Environmental Assessment of the Alaskan Continental Shelf

Annual Reports of Principal Investigators for the year ending March 1977

Volume XI. Receptors — Microbiology

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

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VOLUME I	RECEPTORS -- MAMMALS
VOLUME II	RECEPTORS -- BIRDS
VOLUME III	RECEPTORS -- BIRDS
VOLUME IV	RECEPTORS -- BIRDS
VOLUME V	RECEPTORS -- BIRDS
VOLUME VI	RECEPTORS -- FISH
VOLUME VII	RECEPTORS -- FISH
VOLUME VIII	RECEPTORS -- FISH
VOLUME IX	RECEPTORS -- FISH
VOLUME X	RECEPTORS -- FISH
VOLUME XI	RECEPTORS -- MICROBIOLOGY
VOLUME XII	EFFECTS
VOLUME XIII	CONTAMINANT BASELINES
VOLUME XIV	TRANSPORT
VOLUME XV	TRANSPORT
VOLUME XVI	HAZARDS
VOLUME XVII	HAZARDS
VOLUME XVIII	HAZARDS DATA MANAGEMENT

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Outer Continental Shelf Environmental Assessment Program

Boulder, Colorado

March 1977

U.S. DEPARTMENT OF COMMERCE
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VOLUME XI

RECEPTORS - MICROBIOLOGY

CONTENTS

ANNUAL REPORT

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

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Submitted by:

Ronald M. Atlas, Ph.D.
Principal Investigator
Department of Biology
University of Louisville
Louisville, Kentucky 40208

Tatsuo Kaneko, Ph.D.
Research Associate
Department of Biology
University of Louisville
Louisville, Kentucky 40208

Prepared for:

Outer Continental Shelf Energy Assessment Program
National Oceanic and Atmospheric Administration
Fairbanks Project Office
Fairbanks, Alaska

TABLE OF CONTENTS

Scientific Personnel

Principal Investigator: Ronald M. Atlas

Research Associate: Tatsuo Kaneko

Research Assistant: George Roubal

Research Technicians: Teiko Kaneko
Craig Sholt

I. Summary of Objectives and Conclusions

The objectives of this project were to characterize microbial communities in the Beaufort Sea and to determine the ability of the indigenous microbial community to biodegrade petroleum hydrocarbons. Microbial communities show large seasonal and spacial differences in number and composition of microorganisms. These seasonal and spacial fluctuations appear to be influenced by abiotic determinants other than temperature. The complex nutritional requirements of many of the dominant organisms suggests that functioning of these organisms is dependent on interactions with other organisms, such as phytoplankton. During summer some bacterial populations were widespread in water, but in winter bacterial populations were highly localized. The effects of oil contamination during winter will probably be highly dependent on the site of contamination. The low incidence of ability to utilize hydrocarbons in the dominant microorganisms can be used as a baseline against which the incidence of this property in the microbial community following OCS petroleum development can be measured to monitor hydrocarbon inputs. Measurement of microbial species diversity also appears to hold promise for monitoring inputs of hydrocarbon contaminants. Hydrocarbon biodegradation will probably be initially limited in winter by low numbers of hydrocarbon degrading microorganisms and in late summer severely limiting by abiotic limiting factors.

II. Introduction

This study was conducted to determine the distribution, and to characterize, microbial populations in the Beaufort Sea. Microorganisms are essential components of marine ecosystems. Microbial populations respond rapidly to environmental perturbations. Microbial populations can be used to monitor such perturbations. With specific reference to petroleum development, some microorganisms are capable of biodegrading polluting hydrocarbons, as well as other organic contaminants. Microbial degradation is the main natural process for removal of contaminating petroleum hydrocarbons.

III. Current State of Knowledge

The state of knowledge concerning microbial populations in the Beaufort Sea was summarized in the 1976 annual report. New information developed from this project is described below.

IV. Study Area

Samples were collected in the Beaufort Sea between Pt. Barrow and Prudhoe Bay. Figure 1 shows the locations of sampling sites. During summer 1975 (August-September), samples were collected mainly near Pt. Barrow, including Elson Lagoon and from within Prudhoe Bay. During winter 1976 (March-April) samples were collected along 3 transects from Pt. Barrow, Pitt Pt. and Prudhoe Bay and from within Elson Lagoon and Prudhoe Bay. During summer 1976 (August-September) samples were again collected from the winter 1976 sampling sites during a cruise of the Glacier.

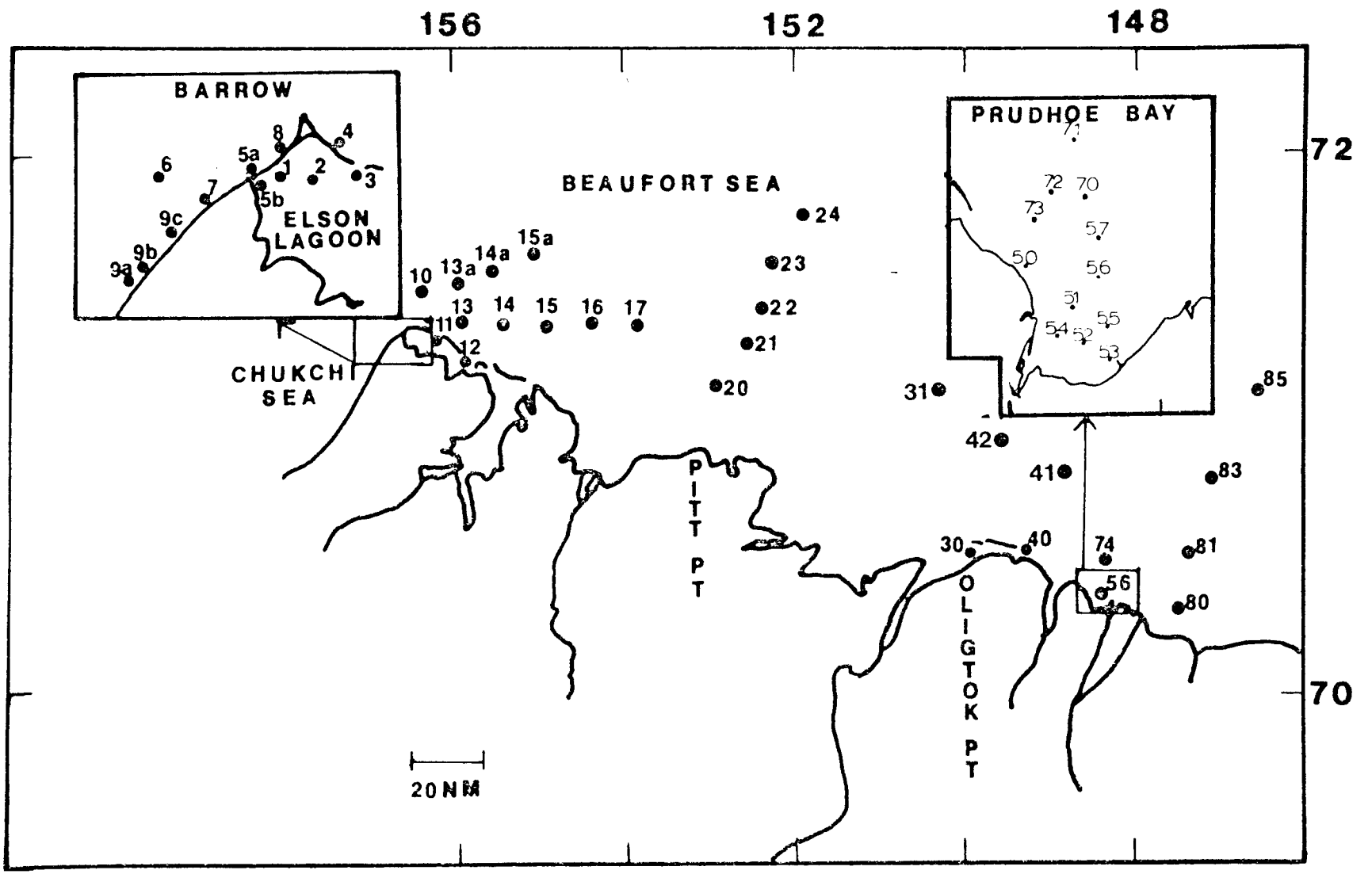


Fig. 1.
Map of sampling area.

V. Methods and Rationale of Data Collection

Several methods were used to enumerate microbial populations in both water and sediment samples. Enumeration procedures included direct and viable counts. Direct counts give the best estimate of the numbers of microorganisms present, but do not distinguish between living and dead microorganisms. Viable counts have the problem of being selective for a given population capable of growth under the enumeration conditions. Viable count procedures were performed to determine the numbers of heterotrophs capable of growth at 4 C and also for heterotrophs capable of growth at 20 C. Even though 20 C is above the temperature at the time of isolation, the optimal growth temperature of microorganisms is usually higher than the temperature of the ecosystem from which they are isolated. Selective procedures were used to enumerate Vibrio spp., a common marine bacterial genus, and to enumerate microorganisms capable of utilizing petroleum hydrocarbons.

In addition to enumeration of microorganisms, microbial populations were extensively characterized by random selection and numerical taxonomic testing of isolates from representative samples. Details of these procedures have been described in previous reports. The characterization includes examination of between 300 and 400 features for each organism. The enzymatic capabilities and nutritional requirements of microbial populations are determined in this testing. Also, the physiological tolerance limits of the tested microorganisms are determined in these tests. Cluster analyses of

these microorganisms were performed to determine the dominant taxonomic groups. The spacial and seasonal distribution of major groups of microorganisms and the diversity of the microbial community were examined.

The ability of the indigenous microbial community to biodegrade petroleum hydrocarbons was determined using ^{14}C labelled hydrocarbon spiked crude oil. To determine the biodegradation potential, water or sediment samples are incubated with ^{14}C spiked crude oil and the production of $^{14}CO_2$ periodically monitored. The amount of $^{14}CO_2$ reflects the ability of the microbial community to remove hydrocarbon contaminants. The hydrocarbon biodegradation potential is determined in part by the numbers and types of microorganisms present and in part by abiotic factors such as concentrations of essential nutrients.

VI. RESULTS

Enumeration

The enumerations of microbial populations are shown as three dimentional histograms (Figs. 2-6). The geographic location of each sample is shown by latitude and longitude in the XY plane. The numbers of microorganisms enumerated are shown by the height of the bar. To aid in gaining a proper perspective for interpretation of these figures, reference lines have been drawn at the base and tops of the bars. These are only reference lines that facilitate comparisons of the heights of the bars.

The direct counts (Fig. 2) showed that total numbers of microorganisms were higher in surfacial sediment than water in both summer and winter and in similar concentrations in ice and water during winter. Total numbers of microorganisms were lower in surface water in winter than in summer. Total numbers of microorganisms were higher in sediment in both winter and summer 1976 than during summer 1975. Significant patterns of spacial variation were not shown in the total counts.

The viable heterotrophs enumerated at 4 C (Fig. 3) were higher in sediment in summer 1976 than in either winter 1976 or summer 1975 samples. No significant spacial variation pattern was shown for sediment. During summer 1975, populations were significantly higher near Prudhoe Bay than near Pt. Barrow in surface water. This pattern was not found however in summer 1976. During winter 1976, viable populations were significantly higher in water within Elson Lagoon then in other areas, including Prudhoe Bay. Viable populations were lower in ice than in water and lower in water during winter than during summer. Viable populations were higher in surface water during summer 1976 than during summer 1975.

The viable heterotrophs enumerated at 20 C (Fig. 4), were significantly higher in both water and sediment samples from the Prudhoe Bay area than near Pt. Barrow during summer 1975. During winter 1976 these populations decreased markedly in water and also decreased in sediment. In sediment in winter there was a decrease in the population away from shore. Mesophilic microorganisms were in very low

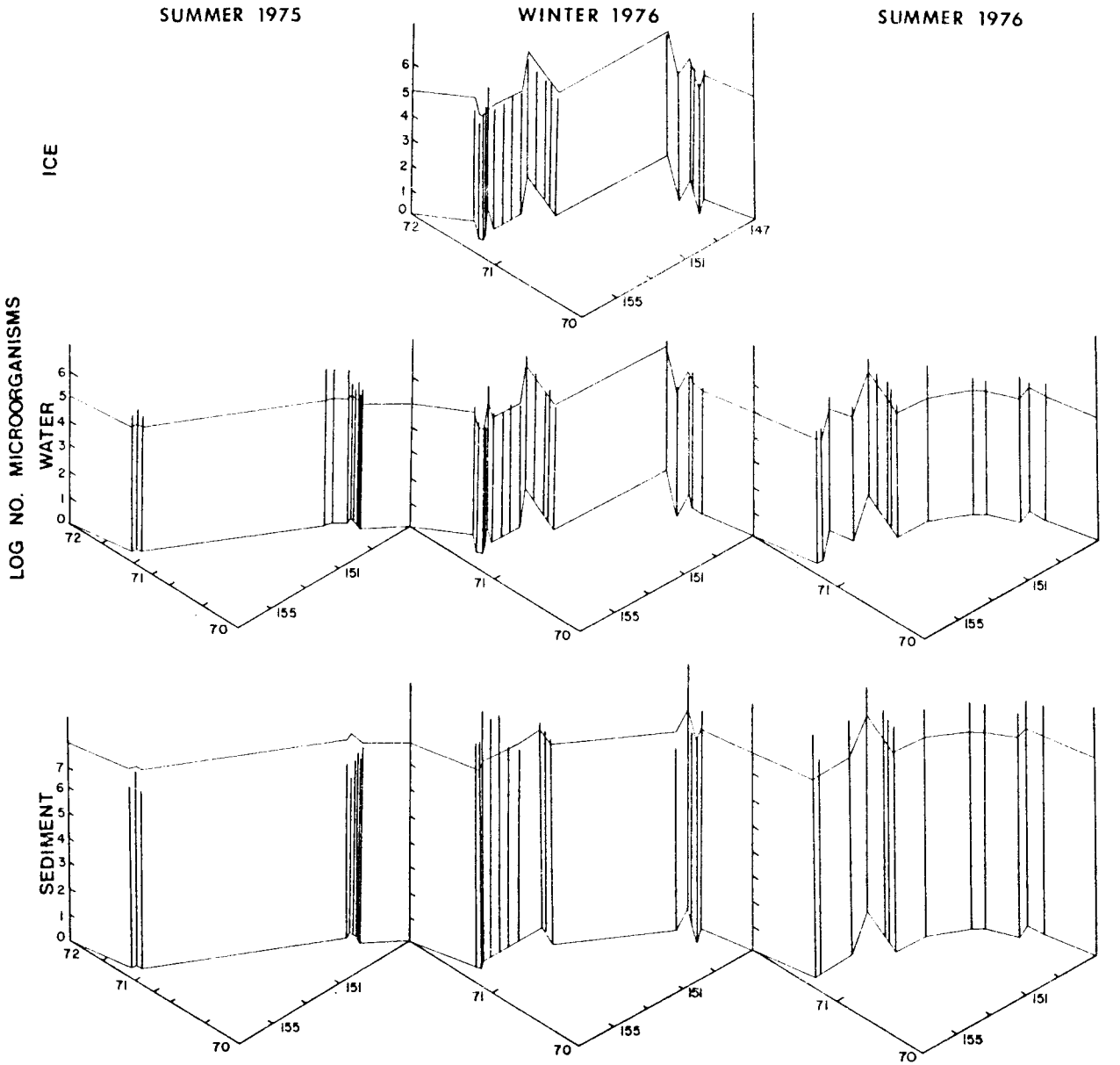


Fig. 2. Enumeration of total microbial populations by direct count procedures.

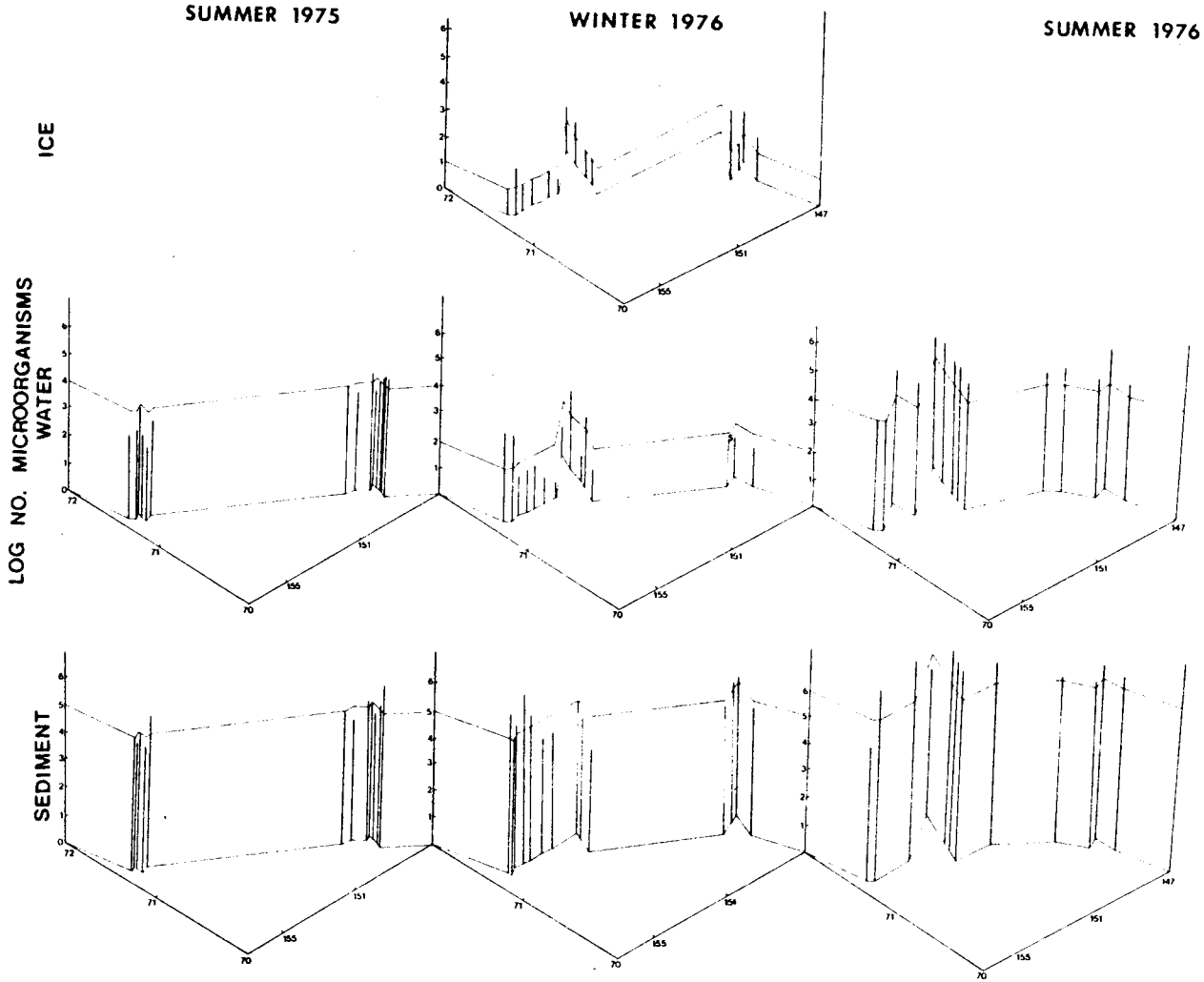


Fig. 3. Enumeration of heterotrophic microbial population at 4 C. (Psychrophilic and psychrotrophic populations)

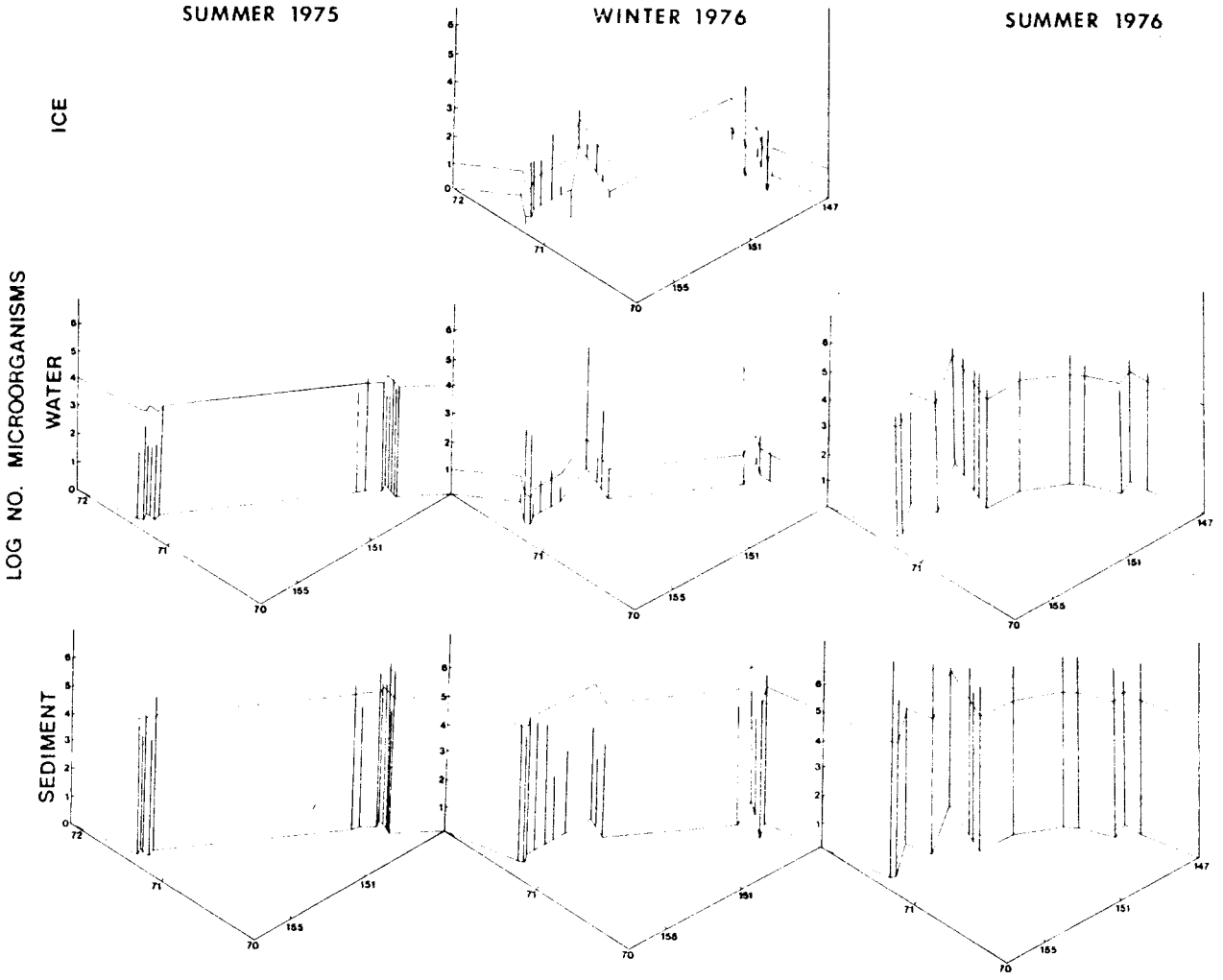


Fig. 4. Enumeration of heterotrophic microbial population at 20 C. (Mesophilic, inc. psychrotrophic populations)

numbers in sea ice. Higher counts were found in both water and sediment in summer 1976 than summer 1975. No spacial variation pattern was apparent in summer 1976.

Unlike the enumerations of viable heterotrophs, populations of presumed Vibrio spp. enumerated at 4 C were higher in both water and sediment during summer 1975 near Pt. Barrow than from Prudhoe Bay (Fig. 5). During winter 1976, counts of Vibrio spp. decreased only slightly in water and increased in sediment. Vibrio spp. were in exceedingly low concentrations in most sea ice samples. During summer 1976 counts of Vibrio spp. in water were lower than in winter or the previous summer. As in the summer 1975, counts in water in summer 1976 were higher near Pt. Barrow and decreased towards Prudhoe Bay. In sediment however, counts were higher in offshore than in nearshore samples and were lower in Elson Lagoon than towards Prudhoe Bay.

Hydrocarbon degrading populations in sediment were higher in samples from near Prudhoe Bay than near Pt. Barrow at all times (Fig. 6). Hydrocarbon degrading populations were higher in summer than winter in both water and sediment. The lowest populations of hydrocarbon degraders were generally found in sea ice. There was a high degree of variability in numbers of hydrocarbon degraders between samples especially in water and ice.

Distribution of Microbial Populations in Relation to Abiotic Parameters

A summary of the means of abiotic parameters is shown in Table

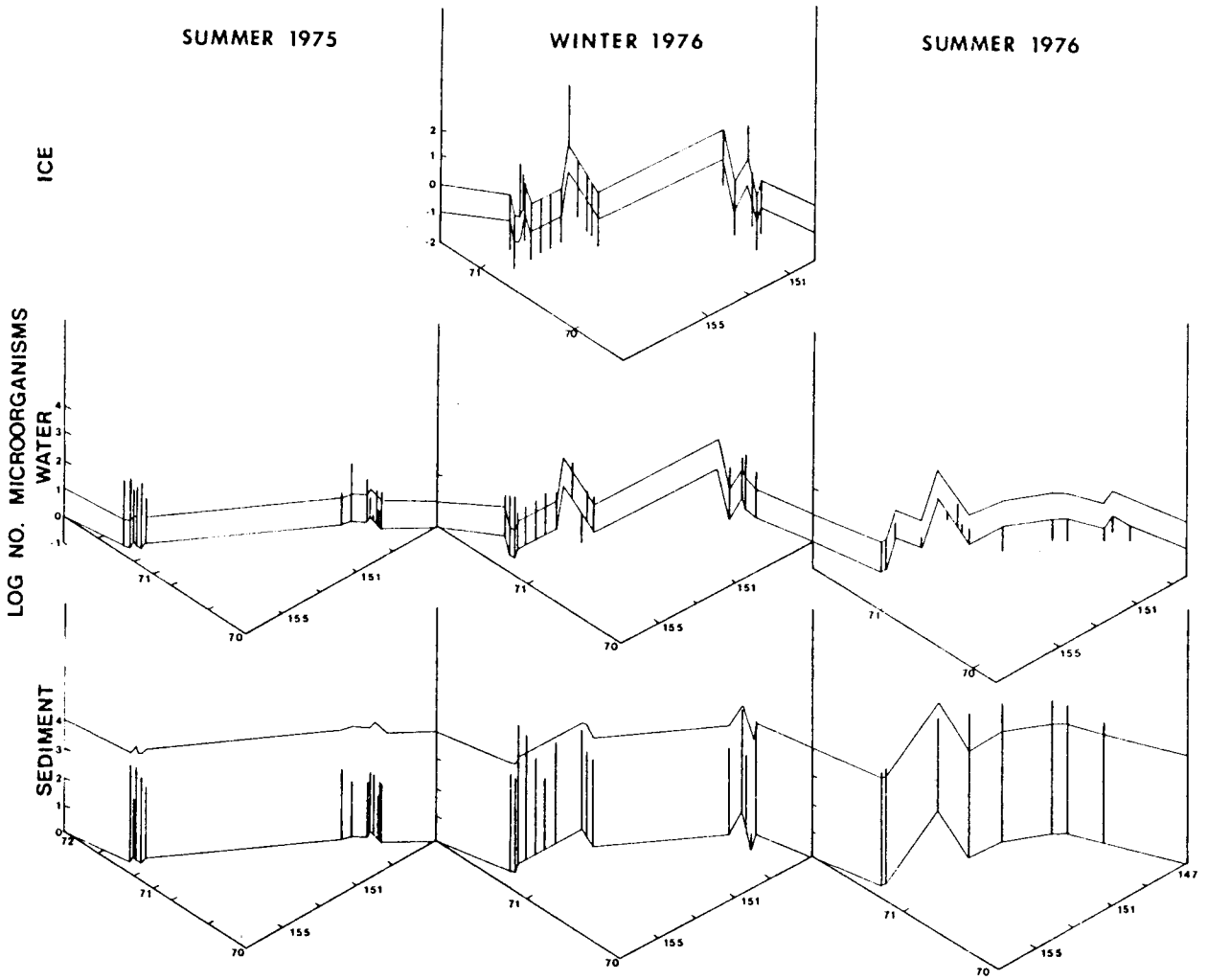


Fig. 5. Enumeration of presumed Vibrio spp. at 4 C.

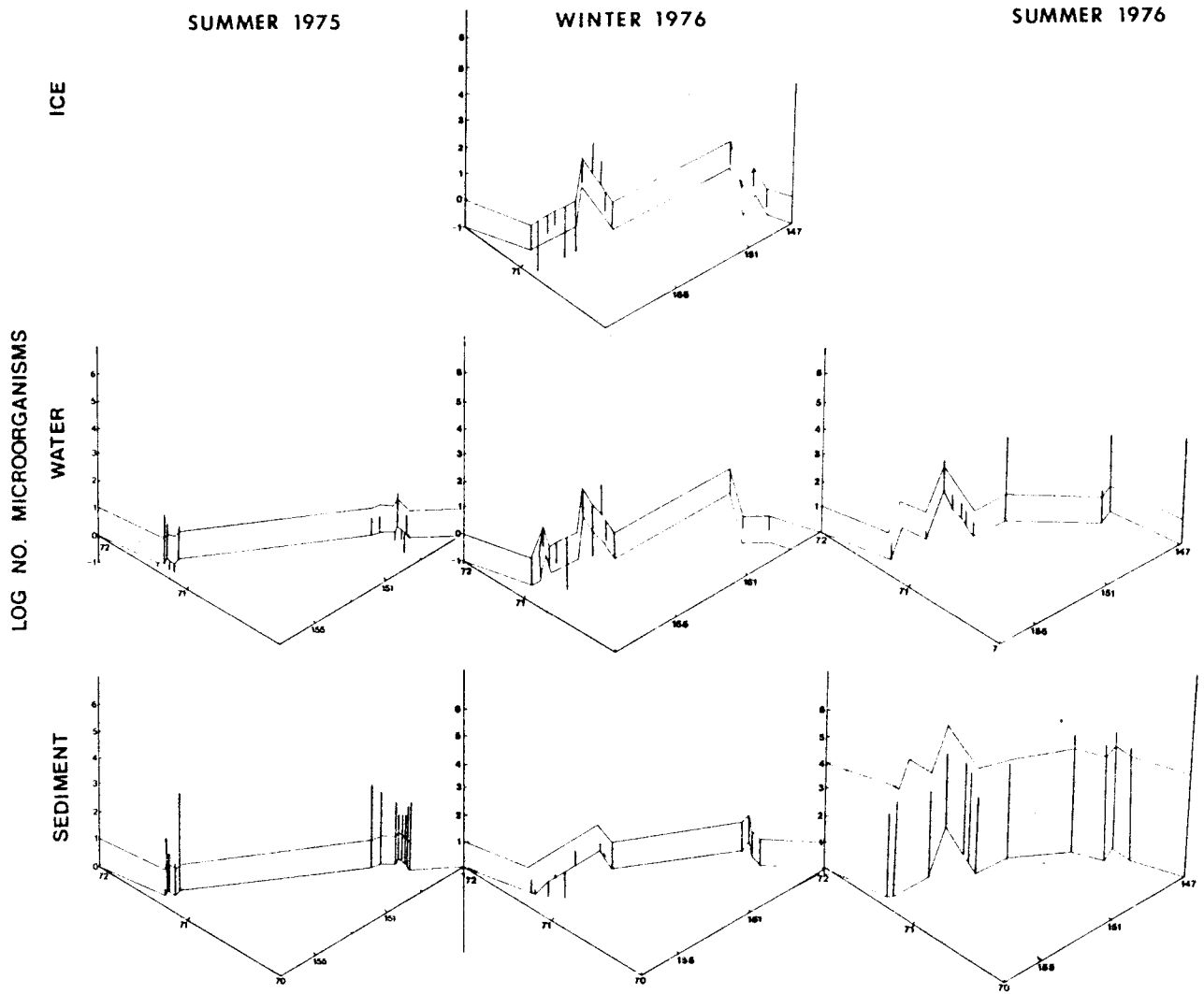


Fig. 6. Enumeration of hydrocarbon degrading microbial population.

1. In water, the mean salinity was lower in summer 1976 than summer 1975 even though samples were collected further from shore in summer 1976. The highest mean salinity and lowest mean temperature in water occurred in winter. The concentration of nitrate ions was significantly higher in winter 1976 than summer 1975. Results of nutrient analyses for summer 1976 have not yet been supplied by Dr. Alexander.

In addition to the mean abiotic parameters shown in Table 1, there were significant spacial variations in abiotic parameters. During both summers, salinities in surface waters were lower near Prudhoe Bay than near Pt. Barrow. In winter, no significant spacial variation in salinity was observed. During summer 1975, temperatures were slightly higher in Prudhoe Bay than in Elson Lagoon. There was no significant difference in concentrations of inorganic nitrogen between samples from Prudhoe Bay and from near Pt. Barrow. Concentrations of phosphate were significantly higher in Elson Lagoon than in Prudhoe Bay during summer 1975. No significant difference in phosphate concentrations though, was seen during winter between samples from near Prudhoe Bay and near Pt. Barrow,including within Elson Lagoon.

During summer, there was a correllation between concentrations of viable bacteria enumerated at 4 C and salinities in surface waters (Figs. 7 and 13). This reflects the lower salinities and higher viable bacterial counts near Prudhoe Bay than near Pt. Barrow. Similarly, the lower phosphate concentrations in Prudhoe Bay

TABLE 1 Average Abiotic Parameters

	SEDIMENT			WATER			ICE
	Summer 1975	Winter 1976	Summer 1976	Summer 1975	Winter 1976	Summer 1976	Winter 1976
Temp. (°C)	1.5	-2.0	0.1	1.1	-1.8	0.6	-2.0
Salinity (°/oo)	-	-	28.6	18.5	24.5	13.8	5.0
$PO_4^{\equiv}$ (µg at/l)	-	-	-	2.2	1.1	-	0.1
NH_4^+ (µg at/l)	-	-	-	1.5	0.5	-	0.3
NO_3^- (µg at/l)	-	-	-	1.2	5.0	-	1.9

than in Elson Lagoon were reflected in a correlation between phosphate concentrations and viable bacteria enumerated at 4 C during summer 1975 (Fig. 8). No correlations were found between direct counts and salinity during either summer (Figs. 9 and 14). During winter, the viable count did not show any significant correlation with salinity (Fig. 10) or phosphate concentration (Fig.11). There was an apparent positive correlation between direct count during winter (Fig. 12),but only a low correlation coefficient. No significant correlation was found in any season between bacterial numbers and temperature, nor between bacterial numbers and concentration of inorganic nitrogen.

General Characterization of Bacterial Populations

General Features

Bacterial isolates were most commonly gram negative rods capable of growth at low temperatures (Table 2). Almost all bacteria isolated at 20 C were psychrotrophs, i.e., also capable of growth at 4 C. Psychrophiles were more frequently isolated in winter than summer. Psychrophiles were more frequently isolated in water than ice during winter. The highest percentage of psychrophiles was found in sediment. Psychrophiles were more frequently isolated from water during summer 1976 than summer 1975. The frequency of isolation of psychrophiles was the same however, in sediment during both summers.

Pigment production was a frequent characteristic of bacteria from summer ice and water and winter ice samples (Table 3, Figs.

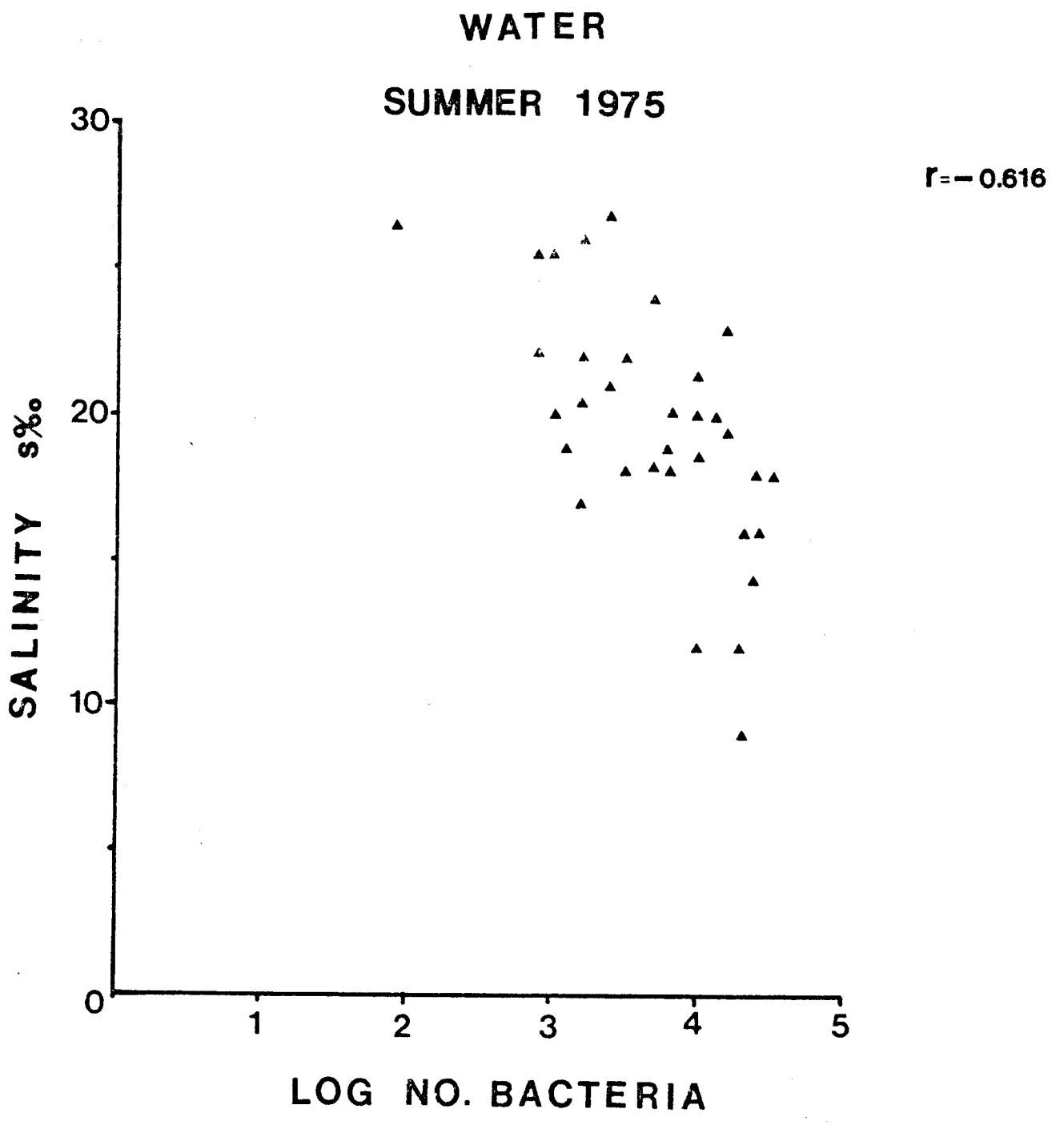


Fig. 7.
Relation of viable 4 C count to salinity in Summer 1975.

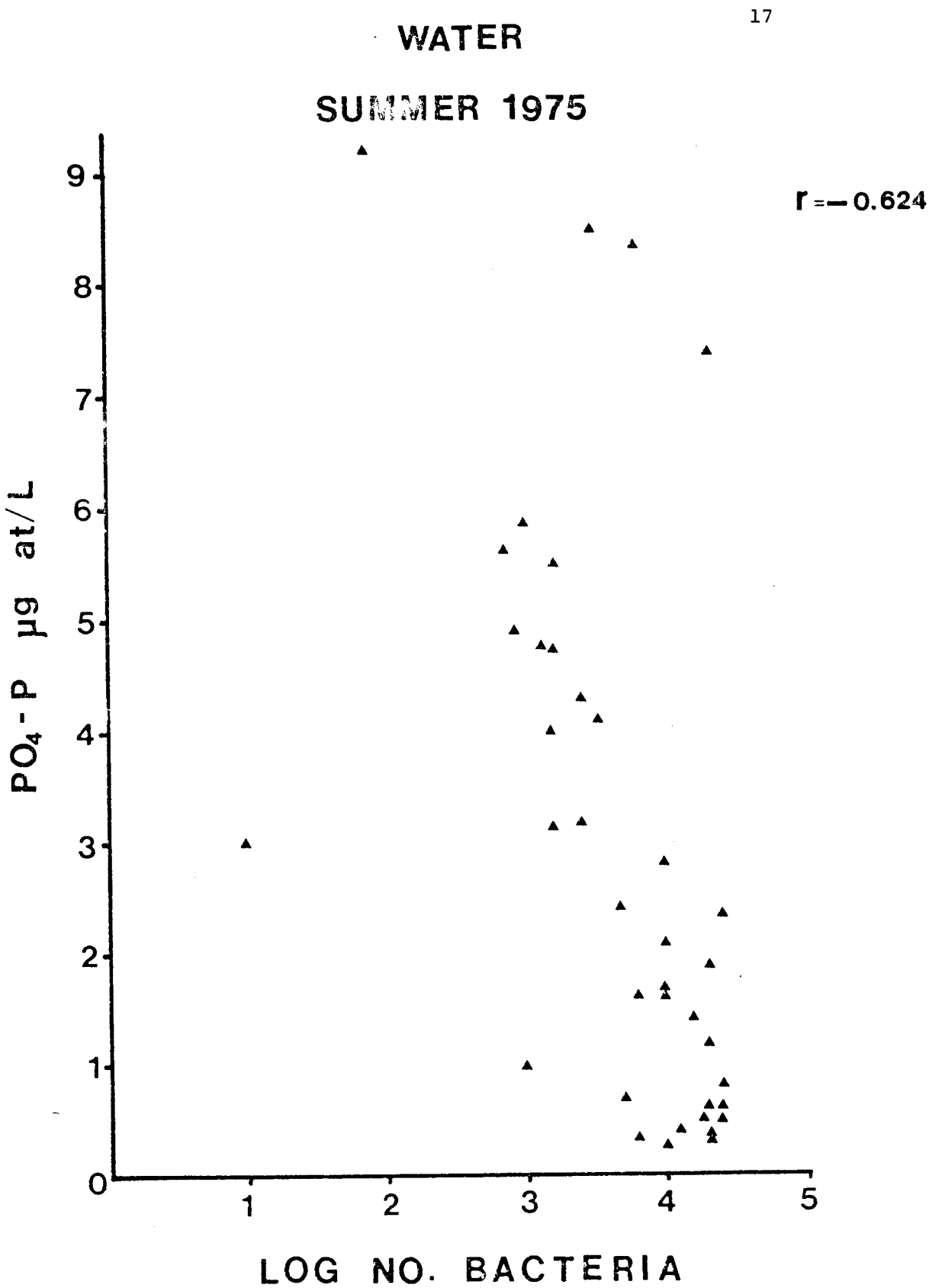


Fig. 8.
Relation of viable 4 C count to phosphate concentration in Summer 1975.

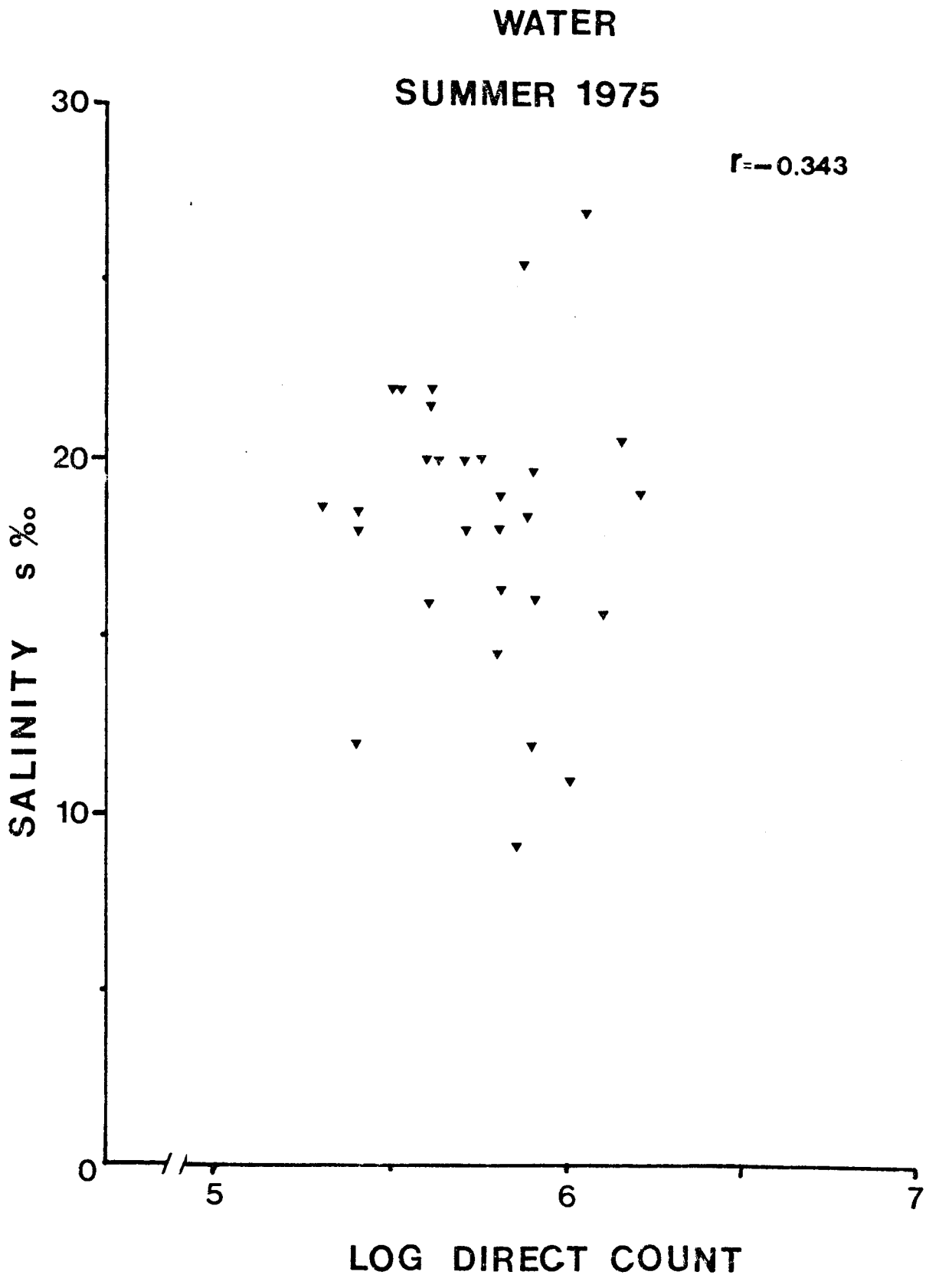


Fig. 9. Relation of total direct count to salinity in Summer 1975.

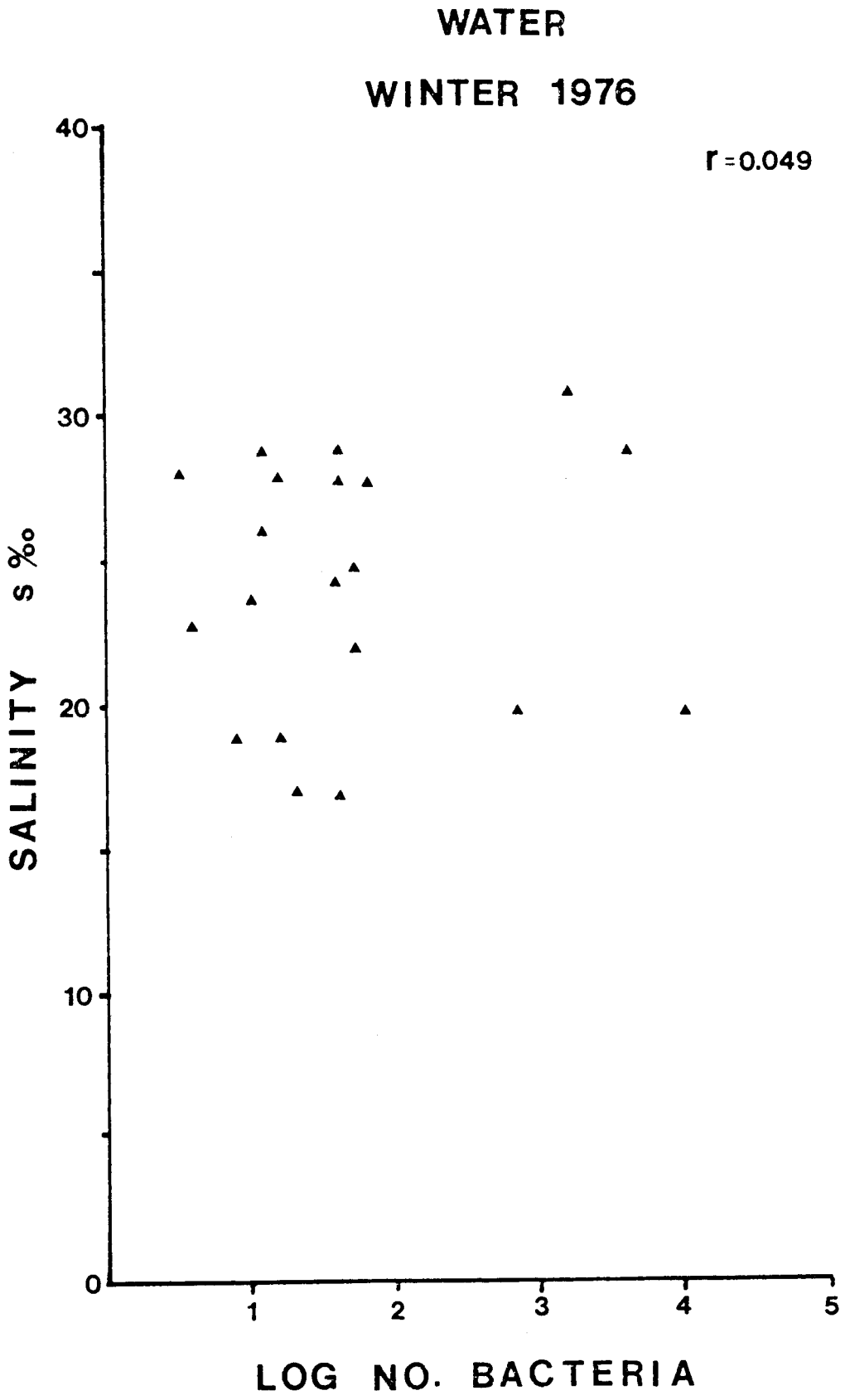


Fig. 10. Relation of viable 4 C count to salinity in Winter 1976.

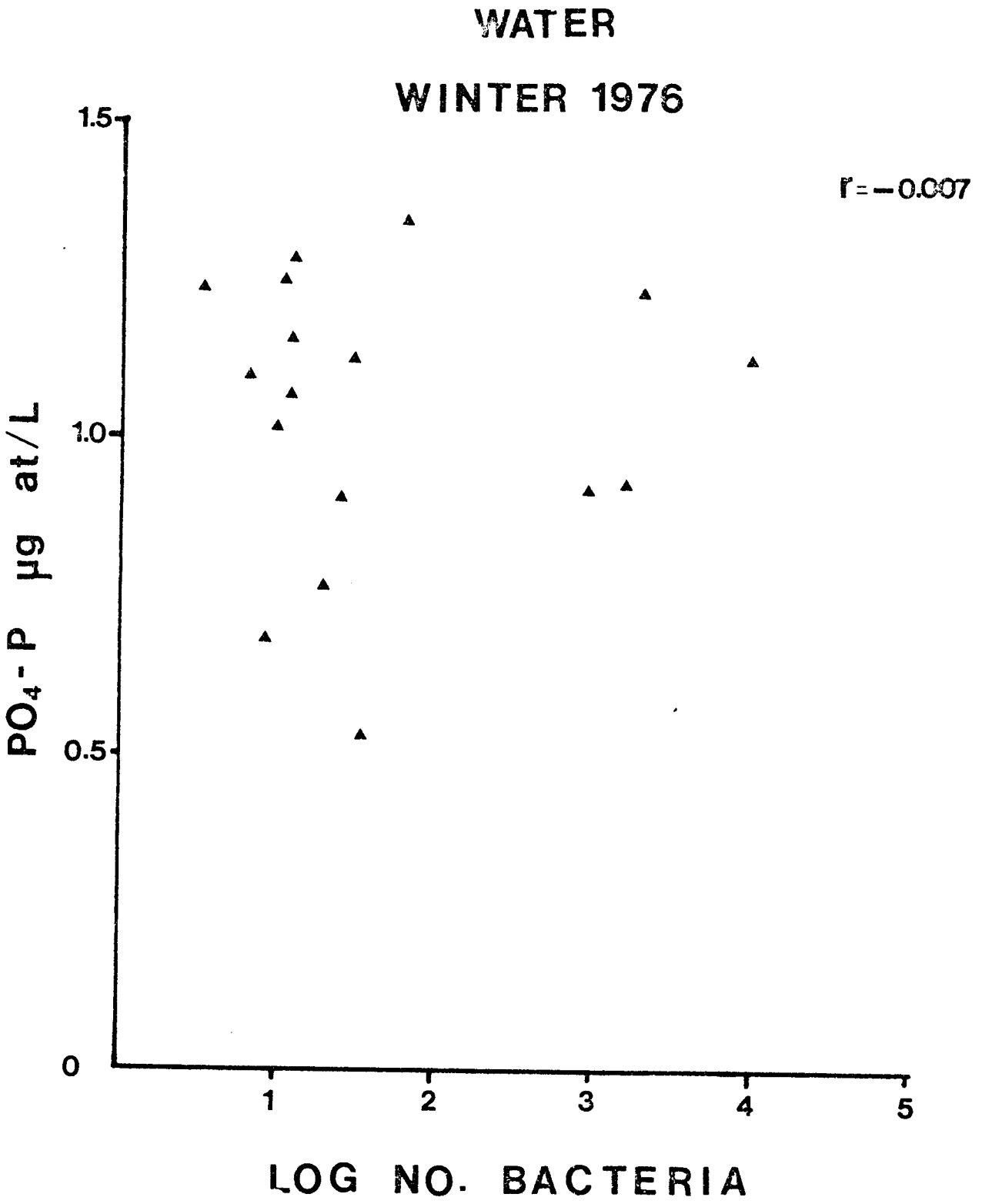


Fig. 11.
Relation of viable 4 C count to phosphate concentration in Winter 1976.

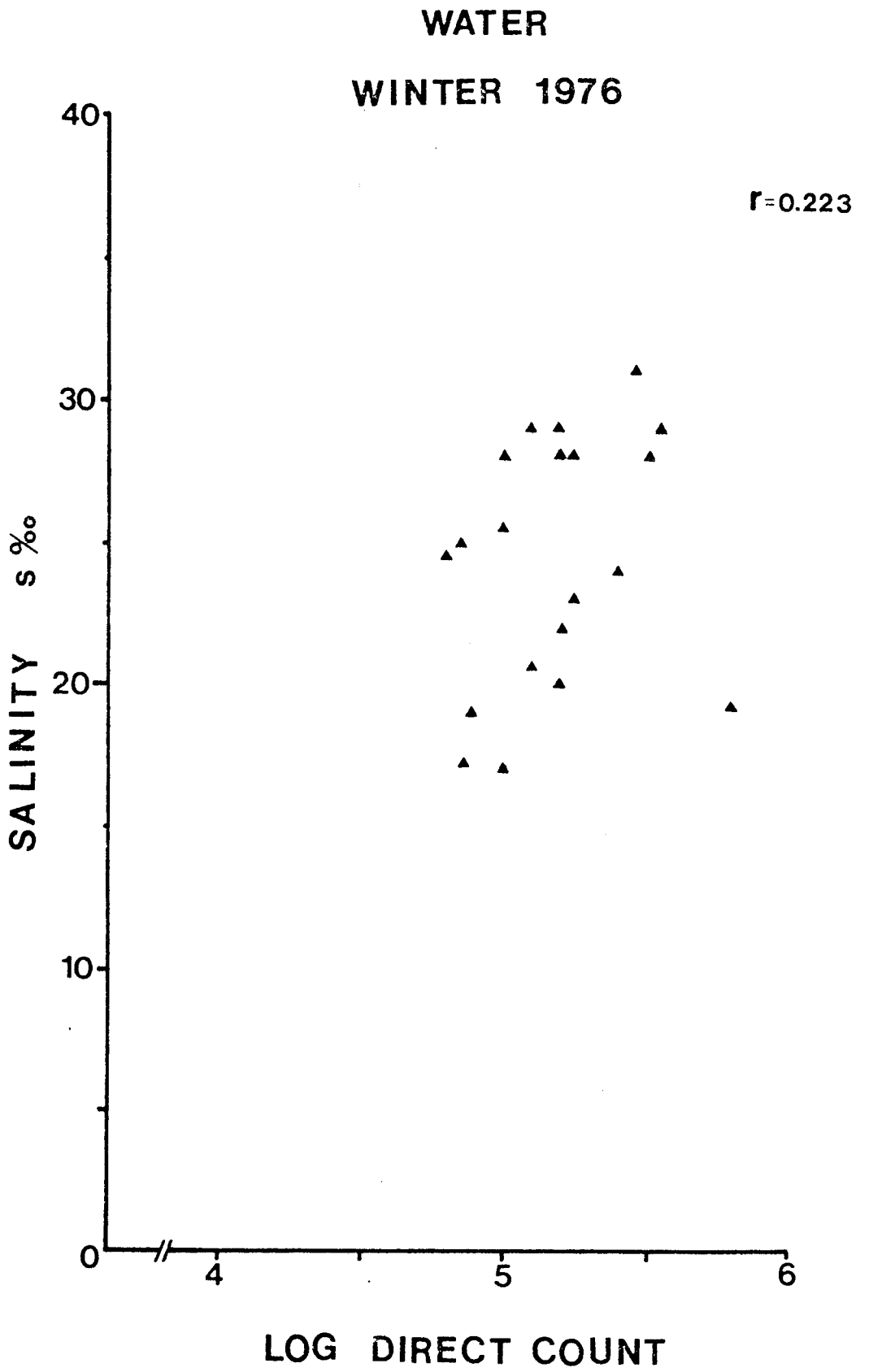


Fig. 12. Relation of total direct count to salinity in Winter 1976.

