth V. Pierce, M.S.
NMFS, NOAA, NWAFC

Role: Histopathologist.

Rod Ramos
NMFS, NOAA, NWAFC

Role: Histology technician.

3. Methods

Two main research activities were performed during the last quarter. Arrangements were made for our participation in the nearshore study of demersal fishes in the northern GOA coordinated by Dr. Murray Hayes of the Race Division of the NWAFC. The other activity concerned further analyses of biological data and tissue specimens taken in 1977 from the northern GOA.

The biological data was recently provided by the RACE Division and involved the length/weight/age data of the normal fish captured in the northern GOA by the Polish R.V. Professor Fiedlecki. This data is presently being tabulated and compared with similar data for diseased fish taken on the same cruise. In addition, a variety of histochemical techniques (Mowry's colloidal iron stain, Fontana-Masson Silver Method, Mayer's Mucicarmine Method, methyl green, and Gomorri's Aniline Blue Stain) were employed to further characterize tissue specimens from tumor-bearing fish from the northern GOA (Preece 1972, Armed Forces Institute of Pathology 1968). Of special interest were tumor-specific X-cells and mononuclear cells which were observed infiltrating certain tumors.

4. Sample Collection Localities

None were obtained

5. Data Collected or Analyzed

a. Number and types of samples

No. of fish from which tissue specimens were taken	46
No. of tissue specimens processed histologically	276

b. Number and types of analyses

No. of histological slides examined microscopically
and interpreted. - 590

XI. Auxiliary Material

A. References Used

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C. Oral Presentations

McCain, B.B.

The Effects of Alaskan Crude Oil on Flatfish, and the Prevalence of Fish Pathology in Alaskan Marine Waters. OCSEAP Program Review, Nov. 29-Dec. 2, 1977, Seattle, Washington.

PROBLEMS ENCOUNTERED -- None

ESTIMATE OF FUNDS EXPENDED

Total spent: \$19.2K

TABLE 1

Data Describing the Prevalence of the Six Major Diseases Found in the GOA During 1977.

Species and Disease	No. of Fish Affected	No. of Fish Examined	Average Disease Frequency (%)	Average Disease Ranged (%)	No. of Hauls With Diseased Fish	Total No. of Hauls Examined
Pollock:						
Pseudobranchial tumors	38	5541	0.7	(0.3-14.3)	9	64
Pacific Cod:						
Pseudobranchial tumors	51	2079	2.5	(1.0-50.0)	25	61
Skin ulcers	18	2079	0.9	(0.6-46.2)	5	61
Rock Sole:						
Epidermal papilloma	3	1945	0.2	(0.0-0.5)	1	23
Flathead Sole:						
Epidermal papilloma	10	2439	0.4	(1.7-16.7)	6	49
Pacific Ocean Perch:						
Epithelioid tumors	15	2466	0.6	(0.2-21.0)	7	36

TABLE 2

FISH SPECIES CAPTURED IN THE GOA DURING 1977

IN WHICH NO DETECTABLE PATHOLOGICAL CONDITIONS WERE IDENTIFIED.

ONLY THOSE SPECIES OF WHICH 50 OR MORE WERE EXAMINED ARE LISTED.

Species	Total Number Examined
Black cod	646
<u>Anaplopoma fimbria</u>	
Arrowtooth flounder	4612
<u>Atheresthes stomias</u>	
Spinyhead sculpin	56
<u>Dasycottus setiger</u>	
Rex sole	2024
<u>Glyptocephalus zachirus</u>	
Arrowhead sculpin	50
<u>Gymnocanthus galeatus</u>	
Yellow Irish lord	271
<u>Hemilepidotus jordani</u>	
Pacific halibut	401
<u>Hippoglossus stenolepsis</u>	
Dover sole	665
<u>Microstomus pacificus</u>	
Great sculpin	100
<u>Myoxocephalus polyacanthocephalus</u>	
Rougeye rockfish	616
<u>Sebastes aleutianus</u>	
Silvergrey rockfish	72
<u>S. brevispinus</u>	
Dusky rockfish	111
<u>S. ciliatus</u>	
Yellowtail rockfish	211
<u>S. flavidus</u>	
Northern rockfish	55
<u>S. polyspinus</u>	
Harlequin rockfish	288
<u>S. variegatus</u>	
Shortspine thornyhead	603
<u>Sebastobolus alascanus</u>	
22 species of which less than 50 were examined	212
Total: 39 species	10,992

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Figure 12. Light micrograph of a epithelioid tumor from the opercular membrane of a POP. X-cells (x) have a variably granular cytoplasm and some are in a state of degeneration. Basement membranes (bm) of X-cell nests are separated by a thin stroma containing capillaries (c). Richardson's Stain, X780.

Figure 13. Electron micrograph of portions of 6 X-cells and 3 envelope cells (E) from a gill-associated epithelioid tumor of a POP. The X-cell nucleus contains a large, central nucleolus (N) with a characteristic hollow area, and numerous nuclear pores (arrows). The X-cell cytoplasm contains a variety of vacuoles and swollen mitochondria (M) with sparse, disintegrating cristae. X22,000. (Courtesy Dr. Joyce Hawkes)

Figure 14. Electron micrograph of a section of an epithelioid tumor of the skin of a POP. Portions of 5 X-cells (X) are bounded by a typical basal lamina (bl). Collagenous and vascular elements of the dermis (D) are evident below the basal lamina. X 7,000
(Courtesy Dr. Joyce Hawkes)

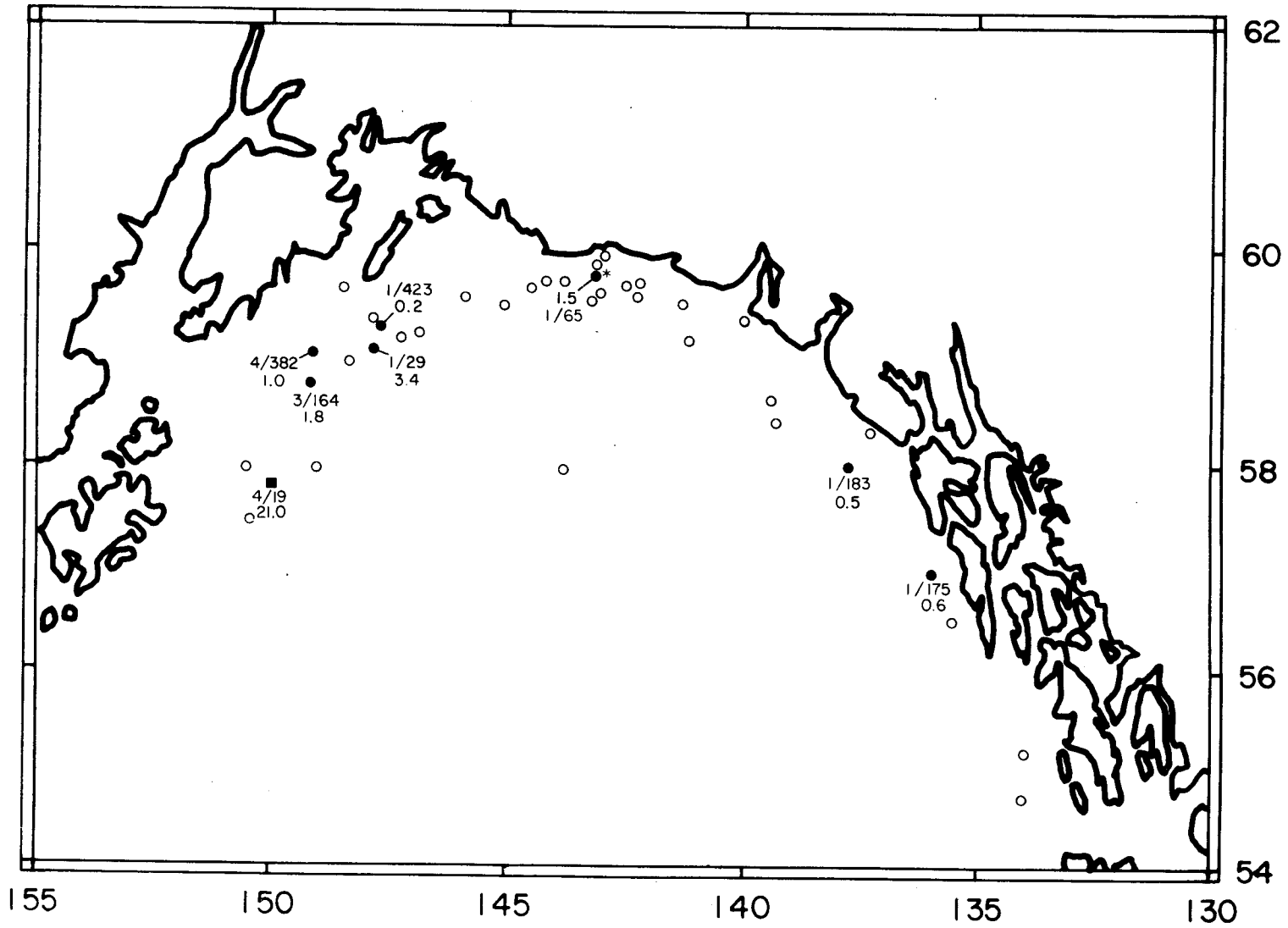


Figure 1.

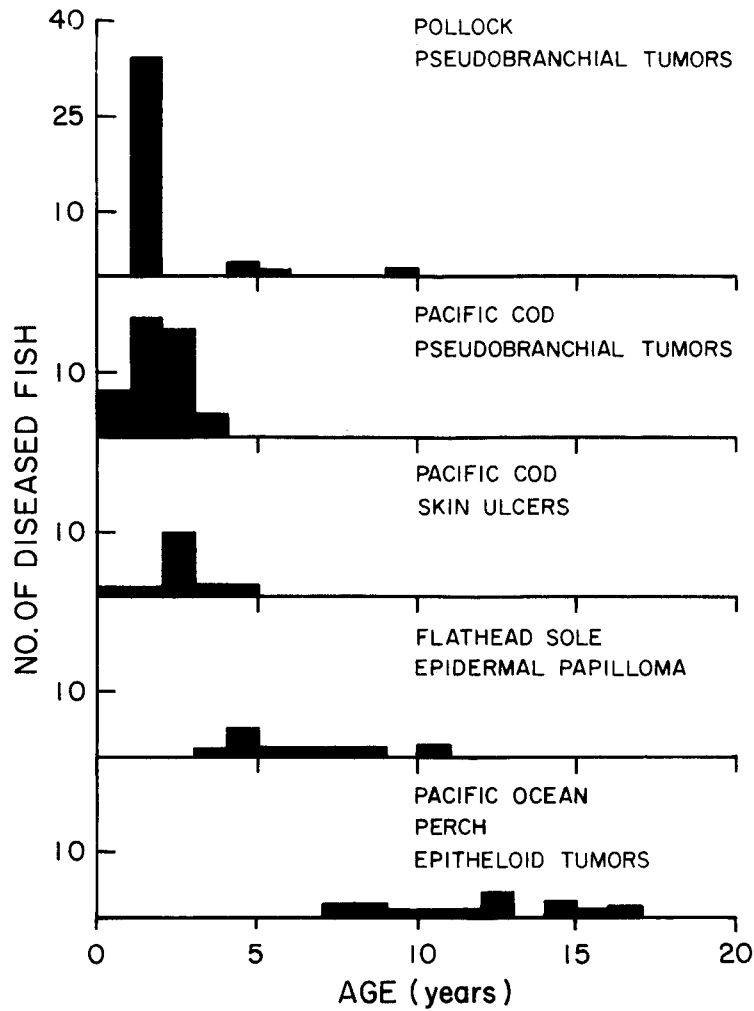


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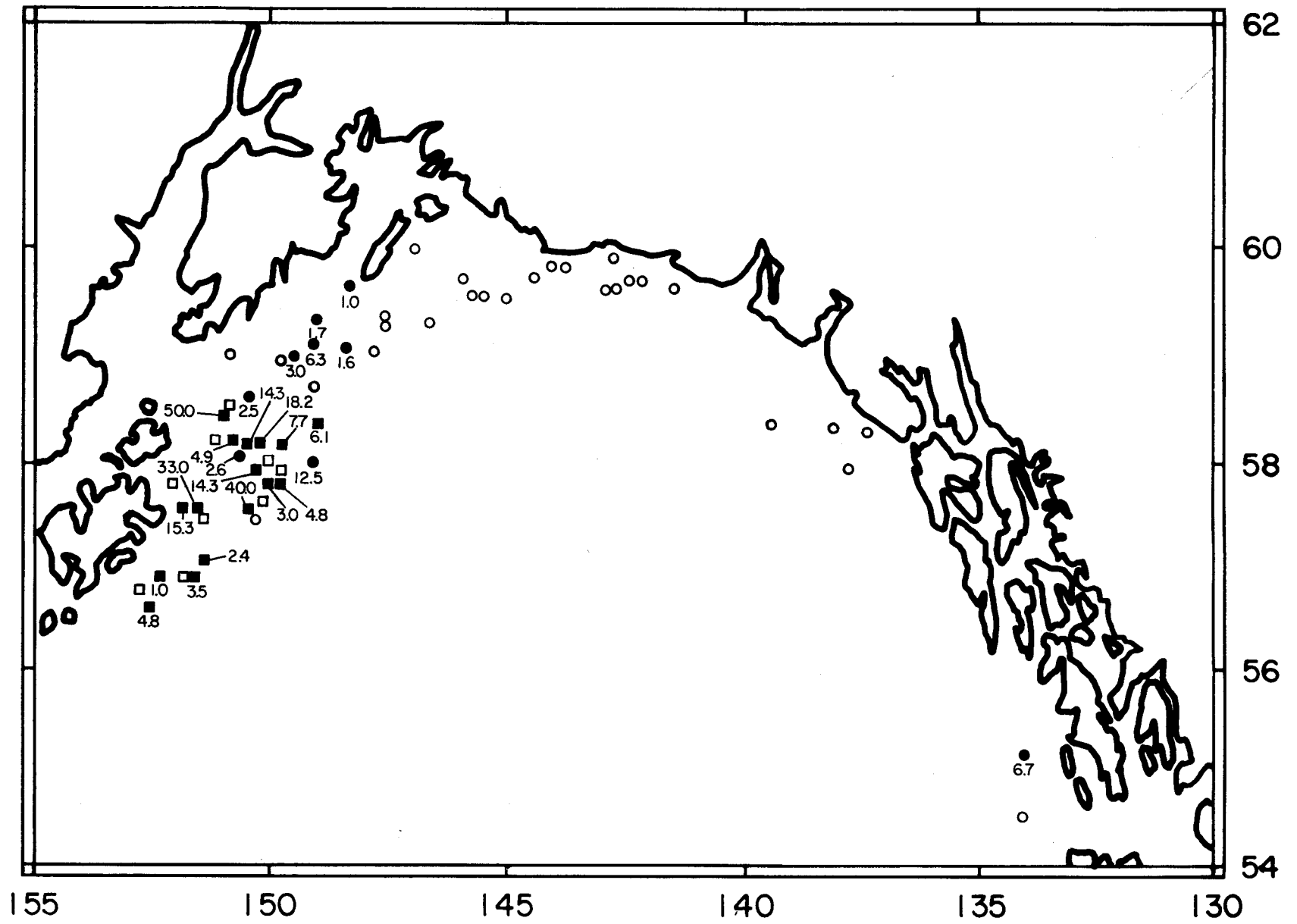


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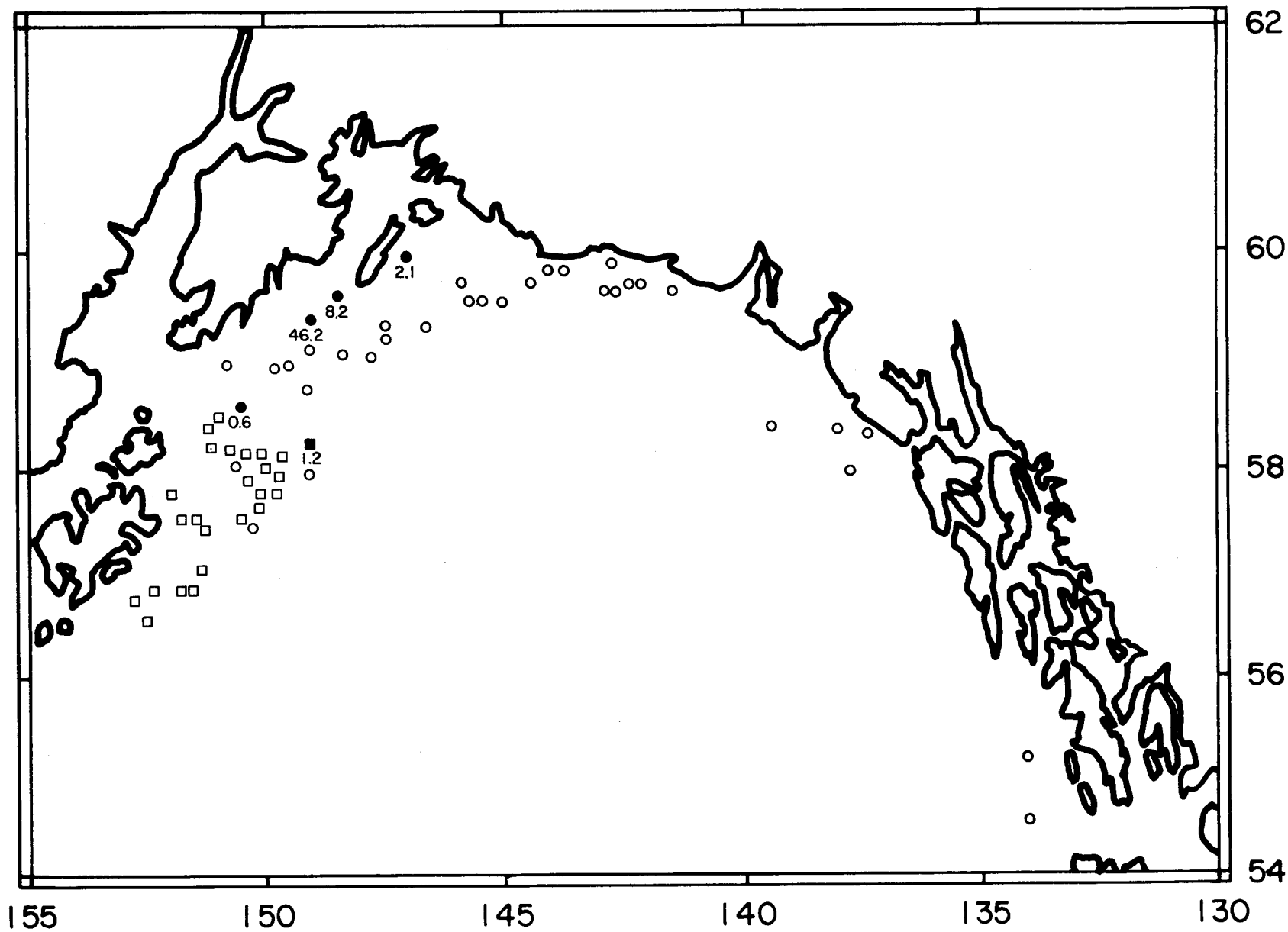


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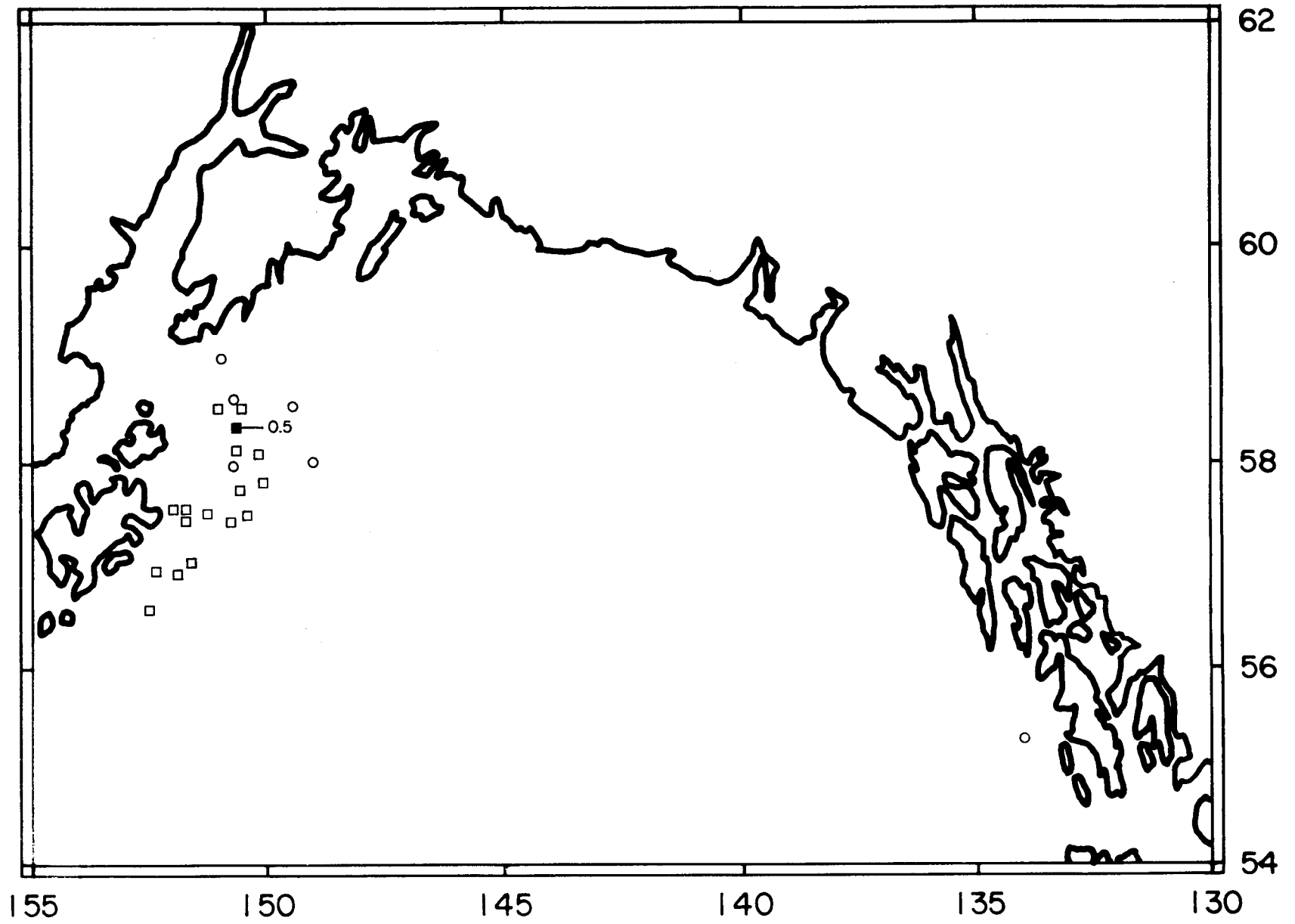


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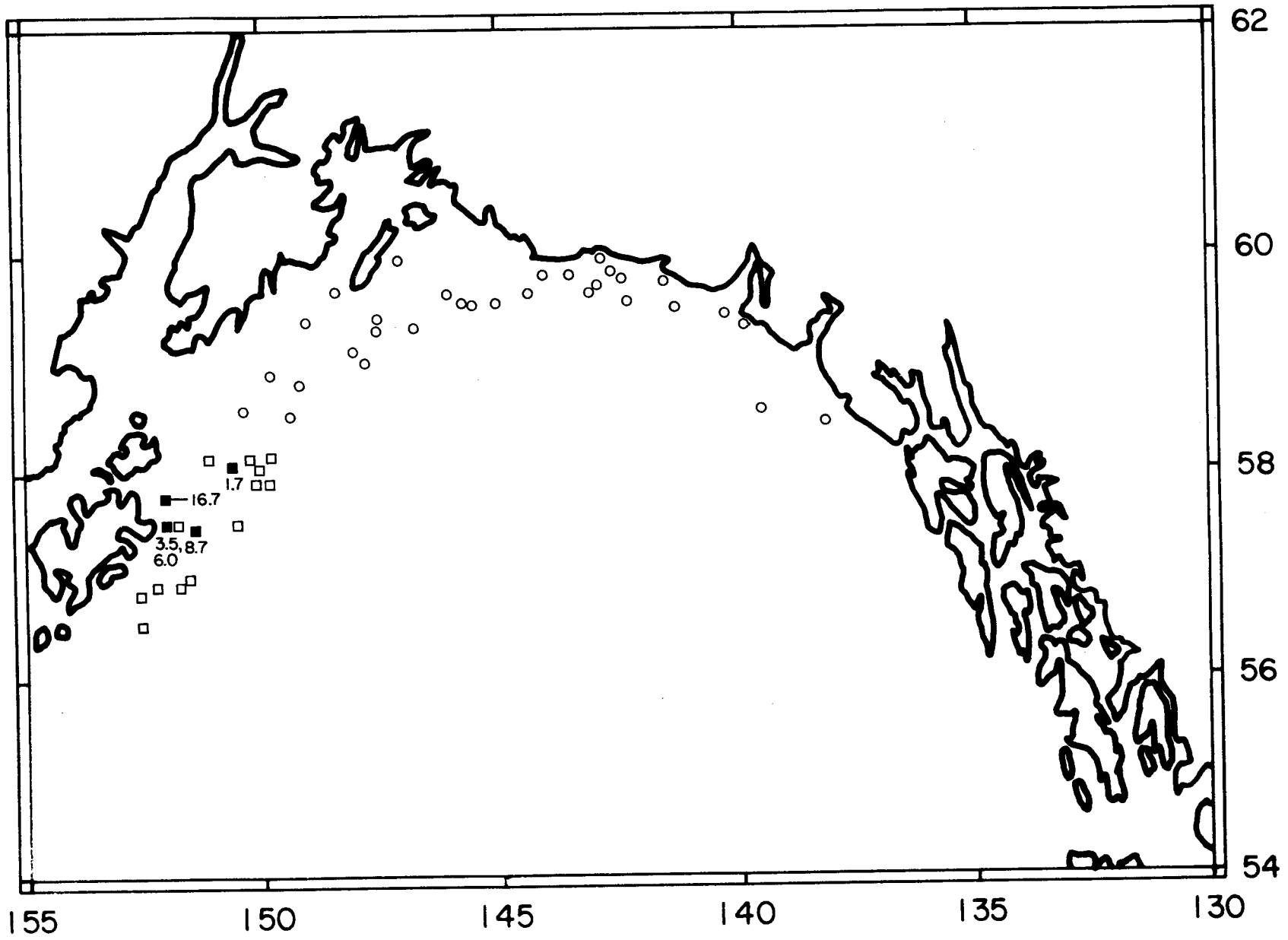


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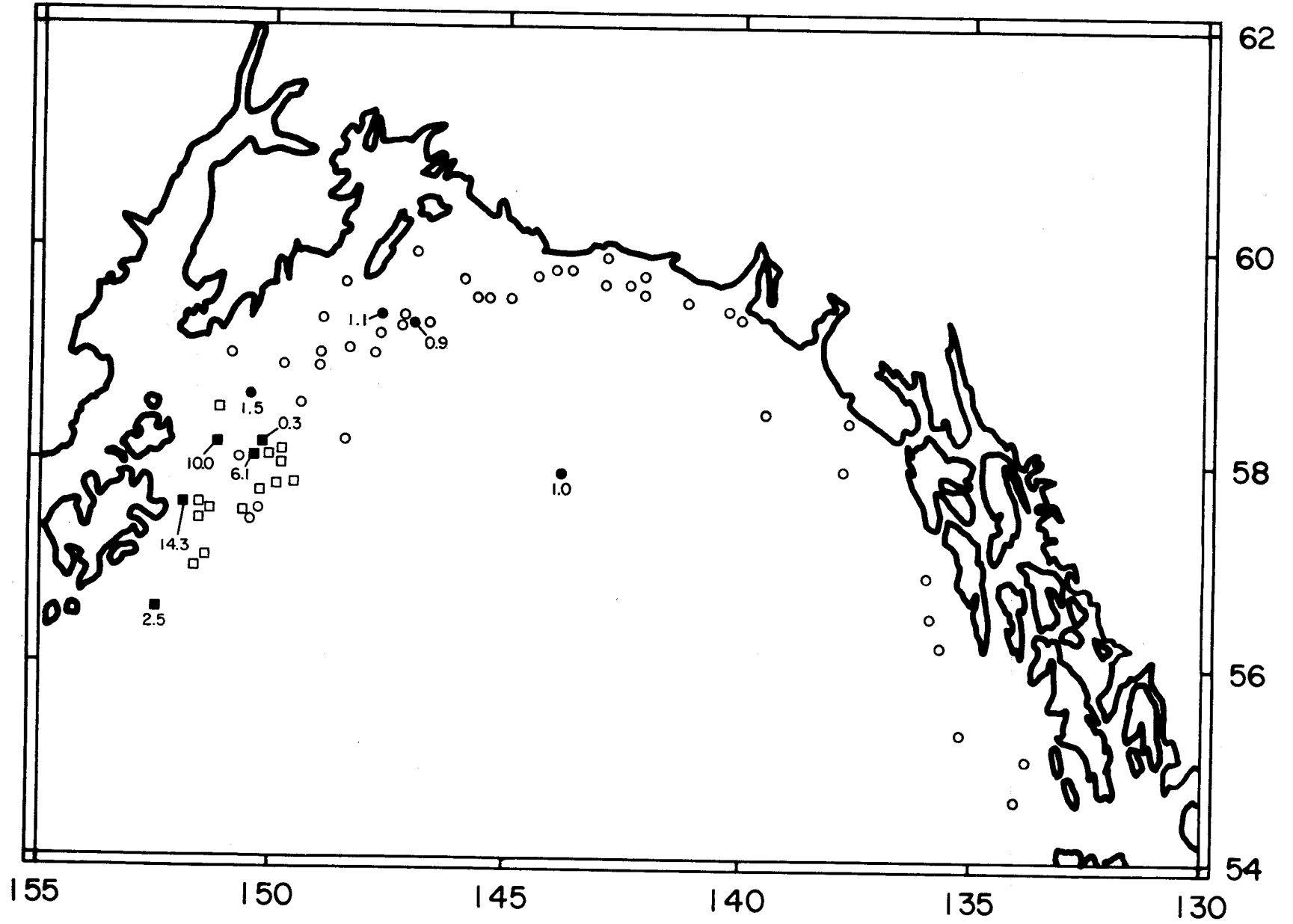


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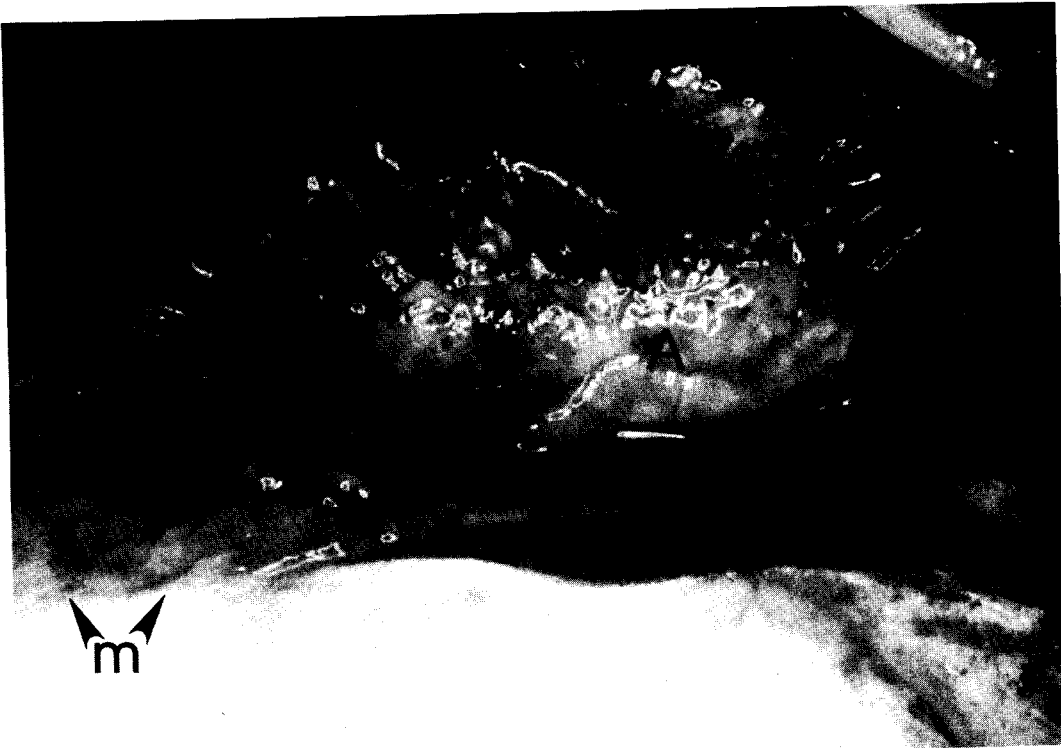


Figure 8.