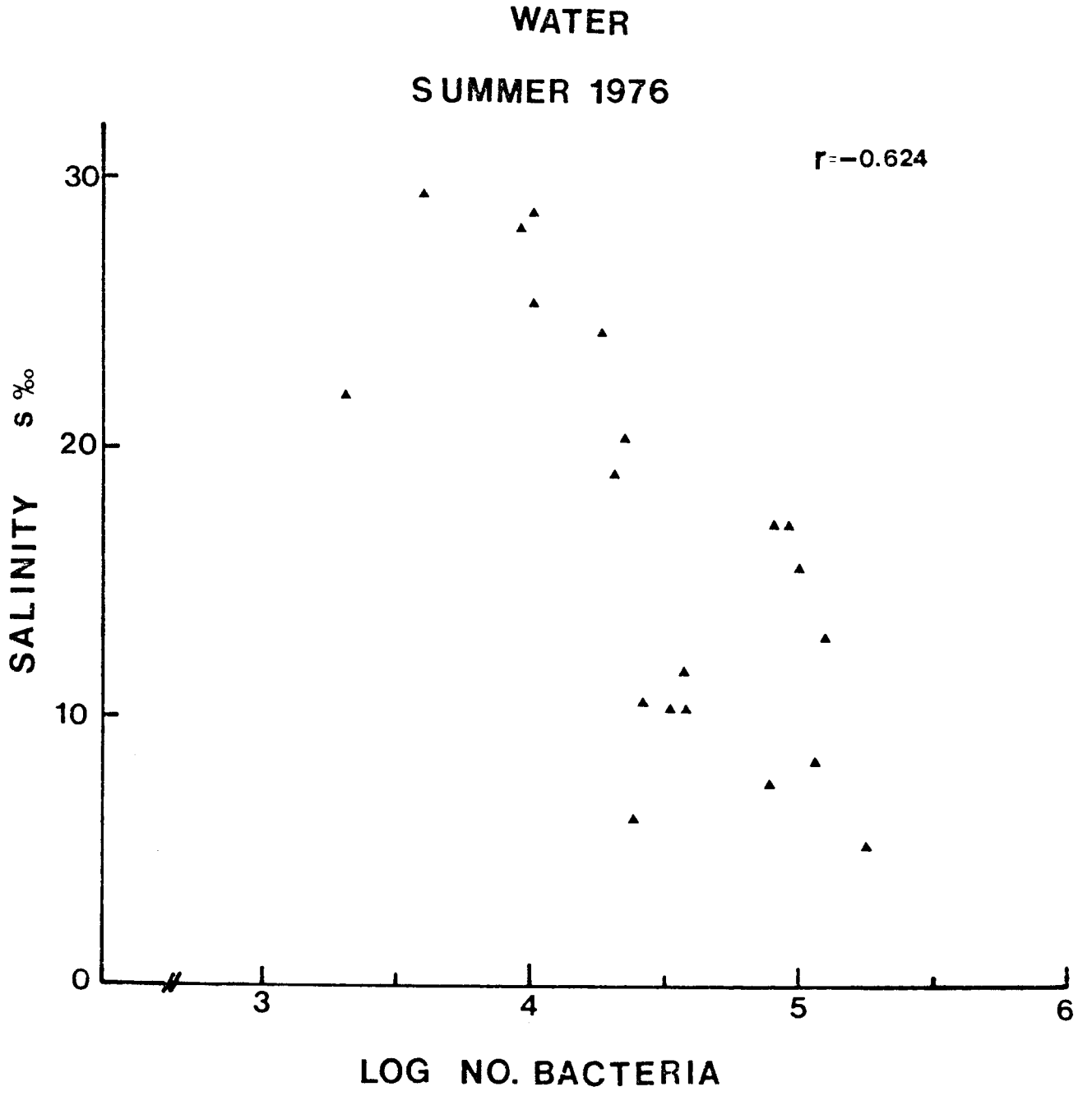


Fig. 13.
Relation of viable 4 C count to salinity in Summer 1976.

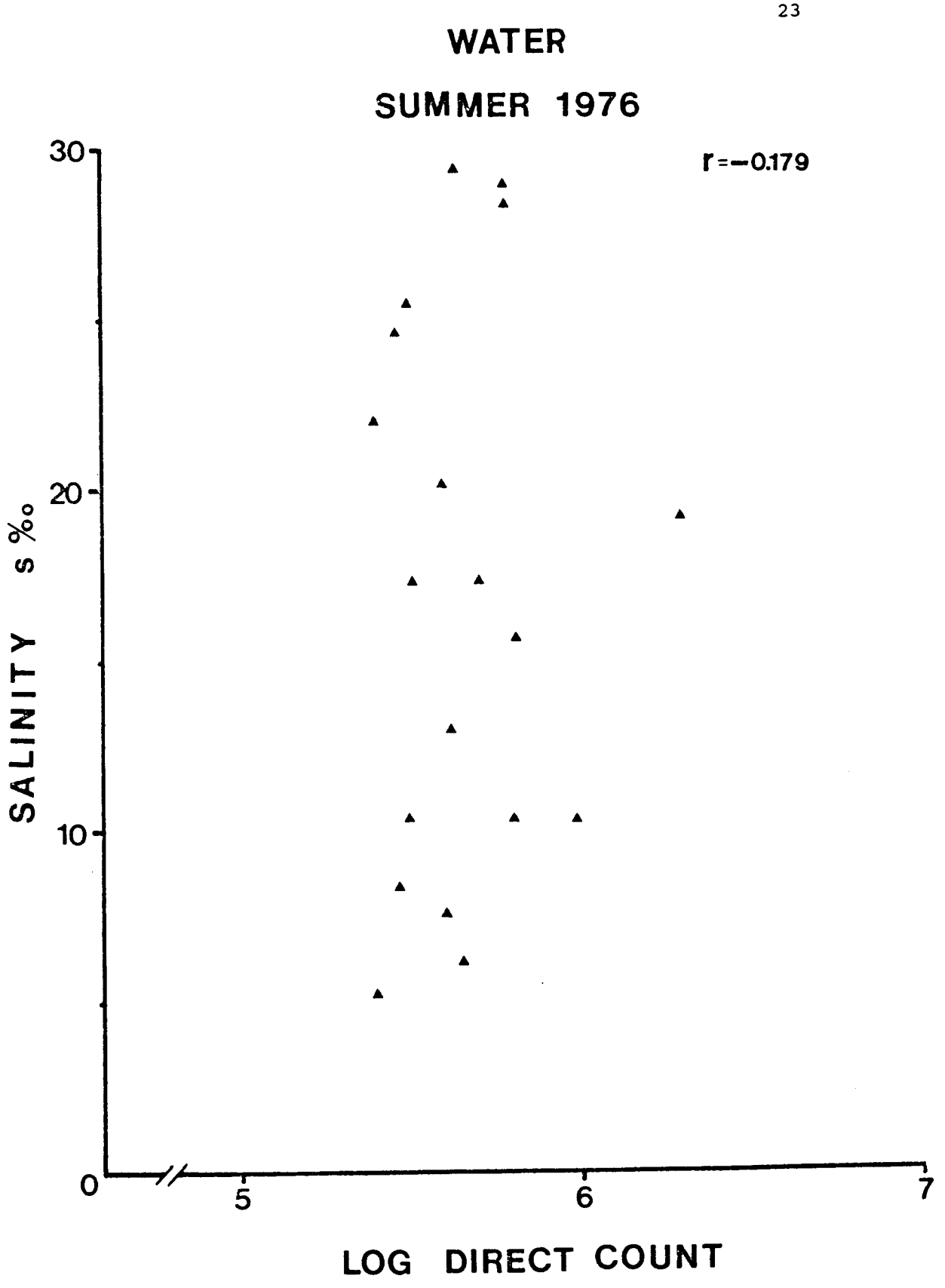


Fig. 14 Relation of total direct count to salinity in Summer 1976.

TABLE 2 General Characteristics of bacterial populations.

		POPULATION ENUMERATED at 4°C			POPULATION ENUMERATED at 20°C	
SEASON	SAMPLE	GRAM NEGATIVE RODS	PSYCHROTROPHS GROW AT 4° & 20°C	PSYCHROPHILES GROW AT 4° BUT NOT 20°C	GRAM NEGATIVE RODS	PSYCHROTROPHS GROW AT 4° & 20°C
SUMMER, 1975	WATER	100	93	7	98	98
	SEDIMENT	100	54	46	99	98
WINTER, 1976	ICE	100	71	29	82	100
	WATER	95	44	56	98	99
	SEDIMENT	100	40	60	97	100
SUMMER, 1976	WATER	100	63	37	-	-
	SEDIMENT	100	56	44	-	-

15-18). Pigment production was much less frequent in water during winter than during summer. Pigment production was much lower in sediment than water or ice during all seasons. A very high percentage of the pigment producing bacteria produced orange colored colonies (Table 3, Fig. 16).

Bacterial Diversity

Bacterial diversity was determined by calculating the species diversity index using the Lloyd, Zar, and Karr modification of the Shannon index. Since bacterial species are very difficult to determine, clusters or phena determined by the numerical taxonomic clustering analyses were used as the equivalent as species. Clusters determined by these procedures are "species" in the sense that they demonstrate a very high degree of phenotypic similarity and are therefore also assumed to be very closely related genetically. Taxonomic clusters had similarities greater than 70%.

The average species diversity indices were higher in sediment than water or ice (Table 4). The average diversity indices of of bacterial populations enumerated at 4 C did not show seasonal variation. The diversity of bacterial populations enumerated at 20 C also did not show seasonal variation in sediment samples but diversity was significantly lower in winter than summer for ice and water samples.

There was a high degree of spacial variation in the diversity indices (Figs. 19-21). There was no pattern to this variation in winter ice samples for either populations enumerated at 4 or 20 C

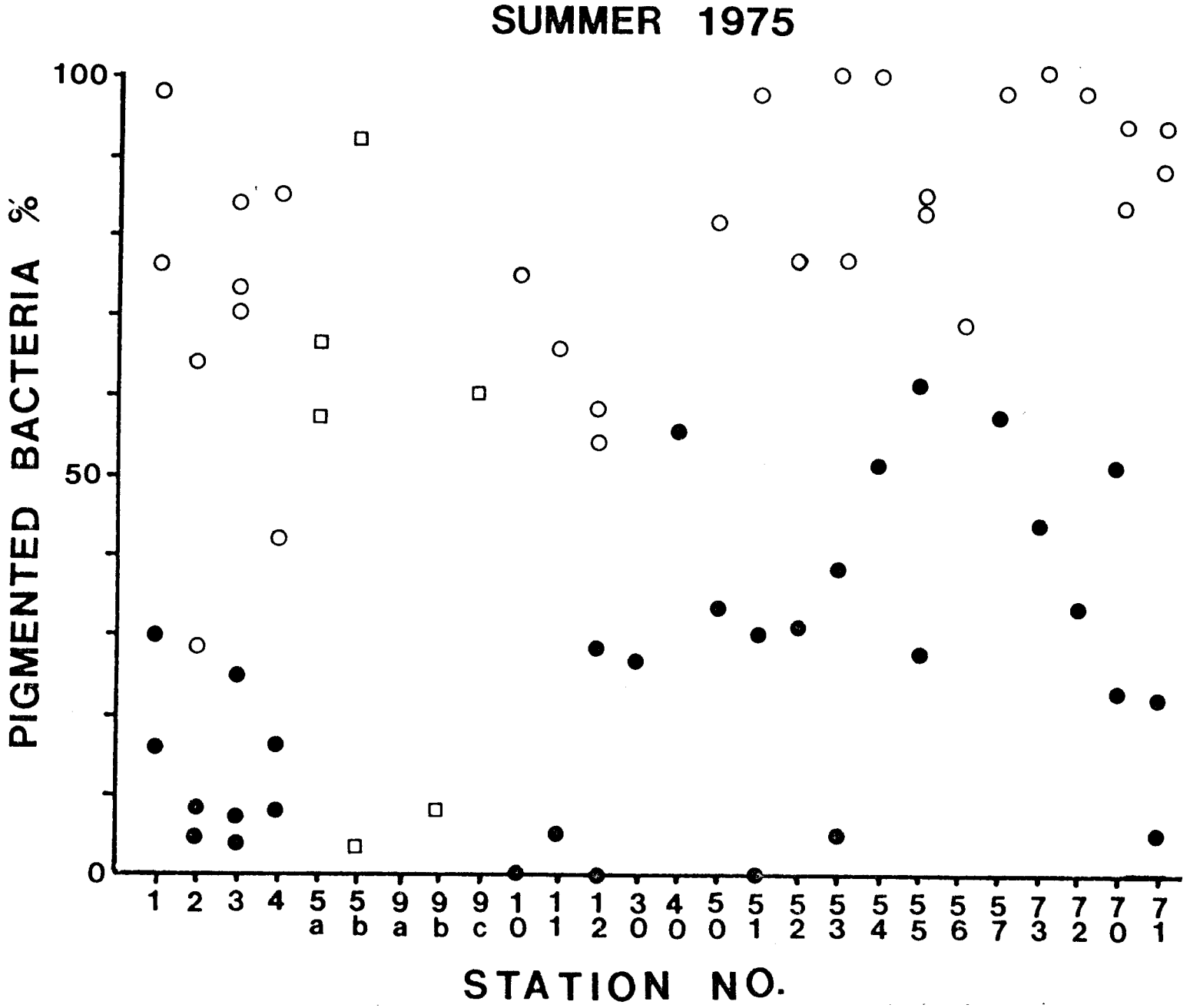


Fig. 15. Distribution of pigment producing bacteria during Summer 1975.

□ = ice; ○ = water; ● = sediment.

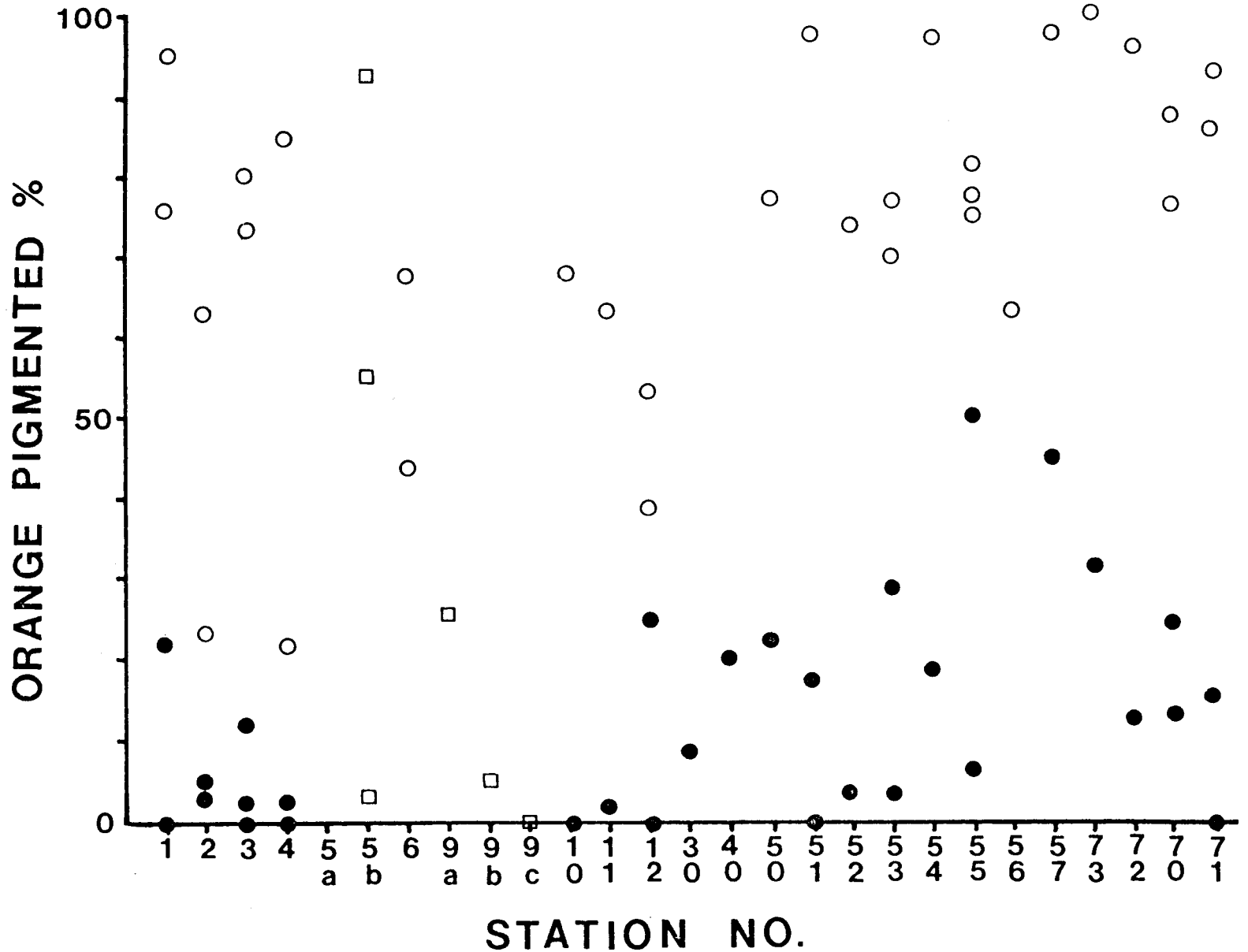


Fig. 16. Distribution of orange pigmented bacteria.
□ = ice; ○ = water; ● = sediment.

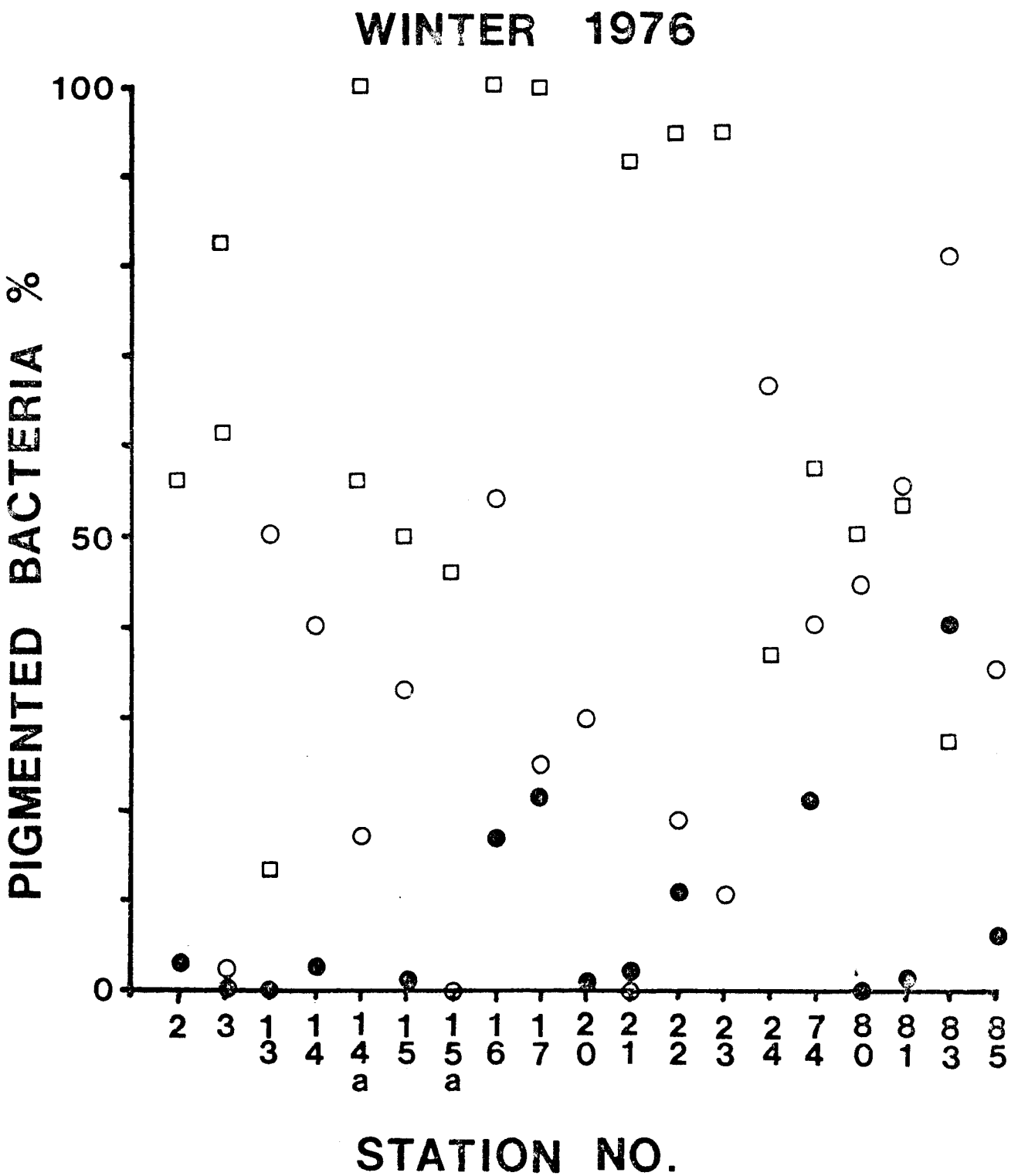


Fig. 17. Distribution of pigment producing bacteria during Winter 1976.

□ = ice; ○ = water; ● = sediment.

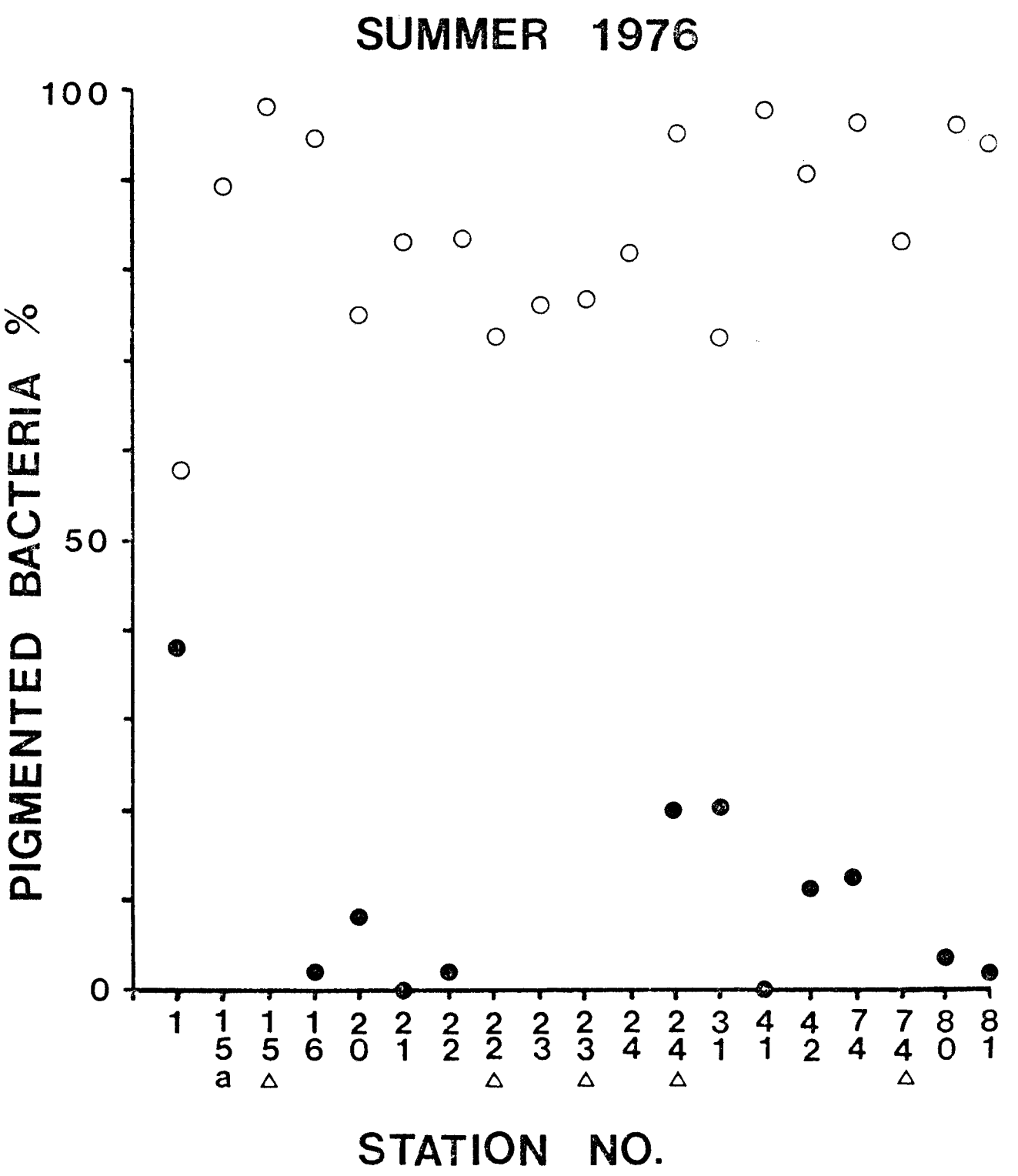


Fig. 18. Distribution of pigment producing bacteria during Summer 1976.

○ = water; ● = sediment.

TABLE 3 Incidence of pigmented and orange pigment producing bacteria*

SEASON	SAMPLE	PB**/TVC**** %	OPB***/TVC %	OPB/PB %
SUMMER, 1975	WATER	78	70	90
	SEDIMENT	27	3	12
	ICE	47	26	56
WINTER, 1976	WATER	30	21	70
	SEDIMENT	9	7	82
	ICE	61	55	90
SUMMER, 1976	WATER	91	88	97
	SEDIMENT	17	13	76

* Based of original plating observations

** PB: Pigmented bacteria

*** OPB: Orange pigment producing bacteria

**** TVC: Viable count at $4^{o}C$

TABLE 4 Average Species Diversity Indexes ($\bar{d}$)

	WATER		ICE		SEDIMENT	
	$4^{o}C$	$20^{o}C$	$4^{o}C$	$20^{o}C$	$4^{o}C$	$20^{o}C$
Summer, 1975	2.3	2.1	-	-	3.5	3.5
Winter, 1976	2.6	1.1	3.0	1.4	3.6	3.5
Summer, 1976	2.4	-	-	-	4.0	-

(Fig. 19). In water samples however, bacterial diversity was lower near Prudhoe Bay than near Pt. Barrow during summer, but higher near Prudhoe Bay than near Pt. Barrow during winter (Fig. 20). The bacterial diversity in sediment samples was relatively constant regardless of site of sample collection (Fig. 21).

There was a lack of correlation between population size and diversity index for ice and sediment samples (Figs. 22 and 24).

In water, however, at population levels below approximately 10^4 viable bacteria/ml, the bacterial diversity index increased with decreasing numbers of bacteria, i.e., the lower the number of bacteria, the greater the diversity (Fig. 23). At population levels of 10^4 viable bacteria/ml or greater, the diversity index was generally between 1 and 3 and was correlated with population size. For equivalent population sizes, bacterial diversity was much lower during winter than during summer in water samples.

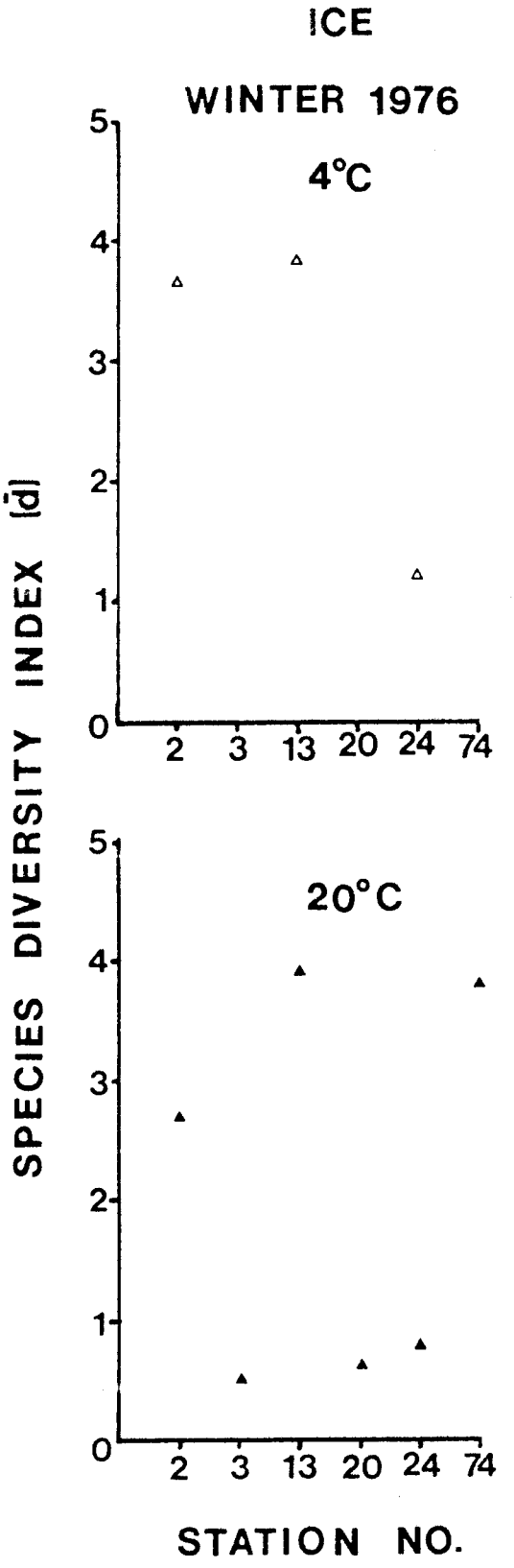


Fig. 19. Spacial variation of bacterial diversity in ice.

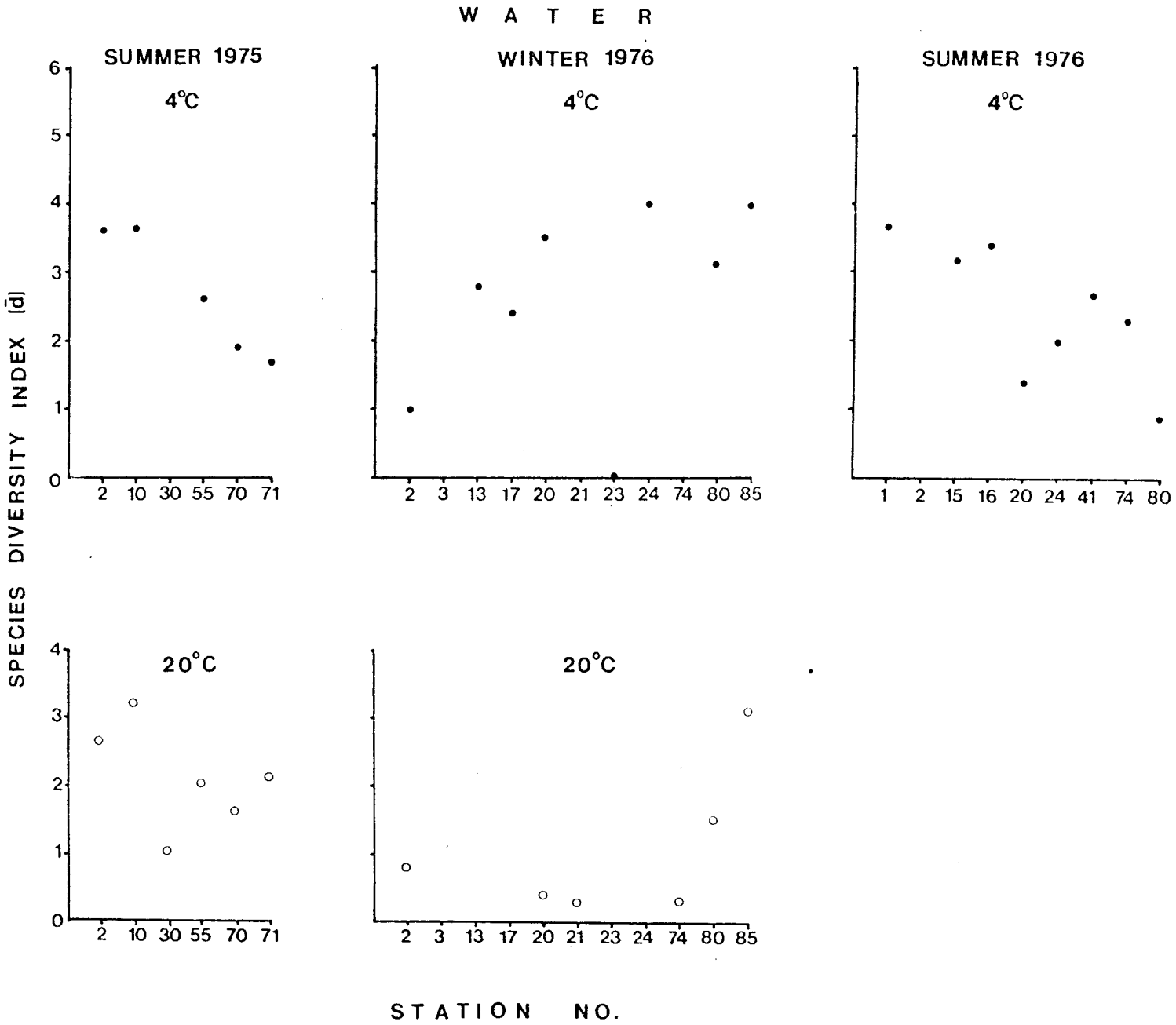


Fig. 20. Spacial variation of bacterial diversity in water.

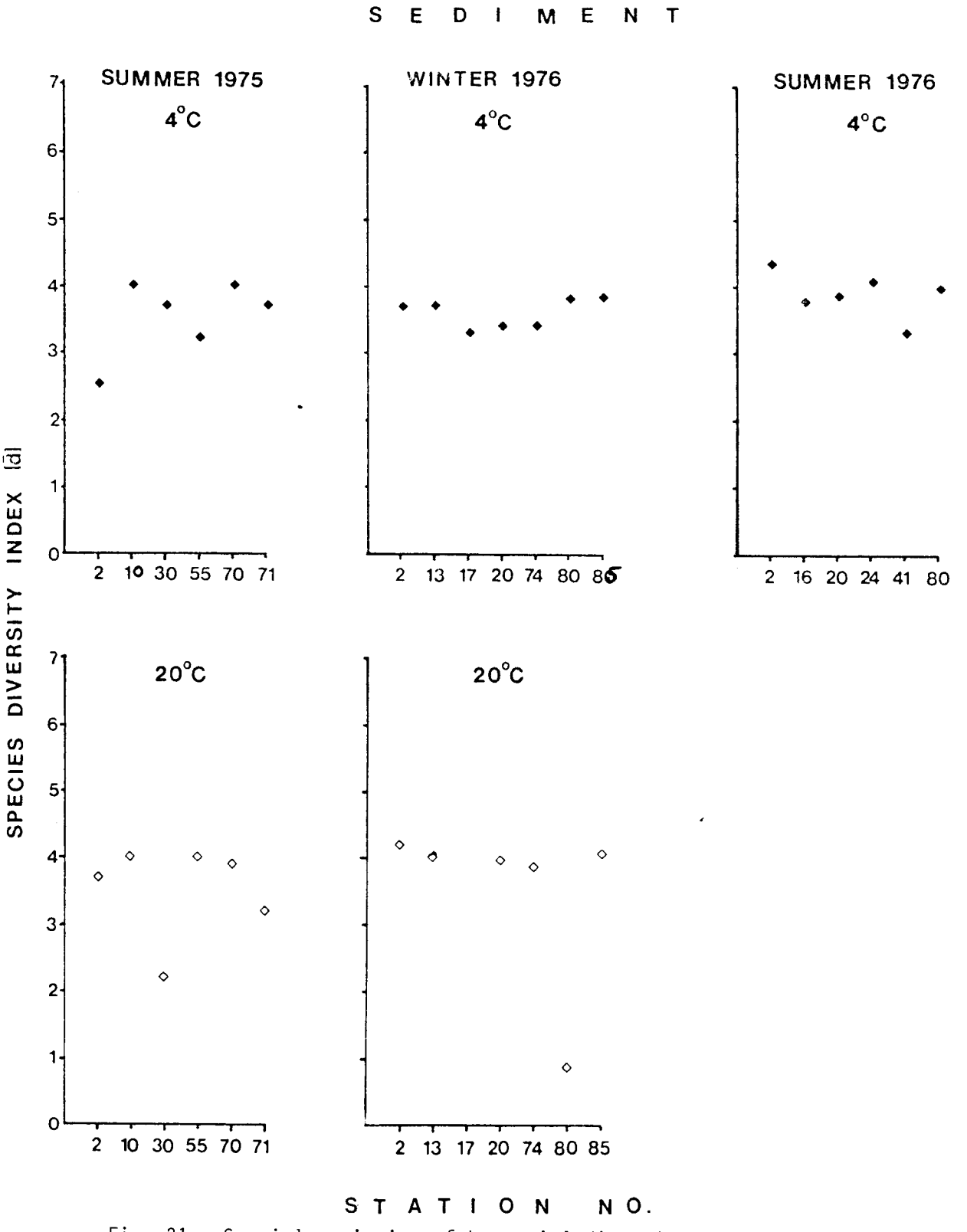


Fig. 21. Spacial variation of bacterial diversity in sediment.

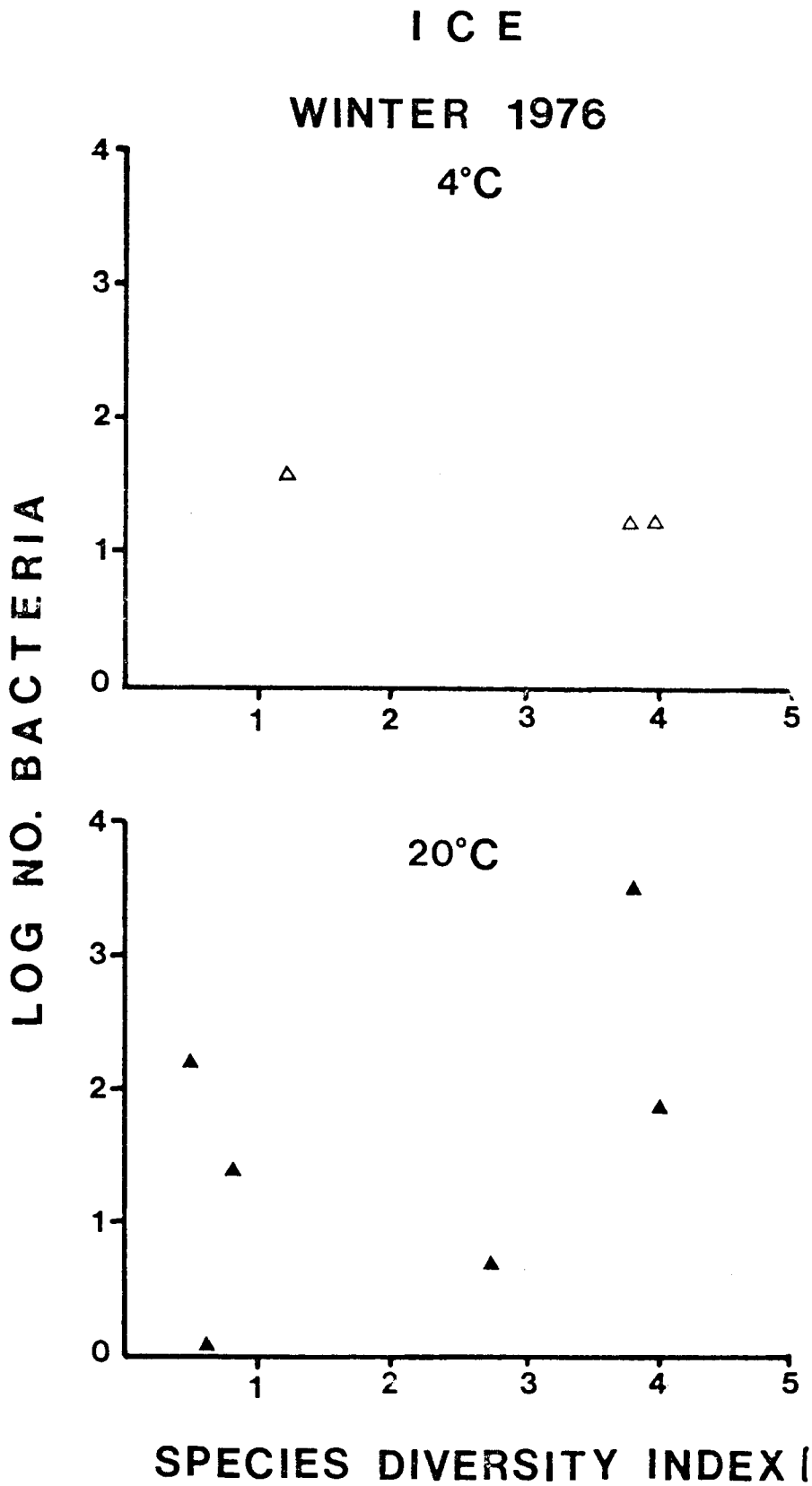


Fig. 22. Relationship between population size and bacterial diversity in ice.

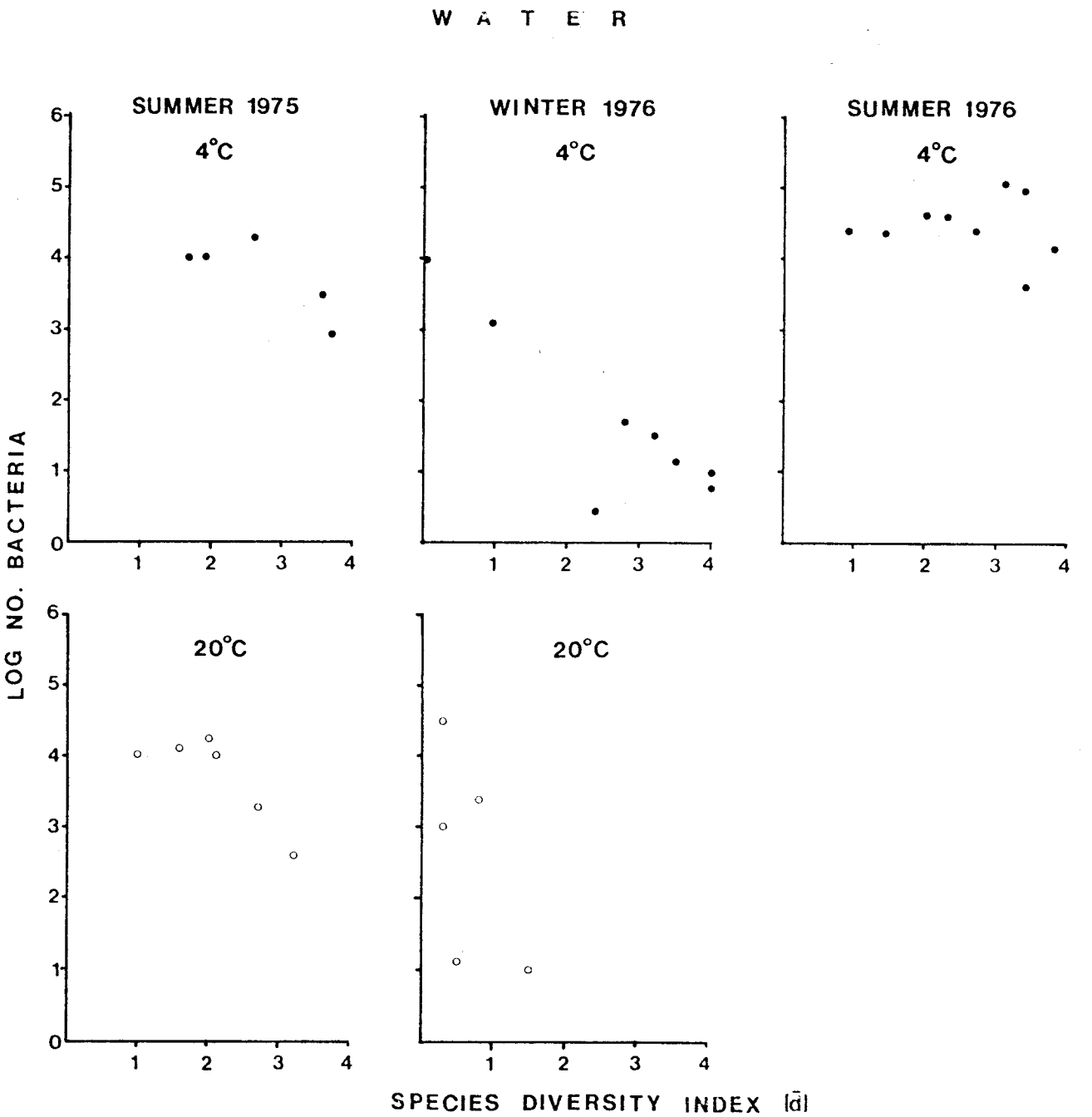


Fig. 23. Relationship between population size and bacterial diversity in water.

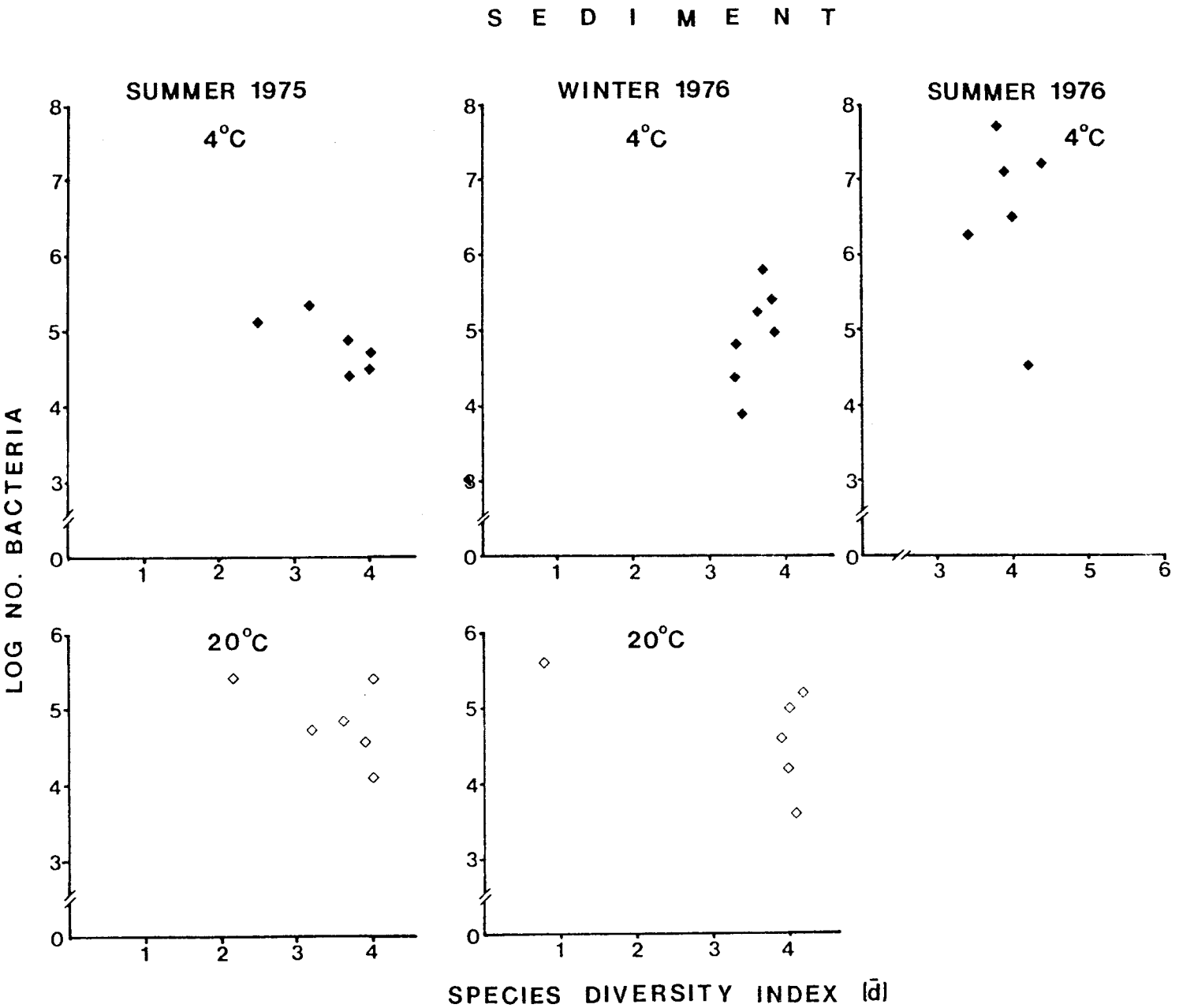


Fig. 24. Relationship between population size and bacterial diversity in sediment.

Spacial and Seasonal Characteristics of Bacterial Populations

Summer 1975

The frequency and distribution of positive features for bacterial populations isolated during summer 1975 are shown in Table 5. Frequency is shown on a scale of 0 to 10, with each unit equal to approximately 10%. A total of 276 bacteria isolated at 4 C, and 277 bacteria isolated at 20 C, were examined. These bacteria were isolated from station 2 in Elson Lagoon, station 10 offshore Pt. Barrow, and stations 55, 70, and 71 in Prudhoe Bay. Station 71 was the furthest offshore station in Prudhoe Bay.

Morphologically, populations isolated from water and sediment at 4 or 20 C were gram negative rods with rounded ends that occurred singly. Pleomorphism was more common in organisms isolated at 4 C (80-90%) than in those isolated at 20 C (40-60%). Curved rods were also more common in sediment organisms isolated at 4 C than in other organisms. Surprisingly, bacteria isolated from water at either 4 or 20 C were nonmotile. Fifty percent of the bacteria isolated from sediment at 4 C were motile. Capsule production was more common in sediment isolates (20-30%) than water isolates (10%). Pigment production, already described (Figs. 15 and 16), was more common in water than sediment isolates.

With respect to physiological features, all isolates could grow at temperatures of 5-15 C. Bacteria isolated at 4 C most frequently had an upper growth temperature of 20 C. Ninety percent of bacteria

Table 5. Features of bacteria isolated at 4 and 20 C from water and sediment at various stations during summer 1975 on a scale of 0 to 10 (0=0%, 10=100% positive results for organisms tested).

	WATER											SEDIMENT												Water Total	Water Total	Sediment Total	Sediment Total
	4°C					20°C						4°C						20°C						4°C	20°C	4°C	20°C
STATION	2	10	55	70	71	2	10	30	55	70	71	2	10	30	55	70	71	2	10	30	55	70	71				
Cell Morphology:																											
Pear shaped	5	7	0	2	3	3	0	5	4	4	2	3	1	1	0	1	3	0	0	2	1	0	1	3	3	2	1
Rod shaped	10	10	10	10	10	10	9	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
Curved	4	4	1	3	2	2	2	2	1	1	0	7	8	6	4	4	6	2	0	8	3	2	5	3	1	6	3
Cocco-bacillary	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0
Pleomorphic	9	8	9	10	10	0	5	8	3	5	4	9	9	8	9	9	3	7	4	8	5	8	5	9	4	8	6
Tapered end(s)	4	1	1	2	2	5	0	2	0	1	0	1	1	1	0	2	1	0	1	0	0	2	0	2	1	1	1
Round end(s)	10	10	10	10	10	9	10	10	9	10	9	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
Square end(s)	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cells:																											
Single	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
Chain	4	3	4	5	6	1	2	3	1	0	2	0	2	5	4	3	1	1	1	1	0	0	0	4	2	3	1
Pair	0	0	0	0	0	1	3	0	1	1	1	0	0	0	0	0	0	1	2	0	1	1	0	0	1	0	1
Irregular	0	4	0	0	0	1	2	2	4	3	0	0	1	0	1	0	0	3	1	1	1	2	0	1	2	0	1
Snapping	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0
Branch	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Filamentous	1	1	5	3	4	0	0	0	1	0	0	0	0	2	1	4	0	0	0	0	0	1	0	3	0	1	0
PHB	2	0	2	2	1	2	0	2	1	0	1	1	1	1	0	1	1	1	2	0	0	2	0	1	1	1	1
Volutin	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	6	1	0	2	0	0	0	2
Capsule	1	1	2	0	3	2	0	1	0	1	0	0	0	0	5	4	4	3	0	1	6	1	0	2	1	1	3	2
Acid fast	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sudan Black B	3	0	2	2	2	3	0	5	1	0	1	1	1	0	0	2	1	0	3	1	1	2	0	2	2	1	1
Gram +	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Gram -	10	10	10	10	10	10	8	10	10	10	9	10	10	10	10	10	10	10	9	10	10	10	10	10	9	10	10
Gram variable	0	0	0	0	0	0	0	0	0	10	0	0	0	0	0	0	0	0	1	0	0	0	0	0	2	0	0
Motility	0	1	0	0	0	0	1	0	0	0	1	7	8	4	2	2	7	0	1	0	1	0	0	0	0	5	0
Fluorescent	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Length:																											
0.5-1.0μ	0	0	0	0	0	1	1	0	1	1	0	0	0	0	0	0	0	2	1	0	0	1	0	0	1	0	1
1.1-2.0μ	2	2	2	3	5	1	1	7	3	5	3	0	0	2	4	1	0	2	3	1	2	1	1	3	3	1	2
2.1-3.0μ	5	5	3	5	3	3	2	1	3	2	4	2	2	4	3	6	2	3	0	3	4	4	2	4	4	3	3

STATION	2	10	55	70	71	2	10	30	55	70	71	2	10	30	55	70	71	2	10	30	55	70	71	Water Total 4°C	Water Total 20°C	Sediment Total 4°C	Sediment Total 20°C
3.1-4.0μ	1	3	4	0	1	1	4	1	2	1	2	6	5	3	2	2	7	1	4	4	2	2	3	2	2	4	3
4.1-5.0μ	1	0	0	0	0	2	1	0	1	1	0	1	4	1	0	1	0	1	1	0	1	2	3	0	1	1	1
5.1-10.0μ	0	0	0	0	1	1	1	0	1	1	0	1	0	0	1	0	0	0	1	1	1	0	0	0	1	0	1
Width:																											
<0.5μ	0	2	1	1	3	1	1	2	0	1	2	0	0	2	0	2	0	5	0	2	1	4	2	1	1	0	2
0.5-1.0μ	9	8	9	9	7	9	9	9	9	9	8	9	9	7	9	8	8	6	10	8	9	6	8	8	9	8	8
1.1-2.0μ	0	0	0	0	0	0	0	0	1	1	0	1	0	1	0	0	1	0	0	0	0	0	0	0	0	1	0
Non-diffusible pigments:																											
Yellow	1	2	0	0	0	1	7	1	1	1	2	1	0	1	1	2	0	7	3	1	3	2	3	1	2	1	3
Orange	5	8	7	8	9	1	1	1	1	2	1	0	0	1	6	4	2	2	2	5	1	3	6	8	1	2	3
Gray	4	1	2	2	1	8	2	8	8	8	7	8	8	6	2	3	7	1	4	3	5	4	1	2	7	6	3
White	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	2	0	1	0	0	0	0	0	0	1	0
Growth in liquid media:																											
Floccular	1	4	0	0	0	4	4	0	1	0	6	0	5	2	1	1	1	7	3	0	2	4	2	1	3	2	3
Ring	1	2	0	0	1	1	2	1	0	0	0	2	2	3	2	0	2	1	1	7	2	3	2	1	7	2	3
Pellicle	1	2	0	0	0	1	2	0	1	0	3	1	6	0	0	1	4	0	1	0	1	2	1	1	1	2	1
Growth even	4	6	3	3	4	4	7	5	4	5	6	7	8	7	6	5	6	3	7	10	8	6	9	4	5	7	7
Growth slight	6	3	9	8	8	7	5	4	9	7	6	2	1	3	5	5	2	7	3	7	4	2	5	7	6	3	4
Growth moderate	3	2	1	2	1	3	4	5	1	3	3	7	7	7	5	3	6	2	7	8	5	7	5	2	3	6	6
Growth heavy	0	0	0	0	0	0	2	0	0	0	1	1	1	0	0	1	2	0	0	0	1	1	0	0	1	1	0
No growth in liquid media	0	0	1	2	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0
Facultative anaerobe	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
Colony morphology:																											
Diameter <1.0mm	10	9	10	8	10	6	6	3	5	4	4	5	2	6	9	8	4	8	5	5	5	5	6	9	5	6	6
Diameter 1.0-2.0mm	0	1	0	0	0	3	3	4	5	7	6	4	5	0	1	1	2	3	3	4	4	3	4	0	5	2	4
Diameter 2.1-6.0mm	0	0	0	0	0	0	1	3	0	0	0	1	3	4	0	1	3	0	2	0	1	2	0	0	1	2	1
Translucent	5	7	8	10	10	0	4	5	8	6	4	3	7	8	8	7	4	5	1	7	6	5	8	8	5	6	5
Transparent	5	2	1	0	0	4	0	1	0	0	0	1	1	0	1	2	0	0	1	2	1	2	1	2	1	1	1
Opaque	0	2	0	0	0	6	6	4	2	5	6	7	1	1	2	2	6	4	8	0	2	4	1	0	5	3	3
Entire	10	10	8	10	10	10	9	10	9	10	9	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
Erose	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lobate	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Undulate	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Raised	0	0	1	0	0	1	1	2	5	1	0	1	1	1	0	0	2	0	0	0	1	1	2	0	2	1	1

STATION	2	10	55	70	71	2	10	30	55	70	71	2	10	30	55	70	71	2	10	30	55	70	71	Water Total 4°C	Water Total 20°C	Sediment Total 4°C	Sediment Total 20°C
Umbonate	0	0	0	0	0	1	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0
Convex	10	10	9	10	10	8	8	8	6	10	9	9	9	8	10	10	8	10	9	9	9	8	7	10	8	9	9
Glistening	10	10	10	10	10	8	8	8	9	8	10	10	10	10	9	10	10	10	8	10	10	9	10	10	9	10	10
Dull	0	0	0	0	0	2	2	2	1	2	0	0	0	0	1	0	0	0	2	0	0	1	0	0	2	0	1
Smooth	10	10	10	10	10	10	9	7	10	8	9	10	10	10	10	10	10	10	10	10	10	7	10	10	9	10	10
Rough	0	0	0	0	0	0	1	3	0	2	1	0	0	0	0	0	0	0	0	0	1	2	0	0	1	0	1
Temperature:																											
5°C	10	10	10	10	10	10	10	10	10	9	9	10	10	10	10	10	10	10	10	10	8	10	10	10	10	10	10
10°C	10	10	10	10	10	10	10	10	10	10	9	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
15°C	10	10	10	10	10	10	10	10	10	9	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
20°C	10	5	10	9	10	10	10	10	10	9	10	3	2	8	8	8	3	10	10	10	10	10	10	9	10	5	10
25°C	3	1	3	2	1	8	10	10	9	9	8	1	0	4	2	3	1	5	8	10	9	8	7	2	9	2	8
37°C	0	0	0	0	0	0	1	0	0	2	1	0	0	0	0	0	0	0	1	0	0	1	0	0	1	0	3
43°C	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
pH:																											
4	0	0	0	0	0	0	6	0	0	5	3	0	0	1	0	0	0	0	3	0	0	1	0	0	2	0	1
5	3	7	6	5	6	5	10	3	3	3	8	7	9	10	9	8	8	5	8	9	7	7	9	5	8	9	8
6	9	9	10	9	8	10	10	10	10	9	10	10	10	10	10	9	9	10	10	10	10	10	10	9	10	10	10
7	10	10	10	10	10	10	10	10	10	9	10	10	10	10	9	10	10	10	10	10	9	10	10	10	10	10	10
8	10	10	10	10	10	10	10	10	10	9	10	10	10	10	9	9	10	10	10	10	10	10	10	10	10	10	10
9	7	9	8	7	7	9	9	9	8	9	8	9	10	9	8	6	8	1	8	3	8	4	4	8	9	8	5
10	0	0	0	0	0	0	2	0	1	1	1	6	7	2	1	2	7	0	0	0	0	0	0	0	1	4	0
Percent NaCl:																											
0	1	3	1	1	0	2	8	2	3	5	3	0	0	4	4	2	0	0	5	3	4	3	3	1	4	2	3
0.5	8	9	10	9	10	9	10	10	9	10	9	3	1	10	9	8	3	4	8	9	10	10	9	9	10	6	8
3	8	9	9	9	8	10	10	10	10	9	9	10	10	10	8	9	8	10	10	10	10	10	10	9	10	9	10
5	7	8	7	8	7	10	9	10	9	8	9	6	8	7	8	7	6	3	10	7	8	5	6	7	9	7	7
7.5	3	3	3	4	1	5	5	8	8	8	3	1	0	1	6	2	2	0	6	4	4	3	3	5	6	2	3
10	3	3	1	0	1	4	4	7	8	6	3	1	0	1	*	2	0	1	8	5	7	6	2	2	5	1	5
15	0	1	0	0	0	0	2	0	0	0	1	0	0	0	5	0	0	0	2	0	0	0	0	0	1	1	0
Hydrolysis of:																											
Starch	1	3	0	0	0	0	7	0	1	2	1	6	6	3	1	1	4	7	4	6	4	5	5	1	2	3	5
Gelatin	4	7	4	7	2	3	3	0	1	2	2	3	6	6	6	7	2	3	4	6	3	5	4	5	2	5	4
Agar	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

STATION	2	10	55	70	71	2	10	30	55	70	71	2	10	30	55	70	71	2	10	30	55	70	71	Water Total 4°C	Water Total 20°C	Sediment Total 4°C	Sediment Total 20°C
Lipase:																											
Tween 20	2	0	3	1	0	4	7	4	4	3	7	7	8	8	3	5	6	4	7	4	4	0	5	1	5	6	4
Tween 80	3	2	2	2	1	7	9	8	9	4	8	7	8	6	5	7	4	4	8	8	8	8	9	2	8	6	8
Alkaline phosphatase	8	8	10	9	10	8	3	8	8	8	7	8	8	8	10	9	9	7	9	9	8	8	8	9	7	9	8
Catalase	9	8	9	10	10	8	10	6	5	7	6	8	5	8	6	8	7	8	10	7	8	9	9	9	7	7	9
Oxidase	0	0	0	0	0	0	0	1	1	0	0	1	3	1	1	0	2	0	2	0	1	1	1	0	0	1	1
Indole production	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Haemolysis (β-)	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
NH_3 production	0	5	0	0	0	4	3	3	3	3	6	7	5	3	2	2	5	5	5	8	6	8	9	1	6	4	7
Reduction NO_3+NO_2	0	1	0	0	0	1	6	1	1	1	3	8	10	7	2	3	9	3	4	2	2	1	2	0	2	7	2
Reduction of NO_2	*	*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Arginine deaminase	5	5	9	2	9	0	0	1	4	4	1	2	4	6	10	5	2	1	0	4	2	3	4	6	2	5	2
Lysine decarboxylase	4	3	0	5	0	1	0	0	0	1	2	3	1	3	2	2	2	0	2	0	0	0	1	2	1	2	1
Ornithine decarboxylase	2	0	0	0	0	1	1	1	0	0	3	1	1	2	1	1	0	3	1	2	3	0	2	0	1	1	2
Acid from:																											
D-Ribose	2	1	0	0	4	3	1	1	3	5	5	6	3	1	2	1	4	1	1	2	2	1	0	1	3	3	1
D-Fructose	1	1	5	0	0	3	2	0	7	1	2	6	3	1	3	1	4	2	0	6	1	2	3	1	1	3	2
D-Glucose (oxidative)	1	2		0	4	1	3	0	1	0	3	8	8	4	5	5	7	5	3	2	5	4	3	2	1	6	4
D-Glucose (fermentative)	3	7	2	0	2	3	3	7	8	6	3	8	9	4	4	5	8	3	3	7	6	5	6	3	5	6	5
Cellobiose	0	2	0	0	0	2	1	0	1	1	1	1	2	1	0	2	2	0	1	1	2	1	0	0	1	1	1
Lactose	0	2	0	0	0	0	2	0	1	1	1	0	0	0	1	0	1	2	0	0	3	2	0	0	1	0	1
Sucrose	2	3	6	3	8	4	2	1	8	7	5	0	0	3	5	3	2	2	3	5	3	3	6	4	5	2	4
D-Mannitol	0	1	0	0	0	0	1	2	1	1	2	0	0	0	2	0	1	2	1	2	1	3	2	0	1	1	2
Gas from glucose	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Antibiotics:																											
Ampicillin, 2µg	9	10	10	10	10	10	8	10	9	10	8	5	5	9	10	9	6	7	8	9	10	10	10	10	9	7	9
Aureomycin, 30µg	8	0	9	10	10	10	9	10	10	10	10	8	10	8	10	9	10	10	10	10	9	0	8	7	10	9	8
Colistin, 10µg	5	1	3	3	5	9	3	8	8	8	8	9	7	5	5	6	7	6	7	10	8	9	7	3	7	7	8
Erythromycin, 2µg	10	10	10	10	10	10	9	10	9	10	9	10	10	9	10	10	10	10	10	10	10	10	9	10	10	10	10
Kanamycin, 30µg	3	0	5	4	3	10	8	10	10	9	9	3	4	7	5	6	5	6	8	9	9	9	9	3	9	5	8
Neomycin, 30µg	5	0	4	4	4	10	3	9	9	9	9	3	8	8	5	6	5	7	7	10	9	9	9	3	8	6	9
Pencillin G, 2 units	8	8	10	10	10	9	8	10	9	9	7	5	2	7	8	7	5	6	7	8	9	9	9	9	9	6	8

STATION	2	10	55	70	71	2	10	30	55	70	71	2	10	30	55	70	71	2	10	30	55	70	71	Water Total 4°C	Water Total 20°C	Sediment Total 4°C	Sediment Total 20°C
Polymyxin B, 300 units	5	3	8	8	6	9	3	8	10	7	8	5	5	10	10	9	8	8	6	9	9	9	8	6	8	8	8
Streptomycin, 20µg	5	1	4	3	4	8	5	9	7	7	7	3	1	3	3	5	2	4	4	3	7	6	5	3	7	3	5
Tetracyclin, 5µg	4	7	6	3	5	1	1	3	2	3	2	3	2	3	4	4	3	3	2	0	3	2	2	5	2	3	2
Oxytetracyclin, 5µg	6	7	7	8	5	2	1	0	2	3	2	3	2	5	5	5	3	8	3	6	4	6	5	7	2	4	5
Carbohydrates & Sugar Derivatives:																											
L-Arabinose	0	0	1	0	2	0	5	0	1	2	2	2	1	0	0	2	0	0	0	0	7	2	1	1	2	1	2
D-Ribose	0	0	0	0	1	0	2	1	0	1	1	5	3	0	1	1	3	0	4	0	0	0	0	0	1	2	1
D-Xylose	1	0	4	2	3	6	5	7	7	7	8	1	3	0	0	2	1	0	3	0	2	3	1	2	7	1	2
L-Rhamnose	0	0	0	0	1	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D-Fructose	1	0	3	4	5	3	7	7	6	8	3	5	3	2	2	3	3	0	4	1	2	3	1	3	6	3	2
D-Galactose	2	2	7	6	6	3	3	6	5	5	5	6	2	2	3	3	2	1	3	1	4	2	1	5	5	3	2
D-Glucose	4	4	8	6	7	10	8	9	8	8	8	8	5	3	5	4	4	1	5	2	7	3	1	6	9	5	3
D-Mannose	2	3	4	3	5	0	5	0	2	2	3	6	3	1	2	1	2	0	0	0	2	2	1	3	2	3	1
L-Sorbose	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
Salicin	0	1	0	0	1	0	1	0	1	0	1	6	1	0	0	1	2	0	2	0	1	2	1	0	1	2	1
Cellobiose	1	0	3	2	4	5	5	8	9	9	7	0	1	0	0	2	2	0	5	2	6	4	2	2	7	1	3
Lactose	0	2	0	0	0	0	2	0	1	0	1	0	0	0	0	1	0	0	0	0	3	1	0	0	1	0	1
Maltose	3	2	6	6	6	3	7	4	2	2	3	7	4	1	5	5	5	0	6	1	3	2	1	5	4	5	2
Sucrose	1	1	5	2	3	0	7	0	1	1	1	0	0	1	2	1	1	0	4	1	2	1	0	2	2	1	1
Trehalose	0	0	0	0	0	0	7	0	1	2	2	0	2	0	1	2	3	0	4	1	1	2	1	0	2	1	2
D-Raffinose	0	0	0	0	0	0	6	0	1	2	1	0	0	0	0	2	0	0	0	0	1	2	2	0	2	0	1
Fatty Acids:																											
Acetate	2	0	3	2	1	8	6	8	8	9	8	1	1	2	2	2	2	1	6	1	5	2	2	2	8	2	3
Propionate	2	0	1	2	1	5	7	7	3	5	6	1	0	2	1	2	0	1	5	1	3	2	1	1	6	1	2
Butyrate	2	0	2	2	1	5	7	7	6	3	6	1	0	3	1	2	1	2	4	2	2	3	2	1	6	1	3
Valerate	0	0	0	1	0	1	7	3	6	4	4	0	0	1	0	0	1	1	3	1	2	1	2	0	4	0	2
Isovalerate	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Caproate	1	0	1	2	1	4	6	8	7	8	5	1	0	2	1	2	1	1	6	3	4	2	2	1	6	1	3
Caprylate	4	0	3	2	1	8	8	9	8	9	7	1	2	6	2	3	2	1	8	3	5	4	3	2	8	3	4
Laurate	0	0	0	1	0	0	4	3	0	7	0	0	0	0	0	0	0	0	0	1	2	0	0	0	2	0	1
Palmitate	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Stearate	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Oleate	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dicarboxylic acids:																											
Malonate	3	0	3	2	1	5	3	8	6	7	5	1	0	1	1	1	1	1	1	0	2	1	0	2	6	1	1
Succinate	0	0	0	0	0	0	3	0	1	0	2	0	0	0	0	0	0	0	3	0	0	0	1	0	1	0	1

STATION	2	10	55	70	71	2	10	30	55	70	71	2	10	30	55	70	71	2	10	30	55	70	71	Water Total 4 °C	Water Total 20 °C	Sediment Total 4 °C	Sediment Total 20 °C
Fumarate	5	0	2	2	4	8	4	8	9	9	10	7	4	5	3	4	5	1	6	3	6	5	3	3	8	5	4
Hydroxyacids:																											
L(+)-Tartarate	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
DL-β-Hydroxybutyrate	3	0	3	2	1	8	7	9	8	9	7	1	0	1	0	2	0	1	6	2	4	3	2	2	8	1	3
DL-Lactate	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
DL-Glycerate	2	0	3	2	1	4	4	7	7	9	8	5	3	2	0	1	1	1	2	0	3	1	1	2	7	2	1
Misc. organic acids:																											
Citrate	3	0	2	1	2	5	3	2	4	5	1	7	2	1	0	2	3	1	5	0	2	1	1	2	3	3	2
α-Ketoglutarate	3	0	3	2	1	8	5	9	8	8	8	1	1	3	2	2	3	1	4	3	6	2	1	2	8	2	3
Pyruvate	4	1	3	3	2	8	8	9	7	9	6	3	7	7	4	5	5	1	6	3	6	4	3	3	8	5	4
Itaconate	0	0	0	0	0	0	2	0	1	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0
Glutarate	3	0	3	2	4	8	4	9	8	9	8	6	3	3	2	2	5	1	3	2	7	2	0	2	8	4	3
Galacturonate	0	0	0	0	1	0	1	0	1	1	2	0	0	0	1	1	0	0	0	0	0	1	0	0	1	0	0
D-Gluconate	0	0	0	0	0	0	3	0	1	1	2	4	2	0	1	1	1	0	3	0	1	1	0	0	1	2	1
2-Ketogluconate	0	0	0	0	0	2	0	0	0	1	0	0	0	0	0	0	0	0	2	0	0	0	0	0	1	0	0
Ascorbic acid	0	0	0	0	0	0	2	0	1	0	1	0	0	0	0	0	0	0	0	0	4	0	0	0	1	0	1
Polyalcohols & glycols:																											
D-Arabitol	0	0	2	1	1	5	1	2	2	4	3	0	0	0	0	2	0	1	1	0	2	0	0	1	3	0	1
D-Mannitol	1	0	2	2	1	5	1	5	7	8	4	1	0	1	1	2	1	1	2	0	3	1	1	1	5	1	1
Dulcitol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D-Sorbitol	1	0	1	1	1	3	1	3	1	2	2	0	0	1	0	2	1	1	2	0	2		0	1	2	1	1
meso-Inositol	0	0	3	1	1	4	1	4	7	7	7	0	0	0	0	0	0	0	2	0	1	1	1	1	5	0	1
Glycerol	4	1	4	2	2	8	3	8	7	9	6	7	5	1	2	2	5	1	3	0	4	4	3	2	7	4	3
1,2-Propane-diol	0	0	0	0	0	0	6	0	0	0	2	0	0	0	0	0	0	0	5	0	0	0	0	0	1	0	1
Alcohols:																											
Ethanol	0	0	0	0	1	0	2	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0
1-Propanol	0	0	0	0	1	0	3	2	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0
2-Propanol	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1-Butanol	0	0	0	0	0	0	5	1	1	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	1	0	0
Non-nitrogenous aromatic and other cyclic compounds:																											
Phenol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2-Phenylethanol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Benzoate	0	0	0	0	0	0	1	0	1	0	1	0	0	0	0	0	0	0	2	0	0	0	0	0	1	0	0
o-Hydroxybenzoate	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0

STATION	2	10	55	70	71	2	10	30	55	70	71	2	10	30	55	70	71	2	10	30	55	70	71	Water Total 4°C	Water Total 20°C	Sediment Total 4°C	Sediment Total 20°C
m-Hydroxybenzoate	3	0	0	0	1	3	4	0	0	2	0	1	0	0	0	2	0	1	4	0	1	1	0	1	2	1	1
p-Hydroxybenzoate	3	0	3	2	1	7	6	8	7	9	7	1	0	1	0	2	0	1	3	0	1	2	1	2	7	1	1
Aliphatic amino acids:																											
Glycine	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
L-Alanine	2	0	2	2	1	7	2	8	7	7	0	1	1	4	1	2	1	1	2	2	0	3	1	1	5	2	2
L-Serine	2	0	1	0	1	5	5	0	0	6	0	0	0	3	0	3	0	1	3	0	1	2	0	1	3	1	1
L-Threonine	1	0	0	0	1	1	1	0	0	1	1	1	1	1	0	1	1	1	2	0	1	1	0	1	1	1	1
L-Leucine	0	0	0	0	0	1	4	0	1	1	1	0	0	0	0	0	0	0	3	0	1	0	0	0	1	0	1
L-Isoleucine	0	0	0	0	0	1	5	1	1	1	1	0	0	1	0	0	0	0	1	0	1	0	4	0	2	0	1
L-Valine	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	1	1	0	0	2	0	0	0	1
L-Aspartate	2	0	4	3	4	2	4	3	2	6	3	5	2	4	3	1	2	1	5	3	4	5	1	3	3	3	3
L-Lysine	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
L-Arginine	2	0	3	2	4	3	3	3	5	6	2	0	0	1	2	1	0	1	1	0	2	0	0	2	4	1	1
L-Ornithine	1	0	2	1	2	4	3	6	4	7	6	1	0	0	1	1	0	1	1	0	2	3	0	1	7	1	1
L-Asparagine	0	0	0	0	0	1	4	1	1	1	2	0	1	2	0	0	0	0	3	1	2	1	0	0	2	1	1
α-Aminobutyrate	3	0	3	2	1	7	2	8	7	7	8	1	0	3	1	2	0	1	2	2	3	3	1	2	7	1	2
L-Cysteine	0	2	0	2	0	2	2	2	4	7	3	0	0	1	0	0	1	0	3	0	2	2	1	1	3	0	1
L-Cystine	1	0	1	2	1	0	2	0	2	0	0	0	0	0	0	0	0	0	0	0	2	1	0	1	1	0	1
L-Methionine	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Amino acids and related compounds containing ring structures:																											
L-Histidine	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0
L-Proline	0	0	1	2	0	4	3	8	4	6	1	1	1	1	1	2	2	1	2	1	2	3	1	1	4	1	2
L-Tyrosine	1	0	0	0	1	1	1	0	0	1	0	1	0	1	0	2	1	1	3	0	1	1	1	0	1	1	1
Phenyalanine	0	0	0	0	0	0	1	0	2	0	0	0	0	0	0	0	0	0	2	0	1	0	0	0	1	0	1
L-Tryptophan	2	0	0	0	1	3	0	0	0	1	0	0	0	0	0	1	0	0	1	0	1	1	0	1	1	0	1
Amines:																											
α-Amylamine	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ethanolamine	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Histamine	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0
Putrescine	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
N-Acetyglucosamine	0	0	0	0	0	0	3	0	1	1	2	6	3	1	2	4	5	0	2	0	2	3	1	0	1	4	1
Misc. nitrogenous compounds:																											
Guanine	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Thymine	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

isolated from water at 4 C could grow at 20 C, but only 50% of bacteria isolated from sediment at 4 C could grow at 20 C. The upper growth temperature for bacteria isolated at 20 C was generally 25 C, but 30% of these isolates could still grow at 37 C.

Bacterial isolates could grow in the pH range 5-9 with the exception of bacteria isolated from water at 4 C, only 50% of which grew at pH 5 and bacteria isolated from sediment at 20 C, only 50% of which grew at pH 9. Bacterial isolates typically grew at sodium chloride concentrations of 0.5-5%. More water isolates were also able to grow at salt concentrations between 5 and 10% NaCl than sediment isolates.

Bacteria isolated from water at 4 C showed only limited ability to hydrolyse gelatin, starch, tween 20 and tween 80. These polymers were more commonly hydrolysed by 4 C sediment isolates and by water and sediment 20 C isolates. Cellulose and agar were not hydrolysed by any isolates. An important ecologic difference between sediment and water 4 C isolates was the ability of 70% of the sediment isolates to reduce nitrate to nitrite, compared to 0% in the water isolates. This is a key step in denitrification. Ammonia production from peptones was not common in 4 C water isolates but was more common in 20 C isolates and 4 C sediment isolates.

When vitamins were supplied as growth factors, the carbohydrates: xylose, glucose, fructose, galactose, cellobiose, maltose and sucrose

were generally utilized by between 20 and 60% of the water and sediment isolates. Carbohydrates were generally more frequently utilized by water isolates than sediment isolates, e.g. glucose was utilized by more than 60% of the water isolates but less than 50% of the sediment isolates.

Fatty acids of chain lengths less than C_{10} were frequently utilized by 20 C water isolates, generally 40-80%. Sediment 20 C isolates also utilized fatty acids, 20-40%. Bacteria isolated at 4 C from water or sediment utilized these fatty acids at 10-30%. Other organic acids, such as citrate, pyruvate, α ketoglutarate, and glutarate, generally also showed the same pattern of utilization. Stearic and oleic acids were not frequently utilized.

Mannitol, inositol and glycerol were utilized by 50-70% of the 20 C sediment isolates. Glycerol was also utilized by 20-40% of the 4 C isolates and 20 C sediment isolates. Other alcohols were only infrequently utilized.

Amino acids were more frequently utilized by 20 C water isolates than 4 C isolates or 20 C sediment isolates. Ornithine, alanine, serine, aspartate, arginine, cysteine and proline were most frequently utilized. Glycine, leucine, isoleucine, valine, lysine, cystine, tyrosine, phenylalanine, and tryptophan were poorly utilized. Other amines were also generally poorly utilized. Only N acetylglucosamine was utilized by 40% of the 20 C sediment isolates.

Hydrocarbons were not utilized by 4 C isolates and were utilized by less than 10% of the sediment isolates.

The growth factor requirements of these organisms will be discussed separately below.

There were some geographic differences in the frequency of positive features observed during summer 1975. Organisms from station 10 differed in some ways from organisms from the other stations. Irregular cell aggregates were seen more frequently at station 10, as was the occurrence of pear shaped and curved rod shaped bacteria. More psychrophiles were found in water from station 10 and in sediment from stations 2 and 10 than other stations. Starch hydrolysis was more common in water at station 10 than at other stations. Nitrate reduction was also more common at station 10 than other stations. Most bacterial 4 C isolates from station 10 failed to grow on media with only vitamins as growth factors, regardless of carbon source. No further comparison can thus be made with organisms from other stations on a substrate by substrate basis. Organisms isolated at 20 C from sediment at station 10, however, generally utilized more substrates than other stations.

As mentioned earlier, pigment production and occurrence of Vibrio spp. differed between stations in Prudhoe Bay and near Barrow. Vibrio spp. were more common near Barrow. Pigmented organisms were more common near Prudhoe Bay.

Winter 1976

Only the feature frequencies of bacteria isolated at 4 C have been examined so far for winter 1976 samples. Table 6 shows the frequency of positive features on a scale of 0 to 10 for water, ice, sediment and beach samples during winter. In general, there were major differences between bacteria isolated from water at different stations, i.e. cloning of phenotypic characteristics occurred in water during winter.

In winter water samples, 40% of the bacteria were motile compared with 0% in summer. Bioluminescence occurred in 10% of the winter water isolates. Orange pigment producing bacteria decreased from 80% in summer to 30% in winter in water. The incidence of capsule production increased from 10% in summer to 40% in winter. The winter water isolates generally had a narrower temperature range than the summer isolates, i.e. more psychrophiles occurred in water in winter. Except at 2 stations, winter water isolates were restricted to a much narrower range of salinity than summer isolates, growing at 3% but not 0.5% or 5% NaCl. At stations 2 and 23, the two exceptions, the bacteria could grow even at 0% NaCl. Also, winter water isolates generally grew at pH 7-9, a narrower range than the summer isolates. Starch and tween 20 were hydrolysed more frequently in water in winter than in summer, but the reverse was true for gelatin hydrolysis. Reduction of nitrate to nitrite occurred at a frequency of

Table 6. Features of bacteria isolated at 4 C from ice, water and sediment at various stations in the Beaufort Sea during winter 1976 on a scale of 0 to 10 (0=0%, 10=100% positive results for organisms tested).

	WATER								ICE			SEDIMENT							Beach	Water Total	Sediment Total	Ice Total
STATION	2	13	17	20	23	24	80	85	2	13	24	2	13	17	20	74	80	85	3a			
Cell Morphology:																						
Pear shaped	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rod shaped	7	2	10	10	10	10	9	9	10	10	10	10	10	10	10	10	10	10	10	8	10	10
Curved	5	0	7	2	0	2	3	0	3	1	1	2	2	3	4	1	2	3	0	2	3	1
Cocco-bacillary	0	0	0	0	0	0	0	0	0	1	8	0	0	0	0	0	0	4	4	0	0	3
Pleomorphic	3	9	8	3	0	9	7	8	9	5	9	7	5	6	7	8	3	4	5	5	6	8
Tapered end(s)	0	1	0	0	0	1	1	2	1	1	0	1	0	0	0	0	0	0	2	0	0	1
Round end(s)	9	10	10	10	10	9	9	9	10	10	10	10	10	10	10	10	10	10	10	10	10	10
Square end(s)	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cells:																						
Single	9	10	10	10	10	9	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
Chain	0	0	1	1	10	2	1	0	2	2	9	0	0	1	3	1	1	1	3	2	1	4
Pair	1	0	0	0	10	0	0	0	3	1	0	0	0	1	1	0	1	0	5	2	0	1
Irregular	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Snapping	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Branched	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Filamentous	0	1	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0
PHB	0	1	0	0	0	1	0	1	1	0	0	0	0	0	0	1	0	0	1	0	0	0
Volutin	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Capsule	0	1	7	3	10	4	4	2	3	6	9	5	7	5	3	4	6	5	9	4	5	6
Acid fast	0	1	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sudan Black B	0	1	0	0	0	3	1	2	2	1	0	0	1	0	0	0	0	0	1	1	0	1
Gram +	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Gram -	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	9	10	10	10	10	10
Gram variable	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Motility	7	4	9	8	0	1	5	4	3	2	0	4	4	9	7	1	6	7	2	4	5	2
Luminescent	0	1	1	3	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Cell Length: <0.5μ	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.5-1.0	0	0	0	0	0	0	6	0	0	0	0	0	0	0	0	1	0	0	4	0	0	0
1.1-2.0	4	4	1	3	10	2	2	6	1	3	1	4	5	2	1	4	4	1	3	5	3	2
2.1-3.0	6	3	8	6	0	6	6	4	7	5	9	5	4	6	8	4	5	7	2	4	6	7

STATION	2	13	17	20	23	24	80	85	2	13	24	2	13	17	20	74	80	85	3a	Water Total	Sediment Total	Ice Total
3.1-4.0	0	0	1	0	0	1	1	1	1	1	0	0	1	3	2	0	1	2	0	0	1	1
4.1-5.0	0	1	1	1	0	0	1	0	1	1	0	1	0	0	0	0	0	1	1	0	0	1
5.1-10	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0
11-15	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
16-100	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cell Width: <0.5μ	1	1	0	0	0	4	2	2	0	1	0	2	0	0	0	1	0	1	1	1	0	0
0.5-1.0	8	6	9	8	10	6	5	8	10	6	10	8	6	10	8	9	9	8	9	7	9	8
1.1-2.0	1	3	1	2	0	1	2	1	0	3	0	1	3	0	2	1	0	1	0	1	1	1
2.1-3.0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
3.1-4.0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
4.1-5.0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Fluorescent Pigment	0	0	3	0	0	0	1	2	2	1	0	2	2	0	1	2	1	0	1	0	1	1
Diffusible Pigments:	0	0	3	2	0	0	1	2	2	1	0	3	2	1	8	2	1	1	1	1	2	1
Blue	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Yellow	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Brown	0	0	0	0	0	0	0	0	0	0	0	0	0	1	4	0	0	1	0	0	1	0
Non-Diffusible Pigments:																						
Pink	0	0	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Brown	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Blue	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Yellow	1	0	1	1	0	0	1	2	1	0	0	1	0	0	0	0	0	0	7	1	0	0
Orange	0	5	0	0	1	4	4	0	4	4	1	1	0	0	1	3	1	1	0	2	1	3
Black	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
White	0	0	0	1	9	0	0	1	0	0	0	0	0	0	0	1	1	0	0	2	0	0
Gray	9	5	8	8	1	5	5	6	5	7	9	0	10	10	8	7	9	9	3	6	9	7
Colong Morphology: Non-diffusible Pigment																						
Center	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	1	1	1	0	0	1	0
Concentric	0	1	1	0	0	0	3	0	1	1	0	0	0	2	0	1	1	0	0	1	1	1
Colony size: <1mm	4	2	0	1	1	6	2	8	8	1	0	1	0	1	0	1	1	1	7	3	1	3
1-2	5	5	0	5	9	4	5	2	1	4	8	2	2	2	8	4	4	2	2	5	3	4
2-6	1	3	1	5	0	0	4	0	1	5	2	7	8	8	0	5	5	7	1	2	7	3
6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

STATION	2	13	17	20	23	24	80	85	2	13	24	2	13	17	20	74	80	85	3a	Water Total	Sediment Total	Ice Total
Translucent	3	7	1	5	2	8	7	8	5	6	1	2	3	10	1	4	7	7	5	5	5	4
Transparent	1	3	0	2	0	1	1	2	4	1	0	3	2	1	0	4	0	0	2	1	1	2
Opaque	6	9	9	3	9	1	2	1	1	2	9	4	5	0	10	3	3	3	3	4	4	4
Entire	4	1	9	8	10	9	8	9	10	8	10	9	8	9	10	9	9	9	10	8	9	9
Erose	0	0	1	0	0	1	1	0	0	0	0	1	1	0	0	0	1	0	0	1	0	0
Filamentous	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lobate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Undulate	6	0	0	2	0	1	2	0	0	1	0	1	2	2	0	2	1	1	0	1	1	0
Spreading	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Convoluted	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
Flat	0	0	1	0	0	0	0	0	1	2	0	1	1	0	1	0	0	2	0	0	1	1
Raised	3	7	9	9	2	9	10	10	7	5	10	7	8	8	9	8	8	8	6	7	8	7
Umbonate	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	1	0	0
Swarming	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Convex	3	3	0	1	9	0	1	0	2	2	0	3	1	3	0	2	1	2	4	2	2	1
Mucoid	1	4	1	0	0	1	2	1	0	1	0	2	2	2	3	1	2	1	0	1	2	0
Glistening	4	10	9	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	9	9	10	10
Dull	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Smooth	3	9	10	10	10	10	10	10	10	10	10	10	10	10	9	10	9	9	10	9	10	10
Rough	8	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Growth in Liquid Media:																						
Floccular	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ring	0	2	2	2	10	0	0	0	2	0	1	0	0	3	2	5	1	1	6	3	2	1
Pellicle	9	7	6	5	0	2	1	0	1	3	0	0	0	0	2	5	2	1	0	3	1	1
Fac. anaerobe	9	10	9	9	10	9	9	10	10	9	10	10	10	10	10	10	9	10	10	10	10	10
Turbidity, even	9	8	7	10	10	8	8	6	9	9	10	10	0	10	7	10	9	10	8	9	9	9
Turb., slight	0	7	6	5	10	8	6	6	8	6	10	10	0	10	3	10	9	8	8	6	8	8
Turb., moderate	9	3	4	5	0	0	3	0	1	4	0	0	0	0	3	0	1	1	3	3	1	2
Turb., heavy	0	0	0	0	0	0	1	4	0	0	0	0	0	0	0	0	0	0	0	1	0	0
No Growth in Liquid Media	1	0	0	0	0	2	0	0	1	0	0	0	10	0	4	0	0	0	0	0	1	0
Antibiotics:																						
Ampicillin, 2µg	1	8	4	1	0	9	6	6	9	4	1	6	6	5	6	5	3	4	5	4	5	5
Chloromycetin, 30µg	1	8	10	9	0	10	10	8	9	10	1	10	10	10	10	10	8	10	8	6	10	7
Aureomycin, 30µg	1	6	7	7	0	9	8	8	10	7	2	9	9	5	6	10	6	7	7	5	7	6

STATION	2	13	17	20	23	24	80	85	2	13	24	2	13	17	20	74	80	85	3a	Water Total	Sediment Total	Ice Total
Colistin, 10µg	1	3	8	9	10	5	7	7	5	6	9	8	10	7	9	7	8	7	3	6	8	7
Erythromycin, 2µg	6	3	4	4	0	7	3	6	5	8	0	7	6	4	4	7	3	4	1	3	5	4
Kanamycin, 30µg	9	2	2	2	10	7	3	6	5	3	9	2	5	3	4	3	4	3	3	6	4	6
Neomycin, 30µg	9	8	3	3	10	7	5	7	3	8	9	3	4	4	7	3	3	4	2	6	4	7
Penicillin, G., 2 units	0	4	4	2	0	7	6	5	7	3	1	4	4	3	3	4	2	3	4	3	3	4
Polymyxin B, 300 units	1	4	8	9	10	5	7	6	4	6	9	8	10	5	9	7	8	7	3	6	8	8
Streptomycin, 5µg	0	0	2	0	0	4	0	4	0	1	0	0	0	0	0	0	1	0	0	1	0	0
Tetracyclin, 5µg	0	6	1	2	0	5	4	4	5	3	6	6	3	3	1	4	1	5	2	3	3	5
Oxytetracyclin, 5µg	0	4	2	2	0	5	4	4	7	4	6	5	2	3	1	2	2	5	0	2	3	6
Hydrolysis:																						
Starch	1	3	6	4	0	0	3	1	4	3	0	3	2	6	9	6	7	9	6	2	6	2
Gelatin	0	2	9	4	3	4	2	3	3	6	0	3	4	6	6	3	3	4	6	3	4	3
Agar	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lipase, Tween 20	7	2	9	6	10	2	5	2	3	8	0	6	7	9	7	6	7	6	3	5	7	4
Lipase, Tween 80	3	2	9	0	10	1	3	1	3	4	10	4	5	6	7	2	6	6	4	2	5	6
Alkaline phosphatase	6	6	8	4	0	1	2	3	5	7	0	5	8	1	1	5	0	1	5	3	3	4
Urease																						
Catalase	10	5	10	8	10	6	9	7	10	5	10	3	1	5	2	7	6	6	10	8	4	8
Oxidase	4	1	8	5	0	0	2	4	5	5	0	1	2	5	4	0	3	5	2	2	3	3
Indole Production	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
NH_3 Production	0	1	4	1	0	1	2	0	0	0	0	1	1	1	1	0	2	2	0	1	1	0
Reduction NO_3 to NO_2	9	6	6	8	0	2	5	0	1	1	0	5	0	9	8	7	7	9	2	4	8	1
Reduction NO_2	7	0	0	0	0	0	2	0	0	1	0	0	0	1	5	3	0	1	0	1	0	0
Arginine deaminase	4	1	7	7	3	3	3	2	1	3	2	2	2	7	5	2	4	5	2	3	4	2
Lysine decarboxylase	0	0	2	2	0	0	2	1	1	2	0	2	3	1	4	1	2	4	0	1	2	1
Ornithine decarboxylase	0	0	2	1	0	0	1	0	1	3	0	2	3	1	3	1	3	1	1	0	2	1

STATION	2	13	17	20	23	24	80	85	2	13	24	2	13	17	20	74	80	85	3a	Water Total	Sediment Total	Ice Total
Acid From:																						
D-Ribose	1	2	3	4	0	1	3	1	0	1	4	2	2	9	1	4	3	4	1	2	3	2
D-Fructose	6	1	3	5	1	1	3	1	0	1	3	2	2	3	1	4	5	5	1	2	3	1
D-Glucose (oxidative)	9	1	4	6	0	4	4	2	2	6	0	7	6	5	4	7	8	6	2	4	6	3
D-Glucose (fermentative)	0	1	4	5	0	3	0	1	1	3	0	6	5	4	3	6	6	6	1	2	5	1
Cellobiose	1	1	0	1	0	1	1	0	1	3	3	4	4	1	1	3	4	2	0	1	3	2
Lactose	0	1	1	1	1	0	1	0	1	1	9	2	3	1	0	2	1	0	0	0	1	4
Sucrose	0	1	0	1	0	0	1	0	1	1	3	1	1	3	2	2	1	1	0	0	1	2
D-Mannitol	0	1	1	1	0	1	2	0	0	1	2	0	1	2	0	1	2	1	0	1	1	1
Temperature:																						
5 C	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
10 C	10	10	10	10	10	7	10	9	10	10	10	10	10	10	10	10	10	10	10	9	10	10
15 C	10	8	9	7	10	5	10	6	10	9	10	7	9	9	7	10	9	8	10	8	8	10
20 C	10	2	2	4	10	3	2	4	7	6	9	4	5	4	2	6	5	2	10	5	4	8
25 C	10	1	1	1	10	2	2	3	7	3	9	2	2	4	0	1	1	1	10	4	1	7
37 C	9	0	0	0	8	1	0	1	0	1	0	1	0	0	0	0	1	0	0	3	0	0
43 C	9	0	0	0	0	0	0	1	0	1	3	0	0	0	0	0	0	0	0	1	0	1
pH:																						
4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
5	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6	0	1	0	5	8	1	3	3	0	1	3	3	1	0	1	6	4	2	1	3	2	1
7	10	9	10	10	10	10	10	10	10	10	9	10	10	10	10	10	10	10	10	10	10	10
8	10	9	10	10	10	9	10	9	10	10	9	10	10	10	10	10	10	10	10	10	10	10
9	10	9	10	10	10	3	10	8	9	9	9	9	10	10	10	10	10	10	9	9	10	9
10	0	0	10	6	10	2	5	2	4	4	9	1	3	7	4	0	6	7	4	4	4	6
Percent NaCl:																						
0	9	0	0	1	10	2	1	2	0	3	9	1	1	0	0	0	0	0	6	4	0	4
0.5	9	3	7	5	10	3	4	5	5	4	9	3	1	2	1	3	1	2	9	6	2	6
3.0	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
5.0	10	7	9	6	3	6	8	6	8	9	3	7	9	8	5	8	8	7	10	6	8	7
7.5	9	1	5	1	0	0	2	2	5	4	0	2	0	0	1	3	1	1	10	2	1	3
10	0	0	1	0	0	0	1	1	1	2	0	1	0	0	0	2	0	0	7	0	0	1
15	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

	WATER								ICE			SEDIMENT							Beach	Water Total	Sediment Total	Ice Total
STATION	2	13	17	20	23	24	80	85	2	13	24	2	13	17	20	74	80	85	3a			
Carbohydrates & Sugar Derivatives:																						
L-Arabinose	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
D-Ribose	0	0	3	2	0	1	3	1	1	0	0	1	1	5	1	0	3	1	0	1	2	0
D-Xylose	0	0	0	0	0	1	0	0	1	0	0	1	0	0	0	1	1	0	0	0	0	0
L-Rhamnose	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0
D-Fructose	0	0	5	4	0	4	4	2	3	4	3	1	1	5	1	1	3	3	5	2	2	3
D-Galactose	0	0	1	1	0	2	0	1	1	0	3	1	0	3	1	1	1	0	0	1	1	1
D-Glucose	1	0	4	3	0	4	5	4	3	2	1	3	4	7	3	1	5	5	6	2	4	2
D-Mannose	0	0	3	3	0	2	3	1	1	0	0	2	0	3	1	1	1	4	1	1	1	0
L-Sorbose	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Salicin	0	0	0	0	0	1	2	0	1	0	0	2	2	3	1	0	2	3	0	0	2	0
Cellobiose	0	0	0	1	0	0	3	1	1	0	0	1	0	0	1	1	1	0	4	1	0	0
Lactose	0	0	0	0	0	0	0	0	2	0	0	1	0	0	0	0	0	0	0	0	0	0
Maltose	0	0	5	1	0	1	2	0	2	0	0	0	0	5	1	0	3	4	2	1	3	0
Sucrose	0	0	0	0	0	1	1	0	1	0	0	0	0	3	0	1	2	1	3	0	1	0
Trehalose	0	0	2	0	0	1	1	0	0	0	0	1	1	1	1	1	2	1	0	0	1	0
D-Raffinose	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0
Fatty Acids:																						
Acetate	7	2	3	1	10	2	4	5	0	0	0	2	2	0	0	2	3	1	0	4	1	0
Propionate	*	*	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0
Butyrate	0	0	3	1	10	4	2	1	1	1	4	0	0	0	0	0	0	0	1	3	0	2
Valerate	0	0	3	0	10	4	2	5	0	1	1	0	1	0	0	0	0	1	1	3	0	1
Caproate	1	0	2	1	10	4	3	4	1	0	0	1	1	0	1	1	3	1	1	4	1	0
Caprylate	0	0	0	0	10	2	2	1	0	0	0	1	1	0	0	0	2	1	1	2	1	0
Laurate	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	1	0	0	0	0
Stearic acid	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Oleic acid	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dicarboxylic Acids:																						
Malonate	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Succinate	*	*	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Fumarate	5	2	4	6	0	2	5	4	0	0	0	4	4	8	4	3	5	5	3	3	5	0

STATION	2	13	17	20	23	24	80	85	2	13	24	2	13	17	20	74	80	85	3a	Water Total	Sediment Total	Ice Total
Hydroxy																						
L(t)-Tartarate	0	0	0	0	0	2	0	1	1	0	0	0	0	0	0	0	0	0	1	0	0	0
DL-β-Hydroxybutyrate	1	0	0	0	10	5	1	1	1	0	0	1	1	0	1	0	0	0	1	3	0	0
DL-Lactate	1	0	0	0	10	5	3	5	2	3	0	1	1	0	1	0	3	1	1	4	1	2
DL-Glycerate	0	0	0	0	0	1	1	0	0	0	0	0	1	0	0	0	1	2	0	0	1	0
Misc. Organic Acids:																						
Citrate	0	0	1	0	0	1	2	0	2	1	0	1	1	1	0	0	2	0	2	1	1	1
α-Ketoglutarate	2	2	3	2	2	4	2	5	1	1	3	1	1	3	2	1	2	1	2	3	1	2
Pyruvate	8	3	9	6	10	3	4	5	2	1	3	3	3	3	2	4	6	3	2	6	3	2
Itaconate	6	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0
Glutarate	1	0	0	0	0	2	4	4	0	0	0	1	1	0	1	2	1	0	0	2	1	0
Galacturonate	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
D-Gluconate	0	0	3	3	0	2	1	0	0	2	3	1	1	1	1	0	2	1	0	1	1	2
2-Ketogluconate	0	0	0	0	0	1	0	0	1	0	3	0	1	0	0	0	0	0	0	0	0	1
Ascorbic acid	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Polyalcohols and Glycols:																						
D-Arabitol	0	0	0	0	0	2	0	1	1	0	0	0	1	0	0	0	0	0	0	0	0	0
D-Mannitol	0	0	1	1	0	2	2	2	1	0	0	0	1	2	0	0	2	1	3	1	0	0
Dulcitol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D-Sorbitol	0	0	0	0	0	2	0	1	0	0	0	0	1	2	0	0	0	0	0	0	0	0
Meso-Inositol	0	0	1	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Glycerol	1	0	2	2	0	3	5	5	1	0	0	3	4	0	2	5	5	3	1	2	3	0
1,2-Propane-diol	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Alcohols:																						
Ethanol	0	0	0	0	10	1	0	0	1	0	0	0	0	0	0	0	0	0	2	2	0	0
1-Propanol	0	0	0	0	3	1	1	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0
2-Propanol	0	0	0	0	10	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
1-Butanol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Non-nitrogenous Aromatic and Other Cyclic Compounds:																						
Phenol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2-Phenylethanol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

STATION	2	13	17	20	23	24	80	85	2	13	24	2	13	17	20	74	80	85	3a	Water Total	Sediment Total	Ice Total
Benzoate	0	0	0	0	10	1	1	0	0	0	0	0	0	0	0	0	1	0	0	2	0	0
o-Hydroxybenzoate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
m-Hydroxybenzoate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
p-Hydroxybenzoate	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Aliphatic Amino Acids:																						
Glycine	0	1	1	2	0	0	1	0	0	1	0	1	0	0	0	0	0	0	0	1	0	0
L-Alanine	0	0	0	1	10	3	2	1	0	0	0	2	1	0	0	0	2	0	0	3	1	0
S-Serine	0	1	3	3	3	2	1	0	1	1	0	1	1	0	0	0	0	0	0	1	0	1
L-Threonine	0	0	2	0	0	1	2	0	0	0	0	1	0	0	0	0	2	0	0	0	0	0
L-Leucine	0	0	0	0	0	1	1	0	0	0	0	0	0	0	1	0	1	1	0	0	0	0
L-Isoleucine	0	0	0	0	0	1	1	0	1	1	0	0	1	0	2	0	1	1	1	0	0	1
L-Valine	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
L-Aspartate	0	1	1	1	2	2	1	1	1	0	0	1	1	1	1	1	3	4	1	1	1	0
L-Lysine	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
L-Arginine	0	0	1	0	0	2	1	0	1	0	0	1	0	0	0	0	2	0	1	0	0	0
L-Ornithine	0	0	1	0	0	1	1	0	1	0	6	1	0	0	0	0	2	0	1	0	0	0
L-Asparagine	0	0	2	0	0	2	0	1	0	0	0	0	1	0	0	0	1	0	1	0	0	0
γ-Aminobutyrate	0	0	0	0	0	2	1	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0
L-Cycstine	0	0	0	0	0	1	1	2	0	0	0	0	1	0	0	0	0	0	0	1	0	0
L-Methionine	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
L-Cystine	0	0	0	0	0	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Amino Acids & Related Compounds Containing Ring Structures:																						
L-Histidine	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
L-Proline	0	1	4	3	10	2	3	2	1	1	0	1	1	0	1	1	2	0	1	3	1	1
L-Tyrosine	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Phenylalanine	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
L-Tryptophan	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Amines:																						
α-Amylamine	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ethanolamine	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Histamine	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Putrescine	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
N-Acetylgluco-amine	0	0	8	4	0	2	3	2	1	1	0	3	3	5	2	1	4	5	1	2	3	1

STATION	2	13	17	20	23	24	80	85	2	13	24	2	13	17	20	74	80	85	3a	Water Total	Sediment Total	Ice Total
Misc. Nitrogenous Compounds:																						
Guanine	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Thymine	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydrocarbons:																						
N-Hexadecane	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
N-Pentadecane	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
omega-Phenyldecane	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pristane	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pentadecylcyclohexane	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
As Carbon and Nitrogen Source:																						
L-Aspartate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
L-Glutamate	9	3	0	7	0	3	6	6	0	0	0	8	6	0	1	3	7	6	0	5	4	0
L-Lysine	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
L-Tryptophan	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ethanolamine	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Medium E:																						
D-Ribose	1	0	3	4	0	2	2	2	2	3	0	2	1	5	3	1	4	2	0	2	2	2
D-Fructose	0	0	2	4	0	2	3	1	5	5	1	1	0	8	3	1	5	3	4	1	3	4
D-Glucose	0	5	4	6	0	4	10	6	5	5	3	8	8	5	4	8	8	7	6	4	7	4
Acetate	4	1	1	4	10	2	5	7	5	0	0	4	6	0	1	5	6	5	5	5	4	0
Glycerate	1	0	2	4	1	3	3	3	1	1	0	1	2	2	2	1	3	3	1	2	2	1
Succ nate	4	1	6	7	0	3	9	6	6	8	0	6	5	8	5	7	7	6	3	4	6	5
DL-Lactate	0	1	2	3	10	5	4	4	6	5	0	2	2	1	3	1	2	2	3	4	2	4
DL-β-Hydroxybutyrate	0	0	0	0	10	1	0	0	1	1	0	1	0	1	0	0	0	0	1	2	0	1
Pyruvate	5	4	10	6	10	1	8	8	6	8	0	5	6	8	5	9	6	6	3	6	7	5
α-Ketoglutarate	4	1	4	7	0	5	4	6	8	9	0	4	4	6	6	5	6	5	5	4	5	6
L-Aspartate	0	6	6	8	10	2	8	7	1	1	0	6	6	1	2	9	7	5	3	1	5	1
L-Glutamate	0	0	6	3	0	1	1	0	2	4	0	1	0	1	1	1	5	1	6	1	1	2
L-Tryptophan	0	0	0	3	0	1	1	0	0	1	0	0	0	0	0	0	0	1	0	1	0	0
L-Lysine	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0

STATION	2	13	17	20	23	24	80	85	2	13	24	2	13	17	20	74	80	85	3a	Water Total	Sediment Total	Ice Total
Medium A:																						
D-Ribose	0	0	2	2	0	1	2	1	1	1	0	1	0	4	1	0	1	2	0	1	1	1
D-Fructose	0	0	3	2	0	2	3	1	1	2	3	1	0	1	1	0	2	2	1	1	1	2
D-Glucose	1	0	2	2	0	2	3	1	1	0	0	2	1	1	1	0	3	3	0	1	1	0
Acetate	8	2	0	2	10	1	2	1	0	0	0	1	1	0	0	0	1	3	0	4	1	0
Glycerol	0	0	2	1	1	1	2	0	1	3	0	1	1	4	2	0	1	3	1	1	1	1
Succinate	7	2	0	4	0	2	3	1	0	0	0	1	1	0	0	0	2	2	0	3	1	0
DL-β-Hydroxy-butyrate	0	0	0	0	10	4	0	1	1	1	0	0	1	0	1	0	1	0	2	2	0	1
DL-Lactate	7	1	3	1	10	2	2	1	2	2	0	1	1	0	1	0	1	3	1	3	1	1
α-Ketoglutarate	6	1	3	3	0	1	2	0	1	1	3	1	1	2	0	0	0	2	1	2	1	2
L-Glutamate	0	1	4	2	0	1	3	1	1	1	0	1	1	2	2	0	1	1	1	1	1	1

greater than 50% in water collected at 6 of the 8 stations, compared to 0% in summer water samples.

Carbohydrates were utilized less frequently in winter water samples than in summer. Fatty acids, on the other hand, were utilized more frequently. Hydrocarbons were not utilized by any of the winter water isolates.

There were large differences in utilization patterns between stations. For example, organisms from water at station 23 extensively utilized fatty acids, a few amino acids and some monoalcohols, but did not utilize any carbohydrates. The alcohols and some of the acids used by organisms at station 23 were only minimally used by organisms from the other stations.

Compared to summer, organisms isolated from sediment showed similar morphological characteristics in winter. As in water, winter sediment isolates showed a narrower tolerance range to pH, 7-9, compared to 5-9 for summer sediment isolates. Both summer and winter sediment isolates showed similar growth ranges for salinity and temperature.

No difference was found between summer and winter frequencies of gelatin or tween hydrolysis in sediment, but starch hydrolysis was more frequent in winter. Ammonia production from peptones was only 10% in sediment in winter compared to 40% in summer. Nitrate reduction occurred as frequently in sediment isolates in winter as summer, 70-80%.

Carbohydrates were used less frequently in winter than summer. Glucose and maltose were the most frequently utilized carbohydrates in winter sediment isolates. In general, fewer acids were utilized in winter sediment isolates. Also, only a few amino acids were utilized and by only relatively few isolates from the winter sediment samples. Hydrocarbons were not utilized by isolates from winter sediment samples.

Organisms isolated from beach sediment had a much higher incidence of pigment production, 70% yellow, compared to benthic sediment samples. Beach sediment isolates also had a higher incidence of capsulated and coccobacillary forms. All organisms isolated from beach sediment grew at temperatures of 5-25 C, pH 7-9 and 0.5-7.5% NaCl. Beach sediment isolates had a lower frequency of lipase production but similar frequencies of starch and gelatin hydrolysis as benthic sediment isolates. Carbohydrates, carboxylic acids, alcohols and amino acids were more frequently utilized by beach sediment isolates. Hydrocarbons were not utilized by beach sediment isolates.

Organisms isolated from ice were examined only from winter samples. More organisms (30%) from ice were coccobacillary than from either water or sediment. About 20% of the organisms from ice were motile. The incidence of orange pigmented bacteria was higher (30%) in ice than winter water or sediment samples. Most of the isolates from ice (70%) were psychrotrophs and 70% could grow at 25 C. The pH

and salt tolerance ranges of ice isolates were similar to winter water isolates. The ability to hydrolyse polymers was similar in ice and in underlying water organisms. Production of ammonia was not observed in organisms from ice and only 10% reduced nitrate to nitrite.

Only 3 carbohydrates, glucose, fructose and galactose, were utilized and only by a small number of isolates from ice. Some amino acids, fatty acids and other organic acids were utilized by about 10-20% of the isolates from ice. No alcohols and no hydrocarbons were utilized by ice isolates.

Growth Factor Requirements

An important area that has emerged from this work is that many of the bacteria in the Beaufort Sea require growth factors. We have classified bacteria as type A requiring no growth factors, B type requiring vitamins, E type requiring amino acids and/or compounds found in yeast extract in addition to vitamins, and U type requiring complex unknown growth factors. The distribution of bacteria relative to their growth factor type is shown in Figs. 25-27.

During summer 1975 the B growth factor type was predominant in water. Very few water isolates could grow without added growth factors. The A growth factor type was more common among 20 C water isolates than 4 C isolates but still a minor portion of the summer population could grow without growth factors. At station 10 a larger percentage of the bacterial population from water required more complex growth factors than just vitamins.

Summer 20 C sediment isolates showed a relatively even overall distribution of A, B and E growth factor types. Summer 4 C sediment isolates also showed a higher relative occurrence of A type and lower predominance of B type than in water. Part of the sediment population had less complex and part more complex growth factor requirements than the water population.