Figure 9.



Figure 10a.



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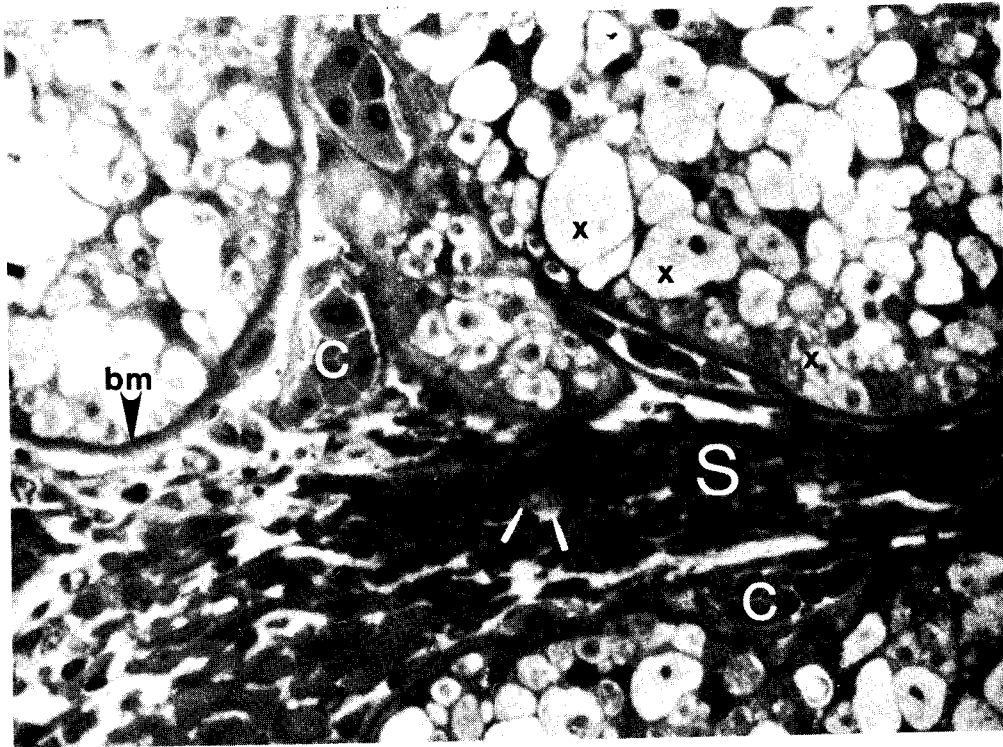


Figure 11.

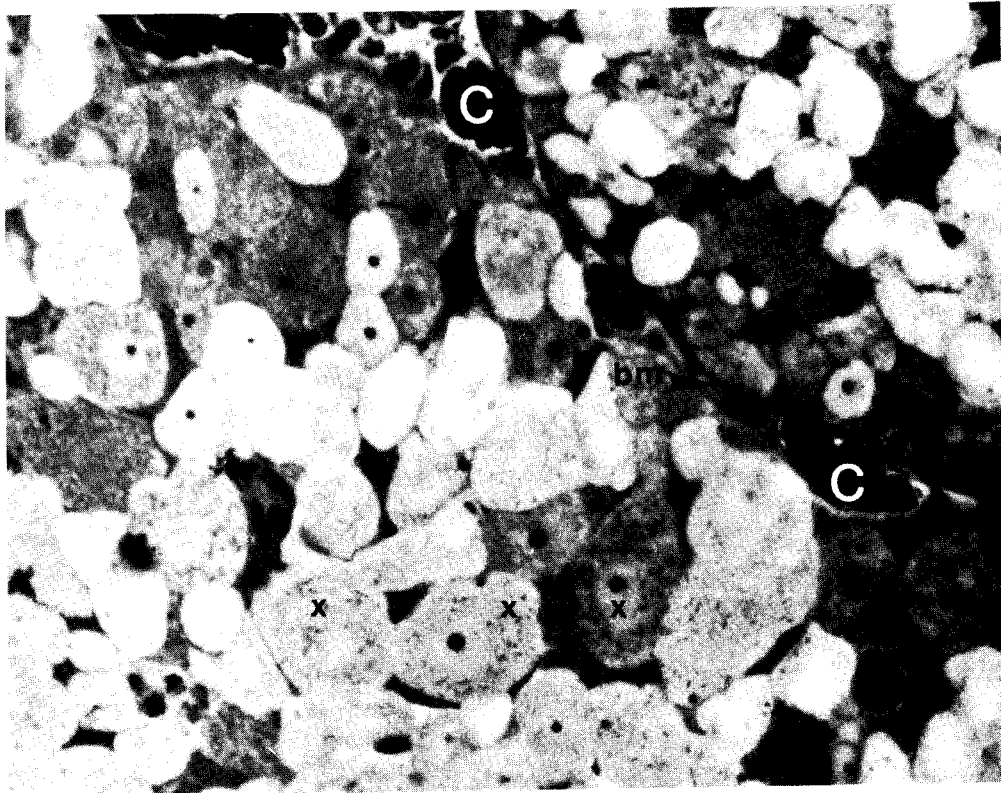


Figure 12.

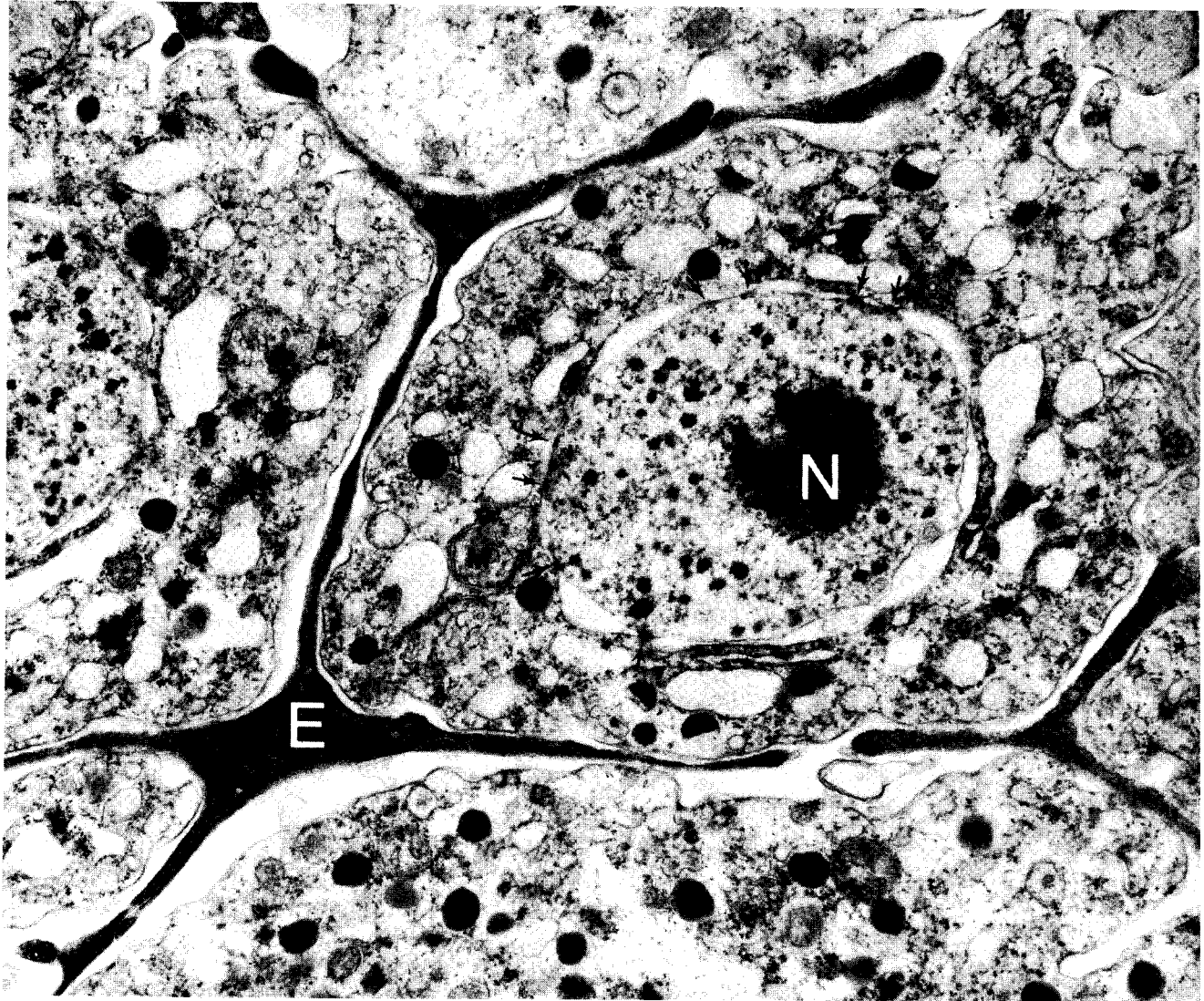


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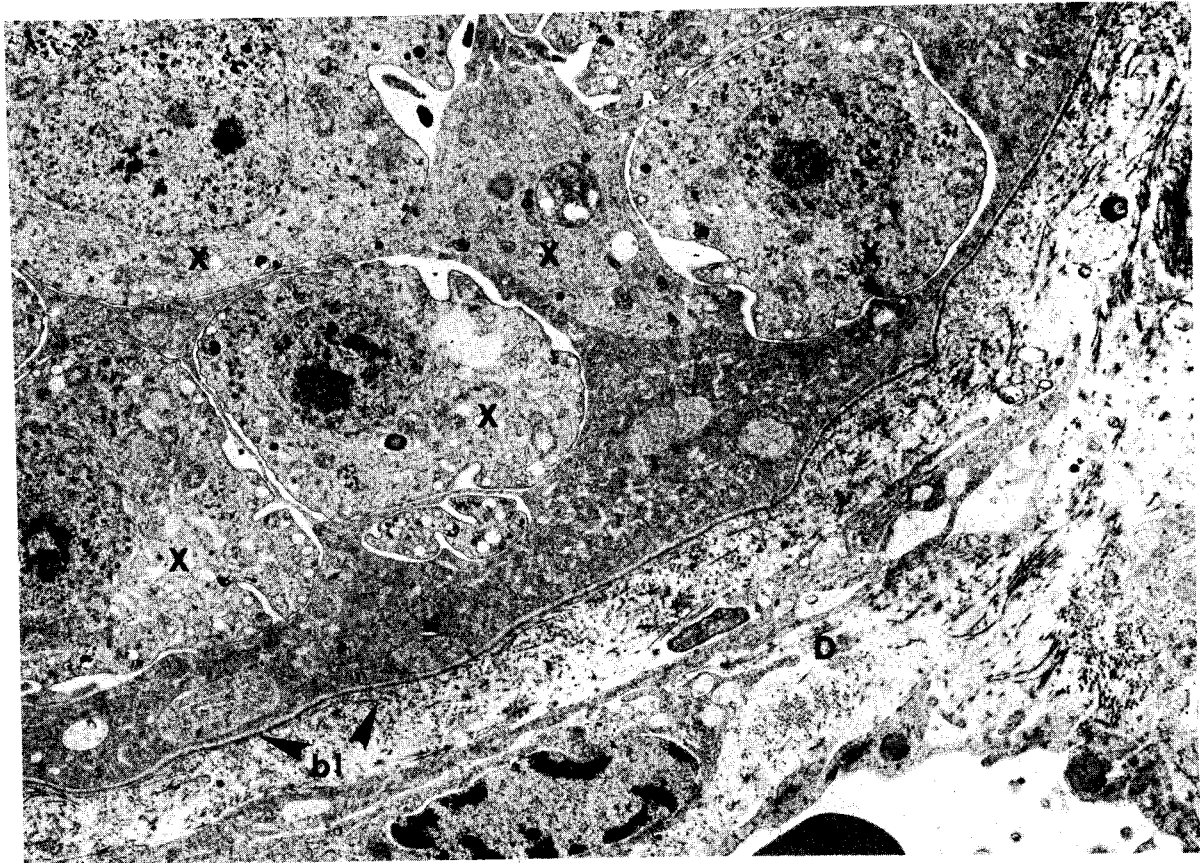


Figure 14.

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ENVIRONMENTAL-ASSESSMENT OF THE
ALASKAN CONTINENTAL SHELF

Bering Sea Ice Edge Ecosystem Study:
Nutrient Cycling and Organic Matter Transfer

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SECTION I

I. SUMMARY OF OBJECTIVES, CONCLUSIONS AND IMPLICATION WITH RESPECT TO OCS OIL AND GAS DEVELOPMENT

The objectives of this study were to determine the seasonal and spatial dynamics of the Bering Sea phytoplankton production in areas subject to future OCS development, with emphasis on the ice-edge community. The compression of annual primary production into a short spring period over much of the Bering Sea shelf implies that hazards associated with development could be particularly critical during short periods of the year. The ice-edge is associated with an intense bloom each spring. This area is also critical to higher trophic levels, and large numbers of birds and mammals are found along the receding seasonal ice. The nature of the transfer of the spring production to the zooplankton population is not clear, and the possibility exists that much of the annual feeding involves detritus from the products of the spring bloom or even previous years. This project (then) was closely coordinated with Dr. Cooney's zooplankton work in order to make preliminary estimates of zooplankton grazing in conjunction with the phytoplankton production work in order to estimate the proportion of production used immediately during the spring period.

We have concluded that the spring bloom results from stabilization of the water column, not so much as a result of salinity gradients or marked temperature gradients, but caused by the presence of ice and its suppressing effect of the extent of mixing. High nutrient conditions and the increased availability of light in the spring, both as a result of ice breakup and increased insolation, result in a short-lived and intense bloom. This bloom follows the ice edge, albeit this is not a clear line across the shelf, and extends approximately 30 to 50 miles

away. Phytoplankton populations within this zone are distinct. The underice production also contributes (epontic algae), but probably to a lesser extent. The extreme seasonal nature of this major production peak and the tendency of the surface layer with its algae to sink below the warmer water away from the ice could result in a downward transport to the benthic regions of oil-contaminated seawater, as well as oil-contaminated plankton, in the event of a major spill, and this may affect the hydrocarbon content of benthic invertebrate populations. We now have a reasonably good estimate of Bering Sea primary productivity and believe that we understand the shelf system sufficiently to make preliminary predictions with respect to possible impacts of OCS development on planktonic populations.

II. INTRODUCTION

A. The Bering Sea has been recognized (from the earliest western explorations) as a productive area of the world sufficient to be of international interest (as early as the Russian American explorations). The initial resource wealth was harvested in furs, then whales and most recently as a major international fishery. The feeding populations of pelagic birds are among the largest in the world and the marine mammal population currently exceeds millions. The success and abundance of these higher trophic level organisms is naturally dependent on the photosynthetic ability of the phytoplankton community to fix the carbon necessary to support these populations. The phytoplankton population together with a small but significant contribution from the coastal *Zostera* beds and other sources of detritus are responsible for the total annual production of the Bering Sea.

While it is obvious that the organic production of the Bering Sea shelf area is high it is remarkable too that this production occurs during a limited portion of the year since the usual winter ice cover and low light levels existent during almost half of the year effectively curtail any net production during the fall and winter months.

Our aim was to study and describe the phytoplankton dynamics of the Bering Sea throughout the year, with particular emphasis on the receding ice edge over the shelf, in order to delineate and describe the basis for the overall Bering Sea ecosystem productivity. In this report, only work through 1976 is included. The 1977 results will be reported later.

B. Specific Objectives

In order to define the phytoplankton dynamics of the relatively vast and unstudied Bering Sea, it is necessary to thoroughly investigate all the factors affecting (limiting or enhancing) phytoplankton growth at different times throughout the year. Our specific objectives are:

1. to study the seasonal variations in primary productivity throughout the Bering Sea together with all associated factors affecting these rates including nutrient concentration, vertical stability of the water column and ice cover effects,
2. to identify, catalogue and quantitatively determine the biomass of the phytoplankton species found in the area of study,
3. to review the literature discussing the phytoplankton of the eastern Bering Sea and to integrate any unpublished data from the study area as might be available,

4. to initiate cooperative studies with the zooplankton program to follow the transfer of organic matter between primary producers and zooplankton and to conduct other experiments to resolve hypothetical questions which may arise during the investigation,
5. to develop a model of the dynamics of the ice edge plankton population,
6. to determine critical areas and times during which disturbance due to development activities could result in serious impact.

C. Relevance to petroleum development

The tremendous primary productivity of the Bering Sea is confined to a relatively short productive period of ice free conditions prior to the onset of low light conditions of fall and winter. Our studies have shown that under certain conditions the bloom is very intense for an extremely short period of time during which the water column is stripped of nutrients, and at least over the major area of the shelf, most of the production occurs during a 3-4 week period. Another feature of this ice edge production zone is that the initial bloom occurs in a very defined layer 5-10 m thick, probably the result of surface ice (chunks) stabilizing the water column. This layer is observed first at the surface and moves deeper away from the ice edge. The surface nature and intensity of this bloom phenomena, given that it represents perhaps most of the annual production for the area, make it particularly vulnerable to an untimely oil spill. The potential for long lasting effects, possibly seriously affecting the entire annual production for the year,

exists to a far greater degree in the vicinity of the receding ice edge.

Secondly, while little production occurs at low light levels in the water column beneath the solid ice pack, production can be very high within areas of loose ice chunks. In fact, fields of broken ice seem to provide the mechanism for the intense surface bloom mentioned earlier. Post-winter high nutrient conditions are capable of supporting a bloom, but less than 1% of the surface radiation penetrates the ice. Following the break-up of the large ice plates into ice chunks (with open spaces between) allowing light penetration, the primed water column becomes productive. Our observations indicate that partial ice coverage, with coincident prevention of wind mixing in the water, is more conducive to an intense burst of phytoplankton activity than is a completely ice free surface.

Additionally, during this period of ice breakup the ice chunk edges which are exposed to light (exposed edges of the ice chunks with their regular exposure to light) allow ice associated phytoplankton to flourish. As these ice edges grate together during storms the slush ice sloughed from the edges, together with associated phytoplankton cells, float on the surface. Such slush fields may cover extensive areas. The plant cells are buoyed up in the well lighted surface area by the ice and are yet exposed to the necessary nutrients. We have observed an area of such slush formed during a night storm to become visibly tinged with the ochre color of diatoms in a single day. Color photos taken from a helicopter clearly show the surface color and the chlorophyll concentration of this slush was very high.

These conditions present another threatening situation. First, if some sort of oil spill or slick were to occur in this area of fragmented

ice, clean-up or containment would be virtually impossible because of the ice itself. Furthermore, the action of the ice would tend to emulsify the floating oil and could possibly result in much higher hydrocarbon concentrations in the water than would otherwise be found. Not only would this have adverse effects on the near surface phytoplankton community, but perhaps more importantly, we have observed this richly productive surface water formed at the ice front to sink beneath the warmer ocean water approaching from the south. This sinking of the surface water would effectively transport dissolved hydrocarbons into the deeper waters perhaps all the way to the bottom where it could affect migrating zooplankton and benthic populations. It appears that while this sinking tongue of cold water remains discrete, not mixing easily with other waters, transport and containment of high hydrocarbon concentrations at depth could result.

III. CURRENT STATE OF KNOWLEDGE

Major studies of primary productivity in the Bering Sea have been carried out over the past few years by McRoy and his colleagues (McRoy *et al*, 1972; McRoy and Goering, 1974). Several cruises by Japanese ships, specifically the *Hakuho Maru*, have added valuable data on a transect basis. In addition, the principal investigator of this project has carried out primary productivity studies during June-July, 1974 on the R/V *Alpha Helix*.

Summer measurements have shown that activity over much of the Bering Sea shelf is extremely low. The earliest measurements were those of Holmes (1958) who measured rates of $11 \text{ mg C/m}^2 \cdot \text{day}$ near the Aleutian Islands. Koblentz-Mishke estimated somewhat higher amounts (Koblentz-

Mishke, 1965), whereas Taniguchi estimated rates of 160 to 630 mg C/m² · day for the eastern Bering Sea. McRoy and his coworkers have estimated 18-867 mg C/m² · day as the range, with an average based on more than 20 stations of 243 mg C/m² · day. Previous winter work detected the under-ice algal component and the ice-edge component of the early season primary productivity, attributing substantial rates to the ice-algae (44 to 95 mg C/m³ · day; McRoy and Goering, 1975). The estimates are based on work done at a much lower level of resolution and intensity than the present OCS work. We feel that the annual estimate suggested by previous workers is low, and some attention needs to be devoted to updating the total estimates based on the new data available from the present program. We expect to accomplish this during the final phases of the project.

Inadequate information exists relative to the role of detritus in the Bering Sea food chain, and its major sources and fate. McRoy (1970) has made estimates of input from sea grass beds, but we have little information on input from rivers on the Alaskan coast, or on the recycling of phytoplankton carbon from the spring production in the form of detritus.

IV. STUDY AREA

In most cases the study area was determined by the location of the ice edge which was emphasized most because of the dynamic phytoplankton activity associated with it. At times when no ice edge was present, stations were distributed throughout the southeastern Bering Sea with an attempt to relocate stations in areas formerly sampled.

Consequently, the data presented in this report covers three ice edge cruises: *Discoverer* Leg I, 1975, where major sampling efforts were conducted northeast of the Pribilofs; *Surveyor* Leg I, 1976, with emphasis southeast of the Pribilofs along the shelf break; and *Surveyor* Leg II, with emphasis west of the Pribilofs and again far to the east in the region of southern Bristol Bay. *Discoverer* Leg II, 1975, was done in conjunction with the benthic sampling program and stations were widely scattered. During the November 1975 *Miller Freeman* cruise, the weather was so marginal that no predetermined cruise track was possible. We sampled when and where weather permitted with a jaunt northward in search of a forming ice front.

Figures 1-4 show the sampling stations for each cruise.

V. SOURCES, METHODS AND RATIONALE OF DATA COLLECTION

All sampling was conducted from the NOAA ships *Discoverer*, *Miller Freeman* and *Surveyor* for a total of six cruises from May 1975 until May 1976. Specific cruise dates were:

1. *Discoverer*, Leg I - May 15-May 30, 1975
2. *Discoverer*, Leg II - June 2-June 19, 1975
3. *Discoverer*, Leg I - August 9-August 28, 1975
4. *Miller Freeman*, Leg II - November 10-November 26, 1975
5. *Surveyor*, Leg I - March 14-April 2, 1976
6. *Surveyor*, Leg II - April 12-April 30, 1976

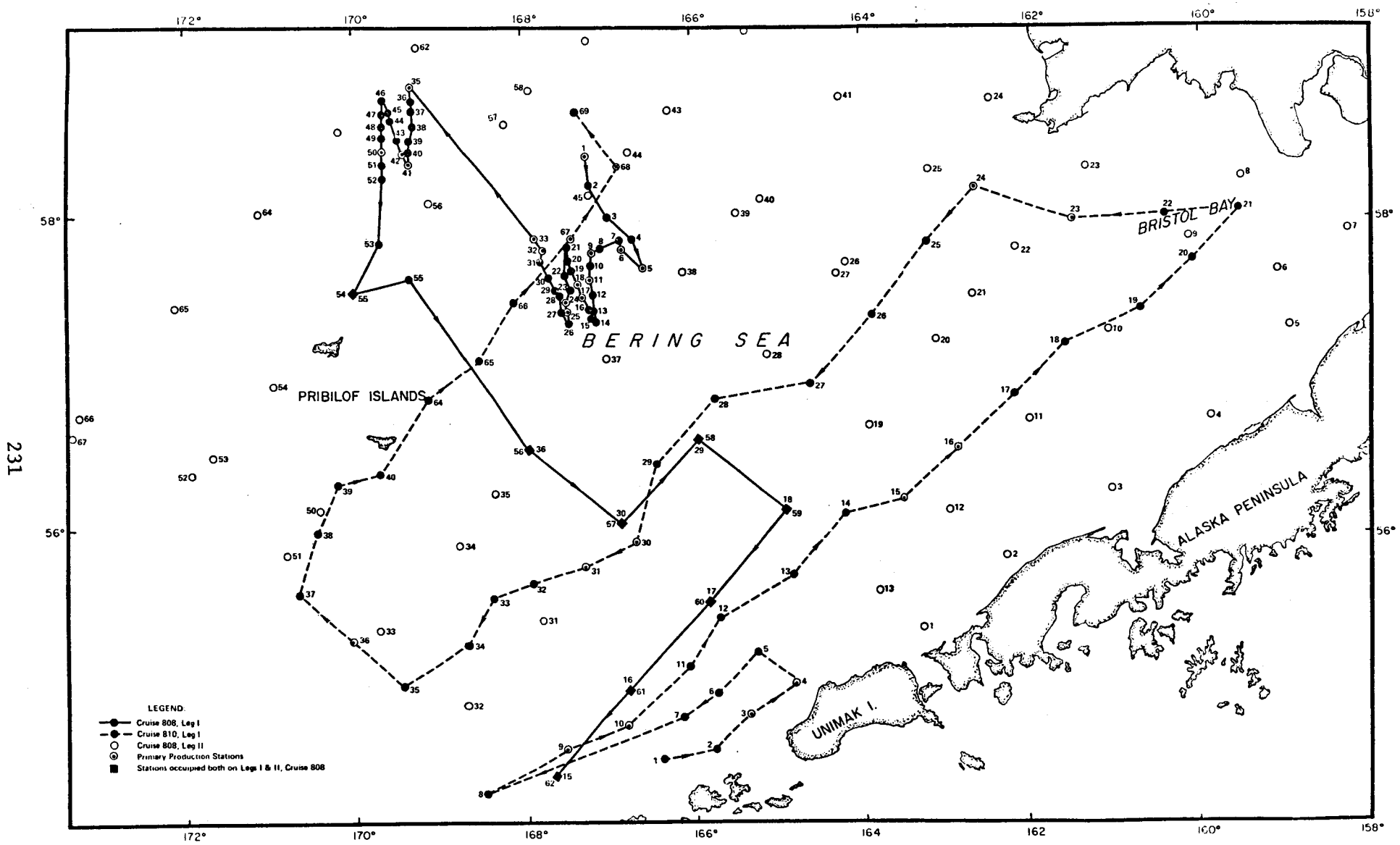


Figure 1. Cruise track for 1975 *Discoverer* cruises.

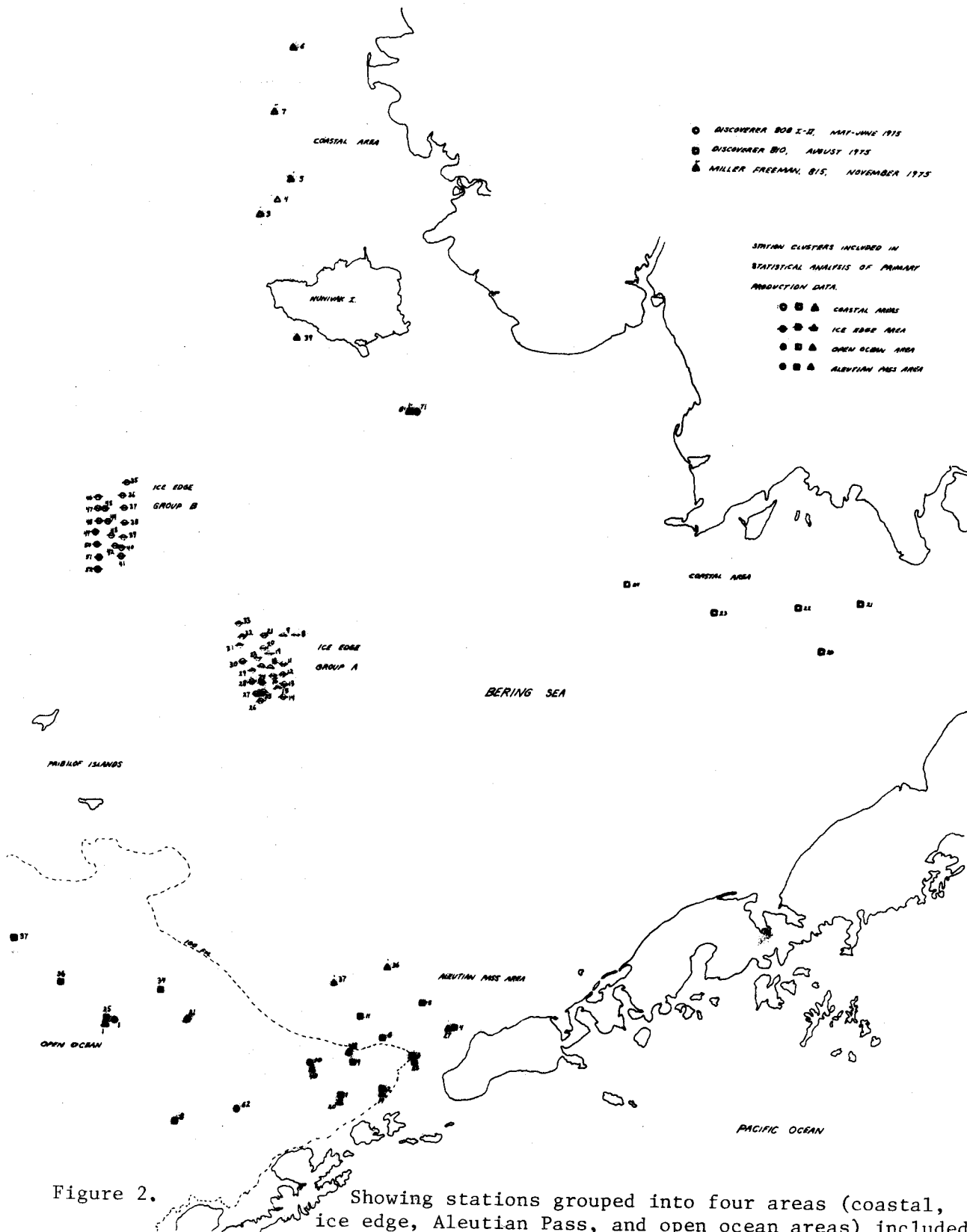


Figure 2. Showing stations grouped into four areas (coastal, ice edge, Aleutian Pass, and open ocean areas) included in the statistical analysis of primary production data, presented in Tables I through VII. Areas include stations occupied during spring, summer, and fall seasons of 1975. Table IX presents data from some of these stations re-occupied at various seasons.