In winter there was a shift to simpler growth factor requirements in water isolates. Organisms not requiring growth factors

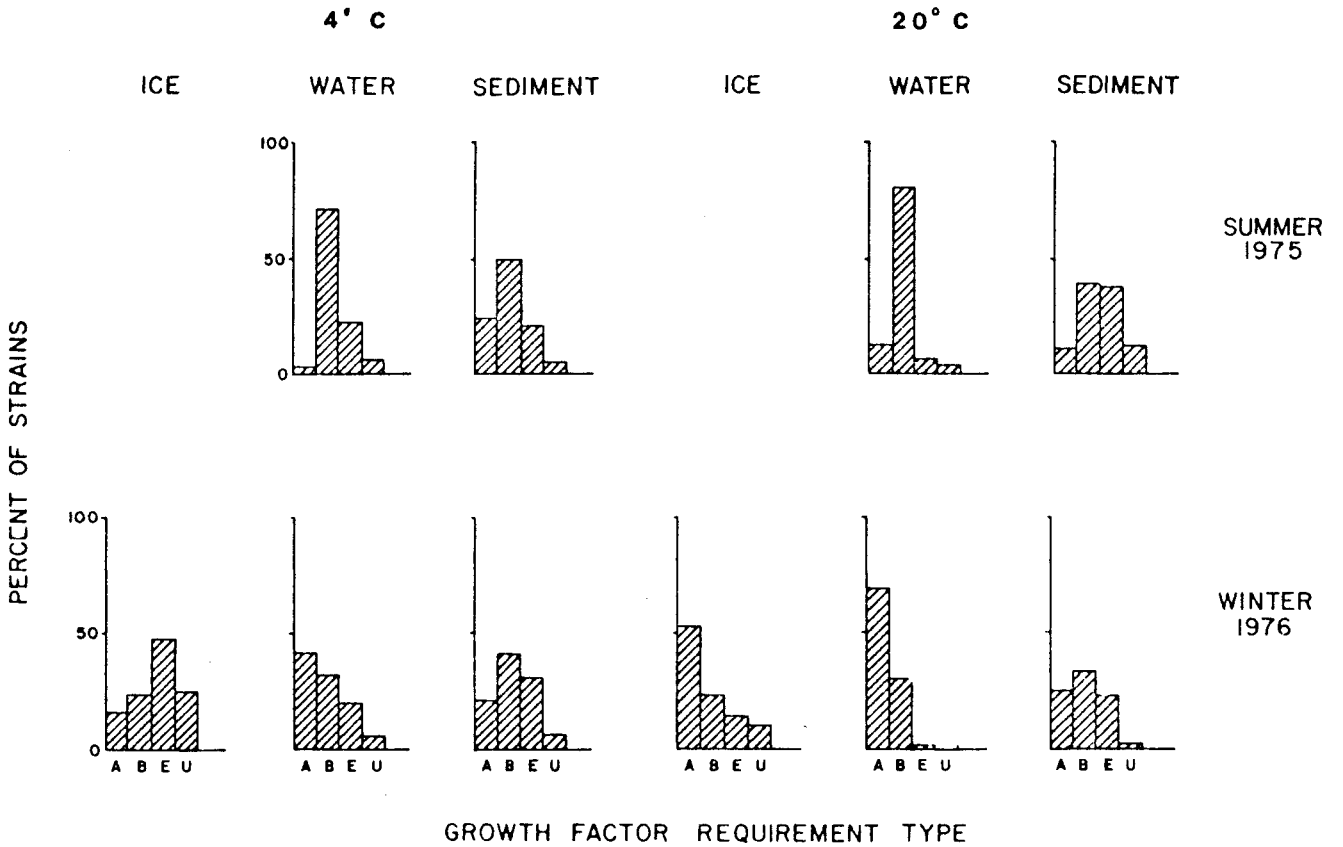


Fig. 25. Comparison of growth factor requirement types for Summer 1975 and Winter 1976 isolates. A type = no growth factors required; B type = require vitamins; E type = require amino acids and/or factors found in yeast extract; U type = require complex unknown growth factors; N type = not tested.

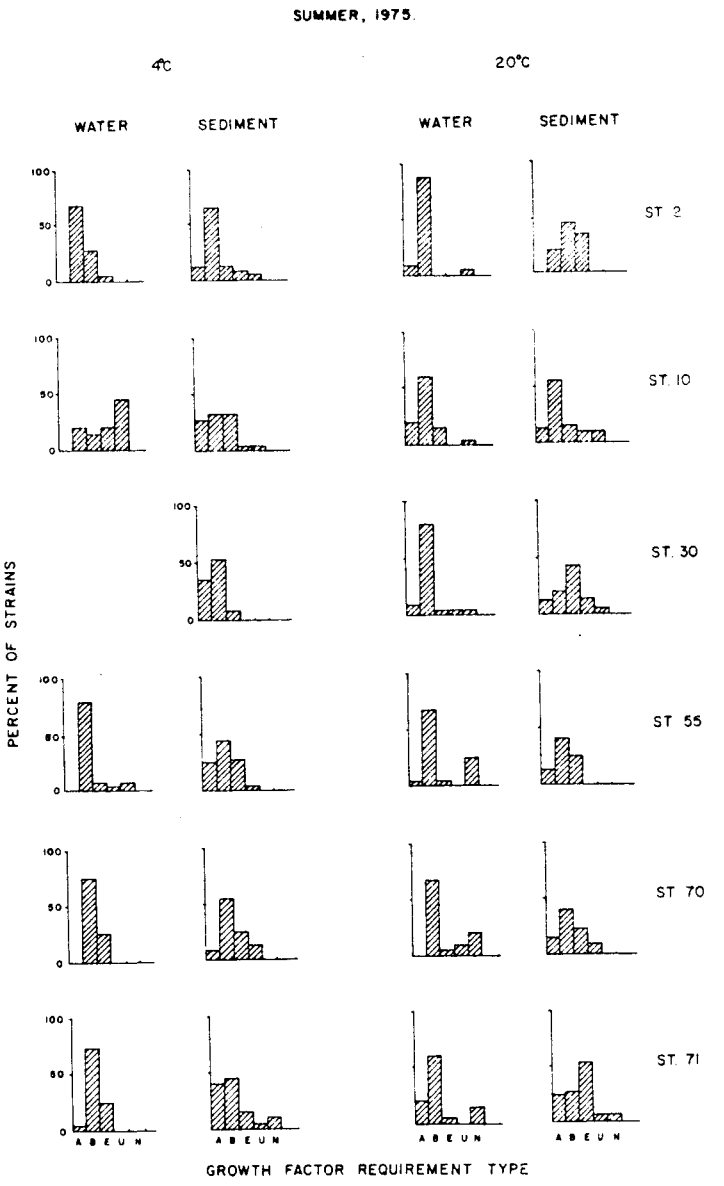


Fig. 26. Growth factor requirement types for Summer 1975 at different stations. See Fig. 25 for explanation of types.

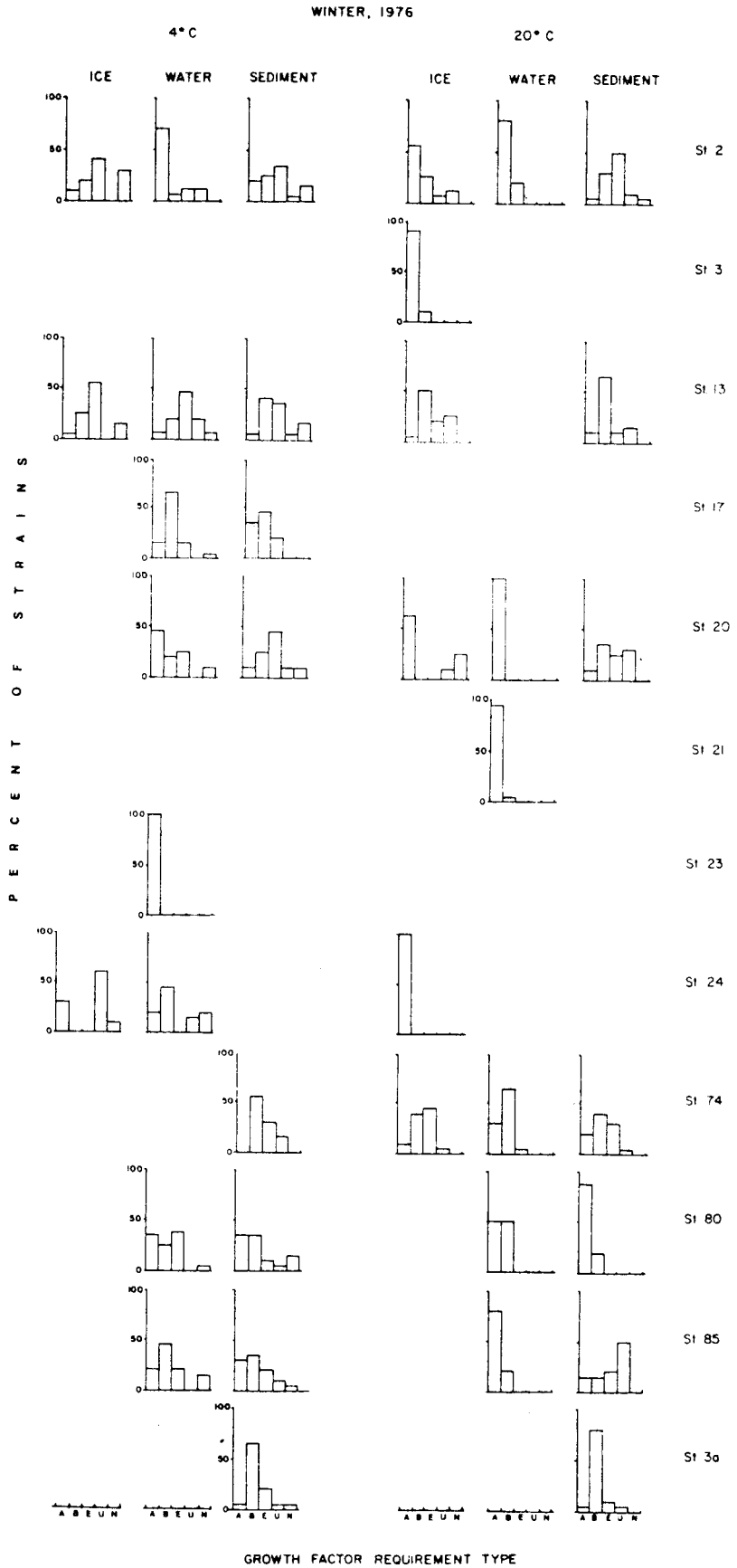


Fig. 27. Growth factor requirement types for Winter 1976 at different stations. See Fig. 25 for explanation of types.

were frequently found in winter samples. Only 4 C isolates from ice showed a predominance of complex growth factor types. The 20 C water and ice isolates showed a predominance of type A organisms. Sediment organisms had similar growth factors in winter as in summer. There were major spacial differences in occurrence of growth factor types during winter but no particular distribution pattern was apparent.

Cluster Analyses

Cluster analyses were performed to find the characteristics and distribution of the major taxonomic groups of bacteria in the Beaufort Sea. Results of cluster analyses using the S_j Jaccard coefficient are shown as simplified dendrograms and cluster triangle matrices in Figs. 28-43. Taxonomic groups are labelled in the figures. The number of taxonomic groups and the percent of strains belonging to clusters are shown in Tables 7-10.

The frequency of occurrence of clusters of a particular size is shown in Table 11. Each population examined had one and only one large cluster with more than 30 strains. Each of the populations studied from summer samples had a cluster with more than 60 strains but none of the winter clusters had more than 40 strains in a cluster. More small size clusters were found in winter and summer 1976 than during summer 1975.

TABLE 7 Number of strains tested showing % belonging to clusters and number of taxonomic groups.

SUMMER, 1975

	Station	No. of strains isolated	No. of strains employed for NT	% of strains belonging to clusters	No. of taxa (taxonomic groups)
4° WATER	2	25	24	41.7	16-17
	10	25	14	14.2	13-24
	55	25	23	78.3	8-10
	70	25	25	96.0	5
	71	25	25	88.0	6
	Total	125	111		
4° SEDIMENT	2	25	24	75.0	10-11
	10	25	24	37.5	18-19
	30	25	24	37.5	17-18
	55	25	25	56.0	14
	70	25	25	40.0	18
	71	25	23	30.4	19-21
	Total	150	145		
	4° GRAND TOTAL	275	256		
20° WATER	2	25	24	58.1	12-13
	10	25	24	41.7	15-16
	30	25	24	91.7	4-5
	55	25	19	63.2	8-14
	70	25	20	80.0	6-11
	71	25	21	61.9	9-13
	Total	150	132		
20° SEDIMENT	2	25	25	52.0	16
	10	25	23	52.2	17-19
	30	25	24	70.8	9-10
	55	25	25	24.0	20
	70	25	25	40.0	18
	71	25	25	48.0	15
	Total	150	147		
	20° GRAND TOTAL	300	279		

TABLE 8 Number of strains tested showing % belonging to clusters and number of taxonomic groups.

WINTER, 1976

	Station	No. of strains isolated	No. of strains employed for NT	% of strains belonging to clusters	No. of taxa (taxonomic groups)
WATER 4°C	2	16	16	81.3	4
	13	15	14	50.0	9-10
	17	20	19	63.2	9-10
	20	20	18	27.8	14-16
	23	20	20	100.0	1
	24	20	16	0	16-20
	80	20	19	68.4	8-9
	85	20	17	11.8	16-19
		Total 151	139		
ICE 4°C	2	20	15	13.3	14-19
	13	20	17	11.8	16-19
	17	10	4	0	
	24	20	18	100.0	2-4
	80	10	2	0	
		Total 80	56		
SEDIMENT 4°C	2	20	17	29.4	14-17
	13	20	17	29.4	14-17
	17	20	20	55.0	12
	20	20	18	38.9	13-15
	74	20	20	55.0	13
	80	20	17	17.6	15-18
	85	20	19	21.1	16-17
		Total 140	128		
BEACH 4°C	3a	Total 20	19	36.8	14-15
		GRAND TOTAL 391	342		

TABLE 9 Number of strains tested showing % belonging to clusters and number of taxonomic groups.

WINTER, 1976

	Station	No. of strains isolated	No. of strains employed for NT	% of strains belonging to clusters	No. of taxa (taxonomic groups)
20°C WATER	2	20	20	100.0	2
	20	10	10	90.0	2
	21	20	20	95.0	2
	74	20	20	95.0	2
	80	20	20	90.0	4
	85	10	10	20.0	9
		Total 100	100		
20°C ICE	2	16	16	87.5	7
	3	10	10	90.0	2
	13	20	20	40.0	16
	20	15	15	100.0	2
	24	20	20	100.0	2
	74	20	20	30.0	16
		Total 101	101		
20°C SEDIMENT	2	20	20	10.0	19
	13	20	20	20.0	18
	20	20	20	25.0	17
	74	20	20	35.0	16
	80	20	20	85.0	4
	85	20	20	30.0	16
		Total 120	120		
20°C BEACH	3a	Total 20	20	40.0	14
		GRAND TOTAL 321	321		

TABLE 10 Number of strains tested showing % belonging to clusters and number of taxonomic groups.

SUMMER, 1976

	Station	No. of strains isolated	No. of strains employed for NT	% of strains belonging to clusters	No. of taxa (taxonomic groups)
4°C WATER	1	25	25	36.0	17
	15a	25	25	68.0	13
	16	25	22	45.5	15-18
	20	25	25	76.0	7
	24a	25	25	72.0	9
	24b	25	24	50.0	15-16
	41	25	24	62.5	11-12
	74	25	25	68.0	10
	80	25	25	84.0	5
		Total 225	221		
4°C SEDIMENT	2	25	23	92.0	22-24
	16	25	25	32.0	20
	20	25	25	48.0	18
	24	25	25	20.0	22
	41	25	25	64.0	10
	80	25	25	40.0	19
		Total 150	148		
		GRAND TOTAL 375	369		

TABLE 11 Size and frequency distribution of clusters

	TOTAL NO. OF CLUSTERS	Frequency distribution NUMBER OF STRAINS									
		2	3-9	10-19	20-29	30-39	40-49	50-59	60-69	70-79	80-89
SUMMER, 1975, 4°C	14	4	5	2	2				1		
SUMMER, 1975, 20°C	13	6	4	1		1					1
WINTER, 1976, 4°C	30	13	12	4	1						
WINTER, 1976, 20°C	30	10	13	3	3	1					
SUMMER, 1976, 4°C	28	15	8	3	1					1	

Summer 1975

As shown in Figs. 28 and 29 a total of 14 clusters were obtained for the 4 C isolates from summer 1975. Sixty-two percent of the isolates fell into one of the clusters. The distribution of the clusters by source, i.e. water vs sediment, is shown in Table 12 and Fig. 44 and by geographic location in Fig. 45. Some of the clusters contained only isolates from water or sediment but the large clusters contained both water and sediment isolates. Fewer but larger clusters were found in water than in sediment.

With the exception of station 10 strains from the large clusters L1, L4 and L6 were found in water at all stations. The incidence of members of L4 and L6 was higher in Prudhoe Bay than Elson Lagoon. Members of cluster L4 were also found in sediment at each station. Organisms in clusters L8 and L9 were found only in sediment at station 10. The clusters found for sediment isolates tended to be site specific.

As shown in Figs. 30 and 31, 13 clusters were obtained for the 20 C isolates for summer 1975. Thirty-two percent of the total strains belonged to a cluster. The distribution of the clusters by source, i.e. water or sediment, is shown in Table 13 and Fig. 46 and by geographic location in Fig. 47. Eighty-five percent of the strains in cluster H7, the largest cluster, came from water. Ninety-two percent of the strains in cluster H4, the second largest cluster,

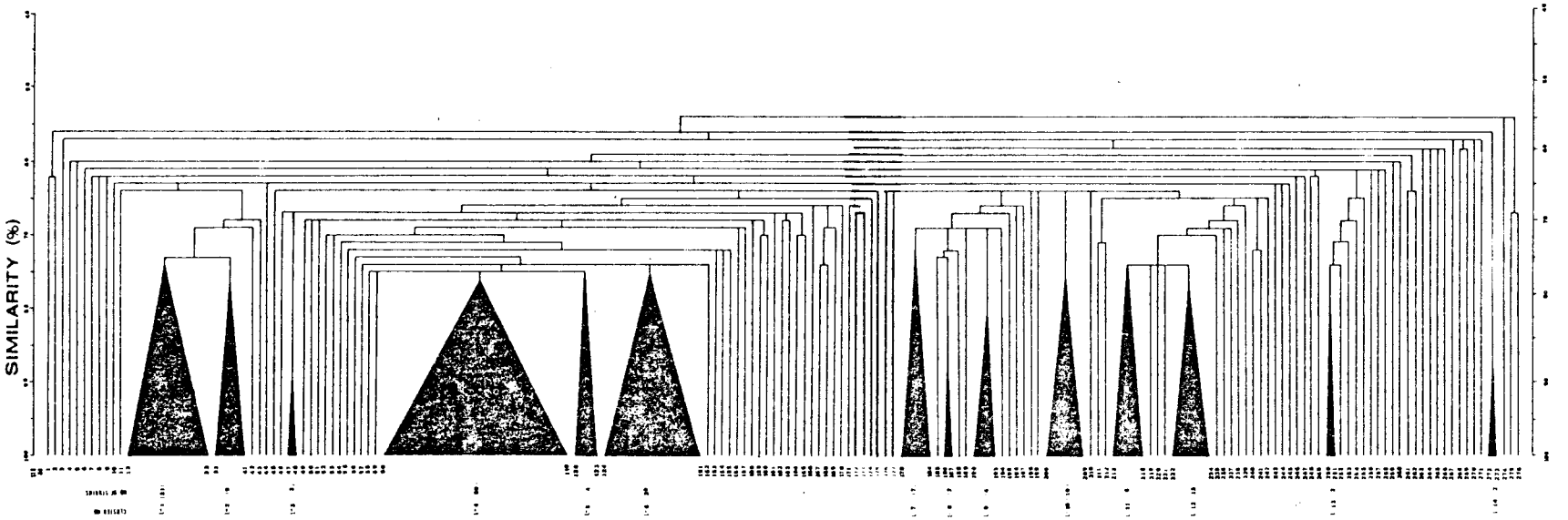


Fig. 28.
Simplified dendrogram for Summer 1975.
4 C isolates - L series

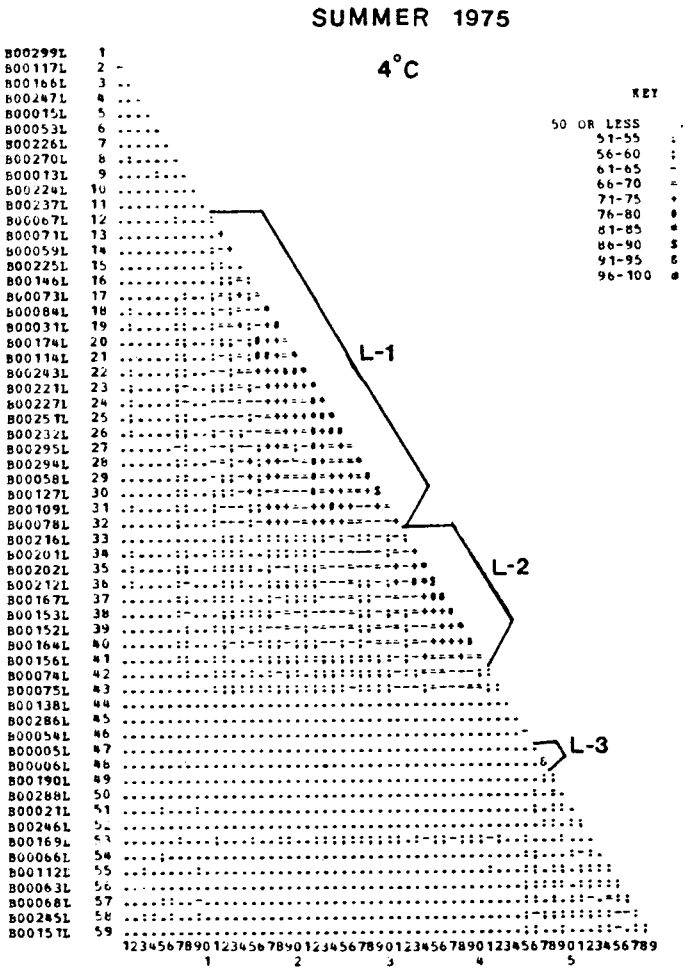


Fig. 29.
Cluster triangle matrix for Summer 1975.
4 C isolates - L series

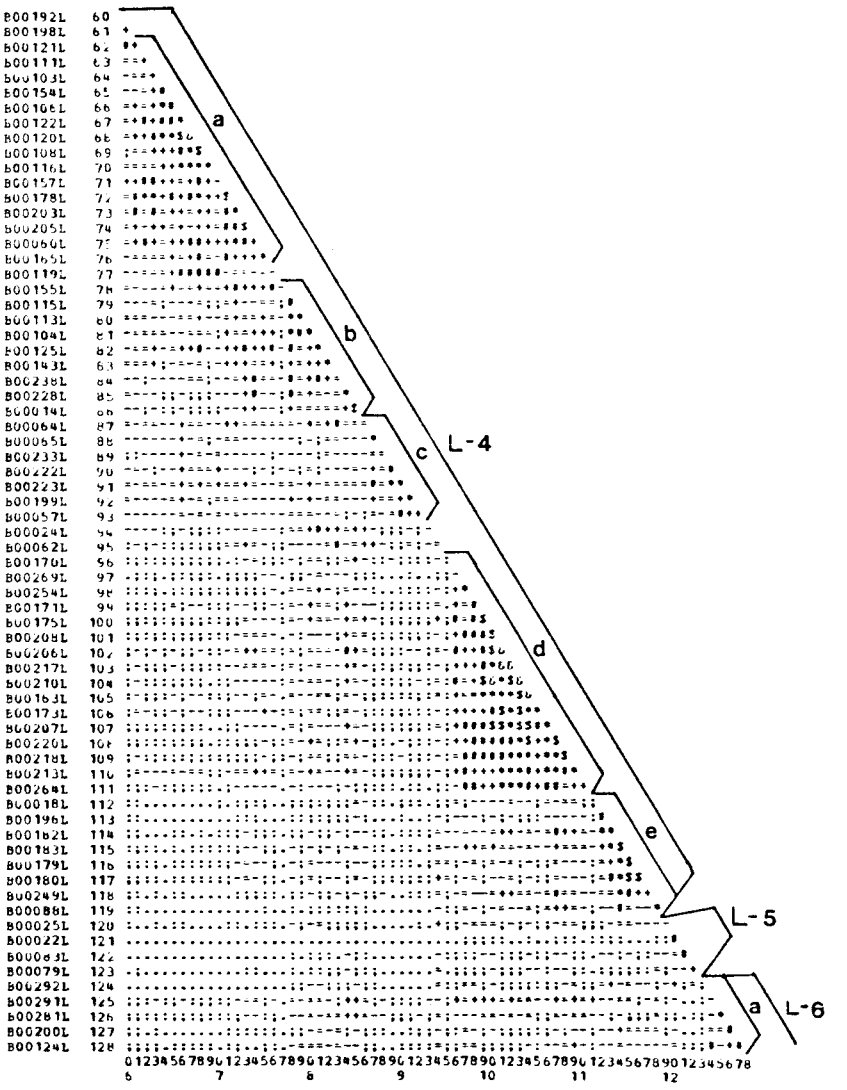


Fig. 29 (cont'd.)

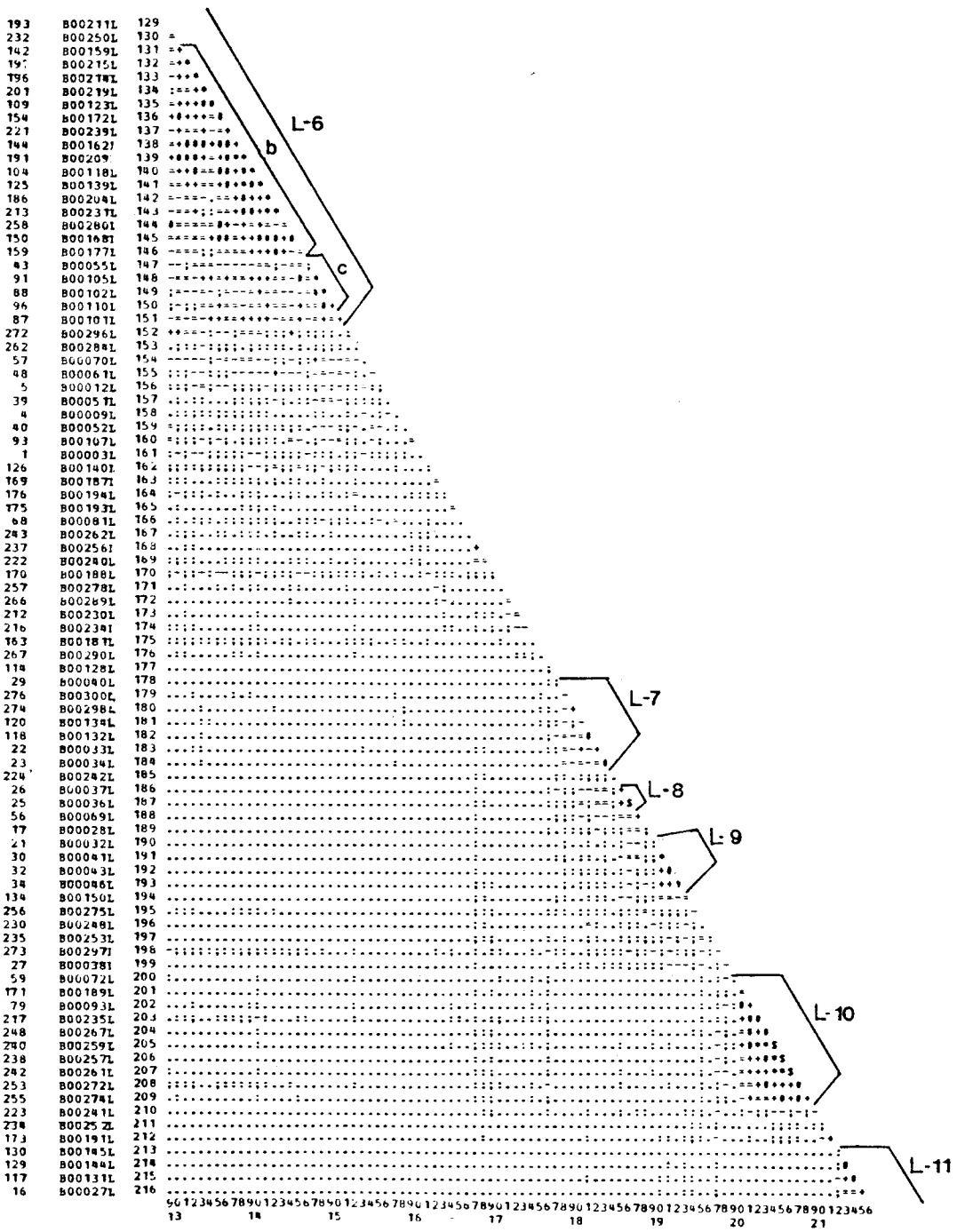
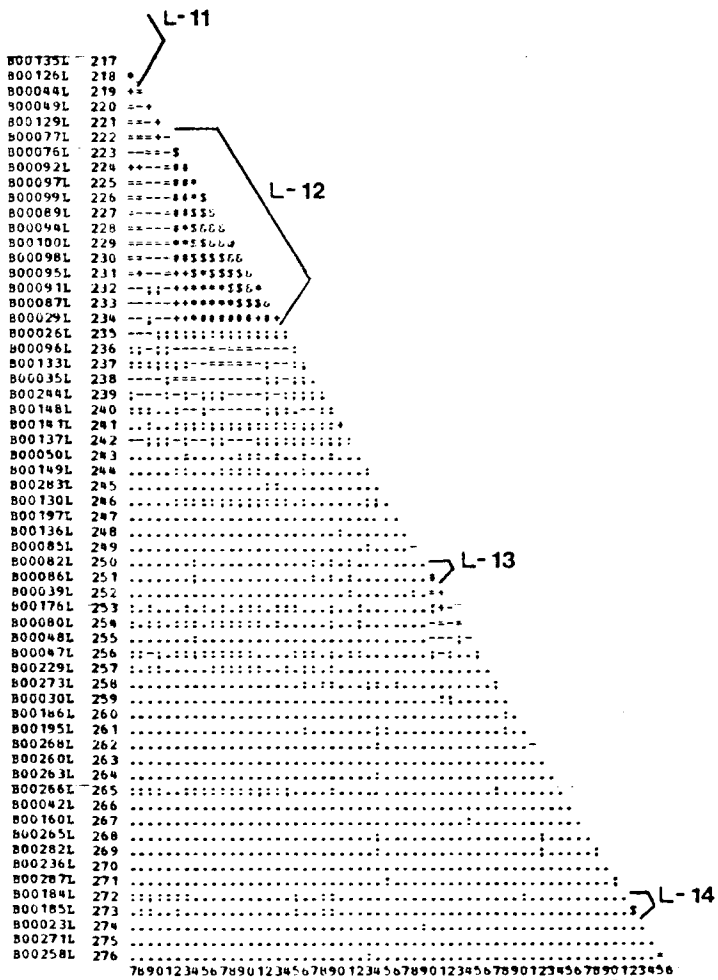


Fig. 29 (cont'd.)


L-11
L-12
L-13
L-14
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B001261 218
B000441 219
B000491 220
B001291 221
B000771 222
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B000871 233
B000291 234
B000261 235
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B000351 238
B002441 239
B001481 240
B001471 241
B001371 242
B000501 243
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B001971 247
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B000821 250
B000861 251
B000391 252
B001761 253
B000801 254
B000481 255
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B002631 264
B002661 265
B000421 266
B001601 267
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B002361 270
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B001851 273
B000231 274
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23
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25
26
27

Fig. 29 (cont'd.)

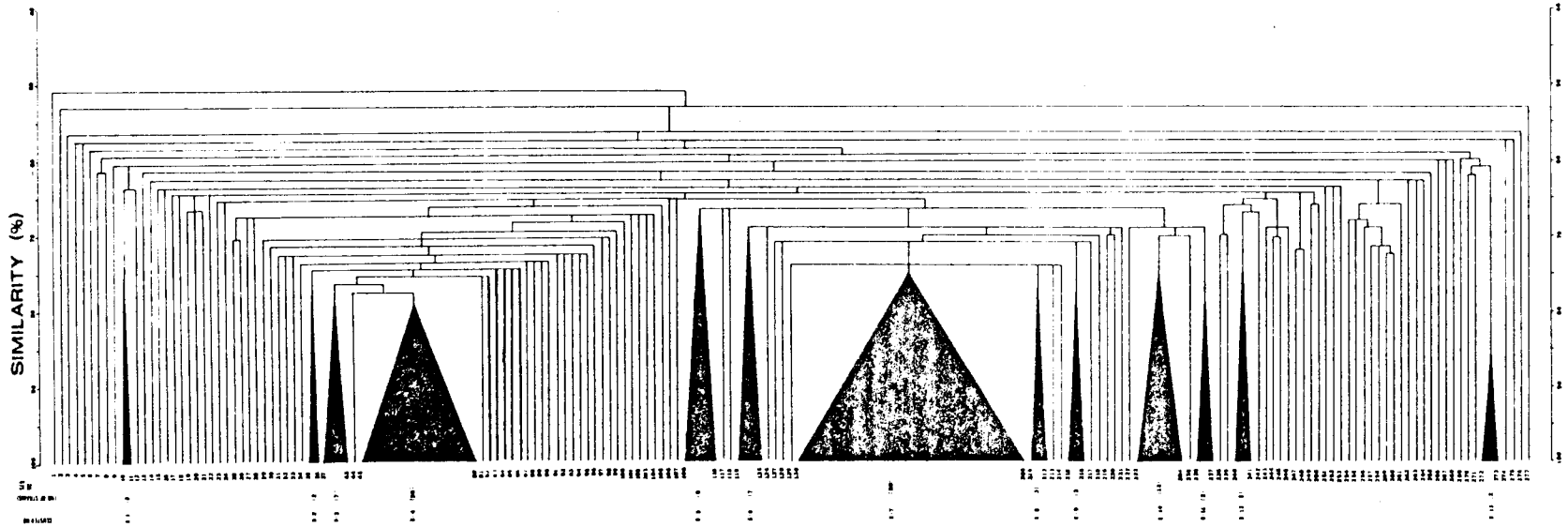


Fig. 30.
Simplified dendrogram for
Summer 1975.
20 C isolates - H series

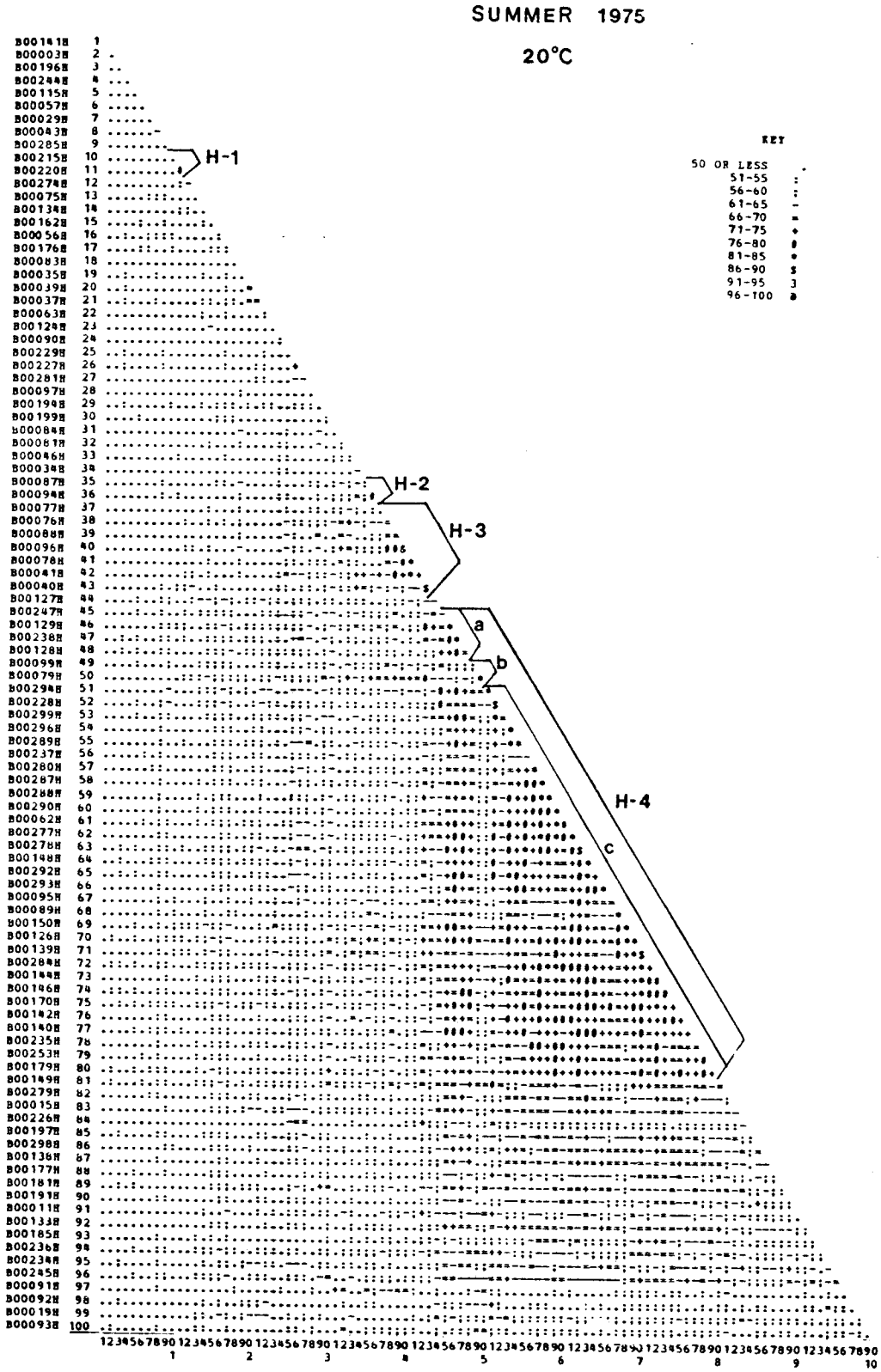


Fig. 31. Cluster triangle matrix for Summer 1975.
20 C isolates - H series

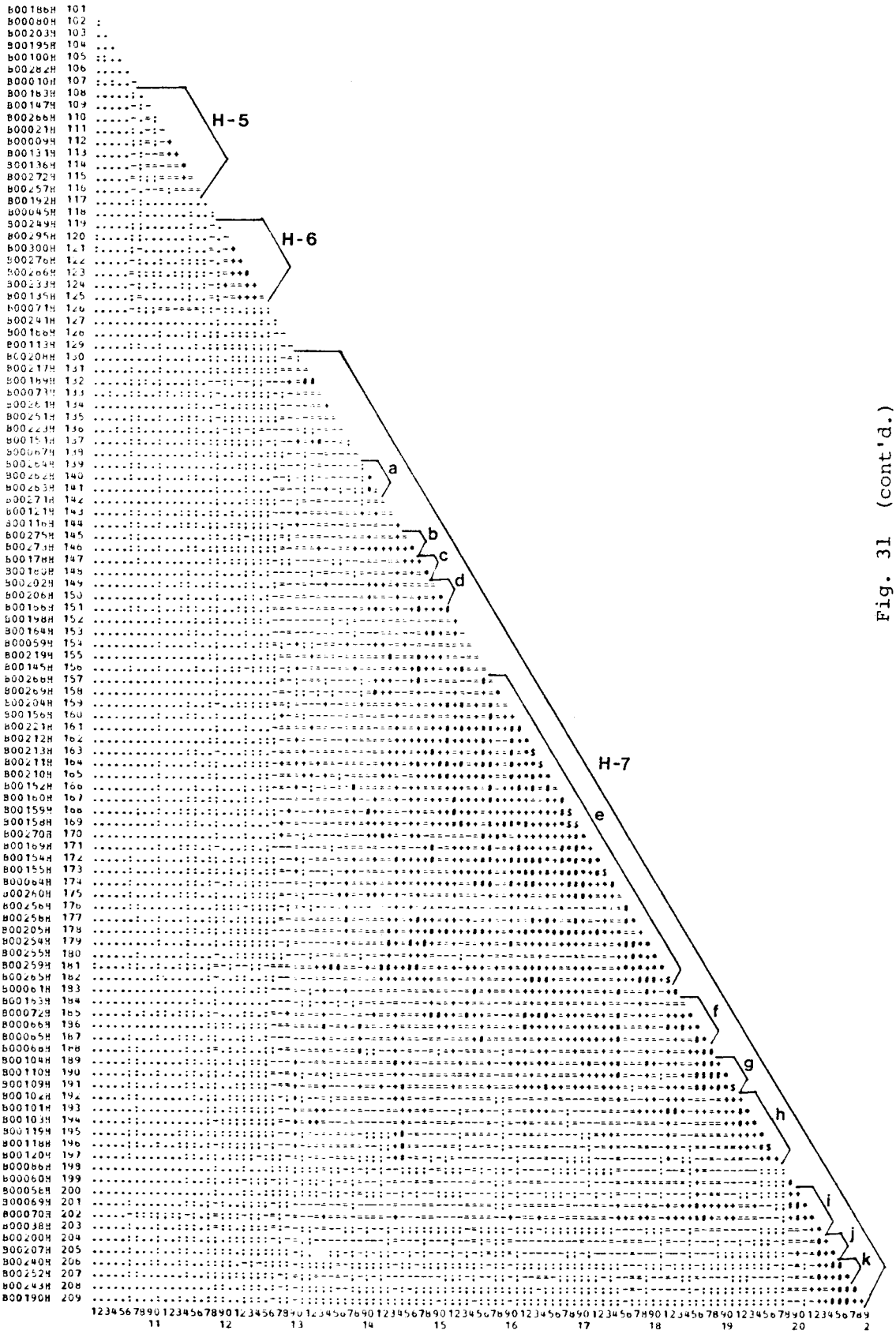


Fig. 31 (cont'd.)

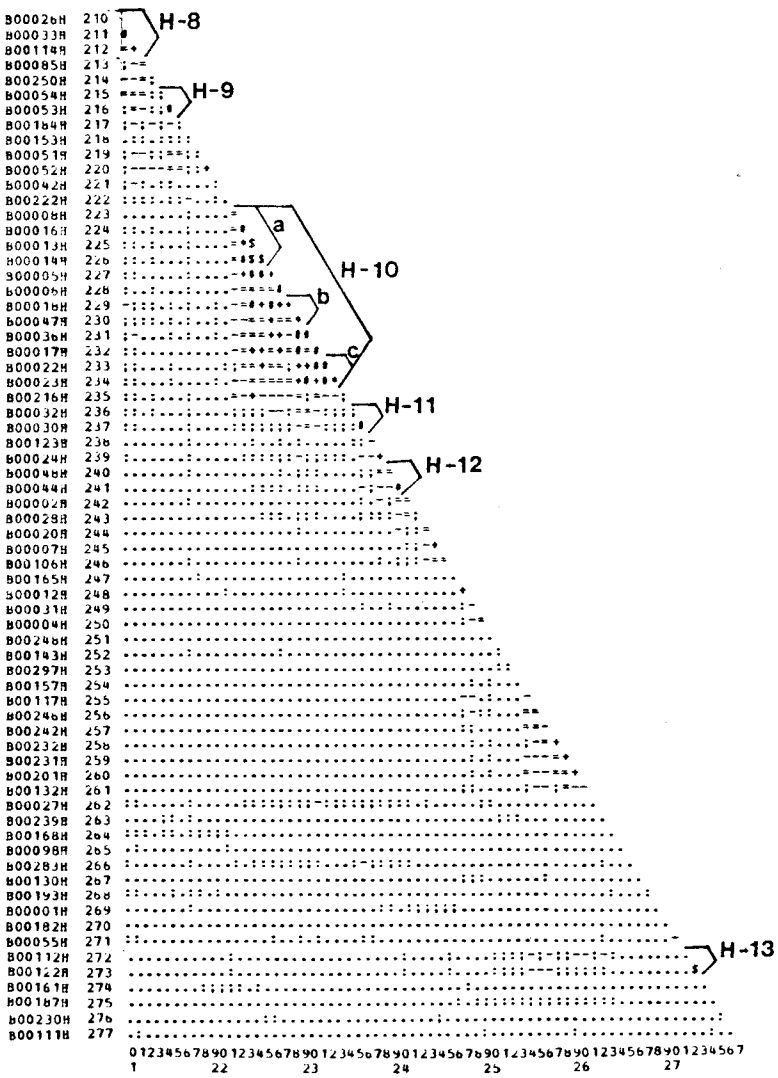


Fig. 31 (cont'd.)

TABLE 12 Source of clusters for summer 1975, 4°C isolates

CLUSTER NO.	NO. OF STRAINS	RATIO TO TOTAL ISOLATES*	WATER ORIGIN %	SEDIMENT ORIGIN %	RATIO TO TOTAL WATER ISOLATES**	RATIO TO TOTAL SEDIMENT ISOLATES***
L-4	60	21.7	66.7	33.3	33.1	12.3
L-6	28	10.1	78.6	21.4	18.2	3.9
L-1	21	7.6	57.1	42.9	9.9	5.8
L-12	13	4.7	0.0	100.0	0.0	8.4
L-10	10	3.6	10.0	90.0	0.8	5.8
L-2	9	3.3	100.0	0.0	7.4	0.0
L-11	6	2.2	0.0	100.0	0.0	3.9
L-7	7	2.5	0.0	100.0	0.0	4.5
L-5	4	1.4	50.0	50.0	1.7	1.3
L-9	4	1.4	0.0	100.0	0.0	2.6
L-3	2	0.7	100.0	0.0	1.7	0.0
L-8	2	0.7	0.0	100.0	0.0	1.3
L-14	2	0.7	0.0	100.0	0.0	1.3
L-15	2	0.7	0.0	100.0	0.0	1.3
TOTAL	170	61.6	51.8	48.2	72.7	52.9

*Total 276 strains

**Total 121 strains

***Total 155 strains

TABLE 13 Source of clusters for summer 1975 20°C isolates

CLUSTER NO	NO. OF STRAINS	RATIO TO TOTAL ISOLATES*	WATER ORIGIN %	SEDIMENT ORIGIN %	RATIO TO TOTAL WATER ISOLATES **	RATIO TO TOTAL SEDIMENT ISOLATES ***
H-7	80	28.8	85.0	15.0	51.5	10.5
H-4	36	12.9	8.3	91.7	2.3	22.6
H-10	12	4.3	83.3	16.7	7.6	1.4
H-5	9	3.2	33.3	67.7	2.3	4.1
H-3	7	2.5	0.0	100.0	0.0	4.8
H-6	7	2.5	0.0	100.0	0.0	4.8
H-8	3	1.1	33.3	67.7	0.8	1.4
H-1	2	0.7	100.0	0.0	1.5	0.0
H-2	2	0.7	0.0	100.0	0.0	1.4
H-9	2	0.7	100.0	0.0	1.5	0.0
H-11	2	0.7	0.0	100.0	0.0	1.4
H-12	2	0.7	0.0	100.0	0.0	1.4
H-13	2	0.7	100.0	0.0	1.5	0.0
TOTAL	166	32.0	53.6	46.4	67.4	53.5

* Total 278 strains

** Total 132 strains

*** Total 144 strains

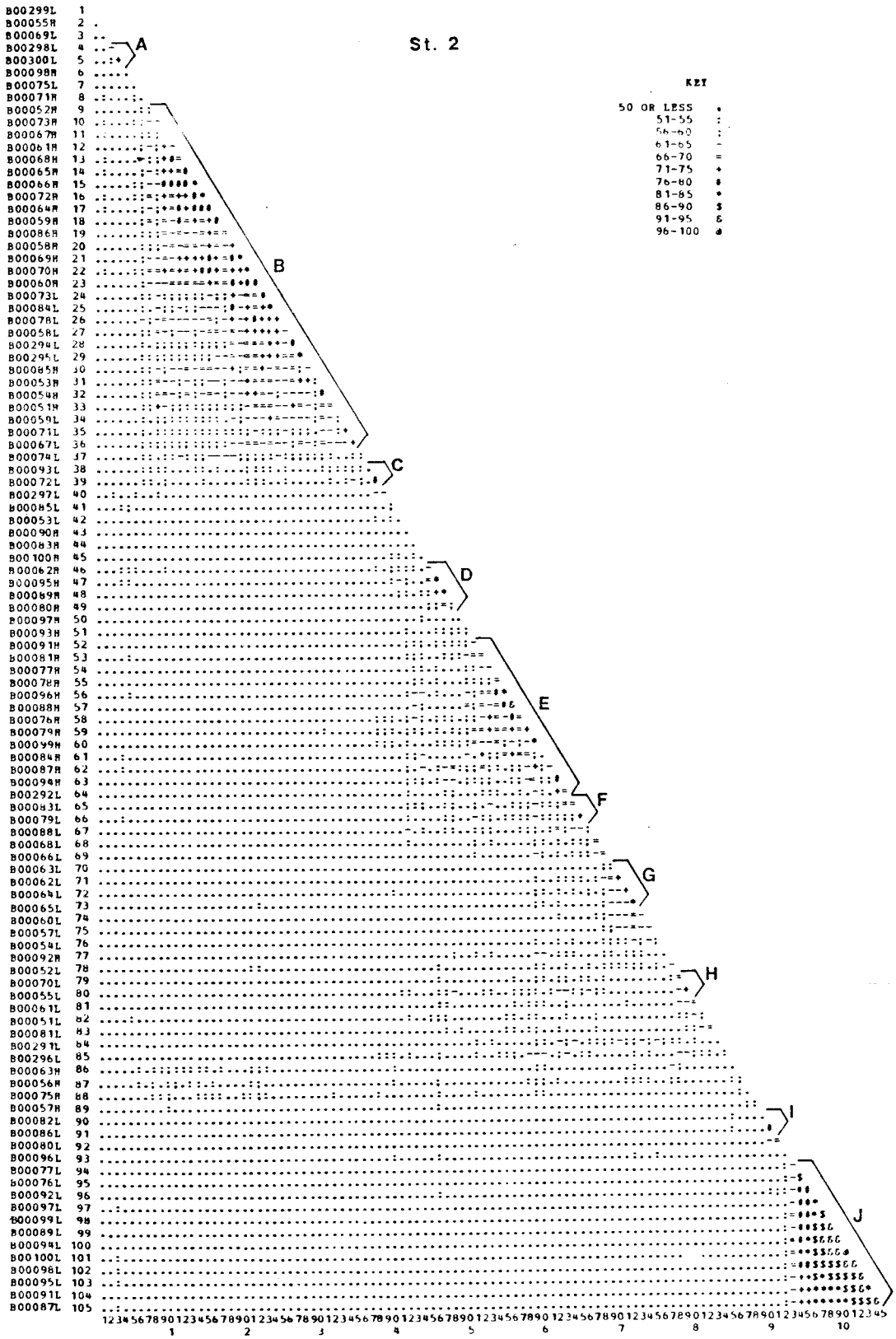


Fig. 32. Cluster triangle for Summer 1975 isolates at Station 2.

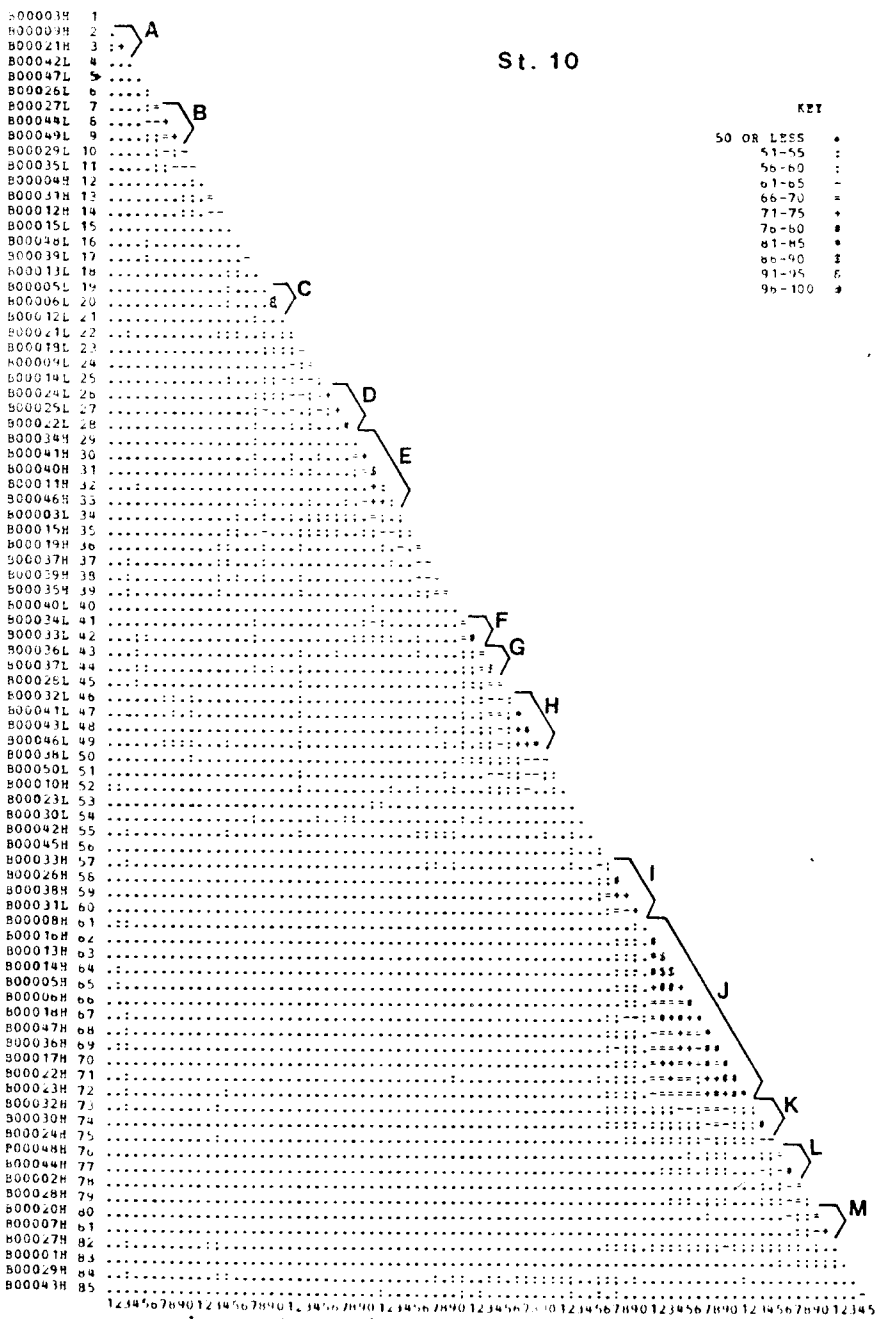


Fig. 33. Cluster triangle for Summer 1975 isolates at Station 10.

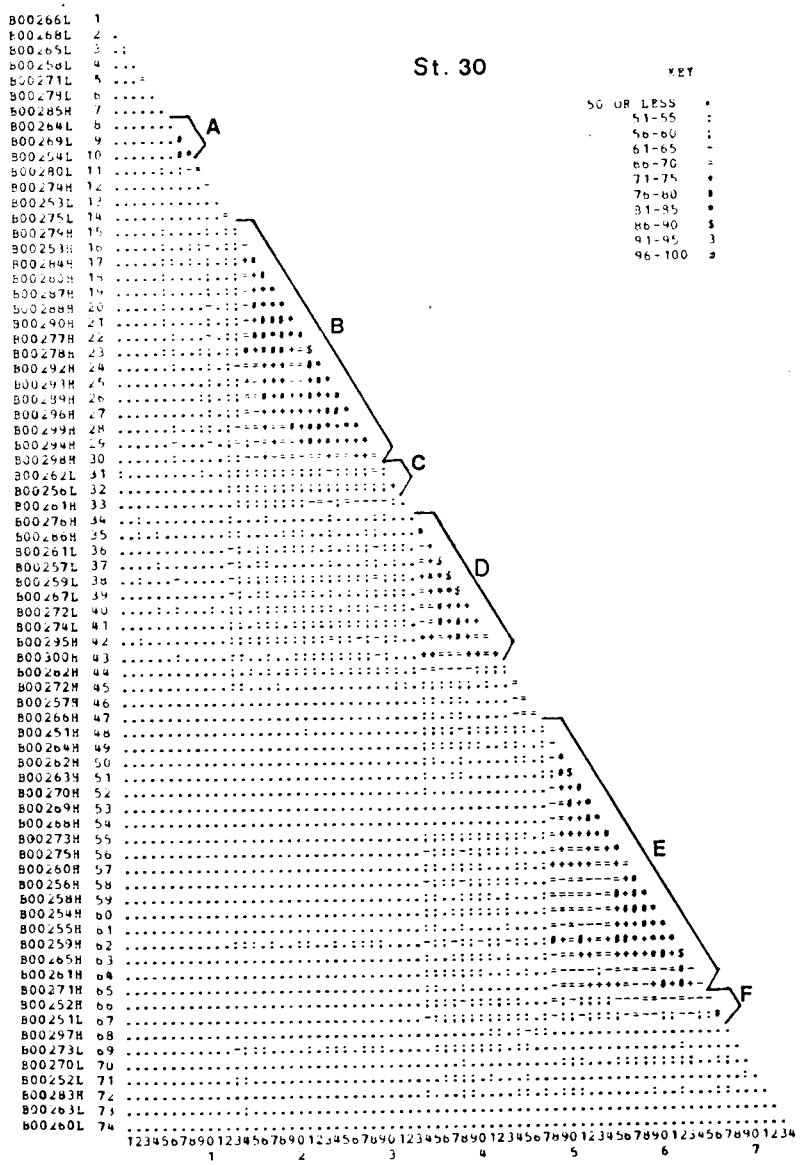


Fig. 34. Cluster triangle for Summer 1975 isolates at Station 30.