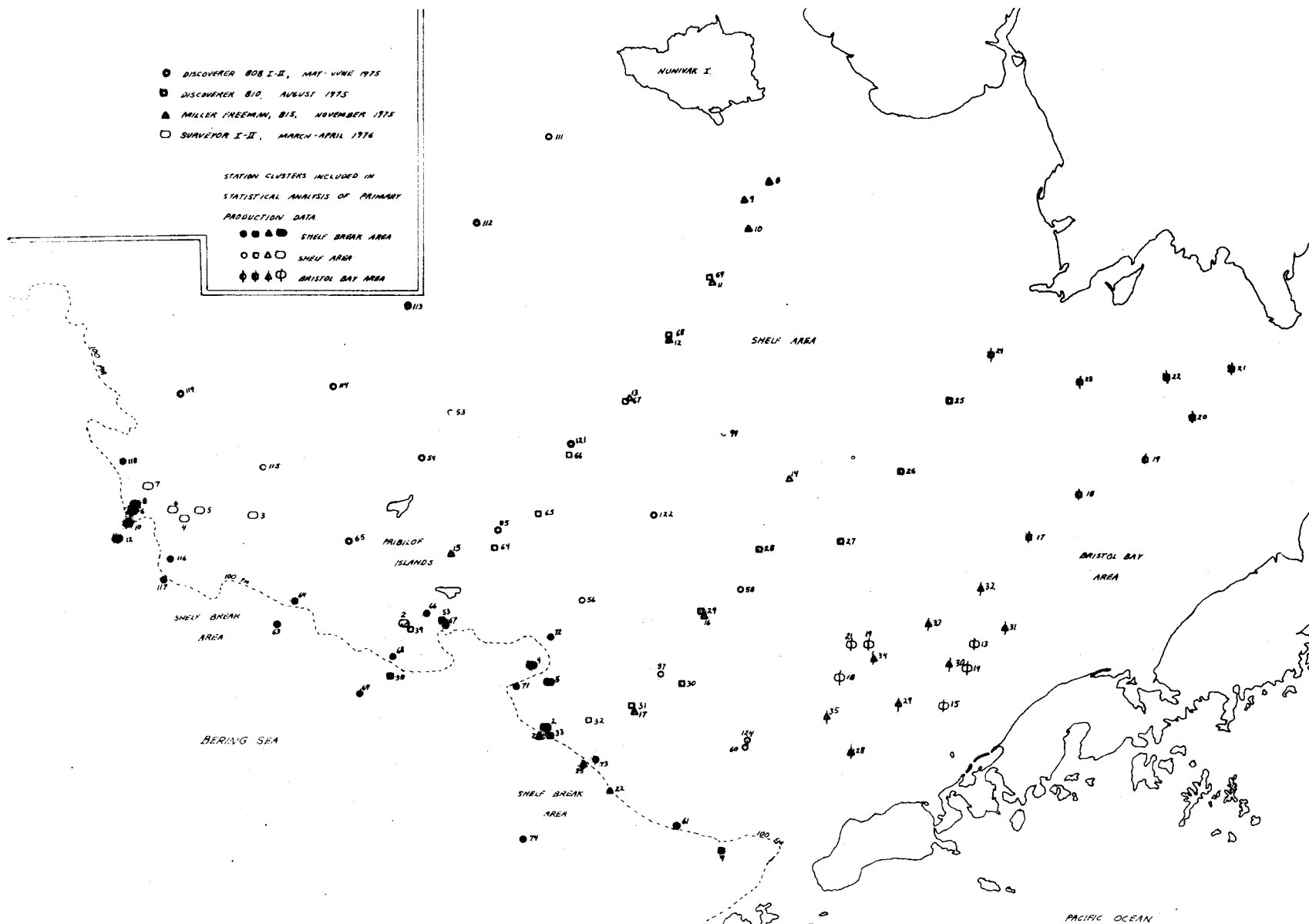


Figure 3. Showing stations grouped into three areas (shelf break, mid shelf, and Bristol Bay areas) included in the statistical analysis of primary production data, presented in Tables I - VII. Areas include stations occupied during spring, summer, and fall of 1975; and spring of 1976. Table IX presents data from some of these stations re-occupied at various seasons.

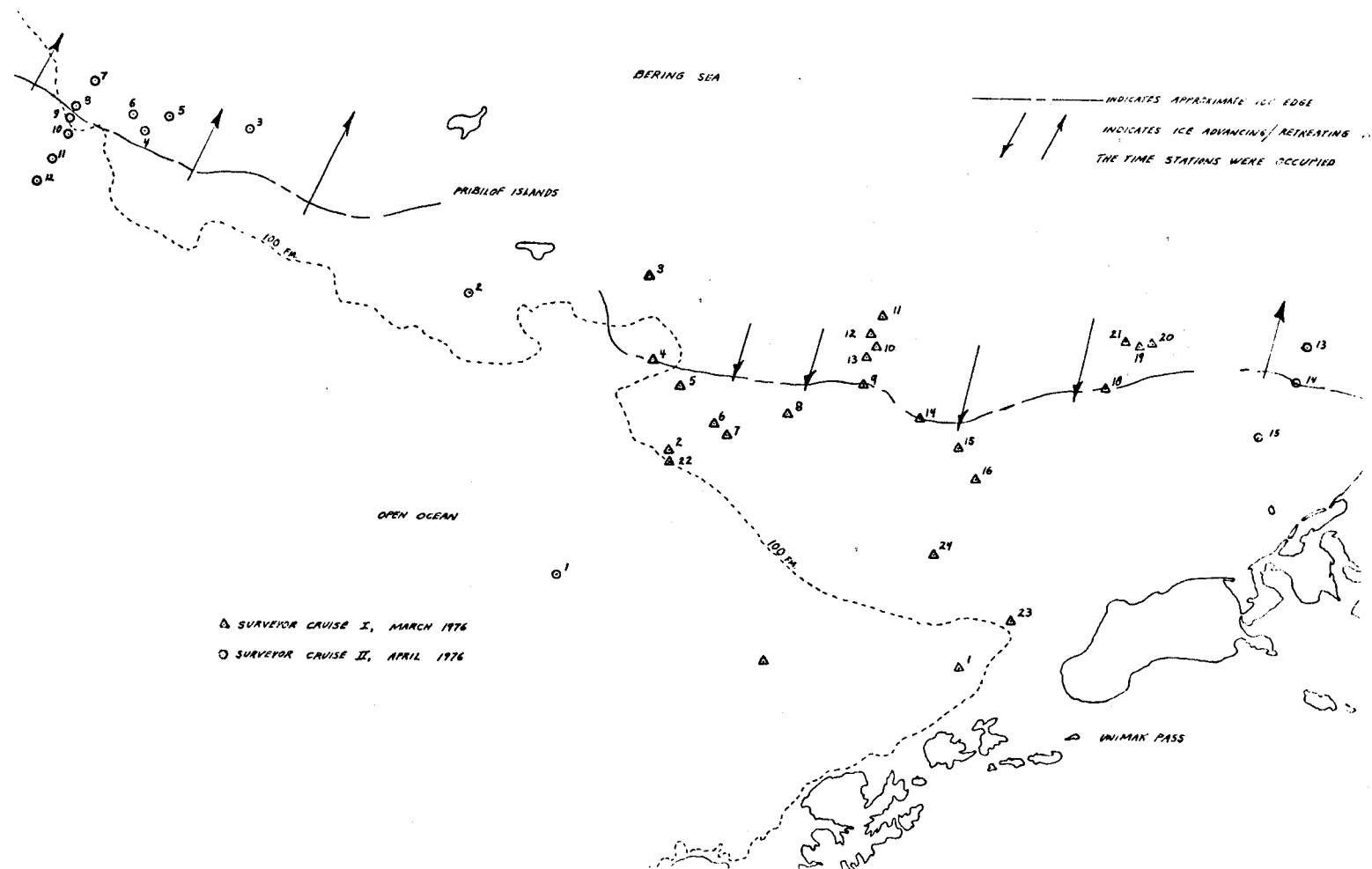


Figure 4. Showing stations occupied during March and April of 1976, in relation to the ice edge, presented in conjunction with Table VIII listing values for chlor (*a*) and carbon uptake per square meter surface area.

All sampling for phytoplankton studies was done with the CTD/Rosette Niskin sampler aboard ship, with the exception of ice samples which were obtained either with a SIPRE corer or with the aid of divers.

The scope of the initial task to describe the phytoplankton dynamics of the Bering Sea with particular emphasis on the ice edge phenomena was great. For although various investigators had made productivity measurements in certain areas at certain times, there had been no seasonal approach designed to follow and describe the entire seasonal dynamics. McRoy and Goering (1974) were able to recognize and describe the ice edge production as significant to the annual production of the Bering Sea, but until the initiation of this study no intensive investigation of the ice edge had been attempted.

The rationale of our data collection involved repeated sampling throughout the year of transects from Unimak Pass toward the ice edge over the shelf break and onto the shelf. At those times when no ice was present, i.e., August 1975 *Discoverer* cruise and November 1975 *Miller Freeman* cruise, those areas which had been sampled during ice edge cruises were emphasized.

In attempting to describe the phytoplankton dynamics of the Bering Sea, it was necessary to distinguish the various productivity regimes for different areas namely: deep water, shelf break and shelf areas in addition to investigating the role of the ice edge.

Micronutrient concentrations were measured throughout the year to depths well below the euphotic zone. Phytoplankton standing crop was estimated by chlorophyll concentration. Specific counts and identification of phytoplankton was done and carbon fixation was measured by the ^{14}C technique. The CTD parameters profiled during the hydrocasts were correlated with the phytoplankton structure.

Methodology

Standard methods were used for data collection with some minor changes to make better use of ship time and to accommodate some peculiarities inherent in high latitude research to make the data more consistent throughout the year. Additionally, as we acquired more sophisticated instrumentation, some of the more laborious techniques were abandoned in favor of more modern technology, although cross calibration of methods was done. Methods are described together with changes below.

Chlorophyll

Initially, 1 l of water from the Niskin bottle was filtered through 47 mm Gelman type A-E glass fibre filters. Under refrigeration the filters were then extracted with 10 ml of 90% spectral grade acetone for 24 hr in the dark. After centrifuging, the absorbance of the acetone-chlorophyll extract was measured with a Beckman DU-2 Spectrophotometer at wavelengths of 630, 645, 665, 750 nm. Chlorophyll *a* concentration was calculated from the equation of Parsons and Strickland.

For the August 1975 *Discoverer* cruise and the November 1975 *Miller Freeman* cruise when the spectrophotometer on board was non-functional, the filters were frozen immediately and absorbance measured following filter grinding and extraction for 24 hr in 90% acetone on a Perkin-Elmer 202 Ultra-Vis recording spectrophotometer at the previously mentioned wavelengths.

For the *Surveyor* cruises of 1976, 1 l of water collected from the Niskin bottle was filtered and extracted as before. After centrifugation the fluorescence of the chlorophyll extract was measured with a Turner

Model III Fluorometer. At representative stations and depths a duplicate sample of 1 l was filtered and frozen. These filters were returned to the laboratory for spectrophotometric analysis as before with the Perkin-Elmer Model 202. Chlorophyll α concentrations were calculated from the Parsons-Strickland equation and the results were used to calibrate the fluorometer.

Nutrients

Water samples for nutrient analysis were filtered through Gelman Type A-E glass fibre filters into aged polyethylene bottles (125 ml) and immediately frozen. Analyses for nitrate, nitrite, ammonia, silicate and phosphate were performed using automated methods on a Technicon Auto-Analyzer II.

Phytoplankton populations

Aliquots from the Niskin bottles were poured into glass jars and preserved with a modified acetic acid Lugol's solution. The Utermöhl inverted microscope technique using a Zeiss phase-contrast inverted microscope and Zeiss counting chambers was used for identifying and counting phytoplankton. To clear diatoms of protoplasmic contents for morphological study of the frustule, samples were placed in a muffle furnace at 560°C for 15-20 minutes. In addition, line drawing of unidentified species were made and photographs taken using an American Optical Differential Contrast Microscope. All phytoplankton samples have been archived for future reference.

Primary productivity

Samples from five depths were placed into glass bottles, dark and light, and to each was added 5 μCi ^{14}C as HCO_3^- . Incubations were done on deck at approximately *in situ* light conditions using neutral density light screens representing 100%, 50%, 25%, 10% and 1% incident light intensities. Surface seawater circulated through the incubator continuously to maintain sea temperature. Following 6 hr incubations, the samples were filtered through a 25 mm HA Millipore filter, rinsed with filtered seawater, the filter dried, and frozen. Upon return to the laboratory the filters were counted on a Picker low background β counter.

For the November *Miller Freeman* cruise the incubations were done somewhat differently. Due to the rigorous weather conditions and the very short daylight hours primary productivities were incubated in a temperature controlled incubation room. Deck light intensities were measured with a light meter and the incubation room light was adjusted to be approximately 80% of the noon radiation on a typically cloudy Bering Sea day. This procedural change enabled us to select stations of interest rather than restricting us to stations where time of arrival permitted daylight incubation.

Furthermore, as attempts were made to integrate marine mammal research with the oceanographic sampling program, it became increasingly difficult to be on station in early morning. Also the great distances transected during the day and/or night left many areas unsampled. It is most certainly unjustified to compare an early morning first light 6 hr incubation with an afternoon or evening incubation. Additionally, the late spring and summer light conditions make it very difficult to decide on uniform incubation times. For these reasons, we feel that

24 hr incubation times are the only solution to provide comparative data and to effectively utilize ship time. Consequently, all primary productivity incubations beginning with the *Surveyor* cruises of 1976 were incubated for 24 hr. During the 1977 field season we set up several comparative 6 hr and 24 hr incubations together with nutrient depletion studies. These data will be reported in the Annual Report for the 1977 field season.

pH and alkalinity were measured to determine the inorganic carbon available. pH was measured by a Coleman portable pH meter, Model 37A. Alkalinity was measured by adding a standard equivalent of HCl and back titrating with standardized NaOH.

Ice sampling

SIPRE cores of the ice were collected from the ice pack in the vicinity of the ship with the aid of small boats and on the *Surveyor* at greater distance from the ship by helicopter. On one cruise ship's divers collected algal samples from beneath and around the edges of floating ice chunks using a 50 ml syringe to collect algae from pockets and depressions with the under ice surface.

Grazing experiments

On *Surveyor* Leg II, experiments were performed to determine the effects of grazing, addition of copper, and addition of oil on the growth rate of the endemic phytoplankton population of the surface waters (0-5 m) at various stations. On site sea water was filtered through 216 μ Nitex netting to remove zooplankters and enriched with a nutrient stock solution to yield a final enrichment of 0.3 $\mu\text{g-at PO}_4\text{-P/l}$ and 4.5 $\mu\text{g-at}$

NO₃-N/ℓ. An initial subsample was preserved with modified Lugol's solution for later identification of the organisms. Initial particle counts were obtained using a Model B Coulter Counter with a 200 μ orifice allowing counts of particles from 4-80 μ diameter. The initial sample system water was subdivided into 1 ℓ poly bottles. To each was added one of the following variables:

1. Grazers (female copepods, *Calanus* : *eshallae*)
2. Copper (CuSO₄) concentrations of 2, 4 or 8 μg Cu⁺⁺/ℓ
3. Oil concentrations of 10 or 30 ppm (μl/ℓ) Prudhoe Crude

These experimental systems were incubated in a deck incubator exposed to surface radiation with a continuous flow of surface seawater to maintain ambient sea temperature. Samples were removed from the bottles at regular intervals for particle counting.

At the termination of some of the experiments, subsamples were again preserved with modified Lugol's solution for phytoplankton identification and counting to be compared with the initial population, and 24 hr primary productivity using H¹⁴CO₃⁻ were run.

One set of experiments (Station 13B, Event #240) used ice algae collected from the bottom of an ice core. A quantity of ice core containing plant material was gently melted and diluted 50:1 with filtered seawater.

Particle counts were done from samples collected at 0, 20 and 50 m at Station 14 (Event #257).

The zooplankters were preserved in formalin, later dried and weighed according to the method of Lovegrove (1966). Particle concentrations were converted to mg wet weight by multiplying their volume by 1.02 g/ml which is taken to be their average density.

Filtering rates of the grazers was calculated according to Rigler (1971):

$$(1) \quad F = \frac{V \ln (C_o / C_t)}{tN}$$

where F = filtering rate (ml/hr/individual)

V = volume ml

C_o = initial particle concentration (mg/l for particles 10-80 μ diameter)

C_t = particle concentration after time t

t = time interval in hr

N = number of grazers

and assuming that the detrital fraction is insignificant.

Growth rates were calculated from:

$$(2) \quad C_t = C_o e^{rt}$$

If the controls exhibited growth or mortality, corrected filtering rates were calculated from:

$$F' = \frac{V \ln (C_o / C_t e^{-rt})}{t \cdot N}$$

This correction implies that growth or mortality occurred immediately prior to the counting.

VI. RESULTS

Field Measurements

This report incorporates some preliminary integration of the results of the various cruises. Stations were divided in groups representing shelf area, shelf break, Bristol Bay area, coastal areas, open ocean station, Aleutian pass area and ice edge station. Tables I through VII compresses data from these areas respectively by depth (where \bar{X} represents the mean

TABLE I
SHELF AREA

A. Cruise 808-I, May 1975

Chlorophyll <i>a</i>	\bar{X}	n	σ	90% C.I.
0	6.92	7	4.67	±2.54
10	7.61	7	4.77	±2.59
20	8.15	7	4.86	±2.64
30	7.88	7	4.55	±2.47
40	6.82	6	4.84	±2.92
Nitrate				
0	1.17	7	2.03	±1.10
10	1.64	7	1.87	±1.02
20	2.76	7	1.74	±0.95
30	4.34	7	2.67	±1.45
40	5.13	7	4.16	±2.26
60	11.49	7	2.72	±1.48
Ammonia				
0	0.13	7	0.11	±0.06
10	0.13	7	0.11	±0.06
20	0.13	7	0.08	±0.04
30	0.24	7	0.21	±0.12
40	0.27	7	0.24	±0.13
60	0.44	7	0.22	±0.12
Silicate				
0	8.14	7	6.47	±3.51
10	8.00	7	5.92	±3.21
20	11.71	7	6.99	±3.80
30	15.86	7	11.45	±6.22
40	17.14	7	7.71	±4.19
60	27.81	7	11.28	±6.13

TABLE I. Continued

B. Cruise 808-II, June 1975, post-bloom period

Chlorophyll α	\bar{X}	n	σ	90% C.I.
0	0.58	10	0.55	± 0.25
10	1.02	10	1.26	± 0.59
20	1.61	10	2.17	± 0.95
30	2.09	10	2.29	± 1.00
50	2.80	10	3.11	± 1.36
Carbon				
0	3.00	2	2.26	± 4.94
10	2.30	2	0.71	± 1.54
20	2.40	2	0.57	± 1.24
30	2.20	2	0	0
50	0		0	0
Nitrate				
0	0.48	4	0.15	± 0.12
10	0.50	4	0.14	± 0.11
20	2.73	4	4.58	± 3.76
30	5.53	4	4.34	± 3.56
50	13.73	4	3.67	± 3.97
Ammonia				
0	0.70	10	0.54	± 0.44
10	0.95	10	0.45	± 0.37
20	0.78	10	0.43	± 0.36
30	1.43	10	1.07	± 0.88
50	0.98	10	0.62	± 0.51
Silicate				
0	3.80	5	6.83	± 4.67
10	3.80	5	6.83	± 4.67
20	6.40	5	10.11	± 6.91
30	11.20	5	10.11	± 6.91
50	22.00	4	7.26	± 5.95

TABLE I. Continued

C. Cruise 810, August 1975

Chlorophyll <i>a</i>	\bar{X}	n	σ	90% C.I.
0	0.78	15	0.73	± 0.25
10	0.93	15	1.02	± 0.36
20	1.33	15	1.03	± 0.36
30	1.14	15	1.08	± 0.38
50	0.50	11	0.46	± 0.19
Carbon				
0	1.73	4	1.23	± 0.94
10	1.58	4	1.28	± 0.98
20	1.77	3	1.01	± 1.09
30	1.03	3	0.67	± 0.73
50	0.10	4	0.12	± 0.10
Nitrate				
0	1.64	14	1.10	± 0.40
10	1.37	14	0.76	± 0.27
20	2.52	13	1.54	± 0.58
30	3.85	12	2.03	± 0.80
50	4.65	11	2.98	± 1.23
Ammonia				
0	0.71	14	0.37	± 0.13
10	0.69	14	0.32	± 0.12
20	1.39	12	0.96	± 0.38
30	1.76	12	1.00	± 0.38
50	1.73	10	0.61	± 0.44
Silicate				
0	9.46	13	3.18	± 1.20
10	10.23	13	3.44	± 1.30
20	10.83	12	2.37	± 0.89
30	14.27	11	5.12	± 2.11
50	16.27	11	8.31	± 3.43

TABLE I. Continued

D. Cruise 815, November 1975

Chlorophyll <i>a</i>	\bar{X}	n	σ	90% C.I.
0	0.93	8	0.67	±0.33
5	1.05	5	0.69	±0.47
10	0.96	8	0.59	±0.29
15	1.66	4	1.07	±0.88
20	0.90	8	0.90	±0.45
30	0.82	5	0.62	±0.42
50	0.47	4	0.36	±0.29
Carbon (1 station)				
0	1.1			
5	1.2			
10	0.5			
15	0.5			
Nitrate				
0	0.34	8	0.11	±0.05
10	0.94	9	1.66	±0.77
20	0.30	8	0.12	±0.06
30	0.95	6	1.69	±1.02
50	1.57	3	2.19	±2.40
Ammonia				
0	0.36	9	0.23	±0.11
10	0.81	9	0.95	±0.44
20	0.24	8	0.21	±0.11
30	0.60	6	0.99	±0.60
50	0.20	3	0.17	±0.19
Silicate				
0	11.78	9	10.91	± 5.09
10	11.33	9	11.82	± 5.52
20	12.13	8	11.29	± 5.67
30	17.00	6	12.36	± 7.72
50	19.10	3	16.47	±18.00

TABLE II

SHELF BREAK

Shelf Break includes stations over shelf break area and within 10 mi of 100 fm contour.

A. Cruise 808-II, June 1975, post-bloom period

Chlorophyll a	\bar{X}	n	σ	90% C.I.
0	7.82	12	5.08	± 2.00
10	7.72	12	4.86	± 1.82
20	9.34	12	4.98	± 1.88
30	9.73	12	5.10	± 1.92
50	3.45	12	2.63	± 0.99
Carbon				
0	8.18	4	3.35	± 2.74
10	7.58	4	4.28	± 3.51
20	7.90	4	3.67	± 3.01
30	6.95	4	4.17	± 3.42
50	0.48	4	0.46	± 0.38
Nitrate				
0	6.23	12	3.13	± 1.23
10	6.02	12	4.43	± 1.67
20	5.90	12	3.21	± 1.26
30	8.42	12	4.35	± 1.64
50	13.84	12	7.03	± 2.65
Ammonia				
0	0.48	12	0.33	± 0.13
10	0.53	12	0.52	± 0.21
20	0.46	12	0.34	± 0.14
30	0.80	12	0.64	± 0.25
50	0.65	12	0.42	± 0.17
Silicate				
0	16.38	13	10.74	± 4.05
10	13.92	13	9.05	± 3.41
20	16.33	12	9.35	± 3.67
30	19.08	13	9.19	± 3.46
50	35.92	13	16.40	± 6.18

TABLE II. Continued

B. Cruise 810, August 1975

Chlorophyll α	\bar{x}	n	σ	90% C.I.
0	2.78	4	1.89	± 1.60
10	2.72	4	1.71	± 1.44
20	2.45	4	1.45	± 1.22
30	0.44	3	0.14	± 0.16
Carbon (1 station)				
0	3.0			
10	1.9			
20	0.5			
30	0.3			
50	0.1			
Nitrate				
0	1.20	3	0.26	± 0.29
10	7.10	4	9.70	± 8.20
20	9.40	4	10.25	± 8.66
30	11.88	4	9.98	± 8.43
Ammonia				
0	0.78	4	0.15	± 0.13
10	0.88	4	0.31	± 0.26
20	1.05	4	0.38	± 0.32
30	1.05	4	0.35	± 0.30
50	0.93	4	0.13	± 0.11
Silicate				
0	6.75	4	1.71	± 1.44
10	15.25	4	11.95	± 10.10
20	31.50	4	39.10	± 33.04
30	35.75	4	36.90	± 31.18
50	42.00	3	41.62	± 39.33

TABLE II. Continued

C. Cruise 815, November 1975

Chlorophyll <i>a</i>	\bar{X}	n	σ	90% C.I.
0	0.2	3	0	0
10	0.2	3	0	0
20	0.2	3	0	0
30	0.2	3	0	0
50	0.2	3	0	0
Carbon (1 station)				
0	0			
10	0.3			
20	0.3			
30	0.2			
50	0			
Nitrate				
0	13.53	3	5.25	±5.74
10	13.93	3	4.92	±5.38
20	14.40	3	3.80	±4.15
30	13.67	3	1.53	±1.67
50	12.60	3	5.29	±5.78
Ammonia				
0	0.53	3	0.32	±0.35
10	0.63	3	0.49	±0.54
20	0.93	3	0.61	±0.67
30	2.03	3	2.48	±2.71
50	0.73	3	0.50	±0.55
Silicate				
0	23.00	3	11.14	±12.17
10	25.33	3	12.22	±13.35
20	25.67	3	10.26	±11.21
30	21.67	3	1.15	± 1.26
50	22.67	3	11.55	±12.61

TABLE III
BRISTOL BAY

A. Cruise 810, August 1975

Chlorophyll α	\bar{X}	n	σ	90% C.I.
0	1.13	8	1.18	± 0.59
10	1.03	8	0.69	± 0.34
20	1.39	8	1.13	± 0.57
30	0.66	8	0.34	± 0.17
50	0.74	7	0.74	± 0.38
Carbon				
0	0.70	2	0.14	± 0.31
10	0.90	2	0.28	± 0.61
20	1.35	2	0.64	± 1.39
30	0.25	2	0.07	± 0.15
50	0.05	2	0.07	± 0.15
Nitrate				
0	2.32	6	2.02	± 1.22
10	1.72	6	1.26	± 0.76
20	3.42	6	2.86	± 1.73
30	5.08	6	2.22	± 1.34
50	4.80	6	3.36	± 2.03
Ammonia				
0	1.18	8	0.80	± 0.40
10	3.03	8	2.83	± 1.42
20	2.86	8	2.57	± 1.29
30	3.49	8	3.58	± 1.79
50	4.20	6	2.18	± 2.54
Silicate				
0	8.75	8	4.23	± 4.39
10	6.50	8	0.93	± 0.46
20	10.00	8	4.87	± 2.44
30	12.50	8	7.11	± 3.57
50	29.17	6	30.92	± 15.52

TABLE III. Continued

B. Cruise 815, November 1975

Chlorophyll <i>a</i>	\bar{X}	n	σ	90% C.I.
0	0.48	8	0.70	± 0.35
10	0.23	7	0.06	± 0.04
20	0.21	8	0.09	± 0.05
30	0.24	7	0.12	± 0.07
50	0.21	7	0.10	± 0.06
Nitrate				
0	5.71	8	2.05	± 1.03
10	8.50	8	3.13	± 1.59
20	8.59	8	3.57	± 1.79
30	6.98	8	4.22	± 2.15
50	6.75	8	2.16	± 1.10
Ammonia				
0	1.06	8	0.91	± 0.46
10	1.38	8	2.49	± 1.25
20	1.29	7	1.34	± 0.73
30	1.53	8	1.68	± 0.84
50	1.25	8	1.35	± 0.68
Silicate				
0	14.25	8	7.32	± 3.68
10	18.13	8	5.62	± 2.82
20	18.50	8	7.71	± 3.87
30	14.00	8	6.57	± 3.30
50	15.25	8	6.73	± 3.38

TABLE IV
COASTAL AREAS

A. Cruise 810, August 1975

Chlorophyll a	\bar{X}	n	σ	90% C.I.
0	1.53	5	1.05	± 0.72
10	2.78	5	3.25	± 2.22
20	3.93	5	3.68	± 2.51
30	4.06	5	3.84	± 2.62
Carbon				
0	2.9	2	1.41	± 3.09
10	2.7	2	1.34	± 2.93
20	2.6	2	1.48	± 3.24
30	1.5	2	0.85	± 1.85
Nitrate				
0	4.16	5	0.05	± 0.04
10	4.20	5	0.17	± 0.12
20	4.22	5	0.13	± 0.09
30	4.28	5	0.25	± 0.17
Ammonia				
0	0.84	5	0.66	± 0.45
10	0.80	5	0.57	± 0.39
20	0.98	5	0.65	± 0.45
30	0.70	5	0.31	± 0.21
Silicate				
0	6.0	5	0	0
10	6.8	5	0.84	± 0.57
20	7.8	4	1.71	± 1.40
30	7.6	5	1.67	± 1.14

TABLE IV. Continued

B. Cruise 815, November 1975

Chlorophyll α	\bar{X}	n	σ	90% C.I.
0	0.33	6	0.13	± 0.08
5	0.40	6	0.16	± 0.10
10	0.35	6	0.13	± 0.08
15	0.66	6	0.45	± 0.27
20	1.31	5	0.47	± 0.29
Nitrate				
0	1.98	5	0.69	± 0.47
5	1.78	5	0.45	± 0.31
10	1.58	5	0.50	± 0.34
15	1.68	5	0.82	± 0.56
20	1.62	5	1.08	± 0.73
Ammonia				
0	1.92	5	0.81	± 0.56
5	2.08	5	1.02	± 0.70
10	1.84	5	0.78	± 0.53
15	2.24	5	1.38	± 0.94
20	1.40	5	0.73	± 0.50
Silicate				
0	16.0	5	2.45	± 1.67
5	16.6	5	4.77	± 3.26
10	15.2	5	4.76	± 3.25
15	14.8	5	3.83	± 2.62
20	14.0	5	4.36	± 2.98

TABLE V
OPEN OCEAN

Cruise 810, August 1975

Chlorophyll α	\bar{X}	n	σ	90% C.I.
0	3.79	5	2.97	± 2.03
10	4.02	4	3.63	± 2.98
20	1.38	5	0.46	± 0.31
30	0.96	5	0.72	± 0.49
50	0.27	3	0.23	± 0.19
Carbon (1 station)				
0	5.5			
10	7.1			
20	0.9			
30	0.4			
50	0			
Nitrate				
0	1.27	3	0.38	± 0.41
10	3.08	5	2.84	± 1.94
20	7.44	5	3.00	± 2.05
30	19.20	5	9.98	± 6.82
50	12.60	3	5.82	± 6.36
100	25.10	4	5.40	± 4.43
Ammonia				
0	1.35	4	0.30	± 0.25
10	1.68	5	0.69	± 0.47
20	1.60	5	0.39	± 0.26
30	2.05	4	0.77	± 0.63
50	1.90	4	1.01	± 0.83
100	1.80	5	1.17	± 0.80
Silicate				
0	5.67	3	0.58	± 0.63
10	8.20	5	6.06	± 4.14
20	15.4	5	5.73	± 3.91
30	53.00	5	36.61	± 25.01
50	21.25	4	7.37	± 6.04