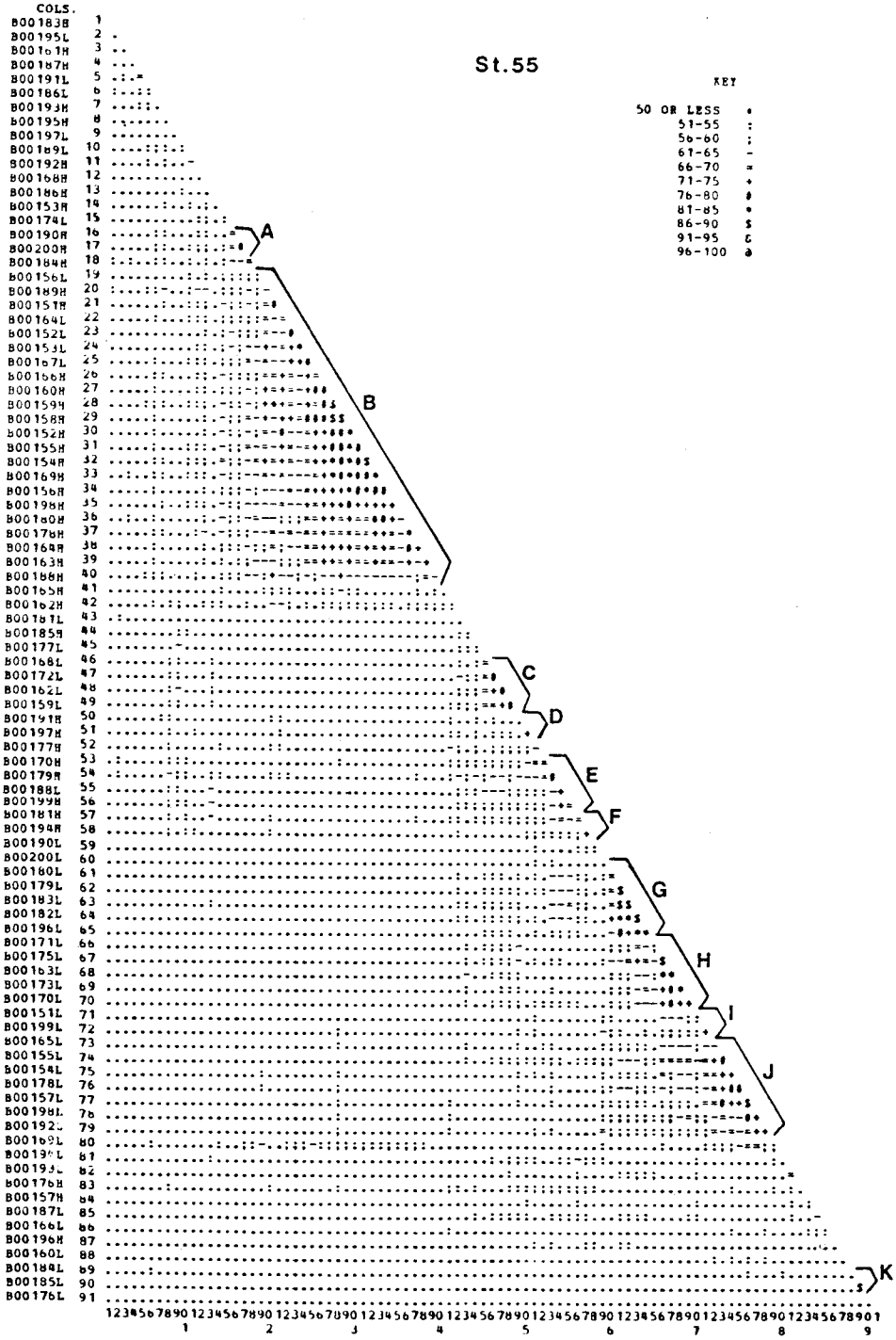


Fig. 35. Cluster triangle for Summer 1975 isolates at Station 55.

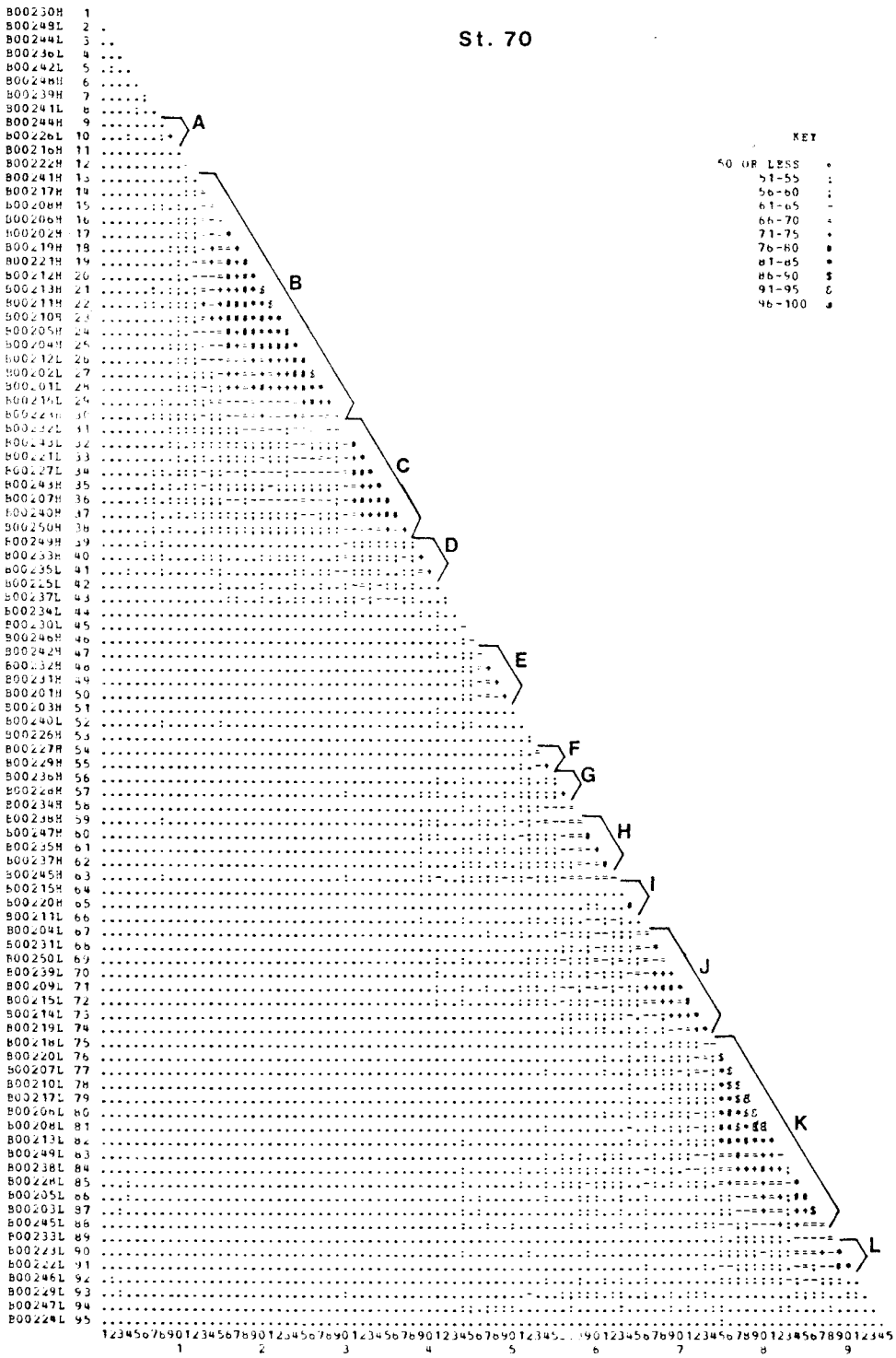


Fig. 36. Cluster triangle for Summer 1975 isolates at Station 70.

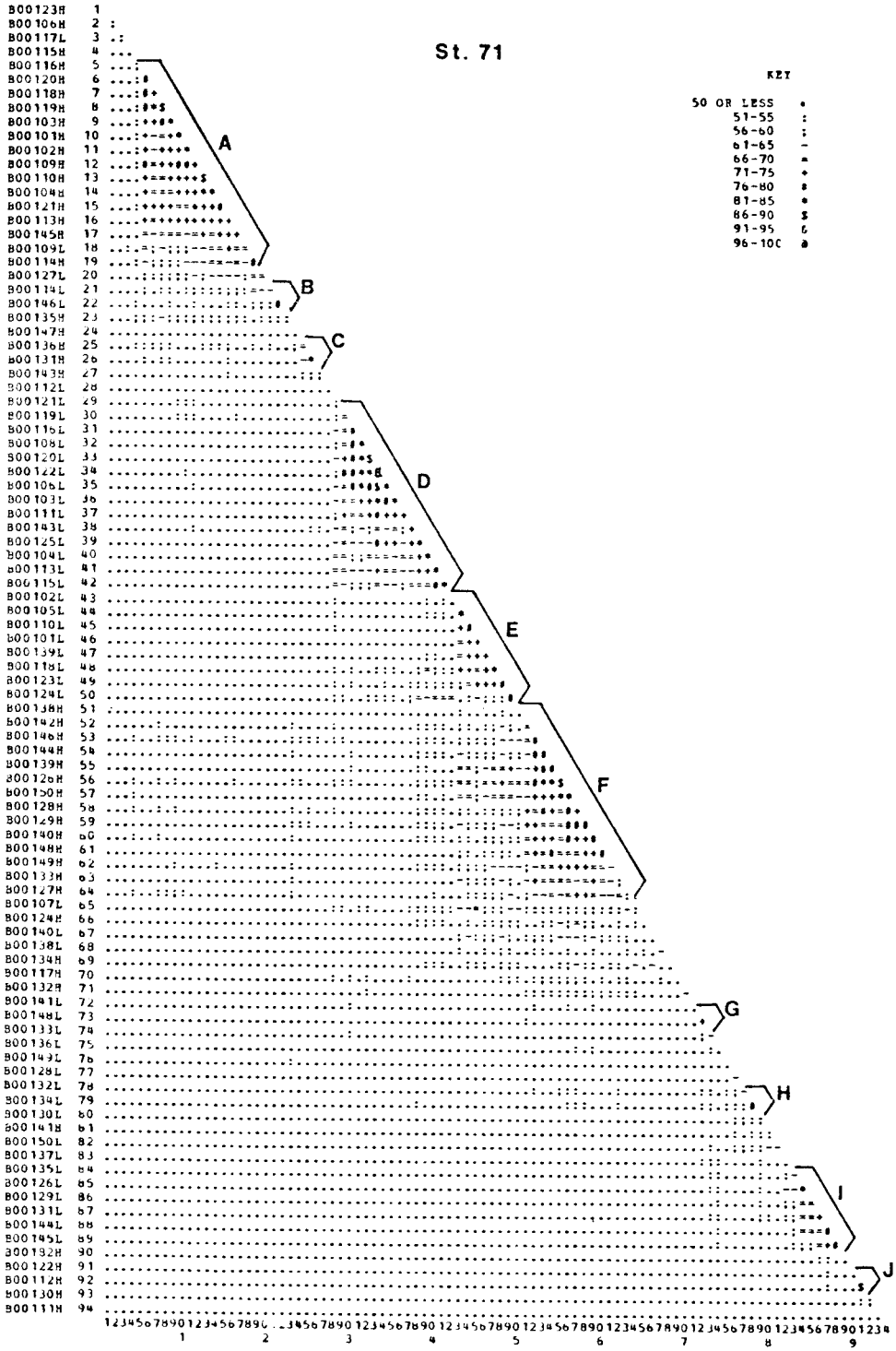


Fig. 37. Cluster triangle for Summer 1975 isolates at Station 71.

TABLE 14 Source of common clusters between 4 and 20 C populations when analyzed by individual station.

		SOURCE OF COMMON CLUSTERS	
STATION	CLUSTER	4°C	20°C
2	B	L-1	H-7
	E	L-6	H-3
10	I	L-1	H-8
30	D	L-10	H-6
	F	L-1	H-7
55	B	L-2	H-7
	E	NONE	NONE
70	A	NONE	NONE
	B	L-2	H-7
	C	L-1	H-7
	D	L-10	H-6
71	A	L-1	H-7

came from sediment. Members of cluster H7 occurred in water at all stations except station 10. Members of cluster H4 occurred in sediment at all stations except station 10. In water from station 10 the only cluster found was H10 and the only other place cluster H10 was found was in sediment at station 10. Sediment at station 10 also contained several small clusters not found at other locations.

When the cluster analyses were performed on a station by station basis using both the 4 C and 20 C isolates several clusters in the 4 C clusters were found to be equivalent to several of the 20 C clusters (Figs. 32-37, Table 14). It would appear that clusters L1, L2, H7 and H8 are closely related. Clusters H3 and L6 appear to be equivalent as do clusters H6 and L10.

Winter 1976

As shown in Figs. 38 and 39 a total of 30 clusters were obtained for the 4 C isolates from winter 1976. Thirteen of these clusters contained only 2 members. The distribution of clusters by source, i.e. ice, water, sediment or beach, is shown in Table 15 and Fig. 48 and by geographic location in Fig. 49. The clusters were both source and site specific. In only 2 cases were clusters of organisms found in water also found in sediment. Isolates from ice also formed their own clusters. None of the clusters showed wide distribution.

As shown in Figs. 40 and 41 a total of 30 clusters were obtained for the 20 C isolates from winter 1976. Ten of these clusters contained only 2 strains. The distribution of the clusters by source,

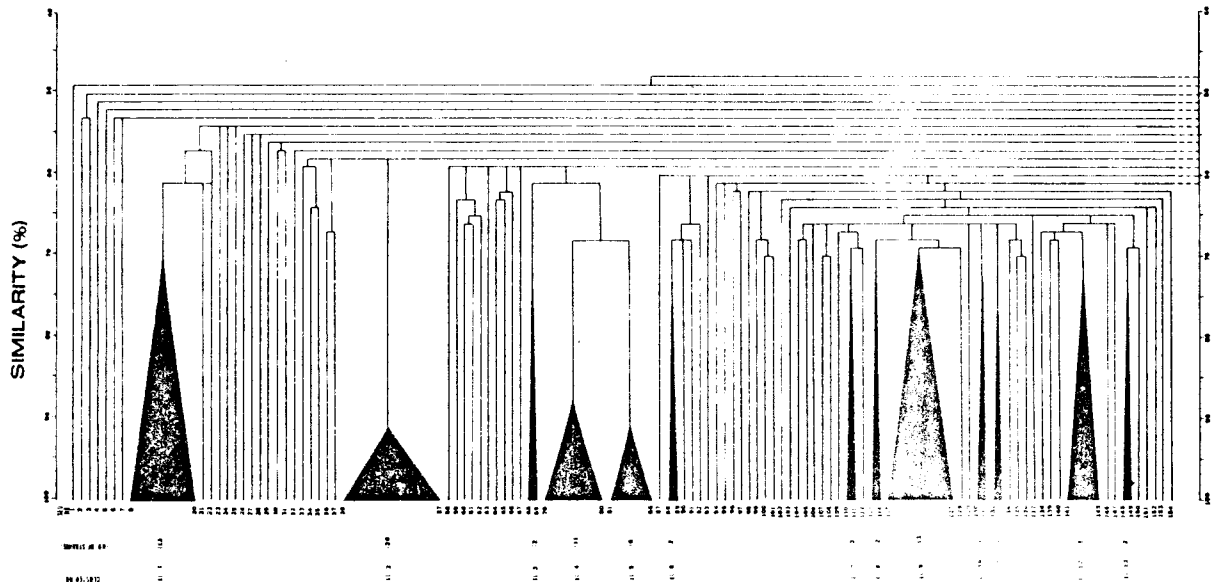


Fig. 38.
Simplified dendrogram for Winter 1976.
4 C isolates - 1L series

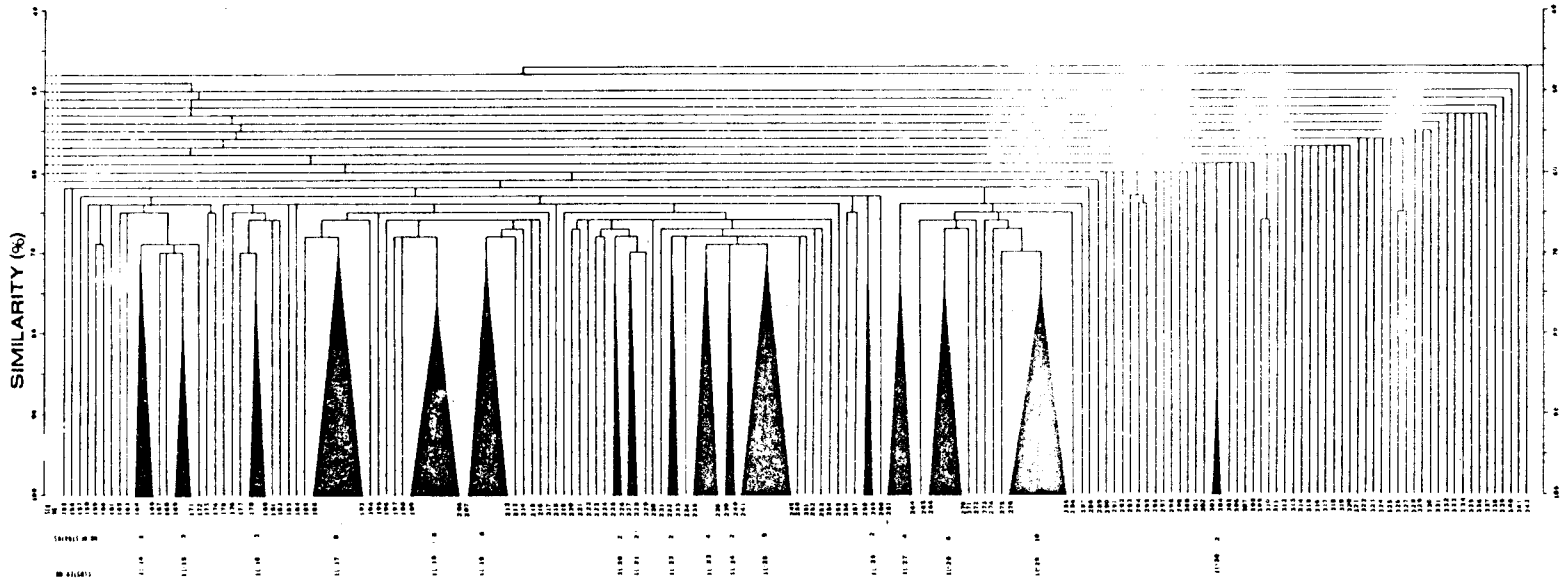


Fig. 38 (cont'd.)

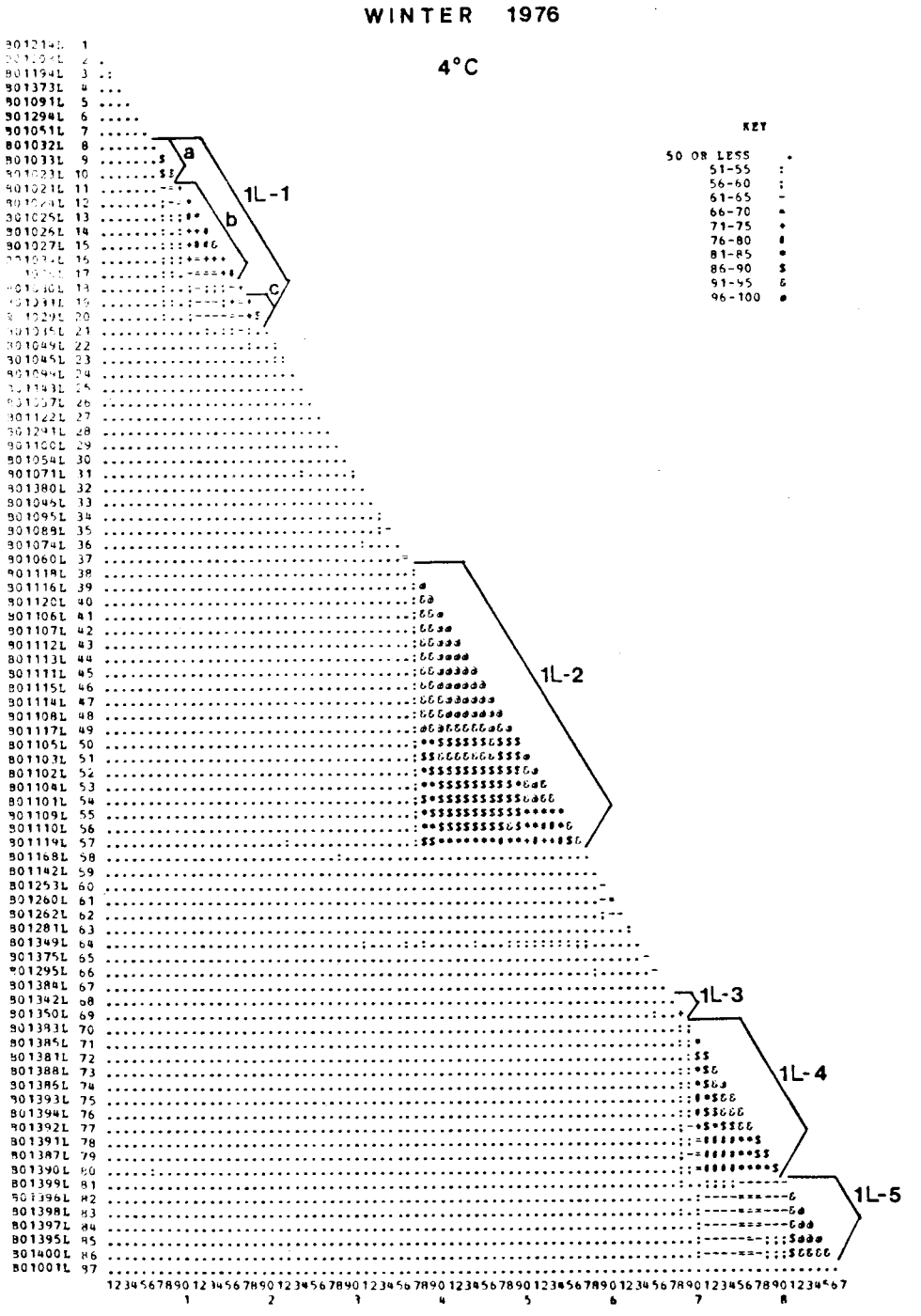


Fig. 39. Cluster triangle for Winter 1976.
4 C isolates - 1L series

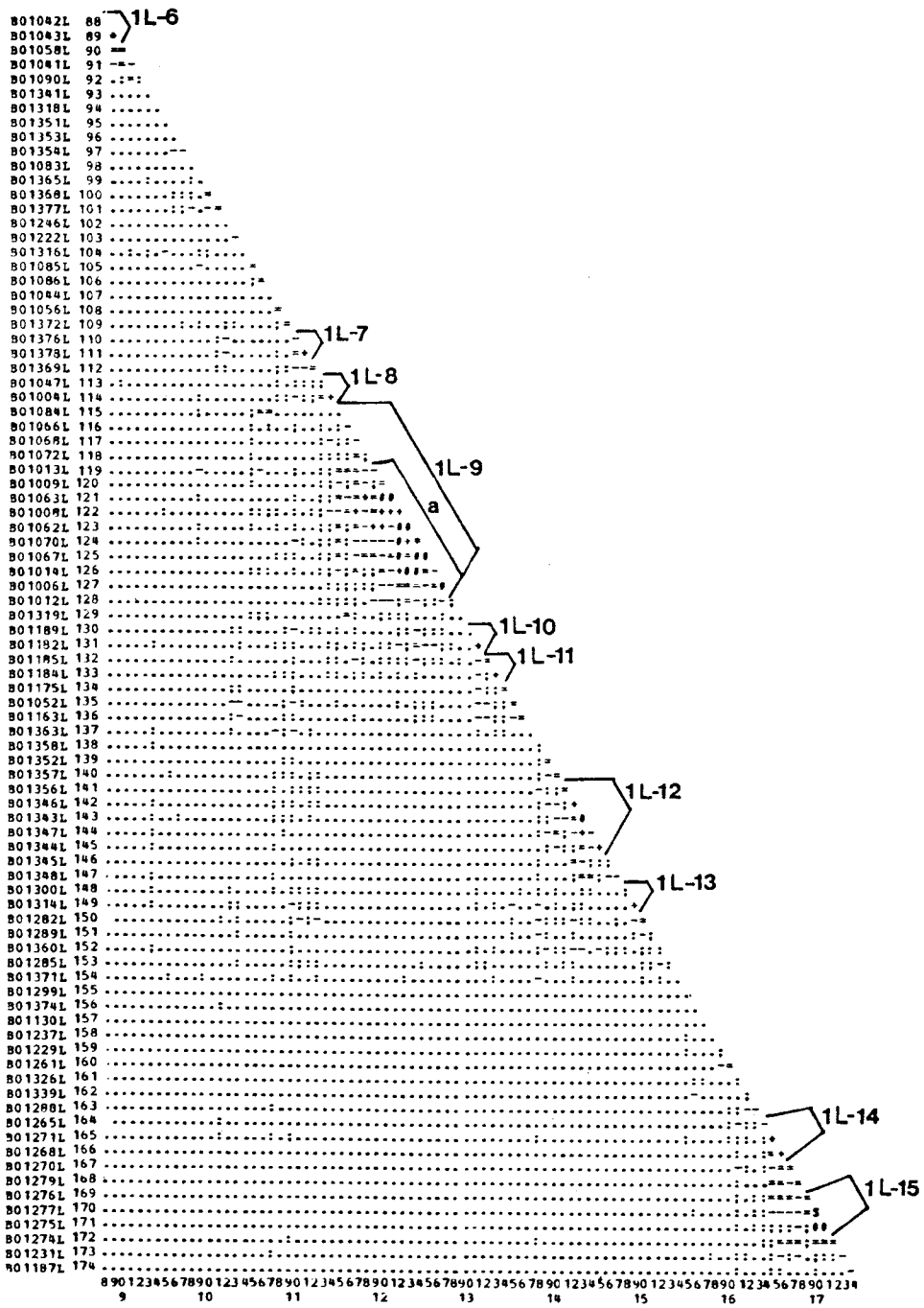
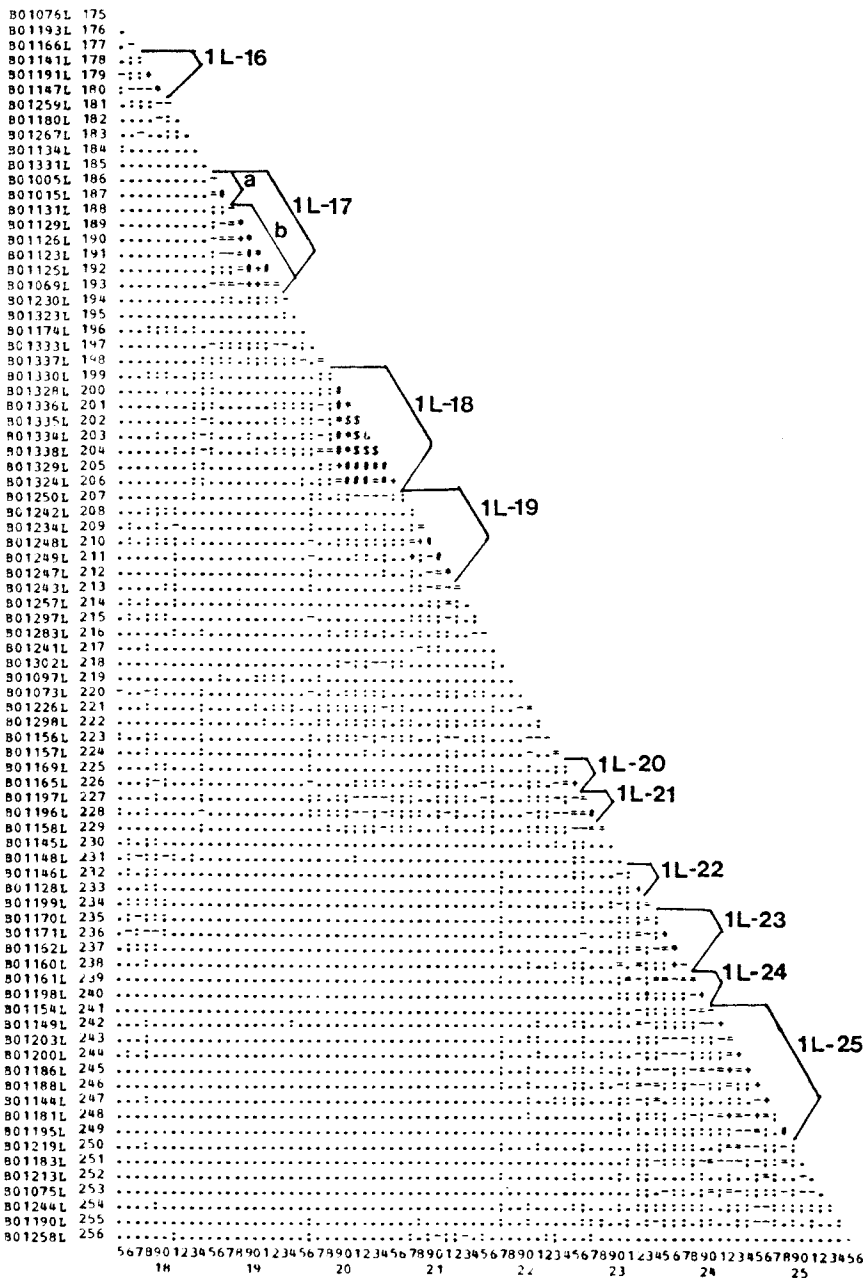


Fig. 39 (cont'd.)


1L-16
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b
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22
23
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25

Fig. 39 (cont'd.)

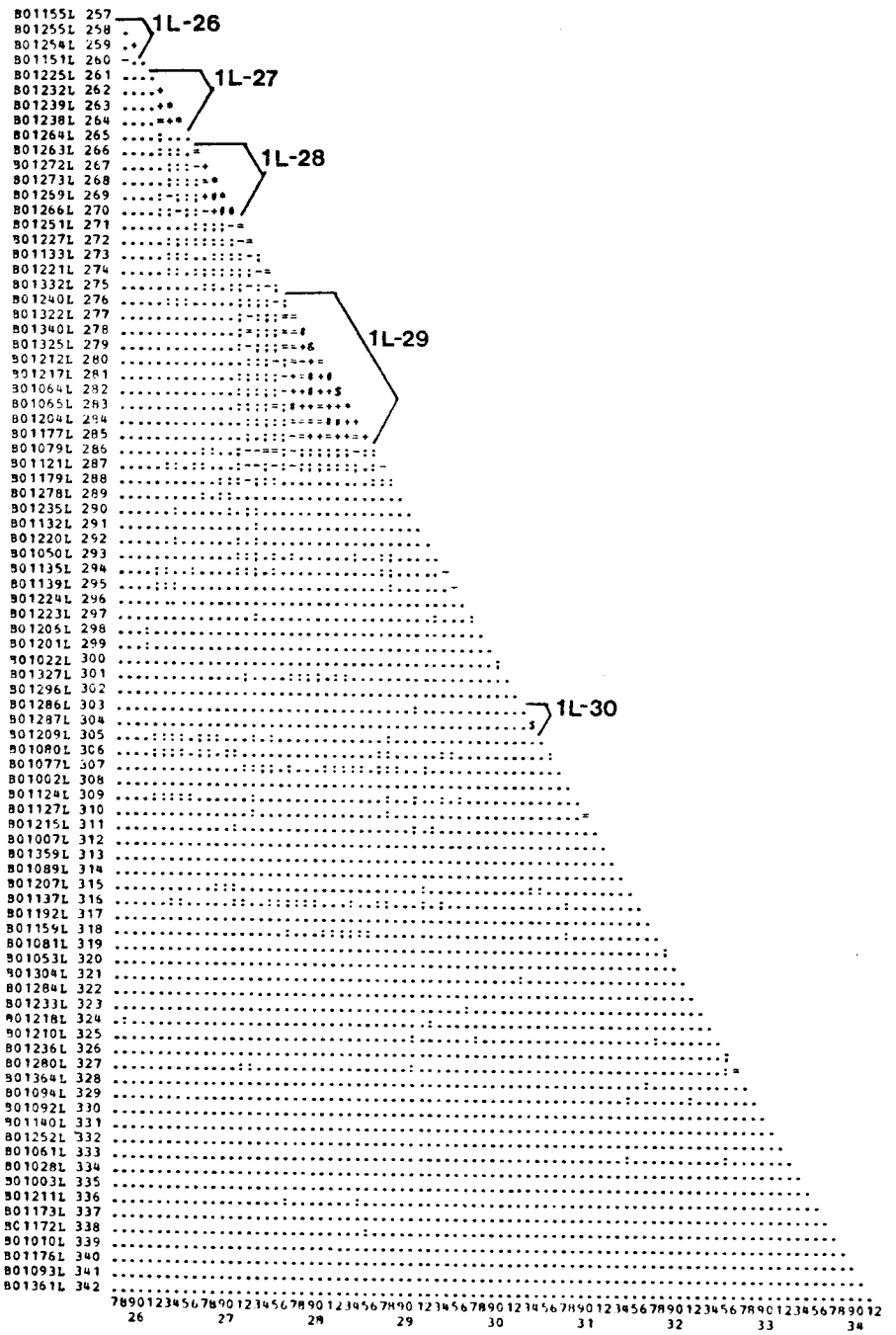


Fig. 39 (cont'd.)

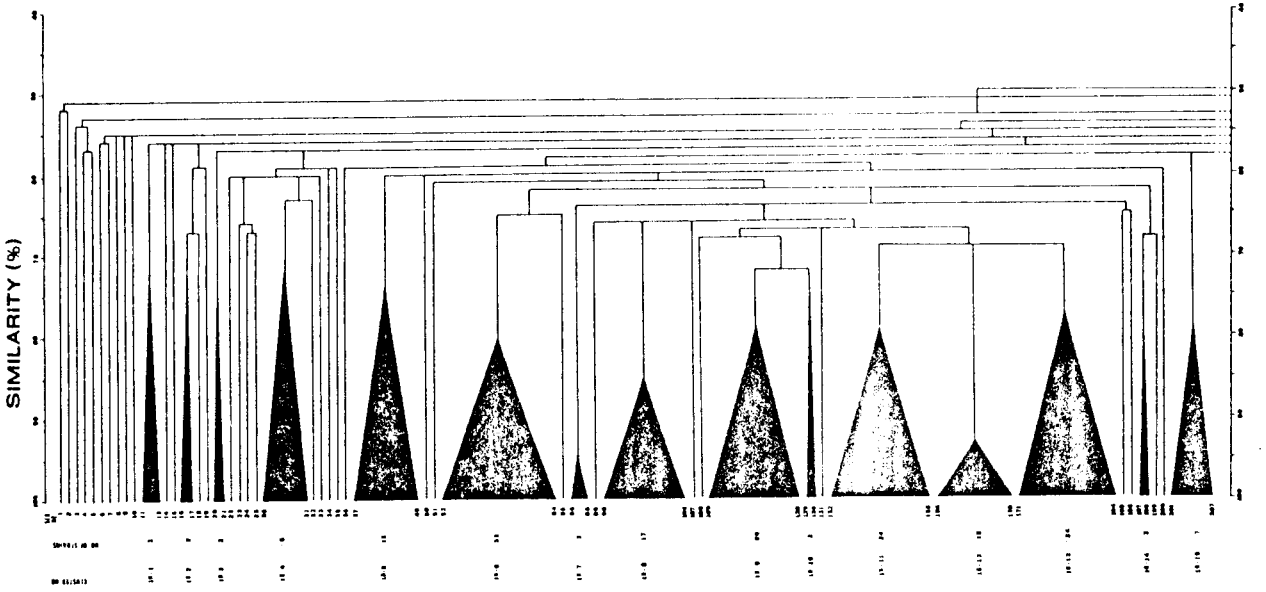


Fig. 40.
Simplified dendrogram for Winter 1976.
20° C isolates - 1H series

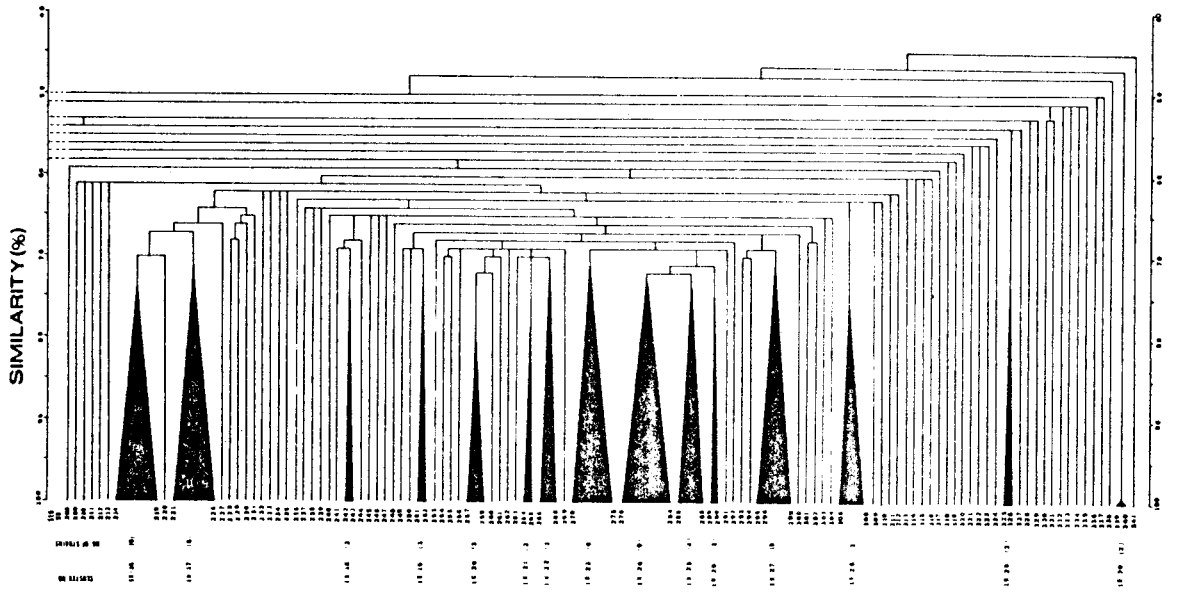


Fig. 40 (cont'd.)

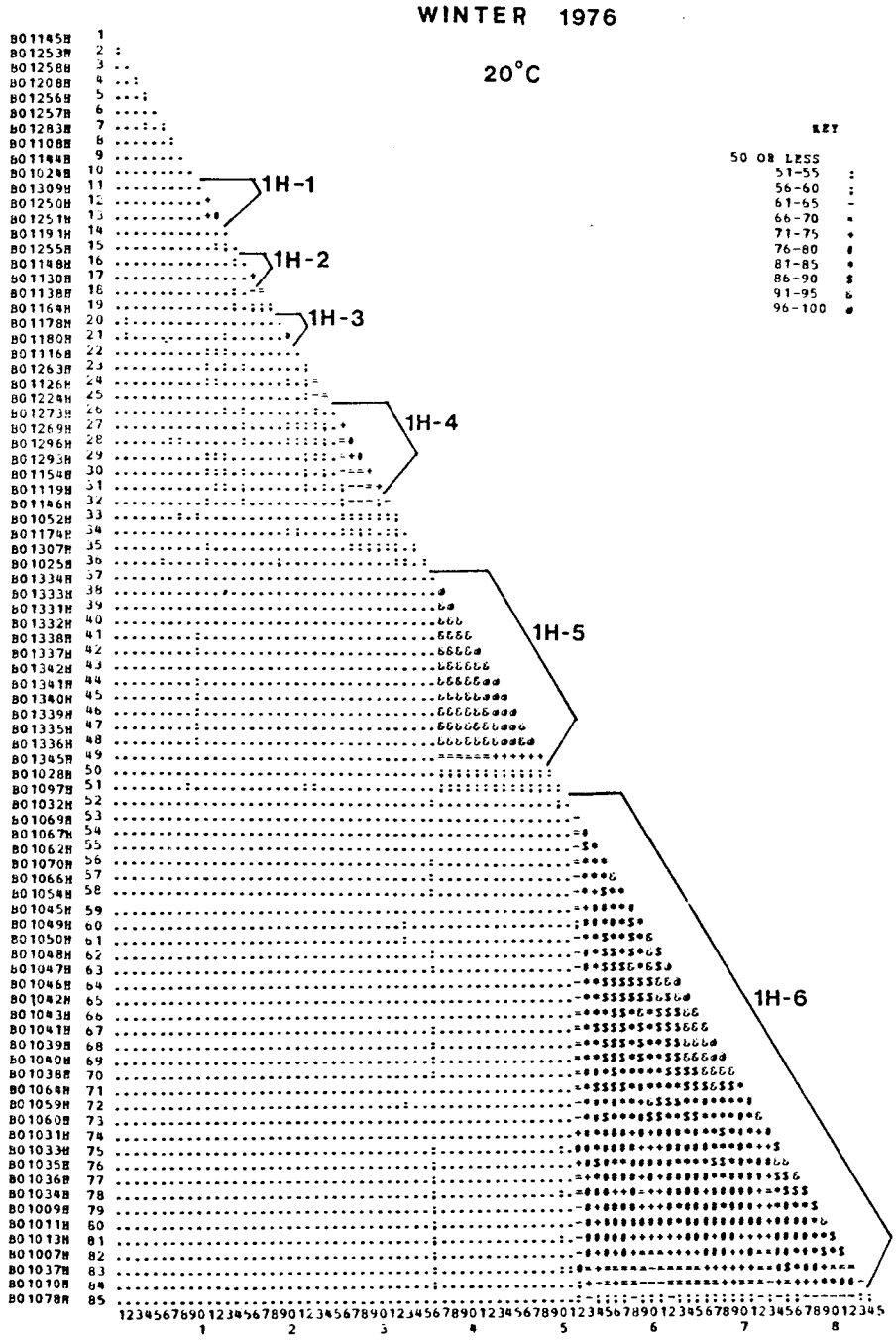


Fig. 41.
Cluster triangle for Winter 1976.
20 C isolates - 1H series

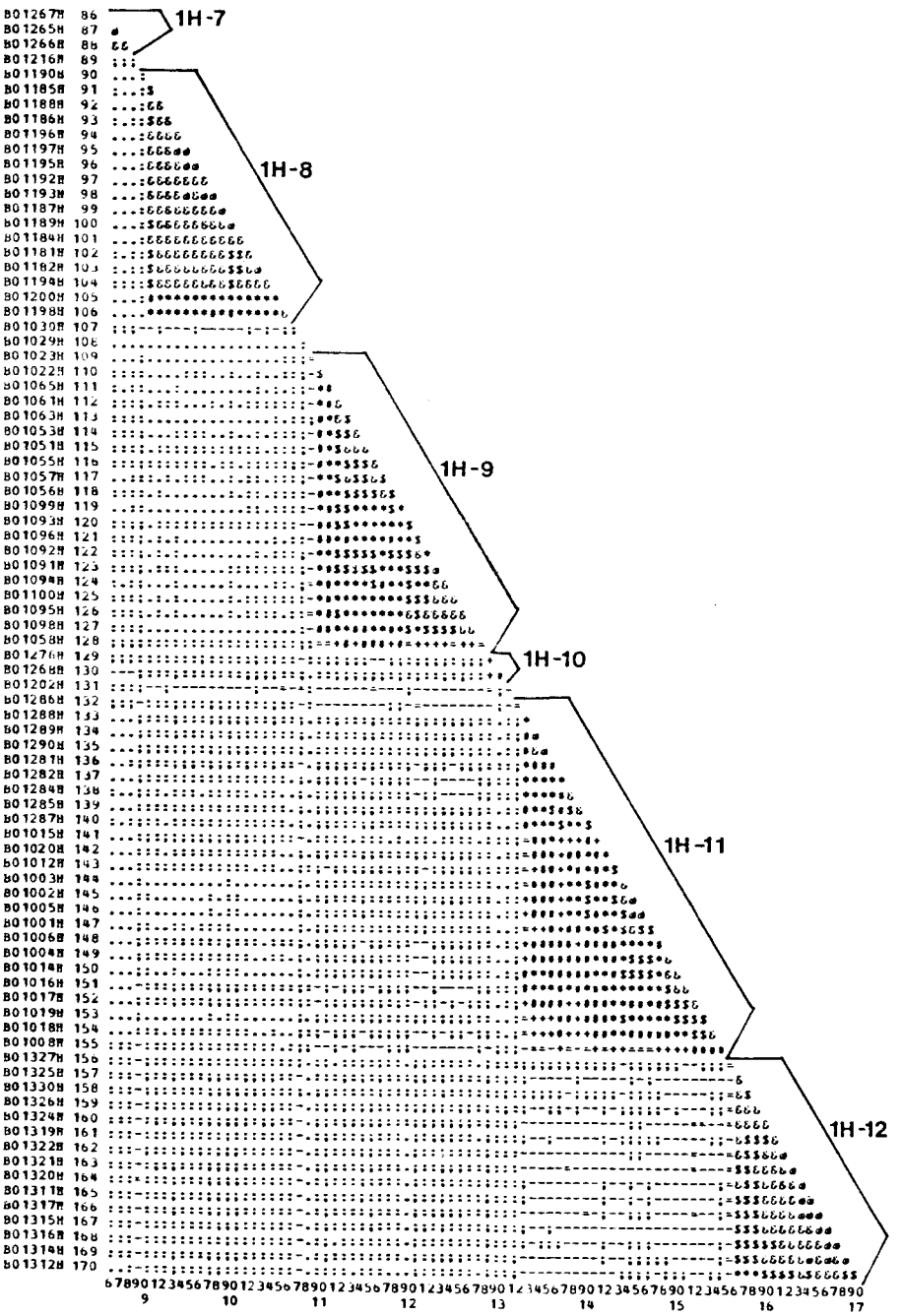


Fig. 41 (cont'd.)

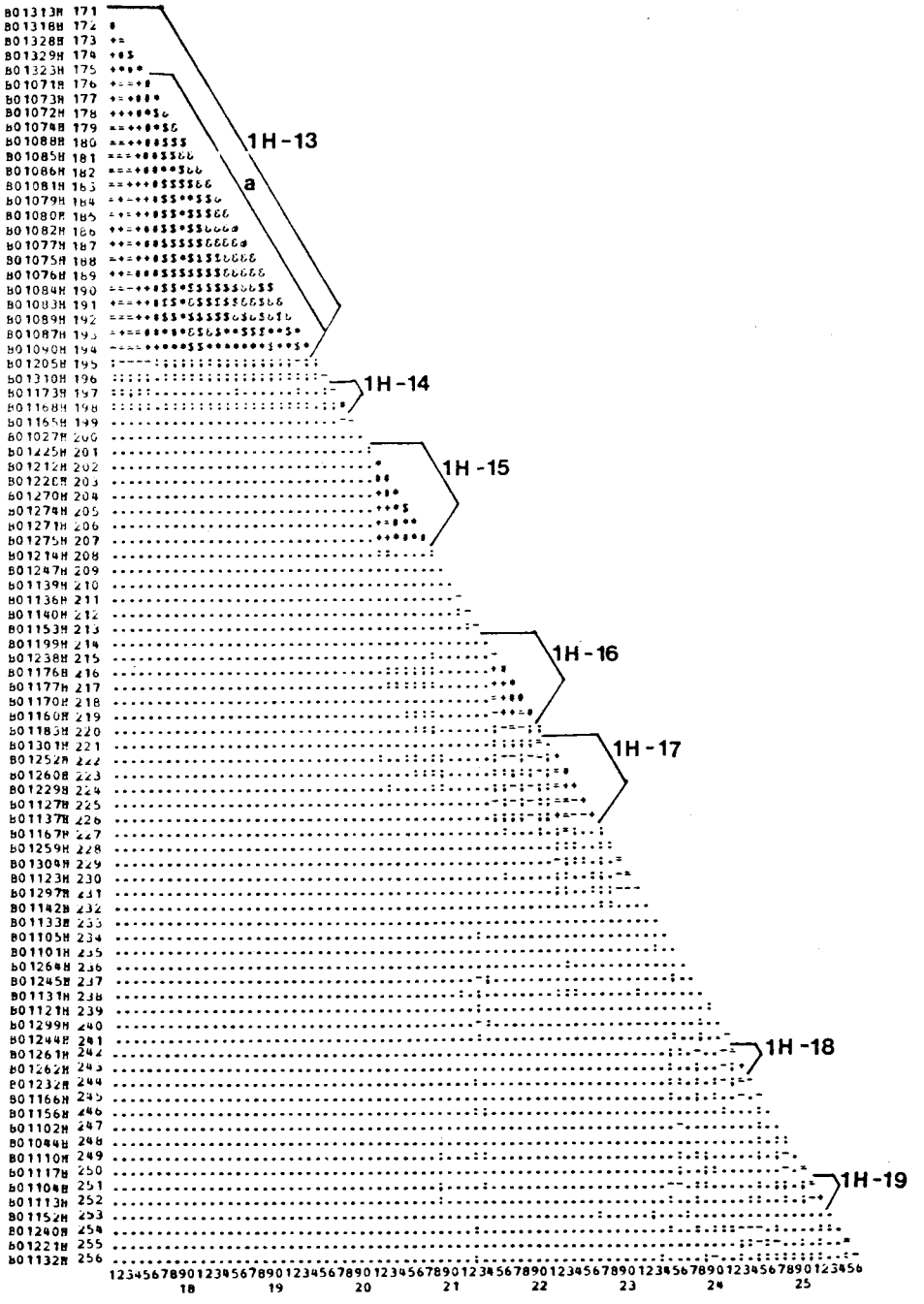
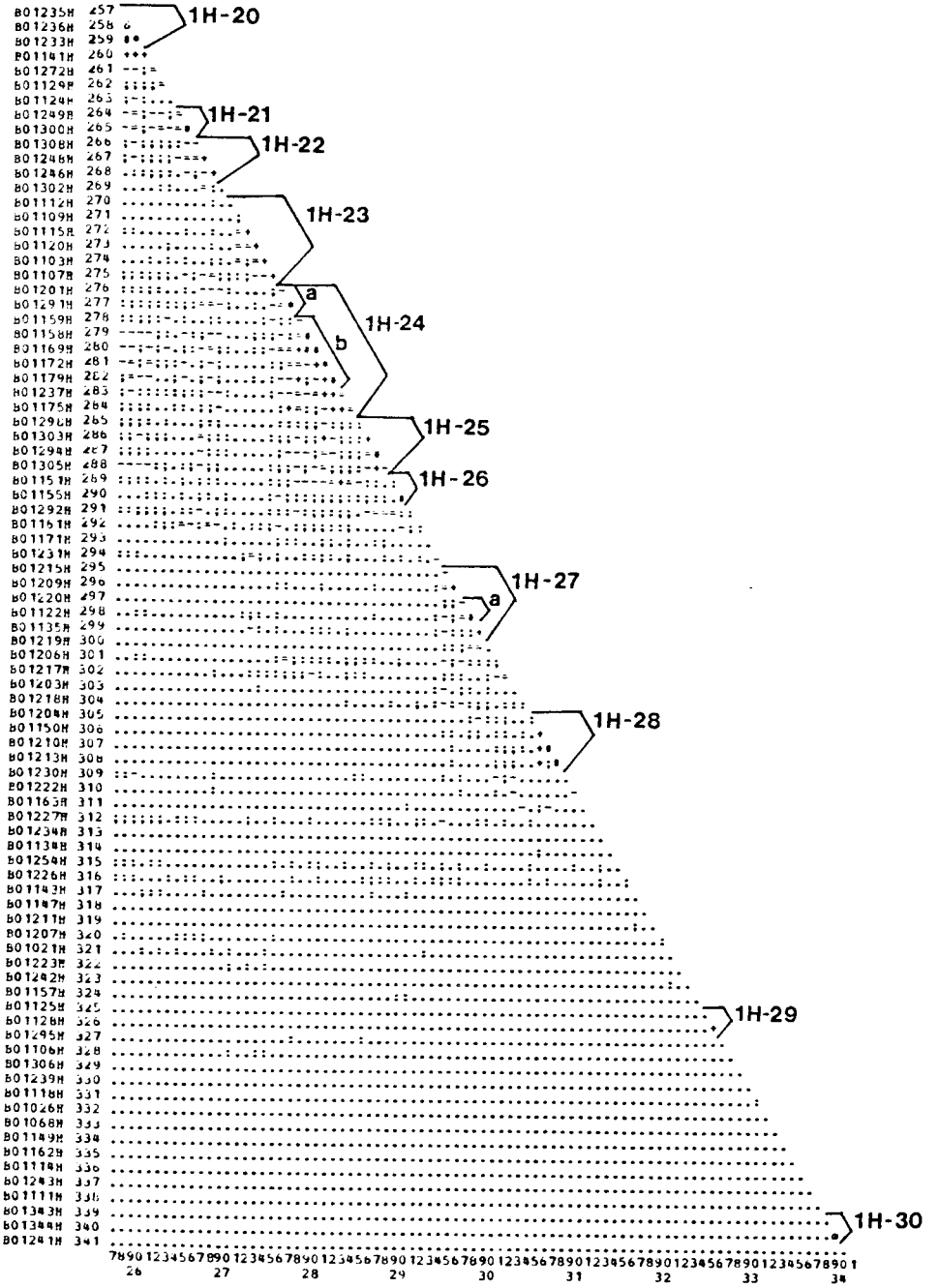


Fig. 41 (cont'd.)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1H-22
1H-23
1H-24
1H-25
1H-26
1H-27
1H-28
1H-29
1H-30
a
b
26
27
28
29
30
31
32
33
34

Fig. 41 (cont'd.)

TABLE 15 Source of clusters for winter 1976 4°C isolates.