TABLE VI

ALEUTIAN PASS AREA

A. Cruise 810, August 1975

Chlorophyll α	\bar{X}	n	σ	I.I.
0	4.17	9	3.18	± 1.48
10	7.19	9	6.09	± 2.84
20	4.05	9	3.20	± 1.49
30	3.00	9	2.46	± 1.15
50	1.68	8	2.14	± 1.08
Carbon				
0	0.70	2	0.42	± 0.93
10	2.93	3	3.14	± 3.43
20	0.70	3	0.52	± 0.57
30	0.47	3	0.29	± 0.32
50	0.10	3	0.10	± 0.11
Nitrate				
0	4.64	9	6.42	± 3.00
10	5.81	9	4.36	± 2.04
20	11.39	9	8.77	± 4.09
30	10.83	9	8.04	± 3.75
50	14.17	9	6.35	± 2.96
Ammonia				
0	0.89	7	0.70	± 0.38
10	1.45	6	0.95	± 0.57
20	2.63	7	1.45	± 0.79
30	2.33	7	0.69	± 0.37
50	2.39	7	0.78	± 0.42
Silicate				
0	6.17	9	3.62	± 1.69
10	13.44	9	10.90	± 5.09
20	17.67	9	8.63	± 4.03
30	13.88	8	13.28	± 6.66
50	32.63	8	25.11	± 12.60

TABLE VI. Continued

B. Cruise 815, November 1975

Chlorophyll α	\bar{X}	n	σ	90% C.I.
0	0.30	8	0.19	± 0.10
10	0.23	8	0.11	± 0.06
20	0.25	8	0.16	± 0.08
30	0.26	8	0.15	± 0.08
50	0.23	8	0.11	± 0.10
Nitrate				
0	10.69	8	6.27	± 3.14
10	8.34	8	6.19	± 3.11
20	7.90	8	5.58	± 2.80
30	9.34	8	6.28	± 3.15
50	11.29	8	6.79	± 3.41
Ammonia				
0	1.04	8	1.12	± 0.56
10	0.94	8	2.58	± 1.29
20	0.69	8	0.96	± 0.48
30	0.89	8	0.91	± 0.46
50	1.14	8	1.30	± 0.65
Silicate				
0	23.38	8	10.23	± 5.13
10	19.75	8	11.07	± 5.55
20	21.00	8	11.71	± 5.88
30	22.88	8	12.55	± 6.30
50	23.50	8	13.41	± 6.73

TABLE VII

ICE EDGE

A. Cruise 808-I, May 1975, Group A

Chlorophyll α	\bar{X}	n	σ	90% C.I.
0	19.53	(23)	9.62	± 2.65
5	18.26	(21)	8.67	± 2.52
10	19.18	(24)	7.53	± 2.03
15	12.41	(24)	7.57	± 2.04
20	11.69	(24)	8.34	± 2.25
30	6.18	(23)	3.30	± 0.91
40	5.20	(23)	2.72	± 0.75
60	4.81	(19)	2.37	± 0.72
Carbon				
0	24.0	8	13.01	± 6.5
5	17.8	7	5.65	± 3.1
10	20.0	8	7.52	± 3.8
15	14.7	8	11.16	± 5.6
20	2.0	8	1.67	± 0.8
Nitrate				
0	0.24	9	0.31	± 0.14
10	0.93	9	1.31	± 0.61
20	9.24	9	5.86	± 2.73
30	13.25	8	3.41	± 1.71
40	14.21	9	2.44	± 1.14
60	13.08	8	3.60	± 1.81
Ammonia				
0	0.38	9	0.20	± 0.09
10	0.44	9	0.25	± 0.12
20	0.46	9	0.29	± 0.13
30	1.11	8	1.01	± 0.51
40	0.92	9	0.83	± 0.39
60	0.66	9	0.72	± 0.34
Silicate				
0	3.67	9	3.54	± 1.65
10	4.69	9	6.15	± 2.87
20	26.89	9	19.17	± 9.20
30	38.01	8	13.91	± 6.86
40	41.78	9	11.82	± 5.52
60	39.63	8	10.97	± 5.50

TABLE VII. Continued

B. Cruise 808-I, May 1975, Group B

Chlorophyll α	\bar{X}	n	σ	90% C.I.
0	19.13	18	5.56	± 1.74
5	21.01	17	9.63	± 3.13
10	20.22	17	6.27	± 2.04
15	18.51	18	7.85	± 2.46
20	13.81	18	6.93	± 2.17
30	5.90	18	7.05	± 2.21
40	3.68	18	1.39	± 0.44
60	3.50	18	1.31	± 0.41
Carbon				
0	13.75	4	6.57	± 5.39
5	19.63	4	11.55	± 9.47
10	19.09	4	3.48	± 2.85
15	13.08	4	8.04	± 6.59
20	0.60	4	0.34	± 0.28
Nitrate				
0	0.52	6	0.83	± 0.50
10	0.33	6	0.35	± 0.21
20	4.72	6	2.39	± 1.44
30	8.98	5	5.91	± 4.04
40	10.53	6	6.15	± 3.71
60	11.56	5	4.95	± 3.38
Ammonia				
0	0.38	6	0.27	± 0.16
10	0.38	6	0.24	± 0.14
20	0.62	6	0.20	± 0.12
30	1.10	5	0.85	± 0.56
40	0.92	6	0.62	± 0.25
60	0.72	5	0.15	± 0.10
Silicate				
0	3.67	6	2.34	± 1.41
10	4.50	6	2.35	± 1.42
20	16.50	6	5.89	± 3.56
30	27.40	5	15.06	± 9.40
40	35.80	6	8.61	± 5.20
60	38.80	5	4.55	± 2.75

value for all stations within a group at a depth). From data presented in this way, the intensity of production at this ice edge station can be seen (Table VII), for example, for further elaboration, see the Discussion below.

Table VIII analyzes the spring bloom and ice edge production regime in greater detail. This presents results for primary productivity and carbon integrated with depth. The high activity well into the ice is evident in March, but this intensifies during the major bloom to extremely high values.

Several stations, or stations in approximately similar location, were occupied more than once. These may be used for seasonal comparison and are shown in Table IX.

A preliminary list of phytoplankton species found in the Bering Sea, with accompanying notes, is found in Table X. Table XI examines the total of all numbers for the 1976 *Surveyor* cruise with respect to distance from the ice.

In order to look more closely at the major groups of phytoplankton in the ice population, a sample from each of the two *Surveyor* cruises is shown in great detail in Table XII.

Table XIII analyzes phytoplankton as percentages by major groups at the various stations of the surface samples.

During cruise 802 (May-June 1977), the phytoplankton population at the ice edge was extremely diverse and showed very high densities. Major species included a large number of diatoms of genera *Thalassiosira*, *Nitzschia* (*Fragilariopsis*) and *Chaetoceros*.

The August 1975 *Discoverer* cruise showed some areas of high diversity and abundance (to 10^6 cells/liter), but with most areas one or two orders of magnitude lower (10^4 or 10^5) and with lower diversity.

TABLE VIII

INTEGRATED CHLOROPHYLL α AND CARBON UPTAKE VALUES FOR *SURVEYOR* I AND II CRUISES ALONG
THE ICE EDGE DURING MARCH AND APRIL 1976

Refer to Figure III for Station Plot and Ice Edge Location

<i>Surveyor</i> I Sta. #	Chlor α $\text{mg}\cdot\text{m}^{-2}$	Carbon $\text{mg}\cdot\text{m}^{-2}\cdot\text{hr}^{-1}$	Sta. relation to ice edge	<i>Surveyor</i> II Sta. #	Chlor α $\text{mg}\cdot\text{m}^{-2}$	Carbon $\text{mg}\cdot\text{m}^{-2}\cdot\text{hr}^{-1}$	Sta. relation to ice edge
1	21.0		Unimak Pass	1	24.4	88.8	Open ocean
2	17.6		50 mi from ice	2	23.5		50 mi from ice
3	24.3		10 mi into ice	3	523.5	480.8	20 mi into ice
4	11.8	11.5	ice edge	4	468.6	302.2	ice edge
5	15.1		5 mi from ice	5	614.4	604.4	20 mi into ice
6	19.1		10 mi from ice	6	429.6	520.1	10 mi into ice
7	13.6		20 mi from ice	7	831.1	725.2	30 mi into ice
8	12.7		10 mi from ice	8	628.1		ice edge
9	12.4		ice edge	9	695.0		ice edge
10	16.4	13.7	10 mi into ice	10	271.6	222.5	10 mi from ice
11	22.0	34.7	30 mi into ice	11	131.5		20 mi from ice
12	31.3	46.7	20 mi into ice	12	50.1		30 mi from ice
13	23.0		5 mi into ice	13	178.2	426.0	20 mi into ice
14	17.9		ice edge	14	73.4	102.7	ice edge
15	17.7		10 mi from ice	15	35.3	56.3	30 mi from ice
16	15.7		20 mi from ice				
17	14.1		10 mi from ice				
18	17.3	22.2	ice edge				
19	22.9	22.9	10 mi into ice				
20	16.9	28.6	10 mi into ice				
21	17.6		10 mi into ice				
22	20.0	33.3	10 mi from ice				
23	11.8		100 mi from ice				
24	18.7	46.0	50 mi from ice				

Note: All Chlor α and Carbon values are integrated from 50 m to the surface from discrete sample values at 0 m, 10 m, 20 m, 30 m and 50 m.

TABLE IX

SEASONAL COMPARISON OF PRIMARY PRODUCTION DATA FROM STATIONS
OF CLOSE PROXIMITY SAMPLED AT VARIOUS TIMESA. Pre- and post-ice edge

Cruise:		808-II	808-I	808-II
Sta. #:		113	46	35
Time:		pre-ice	post-ice	post-ice
Chlor a	0 m	0.17	21.2	12.4
$\mu\text{g}/\ell$	10 m	0.20	20.1	22.9
	20 m	0.26	11.5	5.2
	30 m	0.95	2.2	4.0
	50 m	0.72	2.5	3.5
NO_3/SiO_3	0 m	0.4/1	0.8/6	0/0
$\mu\text{g-at}/\ell$	10 m	0.5/1	0.9/6	0/1
	20 m	0.4/1	4.9/18	6/20
	30 m	8.1/19	10.8/35	6/19
	50 m	10.3/38	10.0/32	3/9

B. Shelf area

Cruise:		808-I	810	815
Sta. #:		20	67	13
Time:		March, ice	August	November
Chlor a	0 m	27.7	0.25	0.62
$\mu\text{g}/\ell$	10 m	22.7	0.48	0.70
	20 m	3.1	0.70	0.58
	30 m	2.3	0.50	0.69
	50 m	1.8	1.35	
NO_3/SiO_3	0 m	0.8/8	2.2/11	0.2/11
$\mu\text{g-at}/\ell$	10 m	0.8/8	2.2/11	5.3/9
	20 m	15.2/50	5.0/13	0.3/11
	30 m	15.4/51	-	4.4/8
	50 m	15.8/53	5.7/14	-

TABLE IX. Continued

C. Mid-shelf area

Comparative transects of Cruise 810, August, and 815, November.

Cruise:		810/815	810/815	810/815	810/815
Sta. #:		67/13	68/12	69/11	70/10
Chlor <i>a</i> μg/l	0 m	0.25/0.6	1.75	1.85/1.35	0.68/2.0
	10 m	0.48/0.7	1.60/0.9	3.20/0.8	1.80/1.7
	20 m	0.70/0.6	1.15/0.8	1.50/0.4	2.20/2.4
	30 m	0.50/0.7	1.10/0.8	2.20/1.8	- / -
	50 m	1.35/ -	1.45/1.09	- / -	- / -
NO ₃ μg-at/l	0 m	2.20/.2	2.20/0.2	2.20/0.3	2.20/0.4
	10 m	2.20/5.3	2.20/1.1	2.20/0.2	2.20/0.3
	20 m	5.00/0.3	2.20/0.2	2.20/0.3	2.20/0.1
	30 m	- /4.4	2.20/0.3	- /0.1	- / -
	50 m	5.70/ -	2.20/ -	- / -	- / -
SiO ₃ μg-at/l	0 m	11/12	11/17	12/3	11/2
	10 m	11/9	11/9	13/3	11/2
	20 m	13/11	11/12	13/3	11/1
	30 m	-/8	11/14	-/3	-/-
	50 m	14/-	13/-	-/-	-/-

D. Open ocean area

Cruise:		810	815	SU-II
Sta. #:		35	1	1
Time:		August	November	April
Chlor <i>a</i> μg/l	0 m	1.40	0.29	0.60
	10 m	1.28	0.30	0.54
	20 m	1.65	0.14	0.44
	30 m	1.08	0.20	0.48
	50 m	0.03	0.25	0.44
NH ₄ μg-at/l	0 m	1.5	0.7	4.7
	10 m	2.7	-	3.5
	20 m	2.1	0.4	3.2
	30 m	2.5	0.3	0.6
	50 m	3.1	0.3	0.5
NO ₃ μg-at/l	0 m	1.7	31.8	27.2
	10 m	1.6	-	20.7
	20 m	7.7	10.6	26.9
	30 m	24.9	18.6	26.7
	40 m	18.1	16.0	26.9

TABLE IX. Continued

D. Open ocean area (cont'd)

Cruise:		810	815	SU-II
Sta. #:		35	1	1
Time:		August	November	April
PO ₄	0 m	5.8	9.1	1.6
μg-at/l	10 m	2.2	-	1.7
	20 m	0.8	2.9	2.0
	30 m	0.8	9.1	1.9
	50 m	0.9	9.4	2.1
SiO ₃	0 m	6.0	44.0	53.0
μg-at/l	10 m	5.0	-	40.0
	20 m	18.0	20.0	53.0
	30 m	81.0	31.0	52.0
	50 m	30.0	25.0	53.0

TABLE X

1975 - 1976 PHYTOPLANKTON SPECIES IN THE BERING SEA

CHRYSOPHYTA

Bacillariophyceae (diatoms)

- Achnanthes* sp. Bory
Actinocyclus roperii (de Bréb.) Grun. ex Van Heurck
Actinoptychus splendens (Shad.) Ralfs ex Pritch.
Amphiprora sp. Ehrenberg
Asterionella glacialis Castr. (*Asterionella japonica* Cleve & Möller)
A. kariana Grun.
Bacteriosira fragilis Gran
Biddulphia aurita (Lyng.) Bréb. & God.
B. longicuris Grev. cf.
Chaetoceros affinis Laud.
C. atlanticus C.
C. borealis Bail.
C. cinctus Gran
C. compressus Laud.
C. concavicornis Mang.
C. convolutus Castr.
C. curvisetus Cl. cf.
C. danicus Cl. (spore)
C. debilis Cl.
C. decipiens Cl.
C. furcellatus Bail.
C. fragilis Meun. cf.
C. gracilis Schütt cf.
C. holsaticus Schütt
C. lacinosus Schütt
C. lorenzianus Grun.
C. radicans Schütt
C. seiracanthas Gran
C. septentrionalis Oestrup
C. similis Cl.
C. socialis Cl.
C. subsecundus (Grun.) Husted
C. wighamii Brighw.
C. spp.
Cocconeis sp. Ehr.
Corethron criphilum Castr. (*Corethron hystrix* Hensen)
Coscinodiscus centralis Ehr.
C. concinnus Ehr.
C. excentricus Ehr.
C. lineatus Ehr.
C. marginatus Ehr.
C. oculus iridis Ehr.
C. radiatus Ehr.
C. spp.

TABLE X. Continued

CHRYSTOPHYTA

Bacillariophyceae (diatoms)

- Cylindrotheca closterium* (Ehr.) Reiman & Lewin (*Nitzschia closterium* Ehr.)
C. gracilis (de Bréb.) Grun.
Dactyliosolen mediterraneus H. Pér.
Denticula seminae Simonsen et Kanaya
Detonula confervacea (Cleve) Gran
Ditylum brightwellii (West) Grun.
Eucampia zodiacus Ehr.
Gyrosigma or *Pleurosigma* sp I
Gyrosigma or *Pleurosigma* sp II
 cf. *Hemiaulus* sp. Ehr.
 cf. *Hyalodiscus* sp. Ehr.
Leptocylindrus danicus Cl.
Liemophora sp. Ag.
Melosira sulcata (Ehr.) Kütz.
M. sp. Ag.
Navicula pelagica (Cleve) (*Stauropsis pelagica* (Cleve) Meun.)
N. transitans Cleve
N. vanhoeffeni Gran (*Stauropsis vanhoeffeni* (Gran) Meun.)
N. spp.
Nitzschia delicatissima Cl. cf
N. frigida Grun.
N. longissima (Bréb.) Ralfs cf.
N. paradoxa (Gmel.) Grun. cf.
N. seriata Cl.
N. subpacifica Hasle
Pleurosigma sp. W. Smith
Porosira glacialis (Gran) Jörg. (*Podosira glacialis* Cleve, *Lauderia glacialis* (Grun.) Cl.
Rhizosolenia alata Brightw.
R. delicatula Cl.
R. fragilissima Berg.
R. hebetata f. *semispina* (Hen.) Gran
R. hebetata f. *hiemalis* Gran
R. setigera Brightw.
R. stolterfothii H. Pér.
R. styliiformis Brightw.
 cf. *Rhabdonema* sp. Kützing
 cf. *Schroderella delicatula* (H. Pér.) Pav.
Skeletonema costatum (Grev.) Cl.
Stephanopyxis nipponica Gran & Yendo
Thalassionema nitzschoides Grun.
Thalassiosira aestivalis (Grun.) Jorg.
T. gravida Cl.
T. hylina (Grun.) Gran

TABLE X. Continued

CHRYSOPHYTA

Bacillariophyceae (diatoms)

Thalassiosira nordenskioldii Cl.
T. polychorda (Gran) Jørg.
T. rotula Meun.
T. spp.
Thalassiothrix frauenfeldii Grun. cf.
Tropidoneis lepidoptera (Greg.) Cl. cf.
unidentified centric diatoms
unidentified pennate diatoms

Chrysophyceae

Dictyocha fibula Ehr.

DINOPHYTA (dinoflagellates)

Ceratium lineatum (Ehr.) Cleve
C. tripos (O.F. Müller) Nitzsch.
Dinophysis norvegica Clap. et. Lachm.
cf. *Gymnodinium* sp. Stein
Oxytoxum sp. Stein
Protoperidinium minisculum (*Peridinium minisculum*)
P. pallidum (Ostenf.) Balech
P. spp.
unidentified dinoflagellates

PRASINOPHYTA (microflagellates)

HAPTOPHYTA

Haptophyceae
Coccolithaceae
Phaeocystaceae
Phaeocystis sp.

CYANOPHYTA

unidentified bluegreens

EUGLENOPHYTA

unidentified euglenoids

Microflagellates of uncertain taxonomic position

CRASPEDOPHYTA

NOTES CONCERNING TAXONOMY SEE SPECIES LIST

CRASPEDOPHYTA (choanoflagellates) were reported in 1975, but recent literature and studies by Leadbeater, B.S.C. and Manton (1974) suggest these organisms should be grouped with the animal kingdom. Parke and Dixon (1976) Checklist of British Marine Algae - Third Revision do not report them. I did not include them in the 1976 counts for those reasons.

The genus *Fragilariopsis* has been synonymized under *Nitzshia* spp. In 1975 it was reported as *Fragilariopsis* but in 1976 and now is reported as *Nitzschia* sp. (Hasle 1972).

The name *Melosira moniliformis* reported in 1975 is incorrect. It may be a *Detonula* but the identification of this diatom is still uncertain.

Thalassiosua hylina known to occur in the Bering Sea has not been separated in 1975 and 1976 data, but noted as *Thalassiosira* spp.

The diatom *Denticula seminae* has been lumped with *Nitzschia* sp. (*Fragilariopsis*) in 1975 and 1976 data. This specie is an important component of the Bering Sea phytoplankton especially during bloom conditions.

TABLE XI

PHYTOPLANKTON (CELLS/LITER) AT VARYING DISTANCES FROM ICE

A. LEG 1 SURVEYOR 1976

LOCATION	IN ICE PACK		OUTSIDE ICE EDGE		AWAY FROM ICE EDGE	
	Station	Cells/Liter	Station	Cells/Liter	Station	Cells/Liter
	4	1.6×10^4	7	7.8×10^4	25	2.6×10^4
DEPTH Om	11	7.96×10^4	15	1.32×10^4		
	12	1.0×10^5	16	-		
	10	3.2×10^4	18	8.24×10^4		
	13	5.0×10^4				
	4	-	7	-	25	3.0×10^4
10m	11	1.06×10^5	15	-		
	12	1.12×10^5	16	2.48×10^4		
	10	2.12×10^4	18	2.72×10^4		
	13	9.4×10^4				
	4	1.52×10^4	7	-	25	-
20m	11	4.8×10^4	15	-		
	12	5.4×10^4	16	1.28×10^4		
	10	-	18	-		
	13	-				

TABLE XI. Continued

B. LEG II SURVEYOR 1976

LOCATION	IN ICE PACK		PARALLEL TO ICE EDGE		JUST OUTSIDE ICE EDGE		AWAY FROM ICE		
	Station	Cells/Liter	Station	Cells/Liter	Station	Cells/Liter	Station	Cells/Liter	
DEPTH	0m	7	6.2×10^5	4	2.7×10^5	10	1.09×10^5	1	8.56×10^4
		8	7.3×10^5	5	2.8×10^5	11	9.52×10^4	2	1.2×10^4
		9	5.42×10^5	6	4.04×10^5	12	9.9×10^4		
		13	4.8×10^5						
	20m	7	-	4	1.85×10^5	10	8.56×10^4	1	3.8×10^4
		8	5.42×10^5	5	3.06×10^5	11	1.23×10^5	2	-
		9	-	6	4.5×10^5	12	8.2×10^4		
		13	-						
	30m	7	-	4	2.12×10^5	10	9.3×10^4	1	-
		8	-	5	3.16×10^5	11	8.6×10^4	2	1.17×10^5
		9	-	6	3.2×10^5	12	4.8×10^4		
		13	-						
40m	7	-	4	2.0×10^5	10	1.1×10^5	1	-	
	8	-	5	-	11	-	2	-	
	9	-	6	-	12	-			
	13	-							
50m	7	-	4	7.68×10^4	10	4.2×10^4	1	-	
	8	-	5	2.0×10^4	11	1.26×10^5	2	1.48×10^4	
	9	-	6	-	12	6.8×10^3			
	13	-							

TABLE XII

SURVEYOR LEG I

20 March 76 Ice Pack Sample Station 4 Om Total Cells/Liter 1.6×10^4

	Cells/Liter
Chrysophyta	
Bacillariophyceae (diatoms)	
<i>Cylindrotheca closterium</i>	3200
<i>Nitzschia</i> sp.	400
<i>Rhizosolenia alata</i>	1200
<i>Thalassiosira</i> spp	800
Dinophyta (dinoflagellates)	
cf. <i>Gymnodinium</i> sp	400
unidentified dinoflagellates	800
Microflagellates	
0-4 μ	6000
5-10 μ	1600
Prasinophyta	<u>1600</u>
TOTAL	16000

TABLE XII. Continued

SURVEYOR LEG II

21 April 76 Ice Pack Sample Station 7 0m Total Cells/Liter 6.2×10^5

	Cells/Liter
Bacillariophyceae (diatoms)	
<i>Bacteriosira fragilis</i>	11200
<i>Chaetoceros compressus</i>	8800
<i>C. debilis</i>	7200
<i>C. gracilis</i> cf.	800
<i>C. socialis</i>	7200
<i>C. wighamii</i> cf.	8000
<i>C. sp.</i>	3200
<i>Cylindrotheca closterium</i>	5600
<i>Eucampia zoodiacus</i>	1600
<i>Licmophora sp.</i>	800
<i>Nitzschia sp. (Fragilariopsis)</i>	188000
<i>N. sp. (Fragilariopsis)</i>	62400
<i>N. sp. (Fragilariopsis oceanica f circularis)</i>	8000
<i>Nitzschia sp.</i>	800
<i>Navicula vanhoeffeni</i>	6400
<i>Navicula sp.</i>	3200
<i>Porosira glacialis</i>	1600
<i>Thalassiosira decipiens</i>	45600
<i>T. gravida</i>	84000
<i>T. nordenskioldii</i>	12000
<i>T. rotula</i>	3200
<i>T. sp.</i>	4000
<i>T. sp.</i>	2400
<i>T. spp.</i>	80800
unidentified pennates	10400
unidentified cells	6400
Dinophyta (dinoflagellates)	
cf. <i>Gymnodinium sp.</i>	9600
cf. <i>Ocyropsis</i>	800
Microflagellates	
0-4 μ	24000
5-10 μ	2400
Prasinophyta	5600

TABLE XIII

COMPOSITION OF THE PHYTOPLANKTON BY MAJOR GROUPS
AT 0m LEG I AND LEG II SURVEYOR MARCH-APRIL 1976

(% of total cells/liter)

LOCATION	LEG I IN ICE PACK		PARALLEL TO ICE EDGE		JUST OUTSIDE EDGE		AWAY FROM ICE	
	Station	% of Total	Station	% of Total	Station	% of Total	Station	% of Total
Phytoplankton Groups †	4				7		25	
Diatoms		35	-			24		11
Dinoflagellates		8	-			41		14
Microflagellates		57	-			34		72
Unknown		0	-			0		3
271 LOCATION	LEG II IN ICE PACK		PARALLEL TO ICE EDGE		JUST OUTSIDE EDGE		AWAY FROM EDGE	
	Station	% of Total	Station	% of Total	Station	% of Total	Station	% of Total
Phytoplankton Groups †	2		4		10		1	
Diatoms		92		96		81		41
Dinoflagellates		2		0		5		2
Microflagellates		5		4		14		52
Unknown		1		0		1		5
	8		5		11		2	
Diatoms		94		92		74		11
Dinoflagellates		1		1		4		6
Microflagellates		5		7		22		83
Unknown		0		0		0		0

By the November *Miller Freeman* cruise diversity was low with microflagellates making up a large part of the phytoplankton population. Dinoflagellates of the genera *Ceratium*, *Dinophysis* and *Peridinium* occurred. Leg I of the *Surveyor* (March 1976) cruise illustrated pre-bloom conditions. Table XII shows that microflagellates and dinoflagellates constitute a large percentage of the phytoplankton population during pre-bloom conditions. Diatoms occur in small numbers and low diversity with members of the genera *Thalassiosira*, *Chaetoceros* and *Cylindrotheca* the most common.

By Leg II (April 1976) of the *Surveyor* cruise phytoplankton had increased in numbers and diversity dramatically from Leg I. (see Table XII). There was an order of magnitude difference in cells/liter reported. Diatoms predominated with *Nitzschia* (*Fragilariopsis*), *Thalassiosira*, and *Chaetoceros* occurring in largest numbers.

Modeling

Both the process of modeling and results from models contribute to research in the marine environment. At the conceptual level, a model provides structure for study planning and data collection. It also provides a framework for organizing literature information. When field data is available, a model is a powerful analysis tool, relating a variety of data points in a biologically meaningful way.

A conceptual model is a description of the structure and internal dynamics of a particular ecosystem, including food chains and nutrient pathways. We have developed a conceptual model for the Bering Sea Shelf with carbon, nitrogen, and silicon units. Pelagic, benthic, and sea ice communities are incorporated. Compartments were selected to emphasize

major material flows, important biological control mechanisms, and commercially fished species. Arranging compartments in a matrix format shows exchanges of N, C, and Si between compartments. For each compartment, inputs are elements in its row of the matrix, and losses are elements in its column. Empty cells indicate potential flow pathways which are known or assumed not to occur.

Constructing a conceptual model as part of developing a study plan has two advantages. First, it insures that the data needed for the model are recognized so that they will be collected. Second, it requires that the model be designed to use information which is measurable. Development of the conceptual model interfaces the expertise of all investigators.

After listing the data needed to model the whole Bering Sea shelf ecosystem as described in the conceptual model, it became apparent that available information was insufficient, and that collection of all the required data was an impossible task within the limits of the present study. Therefore it was decided to concentrate modeling efforts on the sea ice and plankton communities, which can be done well. A revised conceptual model increasing resolution on the plankton by dividing phytoplankton, zooplankton, and micronekton into species groups will assist in planning future field experiments.

Both the conceptual and mathematical levels of a model are statements of a complex hypothesis about ecosystem dynamics. Assumptions which go into a model are made explicit, so that they can be recognized and evaluated objectively. Assumptions will place known limitations on interpretation of final model results. We expect some conceptual difficulties with modeling the ice edge as it traverses various water masses. However,

through use of a model our hypotheses can be tested by comparing field data with model predictions.

In the mathematical model for the ice edge ecosystem, the terms which express flows between compartments will express assumptions on the nature of and the factors controlling material flows. Assumptions, hypotheses, and knowledge of the biology of the system are incorporated into the mathematics.

Computer simulation and sensitivity analysis of the system have not yet been done. Results, when available, will aid in evaluating the effects of perturbations related to OCS development on the marine ecosystem.

Plant Submodel

Field data on phytoplankton in the Bering Sea shelf region show that primary production is greatest near the ice edge in the spring. Production rates averaged $6.6 \text{ gC/m}^2/\text{day}$ at ice edge stations in early May, and $4 \text{ gC/m}^2/\text{day}$ in open waters later in the month. In contrast, production rates averaged only $1.5 \text{ gC/m}^2/\text{day}$ in June, $0.76 \text{ gC/m}^2/\text{day}$ in August, and $0.06 \text{ gC/m}^2/\text{day}$ in November. (These values may deviate from values in Table VIII because of different assumptions but are in fact quite comparable).

Nutrient concentrations in the water column were severely depleted during the spring bloom, particularly at ice edge stations.

The mechanism for simulating production rates in the plant submodel is calculation of a maximum potential photosynthetic rate as a function of available light and chlorophyll concentration, which is decreased as a function of nutrient concentration. Threshold minimum nutrient concentrations cut off production at low nutrient levels. Light and ice movement

are the driving variables in the model. Light available to sea ice algae is modified by snow cover and ice thickness, and light available to phytoplankton is further reduced by the density of the ice algae. The model time step will be 10 days for most of the year, but shortened to a daily time step during the spring bloom to increase resolution.

Input to the plant compartments is photosynthesis. Losses are respiration, sinking, and grazing. Grazing, or the transfer of carbon to zooplankton trophic levels, will be emphasized in plankton model formulation and field studies.

In the plankton model, phytoplankton and zooplankton will be divided into species groups, including those associated with the sea ice. The model will incorporate factors determining primary production and grazing rates.

Grazing Experiments

Of the first five grazing experiments (stations 1, 4, 6, 8 and 13) only one (station 13) resulted in positive filtering rates. An experiment was conducted which consisted of a check of the amount of particulate matter carried over with the zooplankters during transfer into the experimental containers and/or excretion products of the zooplankters. Nineteen *C. marshallae* were transferred directly into 1 l of glass fiber filtered sea water. Counts were obtained after 1 and 5 hrs. After 1 hr there existed 0.21 mg/l total particulate matter with a peak at 58 μm . After 5 hrs the peak had disappeared and the total amount of particulate matter had been reduced to 0.11 mg/l. The amount of particulate matter between 4 and 9 μm had increased. This indicated that large particulate matter was carried

over with the zooplankters and ingested and that fecal matter probably contributed to an increase in small particulate matter.

Grazing results are shown in Table XIV. The mean filtering rate ranged from 5.9 to 7.3 ml/hr/mg dry wt. The filtering rate at station 15 is somewhat higher than the mean for station 13 - 7.3 compared to 6.5. This may be due to the higher concentration of phytoplankton at station 13. The size ranges filtered corresponded to the upper and lower size limits of the blooms.

Figure 5 shows the results for station 13 after 26 hrs of incubation. The phytoplankton size at peak concentration did not change under grazing pressure.

At the conclusion of the grazing experiment at station 15, a 24-hr primary productivity experiment yielded 11.51×10^3 CPM/mg of particles for the control and 8.65×10^3 CPM/mg of particles for the water previously grazed by 6 copepods.

The copepods did not appear to feed on suspended ice algae.

Copper Toxicity

The results of the copper toxicity experiments are shown in Table XV and Figures 6 and 7. A concentration of 2 $\mu\text{g}/\ell$ had no observable effect on large phytoplankton (10-80 μm) in ice-free and ice-flow areas. Above 2 $\mu\text{g}/\ell$ growth rate was inhibited. No differences were noted in the tolerances of algae from ice-free and ice-flow areas since the curves in Figure 6 parallel each other.

Mean carbon uptake rates per unit biomass for total phytoplankton from ice-flow areas do not appear to be influenced by copper concentrations. This suggests that enzyme and/or transport systems other than the

TABLE XIV

MEAN FILTERING RATE, SIZE AND SIZE RANGE FILTERED AT FIVE STATIONS
IN THE BERING SEA FOR *CALANUS MARSHALLAE*

Station	Number of copepods	Mean length (μm)	Mean dry wgt. (mg/copepod)	Size range filtered (μm)	<u>mls/hr·Individual⁻¹</u>		<u>mls/hr·mg dry wgt.⁻¹</u>	
					Uncorrected	Corrected	Uncorrected	Corrected
13	6	-	0.56	18-90	2.0	3.9 ⁽¹⁾	3.6	7.0 ⁽¹⁾
13	20	4.4	0.59	18-90	2.9	3.5 ⁽¹⁾	4.9	5.9 ⁽¹⁾
13B	20	4.4	0.56	-	0.24	-	0.43	-
277 15	6	4.5	0.60	23-90	7.3	4.4 ⁽²⁾	12.2	7.3 ⁽²⁾

(1) Corrected for particle growth rate of $.012 \text{ hr}^{-1}$

(2) Corrected for particle mortality rate of $.018 \text{ hr}^{-1}$

n.b. Stations 13 and 13B used unwashed copepods

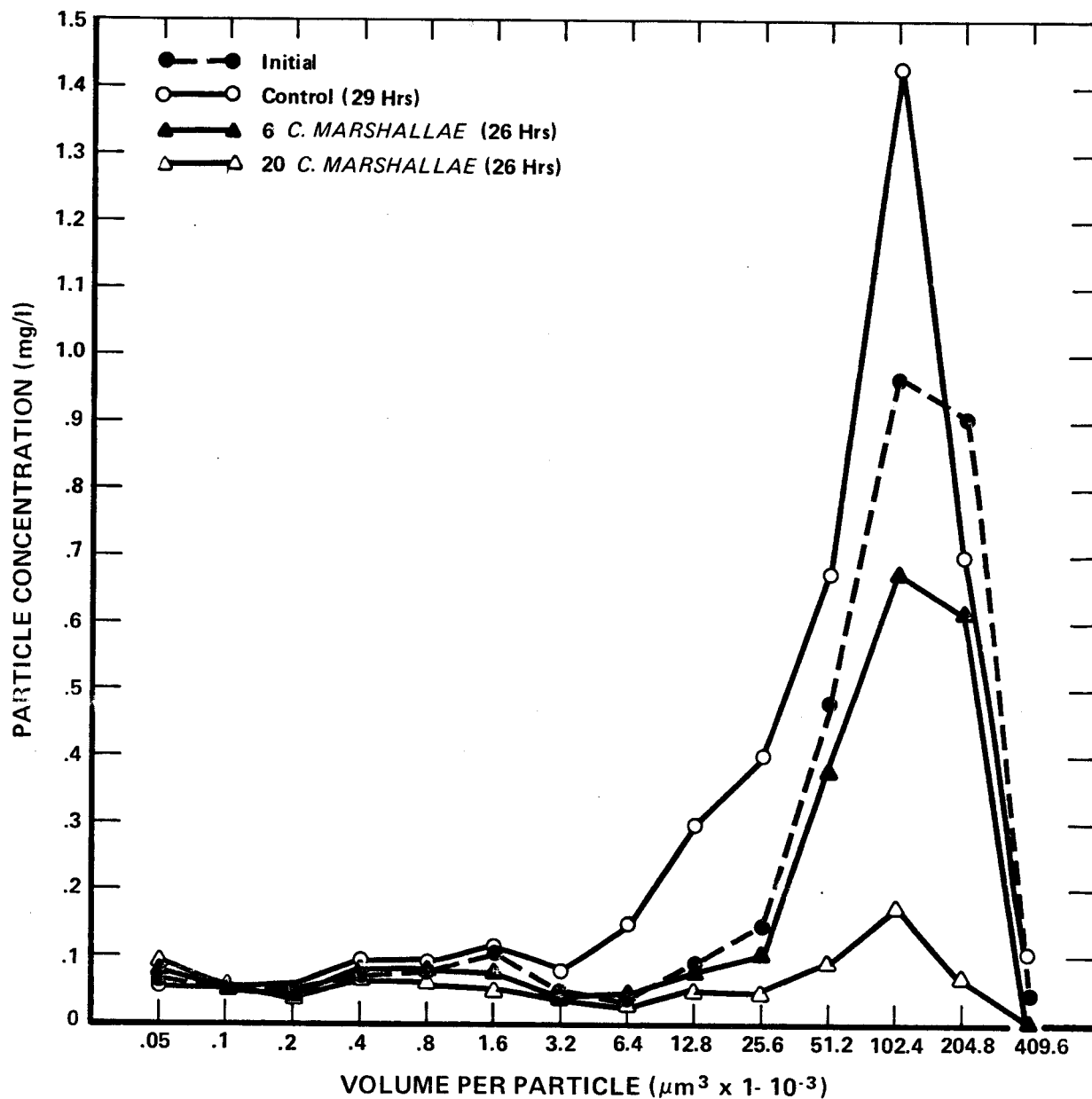


Figure 5. Particle concentration vs. particle size for station 13 grazing experiment.

TABLE XV
 PHYTOPLANKTON GROWTH RATES (hr^{-1}) AT FOUR COPPER CONCENTRATIONS
 FOR SIX STATIONS IN THE BERING SEA ⁽¹⁾

Station	Copper concentration ($\mu\text{g}/\ell$)				Length of Expt (hrs)
	0	2	4	8	
1	.0069	-.0021	.0037	-.0025	105
2	.0018	.0108	.0037	.0026	151
4	.0071	.0078	.0061	.0013	126.5
6	.0057	.0059	.0058	.0055	130.5
8	.0052	.0048	.0060	.0059	132
13	.0086	.0089	.0082	.0073	105.5
Mean 1+2 (ice free)	.0044	.0044	.0037	.0001	-
Mean 4, 6, (ice flow)	.0067	.0069	.0065	.0050	-
Mean all stations	.0059	.0060	.0056	.0034	-

(1) Phytoplankton were 10-80 μm in diameter.

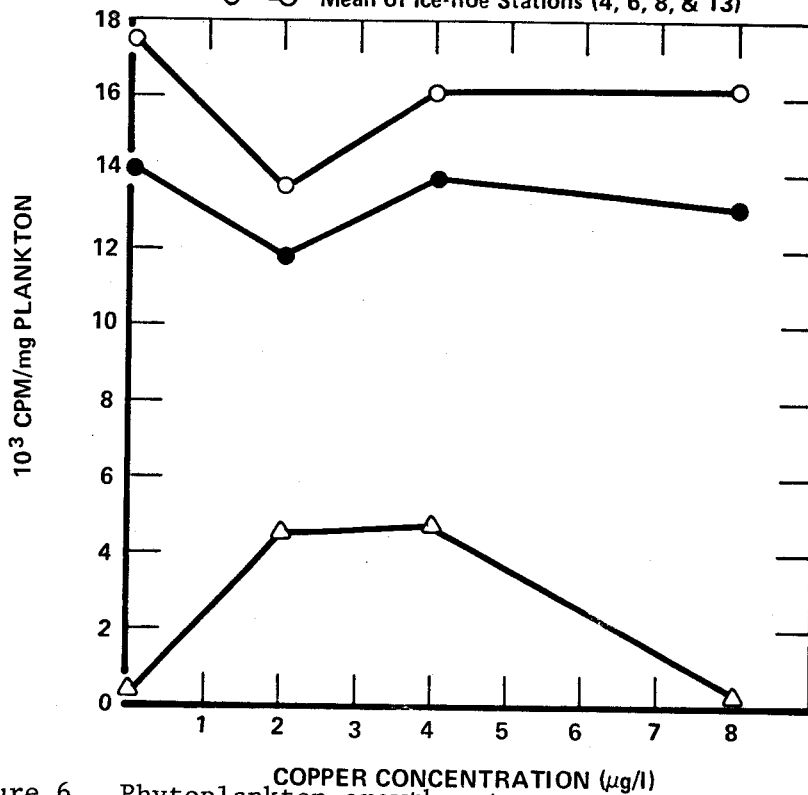
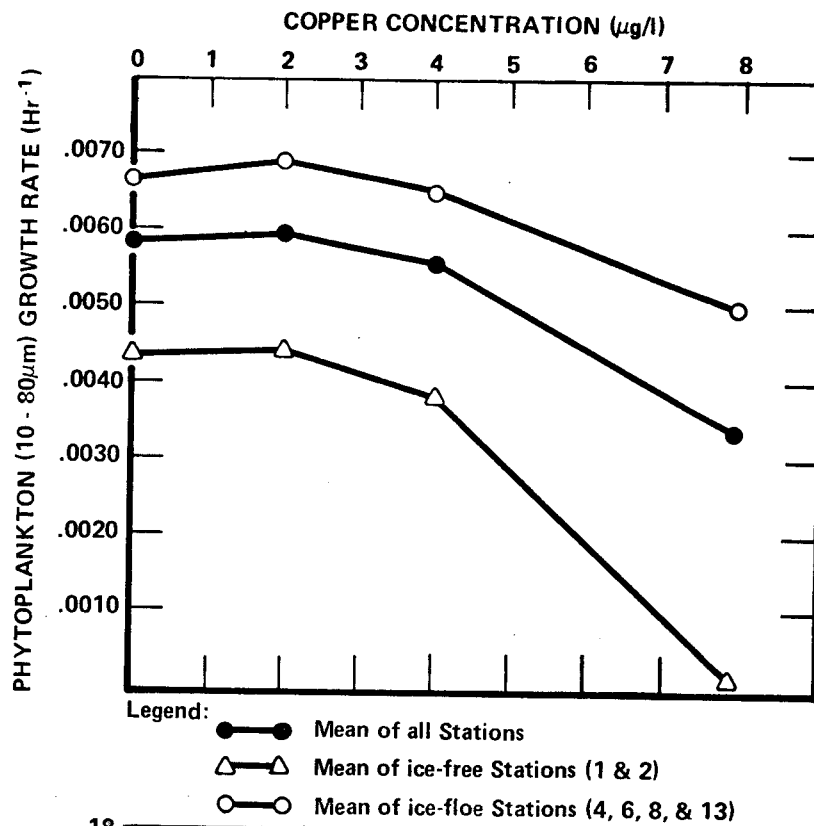


Figure 6. Phytoplankton growth rate vs. copper concentration for phytoplankton 10-80 µ diameter.

Figure 7. Twenty-four hour primary productivity for total phytoplankton at four concentrations of copper. The planktons were first subjected to copper for periods ranging from 105-151 hours.

photosynthetic system are influenced by copper resulting in a decrease in growth rate. Carbon uptake by phytoplankton from an ice-free station was stimulated at low copper concentrations.

A typical set of experimental data is shown in Figure 8. Particle size at peak concentration does not decrease with increasing copper concentration.

Oil Toxicity

Results of the oil toxicity experiments are shown in Table XVI and Figures 9 and 10. Concentrations of 10 and 30 ppm oil inhibited the growth rates of large phytoplankton (10-80 μm) from ice-flow areas. In contrast, the mean growth rate for phytoplankton in ice-free areas increased at 10 ppm but was inhibited at 30 ppm.

A typical set of experimental data is shown in Figure 11. Particle size at peak concentration decreased with increasing oil concentration.

Figure 9 also shows the growth rate of suspended ice algae at 0, 10 and 30 ppm of Prudhoe crude oil. Growth rate decreased with increasing oil concentration.

The results of grazing experiments carried out in 1977 on the *Discoverer* are presented separately as Appendix I.

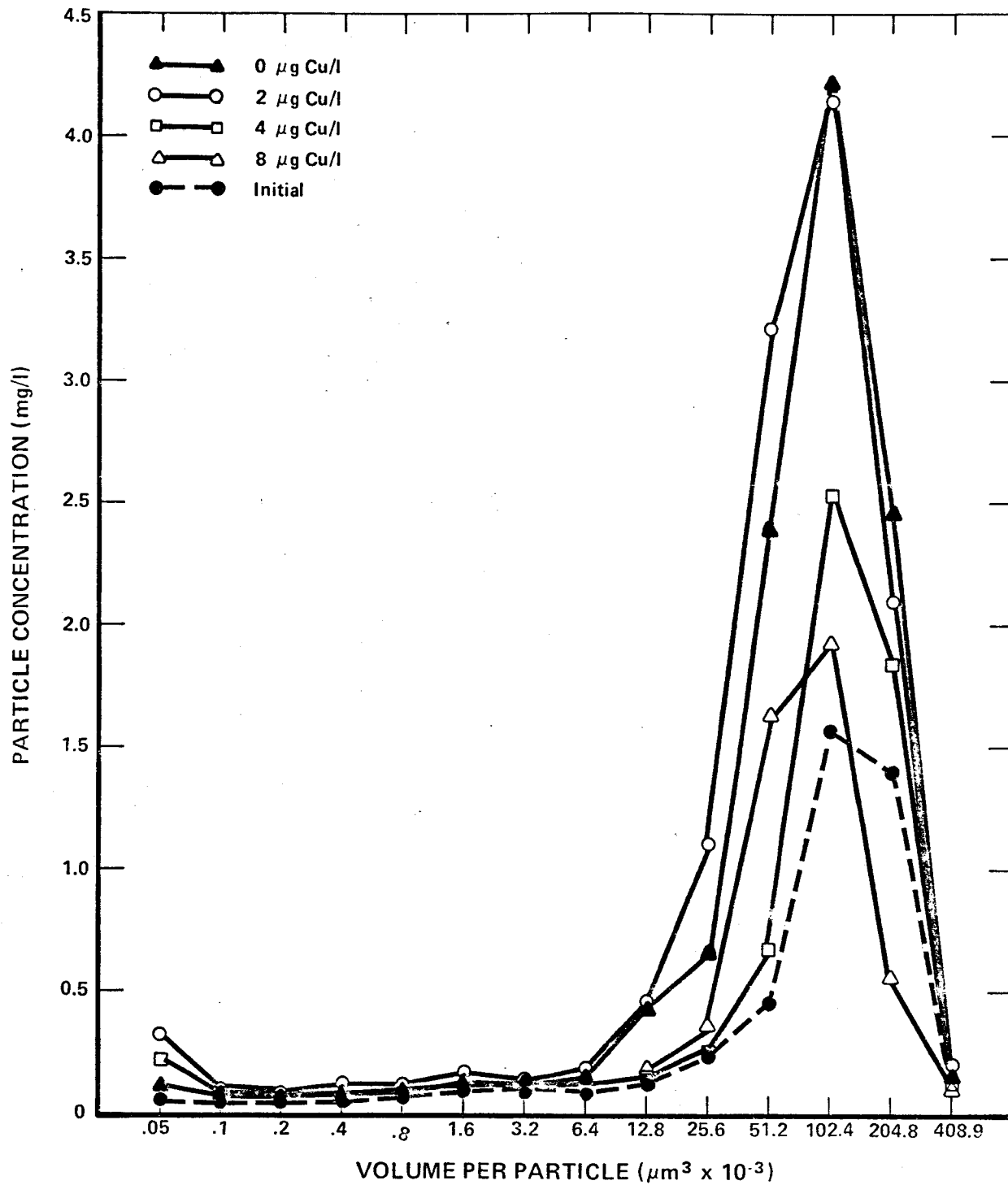


Figure 8. Particle concentration vs. particle size at Station 4 at four copper concentrations after 126.5 hours.

TABLE XVI
 PHYTOPLANKTON GROWTH RATES (hr^{-1}) AT THREE CONCENTRATIONS OF
 CRUDE OIL FOR SIX STATIONS IN THE BERING SEA ⁽¹⁾

Station	Oil concentration (ppm)			Length of Expt (hrs)
	0	10	30	
1	.0069	.0115	-.0019	105
2	.0018	0	.0002	151
4	.0071	-.0045	-.0087	126.5
6	.0057	.0052	-.0046	130.5
8	.0052	.0043	.0004	132
13	.0086	.0004	-.0060	105.5
Mean 1+2 (ice free)	.0044	.0058	-.0009	-
Mean 4, 6, 8, 13 (ice flow)	.0067	.0014	-.0047	-
Mean all stations	.0059	.0028	-.0034	-

(1) Phytoplankton were 10-80 μm in diameter.

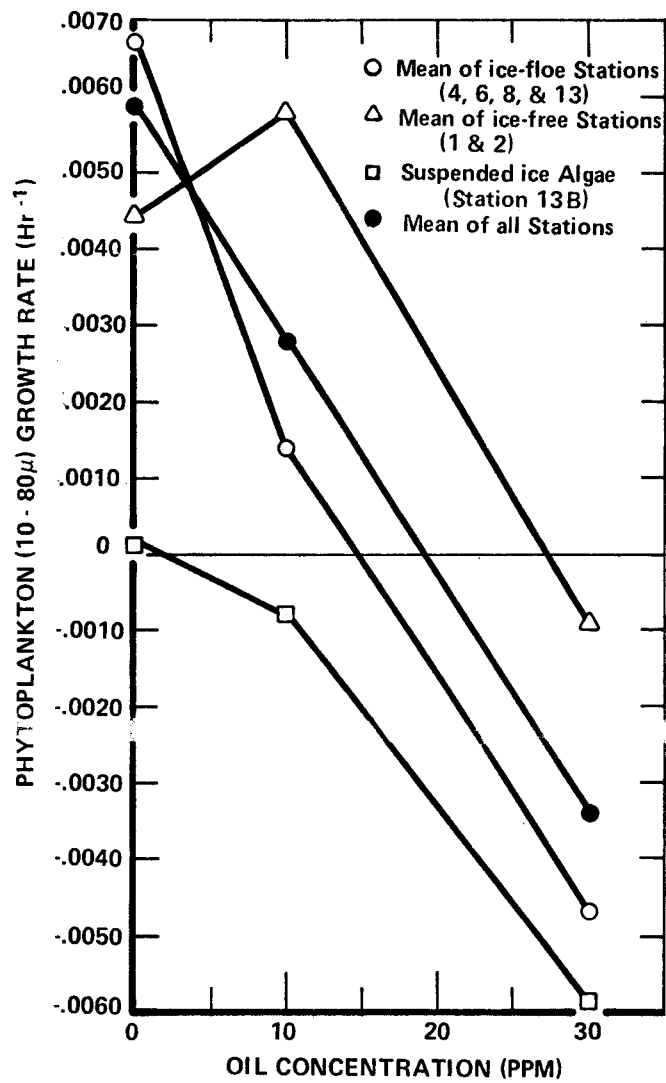


Figure 9. Phytoplankton growth rate vs. oil concentration for plankton 10-80 μ m diameter.

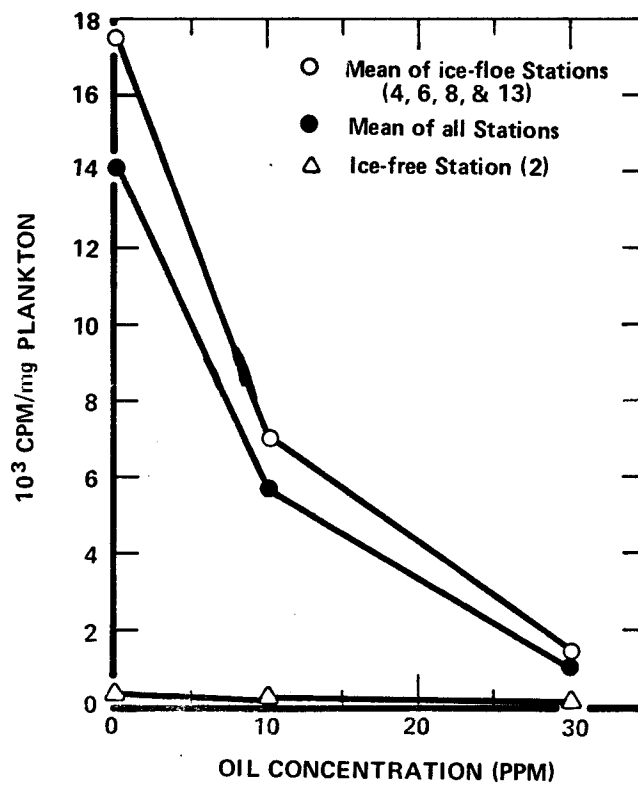


Figure 10. Twenty-four hour primary productivity for total phytoplankton at three concentrations of Prudhoe crude oil. The plankton populations were first subjected to oil for periods ranging from 150-151 hours.

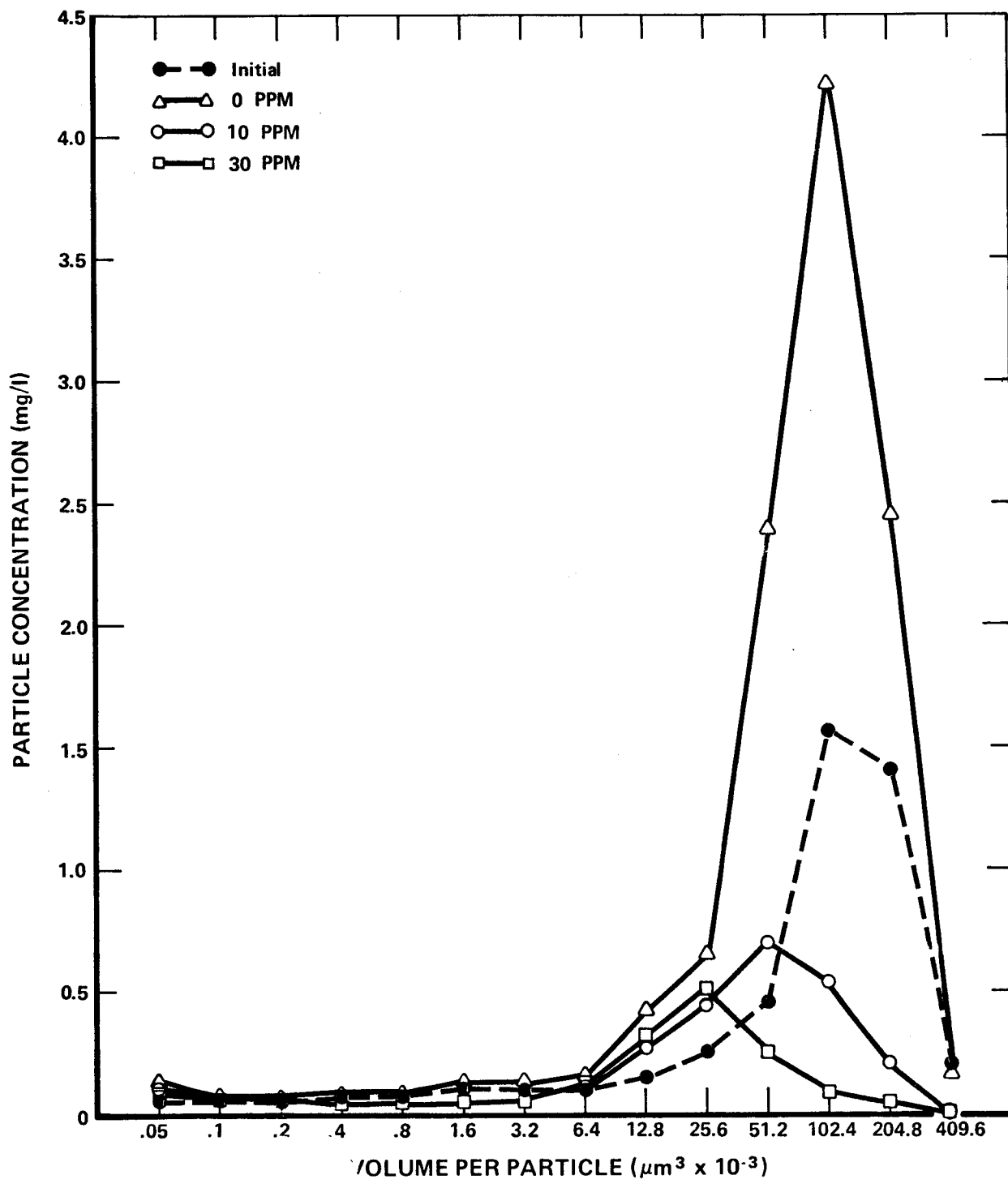


Figure 11. Particle concentration vs. particle size at Station 4 for three concentrations of crude oil after 126.5 hours.

VII. DISCUSSION

The ice edge bloom

The initial sampling of the ice edge zone revealed an extremely high standing crop of phytoplankton and high rates of carbon fixation. Surface chlorophyll values frequently exceeded 20 mg/m^3 and productivity frequently exceeded $25 \text{ mg C/m}^3 \cdot \text{hr}$. These are extremely high values and such rates of production must be limited to short periods of time. Re-sampling of the area where these results were obtained on Leg I of the *Discoverer* cruise showed that surface chlorophyll values had declined to an average of less than 1 mg/m^3 in less than three weeks. Nutrient levels observed during the bloom and again three weeks later were very low with nitrate concentrations - less than $1 \text{ } \mu\text{g-at/l}$ at both times and silicate concentrations dropping from $6 \text{ } \mu\text{g-at/l}$ to $1 \text{ } \mu\text{g-at/l}$ during the period. Obviously, the system is nutrient-limited soon after the initiation of the bloom. Low concentrations of ammonia were observed indicating that *in situ* regeneration of available nitrogen is not occurring significantly or at least that utilization equals regeneration. Diatoms comprise the greatest percentage of the phytoplankton population at the ice edge during a bloom. These diatoms at the ice front are considerably smaller than those found in the warmer open water due probably to the rapid rate of reproduction and perhaps to adaptations to the extreme cold water. This small organism size would greatly accelerate the sinking rate of the cells as the water warmed. Another perhaps major phenomena to carry cells downward out of the euphotic zone, thus reducing the possibility of any nutrient regeneration, will be discussed later. Apparently, the ice edge bloom begins when sufficient

light becomes available and progresses rapidly until nutrients are depleted.

The initial supply of available nutrients over the shelf when the ice begins to recede would appear to determine the gross production at the ice front. Since the initial cruise occurred well into the bloom we did not obtain any pre-bloom nutrient concentrations until the following spring when the *Surveyor* Leg I cruise arrived at the ice edge prior to the initiation of the bloom. At that time nutrient concentrations were observed to be very uniform throughout the water column except near the bottom and also uniform over the entire shelf area. At that time the water column over the shelf had nitrate concentrations of approximately 17.5 $\mu\text{g-at}/\ell$ in contrast to the waters beyond the shelf break where nutrient concentrations were considerably higher. Since we observed near total depletion of nutrients following the bloom we can assume that potential production within the euphotic zone is a function of the initial nutrient supply, i.e., 17.5 $\mu\text{g-at NO}_3$ and 34 $\mu\text{g at SiO}_3$ assuming that there is no immediate *in situ* regeneration.

Rapidly dividing algal cells - like diatoms during a spring bloom situation - have relatively low C:N ratios, 6.5:1, indicating high protein content (Russel-Hunter). Since the bloom conditions we observed showed extremely rapid growth rates we can assume that high protein cells are being produced - a situation highly favorable to the higher trophic levels and perhaps a factor in the overall high production of the Bering Sea.

The initial supply of 17.5 $\mu\text{g-at}/\ell$ $\text{NO}_3\text{-N}$ would then permit a potential fixation of approximately 1.6 g of C/ m^3 . With an average observed rate of C fixation of 25 $\text{mg}/\text{m}^3/\text{hr}$ and assuming a productive day length of 12 hr

we arrive at the conclusion that under ideal conditions the ice edge bloom might be complete within 5-6 days. For a variety of reasons the bloom time is probably a little longer than this estimate, but it can be of extremely short duration. We know from our own work and from Goering and McRoy (1972) that very little production occurs under the solid ice pack, one reason alone being that less than 1% of the sub-surface light penetrates the ice cover. Yet we have also observed nearly complete nitrogen depletion very shortly after the ice pack has broken up sufficiently to allow light penetration but still represents 30-50% coverage indicating that the bloom does occur rapidly with great intensity.

The Ice

The normal limit of ice cover in the Bering Sea is the shelf break with the shelf area itself being ice covered by seasonal ice. Although significant cell concentrations are found within the ice they are restricted to a relatively narrow band and while these epontic algal growths may act to seed the water column they are probably not major contributors to the annual production when compared to the potential of the water column.

Microscopic observation of ice core samples shows the cells to exist in distinct channels within the ice structure. Melting of the ice causes the cells to be flushed from the channels. During the late stages of ice disintegration cells are not found within the ice.

These populations are difficult to study *in vivo* because of the susceptibility to lysis when the ice melts. Maintaining incubation

temperatures such that the ice does not melt and alter the salinity is beyond the scope of a survey approach. Attempts to incubate samples *in situ* were unsuccessful. Therefore, we have no reliable estimates of the actual production rates within the ice. Recently we have observed that less than 1% of the subsurface light is available beneath the ice, and suspect the production is low. When the ice and snow cover becomes sufficiently thin to allow more light to penetrate, the cells have already been flushed from the ice during the melt.

Yet, the ice edge does exhibit abnormally high primary production under certain circumstances. We believe that the major effect of the receding ice edge on the productivity regime results from a stabilization of the water column by dampening the effects of wind mixing.

The Bering Sea is an area of frequent storms and the sea state is usually active. Considering the low angle of the sun it is probable that wind mixing effects do inhibit production by transporting the cells out of the euphotic zone. As already mentioned total ice cover severely reduces the light available to the water column. With the advent of milder temperatures, so that refreezing does not occur, the ice edge gradually breaks up into smaller flows from sea and swell action. But these ice flows do not melt rapidly and a system of ice flows with openings develops and characterizes the receding ice front throughout its retreat. It is this physical system which so enhances productivity. The partial ice cover effectively prevents wind mixing while the open spaces between the ice allow sufficient light penetration to initiate a bloom. It is this stabilized system that promotes the development of the strongly stratified bloom in the surface waters of the ice front. The surface bloom

is at times sufficiently intense in the upper waters to severely light limit the deep waters.

The extent and nature of the ice cover may strongly influence the nature of the productivity regime. The ice edge system is too complex and variable for us to define all of its aspects from the two seasons we observed it. The most recent cruises, spring 1977, provided the opportunity to observe the shelf situation when less than normal ice coverage existed due to the mild winter. Those areas over the shelf which had not been ice covered and therefore exposed to the available light but also to wind mixing showed a less intense bloom of a more "normal" distribution throughout a greater depth of the water column. Thus, it appears that an ice edge effect is to induce a very intense stratified bloom which causes rapid nutrient depletion of the water, while the non-ice covered areas demonstrate a slower rate of production perhaps over a longer period of time. Not enough data is yet available to estimate which of the two regimes is ultimately the most productive on an annual basis. If the protein content of these rapidly growing ice edge diatoms is significantly greater than in a slow growth regime as Russel-Hunter (1970) suggests, then we may assume that the energy transfer to higher trophic levels is more efficient for the ice edge algae than the open water system.