CLUSTER NO.	NO. OF STRAINS	RATIO TO TOTAL ISOLATES *	WATER ORIGIN %	ICE ORIGIN %	SEDIMENT ORIGIN %	BEACH SAMPLE ORIGIN %	RATIO TO TOTAL WATER ORIGIN **	RATIO TO TOTAL ICE ORIGIN ***	RATIO TO TOTAL SEDIMENT ORIGIN****	RATIO TO BEACH SAMPLE ORIGIN*****
1L-2	20	5.8	100.0	0.0	0.0	0.0	14.3	0.0	0.0	0.0
1L-1	13	3.8	100.0	0.0	0.0	0.0	9.3	0.0	0.0	0.0
1L-9	13	3.8	100.0	0.0	0.0	0.0	9.3	0.0	0.0	0.0
1L-4	11	3.2	0.0	100.0	0.0	0.0	0.0	19.6	0.0	0.0
1L-29	10	2.9	50.0	0.0	50.0	0.0	3.6	0.0	3.9	0.0
1L-25	9	2.6	0.0	0.0	100.0	0.0	0.0	0.0	7.0	0.0
1L-17	8	2.3	100.0	0.0	0.0	0.0	5.7	0.0	0.0	0.0
1L-18	8	2.3	100.0	0.0	0.0	0.0	5.7	0.0	0.0	0.0
1L-5	6	1.8	0.0	0.0	0.0	100.0	0.0	0.0	0.0	31.6
1L-19	6	1.8	0.0	0.0	100.0	0.0	0.0	0.0	4.7	0.0
1L-12	5	1.5	0.0	0.0	0.0	100.0	0.0	0.0	0.0	26.3
1L-28	5	1.5	0.0	0.0	100.0	0.0	0.0	0.0	3.9	0.0
1L-23	4	1.2	0.0	0.0	100.0	0.0	0.0	0.0	3.1	0.0
1L-14	3	0.9	0.0	0.0	100.0	0.0	0.0	0.0	2.3	0.0
1L-27	3	0.9	0.0	0.0	100.0	0.0	0.0	0.0	2.3	0.0
1L-15	3	0.9	0.0	0.0	100.0	0.0	0.0	0.0	2.3	0.0
1L-16	3	0.9	0.0	0.0	100.0	0.0	0.0	0.0	2.3	0.0
1L-3	2	0.6	0.0	0.0	0.0	100.0	0.0	0.0	0.0	10.5
1L-6	2	0.6	100.0	0.0	0.0	0.0	1.4	0.0	0.0	0.0
1L-7	2	0.6	0.0	0.0	0.0	100.0	0.0	0.0	0.0	10.5
1L-8	2	0.6	100.0	0.0	0.0	0.0	1.4	0.0	0.0	0.0
1L-10	2	0.6	0.0	0.0	100.0	0.0	0.0	0.0	1.6	0.0
1L-11	2	0.6	0.0	0.0	100.0	0.0	0.0	0.0	1.6	0.0
1L-13	2	0.6	0.0	100.0	0.0	0.0	0.0	3.6	0.0	0.0
1L-20	2	0.6	0.0	0.0	100.0	0.0	0.0	0.0	1.6	0.0
1L-21	2	0.6	0.0	0.0	100.0	0.0	0.0	0.0	1.6	0.0
1L-22	2	0.6	50.0	0.0	50.0	0.0	0.7	0.0	0.8	0.0
1L-24	2	0.6	0.0	0.0	100.0	0.0	0.0	0.0	1.6	0.0
1L-26	2	0.6	0.0	0.0	100.0	0.0	0.0	0.0	1.6	0.0
1L-30	2	0.6	0.0	100.0	0.0	0.0	0.0	3.6	0.0	0.0

* Total 342 strains, ** Total 139 strains, *** Total 56 strains, **** Total 128 strains, *****Total 19 strains

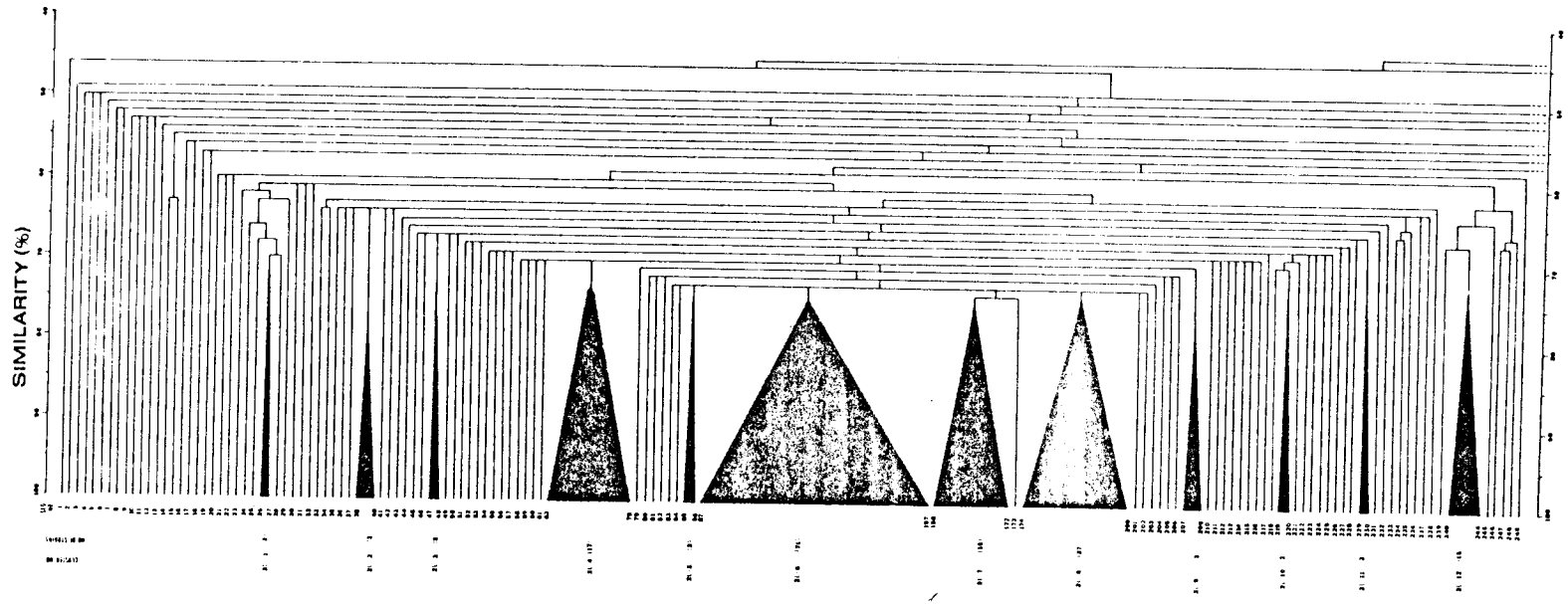


Fig. 42.
Simplified dendrogram for Summer 1976.
4 C isolates - 2L series

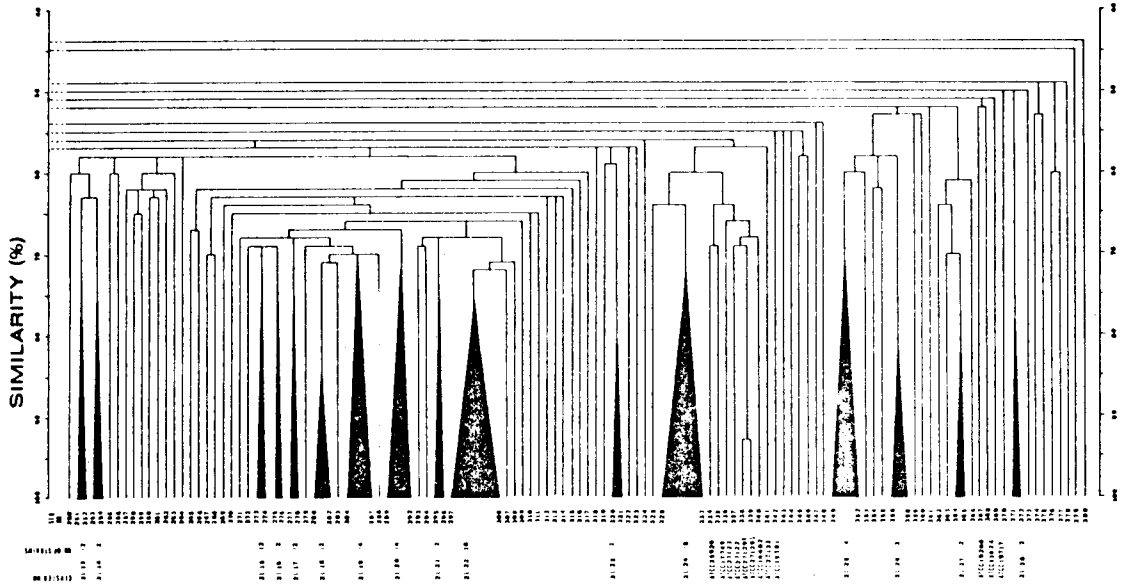


Fig. 42 (cont'd.)

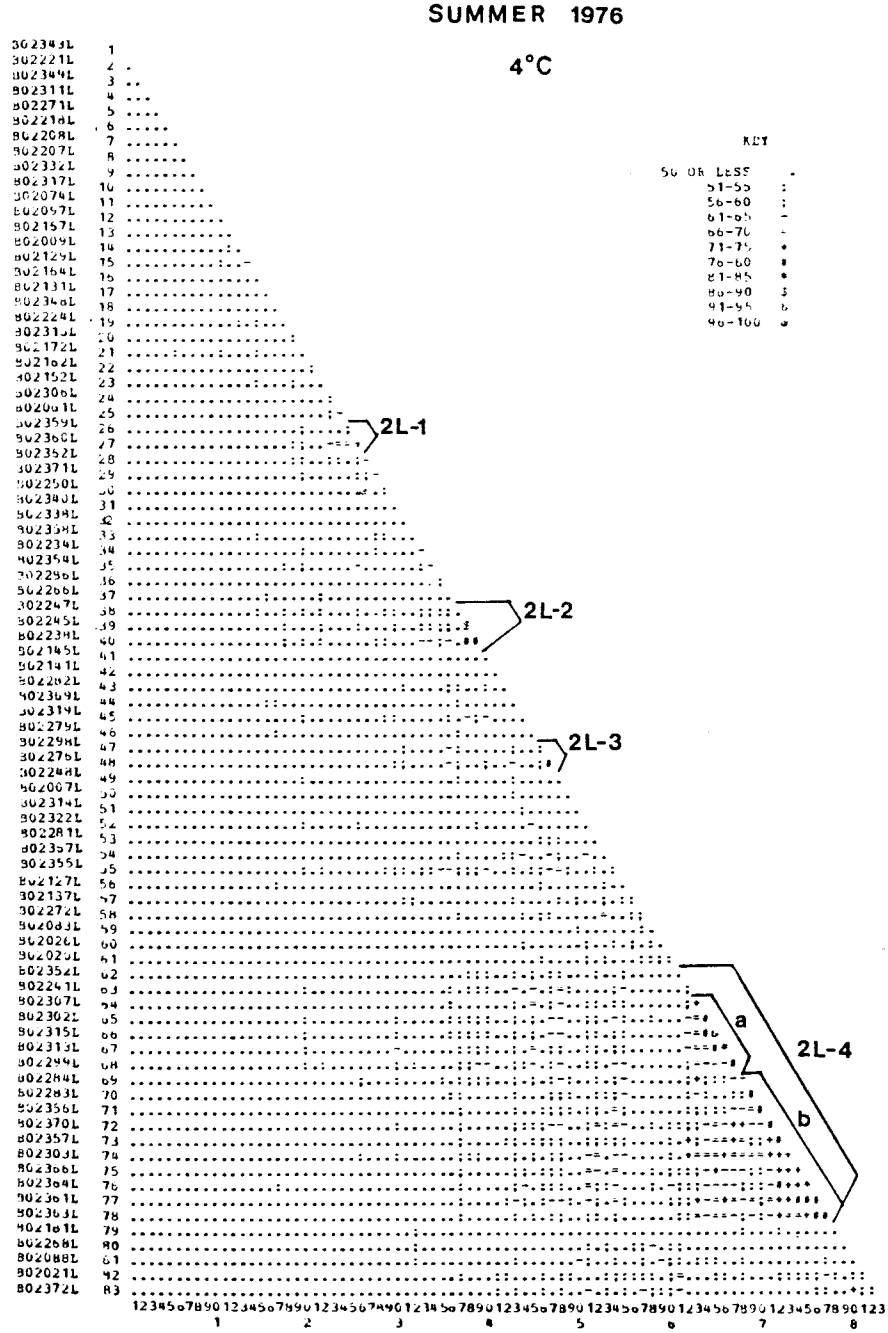


Fig. 43. Cluster triangle for Summer 1976.
4 C isolates - 2L series

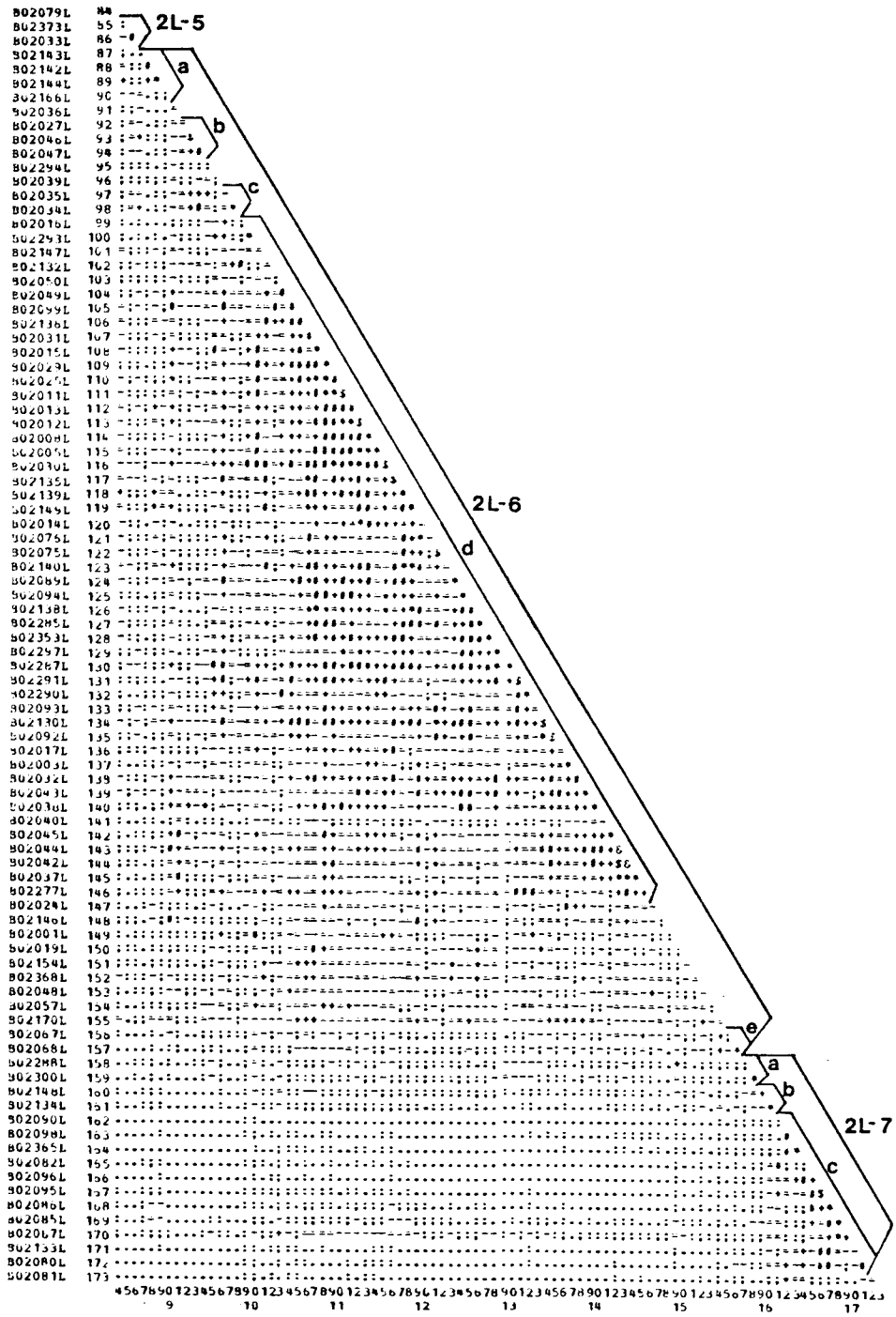


Fig. 43 (cont'd).

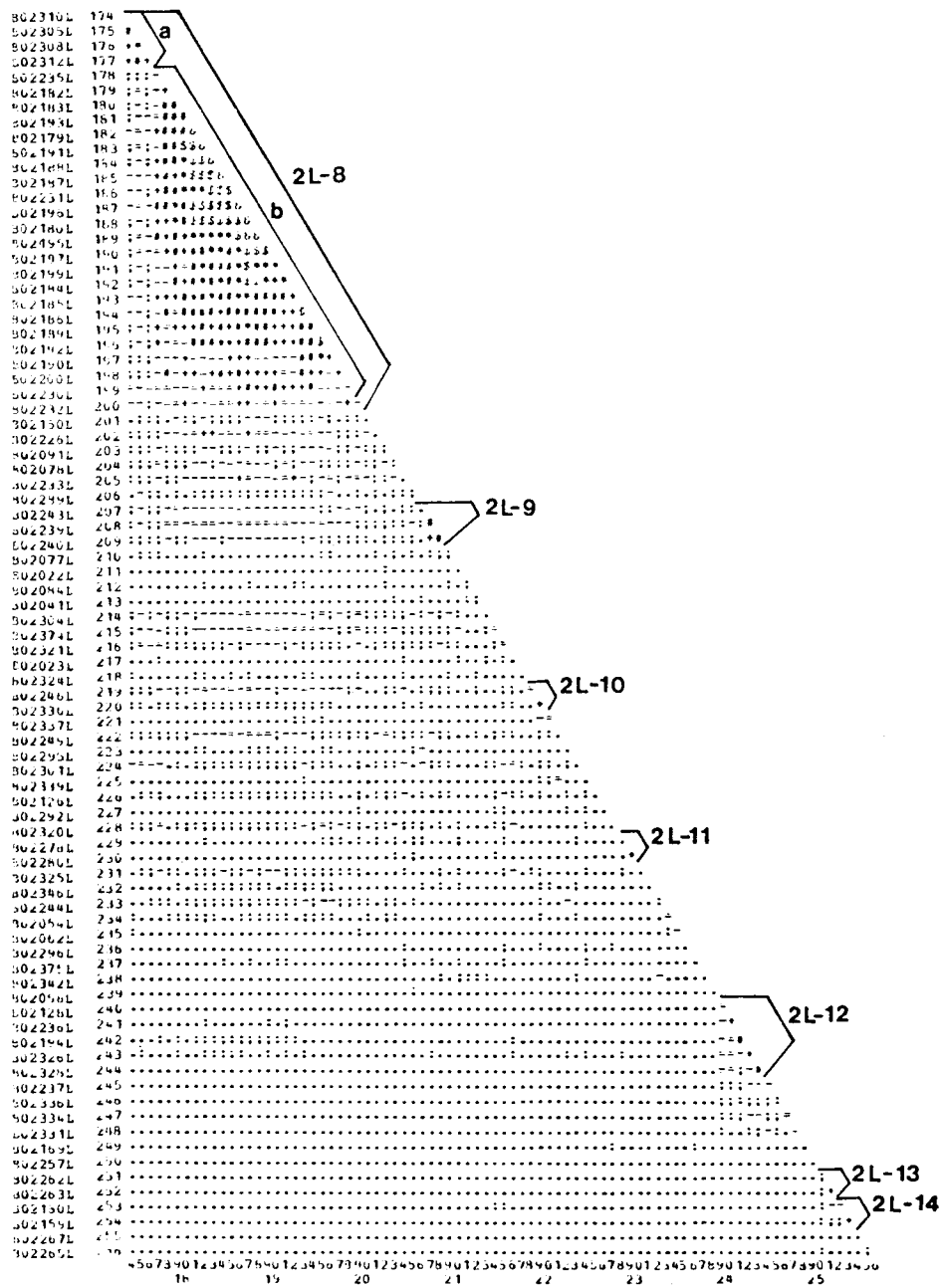


Fig. 43 (cont'd.)

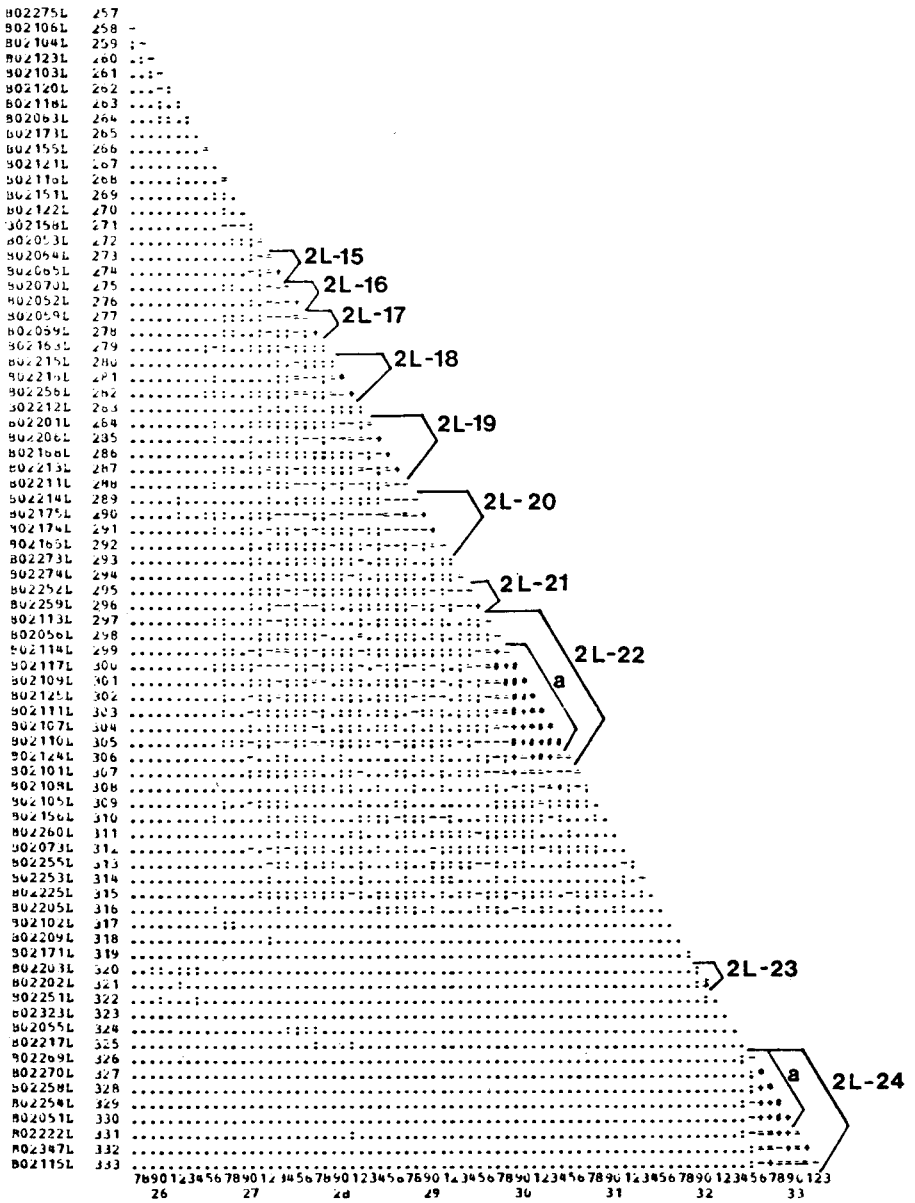
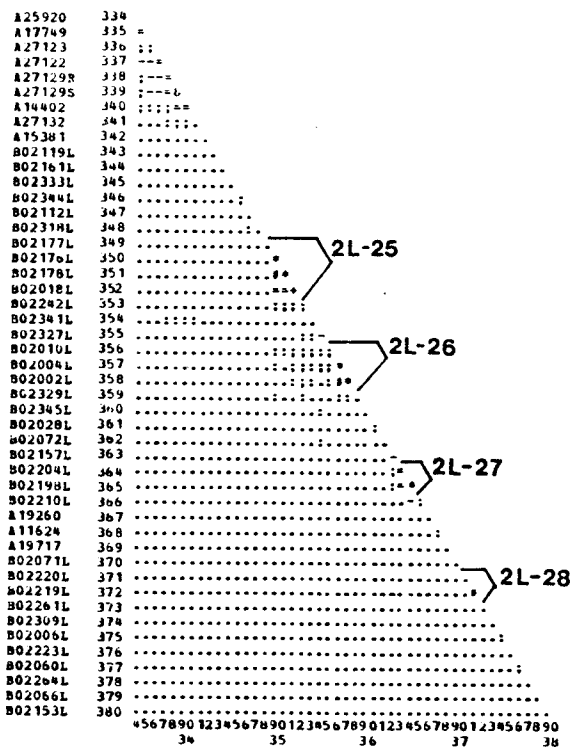


Fig. 43 (cont'd.)


A25920 334
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A27123 336
A27122 337
A27129R 338
A27129S 339
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A27132 341
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B021611 344
B02333L 345
B02344L 346
B021121 347
B02311L 348
B02177L 349
B02170L 350
B02178L 351
B02001L 352
B02242L 353
B02341L 354
B02327L 355
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B02284L 378
B02066L 379
B02153L 380
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2L-26
2L-27
2L-28
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34 35 36 37 38

Fig. 43 (cont'd.)

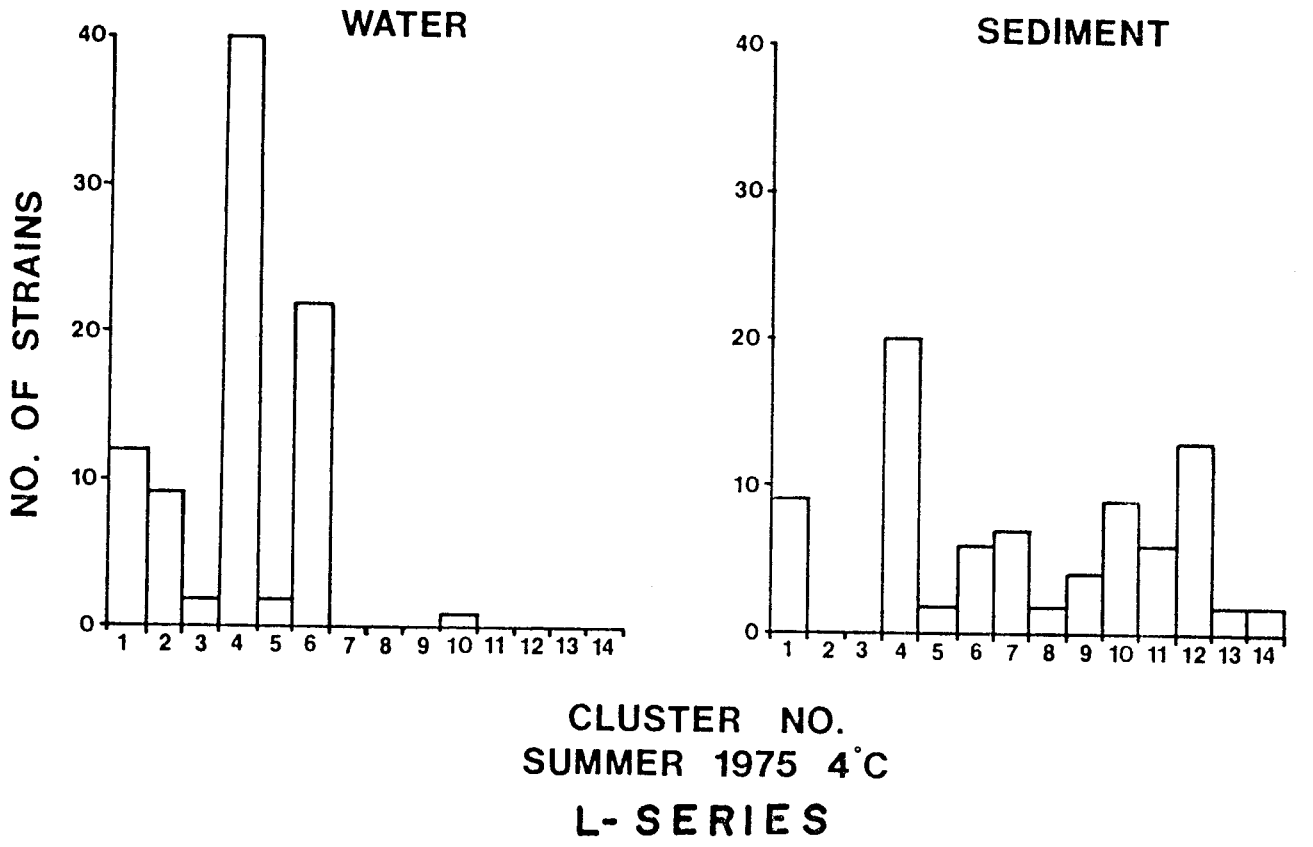

Fig. 44 Cluster distribution of Summer 1975 4 C isolates.

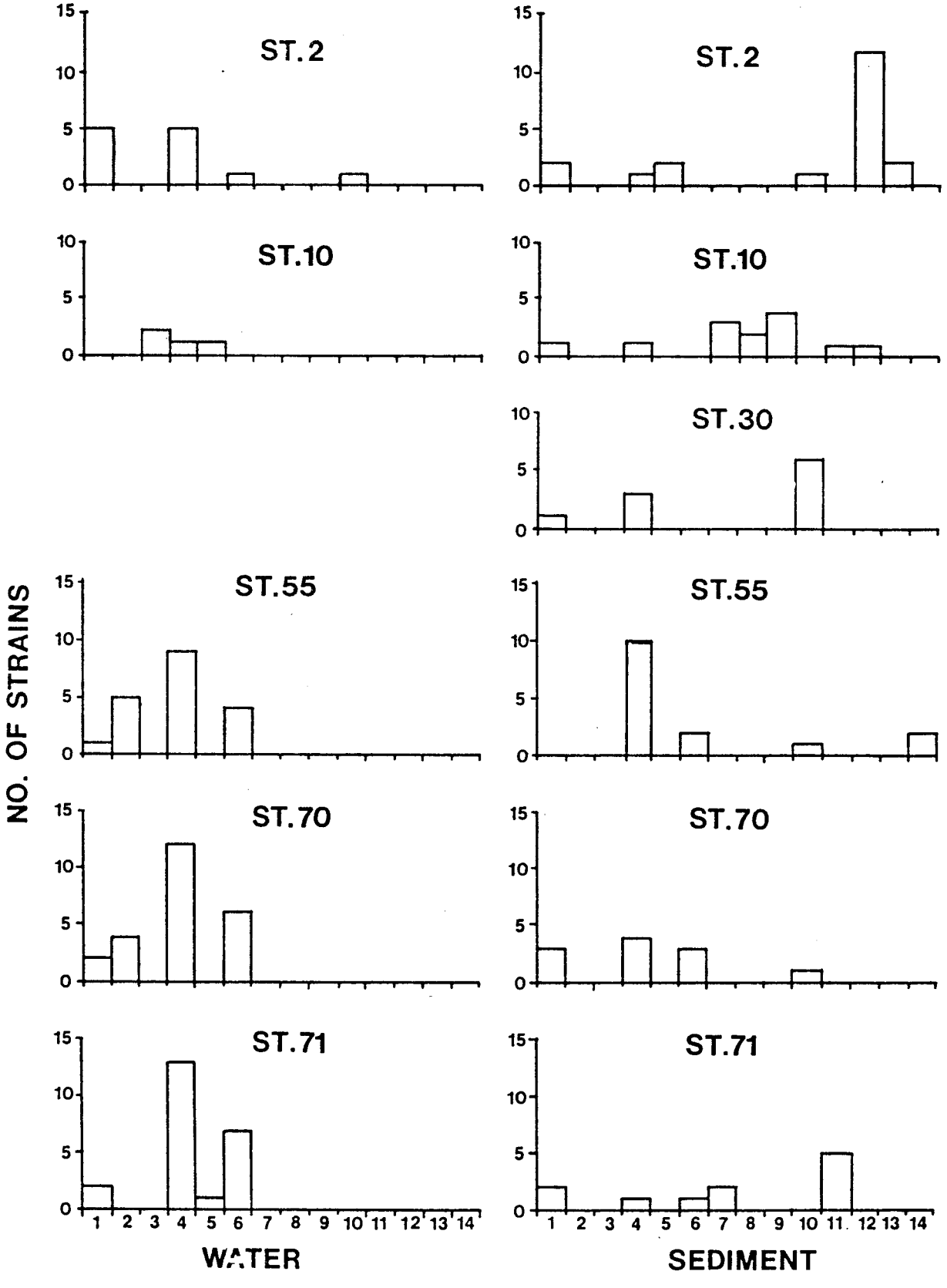

Fig. 45. Cluster distribution of Summer 1975 4 C isolates by station.

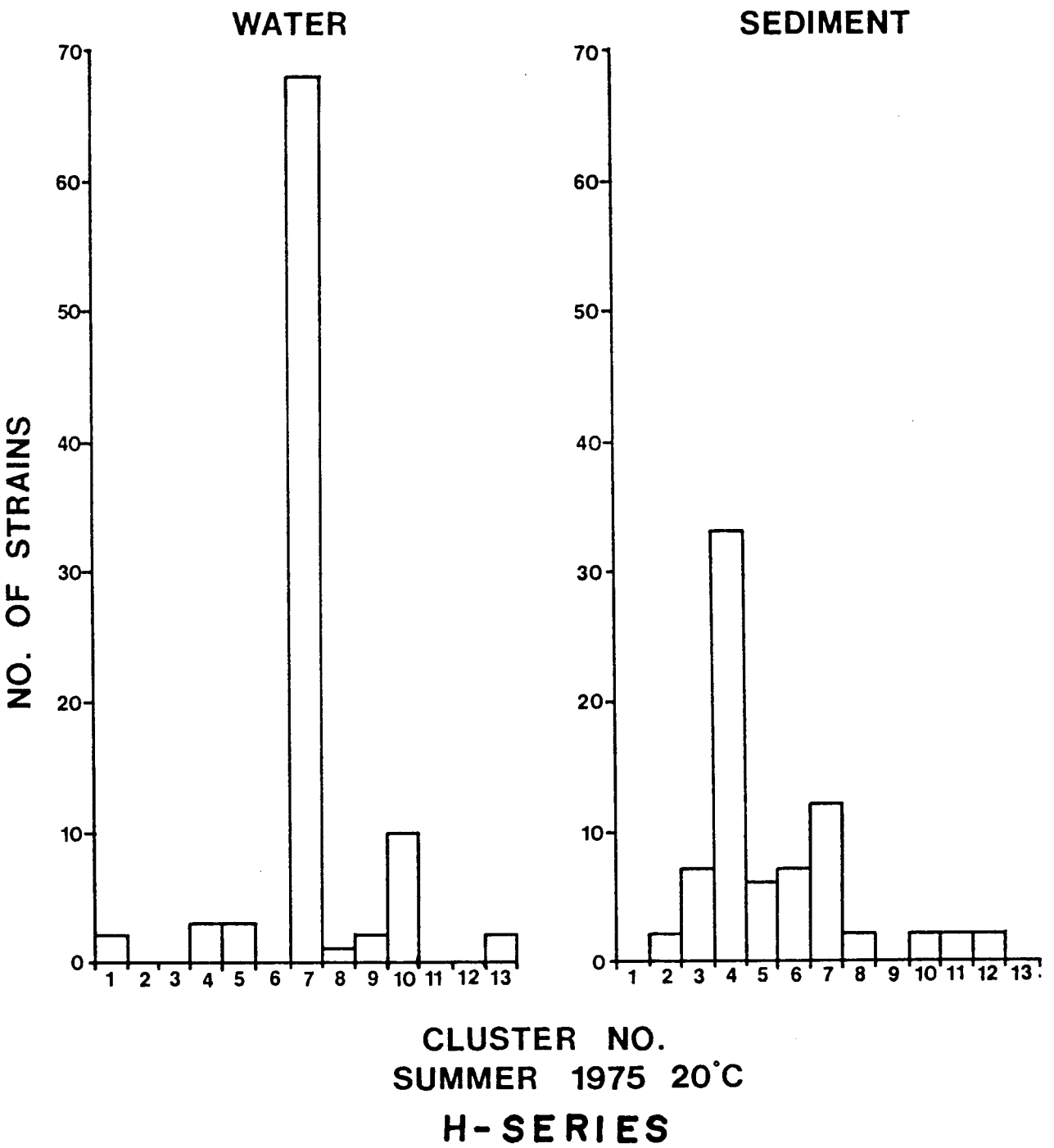

Fig. 46. Cluster distribution of Summer 1975 20 C isolates.

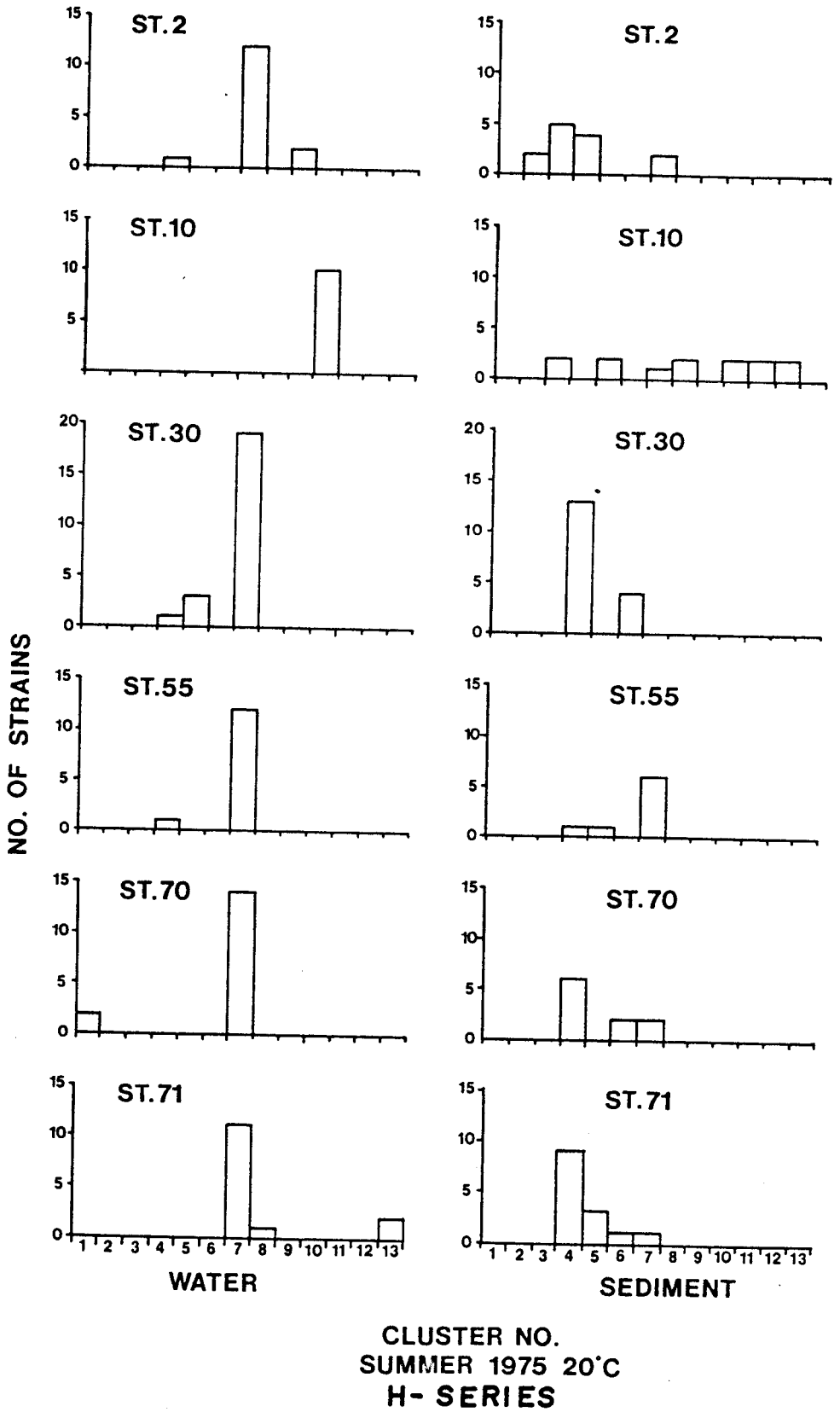

Fig. 47. Cluster distribution of Summer 1975 20 C isolates by station.

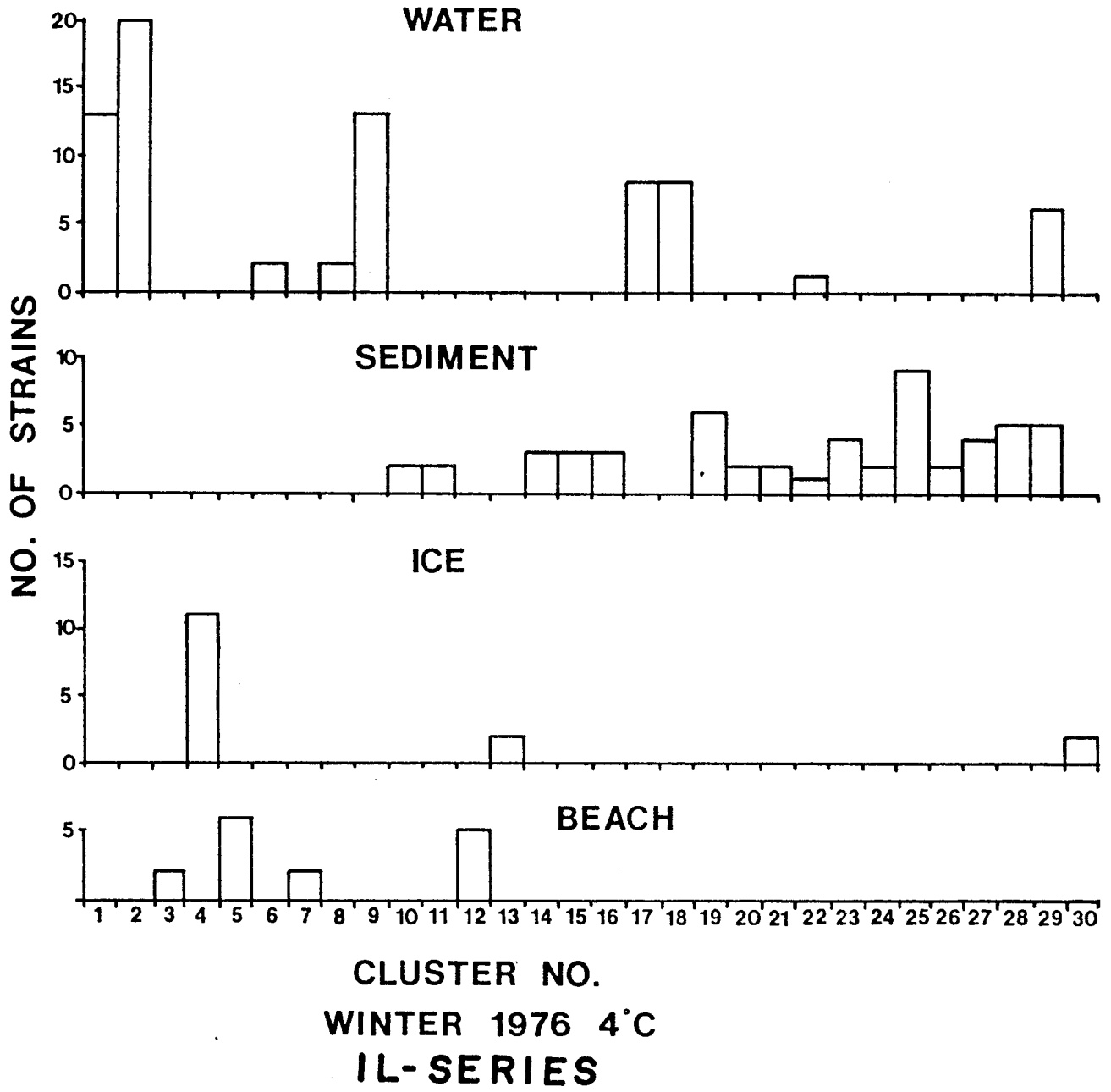

Fig. 48. Cluster distribution of Winter 1976 4 C isolates.

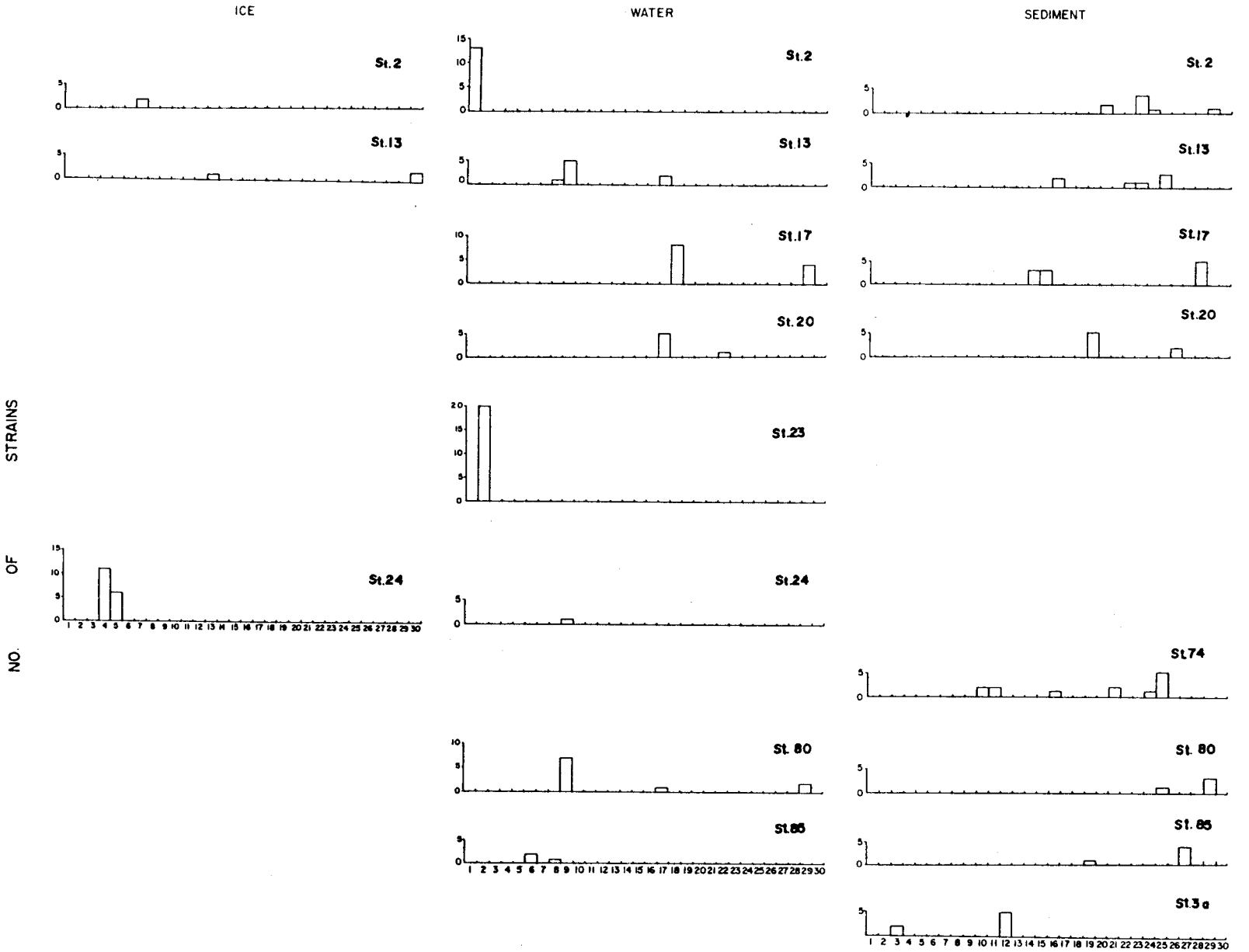

Fig. 49. Cluster distribution of Winter 1976 4 C isolates by station.

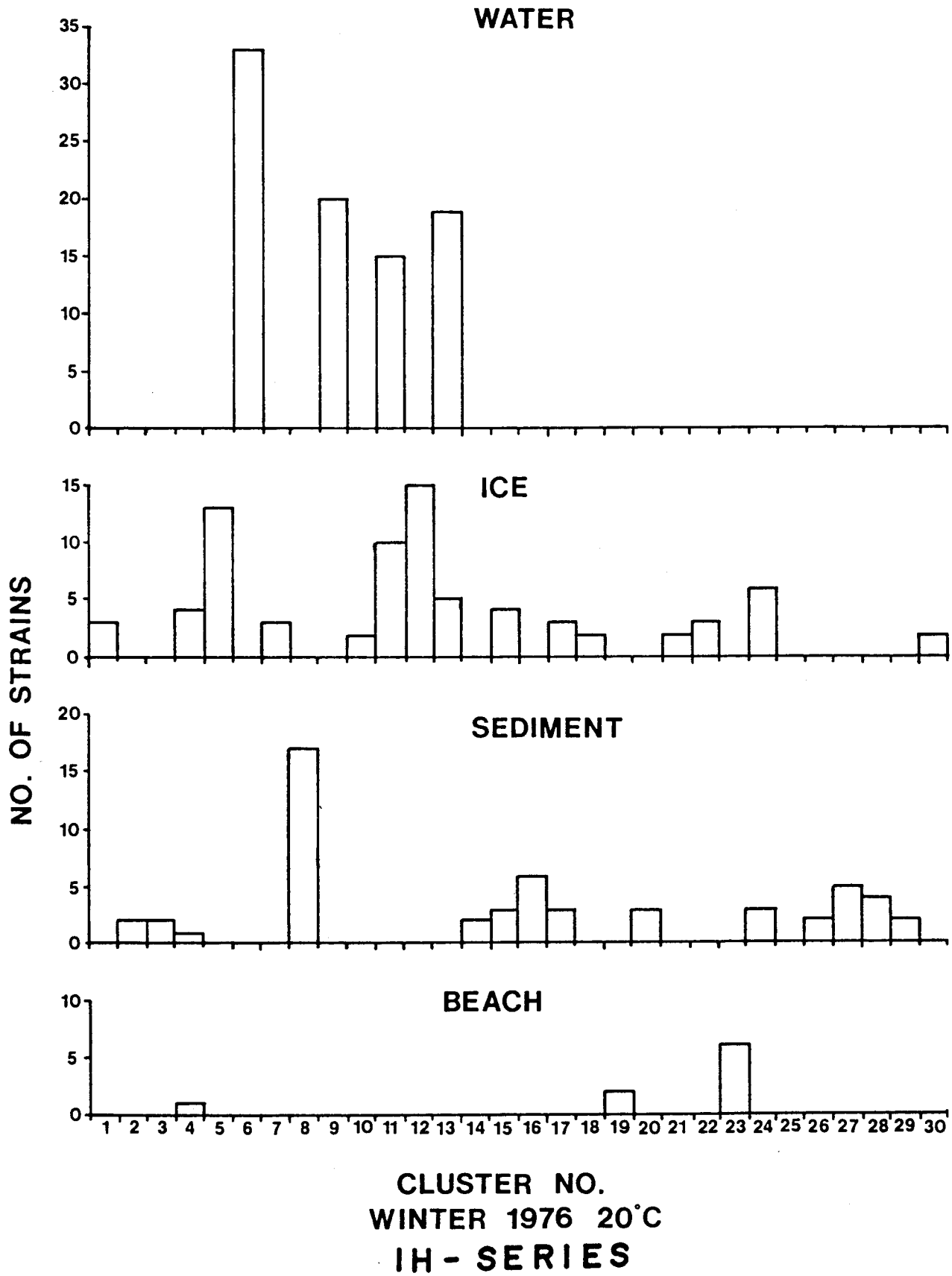

Fig. 50. Cluster distribution of Winter 1976 20 C isolates.

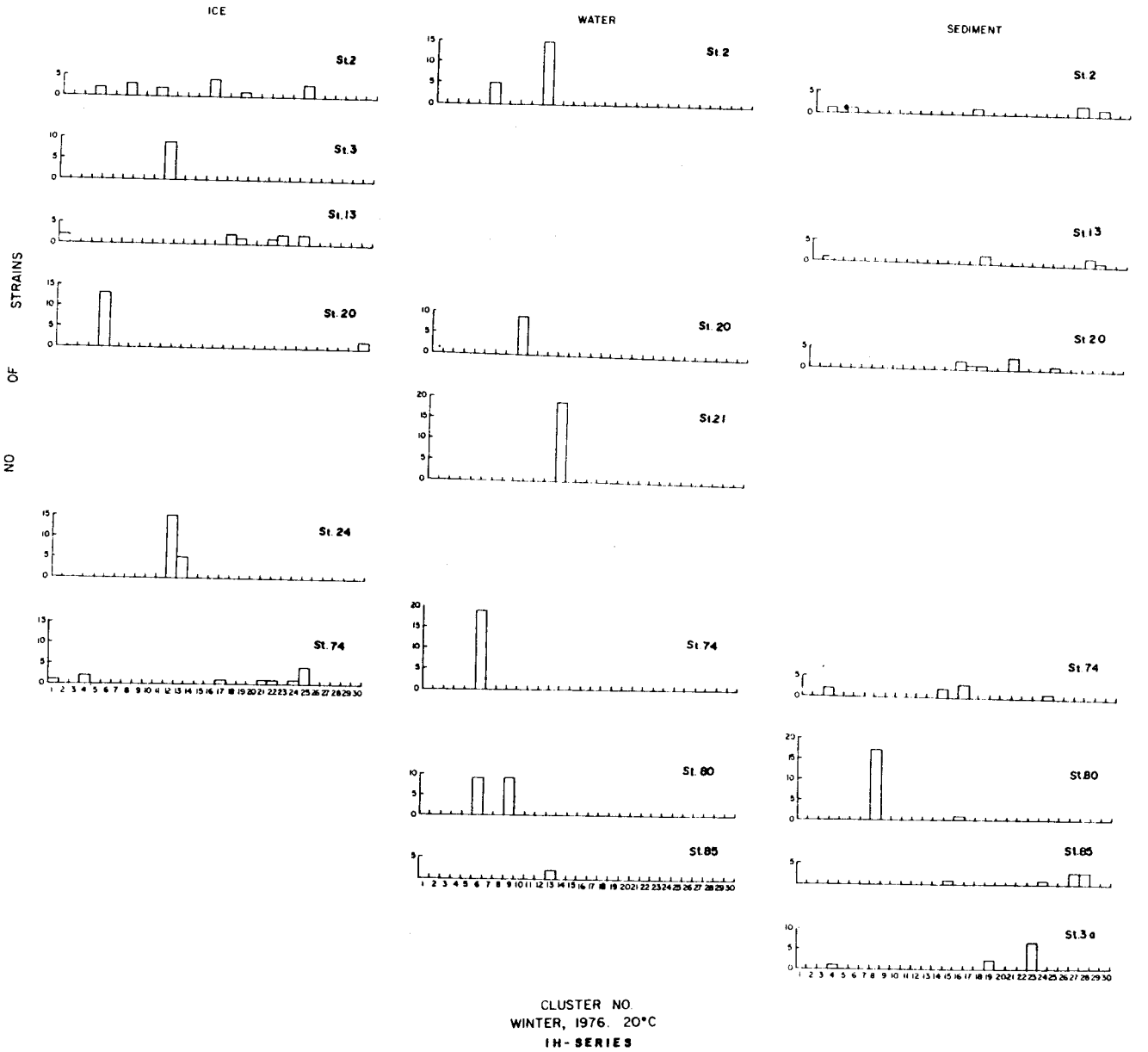

Fig. 51. Cluster distribution of Winter 1976 20 C isolates by station.

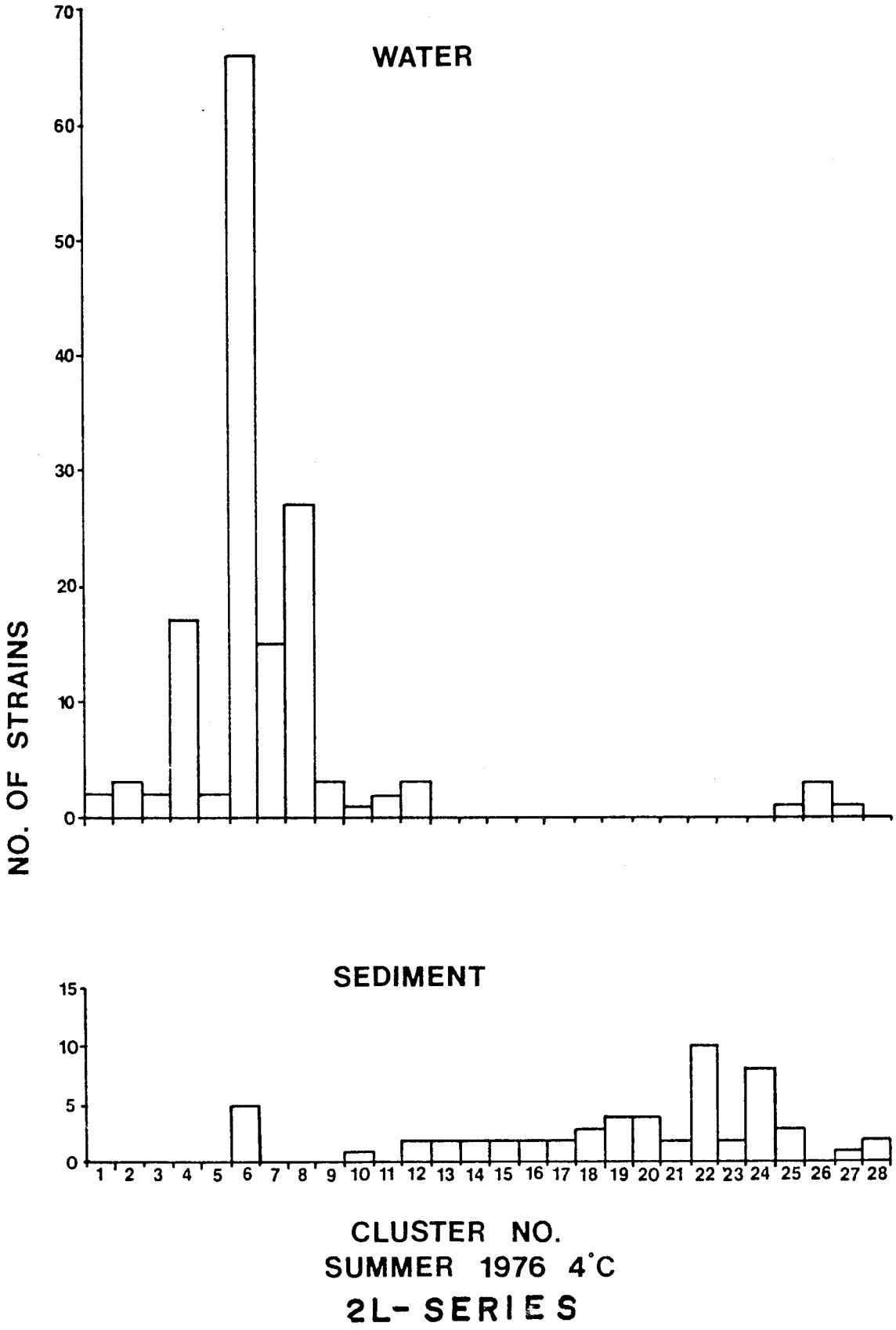

Fig. 52. Cluster distribution of Summer 1976 4 C isolates.

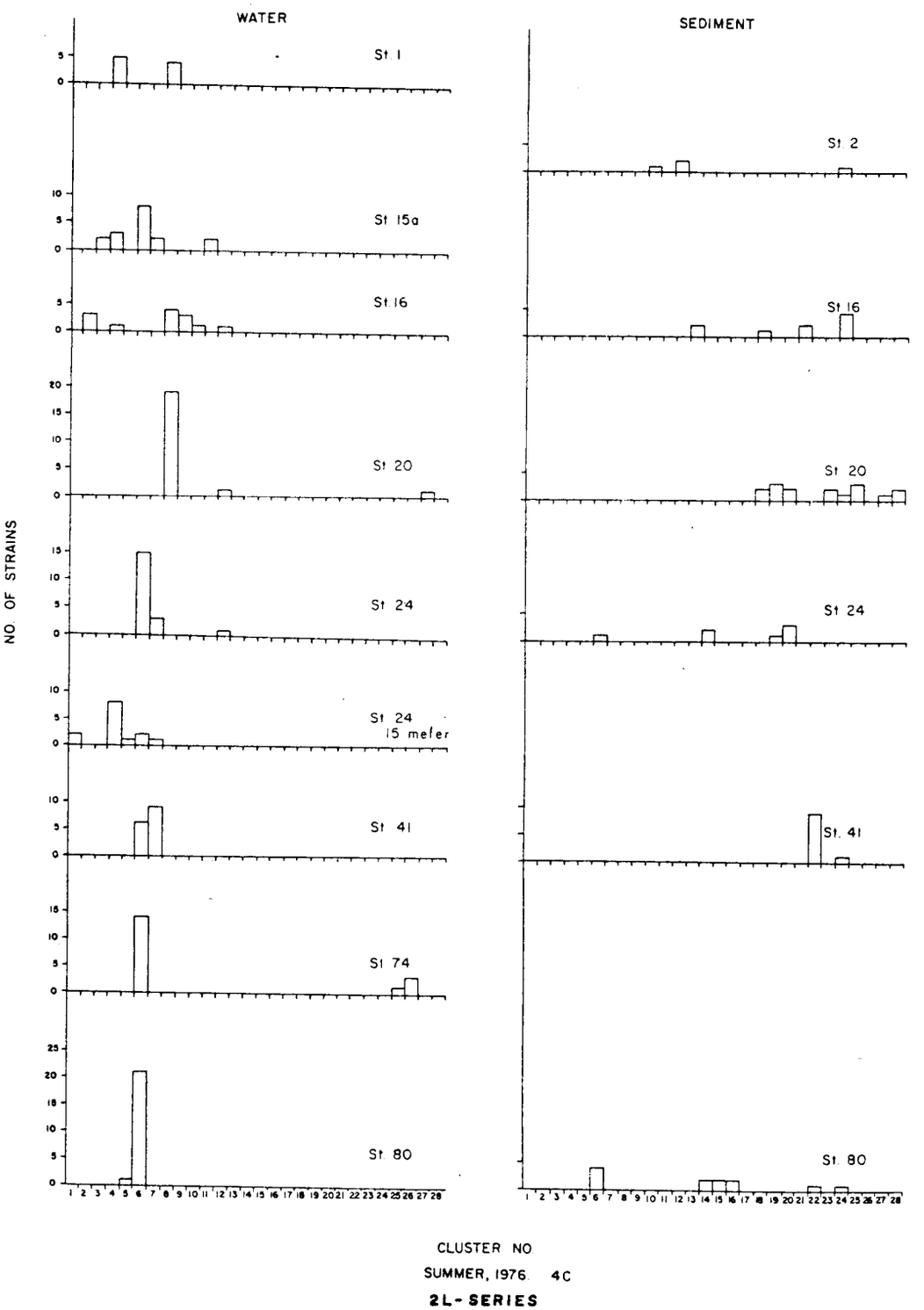

Fig. 53. Cluster distribution of Summer 1976 4 C isolates by station.

TABLE 16 Source of clusters, for winter 1976 20°C isolates

CLUSTER NO.	NO. OF STRAINS	RATIO TO TOTAL ISOLATES *	WATER ORIGIN %	ICE ORIGIN %	SEDIMENT ORIGIN %	BEACH SAMPLE ORIGIN %	RATIO TO TOTAL WATER ORIGIN **	RATIO TO TOTAL ICE ORIGIN ***	RATIO TO TOTAL SEDIMENT ORIGIN****	RATIO TO BEACH SAMPLE ORIGIN*** **
1H-6	33	9.6	100.0	0.0	0.0	0.0	33.0	0.0	0.0	0.0
1H-11	24	7.0	62.5	37.5	0.0	0.0	15.0	8.9	0.0	0.0
1H-13	24	7.0	79.1	20.9	0.0	0.0	19.0	5.0	0.0	0.0
1H-9	20	5.9	100.0	0.0	0.0	0.0	20.0	0.0	0.0	0.0
1H-8	17	5.0	0.0	0.0	100.0	0.0	0.0	0.0	14.2	0.0
1H-12	15	4.4	0.0	100.0	0.0	0.0	0.0	14.9	0.0	0.0
1H-5	13	3.8	0.0	100.0	0.0	0.0	0.0	12.9	0.0	0.0
1H-24	9	2.6	0.0	66.6	33.3	0.0	0.0	5.9	2.5	0.0
1H-15	7	2.1	0.0	57.1	42.9	0.0	0.0	4.0	2.5	0.0
1H-4	6	1.8	0.0	66.6	16.7	16.7	0.0	4.0	0.8	5.0
1H-16	6	1.8	0.0	0.0	100.0	0.0	0.0	0.0	5.0	0.0
1H-17	6	1.8	0.0	50.0	50.0	0.0	0.0	3.0	2.5	0.0
1H-23	6	1.8	0.0	0.0	0.0	100.0	0.0	0.0	0.0	30.0
1H-27	5	1.5	0.0	0.0	100.0	0.0	0.0	0.0	4.2	0.0
1H-25	4	1.5	0.0	100.0	0.0	0.0	0.0	4.0	0.0	0.0
1H-28	4	0.9	0.0	0.0	100.0	0.0	0.0	0.0	3.3	0.0
1H-1	3	0.9	0.0	100.0	0.0	0.0	0.0	3.0	0.0	0.0
1H-7	3	0.9	0.0	100.0	0.0	0.0	0.0	3.0	0.0	0.0
1H-19	3	0.9	0.0	0.0	0.0	100.0	0.0	0.0	0.0	15.0
1H-20	3	0.9	0.0	0.0	100.0	0.0	0.0	0.0	2.5	0.0
1H-22	3	0.9	0.0	100.0	0.0	0.0	0.0	3.0	0.0	0.0
1H-2	2	0.6	0.0	0.0	100.0	0.0	0.0	0.0	1.6	0.0
1H-3	2	0.6	0.0	0.0	100.0	0.0	0.0	0.0	1.6	0.0
1H-10	2	0.6	0.0	100.0	0.0	0.0	0.0	2.0	0.0	0.0
1H-14	2	0.6	0.0	0.0	100.0	0.0	0.0	0.0	1.6	0.0
1H-18	2	0.6	0.0	100.0	0.0	0.0	0.0	2.0	0.0	0.0
1H-21	2	0.6	0.0	100.0	0.0	0.0	0.0	2.0	0.0	0.0
1H-26	2	0.6	0.0	0.0	100.0	0.0	0.0	0.0	1.6	0.0
1H-29	2	0.6	0.0	0.0	100.0	0.0	0.0	0.0	1.6	0.0
1H-30	2	0.6	0.0	100.0	0.0	0.0	0.0	2.0	0.0	0.0

* Total 341 strains, ** Total 100 strains, *** Total 101 strains, **** Total 120 strains, ***** Total 20 strains.

TABLE 17 Source of clusters for summer 1976 4°C isolates.

CLUSTER NO.	NO. OF STRAINS	RATIO TO TOTAL ISOLATES *	WATER ORIGIN %	SEDIMENT ORIGIN %	RATIO TO TOTAL WATER ISOLATES **	RATIO TO TOTAL SEDIMENT ISOLATES***
2L-6	71	19.2	93.0	7.0	29.9	3.4
2L-8	27	7.3	100.0	0.0	12.2	0.0
2L-4	17	4.6	100.0	0.0	7.7	0.0
2L-7	15	4.1	100.0	0.0	6.8	0.0
2L-22	10	2.7	0.0	100.0	0.0	6.7
2L-24	8	2.2	0.0	100.0	0.0	5.4
2L-12	5	1.4	60.0	40.0	1.4	1.4
2L-19	4	1.1	0.0	100.0	0.0	2.7
2L-20	4	1.1	0.0	100.0	0.0	2.7
2L-25	4	1.1	25.0	75.0	0.4	2.0
2L-2	3	0.8	100.0	0.0	1.4	0.0
2L-9	3	0.8	100.0	0.0	1.4	0.0
2L-18	3	0.8	0.0	100.0	0.0	2.0
2L-26	3	0.8	100.0	0.0	1.4	0.0
2L-1	2	0.5	100.0	0.0	0.9	0.0
2L-3	2	0.5	100.0	0.0	0.9	0.0
2L-5	2	0.5	100.0	0.0	0.9	0.0
2L-10	2	0.5	50.0	50.0	0.5	0.7
2L-11	2	0.5	100.0	0.0	0.9	0.0
2L-13	2	0.5	0.0	100.0	0.0	1.4
2L-14	2	0.5	0.0	100.0	0.0	1.4
2L-15	2	0.5	0.0	100.0	0.0	1.4
2L-16	2	0.5	0.0	100.0	0.0	1.4
2L-17	2	0.5	0.0	100.0	0.0	1.4
2L-21	2	0.5	0.0	100.0	0.0	1.4
2L-23	2	0.5	0.0	100.0	0.0	1.4
2L-27	2	0.5	50.0	50.0	0.5	0.7
2L-28	2	0.5	0.0	100.0	0.0	1.4

* Total 369 strains, ** Total 221 strains. *** Total 148 strains

TABLE 18 Source of main clusters of orange color producing organisms.

CLUSTER	NO. OF STRAINS	SUMMER, 1975		WINTER, 1976	
		4°C	20°C	4°C	20°C
A	2	0	2	0	0
B	6	0	0	0	6
C	3	0	0	0	3
D	2	0	0	0	2
E	2	0	0	2	0
F	2	0	0	2	0
G	10	0	0	0	10
H	2	0	0	0	2
I	2	0	0	0	2
J	3	0	0	0	3
K	2	0	0	0	2
L	2	0	0	2	0
M	9	0	0	9	0
N	25	0	25	0	0
O	3	3	0	0	0
P	20	20	0	0	0
Q	61	59	2	0	0
R	2	2	0	0	0
S	2	2	0	0	0
T	2	0	2	0	0
TOTAL	162	86	31	15	30

i.e. ice, water, sediment and beach is shown in Table 16.and Figure 50 and by geographic distribution in Figure 51. There were no common clusters between sediment and water isolates but there were several large clusters that contained both ice and water isolates and several smaller clusters that contained ice and sediment isolates. Beach sediment isolates did not cluster with benthic sediment isolates. As was seen for the Winter 4 C isolates, the occurrence of clusters was highly site specific. None of the clusters showed wide distribution.

Summer 1976

As shown in Figures 42 and 43, a total of 28 clusters were obtained for the 4 C isolates from Winter 1976. Fifteen of these clusters had only two members. About 40% of the strains tested produced orange pigments and fell into two clusters.

The distribution of the clusters by source, i.e. water or sediment, is shown in Table 17 and Figure 52 and by geographic distribution in Figure 53. There was less mixing of sediment and water isolates in the same cluster than seen in Summer 1975. The large clusters were generally either 100% of sediment or 100% of water origin. Isolates of cluster 2L6 were widespread, occurring in two-thirds of the water samples. Organisms of cluster 2L8 were found in the three sites where isolates of cluster 2L6 were not found and in none of the samples where members of cluster 2L6 were found. Sediment at Station 41, which is influenced by the Colville River,

contained a cluster not found elsewhere and had no common clusters with other sediment samples.

Orange Pigment Producing Isolates

Because of the high incidence of orange pigment producing bacteria in water during summer, separate cluster analyses were performed for all isolates that produced orange pigments isolated during Summer 1975 and Winter 1976 at both 4 and 20 C. Summer 1976 isolates that produced orange pigments were not available at the time these analyses were run. Figure 54 shows that a total of 20 clusters were found representing 59% of the orange pigment producing organisms tested. Organisms that failed to cluster have been excluded from this figure. The source of the clusters is shown in Table 18. There was surprisingly no commonality between clusters from Summer and Winter isolates.

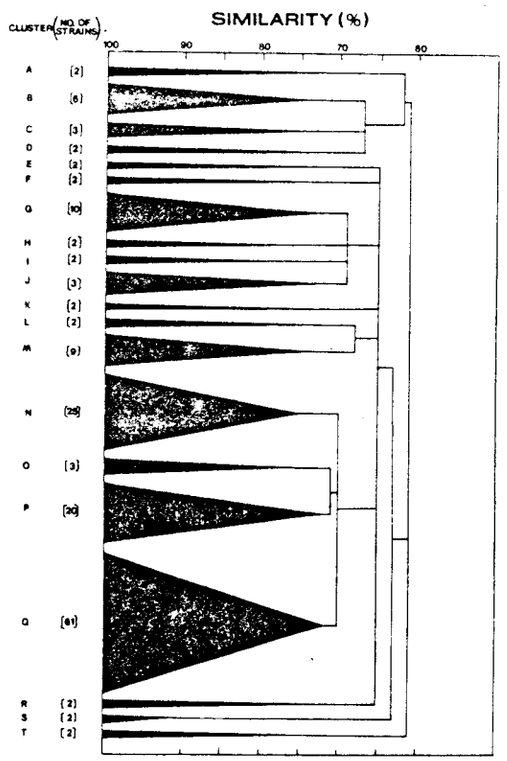

Fig. 54.
Simplified dendrogram for orange pigment producing organisms isolated during Summer 1975 and Winter 1976.

Features of clusters of 1975 isolates

Only the clusters obtained from the 1975 isolates have been thoroughly characterized. Table 19 shows the detailed feature analysis. Below the prominant features of the major clusters are described. Clusters with only a few members are not described below.

Cluster L-1:

(i) Morphological characteristics: Gram-negative rods. Cell shapes were very peculiar and highly pleomorphic. Two types of cells were found. The first type was comma shaped cell with the length of 3-4μ, and width 0.6-0.8μ. The other type was very large, often 10μ or more, and was swollen on one end and tapered on the other end. Some cultures had two types of cells. Some had only one type.

Cells contained poly-β-hydroxybutyric acid inclusions. Cells occurred singly. Cells were not motile.

The colonies were rather small, diameter ca. 0.2mm, white-grayish, glistening, translucent, convex, with entire edge. Most grew slightly in liquid media.

(ii) Physiological characteristics: All members of this cluster grew at temperatures 5-20°C, many also grew at 25°C. Optimal pH was pH 7-8, and opitmal NaCl concentration was 3% but many also grew at 0.5 and 5.0%.

(iii) Biochemical characteristics: Most of the isolates failed to produce acid from carbohydrates. Starch, gelatin, tween 20 and tween 80 were not hydrolyzed. Alkaline phosphatase positive. Catalase positive.

(iv) Antibiotic sensitivity: Sensitive to 15 of 20 antibiotics.

(v) Nutritional characteristics: When vitamins were supplied as growth factors the following substrates were utilized by 80% of the organisms of this cluster; glucose, acetate, propionate, caprylate, fumarate, β-hydroxybutyrate, citrate, α-ketoglutarate, pyruvate, glutarate, glycerol, p-hydroxybenzoate, β-alanine, serine, threonine, glutamate, γ-aminobutyrate.

Their growth factor requirement was type B.

(vi) Distribution: Organisms in this cluster were found at almost all stations, in both water and sediment.

Cluster L-2:

(i) Morphological characteristics: Gram negative, rods. Cells occur singly, sometimes also in chains. Cells are pleomorphic, length varies but typically about 2μ length and 0.8-1.0μ width. Poly-β-hydroxybutyric acid inclusions often occur. Non-motile. Colonies are small, 0.1 mm in a diameter, grey, glistening, smooth, convex, with entire edge. Slight growth in liquid media.

(ii) Physiological characteristics: All grow at 5-20°C, and many also grow at 25°C. The pH range for growth is pH 6-9. Range of salt tolerance 0.5-5.0% NaCl.

(iii) Biochemical characteristics: No acid was produced from carbohydrates. No hydrolysis of polymers. Alkaline phosphatase positive, catalase positive, but very weak. Oxidase negative.

(iv) Antibiotic sensitivity: Sensitive to 11 of 19 anitbiotics

(v) Nutritional characteristics: The following substrates were utilized by more than 80% of organisms in this cluster. Xylose, Glucose, Cellobiose, acetate, caprylate, malonate, β-hydroxybutyrate, α-ketoglutarate, pyruvate, γ-aminobutyrate. In addition L-Alanine, L-Aspartate, L-Arginine, L-Cysterine, L-cystine, L-proline were utilized on the nitrogen deficient media.

Growth factor requirement type B.

(vi) Distribution: Only found in water samples from station 55 and 70.

Cluster L-4:

(i) Morphological characteristics: Gram negative rods, with rounded ends, highly pleomorphic. 1.2-1.5μ in length, 0.6-0.8μ in width. Some cells, however, greater than 20μ. Cells occur singly but also in chains. Round body (or spherical body) formation is very common especially in old cultures. Protrusion often occurs from round bodies. Non-motile. Colony ca 0.5 mm in a diameter, orange pigmented, translucent, glistening, smooth, convex. Slight growth in liquid media.

(ii) Physiological characteristics: The range of temperature, pH and NaCl concentration for growth are 5-20°C, pH 5-9, and 0.5-7.5% NaCl.

(iii) Biochemical characteristics: Acid was not produced from D-glucose, but was produced from sucrose. Gelatin was hydrolyzed. No amylase nor lipase produced. Alkaline phosphatase positive, catalase positive, oxidase negative, typically Arginine diaminase positive.

(iv) Antibiotic sensitivity: Sensitive to only 5 of 13 antibiotics.

(v) Nutritional characteristics: Following substrates were utilized by 50-80% of isolates. D-galactose, D-glucose, D-mannose, maltose, L-glutamate.

Growth factor type B

(vi) Distribution: Organisms of this cluster were found in all samples at all stations, however, the incidence of these organisms was much higher in water samples.

Cluster L-5:

(i) Morphological characteristics: Gram negative, curved comma shaped rods. Ca 3-4μ long and 0.8μ wide, round bodies were common. Cells occur singly. Their colonies are small (0.1 mm in a diameter), yellow pigmented, translucent, convex, entire, glistening, smooth. Slight growth in liquid media. Nonmotile.

(ii) Physiological characteristics: Range of temperature, pH and NaCl concentration for their growth are 5-15°C, pH 6-9, and 3-5% NaCl.

(iii) Biochemical characteristics: Acids were not produced from carbohydrates. Alkaline phosphatase positive, catalase weakly positive, oxidase negative.

(iv) Antibiotic sensitivity: Sensitive to six of 13 antibiotics.

(v) Nutritional characteristics: Only α-ketoglutarate was utilized by 3 of 4 strains on media E. No other substrates were utilized on any base of media. Growth factor type E or U.

(vi) Distribution: Mainly from sediment sample at st. 2.

Cluster L-6:

(i) Morphological characteristics: Morphologically similar to Cluster L-4. However, very small rod shaped cells, length 0.8-1.0μ and width 0.4-0.6μ were often found. Orange pigments produced. Nonmotile.

(ii) Physiological characteristics: Grew at temperatures between 5 and 20°C, the range of the pH and NaCl concentration were narrower than Cluster L-4, pH 6-8, 0.5-3.0% NaCl.

(iii) Biochemical characteristics: Acid was produced from D-glucose aerobically. Alkaline phosphotase positive. Catalase positive, oxidase negative.

(iv) Antibiotic sensitivity: Sensitive to 13 of 18 antibiotics.

(v) Nutritional characteristics: Only α-ketoglutarate was utilized on medium E. Carbohydrates were utilized by a very limited number of organisms on media E.

Growth factor requirement E or U.

(vi) Distribution: The organisms of the cluster were widely distributed, except at st. 10. Especially high incidence in water at Prudhoe Bay.

Cluster L-7:

(i) Morphological characteristics: Gram-negative straight or curved rod-shaped organisms, two types were seen, the first type was broader, size ca 2-2.5μ long and 1.0μ wide, the second type was more slender, 0.6-0.8μ wide and 2.5-3.0μ long. Irregular pleomorphic shapes often developed. Cells occur singly. Nonmotile. Their colonies were white-grayish. Opaque, entire, glistening, smooth and convex. Colony size was 2.5-3.0 mm in a diameter. Pellicle was formed in liquid media.

(ii) Physiological characteristics: Range of temperature and NaCl concentration for growth 5 - 15°C, and 3 to 5% NaCl, respectively. Growth at pH 5-10.

(iii) Biochemical characteristics: Acid was formed from D-glucose under anaerobic condition, therefore they were fermentative. No gas from carbohydrates was formed. Starch, gelatin, Tween 20 and Tween 80 were hydrolyzed, alkaline-phosphatase positive, NO_3 to NO_2 positive, oxidase negative, catalase negative.

(iv) Antibiotic sensitivity: Sensitive to 7 of 13 antibiotics.

(v) Nutritional characteristics: No substrates were utilized on Media B. Fumarate, lactate and α-ketoglutarate were utilized on media E.

Growth factor requirement was type E.

(vi) Distribution: Found in sediment samples from sts. 10 and 71.

Cluster L-9:

(i) Morphological characteristics: Cells were gram-negative, typically large 4-5μ long by 1μ wide, straight rods. Some smaller rods also occur, 1.5-2.0μ long, and 0.6μ wide. Cells occur singly and in chains. Motility positive in three of four. Colonies were grayish, translucent, convex, entire, glistening and smooth. Colony diameter 1.8-2.0 mm. Flocculation and pellicle occurred in liquid media.

(ii) Physiological characteristics: Range of temperature and NaCl concentration 5-15°C, and 3-5% NaCl. The range of pH was pH 5-9.

(iii) Biochemical characteristics: Acids were produced from D-glucose, both aerobically and anaerobically. No gas was produced. They were facultative anaerobes. Gelatin, tween 20 and tween 80 were hydrolyzed, but not starch. Alkaline phosphatase positive. Catalase positive. Oxidase negative. Ammonia was produced from peptones. NO_3 was reduced to NO_2.

(iv) Antibiotic sensitivity: Sensitive to 5 of 13 antibiotics.

(v) Nutritional characteristics: Only pyruvate was utilized on media B, however, D-fructose, D-glucose, succinate, fumarate, DL-lactate were also well utilized on media E.

Growth factor requirement was type B or type E.

(vi) Distribution: Only found in the sediment from station 10.

Cluster L-10:

(i) Morphological characteristics: Cells were gram-negative, rod-shaped, often slightly curved. Elongated cells often occur. Two types of cells were found, the first type 2.2-2.5μ in length, 1.0μ in width. The second type 1-1.5μ in length, 0.6μ in width. Cells occur singly and in chains with many small cells. Generally capsulated. Non-motile. The

colonies were small (0.1-0.3 mm in a diameter), gray, translucent, convex, entire, glistening and smooth. Poor growth in liquid media.

(ii) Physiological characteristics: Range of temperature, pH and NaCl concentration for their growth, between 5-25^{o}C, pH 5-9, and 0 - 3% NaCl.

(iii) Biochemical characteristics: No acid or gas produced from carbohydrates. Starch and gelatin not hydrolyzed. Tween 20 hydrolyzed. Catalase positive. Oxidase negative. NO_3 reduced to NO_2.

(iv) Antibiotic sensitivity: Sensitive to 16 of 18 antibiotics.

(v) Nutritional characteristics: Substrates utilized by 80% of organisms in this cluster: caprylate, pyruvate, alanine, aspartate, γ-aminobutyrate. In addition to these substrates, L-glutamate & L-asparagine were utilized on nitrogen deficient media.

Growth factor requirement type A.

(vi) Distribution: Found in sediment, at all stations except stations 10 and 71.

Cluster L-11:

(i) Morphological characteristics: Gram-negative, straight or curved rods. Two types of cells were found. The first type 2-3μ long x 1μ wide. The second type 2-3μ long, 0.6-0.8μ wide. Cells occur singly. Motile. Colonies were white, translucent, convex, entire, glistening and smooth. Pellicle was often formed in liquid media.

(ii) Physiological characteristics: The range of temperature and NaCl concentration for their growth were 5-15^{o}C and 3-5% NaCl respectively. The pH for the growth was between 5 and 10.

(iii) Biochemical characteristics: Acids were produced from D-fructose D-glucose aerobically and anaerobically. No gas was produced. No hydrolysis of starch, gelatin , tween 20 or tween 80. Alkaline phosphatase positive. Catalase positive. Oxidase negative. Ammonia was produced from peptones. NO_3 reduced to NO_2. Arginine diaminase positive.

(iv) Antibiotic sensitivity: Sensitive to 5 of 13 antibiotics.

(v) Nutritional characteristics: Following substrates were utilized on media B: ribose, fructose, glucose, maltose, fumarate, citrate, α-ketoglutarate, pyruvate, glutarate, glycerol, arginine, N-acetylglucosamine. In addition to these substrates, L-threonine, L-aspartate, L-glutamate, L-cysteine, L-proline and allantoin were utilized on nitrogen deficient media.

Growth factor requirement type A.

(vi) Distribution: Found in sediment at station 55.

Cluster L-12:

(i) Morphological characteristics: Gram-negative rods. Cells were straight and curved, two types of cells were found, the first type was 2-3μ long x 1μ wide, the other was slender, 3-4μ long x 0.6-0.8μ wide. Motile. Cells occur singly. The colonies were gray, opaque, convex, entire, glistening and smooth. Colony diameter of 1.0-2.0 mm. Moderate growth was observed in liquid media.

(ii) Physiological characteristics: The range of temperature, pH and NaCl concentration were, 5-15°C, pH 5-10, 3-5% NaCl.

(iii) Biochemical characteristics: Acids were produced from D-fructose & D-glucose both aerobically and anaerobically. No gas was produced. Starch, tween 20 and tween 80 were hydrolyzed. Catalase positive. Oxidase negative. NO_3 was reduced to NO_2.

(iv) Antibiotic sensitivity: Sensitive to 5 of 12 antibiotics.

(v) Nutritional characteristics: Following substrates were utilized on media B: D-ribose, D-fructose, D-galactose, D-glucose, D-mannose, salicin, maltose, fumerate, DL-glycerate, citrate, glutarate, glycerol, L-aspartate and N-acetylglucosamine. In addition to these substrates, L-glutamate and L-asparagine, were utilized on nitrogen deficient media.

(vi) Distribution: Sediment at station 10 and 12.

Cluster H-3:

(i) Morphological characteristics: Gram-negative straight rods, 0.6-1.0μ long x 0.4μ wide. Cells occur singly. Non-motile. Colonies were yellow, opaque, convex, entire, glistening, smooth. Colony diameter was about 1 mm.

(ii) Physiological characteristics: The range of temperature, pH and NaCl concentration for their growth were, 5-20°C, pH 6-8, and 3.0% NaCl.

(iii) Biochemical characteristics: Acid and gas were not produced. Starch was hydrolyzed. Gelatin and tween not hydrolysed, catalase positive, alkaline phosphatase negative, oxidase negative, ammonia was produced from peptones.

(iv) Antibiotic sensitivity: Sensitive to 10 of 30 antibiotics.

(v) Nutritional characteristics: No substrates were utilized on defined media.

Growth factor type U.

(vi) Distribution: Found in sediment collected from stations 2 and 10 only.

Cluster H-4:

(i) Morphological characteristics: Gram-negative rods. Cells were straight or curved, often comma shaped. Highly pleomorphic with very large cells as well as round bodies often seen. Typical cells were 2.5-3.5μ long x 0.8-1.0μ wide. Cells occur singly. Nonmotile.

Colonies were orange, translucent or transparent, convex, entire, glistening and smooth. Colony diameter was 1.0-2.0 mm. Moderate growth in liquid media.

(ii) Physiological characteristics: Organisms grew at temperatures between 5 and 25°C, pH 5-8, and 0.5 and 3% NaCl.

(iii) Biochemical characteristics: Acid produced from glucose and sucrose both aerobically and anaerobically. Starch and tween 80 were hydrolyzed. Most hydrolyzed gelatin. Alkaline phosphatase positive, most catalase positive. Ammonia produced from peptone.

(iv) Antibiotic sensitivity: Sensitive to 11 of 13 antibiotics.

(v) Nutritional characteristics: Most utilized L-glutamate on nitrogen deficient media, and succinate, fumarate, lactate and α-ketoglutarate on media E.

Growth factor requirement type E.

(vi) Distribution: Organisms were distributed at all stations except station 10.

Cluster H-5:

(i) Morphological characteristics: Gram-negative, very small rods. 0.8-1.2μ x 0.4-0.6μ. Cells often elongated. Cells occur singly and in chains. Colonies were small, 0.5-0.8 in a diameter. Colonies yellow, translucent, convex, entire, glistening and smooth. Moderate growth in liquid media.

(ii) Physiological characteristics: The range of temperature, pH and NaCl concentration were, 5-25°C, pH 5-9, 0-10% NaCl.

(iii) Biochemical characteristics: No acid or gas produced from carbohydrates. Lipase positive. Alkaline phosphatase positive. Catalase negative. Oxidase negative. Ammonia produced from peptones.

(iv) Antibiotic sensitivity: Sensitive to 7 of 13 antibiotics.

(v) Nutritional characteristics: The following substrates were generally utilized: D-glucose, propionate, butyrate, valerate, isovalerate, caprylate, fumarate, p-hydroxybenzoate, itaconate, 1-butanol, isoleucine. In addition, L-leucine, L-glutamate, L-cysteine, L-cystine, L-proline, L-tyrosine were utilized on nitrogen deficient media.

Growth factor requirement was type A.

(vi) Distribution: Found at stations 10, 30, 55 and 71.

Cluster H-6:

(i) Morphological characteristics: Gram-negative rods. Cells were highly or moderately curved. Cells 2-5μ x 0.6-0.8μ. Cells occur singly. Nonmotile.

Colonies were gray, entire, convex, glistening and smooth. Colony diameter was 0.5-1.0 mm. In most cases, moderate growth and ring formation were observed in liquid media.

(ii) Physiological characteristics: The range of temperature, pH and NaCl concentration were 5-25°C, pH 5-9, 0-3% NaCl.

(iii) Biochemical characteristics: No acid and gas were produced from carbohydrates. Tween 20 was hydrolyzed. No hydrolysis of starch, gelatin or tween 80. Alkaline phosphatase positive, catalase positive. Ammonia was produced from peptones. NO_3 reduced to NO_2.

(iv) Antibiotic sensitivity: Sensitive to all antibiotics except tetracycline.

(v) Nutrutional characteristics: The following substrates were utilized by most organisms on media B: acetate, caprylate, fumarate, pyruvate, alanine, aspartate, glutarate, γ-amino-butyrate. In addition to these substrates, L-serine, L-arginine, L-asparagine, L-proline were utilized on nitrogen deficient media.

Growth factor requirement type B.

(vi) Distribution: Organisms of this cluster were found in sediment samples collected from Stations 30, 70 and 71.

Cluster H-7:

(i) Morphological characteristics: Gram-negative rods. Morphologically very heterogenous. The most common shape was pear shaped. Often elongated cells as well as ordinary shaped rods. Cells 1.5-3.0μ x 0.8-1.0μ in width. Some long cells > 10μ. Some cells had poly-β-hydroxybutyrate inclusions. Cells occurred singly and in pairs. Colonies were gray, translucent, convex, entire, glistening and smooth, 0.5-1.0 mm in a diameter, in most cases. Slight growth in liquid media.

(ii) Physiological characteristics: The range of temperature, pH and NaCl concentration were 5-25°C, pH 6-9, and 0.5-5% NaCl.

(iii) Biochemical characteristics: Acid was produced from D-glucose anaerobically, by most of organisms (60%). Only tween 80 was hydrolyzed. Alkaline phosphatase positive. Catalase was produced by 55% of organisms. Other biochemical responses were negative.

(iv) Antibiotic sensitivity: Sensitive to all antibiotics except tetracycline.

(v) Nutritional characteristics: The following substrates were utilized by more than 80% of the organisms: xylose, glucose, cellobiose, acetate, caprylate, fumarate, p-hydroxybenzoate, alanine, L-glutamate, γ-aminobutyrate. In addition to these substrates, L-aspartate, L-arginine, L-cysteine, L-cystine, L-proline were utilized on nitrogen deficient media.

Growth factor requirement type B.

(vi) Distribution: Organisms of the cluster were found from almost all samples, especially from water samples from all stations except station 10.

Cluster H-10:

(i) Morphological characteristics: Gram-negative relatively large straight rods but highly pleomorphic and irregular shaped. Cells often had protrusions or branches. Occurs singly and in chains. Cells were mostly 3-5μ x 0.8-1.2μ. Non-motile. Colonies were yellow, opaque, highly convex, entire and smooth. Center of the colony was dark yellow. Colony diameter ca 1.0 mm. Organisms grew slightly in liquid media.

(ii) Physiological characteristics: The range of temperature, pH and NaCl concentration for their growth or tolerance were, 5-20°C, pH 4-9, and 0-5% NaCl.

(iii) Biochemical characteristics: No acid and gas were produced from carbohydrates. Only tween 80 was hydrolyzed. Alkaline phosphatase negative, catalase positive, NO_3 was reduced to NO_2.

(iv) Antibiotic sensitivity: Sensitive to 7 of 13 antibiotics.

(v) Nutritional characteristics: The following substrates were utilized by more than 80% or organisms: arabinose, fructose, glucose, cellobiose, maltose, sucrose, trehalose, raffinose, acetate, propionate, butyrate, valerate, isovalerate, caprylate, palmitate, p-hydroxybenzoate, α- ketoglutarate, pyruvate, 1,2-propane-diol, m-or p-hydroxybenzoate, serine, glutamate, and histidine. In addition to these substrates, L-aspartate, L-asparagine, and L-proline were utilized on nitrogen free media.

Growth factor requirement type B.

(vi) Distribution: Organisms of this cluster were isolated exclusively from station 10.

Table 19 Features of dominant clusters of bacterial populations isolated at 4 C and 20 C during summer 1975

+ = >80% positive
D = 51-79%
d = 21-50%
- = <20%
* = not tested or results could not be determined

	4 C ISOLATES L-SERIES														20 C ISOLATES H-SERIES												
CLUSTER	1	2	3	4	5	6	7	8	9	10	11	12	13	14	1	2	3	4	5	6	7	8	9	10	11	12	13
NO. OF STRAINS	21	9	2	60	4	28	7	2	4	10	6	13	2	2	2	2	7	36	9	7	80	3	2	12	2	2	2
Cell morphology:																											
Pear shaped	-	+	d	-	d	d	-	-	-	+	d	-	-	-	-	-	-	-	-	d	d	d	-	-	-	-	-
Rod shaped	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	d	+
Curved	d	d	-	-	+	d	D	+	-	+	+	-	-	d	-	-	-	+	d	D	-	-	+	d	-	-	-
Tapered end (s)	+	d	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-
Rounded end (s)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	d	+	+
Square end (s)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Pleomorphic	+	D	+	+	+	+	+	+	+	-	+	+	d	+	+	+	d	+	D	d	d	D	+	+	-	-	-
Cocco-bacillary	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	d	d	-
Length:																											
0.5-1.0 μ	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	D	-	-	-	-	-	-	-	-	-	-
1.0-2.0 μ	-	d	-	d	-	-	-	-	-	-	-	-	-	-	-	d	-	-	D	d	d	-	-	-	-	d	d
2.1-3.0 μ	-	d	d	d	d	d	d	d	-	d	d	-	-	-	d	-	-	d	d	-	d	-	d	-	-	-	d
3.1-4.0 μ	-	d	-	-	d	-	d	d	d	d	d	D	+	+	d	-	-	d	-	-	-	+	-	d	-	-	-
4.1-5.0 μ	d	-	-	-	-	-	-	-	-	-	-	-	-	-	-	d	-	d	-	-	-	-	-	d	-	-	-
5.1-10.0 μ	D	-	-	-	d	-	-	-	-	-	-	-	-	-	-	-	-	-	-	d	-	-	d	-	-	-	-
Width:																											
< 0.5 μ	-	-	-	-	d	d	-	-	-	-	-	-	-	-	-	-	D	-	d	d	-	-	-	-	-	-	-
0.5-1.0 μ	+	+	+	+	d	D	+	+	+	+	+	+	+	d	+	+	d	+	D	+	+	+	+	+	+	+	+
1.1-2.0 μ	-	-	-	-	-	-	-	-	-	-	-	-	-	d	-	-	-	-	-	-	-	-	-	-	-	-	-
PHB	+	D	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-
Capsule	d	-	-	d	-	d	d	-	-	D	-	-	-	-	d	+	-	-	-	-	-	-	+	-	-	-	-
Sudan Black	+	D	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	d	d	+	+	-	-	-	-
Gram -	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	d	d	d
Motility	-	-	-	-	-	-	+	+	D	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+
Cell arrangements:																											
Single	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Chains	-	d	-	D	-	D	-	-	d	d	-	-	-	-	-	-	-	-	-	-	-	d	-	d	-	d	-
Irregular	-	-	+	-	-	-	-	-	d	-	-	-	-	-	-	+	-	-	-	-	d	-	-	d	d	d	-
Filament	-	d	-	d	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 19 continued

CLUSTER	1	2	3	4	5	6	7	8	9	10	11	12	13	14	1	2	3	4	5	6	7	8	9	10	11	12	13
NO. OF STRAINS	21	9	2	60	4	28	7	2	4	10	6	13	2	2	2	2	7	36	9	7	80	3	2	12	2	2	2
Colony:																											
Translucent	D	D	+	+	D	+	-	+	+	+	D	-	+	+	+	+	d	d	+	+	d	-	d	-	d	-	-
Transparent	d	d	-	-	d	-	-	-	-	-	-	-	-	-	-	-	-	d	-	-	-	d	-	-	-	-	-
Opaque	-	-	-	-	-	-	+	-	-	-	d	+	-	-	+	+	D	-	+	+	d	+	-	+	-	+	+
Entire	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	D	+	+	+	+	-
Convex	+	+	+	+	+	+	+	+	d	+	+	+	d	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Raised	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
Glistening	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	d	+	D	+	+	+
Smooth	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
Mucoid	-	-	-	-	-	-	d	-	d	-	-	-	-	-	-	-	-	d	-	d	-	-	-	-	-	-	-
Colony size:																											
<1.0 mm	+	+	+	+	+	+	-	-	d	+	-	-	+	+	-	+	+	d	+	+	d	D	d	+	+	d	-
1.1-2.0 mm	-	-	-	-	-	-	-	+	D	-	D	D	-	-	+	-	-	D	-	-	d	d	d	-	-	-	+
2.0-6.0 mm	-	-	-	-	-	-	+	-	-	-	D	d	-	-	-	-	-	-	-	-	-	-	-	-	-	d	-
Pigment:																											
Gray	+	D	-	-	-	-	+	+	+	+	d	+	+	-	-	-	-	d	-	-	+	+	+	-	+	+	-
White	-	-	-	-	-	-	-	-	-	-	D	-	-	+	-	-	-	-	-	-	-	-	-	-	-	+	-
Non. dif. Yellow	-	-	-	-	D	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-	+	-	-	+
Orange	-	d	+	+	d	+	-	-	-	-	-	-	-	-	+	+	-	D	-	-	-	-	-	-	-	-	-
dif. Yellow	-	-	-	-	-	-	-	d	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Growth in liquid media:																											
No growth	-	d	-	-	d	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Floccular	-	-	+	-	-	-	-	+	+	-	d	-	-	-	*	+	D	-	d	-	-	d	d	d	-	-	+
Ring	-	-	-	-	-	-	-	-	-	-	d	d	-	-	*	-	-	d	D	d	-	-	-	-	-	+	-
Pellicle	-	-	+	-	-	-	+	+	+	-	D	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
Turbidity:																											
even	d	-	+	d	-	d	d	+	+	+	-	+	-	-	+	-	d	+	+	+	d	D	D	d	+	+	+
slight	D	+	-	D	+	+	d	-	-	d	-	-	d	+	+	+	D	d	-	d	D	+	+	+	-	-	-
moderate	d	-	-	d	-	-	d	-	+	d	+	+	-	-	-	-	d	D	D	D	d	-	-	-	+	d	+
heavy	-	-	+	-	-	-	-	+	-	-	-	-	d	-	-	-	-	-	-	d	-	-	-	-	-	d	-
Facultative anaerobic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Table 19 continued

CLUSTER	1	2	3	4	5	6	7	8	9	10	11	12	13	14	1	2	3	4	5	6	7	8	9	10	11	12	13
NO. OF STRAINS	21	9	2	60	4	28	7	2	4	10	6	13	2	2	2	2	7	36	9	7	80	3	2	12	2	2	2
Temperature:																											
5° C	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*	+	+	+	+	+	+	+	+	+	+	+	+
10° C	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
15° C	+	+	+	+	+	+	D	+	+	+	+	+	+	+	*	+	+	+	+	+	+	+	+	+	+	+	+
20° C	+	+	+	+	-	+	-	-	-	+	-	-	-	-	*	+	+	+	+	+	+	+	+	+	+	+	+
25° C	D	D	-	-	-	-	-	-	-	+	-	-	-	-	-	-	d	+	+	+	+	D	d	+	+	+	+
37° C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	d	-	-	+
43° C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
pH 4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*	*	-	-	-	-	-	-	-	+	-	+	+
5	d	-	+	+	d	d	+	+	+	+	+	+	d	+	-	-	d	+	+	+	d	+	+	+	+	+	+
6	D	+	+	+	+	D	+	+	+	+	+	+	+	+	*	+	+	+	+	+	+	+	+	+	+	+	+
7	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
8	+	+	+	+	+	D	+	+	+	+	+	+	+	+	*	+	+	+	+	+	+	+	+	+	+	+	+
9	-	+	+	+	+	-	+	+	+	+	+	+	+	+	*	-	-	-	D	+	+	+	+	+	+	+	-
10	-	-	-	-	-	-	D	+	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
NaCl 0.0%	-	d	+	-	-	-	-	-	-	+	-	-	-	+	-	-	-	d	+	+	-	d	-	+	+	+	+
0.5%	D	+	+	+	-	+	-	-	-	+	-	-	+	+	+	d	-	+	+	+	+	D	d	+	+	+	+
3.0%	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
5.0%	D	+	+	+	+	-	+	+	+	-	+	D	-	+	-	+	-	D	+	-	+	+	+	+	+	+	+
7.5%	d	d	+	D	-	-	d	-	-	-	-	-	-	+	-	-	-	d	D	-	D	D	d	d	-	+	+
10.0%	d	-	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-	d	+	-	D	d	-	-	+	+	+
15.0%	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-
Biochemistry:																											
Acid from																											
D-Ribose	-	-	-	-	-	-	-	-	-	-	D	+	-	+	-	-	-	-	-	-	d	-	d	-	-	-	+
D-Fructose	-	-	-	d	-	-	-	-	-	-	+	+	-	+	-	-	-	-	*	-	d	-	-	-	-	-	+
D-Glucose (oxid.)	d	-	+	-	-	D	d	-	+	-	+	+	+	+	*	*	-	D	-	-	-	-	-	-	d	-	+
D-Glucose (fermt.)	d	-	+	-	-	d	D	d	+	-	+	+	+	+	-	-	-	D	d	-	D	d	-	-	d	-	+
Cellobiose	-	-	d	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
Lactose	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
Sucrose	-	-	+	D	-	d	-	-	-	-	-	-	-	+	-	-	-	D	-	-	d	-	-	-	-	-	-
D-Mannitol	-	-	-	-	-	-	-	-	-	-	d	-	-	-	-	d	-	d	-	-	-	-	-	-	-	-	d
Gas from Glucose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 19 continued

CLUSTERS	1	2	3	4	5	6	7	8	9	10	11	12	13	14	1	2	3	4	5	6	7	8	9	10	11	12	13
NO. OF STRAINS	21	9	2	60	4	28	7	2	4	10	6	13	2	2	2	2	7	36	9	7	80	3	2	12	2	2	2
Hydrolysis of:																											
Starch	-	-	+	-	-	-	+	+	-	-	-	+	+	-	-	d	+	+	-	-	-	-	-	-	-	+	-
Gelatin	-	d	+	D	-	d	+	+	+	-	d	d	-	+	d	+	-	D	-	-	-	-	-	-	-	+	-
Chitin	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Cellulose	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Agar	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Lipase:																											
Tween 20	-	d	-	-	-	d	+	+	+	+	d	+	-	-	d	-	-	-	+	+	d	D	-	d	-	+	+
Tween 80	d	D	-	-	d	-	+	+	+	d	-	+	-	+	-	-	-	+	+	d	+	+	d	+	+	+	-
Alkaline phosphatase	+	+	+	+	+	+	+	+	+	D	+	D	d	+	+	+	-	+	D	D	+	D	+	-	d	+	-
Urease	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Catalase	+	D	+	+	D	+	-	-	+	+	+	+	-	+	+	*	D	D	d	+	D	D	+	+	+	+	+
Oxidase (Kovac's)	-	-	-	-	-	-	d	-	-	-	-	-	-	-	-	*	-	-	-	-	-	-	-	-	-	-	-
Indole production	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*	*	-	-	-	-	-	-	-	-	-	-	-
MR	-	-	-	-	-	-	-	-	-	-	D	-	-	-	*	*	*	d	-	-	-	-	-	-	-	-	-
VP	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*	*	*	-	-	-	-	-	-	-	-	-	-
Haemolysis (β-)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*	-	-	-	-	-	-	-	-	-	-	-	-
Production of NH_3	-	-	+	-	-	-	-	+	+	-	+	-	-	-	+	-	-	+	+	+	-	d	-	-	d	+	+
$NO_3 \rightarrow NO_2$	-	-	-	-	-	-	+	+	+	+	+	+	+	+	-	-	+	-	-	+	-	d	-	+	+	+	+
Reduction of NO_2	-	-	-	-	-	-	d	+	-	d	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Arginine deaminase	d	D	-	D	D	D	-	-	-	d	+	d	-	+	+	-	d	d	d	d	-	-	-	-	-	-	-
Lysine decarboxylase	d	-	-	-	-	-	-	-	-	d	-	D	-	-	*	-	-	-	-	-	-	d	-	-	-	-	-
Ornithine decarboxylase	-	-	-	-	d	-	d	-	d	-	-	-	-	-	*	d	-	d	d	-	-	d	-	-	-	-	-
Antibiotics:																											
Ampicillin, 2 µg	+	+	+	+	+	+	D	+	-	+	-	-	+	d	*	+	D	+	+	+	+	+	+	+	+	-	-
Ampicillin, 10 µg	+	+	*	*	*	+	*	*	*	+	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Chloromycetin, 30 µg	-	-	*	*	*	*	*	*	*	*	*	*	*	*	*	*	+	+	d	+	+	+	+	-	-	d	d
Chlorotetracyclin, 30 µg	+	+	*	*	*	+	*	*	*	+	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Colistin 2 µg	+	-	*	*	*	+	*	*	*	+	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Colistin, 10 µg	+	d	-	-	-	D	+	+	-	+	+	+	+	+	*	*	+	+	d	+	+	+	+	-	-	d	d
Erythromycin, 15 µg	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*	*	+	+	+	+	+	+	+	+	+	+	+
Kanamycin, 5 µg	+	+	-	-	-	+	-	-	-	+	-	*	*	*	*	*	+	+	+	+	+	+	+	+	+	+	d
Kanamycin, 30 µg	+	+	-	-	-	D	+	-	+	+	-	-	+	+	*	*	+	+	+	+	+	+	+	+	+	-	+
Neomycin, 5 µg	+	-	*	*	*	+	*	*	*	+	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Neomycin, 30 µg	+	+	-	-	-	+	+	+	+	+	-	-	+	+	*	*	+	+	d	+	+	+	+	-	-	d	+
Nitrofurantoin, 300 µg	+	D	d	+	+	+	+	d	+	+	+	+	+	+	*	*	+	+	+	+	+	+	+	-	-	d	+

Table 19 continued

CLUSTER	1	2	3	4	5	6	7	8	9	10	11	12	13	14	1	2	3	4	5	6	7	8	9	10	11	12	13
NO. OF STRAINS	21	9	2	60	4	28	7	2	4	10	6	13	2	2	2	2	7	36	9	7	80	3	2	12	2	2	2
Antibiotics:																											
Novobiocin, 5 µg	+	+	+	+	-	+	+	+	d	+	+	+	+	d	*	-	+	+	+	+	+	+	+	+	+	+	-
Oxytetracyclin, 5 µg	D	-	+	D	+	D	-	-	-	d	-	-	+	d	*	*	*	*	*	*	*	*	*	*	*	*	*
Penicillin G, 2 units	+	+	*	+	+	+	d	-	-	+	-	-	+	d	*	*	+	+	+	+	+	+	+	+	-	-	-
Polymyxin B, 300units	+	+	-	-	-	D	+	+	+	+	+	+	+	+	*	*	+	+	-	+	+	+	+	-	-	-	+
Streptomycin, 20 µg	+	D	-	-	-	+	-	-	d	+	-	-	+	-	*	d	d	d	D	+	+	+	+	+	+	-	-
Tetracyclin, 5 µg	d	d	+	d	+	D	-	-	-	d	-	-	+	+	*	-	d	-	-	d	-	-	-	-	-	-	-
Tetracyclin, 30 µg	D	+	-	*	*	+	*	*	*	+	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Carbohydrates and sugar derivatives:																											
L-Arabinose	-	-	-	-	-	-	-	-	-	-	-	-	d	-	*	-	-	-	-	-	-	-	d	+	-	-	+
D-Ribose	-	-	-	-	-	-	-	-	-	-	+	+	d	+	*	-	-	-	-	-	d	-	D	D	+	+	+
D-Xylose	d	+	-	-	-	-	-	-	-	-	d	-	-	-	*	-	-	-	-	-	+	-	+	D	+	d	+
L-Rhamnose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*	-	-	-	-	-	-	-	-	-	-	-	+
D-Fructose	-	D	-	d	-	-	-	-	-	-	+	+	-	+	*	-	-	-	-	-	D	d	-	+	+	d	+
D-Galactose	-	D	+	D	-	-	-	-	-	-	D	+	+	+	*	-	-	-	-	-	D	-	-	d	+	d	+
D-Glucose	+	+	+	D	-	-	-	-	d	-	+	+	+	+	*	-	-	-	D	-	+	D	+	+	+	+	+
D-Mannose	-	-	+	D	d	-	-	-	-	-	D	+	+	-	*	-	-	-	-	-	-	-	-	D	-	-	+
L-Sorbose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*	-	-	-	-	-	-	-	-	-	-	-	-
Salicin	-	-	-	-	-	-	-	-	-	-	-	+	+	-	*	-	-	-	-	-	-	-	-	-	d	d	d
Cellobiose	-	+	-	-	-	-	d	-	-	-	d	-	-	-	*	-	-	-	d	-	+	d	+	+	+	D	+
Lactose	-	-	+	-	-	-	-	-	-	-	-	-	-	-	*	-	-	-	-	-	-	d	-	-	-	-	-
Maltose	d	-	+	D	-	d	-	-	-	-	+	+	+	+	*	-	-	-	-	-	-	-	+	+	+	+	+
Sucrose	-	-	+	d	-	-	-	-	-	-	-	-	-	+	*	-	-	-	-	-	-	-	-	+	+	+	-
Trehalose	-	-	-	-	-	-	-	-	-	-	D	-	-	-	*	-	-	-	-	-	-	-	-	+	+	+	+
D-Raffinose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*	-	-	-	-	-	-	-	-	+	-	-	-
Fatty acids:																											
Acetate	+	+	-	-	-	-	-	-	-	-	-	-	-	+	*	-	-	-	-	D	+	+	+	+	+	+	-
Propionate	+	d	-	-	-	-	-	-	-	-	-	-	-	-	*	-	-	-	D	d	D	+	-	+	+	+	-
Butyrate	D	D	-	-	-	-	-	-	-	-	-	-	-	-	*	-	-	-	+	d	D	+	-	+	+	+	-
Valerate	-	d	-	-	-	-	-	-	-	-	-	-	-	-	*	-	-	-	+	-	d	d	-	+	D	+	-
Isovalerate	d	-	-	-	-	-	-	-	-	-	-	-	-	-	*	-	-	-	+	d	d	d	d	+	+	+	-
Caproate	D	d	-	-	-	-	-	-	-	-	-	-	-	-	*	*	*	*	*	*	*	*	*	*	*	*	*
Caprylate	+	+	-	-	-	-	-	-	-	+	-	-	-	-	*	-	-	-	+	+	+	+	+	+	+	+	-
Laurate	-	d	-	-	-	-	-	-	-	-	-	-	-	-	*	-	-	-	-	-	d	-	-	d	-	-	-
Palmitate	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*	-	-	-	-	-	-	-	-	+	-	d	-

Table 19 continued

CLUSTER	1	2	3	4	5	6	7	8	9	10	11	12	13	14	1	2	3	4	5	6	7	8	9	10	11	12	13
NO. OF STRAINS	21	9	2	60	4	28	7	2	4	10	6	13	2	2	2	2	7	36	9	7	80	3	2	12	2	2	2
Dicarboxylic acids:																											
Malonate	D	+	-	-	-	-	-	-	-	-	d	-	-	-	*	-	-	-	-	-	D	d	+	d	-	-	-
Succinate	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*	-	-	-	-	-	-	d	-	d	+	+	+
Maleate (Maleic acid)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*	-	-	-	-	-	-	-	-	-	-	-	-
Fumarate	+	D	-	-	-	-	d	-	d	D	+	+	-	+	*	-	-	-	D	+	+	+	+	D	D	+	+
Hydroxyacids:																											
L(+)-Tartarate	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*	-	-	-	-	-	-	-	-	-	-	-	-
DL-β-Hydroxy-butyrate	+	+	-	-	-	-	-	-	-	-	-	-	-	-	*	-	-	-	+	d	+	+	+	+	+	+	-
DL-Lactate	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*	-	-	-	-	-	-	d	-	-	-	-	d
DL-Glycerate	d	+	-	-	-	-	-	-	-	-	d	+	-	-	*	-	-	-	-	-	+	-	-	D	-	d	+
Misc. organic acids:																											
Citrate	+	d	-	-	-	-	-	-	-	-	+	+	-	-	*	-	-	-	-	-	d	D	+	-	+	+	d
α-Ketoglutarate	+	+	-	-	-	-	-	-	-	D	+	-	-	-	*	-	-	-	-	-	d	+	+	+	-	+	-
Pyruvate	+	+	-	-	-	-	d	-	+	+	+	d	-	+	*	-	-	-	+	+	+	+	+	+	+	+	+
Itaconate	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*	-	-	-	-	-	-	-	-	d	-	-	-
Glutarate	+	+	-	-	-	-	-	-	-	d	+	+	-	+	*	-	-	-	d	d	+	+	+	-	-	+	d
Galacturonate	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*	-	-	-	-	-	-	-	-	-	-	-	+
D-Gluconate	-	-	-	-	-	-	-	-	-	-	d	D	-	+	*	-	-	-	-	-	-	-	-	-	+	d	+
Polyalcohols and glycols:																											
D-Arabitol	D	d	-	-	-	-	-	-	-	-	-	-	-	-	*	-	-	-	-	-	d	-	-	-	d	-	-
D-Mannitol	D	D	-	-	-	-	-	-	-	-	d	-	-	+	*	-	-	-	-	-	D	+	-	-	-	d	+
Dulcitol	-	-	-	-	-	-	-	-	-	-	-	d	-	-	*	-	-	-	-	-	-	-	-	-	-	-	-
D-Sorbitol	D	d	-	-	-	-	-	-	-	-	-	d	-	-	*	-	-	-	-	-	-	d	-	-	+	d	-
meso-Inositol	-	D	-	-	-	-	-	-	-	-	-	-	-	-	*	-	-	-	-	-	D	-	-	-	d	+	+
Glycerol	+	+	-	-	-	-	-	-	d	-	+	+	-	+	*	-	-	-	-	-	+	d	+	-	-	+	+
1,2-Propanediol	-	-	-	-	-	-	-	-	-	-	d	-	-	-	*	-	-	-	d	-	-	D	+	+	+	+	-
Alcohols:																											
Ethanol	-	-	-	-	-	-	-	-	-	-	d	-	-	-	*	-	-	-	-	-	-	-	-	-	-	+	-
1-Propanol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*	-	-	-	-	-	-	-	-	d	-	+	-
2-Propanol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*	-	-	-	-	-	-	-	-	-	-	-	-
1-Butanol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*	-	-	-	D	-	-	-	-	d	-	D	-