VIII. CONCLUSIONS

Our studies of primary productivity related to the edge of the seasonal ice pack in the Bering Sea enable us to draw the following tentative conclusions:

1. The major effects of the ice field appear to be in limiting light energy to the water column and reducing windmixing at the surface. This means that water column plant production is probably negligible until the pack begins to break up. While loose ice is present at the sea surface (in the retreating edge zone) it tends to stabilize the wind mixed layer and hence, greatly enhances the opportunity for rapid plant production. With reduced mixing, ample light, and nutrients, an exceedingly intense bloom of short duration often occurs. This band of production follows the ice northward in the late spring.
2. The very cold ice-related water tends to sink away from the surface as warming progresses. We present evidence that algal populations also sink with the water mass. The ramifications of oil contamination are obvious, particularly since a surface spill could become incorporated and carried to depth with the sinking algal cells to enter benthic food webs on the sea bed.

IX. NEEDS FOR FURTHER STUDY

With respect to our current work, we still have some mop-up work to do on the phytoplankton taxonomy. Certain diatom species cannot be properly identified without clearing them (oxidizing them of protoplasmic contents) because taxonomy is based on the morphology of the frustule and cell contents obscure the frustule markings. We have been using a muffle furnace set at 560°C for 15-20 minutes to clear diatoms for taxonomic study (Zoto *et al.*, 1973). In addition, line drawings are made of unidentified species and photographs have been taken using an American Optical Differential Contrast Microscope.

For further analysis of the phytoplankton data we plan to use cluster analysis techniques to delineate changes in community structure between station, depths and seasons. Changes in community structure may correlate with changes in other parameters such as light intensity, nutrient concentration, etc. In addition, we may look at the diversity of communities and how they change spatially and seasonally. Differences between the ice communities and phytoplankton in open water will be studied. Our preliminary completion runs show promise.

With respect to the *in situ* dynamics of phytoplankton, the fate of the cells from the spring bloom, the transfer to grazers of the ice algae and the ice edge cells, and the role of detritus in the Bering Sea food chain, all remain to be clarified.

X. SUMMARY OF JANUARY - MARCH QUARTER

A. Laboratory Activities

1. Data synthesis from all 1977 cruises has been completed.
2. Report preparation and computer interpretation of station data.
3. Drafting of station plots.
4. Cluster analysis work on phytoplankton population initiated with preliminary successful runs of the program.

Personnel involved: T. Chapman, G. Mimken, L. Schandelmeier, L. Molot, V. Alexander.

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SECTION I - APPENDIX I

REPORT ON ZOOPLANKTON COMMUNITY GRAZING EXPERIMENTS CONDUCTED
ON BOARD THE NOAA SHIP, *DISCOVERER*, IN THE BERING SEA,
MAY-JUNE 1977

Lewis Molot

REPORT ON ZOOPLANKTON COMMUNITY GRAZING EXPERIMENTS CONDUCTED
ON BOARD THE NOAA SHIP, *DISCOVERER*, IN THE BERING SEA,
MAY-JUNE 1977

METHODS

Two types of grazing experiments were performed during a cruise to the Bering Sea outer continental shelf in May and June 1977, one monospecific, using various herbivorous species, and the other using natural assemblages.

Single Species Grazing

Surface sea water was filtered through 211 μm Nitex netting and subdivided into one litre poly bottles. Animals were gathered from a vertical net tow, transferred to a beaker of filtered sea water and left in the dark at 4°C for 12 hours. The animals were then added to the poly bottles in various concentrations. Initial particle counts in the range 4-80 μm were obtained using a Model B Coulter Counter (Coulter Electronics, Inc.) with a 200 μm orifice tube before incubation at 4°C under a dim incandescent bulb. The bottles were removed at regular intervals for particle counting. Initial and final samples of some experiments were preserved with lugol for phytoplankton species identification. Primary productivities (24 hr) were incubated under the same conditions at the conclusion of some experiments. Dry weights were measured on a Cahn Electrobalance after the animals had been rinsed in distilled water for a few seconds, dried in a vacuum oven at 60°C for 18 3/4 hrs, and cooled in a vacuum dessicator for 1 1/2 hours.

Community Grazing

Each set of experiments consisted of three 20-litre cubetainers filled with surface layer sea water. One cubetainer had been filtered through

211 μm Nitex netting and was used as a control, another cubetainer contained unfiltered surface layer water and the third contained a salted community of zooplankton, which had been obtained by towing a 1-m vertical net slowly through the water column, closing it before it entered the euphotic zone, and transferring the animals to the cubetainer. The cubetainers were incubated at 4°C under a dim incandescent bulb. Samples were taken regularly for particle counts. At the end of each experiment, the zooplankton were preserved in 10% formalin for identification. Twenty-four hour primary productivities were run at the conclusion of some experiments.

Filtering rates were calculated using (Coughlan 1969) -

$$F = \frac{\ln (C_o / C_t e^{-Rt})}{t} \quad (1)$$

where F = filtering rate (mls/hr/litre of community) of particles 10-80 μm ,

t = time intervals (hrs)

C_o = initial particle concentration (mg wet wgt/l) of particles 10-80 μm ,

C_t = final particle concentration (mg wet wgt/l) of particles 10-80 μm ,

R = instantaneous growth rate (hr^{-1}) as measured from the growth of particles 10-80 μm in the control.

Ingestion rates were calculated directly from volume counts by,

$$I = (C_o - C_t) / t \quad (2)$$

This assumes that R = 0.

Particle concentrations were converted to mg wet weight by assuming a mean density of 1g/ml.

Results

The data are presented in Tables I-II and in Figures 1-9. The algal blooms at stations 9, 10, 26 and 27 occurred within the broken ice of the receding ice edge. The blooms were primarily diatoms with peak diameters in the window 51-64 μm . The bloom at station 39, which occurred much farther south in the open water near Unimak Pass, was primarily Haptophyta (*Phaeocystis* sp.) with a peak diameter in the window 5-6.3 μm . Grazing rates of particles 4-10 μm at station 39 could not be calculated due to high background counts induced by line voltage surges.

Single Species Grazing

Single species grazing rates are presented in Table II. Maximum rates occurred during the first counting interval, probably because of the prior starvation period in filtered seawater. Calculated filtering rates for the first interval in ml/hr·mg dry weight were 26.2 for *Metridia lucens*, 32.4 for *Pseudocalanus* sp. and 5.2 for *Calanus glacialis*. The latter agrees well with the data obtained for *Calanus marshallae* in the ice edge (range 5.9-7.3) in April 1976.

Primary productivity data are given in Table VIII for *M. lucens*. There is little difference in CPM/mg wet weight between ungrazed and grazed algal populations, suggesting that growth rates are unaffected by grazing during the course of the experiment.

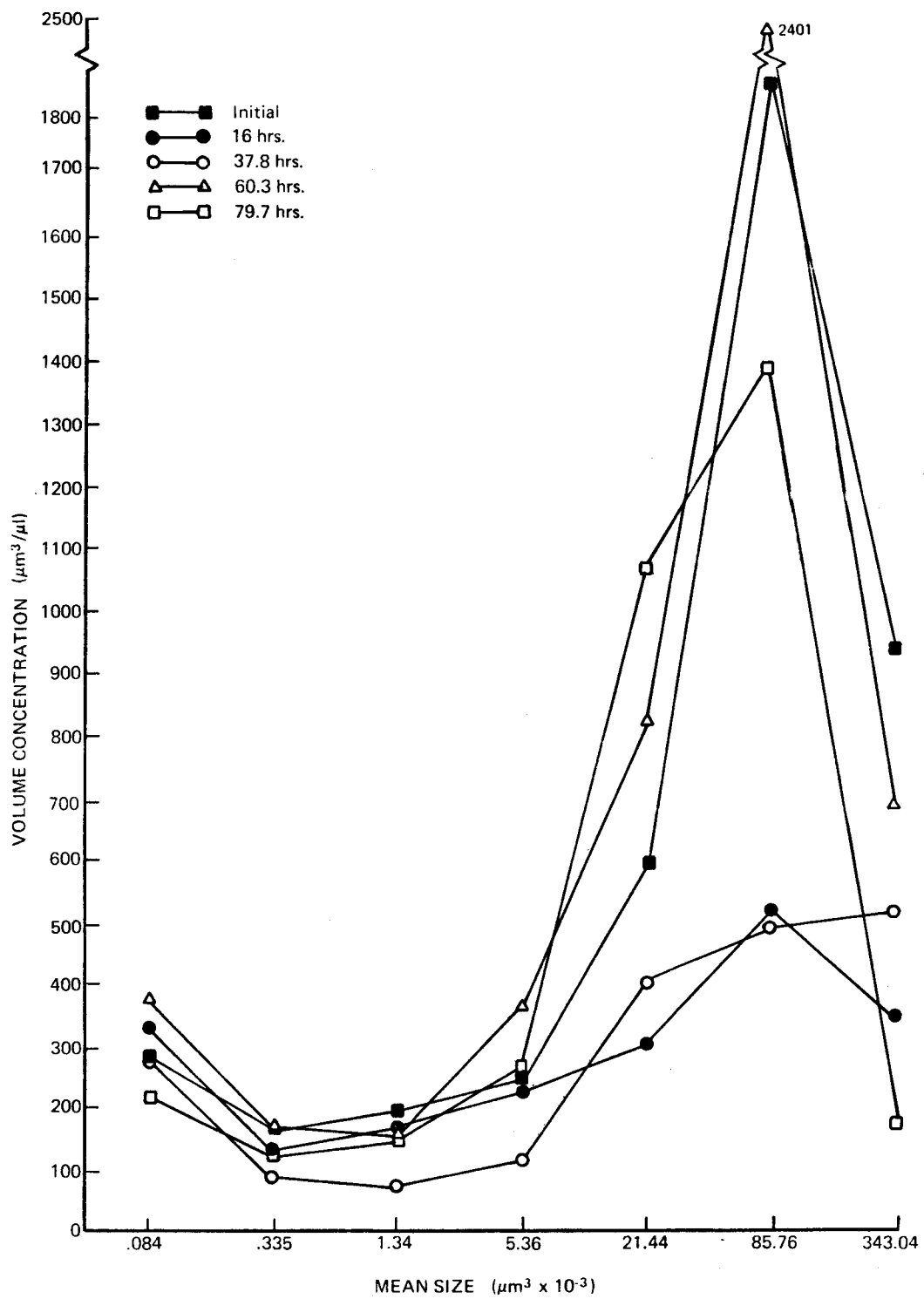
Mean dry weights per animal are given in Table III for the three grazing species used.

TABLE I
STATION LOCATION, ICE CONDITION AND DATE

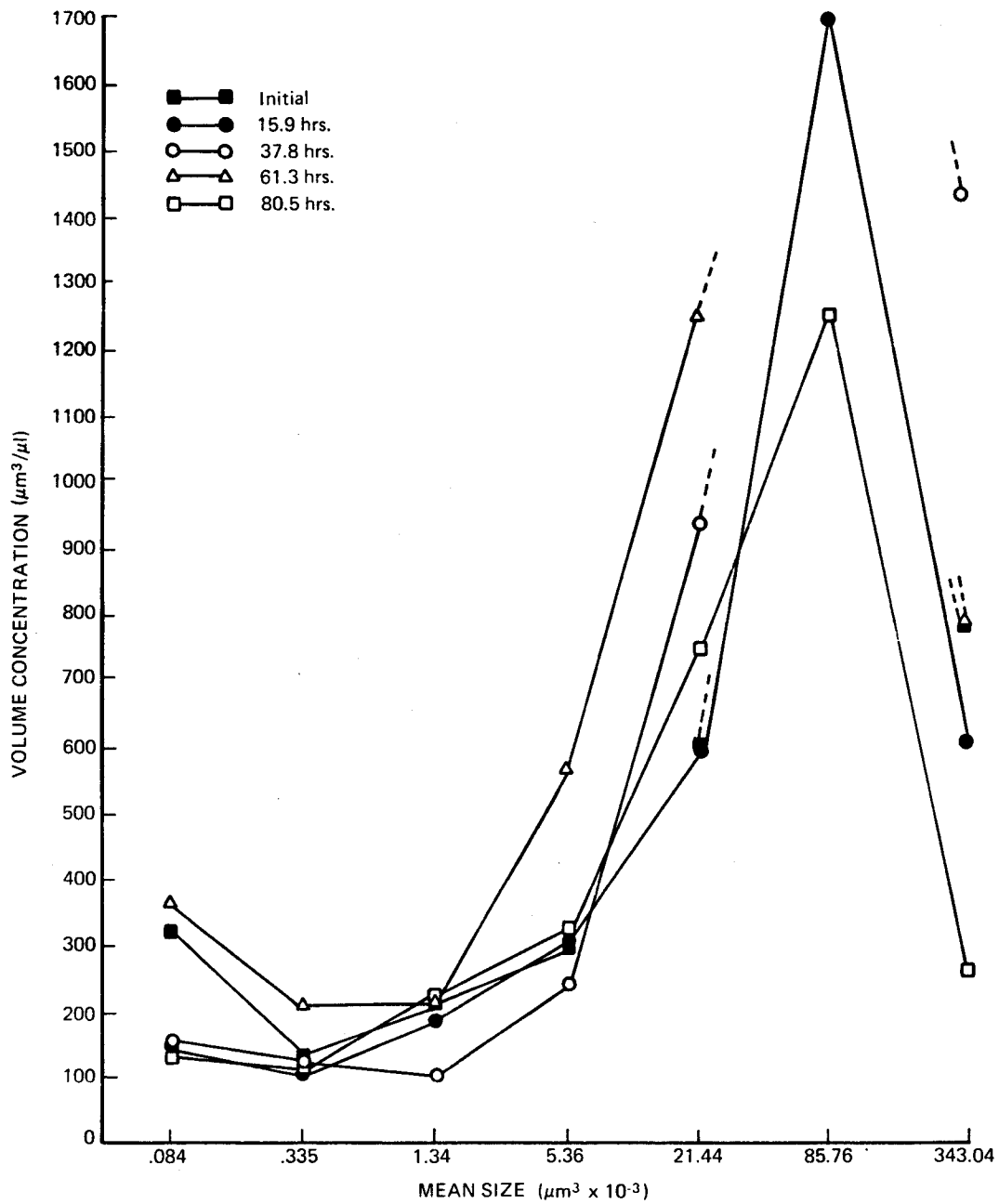
Station	Ice Condition	Latitude	Longitude	Date
9	ice	60°37.0'	174°30.3'	May 26/77
10	ice	60°39.0'	174°33.8'	May 27/77
26	ice	60°57.5'	170°51.8'	June 4/77
27	ice	60°40.5'	169°32.3'	June 6/77
39	ice free	55°08.1'	166°05.1'	June 9/77

TABLE II
 SINGLE SPECIES' FILTERING RATES (ml/hr•mg DRY WGT)

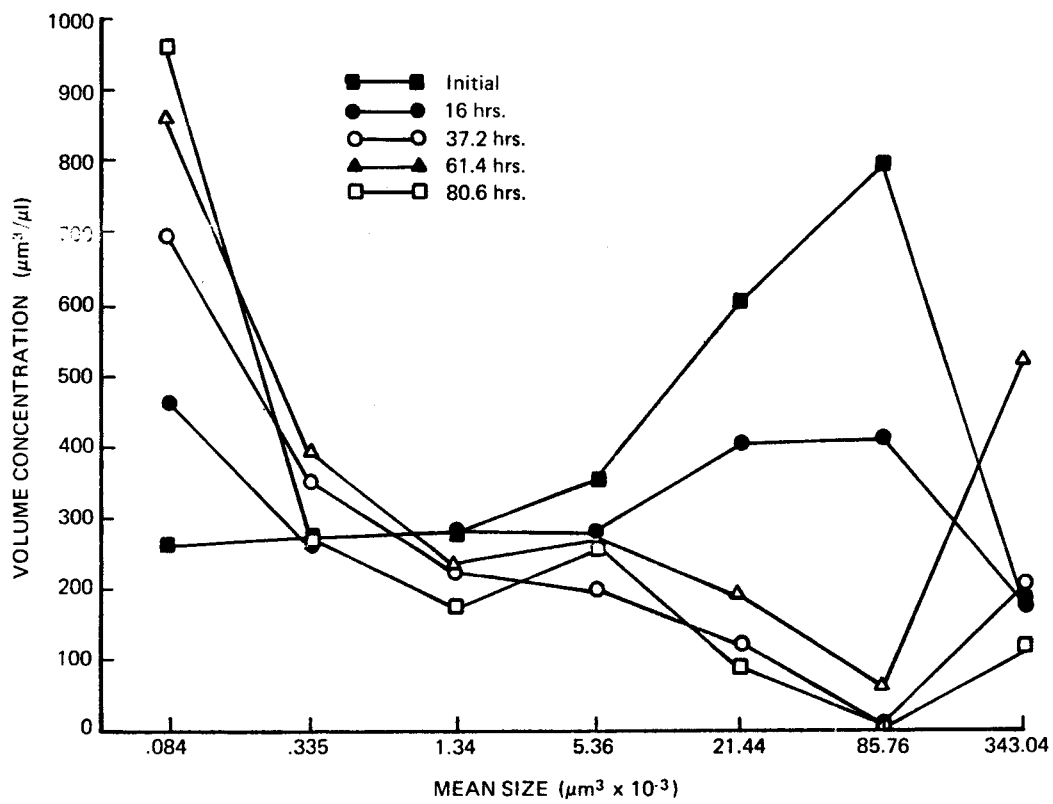
Station	Interval	Length of Interval (hrs)	Number of animals per bottle				
			5	6	10	19	Mean
9 (<i>Metridia lucens</i>)	1	13.6	60.7	17.1	21.0	11.1	27.5
	2	10.1	0	0	0	0	0
	ave	23.7	4.6	0	7.1	5.1	4.2
			4	11	18	Mean	
10 (<i>M. lucens</i>)	1	15.3	27.1	18.8	28.4	24.8	
	2	19.3	0	7.7	0	2.6	
	ave	37.4	4.5	12.5	11.8	9.6	
			11	22	Mean		
26 (<i>Calanus glacialis</i>)	1	24.4	4.8	5.5	5.2		
				55			
27 (<i>Pseudocalanus</i> sp.)	1	23.0	32.4				
	2	25.6	16.7				
	ave	48.6	25.2				



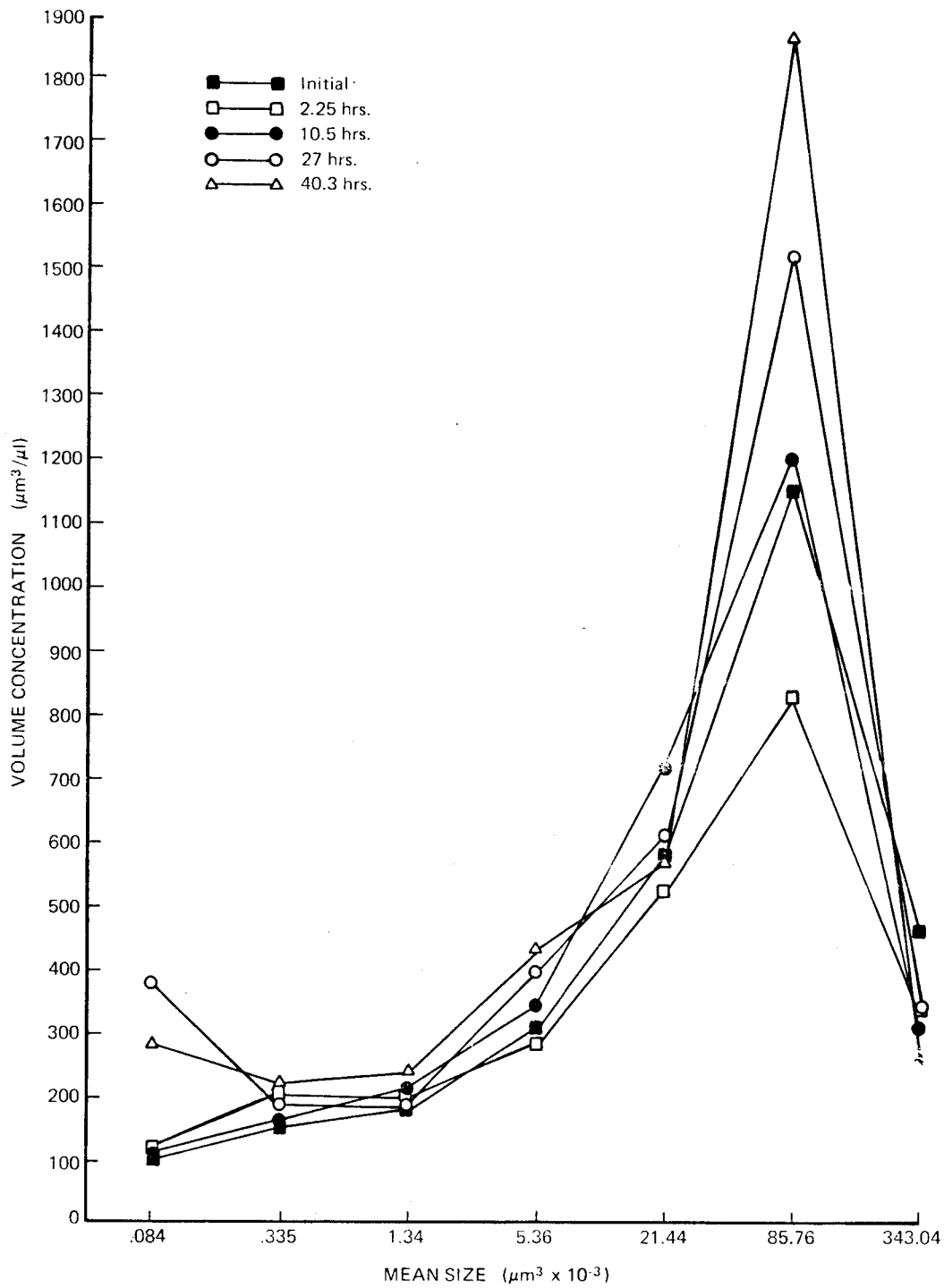
Appendix I - Figure 1. Community grazing Station 9A, control.



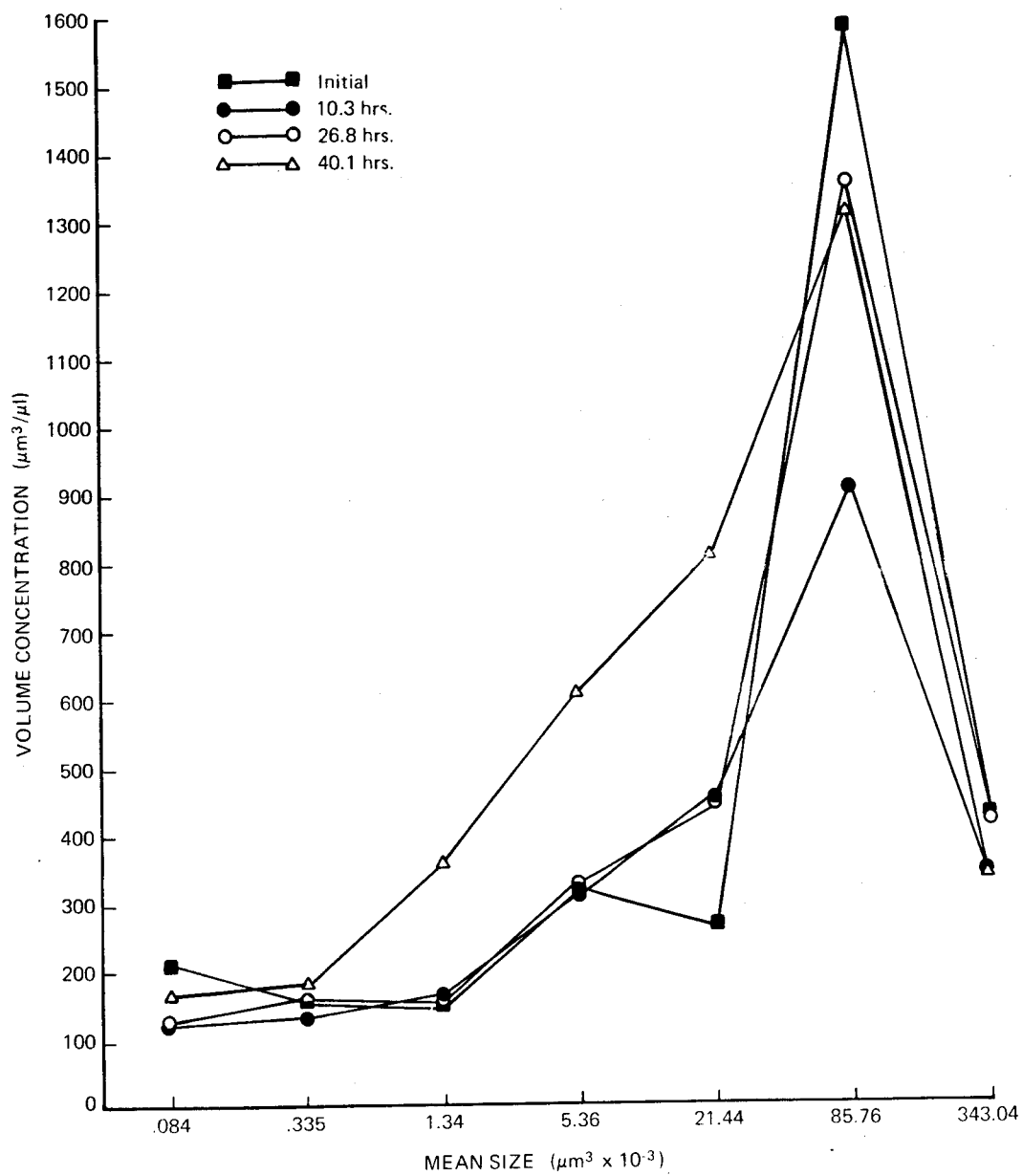
Appendix I - Figure 2. Community grazing experiment Station 9A unsalted.



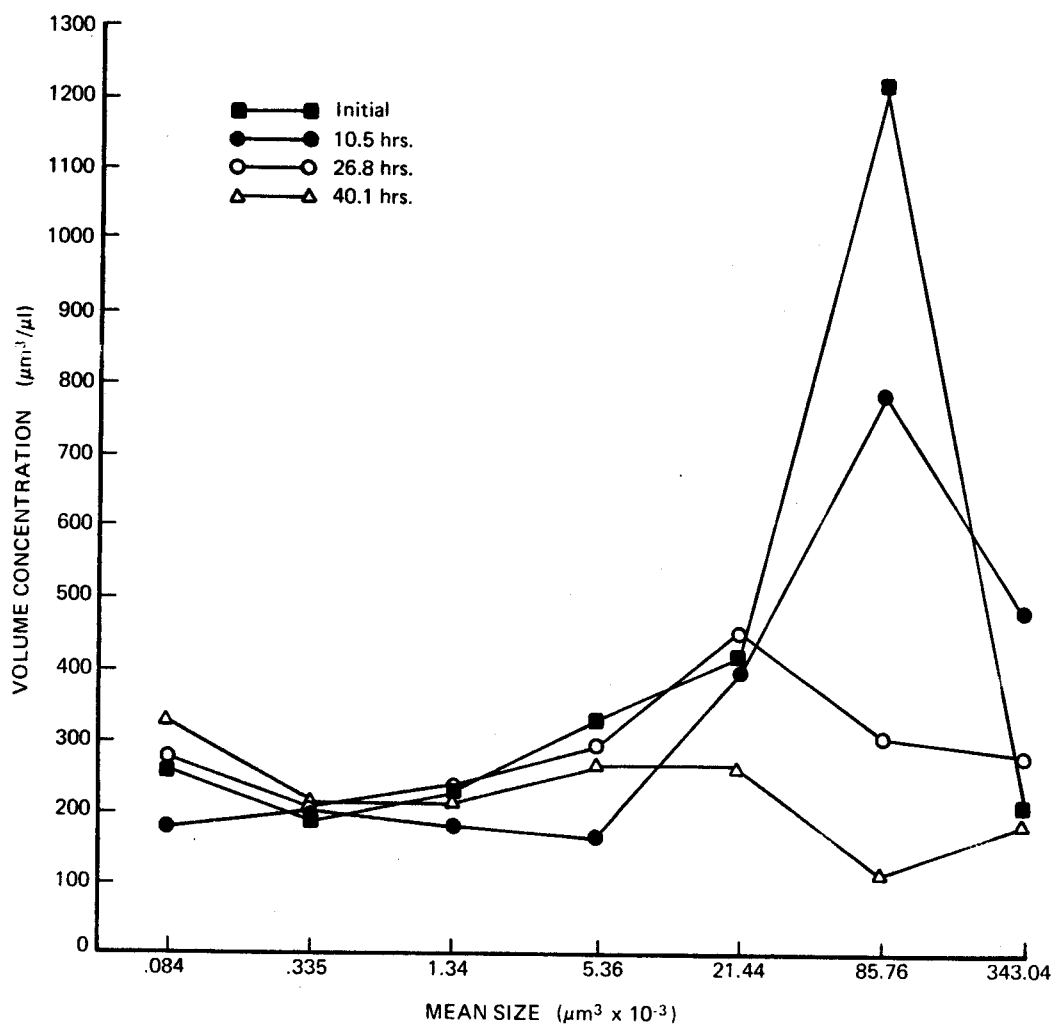
Appendix I - Figure 3. Community grazing Station 9A salted.



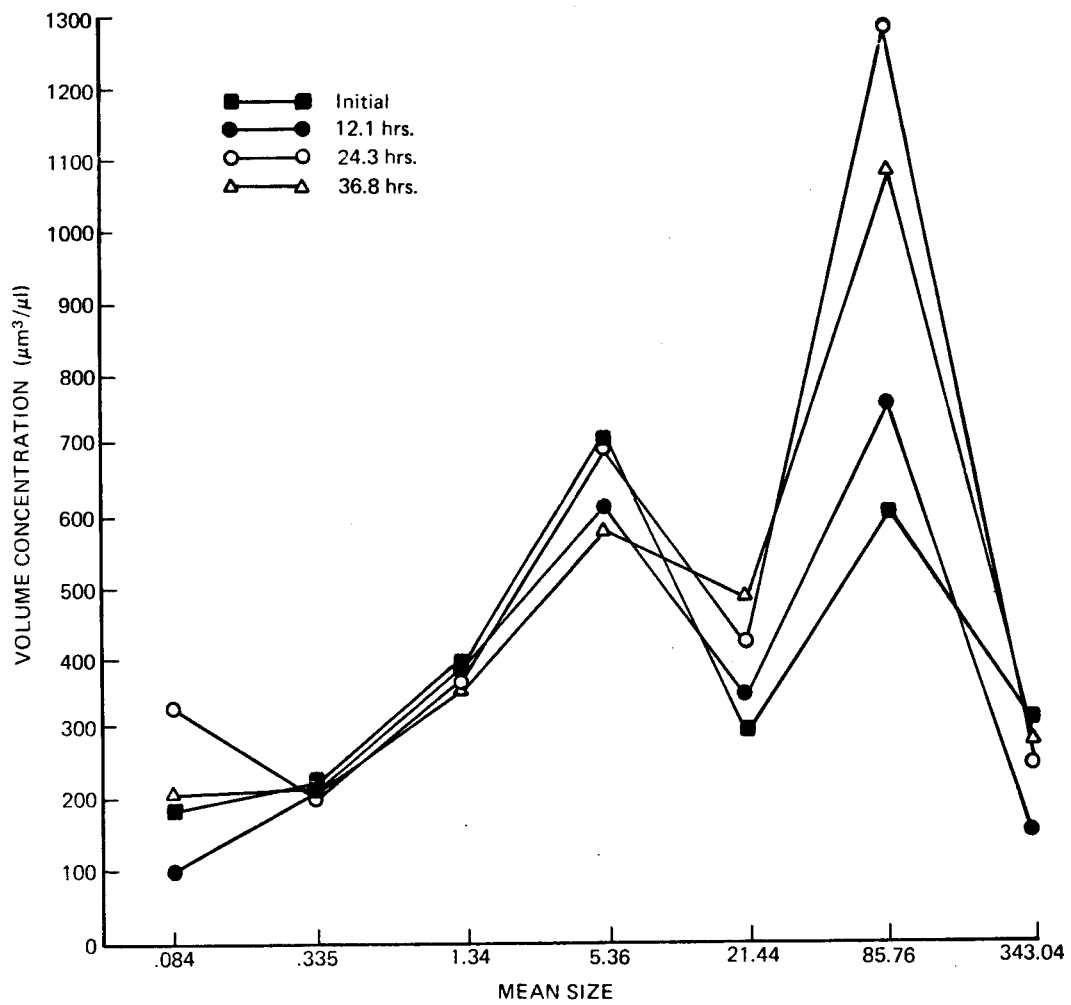
Appendix I - Figure 4. Community grazing Station 26, control.



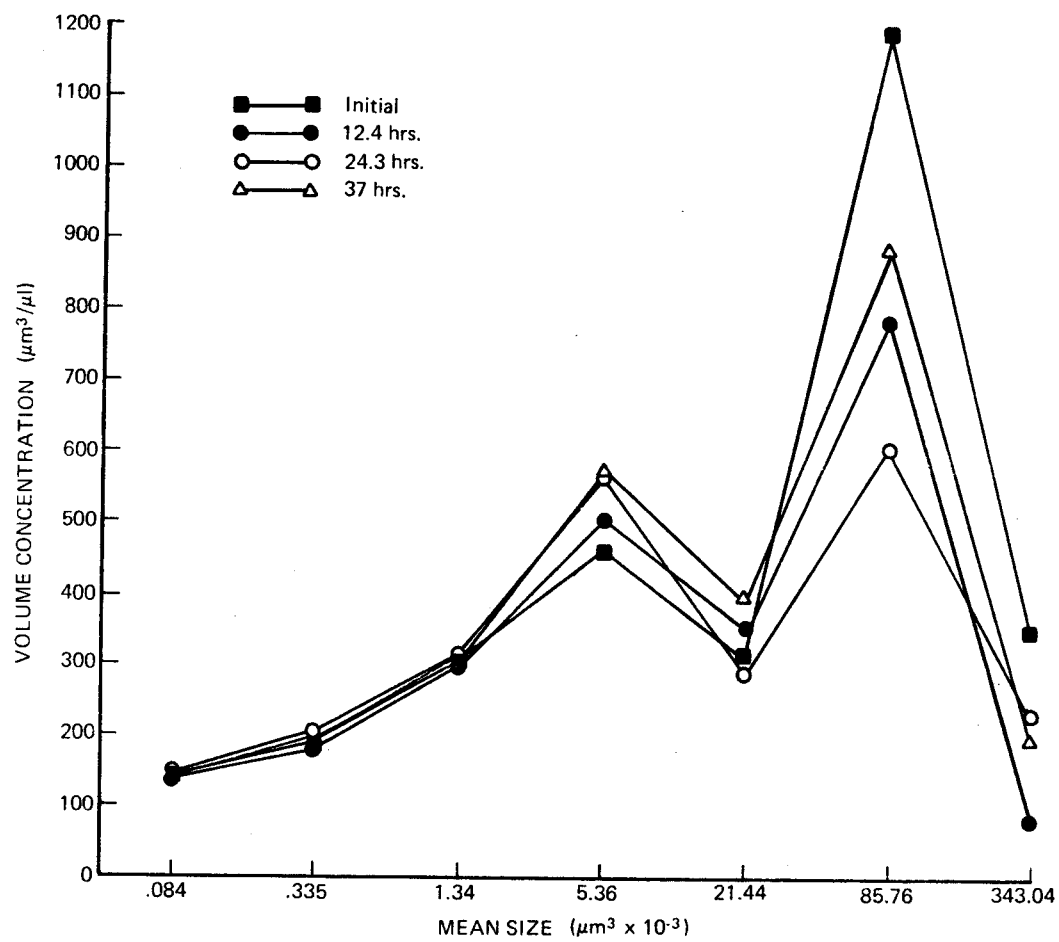
Appendix I - Figure 5. Community grazing Station 26, unsalted.



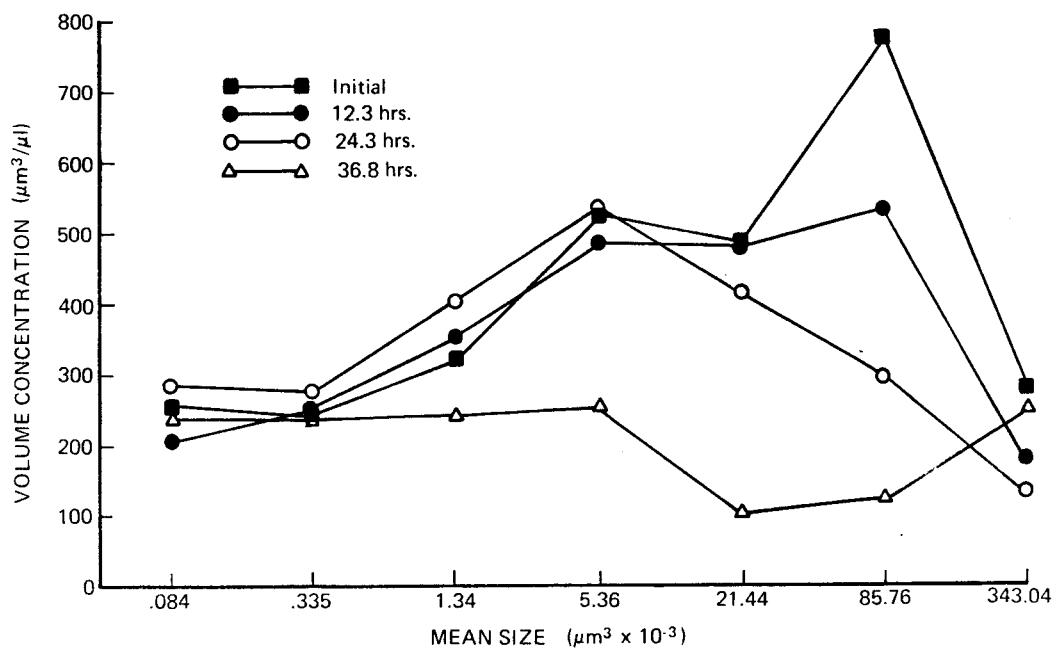
Appendix I - Figure 6. Community grazing Station 26, salted.



Appendix I - Figure 7. Community grazing Station 27, control.



Appendix I - Figure 8. Community grazing size distribution for Station 27, unsalted.



Appendix I - Figure 9. Community grazing Station 27, salted.

TABLE III
SPECIES DRY WEIGHTS (mg/ANIMAL)

Species	Range	Mean
<i>Metridia lucens</i>	.094 - .139	.121
<i>Calanus glacialis</i>	.420 - .436	.428
<i>Pseudocalanus</i> sp.	-	.011

Community Grazing

Sampling depth, time of day and tow volume are presented in Table IV. Sampling was done as close as possible to midnight in order to maximize the number of animals in the unsalted experiment.

Community filtering rates of particles 10-80 μm diameter are given in Table V. After 37-40 hours, the unsalted communities of stations 9A, 26 and 27 showed little or no grazing (0, 0, .008 $\ell/\text{hr}\cdot\ell$ respectively, corrected for growth of algae) while the salted community had filtering rates of .006, .027 and .031 $\ell/\text{hr}\cdot\ell$ respectively. Note that filtering rates are equivalent to hourly turnover rates. Results from station 39 are ignored because line voltage problems prevented counting of the bloom windows.

The species composition of the zooplankton can be used to calculate filtering rates based on single-species experiments. The results are presented in Table VI. One would expect the single-species derived results to be lower than the community-derived rates since the presence of raptors would decrease the number of grazers in the cubetainer and only the final zooplankton composition is available. However, the reverse is true as single-species derived rates are 5-40 times higher than community-derived rates.

Algal size distributions for the experiments are shown in Figures 1-9. The blooms in the salted cubetainers were grazed down to baseline levels within 40 hours. This is verified by primary productivity measurements (Table VIII) which showed no C-14 uptake by the salted community phytoplankton.

Community ingestion rates are shown in Table VII. The salted rates for stations 9A, 26 and 27 after 37-40 hours were 1.96, 1.70 and 2.00 $\mu\text{g C}/\text{hr}\cdot\ell$. Assuming a 10 hour feeding period, this amounts to only 20 $\mu\text{g C}/\text{day}\cdot\ell$.

TABLE IV

SAMPLING DEPTH, TIME, AND TOW DISTANCE OF
COMMUNITY GRAZING EXPERIMENTS

Station	Water Sample Depth (m)	Depth of Main Chlorophyll <i>a</i> Peak (m)	Time of Collection	Vertical Tow Distance (m)	Tow Volume (m ³)
9	12	12	~0500	-	-
9A	10	12	2130	90-48	33.0
26	5	5	~midnight	50-12	29.8
27	12	0	~midnight	40-12	22.0
39	8	8	0600	75-30	35.3

TABLE V
COMMUNITY FILTERING RATES (l/hr·l COMMUNITY)

Station	Interval	Length of interval (hrs)	Filtering Rate*	
			Unsalted	Salted
9	1	14.5	.001(0)	-
9A	1	16.0	0(.014)	0(.022)
	2	21.5	0(0)	.036(.035)
	3	23.9	.039(0)	.024(0)
	4	19.2	.018(.037)	.014(.026)
	ave	80.5	.015(.005)	.001(.015)
	ave (1+2)	37.5	0(0)	.006(.029)
26	1	10.5	.026(.022)	.021(.017)
	2	16.4	0(0)	.019(.017)
	3	13.3	0(0)	.041(.030)
	ave	40.2	0(0)	.027(.021)
27	1	12.4	.020(.021)	.013(.013)
	2	11.9	.025(.006)	.040(.016)
	3	12.6	0(0)	.041(.048)
	ave	37.0	.008(.003)	.031(.026)
39	1	13.8	.005(.010)	0(.002)
	2	12.2	0(.005)	0(.026)
	ave	25.9	0(.007)	0(.013)

* Filtering rates without control growth rate correction are in parentheses.

TABLE VI

ZOOPLANKTON SPECIES COMPOSITION AND COMMUNITY FILTERING RATES
BASED ON SINGLE SPECIES EXPERIMENTS

	Station 9A		Station 26		Station 27	
	No/20ℓ	Filtering Rate (ℓ/hr·ℓ)	No/20ℓ	Filtering Rate (ℓ/hr·ℓ)	No/20ℓ	Filtering Rate (ℓ/hr·ℓ)
<u>Grazers</u>						
<i>Pseudocalanus</i> sp.	6520	0.116	15360	0.273	3440	0.061
<i>Acartia longiremis</i>	80	0.001	280	0.005	120	0.002
<i>Calanus glacialis</i>	160	0.018	240	0.027	920	0.102
<i>Metridia lucens</i>	2080	0.330	-	-	-	-
Total		0.465		0.305		0.165

Major Raptors

<i>Sagitta elegans</i>	2640	3360	1960
<i>Bathymedon</i> sp.	760	320	-
<i>Thysanoessa raschii</i>	-	440	-

Ratio of *Sagitta*/*Pseudocalanus*/*Acartia*/*Calanus*/*Metridia* numbers

9A: 33/81.5/1/2/26
 26: 14/64/1.2/1/0
 27: 16.3/28.7/1/7.7/0

TABLE VII
COMMUNITY INGESTION RATES*

Station	Interval	Unsalted ingestion rate		Salted ingestion rate	
		mg wet wgt/hr·ℓ	μg C-12/hr·ℓ**	mg wet wgt/hr·ℓ	μg C-12/hr·ℓ**
9	1	0	0	-	-
9A	1	.051	2.55	.041	2.05
	2	0	0	.038	1.90
	3	0	0	0	0
	4	.149	7.45	.031	1.55
	ave (80.5 hrs)	.017	0.85	.019	0.95
	ave (1+2)	0	0	.039	1.96
26	1	.053	2.65	.038	1.90
	2	0	0	.029	1.45
	3	0	0	.037	1.85
	ave (40.2 hrs)	0	0	.034	1.70
27	1	.049	2.45	.030	1.50
	2	.001	0.05	.029	1.45
	3	0	0	.060	3.00
	ave (37.0 hrs)	.007	0.35	.040	2.00
39	1	.007	0.35	.003	0.15
	2	.004	0.20	.033	1.65
	ave (25.9 hrs)	.006	0.30	.017	0.85

* $I = (C_o - C_t)/t$, assuming no growth of algae

** Assuming 5% of wet weight is carbon

TABLE VIII
FINAL C-14 UPTAKE (CPM/mg WET WGT)

Experiment	Number of animals					Mean (5, 6, 10, 19)
	0	5	6	10	19	
9 - <i>M. lucens</i>	2447	4436	1459	1615	1496	2252
	0	4	11	18		Mean (4, 11, 18)
10 - <i>M. lucens</i>	1305	1813	1718	1618		1716
	0			Unsalted		Salted
26	3744			3852		0
27	2445			2427		0

Carbon-12 uptake measurements for the ice edge stations ranged from 0 to 1400 $\mu\text{g C/day}\cdot\ell$. Hence the salted communities were ingesting only 2% of the daily net primary productivity. Unsalted community ingestion was barely measurable.

Discussion

The salted community grazing rates were lower than the potential rates derived from single species data. This can probably be attributed to extreme concentrations of grazers and their main predator, *Sagitta elegans*. Perhaps the grazers were resource limited and/or they spent a great deal of time avoiding predators since the cubetainers were incubated under constant light. Although grazing was lower than expected, it exceeded productivity and the phytoplankton concentrations were reduced to virtually nothing after about forty hours.

The zooplankton composition of a vertical tow was used to derive a mean hourly filtering rate of 0.61 ml/hr $\cdot\ell$ for the euphotic zone to a depth of 12 meters (Table IX). The zooplankton concentrations were very low and probably were not resource limited, nor seriously inhibited by predators in contrast to salted population since most of their feeding would occur in the dark (assuming that *Sagitta* is visually oriented). Using a mean algal concentration of 1.38 mg wet weight/ ℓ in the 12 meter water column, a 10 hour feeding period, and a carbon conversion factor of .05, the mean daily grazing amounts to only 0.42 $\mu\text{g C/day}\cdot\ell$. This is insignificant in comparison to daily primary productivity which ranged from 0-1400 $\mu\text{g C/day}\cdot\ell$.

Grazing rates of unsalted communities may be measurable during non-bloom conditions since the change in initial particle concentration may be significant. During an intense bloom at low grazing rate, $C_o/C_t = 1$. During

TABLE IX

MEAN COMPOSITION OF ZOOPLANKTON IN TWO ONE-METER VERTICAL
TOWS SAMPLED FROM 0-12 M AT 0220, JUNE 6/77 AND
CALCULATED FILTERING RATES

	Total mean number	Mean number/ℓ	Filtering rate ml/hr·ℓ
<u>Grazers</u>			
<i>Pseudocalanus</i> sp.	6340	0.673	0.239
<i>Acartia longiremus</i>	5800	0.616	0.219
<i>Calanus glacialis</i>	640	0.068	<u>0.151</u>
		Total	0.61 ml/hr·ℓ
<u>Major Predators</u>			
<i>Sagitta elegans</i>	9190	0.976	-

non-bloom conditions however, it may be that $C_o/C_t \gg 1$. and filtering and ingestion rates would be measurable.

Recommendations

1) We must verify that single-species derived rates are valid before we can come to conclusions regarding crowding and energy transfer to the zooplankton community. If a number of salted community experiments were conducted using various degrees of salting, then an optimum concentration or tow length would be indicated by that salted community having a grazing rate equal to or greater than the corresponding single-species derived rate. Any concentration less than the optimum would probably not be sufficient to allow measurement and any concentration greater would probably include significant crowding effects. I suspect that the tow distance may only be one or two meters. The ingestion rate may then be divided by the zooplankton salting factor to arrive at an unsalted ingestion rate. It may only be necessary, however, to apply single species rates to natural zooplankton concentrations to arrive at valid ingestion rates.

2) Daily ingestion rates for the water column are complicated by the vertical migration of the zooplankton. It will be necessary to know the residence time of the grazers in the euphotic zone. Perhaps a series of experiments can be conducted in which grazing is measured at several depths in the water column over a 24 hour period. *In situ* light intensities at the time of sampling would need to be simulated.

3) A shaking device will be necessary to prevent algae from settling to the bottom of the cubetainers. This suggests the use of smaller cubetainers, say 10 l, (which will affect the tow distance) for ease of handling.

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APPENDIX A

PARTICLE SIZE DISTRIBUTIONS FOR 23 STATIONS
IN THE BERING SEA, MAY-JUNE 1977

APPENDIX TABLE I

PARTICLE SIZE DISTRIBUTIONS AT 0 METERS IN THE BERING SEA,
MAY-JUNE 1977

Station	Ice condition	Peak size (μm)	Abundance of 10-100 μm (mg wet wgt/l)
1	ice free	40-64*	3.87
2	ice free	40-64*	4.18
3	ice free	25-40*	2.37
4	ice free	40-64*	1.82
6	ice free	40-64*	2.67
8	ice	40-64*	1.74
9	ice	40-64*	3.27
10	ice	51-64	2.72
11	ice edge	51-64	3.25
13	ice edge	40-51	2.22
16	ice free	51-64	0.88
17	ice free	40-51	0.80
18	ice free	51-64	2.38
20	ice free	32-40	1.35
22	ice free	32-40	0.43
23	ice	51-64	1.13
26	ice	51-64	2.48
27	ice	51-64	2.16
28	ice	51-64	1.48
32	ice free	40-51	1.13
34	ice free	51-64	0.67
39	ice free	5-6.3	0.75 (0.45 in the 4-10 μm)
40	ice free	40-64	0.69

* Counted with 7 window resolution. Stations 10-40 counted with 13 window resolution.

APPENDIX TABLE II

PARTICLE SIZE DISTRIBUTIONS AT DEPTH OF MAXIMUM CHLOROPHYLL *a*
IN THE BERING SEA, MAY-JUNE 1977

Station	Ice condition	Depth of max CHL <i>a</i>	Depth of count (m)	Peak size (μ m)	Abundance of 10-100 μ m (mg wet wgt/l)
1	ice free	5	10	40-64*	3.49
2	ice free	5	10	40-64*	4.11
3	ice free	-	10	40-64*	2.48
4	ice free	0	0	40-64*	1.83
6	ice free	0-42	20	40-64*	4.31
8	ice	0-10	0	40-64*	1.74
9	ice	12	10	40-64*	3.78
10	ice	-	10	51-64	4.83
11	ice edge	20	20	40-51	3.34
13	ice edge	20	20	40-51	3.07
16	ice free	42	40	51-64	1.58
17	ice free	35	40	51-64	1.73
18	ice free	50	50	32-40	2.22
20	ice free	45	30	25-32	1.90
22	ice free	57	50	32-40	1.47
23	ice	5	5	32-64	0.88
26	ice	5	5	51-64	2.79
27	ice	0	0	51-64	2.16
28	ice	0, 12	0, 10	51-64, 51-64	1.48, 2.10
32	ice free	10	10	51-64	1.50
34	ice free	30	30	40-51	1.15
39	ice free	8	10	5.0-6.3	0.56 (0.86 in 4-10 μ m)
40	ice free	10, 30	10, 30	32-40, 4-5	0, 63, 0.33 (0.2 in 4-10 μ m at 30 m)

* Counted with 7 window resolution. Station 10-40 counted with 13 window resolution.

SECTION II

I. SUMMARY

This report describes progress made towards stated program goals and presents a bibliography (Section II, Appendix I) in fulfillment of task A-22 as applied to the Bering Sea: "to summarize the existing literature and unpublished data on the transfer of synthesized organic matter to zooplankton, micronekton, and ichthyoplankton." The finished product goes beyond the original task description with the inclusion of references on the following subjects: uptake of organic and inorganic nutrients by primary producers; primary production; trophic interactions, distribution, and population dynamics of marine mammals and sea birds; trophic interactions, population dynamics, and distribution of benthic invertebrates and fishes; the taxonomy and ecology of parasites, of invertebrates and vertebrates; microbial decomposition of marine organic matter.

II. INTRODUCTION

In 1977 studies by Alexander and Cooney of the ice edge ecosystem were merged into a single research unit, No. 427. This work was directed toward a quantitative understanding of the processes of primary productivity, nutrient cycling, and organic matter transfer at the edge of the seasonal ice pack in the Bering Sea. We proposed to model the lower trophic levels of this system in an attempt to better understand the partitioning of organic matter utilized on the underice, in the water column, and on the seabed. It was my purpose to describe the grazing community adjacent to and under the edge zone of the seasonal pack, and to then conduct the experiments necessary to estimate grazing losses in the water column. The preliminary observations of distribution and abundance were

accomplished during this funding period; proposed measures of particle ingestion by the grazing community were planned for FY 78.

III. CURRENT STATE OF KNOWLEDGE

Cooney (1976, 1977, 1978) reviewed the literature pertaining to zooplankton and micronekton in the southeastern Bering Sea. While not exhaustive, these reviews and final report represent an overview of the animal plankton and micronekton assemblages, their composition and the distribution and abundance of dominant species. Some preliminary notions concerning the effect of the ice pack were presented.

IV. STUDY AREA

Samples were obtained in the edge zone of the seasonal ice pack March 15-April 4 and April 14-May 3, 1977 aboard the NOAA vessel *Surveyor*. Additional collections were made between June 20 and July 11 in the Norton Sound and southern Chukchi Sea from the *Discoverer*.

V. SOURCES, MATERIALS, METHODS

The bibliography prepared as this report was compiled from library searches accomplished at the University of Alaska (Fairbanks), the University of Washington (Seattle), and Oregon State University (Corvallis) by Mr. Al Adams.

Field data were collected as per Task Order No. 1.

VI. RESULTS

Over 1500 references have been accumulated addressing Task Order A-22 as pertaining to the southeastern Bering Sea and north Pacific Ocean (see Section II, Appendix I).

The results of zooplankton data collected in the field will appear as the Final Report of this project, September, 1978. Funding delays have prevented the final processing and assessment of collections made in the spring and summer of last year.

VII. SUMMARY OF FOURTH QUARTER OPERATIONS

The final processing of samples obtained aboard the *Discoverer* (June-July, 1977) were begun, together with statistical studies of data processed for the spring cruise. This material address both the question of the nearshore zones along the northern shelf, and details of the distribution of dominant species adjacent to and under the ice pack in the southern shelf area. The synthesis of this information will form the content of the Final Report of the project.

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SECTION II - APPENDIX I

A bibliography of references pertaining to the following subjects as applying to natural phenomena occurring in the north Pacific Ocean: uptake of organic nutrients by primary producers, primary production; trophic interactions, distributions, and population dynamics of marine mammals and sea birds; trophic interactions; population dynamics, and distribution of benthic invertebrates and fishes; the taxonomy and ecology of parasites of invertebrates and vertebrates; and microbial decomposition of organic matter.

In general, most of the references apply primarily to the Bering Sea, Chukchi Sea, and northern north Pacific Ocean (with the Kurile-Kamchatka Trench as a southern boundary in the west and the Gulf of Alaska as a southern boundary in the east). Some species with particularly widespread distributions have been studied in northern waters of the north Atlantic Ocean, Canadian and Siberian Arctic Ocean, and the Barents Sea. Because this literature represents a significant contribution to an understanding of the ecology of marine organisms (which are found in the Bering Sea) it has been included in the bibliography.

Some omissions were unavoidable due to time constraints upon the total number of citations that could be examined. This is especially true of literature concerning commercial harvests and taxonomy of marine mammals and fish, taxonomy and productivity of sea birds, and taxonomy of benthic invertebrates. Popular reading materials (such as newspapers and non-scientific magazines) were not searched.

The bibliography is arranged alphabetically by author. Most titles are in English, however, a number of Russian titles have only been transliterated. Some references which were cited in the literature were not

available for examination and could not be varified. In these cases the inclusive page numbers are generally missing; however, such articles have been retained in the bibliography due to their pertinence.

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NUTRIENT DYNAMICS IN NEARSHORE UNDER-ICE WATERS

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I. SUMMARY OF OBJECTIVES, CONCLUSIONS AND IMPLICATIONS WITH RESPECT TO OCS OIL AND GAS DEVELOPMENT

Objectives

The overall objective of RU 537 is to describe the principal processes supplying energy (i.e. fixed carbon) to the biota of the Beaufort Sea coastal zone and to relate the various nutrient chemistry regimes observed to this production of energy. Both terrestrially derived and offshore derived nutrient sources are considered. The information obtained is for integration into the overall structure of the LGL Barrier Island study group, RU 467. Their modelling effort will relate this information to: 1) description of the overall ecosystem, 2) possible OCS direct impacts on the nearshore biota by offshore oil and gas development and 3) possible impacts on the nearshore biota caused by "upstream effects" on land which would change the character of terrestrial input of nutrients and/or carbon to the marine ecosystem via erosional processes or runoff.

Conclusions

The conclusions to date present a picture which is at first startling but upon closer scrutiny is a reasonable, if unusual, pattern for a marine ecosystem. The preliminary estimates of energy input to the nearshore marine biota shows that within approximately 10 km of shore, over 75% of the carbon is derived from the land and that this carbon is composed primarily of peat-like material that has been accumulating on land for up to 12,000 years. Thus the nearshore marine biome is apparently a "fossil fuel" subsidized ecosystem wherein the meager annual primary production by ice algae and phytoplankton is heavily supplemented by organic carbon eroded from coastal peat bluffs and transported by river flow into the coastal zone.

Implications with respect to oil and gas development

The implications of oil and gas development on the primary production and secondary production are mostly indirect but potentially great. Development which would increase or decrease shoreline erosion or significantly alter the rate of riverbank erosion or runoff characteristics could be expected to show corresponding impacts on the nearshore zone. Refinement of our assessments as to the magnitude of these impacts is continuing.

II. INTRODUCTION

General nature and scope of study

This research unit originally proposed to look at a very specific aspect of the nearshore primary production regime, namely, the contribution of ice algae, and the effects of thermohaline convection in supplying nutrients to ice algae populations. Since that time the importance of detritus became more evident and this research unit began to consider the possible magnitude of its significance through the use of data collected previously by the author. These results, summarized in the last quarterly report (October-December 1977), and refined below, show the approximate magnitudes of carbon inputs to the Simpson Lagoon ecosystem and set forth an outline of isotopic techniques that would allow determination of the relative inputs of peat carbon versus modern primary production carbon. Currently, a proposal modification is being presented to NOAA/OCSEAP to provide the support necessary to perform these additional tasks. If approved, RU 537 will expand its coverage from assessing the primary production and nutrient chemistry of the nearshore zone to include the appraisal of all energy sources entering the Beaufort Sea coastal system and will look at the subsequent flow of energy into the higher trophic levels of the Beaufort Sea.

Specific objectives

The specific objectives of this research unit are as follows:

1. Establish mass balance relationships for particulate and dissolved nitrogenous nutrients beneath the winter ice cover in the nearshore Beaufort Sea.
2. Compare standing stocks of epontic algae in relation to under-ice water circulation.
3. Collect data delineating temporal and spatial variability in ice algae blooms in the nearshore Beaufort Sea.

The data requirements of the LGS-Barrier Island study group have identified and necessitated the inclusion of the following objectives relating to the nutrient and energy inputs to the coastal marine ecosystem.

4. Determine the total inputs of energy to the coastal ecosystem including allochthonous carbon and nitrogen entering the system via terrestrial runoff and coastal erosion.
5. Relate the observed patterns in nutrient availability over the annual cycle to the heterotrophic utilization of detrital carbon within the coastal ecosystem.
6. Determine to what extent the detrital carbon is passed up the food chain and the relative significance of the various energy inputs to specific higher organisms in the coastal Beaufort Sea.

Relevance to problems of petroleum development

A detrital based ecosystem such as may be present along large portions of the Beaufort Sea could be readily altered directly or indirectly through OCS related petroleum development. A summary of the specific impacts which

might affect the various sources of energy into the ecosystem would include, by type:

Ice algae productivity:

1. Oil spills on or under the spring ice cover would diminish primary production through either phytotoxic effects or by attenuation of light passing through the ice sheet.
2. Alteration of bottom topography by dredging channels or the construction of causeways could alter ice algae production by changing patterns of thermohaline convective flow beneath the ice cover. Prevention of brine drainage by closing off deeper channels would lead to brine accumulation on the bottom which could seriously impact both fauna and flora.

Phytoplankton production: Open water primary production would be most sensitive to such impacts as phytotoxicity resulting from oil spills. The rapid lateral flushing of water along the Beaufort Sea coast may, however, serve to minimize this aspect of potential impact.

Detrital-based production and heterotrophic productivity: Impacts upon the heterotrophic organisms that depend upon eroded and transported peat materials as their energy source would occur primarily through OCS related developments that impinged upon the sources of detritus. Such procedures such as shoreline stabilization could alter the food base by elimination of eroded materials. Causeway construction could change wave energy regimes and thus decrease shoreline erosion. Stabilization or channelizing of streambeds might add to or subtract from the total organic load carried by runoff waters. The present lack of knowledge concerning the role of detrital

based production in the overall food web of the Beaufort Sea makes assessment of the potential impacts speculative at this time.

III. CURRENT STATE OF KNOWLEDGE

Primary production

In comparison to the warmer waters along the more southern Alaskan coastlines, the Beaufort Sea supports a relatively sparse biota. No appreciable harvests of renewable marine resources are made with the exception of small commercial fisheries operated principally by native communities in the estuaries along the coast and seasonal harvesting of bowhead whales at Point Barrow. The zone of maximum biological productivity is confined to a relatively narrow strip along the coast wherein the interaction of terrestrial influences ameliorates and somewhat enhances the sparse oceanic regime.

The primary production supporting the pelagic community occurs in two distinct phases in the Beaufort Sea (and other polar waters). The initial algal bloom in the spring occurs well before the >2 m ice cover has begun to melt but after the returning daylight reaches critical intensities sufficient to supply the necessary energy beneath the ice (Appolonio, 1965; Bunt, 1963). Attached, or epontic algal populations grow on the ice-water interface and thrive until the melt begins around the beginning of June. Estimates of the carbon fixed during this period range from about $1 \text{ gm/m}^2\text{-yr}$ in the shallow Prudhoe Bay area (Horner *et al.*, 1974) to $5 \text{ gm/m}^2\text{-yr}$ off Point Barrow (Clasby *et al.*, 1976). Little is known of the distribution or spatial variability of ice algae populations along the Beaufort Sea coast.

As the ice cover melts, phytoplankton production assumes the major role in energetic input although the stability of the water column caused by the melting of the nutrient-poor ice cover hinders the advection of deep water nutrients to the photic zone. Only in limited areas near Barter Island has Hufford (1974) identified possible upwelling of deep waters. As a result, primary production by phytoplankton is low and estimates range from $<10 \text{ g C/m}^2\text{-yr}$ in the central arctic Ocean (English, 1961) to about $20 \text{ g C/m}^2\text{-yr}$ on the coastal zone near Barrow (Alexander *et al.*, 1974).

Input of terrestrial carbon to the nearshore coastal zone

The enhancement of biological activity in the proximity of land has been long recognized and attributed to various factors among which are the provision of suitable habitat for both benthic flora and fauna, substrate for macrophytes and input of terrigenous nitrogen, phosphorus and carbon via runoff from land. The arctic coastline provides very limited habitat for macrophytes or benthic infauna due to the 2 m freeze depth which effectively eliminates the shallow nearshore zone as a year-round environment for marine organisms. Again, in the deeper water, ice scouring creates sufficient habitat disturbance to account for the paucity of observed infauna. Below the 2 m contour in the bays and lagoons, however, large standing stocks of invertebrates - amphipods, mysids and isopods - are common and estimates by the LGL-Barrier Island Study (RU 467) personnel place the biomass at approximately 20 g/m^2 dry weight in Simpson Lagoon. These invertebrates are commonly found in close association with eroded organic material from the shoreline and studies by Broad (RU 356) have shown that certain gammarid amphipods and saduria do ingest and degrade the peat. This ingestion is probably accompanied by the removal

and digestion of heterotrophic microflora and microfauna that are attached to the peat particles. Although it is known that the detrital material is ingested and that large numbers of invertebrates are associated with the organic material, as yet no conclusive evidence has been found indicating that the peat carbon is being assimilated either directly or indirectly by invertebrates or is being carried up the food chain to higher organisms such as fish or birds.

By using data obtained by Lewellen (1973) and the author during an earlier study of the Simpson Lagoon shoreline, erosion rates and the resulting quantities of carbon and nitrogen washed into the lagoon were estimated for the shoreline between Oliktok Point and Beechey Point. These estimates have been expanded by Cannon and Rawlinson (RU 530) to include all of Simpson Lagoon and are presented in Section VI. Further estimates on the total input of allochthonous carbon to the Beaufort Sea have been made by the author and S. Rawlinson (RU 530) which show that approximately 75% of the total carbon input is terrestrially derived. The implications of this compartmentalization of the energy input to the marine ecosystem are discussed in Section VI.

IV. STUDY AREA - BEAUFORT SEA - 100%

The study area for this project has been shifted from the originally proposed Elson Lagoon-Dease Inlet area near Pt. Barrow to Simpson Lagoon approximately 60 km west of Prudhoe Bay. This shift in siting was made to allow integration with the tasks being undertaken by the LGL-Barrier Island Study group. The principal data collection effort and detailed analyses on primary production and heterotrophic production will be made in this area. However, in conjunction with RU 530, estimates of terrestrial

input of carbon along the entire Beaufort Sea coast via runoff and erosion will be undertaken on a much less detailed program.

V. SOURCES, METHODS AND RATIONALE OF DATA COLLECTION

Primary production by epontic ice algae in Elson Lagoon near Point Barrow

The sampling program for ice algal production and spatial distribution occurs during the spring months and involves sampling the ice-water interface before and after the ice algae bloom. The first sampling period is during early April and yields the water chemistry data representing the maximum nutrient concentrations and salinities of the annual cycle. Ice cores and water samples are taken as logistics and weather allow. Samples are filtered and analyzed for inorganic nutrients and particulate nitrogen to yield a total nitrogen budget for the water column. Sample locations are located to give spatial data yielding densities and distribution of ice algae.

Delays in funding of RU 537 prevented an extensive sampling program in Spring 1977 and very limited data were collected in the Point Barrow area. Extremely poor weather and lack of available helicopter support restricted sampling during the bloom to a single transect of Elson Lagoon. However, the data obtained will serve as a base for the Spring 1978 program near Simpson Lagoon.

Analytical methods employed for nitrate, ammonia and phosphate analyses are similar to those utilized by Alexander *et al.* (1974) for their ice algae studies. Dissolved organic nitrogen was run using the ultraviolet photo-oxidation technique employed by Schell (1974). Particulate nitrogen analyses were run on glass fiber filters containing aliquots of the melted ice cores

or underlying water. The filtered samples were burned and the evolved nitrogen gas measured using a Coleman Nitrogen Analyzer.

By establishing detailed nitrogen budgets for the water column and ice column before and after the epontic algal bloom it is possible to determine two important facets of the nearshore productivity regime. First, the total standing stocks of ice algae (and assimilated nitrogen) can be quantitatively described for the nearshore zone and the validity of extrapolating primary production measurements obtained by Clasby *et al.* (1976) at Point Barrow to include other areas of the Beaufort Sea coast can be determined. Second, by establishing budgets of nitrogen in the dissolved and particulate phase, the importance of thermohaline convective flow as a nutrient input in the nearshore zone can be estimated. This hypothesis states that enhanced primary production by ice algae can be expected in the 2-5 m depth zone in the late Spring and the measurement of total particulate and inorganic nitrogen in the ice/water column offers the most direct test of whether or not this enhancement occurs.

Utilization of detrital carbon and transfer of detrital carbon in the food web

The magnitude of carbon input to the nearshore zone of Simpson Lagoon (Fig. 1) required that the effects of this energy source be evaluated in respect to the inputs of primary production. Detrital input occurs through essentially two sources - coastal erosion and runoff from the tundra. Thus assessment of these inputs becomes a geomorphological problem for the former source and a hydrological problem for the second. Chemical data on the eroded tundra have been previously obtained by Schell (1975) and new

ENERGY SOURCES FOR SIMPSON LAGOON - BARRIER ISLAND ECOSYSTEM

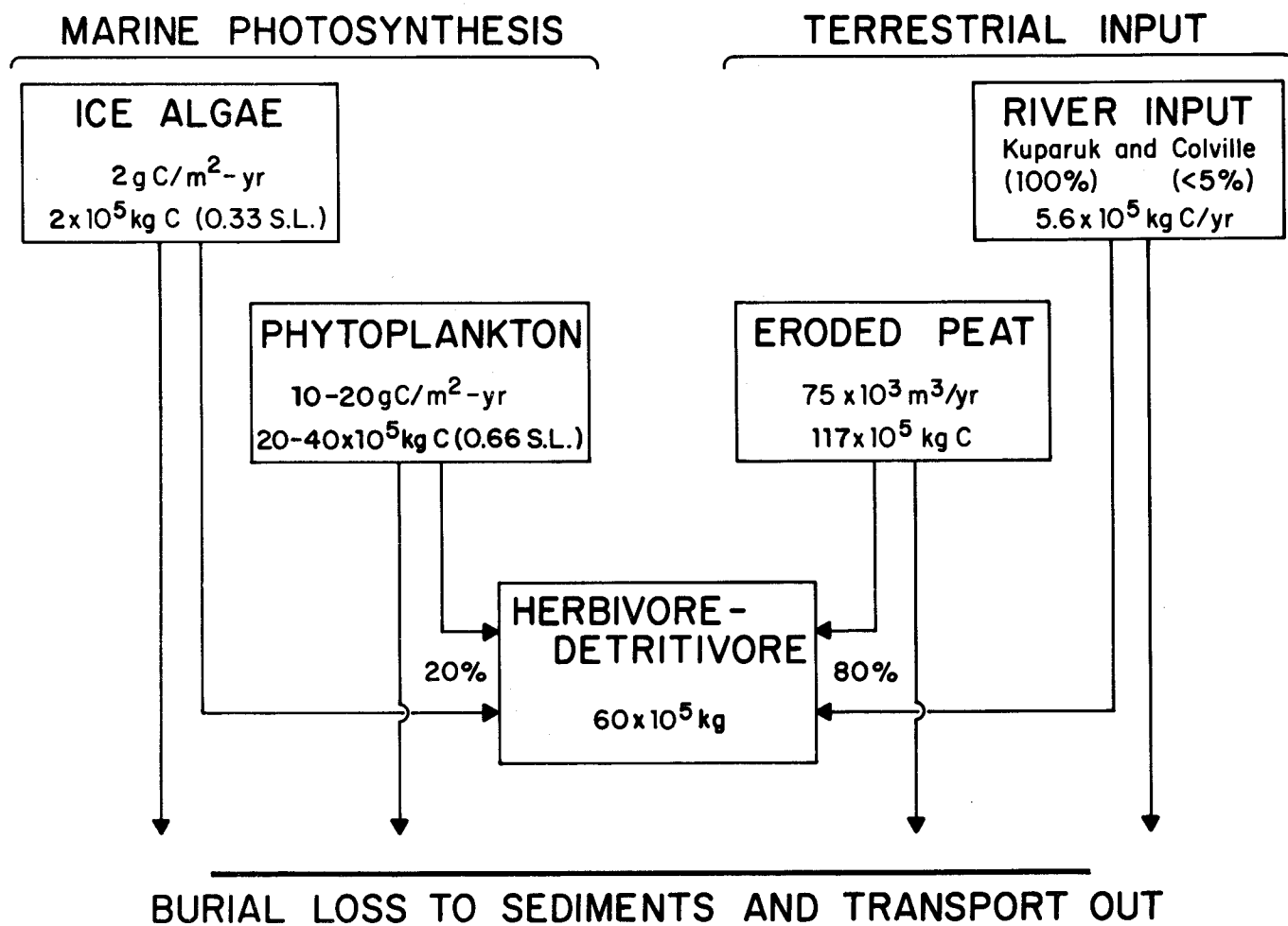


Figure 1. Carbon input to Simpson Lagoon.

erosional data are being determined by Cannon and Rawlinson (RU 530). Total organic carbon data for the Colville River waters have been kindly provided by the US Geological Survey (Charles Sloan, personal communication) and flow data are available from the literature (Arnborg *et al.*, 1967; Walker, 1974). Nitrogen data has been collected by Schell (RU 537) and will be incorporated with the above to yield quantitative inputs of nitrogen and carbon by the Colville River to the nearshore zone.

The utilization of detrital organic carbon by heterotrophs and the further transfer of this carbon into the food web is being investigated through the use of carbon isotope ratios in the various coastal marine living and non-living organic materials. Figure 2A shows the three fractions that would comprise the organic carbon of a detritivore or the predators of detritivores. The proposed analytical techniques to identify these fractions are shown in Figure 2B. If the carbon in the eroded peat materials of the shoreline are incorporated to a significant extent in the food web of heterotrophic microorganisms and these are then consumed and assimilated by benthic invertebrates such as amphipods, isopods and mysid shrimp, the isotopic abundances in the higher organisms should generally reflect the food source. Some species of these benthic invertebrates are known to comprise a large fraction of the diets of higher organisms found in the coastal Beaufort Sea and thus the potential exists for detrital carbon to constitute a large fraction of the energy supply to the ecosystem.

Radiocarbon dating will be used to delineate the fraction of peat carbon in the organisms. Eroded peat in Simpson Lagoon has a mean radiocarbon age of about 4000-5000 years B.P. if the radiocarbon dates given by Lewellen (1973) are representative of the basal peat layer in the Simpson Lagoon area. It is assumed that the peat has been accumulating at

DETERMINATION OF HERBIVORE- DETRITIVORE CARBON SOURCE

IS CARBON SOURCE
RECENT OR OLD ?

HERBIVORE-
DETRITIVORE BIOMASS

IS CARBON SOURCE
MARINE OR TERRESTRIAL ?

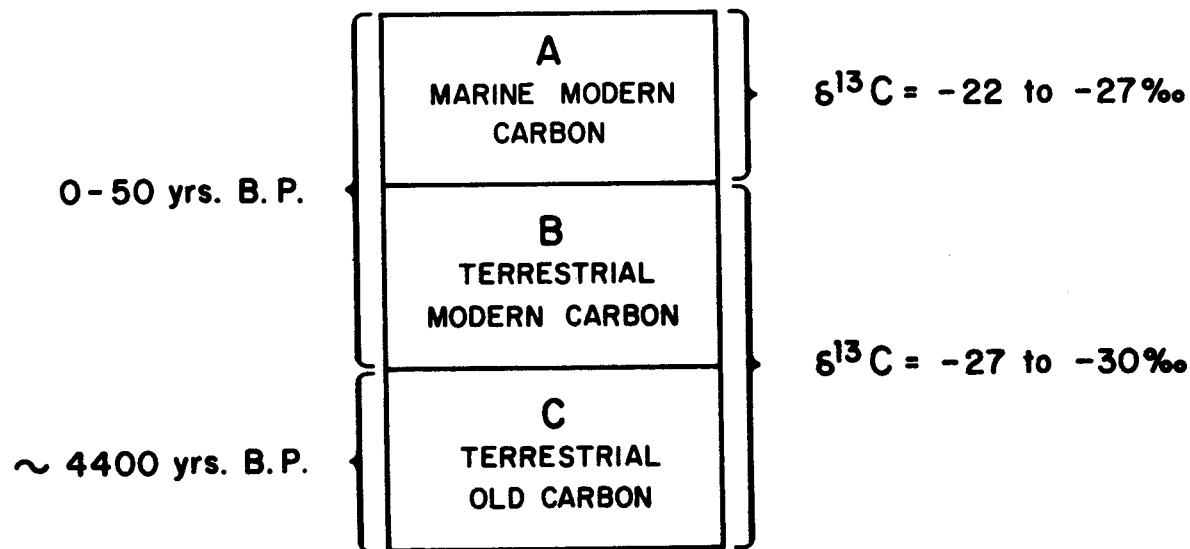


Figure 2A. Fractionation of carbon in nearshore Beaufort Sea fauna and characteristics of carbon isotopes from each source.

RADIOCARBON DATING

$$\% C = \left[\frac{\text{Mean peat age} - \text{Sample age}}{\text{Mean peat age}} \right] \times 100$$

$$\begin{aligned} \% A + B &= 100 - C \\ &= \text{percent modern carbon} \\ &\quad (\text{marine and terrestrial}) \end{aligned}$$

STABLE ISOTOPE RATIOS

$$\% A = \frac{{}^{12}\text{C}/{}^{13}\text{C}(\text{terr.}) - {}^{12}\text{C}/{}^{13}\text{C}(\text{sample})}{{}^{12}\text{C}/{}^{13}\text{C}(\text{terr.}) - {}^{12}\text{C}/{}^{13}\text{C}(\text{marine})} \times 100$$

$$\begin{aligned} \% B &= 100 - (A + C) \\ &= \text{carbon derived from modern terrestrial} \\ &\quad \text{sources.} \end{aligned}$$

Figure 2B. Techniques for quantitation of carbon input sources shown in Figure 2A.

a constant rate to present. As yet no age has been obtained for carbon transported by the Colville River but the surficial nature of runoff in a permafrost environment such as the North Slope would suggest a modern date somewhat tempered by peat addition via riverbank erosion and collapse.

Stable isotope techniques allow the discrimination of food sources in ecosystems where the source materials (primary producers) have significantly different $^{12}\text{C}/^{13}\text{C}$ ratios. By comparing $^{12}\text{C}/^{13}\text{C}$ ratios of organisms at different trophic levels the food sources of the higher organisms can be apportioned. This technique has been used by McConnaughey (1978) to study the detrital input of eelgrass beds in Izembek Lagoon to the fauna of the lagoon and nearshore Bering Sea. Application of this technique is shown in Figure 2B and will be investigated as a method to separate terrestrial and marine contributions to the nearshore fauna. Although the method is acknowledged to be less sensitive than ^{14}C dating, the applicability to modern carbon sources increases its desirability. Analytical cost is low compared to ^{14}C dating.

VI. RESULTS AND DISCUSSION

Primary production by epontic ice algae

Ice algae primary production data for 1977 were obtained for only one transect of Elson Lagoon at Point Barrow due to logistic problems and very poor flying weather which necessitated travel by tracked vehicle. Three stations were selected across the lagoon ranging from relatively deep water near the Eluitkak Pass entrance to the lagoon, another near mid-lagoon and the third approximately one kilometer from the mainland shore. Particulate nitrogen data for these stations are listed in Table I

TABLE I

PARTICULATE NITROGEN CONCENTRATIONS IN ELSON LAGOON AND
DEASE INLET ICE AND WATER, SPRING 1977

Dease Inlet 31 March 1977					
Station (mgN/m ³)	1 71°13'N 155°28'W	2 71°14'N 155°49'W	3 71°09'N 155°24'W	4 71°02'N 155°25'W	5 70°53'N 155°42'W
Top ice	14	70	67	196	-
Center ice	23	9	49	12	23
Bottom ice	52 (10.6) ¹	39 (7.7)	47 (2.1)	36 (1.7)	28 (8.2)
Under-ice water	27	38	53	18	26
Elson Lagoon 23 May 1977					
Station (mgN/m ³)	1 71°21.5'N 156°21.0'W	2 71°21.1'N 156°24.0'W	3 71°20.6'N 156°28.0'W		
Top ice	70	-	170		
Center ice	83	60	2		
Bottom ice	1199 (69.4)	140 (16.3)	340 (35.7)		
Under-ice water	48	40	22		

¹Value in parentheses are mgN/m² of particulate nitrogen at the water-ice interface.

and compared to data obtained in April 1977 from Dease Inlet. The concentrations are expressed in mg N/liter of the melted core sections and for bottom ice, as mg N/m² since the ice algae are present as a discrete layer at the ice-water interface. The much more restricted circulation within Dease Inlet precludes direct comparison of nutrient chemistry in the underlying water column with that of Elson Lagoon and the value of these data in the ice algae study is to give a range of particulate nitrogen values found in the ice column. The high values occasionally present in the top ice were due to bands of detrital material frozen into the ice following storms in the fall. The nutrient data on nitrate, ammonia and phosphate concentrations beneath the ice were useful in assessing heterotrophic activity beneath the ice as discussed in the following section.

The particulate nitrogen values measured in Elson Lagoon are considerably less than those found beneath sea ice on the Chukchi Sea off Point Barrow (V. Alexander, personal communication). Either the nutrient chemistry regime is sufficiently different or the algal bloom had not progressed to the same intensity as when the Chukchi Sea samples were taken. The relatively high ammonia and nitrate concentrations (approximately 5 µg-atoms N/liter) indicate that the latter condition was probably the case. The weather had been cold and overcast for several days prior to sampling and no snow melt had occurred. Typically, ice algae populations reach maximum density just before the ice sheet begins to melt, around 1 June. The 1978 field season and sampling effort will be scheduled to optimize the collection of the maximum ice algae densities.

Carbon input to the nearshore marine zone from
terrestrial runoff and coastal erosion

Estimation of erosional input of nitrogen to Simpson Lagoon was first attempted by Schell (1975) using data obtained by Lewellen (1973). Coastal erosion rates were determined through the use of coastline aerial photography made in 1955 and again in 1972. Coastline retreat was utilized with field data on average shoreline relief and soil types to calculate total organic matter and nitrogen being eroded on an annual basis. This technique has been expanded by Rawlinson (RU 530) and the results are shown in Figure 1 which gives the relative carbon inputs to Simpson Lagoon. Approximately 80% of the carbon input to the lagoon system is from terrestrial runoff and 75% is derived from peat eroded from the shorelines. This material, which has an average C:N atom ratio of about 18.8 also represents an input of approximately 730 metric tons of fixed nitrogen to the lagoon system.

When extending these calculations to include the entire Alaskan Beaufort Sea coast, a mean erosional rate of 1.75 m/year and a coastal relief of 1.5 m was assumed, of which 1.0 m was peat materials. From the data available on the Elson Lagoon coastline near Barrow, and the Simpson Lagoon area, the estimates are felt to be about representative. The carbon budget for the nearshore zone as a whole is shown in Figure 3. As in Simpson Lagoon, over 75% of the carbon input is from terrigenous material with 22% derived from marine primary production. Coastline erosion is responsible for approximately half of the total carbon input. Accompanying this approximately 4.5×10^6 tons of carbon is an estimated 2.7×10^4 tons of nitrogen which, after mineralization by heterotrophic oxidation of the carbon, should supply a large fraction of the nitrogen

BEAUFORT SEA COASTAL ZONE ENERGY INPUT 10^8 kg C/year

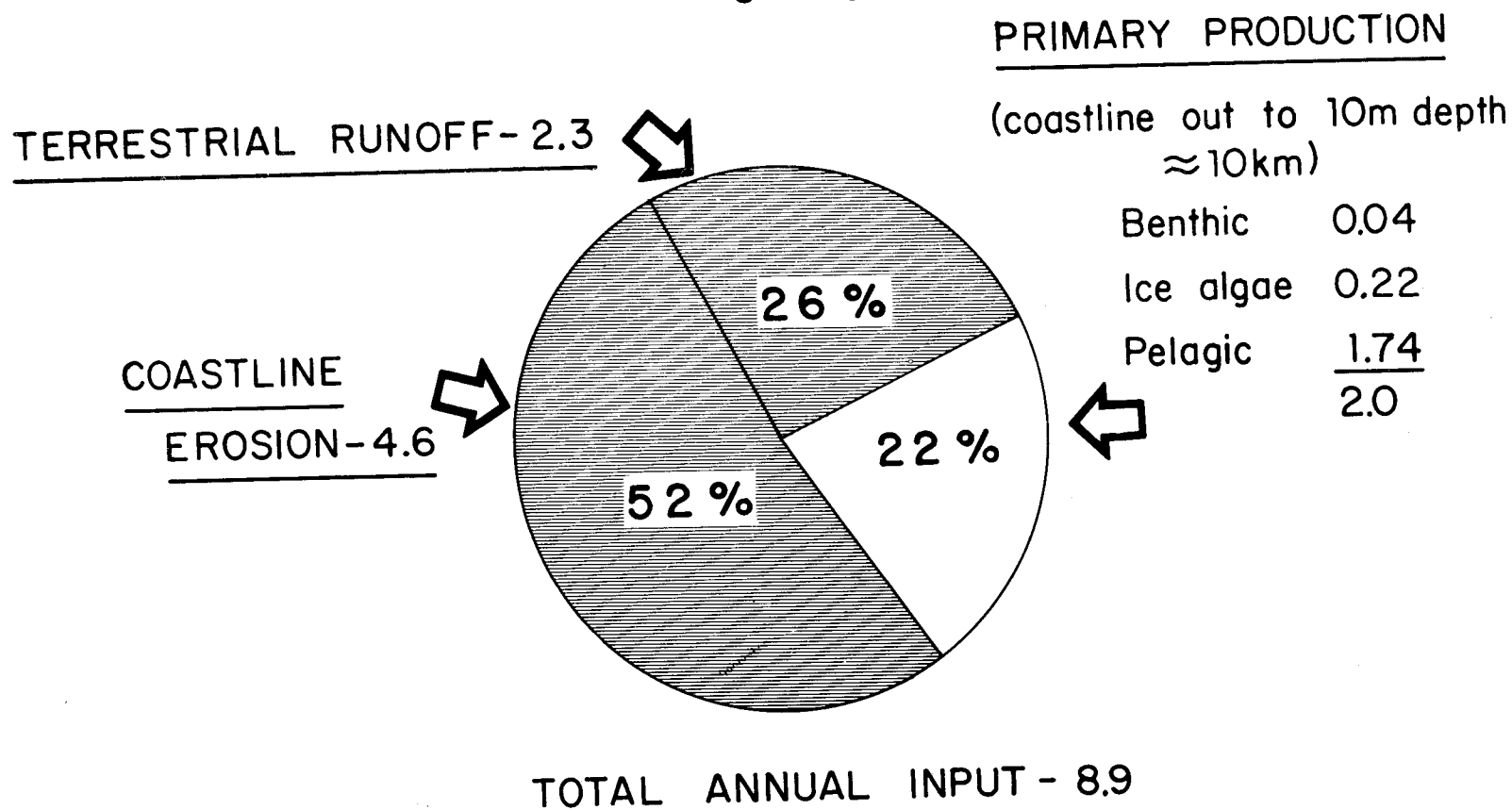


Figure 3. Carbon input to the Alaskan Beaufort Sea coastal zone.

requirements of the nearshore primary producers. The refinement of these estimates of input quantities and nitrogen content will be a major concern during the next year.

Food webs of the coastal Beaufort Sea

The epifauna of the nearshore zone have not been tested using the techniques described above to ascertain the relative amounts of carbon being derived from terrigenous sources versus marine primary production. The predominant species of amphipods that are in close association with the peat (gammarids) have not been found in large percentages in the gut contents of the fish and birds being studied by LGL personnel. To date no data exist which define the role of these nearshore invertebrates in the trophic dynamics of the coastal zone. Only one radiocarbon date has been obtained on a Colville River anadromous fish (*Coregonus autumnalis*) and that specimen was completely modern in radiocarbon age. The dietary information regarding this fish indicates that it feeds upon pelagic invertebrates and thus no conclusions regarding the detrital food web are yet possible. The proposal modification submitted to NOAA by RU 537 will allow a much more detailed compilation of nearshore trophic system data.

Circumstantial evidence for intense heterotrophic activity in the eroded peat is available through re-evaluation of existing Beaufort Sea data. The peat material contains a relatively high nitrogen content but is extremely phosphate deficient. When eroded or transported into the marine waters which contain a high concentration of phosphate, it would be expected that heterotrophic activity would yield measurable changes in the nitrogen-phosphorus ratios in the water through uptake of the

limiting nutrient. Figure 4 shows the nitrogen/phosphorus ratios present in the water beneath the ice in Dease Inlet during April, 1973 (Schell, 1975). The water column throughout the inlet and up the delta channels of the Meade River was saline as all freshwater inflow ceases by late fall. A pronounced phosphate depletion is apparent as the stations progress toward the head of the inlet without a corresponding depletion in nitrogen. The author believes that this phosphate consumption is due to intense heterotrophic activity in the detritus and since thermo-haline convective processes are active throughout the winter, the eroded terrestrial material must act as sink for phosphorus. The fate of this phosphorus is unknown. If incorporation into the sediments does not occur then at some period of the year, as yet unknown, regeneration and transport outward must be active. The proposed modifications to RU 537 would include study of this problem.

VII. CONCLUSIONS

Much of the work to be accomplished by this research unit has only begun and the conclusions drawn below must be regarded as tentative and undergoing modification as new data is acquired. Specifically, several conclusions regarding the primary production and energetics of the near-shore may be stated:

1. Primary production by ice algae and phytoplankton are a minor fraction of the total carbon input to the nearshore marine ecosystem. No implication as to the significance of marine primary production versus terrestrial input in trophic energetics can be drawn at this time.
2. The offshore waters of the Beaufort Sea are strongly nitrogen limited in respect to phytoplankton nutrition. In the immediate nearshore

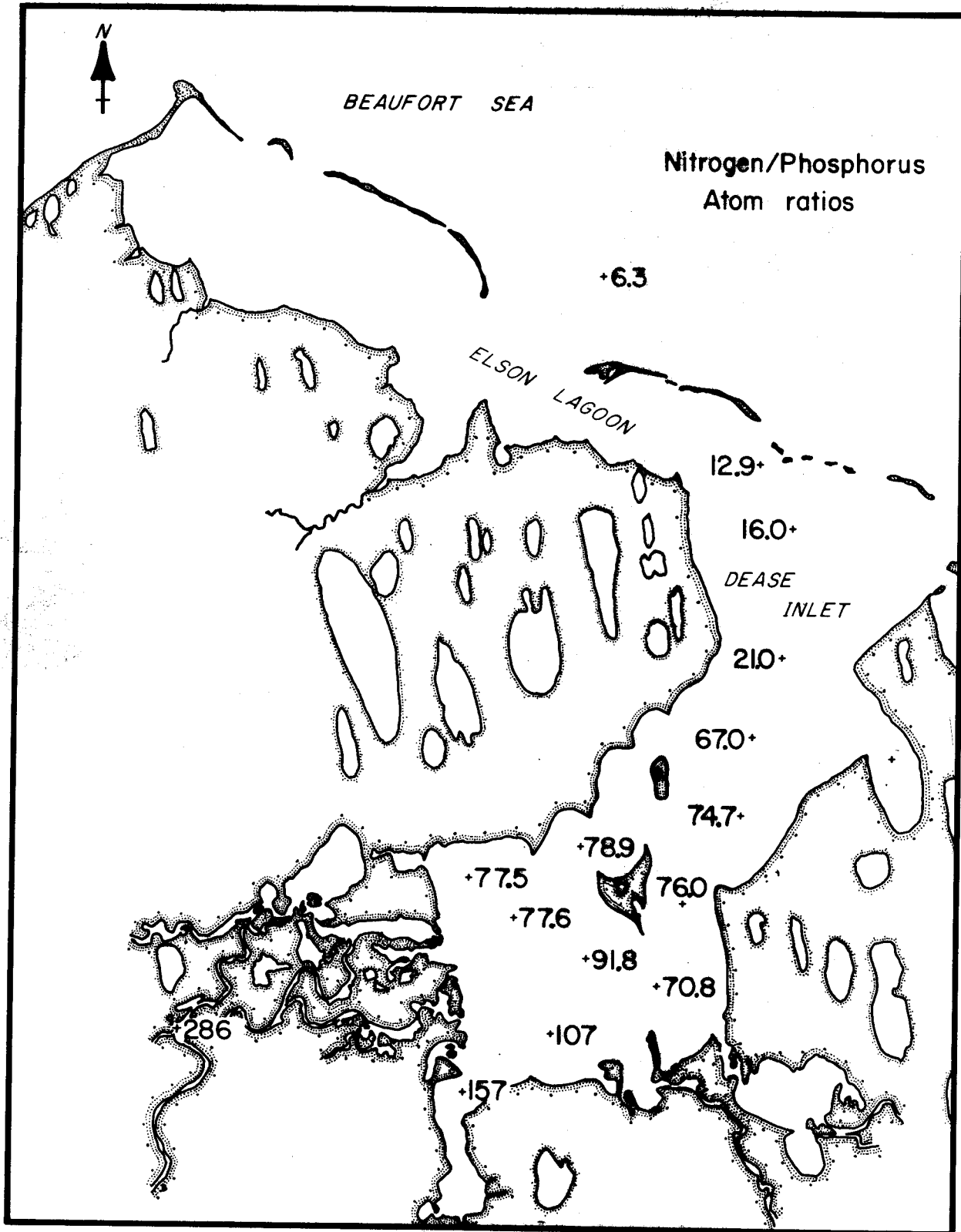


Figure 4. Dease Inlet nitrogen/phosphorus atom ratios in under-ice water, April 1973. Saline waters had intruded to the head of the Meade River delta (lower left) (from Schell, 1975).

zone, however, phosphate appears to be the limiting nutrient.

3. Nutrient regeneration and standing stocks of nutrients in the nearshore water column appear to be controlled by thermohaline convection and heterotrophic utilization during winter months and by primary producers during late spring and summer.
4. Environmental impacts on basic trophic energetics related to OCS development must be assessed with regard to the factors controlling the input of terrestrial organic material as well as factors controlling marine primary production.
5. Important higher organisms (i.e. birds, fish and mammals which utilize the nearshore environment as habitat must be investigated by individual species to determine the relative importance of detrital carbon versus primary production as the ultimate energy source within the nearshore marine ecosystem.

VIII. NEEDS FOR FURTHER STUDY

The various problems delineating the critical areas of study have been described above with the appropriate techniques to obtain the required data.

IX. SUMMARY OF FOURTH QUARTER ACTIVITIES

1. Ship/field trips

None

2. Scientific party

Not applicable

3. Methods

Not applicable

4. Sample localities

Not applicable

5. Data collected or analyzed

Particulate nitrogen analyses for all 1977 samples were completed and the data processed.

Meetings

The LGL-Barrier workshop was held in Vancouver on 2-5 December 1977. Techniques for carbon isotope studies were presented as a means of elucidating relative carbon inputs to the food webs of the nearshore Beaufort Sea.

A scientific presentation and review of data obtained to date was given to BLM and NOAA/OCSEAP personnel in Anchorage on 7 March 1978.

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