Table 19 continued

CLUSTER	1	2	3	4	5	6	7	8	9	10	11	12	13	14	1	2	3	4	5	6	7	8	9	10	11	12	13
NO. OF STRAINS	21	9	2	60	4	28	7	2	4	10	6	13	2	2	2	2	7	36	9	7	80	3	2	12	2	2	2
Non-nitrogenous aromatic and other cyclic compounds:																											
Phenol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*	-	-	-	-	-	-	-	-	-	-	-	-
2-Phenylethanol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*	-	-	-	-	-	-	-	-	-	-	-	-
Benzoate	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*	-	-	-	-	-	-	-	-	-	+	+	-
o-Hydroxybenzoate	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*	-	-	-	-	-	-	-	-	-	-	+	-
m-Hydroxybenzoate	D	-	-	-	-	-	-	-	-	-	-	-	-	-	*	-	-	-	-	-	-	+	+	+	d	-	-
p-Hydroxybenzoate	+	+	-	-	-	-	-	-	-	-	-	-	-	-	*	-	-	-	-	-	+	+	+	+	d	-	-
Aliphatic amino acids:																											
Glycine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*	-	-	-	-	-	-	-	-	-	-	-	-
L-Alanine	+	D	-	-	-	-	-	-	-	+	-	-	-	+	-	-	-	-	-	+	+	+	+	-	+	d	-
β-Alanine	D	D	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-
L-Serine	+	d	-	-	-	-	-	-	-	d	D	-	-	-	-	-	-	-	-	d	d	+	+	+	+	+	-
L-Threonine	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	d	-	-
L-Leucine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	d	-	-	-	-	d	+	+	-
L-Isoleucine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	D	-	-	-	-	D	+	+	-
L-valine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	d	-
L-Aspartate	d	D	d	-	-	-	-	-	-	+	D	+	-	-	*	-	-	-	-	+	d	+	d	d	+	d	d
L-Glutamate	+	+	-	d	-	-	-	-	-	-	-	-	-	-	*	-	-	-	d	+	+	+	+	+	+	+	+
L-Lysine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*	-	-	-	-	-	-	-	-	-	-	-	-
L-Arginine	d	D	-	d	-	-	-	-	-	-	+	-	-	-	*	-	-	-	-	-	d	-	-	d	-	+	+
L-Ornithine	D	D	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	d	D	d	-	D	d	d	-
L-Asparagine	d	-	-	-	-	-	-	-	-	d	-	-	-	-	*	-	-	-	-	-	-	d	d	d	+	+	+
γ-Aminobutyrate	+	+	-	-	-	-	-	-	-	+	-	-	-	-	*	-	-	-	-	+	+	d	d	-	-	d	+
L-Cysteine	d	d	-	-	-	-	-	-	-	-	D	-	-	-	*	-	-	-	d	-	d	+	+	-	-	d	-
L-Cystine	d	D	-	-	-	-	-	-	-	-	-	-	-	-	*	-	-	-	-	-	-	-	-	d	-	-	-
L-Methionine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*	-	-	-	-	-	-	-	-	-	-	-	-
Amino acids and related compounds containing a ring structure:																											
L-Histidine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	d	+	+
L-Proline	d	D	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	d	d	D	-	-	-	-	+	+
L-Tyrosine	d	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-
Phenylalanine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-
L-Tryptophan	D	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-

Table 19 continued

CLUSTER	1	2	3	4	5	6	7	8	9	10	11	12	13	14	1	2	3	4	5	6	7	8	9	10	11	12	13
NO. OF STRAINS	21	9	2	60	4	28	7	2	4	10	6	13	2	2	2	2	7	36	9	7	80	3	2	12	2	2	2
Amines																											
α-Amylamine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ethanolamine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*	-	-	-	-	-	-	-	-	-	-	-	-
Histamine	-	-	-	-	-	-	-	-	-	d	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-
Putrescine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-
Tryptamine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	d	+	+
N-Acetylglucos-amine	d	-	-	-	-	-	-	-	-	-	+	+	+	+	*	-	-	-	-	-	-	-	-	-	-	-	-
Misc. nitrogenous compounds:																											
DL-Carnitine	-	d	-	-	-	-	-	-	-	-	d	-	-	-	*	-	-	-	-	-	d	-	-	-	-	-	-
Hippurate	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	d	+	+	-
Sarcosine	-	d	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Allantoin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Guanine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Thymine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Hydrocarbons:																											
N-Hexadecane	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-
N-Octadecane	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-
N-Pentadecane	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-
Phenanthrene	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
omega-Phenyldecane	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-
Pristane	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-
Pentadecylcyclo-hexane	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-
N-Dotriaconitane	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1-Phenyltriacetane	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-
2-Ethylnaphthalene	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-
As nitrogen and carbon source:																											
Glycine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	D	-	-	-
L-Alanine	+	+	-	-	-	-	-	-	-	+	-	-	-	+	*	*	-	-	-	-	-	-	*	*	*	*	*
L-Serine	D	d	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	D	d	-	+	d	+	+	-
L-Threonine	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	+	-	D	+	-	-
L-Leucine	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	d	D	+	d	+	+	-

Table 19 continued

CLUSTER	1	2	3	4	5	6	7	8	9	10	11	12	13	14	1	2	3	4	5	6	7	8	9	10	11	12	13
NO. OF STRAINS	21	9	2	60	4	28	7	2	4	10	6	13	2	2	2	2	7	36	9	7	80	3	2	12	2	2	2
L-Isoleucine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	D	-	-	-	-	-	+	+	-
L-Valine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	d	-
L-Aspartate	+	+	-	d	-	-	-	-	-	+	+	-	-	-	-	-	d	d	d	+	+	+	+	+	+	+	+
L-Glutamate	+	+	+	D	-	d	-	+	d	+	+	+	-	+	-	-	-	D	+	+	+	+	+	+	+	+	+
L-Lysine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
L-Arginine	+	+	-	-	-	-	-	-	-	D	-	D	-	-	-	-	-	-	-	+	+	+	+	d	-	+	+
L-Asparagine	+	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	+	d	+	+	+	+	+	+
L-Cysteine	+	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	D	-	+	+	-	d	-	-	-
L-Cystine	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	D	-	+	-	-	d	-	-	-
L-Methionine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
L-Histidine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+
L-Proline	+	+	+	-	-	-	-	-	d	D	+	-	-	+	-	-	-	-	+	+	+	+	+	+	+	+	+
L-Tyrosine	D	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	D	-	-	+	d	-	-	+	-
L-Tryptophan	D	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-
L-Phenylalanine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Urea	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ethanolamine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Histamine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Allantoin	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Guanine	-	d	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Thymine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
Medium A:																											
D-Ribose	-	-	-	-	-	-	-	-	-	-	+	-	-	+	*	*	*	*	*	*	*	*	*	*	*	*	*
D-Fructose	-	-	-	-	-	-	-	-	-	-	+	-	-	+	*	*	*	*	*	*	*	*	*	*	*	*	*
D-Glucose	-	-	-	-	-	-	-	-	-	-	+	-	d	+	-	-	-	-	-	-	-	-	-	-	-	+	+
Acetate	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
Succinate	-	-	-	-	-	-	-	-	-	D	+	-	-	+	*	*	*	*	*	*	*	*	*	*	*	*	*
Fumarate	-	-	-	-	-	-	-	-	-	+	-	-	-	+	*	*	*	*	*	*	*	*	*	*	*	*	*
β-Hydroxybutyrate	-	-	-	-	-	-	-	-	-	d	-	-	-	-	-	-	-	-	D	d	-	-	-	-	-	D	-
DL-Lactate	-	-	-	-	-	-	-	-	-	D	-	-	-	+	*	*	*	*	*	*	*	*	*	*	*	*	*
Pyruvate	-	-	-	-	-	-	-	-	-	-	d	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
α-Ketoglutarate	-	-	-	-	-	-	-	-	-	d	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
D-Gluconate	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
Glycerol	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+

Table 19 continued

CLUSTER	1	2	3	4	5	6	7	8	9	10	11	12	13	14	1	2	3	4	5	6	7	8	9	10	11	12	13
NO. OF STRAINS	21	9	2	60	4	28	7	2	4	10	6	13	2	2	2	2	7	36	9	7	80	3	2	12	2	2	2
Medium E:																											
D-Ribose	d	+	-	-	-	-	-	-	-	d	D	+	+	+	-	-	-	-	d	-	D	+	+	d	+	+	+
D-Fructose	d	D	+	d	d	d	-	-	D	-	+	+	+	+	-	-	-	-	-	-	D	+	-	+	+	+	+
D-Glucose	D	D	+	d	d	d	-	-	D	d	+	+	+	+	*	*	-	d	-	d	d	+	+	+	+	+	+
Acetate	D	D	-	-	-	-	-	-	-	-	+	-	-	+	-	-	-	-	d	-	D	+	-	D	d	+	+
Succinate	D	+	+	d	d	d	d	+	D	-	+	+	-	+	-	+	-	D	D	-	+	+	+	+	+	+	+
Fumarate	+	+	+	D	d	d	+	+	+	+	+	+	-	+	-	d	-	+	+	+	+	+	+	+	+	+	+
DL-Lactate	+	-	+	+	d	d	+	+	+	+	+	+	-	+	-	-	-	D	D	+	+	+	-	+	-	-	+
β-Hydroxybutyrate	D	D	-	d	-	-	-	-	-	-	-	-	-	-	*	-	-	-	d	-	D	+	+	+	+	+	-
Pyruvate	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	D	+	d	+	+	d
D-Gluconate	+	D	-	D	-	-	-	-	-	+	-	d	-	-	*	*	-	d	-	d	d	+	+	+	+	+	+
α-Ketoglutarate	+	+	+	D	D	+	+	+	d	+	+	+	-	+	-	+	-	+	D	+	+	+	+	+	+	+	+
Glycerol	-	+	+	-	-	-	-	-	d	+	+	-	-	-	-	+	-	-	d	-	D	+	-	d	+	+	+

Hydrocarbon Biodegradation Potential

In addition to the extensive characterization of heterotrophic microbial populations described in the previous sections the potential of the indigenous populations to degrade petroleum hydrocarbons was examined.

Figure 55 shows the results of the hydrocarbon biodegradation potential measure as a three dimentional histogram. The height of the bars shows the units of activity. Units of activity represent CPM $^{14}CO_2$ produced during nine weeks incubation at 5°C. Two reference lines are provided at 0 and 3000 units to aid in gaining proper perspective. In winter the highest activities were found in water. The highest biodegradation potentials in water were found off Barrow. High biodegradation potential in water also occurred near Prudhoe Bay. High biodegradation potentials were also found in sediment. The lowest biodegradation potentials in winter occurred in ice.

Surprisingly only minimal biodegradation potentials were found in summer water and sediment samples. There was no correllation between numbers of hydrocarbon utilizing bacteria and the biodegradation potential. The highest numbers of hydrocarbon degrading bacteria were found in summer when the biodegradation potentials were lowest. It would appear that summer hydrocarbon biodegradation potentials were restricted by some limiting factor. Inorganic nitrogen nutrients are probably in lowest available concentration in late summer following extensive phytoplankton assimilation. Nitrogen nutrients are essential for microbial oil degradation.

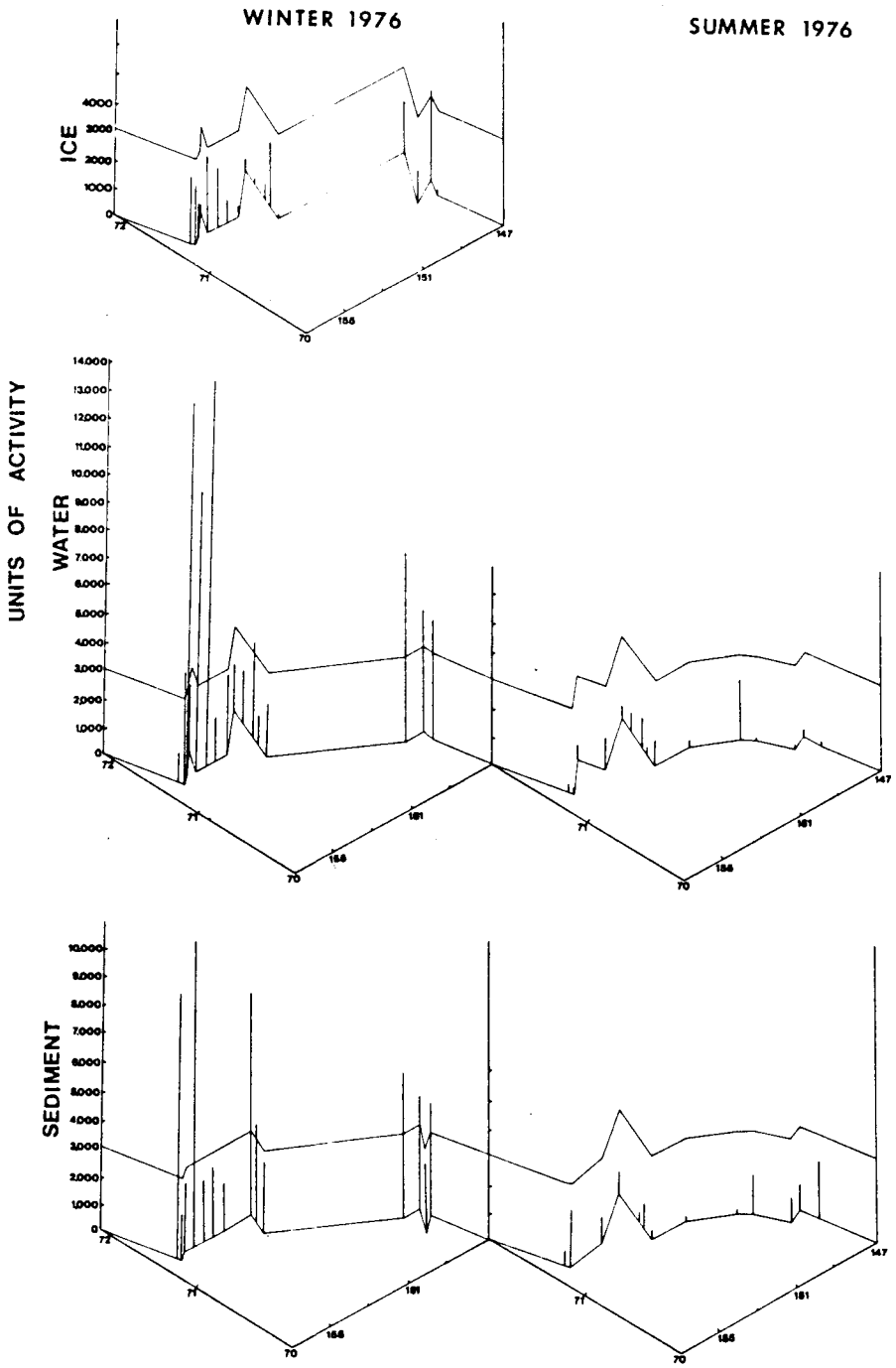

Fig. 55 Hydrocarbon biodegradation potential.

Separate studies have been conducted to examine the compositional changes in oil exposed to indigenous microorganisms of the Beaufort Sea. The results of these studies were recently summarized at the NOAA sponsored Fate and Effects Symposium in Seattle. Basically oil spilled on water during early summer can be expected to undergo slow biodegradation with 30% lost by the end of summer. The relative class composition of oil after one summer exposure can be expected to be similar to oil shortly after spillage. Degradative losses from oil spilled under ice in late winter will be minimal. During one month exposure only 10% of oil under ice can be expected to be lost. Ice cover will restrict abiotic losses of the oil.

VII. DISCUSSION AND CONCLUSIONS

Bacterial populations showed decreases in water during winter. Bacteria may be sedimented with dense water formed from salt and particle exclusion during ice formation. The correlation of viable bacteria with salinity in summer suggests that the sizes of bacterial populations are highly dependent on the source of the water. Bacterial diversity in water is higher in summer than winter and shows opposite spacial variation patterns in the two seasons. Bacterial populations were lower in the ice dominated summer of 1975 than the milder summer of 1976 suggesting that summer ice conditions are critical in determining bacterial population levels. The occurrence of orange pigmented bacteria in summer appears to be ecologically significant. The nutritional requirements of these orange pigmented bacteria suggests that they may be dependent on the spring phytoplankton bloom.

During summer bacterial populations in water show wide distribution. In winter bacterial populations in water show cloning and appear to be more site specific than in summer. Bacteria in ice also appear to be highly site specific.

Viable bacterial populations in sediment did not show decreases in winter as occurred in water. Bacterial populations in sediment were more diverse than in water and did not show spacial variation patterns in bacterial diversity. Bacterial populations in sediment were less tolerant of changes in temperature than populations in water.

The hydrocarbon biodegradation potential was severely limtied in late summer probably due to an abiotic limiting factor such as available nitrogen. During late winter hydrocarbon degrading microorganisms were in low concentrations indicating initial rates of hydrocarbon biodegradation would be slow. Winter biodegradation potentials were relatively high indicating that winter oil biodegradation can occur. The fate of oil in the Beaufort Sea is likely to be highly dependent on the site and time of year of contamination.

VIII. STUDY NEEDS

The features of microorganisms isolated during summer 1976 remain to be analysed. Also numerous hydrocarbon degrading microorganisms isolated during winter and summer 1976 have been tested but cluster and feature frequency analyses of the hydrocarbon degrading populations have not yet been completed. The characterization of these microbial populations are important study needs which are presently being carried out.

With respect to future study needs long term studies of the fate of oil spilled in the Beaufort Sea are needed. No concern has been given to the fate of oil that may enter sediment in the Arctic. Plans to pipe oil onshore through buried pipes make such studies essential. Also long term studies on the abiotic and biotic changes in oil stranded under ice are needed.

Microbial communities in the Beaufort Sea in U. S. areas east of Prudhoe Bay have never been examined. Such studies should be carried out. Extensive characterization of microbial communities should also be carried out in areas of active OCS oil development activity.

Effects studies on essential microbial biogeochemical cycling processes such as nitrogen fixation and denitrification also need to be performed.

IX. SUMMARY OF 4TH QUARTER ACTIVITIES

No field activities were performed during the 4th Quarter. Laboratory activities consisted of data analyses presented in this report.

ANNUAL REPORT

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

RU #30

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Submitted by:

Ronald M. Atlas, Ph.D.
Principal Investigator
Department of Biology
University of Louisville
Louisville, Kentucky 40208

Prepared for:

Outer Continental Shelf Energy Assessment Program
National Oceanic and Atmospheric Administration
Juneau Project Office
Juneau, Alaska

Personnel

Principal Investigator:	Ronald M. Atlas
Research Associates:	James Hauxhurst Tatsuo Kaneko
Research Assistant:	George Roubal
Research Technicians:	Teiko Kaneko Craig Short Linda Haag Khosrow Jaberizadeh

I. Summary of Objectives and Conclusions

The objectives of this project were to characterize microbial communities in the Gulf of Alaska and Cook Inlet. There were differences in the microbial communities in the different areas sampled but since only one sampling was made in each area at different times of the year, it is not possible to separate spacial from seasonal variations. Numbers of microorganisms in water in all areas of the Gulf of Alaska were very low, more typical of mid-ocean than continental shelf areas. Analyses have not been completed to the point that a baseline exists against which changes in the microbial community following OCS petroleum development can be made. Hydrocarbon biodegradation potentials in Lower Cook Inlet in late summer were unexpectedly lower than in the Northeast Gulf of Alaska in winter.

II. Introduction

This study was conducted to determine the distribution, and to characterize, microbial populations in the Gulf of Alaska and Cook Inlet. Microorganisms are essential components of marine ecosystems. Microbial populations respond rapidly to environmental perturbations. Microbial populations can be used to monitor such perturbations. With specific reference to petroleum development, some microorganisms are capable of biodegrading polluting hydrocarbons, as well as other organic contaminants. Microbial degradation is the main natural process for removal of contaminating petroleum hydrocarbons.

III. Current State of Knowledge

The state of knowledge concerning microbial populations in the Gulf of Alaska was summarized in the 1976 annual report. New information developed from this project is described below.

IV. Study Area

Samples were collected in the Northwest Gulf of Alaska during late summer 1975 (September-October). Figure 1 shows the sample site locations. During winter 1976 (March) samples were collected in the northeast Gulf of Alaska. Figure 2 shows the sites of sample collection in NEGOA. During the late summer 1976 (October), samples were collected in Cook Inlet. The sampling sites in Cook Inlet are shown in Fig. 3.

V. Methods and Rationale of Data Collection

Several methods were used to enumerate microbial populations in

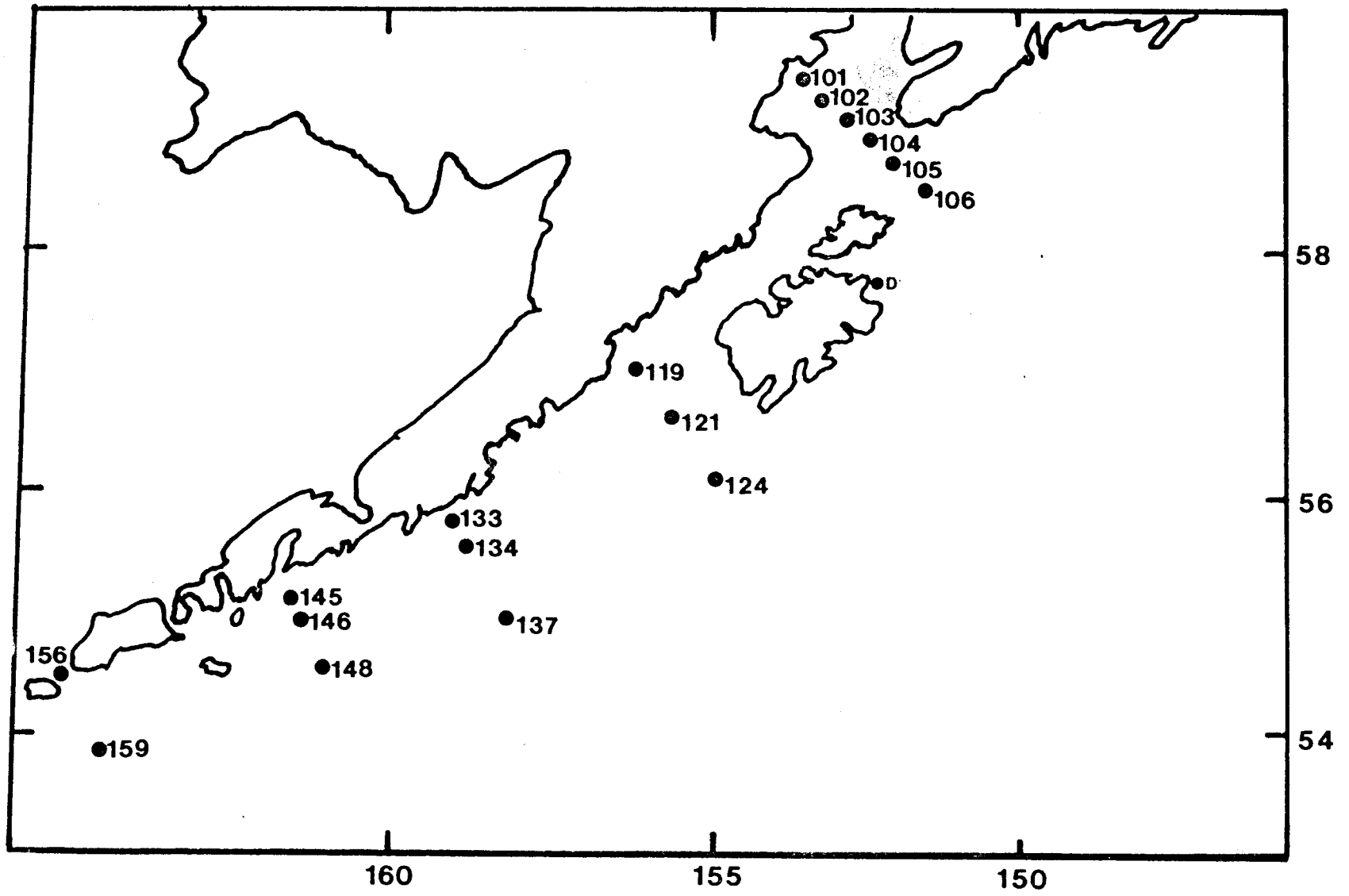

Fig. 1.
Map of stations sampled in Northwest Gulf of Alaska.

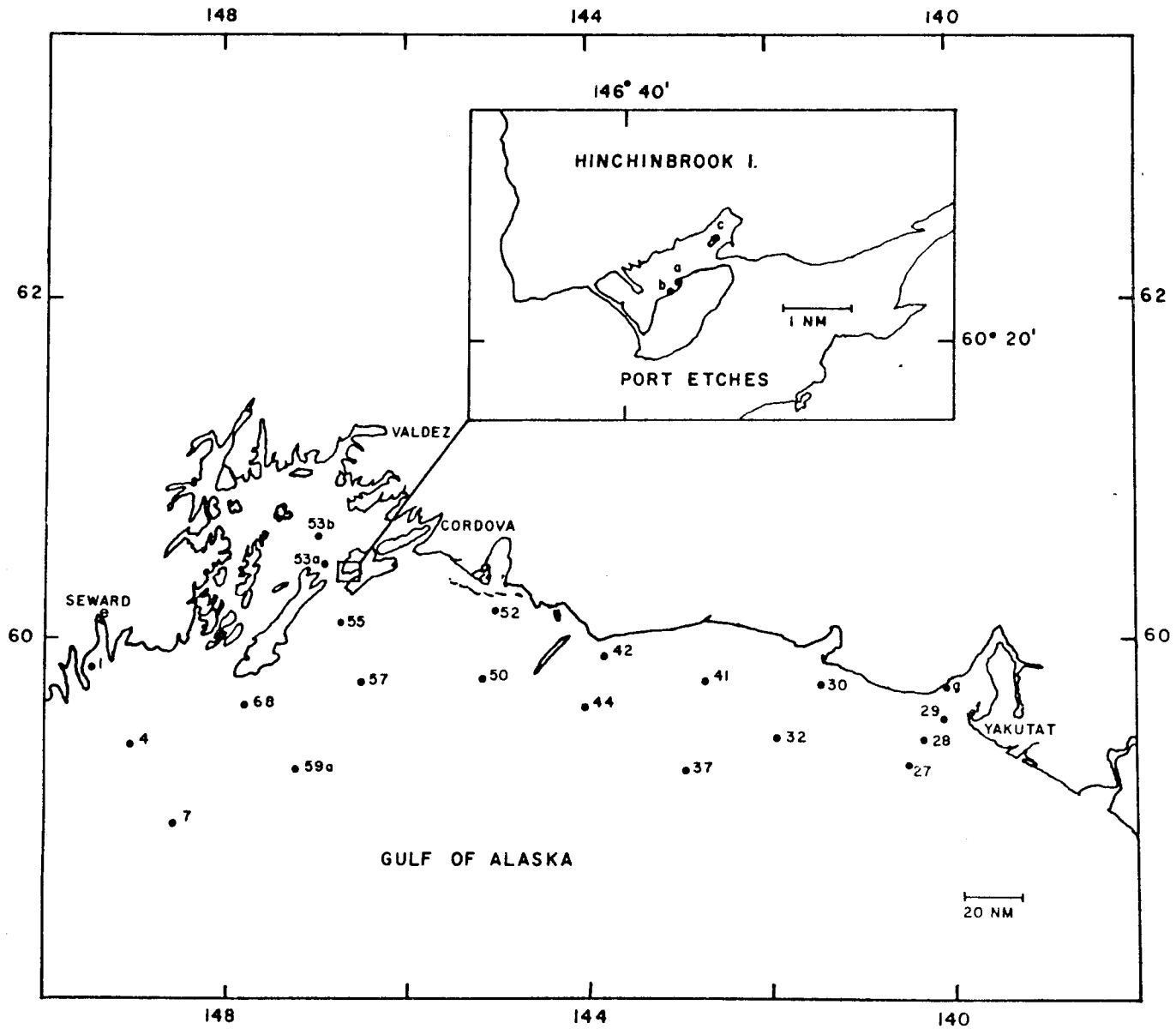

Fig.2
Map of stations sampled in North-east Gulf of Alaska.

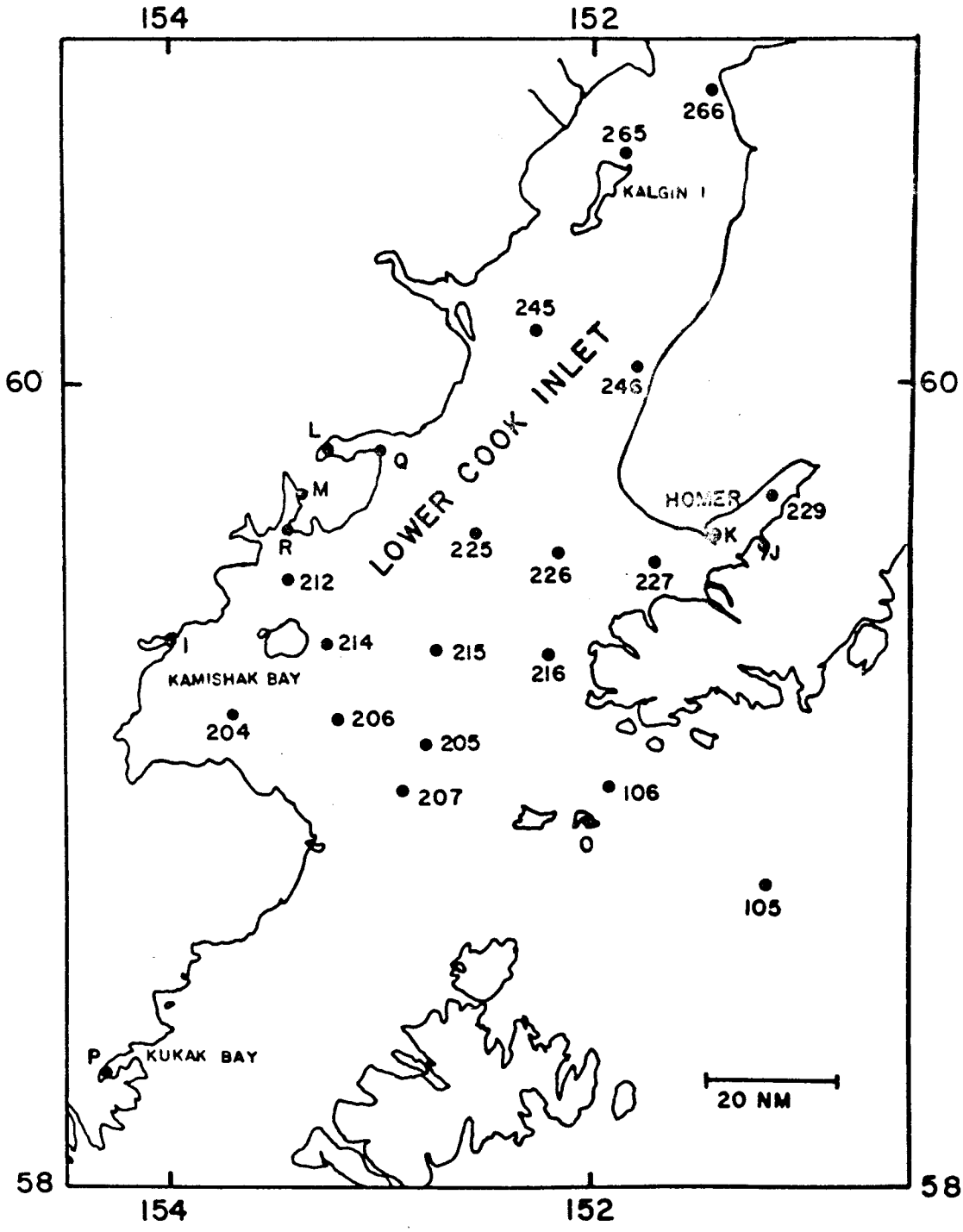

Fig.3.
Map of stations sampled in
Cook Inlet.

both water and sediment samples. Enumeration procedures included direct and viable counts. Direct counts give the best estimate of the numbers of microorganisms present, but do not distinguish between living and dead microorganisms. Viable counts have the problem of being selective for a given population capable of growth under the enumeration conditions. Viable count procedures were performed to determine the numbers of heterotrophs capable of growth at 4 C and also for heterotrophs capable of growth at 20 C. Even though 20 C is above the temperature at the time of isolation, the optimal growth temperature of microorganisms is usually higher than the temperature of the ecosystem from which they are isolated. Selective procedures were used to enumerate Vibrio spp., a common marine bacterial genus, and to enumerate microorganisms capable of utilizing petroleum hydrocarbons.

In addition to enumeration of microorganisms, microbial populations were extensively characterized by random selection and numerical taxonomic testing of isolates from representative samples. Details of these procedures have been described in previous reports. The characterization includes examination of between 300 and 400 features for each organism. The enzymatic capabilities and nutritional requirements of microbial populations are determined in this testing. Also, the physiological tolerance limits of the tested microorganisms are determined in these tests. Cluster analyses of these microorganisms were performed to determine the dominant taxonomic groups. The spacial and seasonal distribution of major groups

of microorganisms and the diversity of the microbial community were examined.

The ability of the indigenous microbial community to biodegrade petroleum hydrocarbons was determined using ^{14}C labelled hydrocarbon spiked crude oil. To determine the biodegradation potential water or sediment samples are incubated with ^{14}C spiked crude oil and the production of $^{14}CO_2$ periodically monitored. The amount of $^{14}CO_2$ reflects the ability of the microbial community to remove hydrocarbon contaminants. THe hydrocarbon biodegradation potential is determined in part by the numbers and types of microorganisms present and in part by abiotic factors such as concentrations of essential nutrients.

VI. Results

Enumeration

The enumerations of microbial populations are shown as three dimentional histograms (Figs. 4-17). The geographic location of each sample is shown by latitude and longitude in the XY plane. The numbers of microorganisms enumerated are shown by the height of the bar. To aid in gaining a proper perspective for interpretation of these figures, reference lines have been drawn at the base and tops of the bars. These are only reference lines that facilitate comparisons of the heights of the bars.

The direct counts showed that total numbers of microorganisms were higher in surfacial sediment than water in both summer

and winter. The direct counts showed no spacial variation pattern in the northwest Gulf of Alaska during summer 1975, nor within Cook Inlet during summer 1976 (Figs. 4 and 13). Direct counts in the northeast Gulf of Alaska during winter 1976 (Fig. 8) were lower than the summer Cook Inlet samples in both water and sediment. The winter NEGOA sediment samples direct counts were slightly lower in the eastern portion of the transect than in the westernmost stations.

The viable counts enumerated from water at 4 C in the Northwest Gulf of Alaska during summer 1976 showed a definite pattern of increasing counts moving from Kodiak Island to the Aleutians Unimak Pass (Fig. 5). This pattern was also very apparent in the populations enumerated at 20 C (Fig. 6). Not enough sediment samples were collected in the northwest Gulf of Alaska to establish a spacial pattern of population densities. The viable counts in the Northeast Gulf of Alaska during winter were lower than those found in the northwest Gulf of Alaska during summer (Figs. 9 and 10). The bacterial populations were very low in most water samples collected in the NEGOA region during winter. Also, viable counts of bacteria at both 4 and 20 C were very low in winter sediment samples in NEGOA, particularly samples collected in deeper offshore areas. Within Cook Inlet, viable counts of microorganisms were variable (Figs. 14 and 15). Populations in water above Kalgin Island were higher than in the lower portions of Cook Inlet. The population in water collected in Kennedy Entrance was higher than other locations in lower Cook In-

let. The lowest viable populations in both water and sediment were found in the center of Cook Inlet at stations 225 and 226. Viable counts at these two stations were an order of magnitude lower than the mean.

Vibrio species were in very low concentrations in water in the northeast Gulf of Alaska during winter (Fig. 11). Counts of Vibrio species were higher in sediment collected in the western portion of the NEGOA region than in the eastern portion of this region. Counts of Vibrio species were higher in summer water and sediment samples from Cook Inlet than in the winter NEGOA samples (Fig. 16). In water the highest counts of Vibrio species were found in the upper regions of Cook Inlet.

Numbers of hydrocarbon degraders were low in many of the Gulf of Alaska samples. In water samples collected in the northwest Gulf of Alaska in summer, concentrations of hydrocarbon degraders were higher just south of Kodiak Island and near Unimak Pass than other areas (Fig. 7). In the winter Northeast Gulf of Alaska samples, numbers of hydrocarbon degraders were low in both water and sediment samples (Fig. 12). Counts of hydrocarbon degraders were significantly higher within Cook Inlet during summer than in the NEGOA region during winter especially in sediment samples (Fig. 17). Hydrocarbon degrading microorganisms were not found in higher concentrations in the inner portion of Cook Inlet than near the entrance to the inlet.

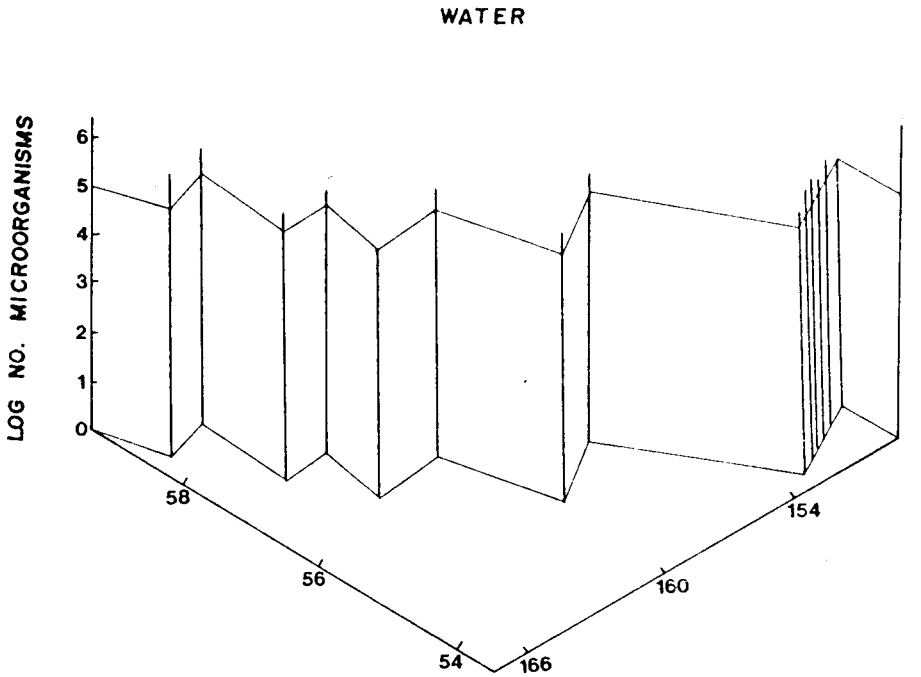

Fig. 4.
Direct counts of bacteria in North
west Gulf of Alaska during Summer
1975.

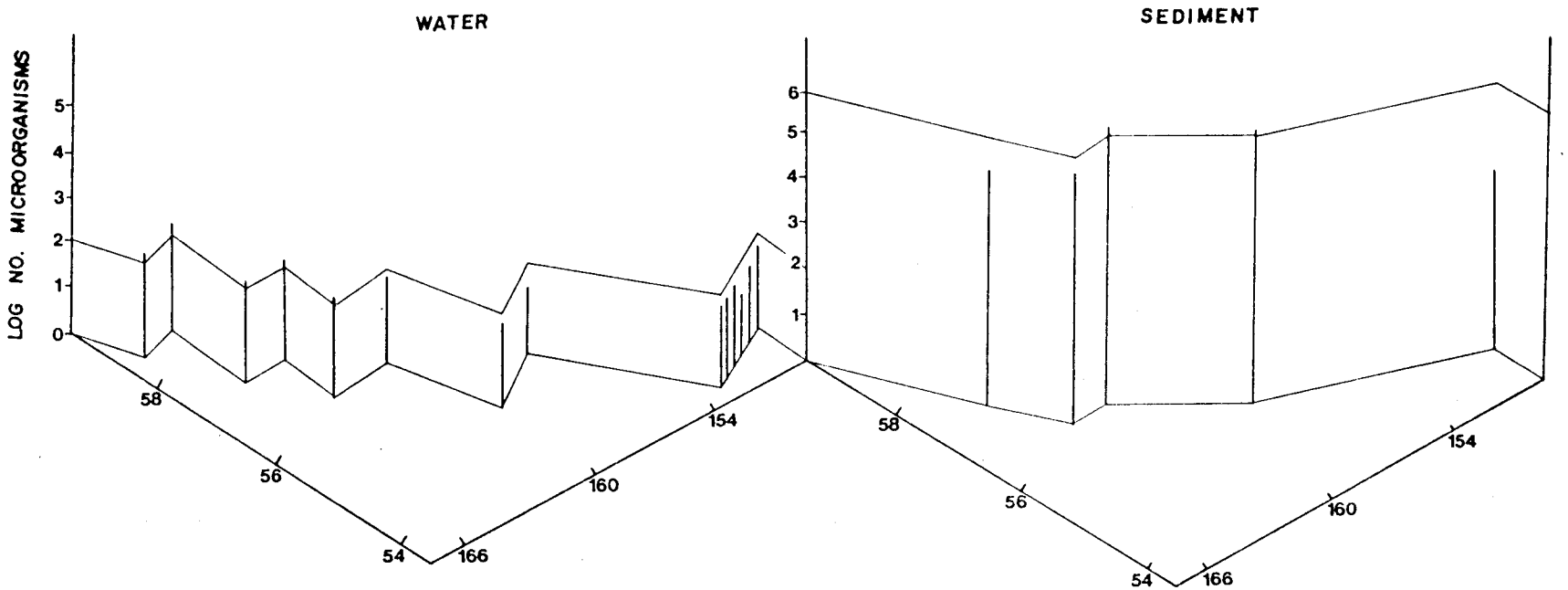

Fig. 5. Viable bacterial populations enumerated at 4°C from Northwest Gulf of Alaska during summer 1975.

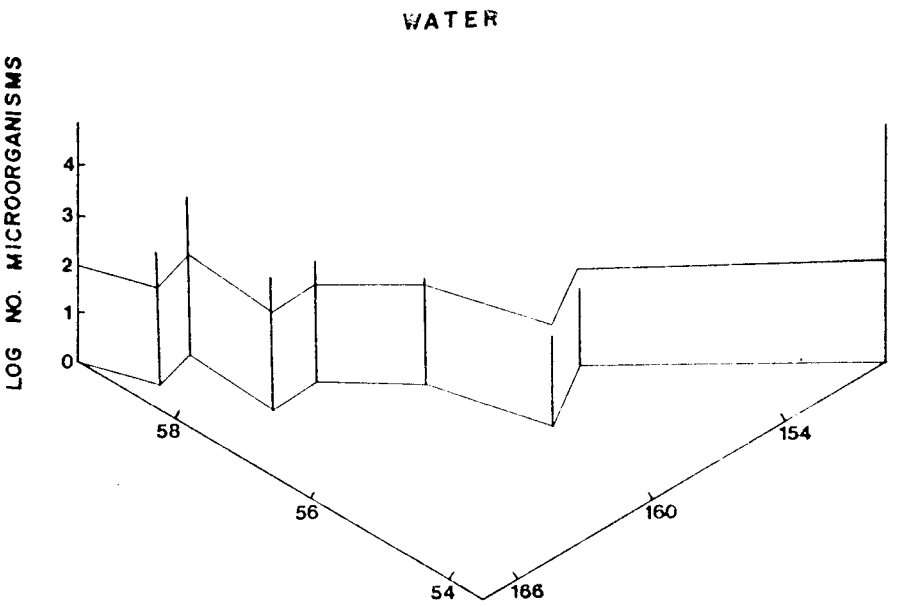

Fig. 6.
Viable bacterial populations enumerated at 20 C from Northwest Gulf of Alaska during Summer 1975.

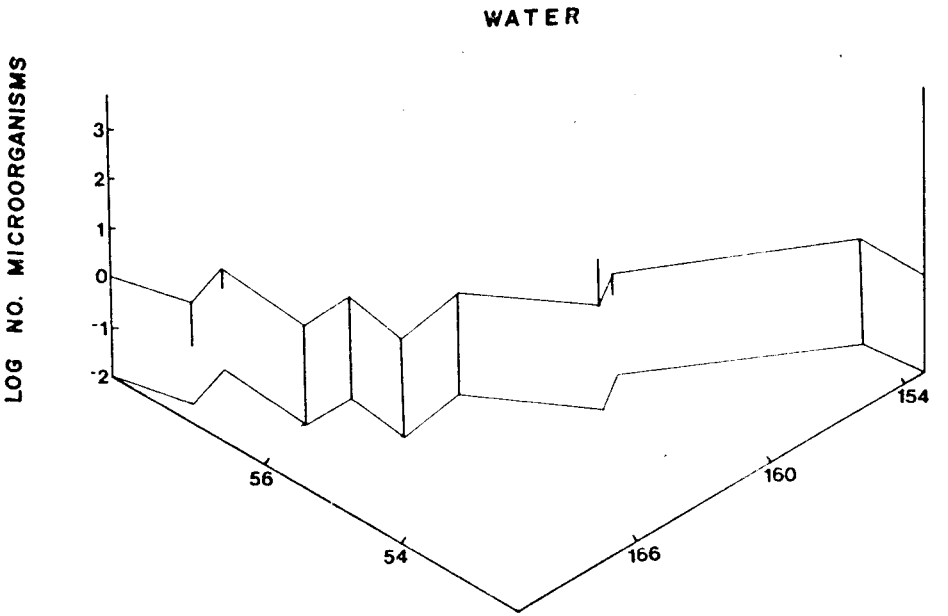

Fig. 7. Counts of hudrocarbon degraders in Northwest Gulf of Alaska during summer 1975.

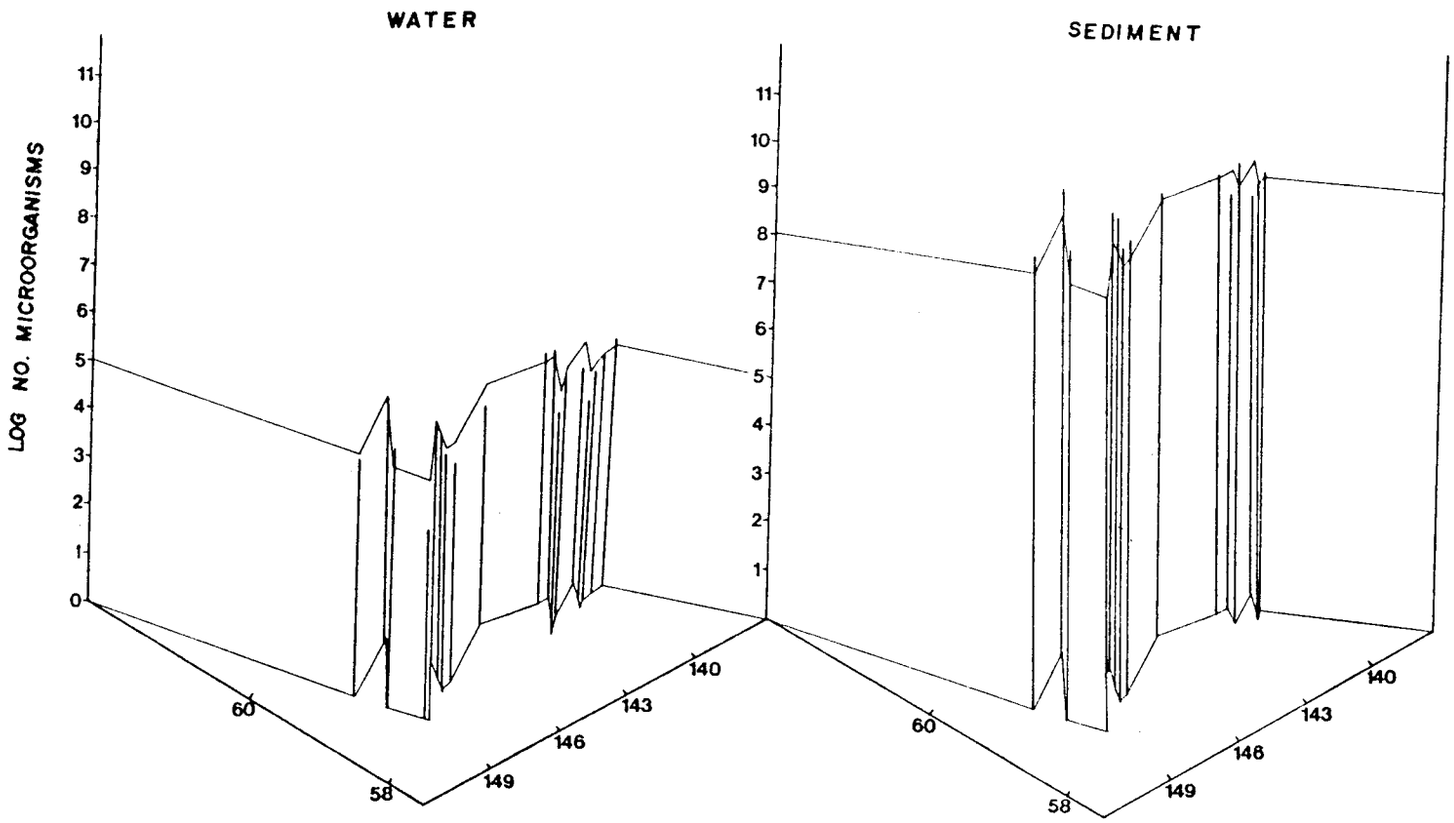

Fig. 8. Direct counts of bacteria in Northeast Gulf of Alaska during winter 1976

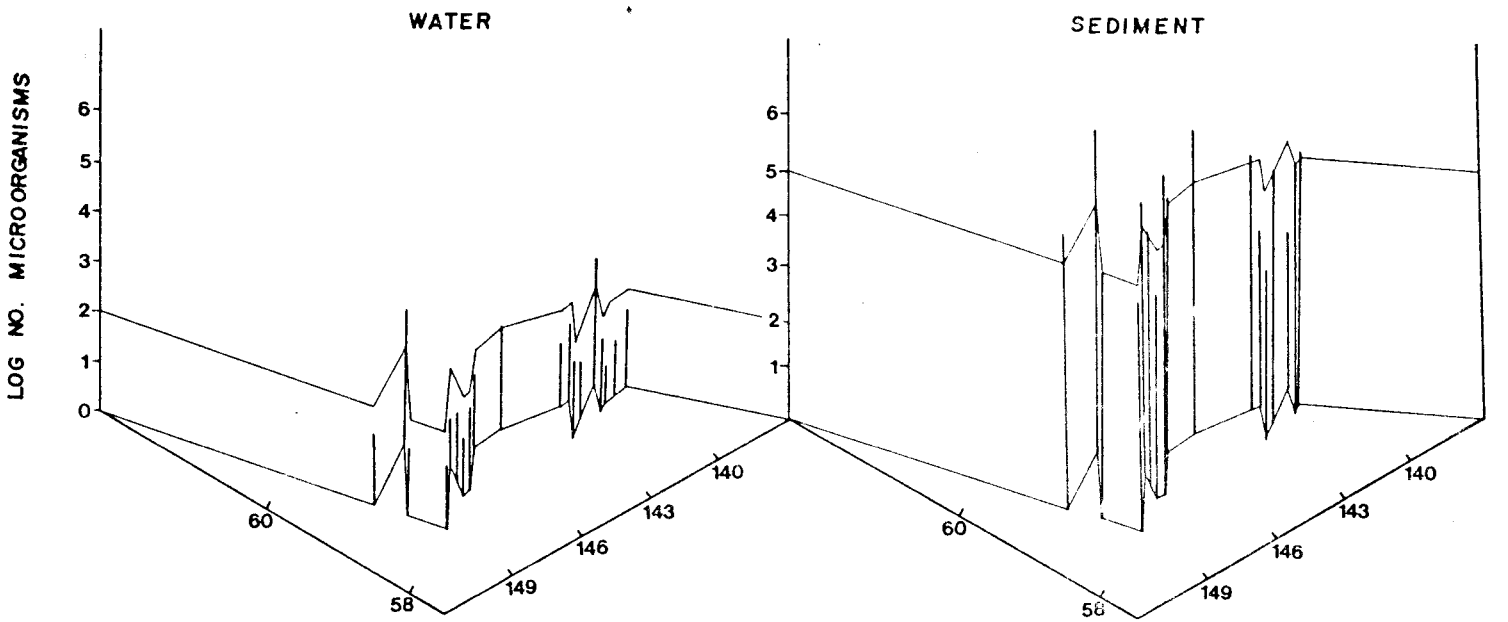

Fig. 9.
Viable bacterial populations enumerated at 4 C from Northeast Gulf of Alaska during Winter 1976.

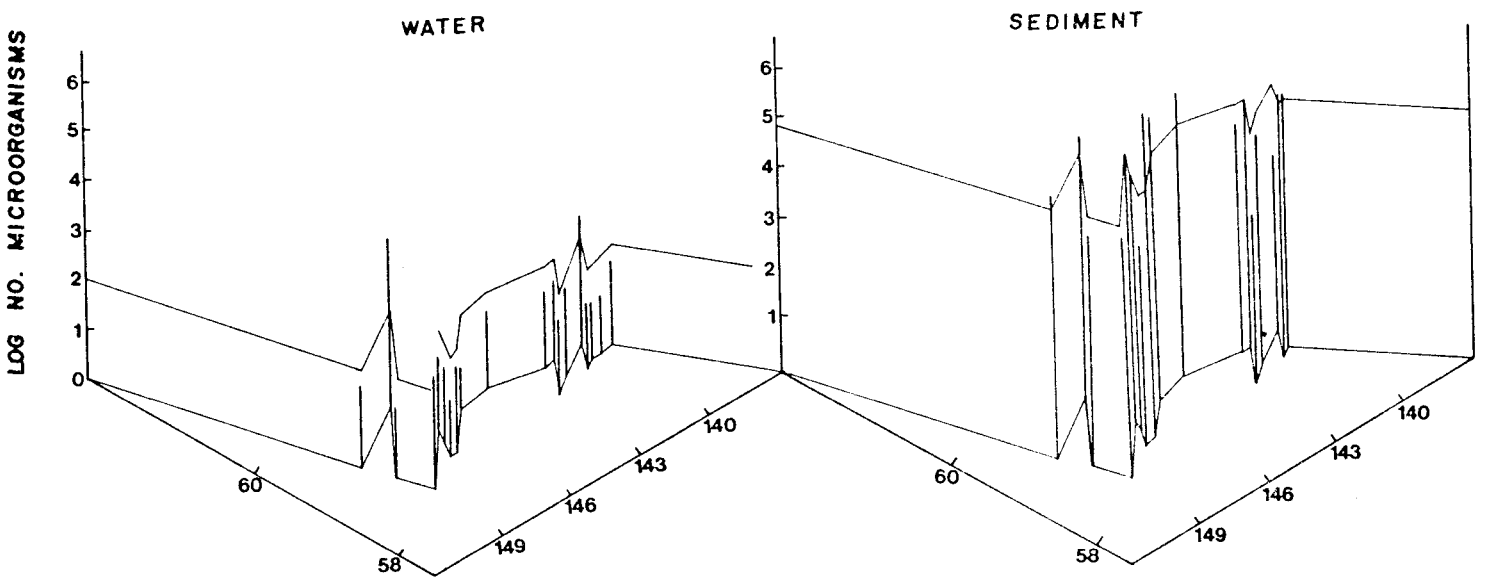

Fig. 10.
Viable bacterial populations enumerated at 20 C from Northeast Gulf of Alaska during Winter 1976.

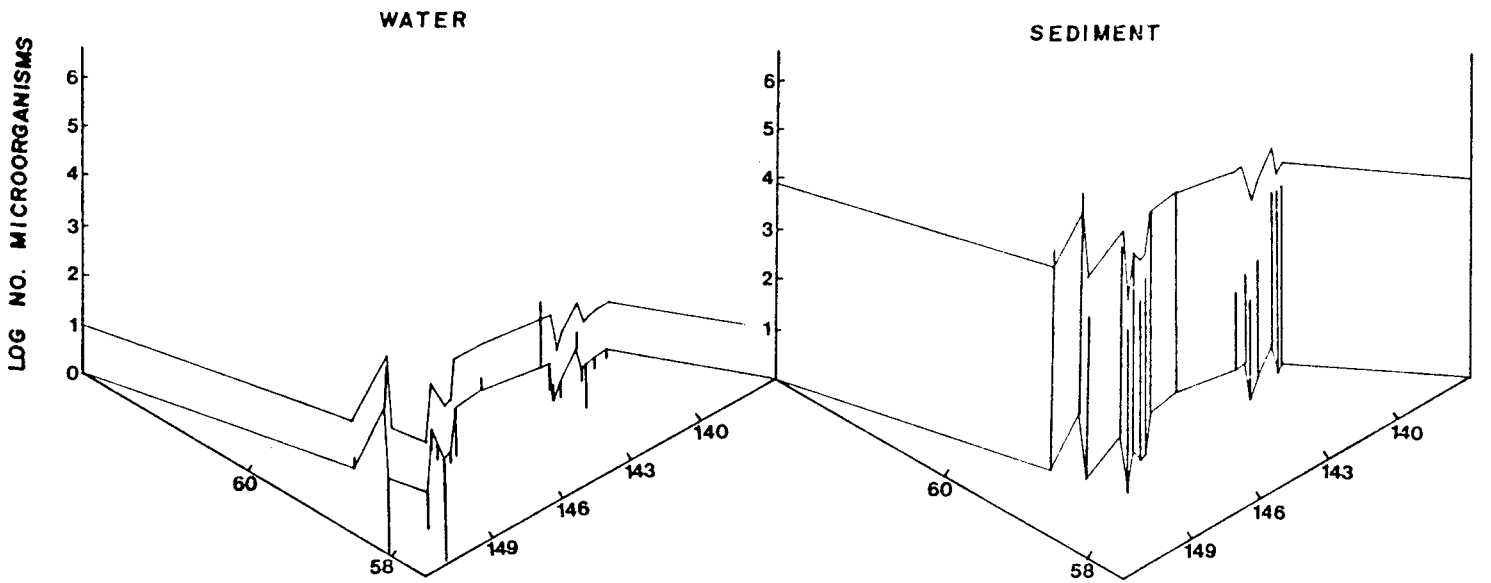

Fig. 11.
Counts of Vibrio spp. in
Northeast Gulf of Alaska during
Winter 1976 enumerated at 4 C.

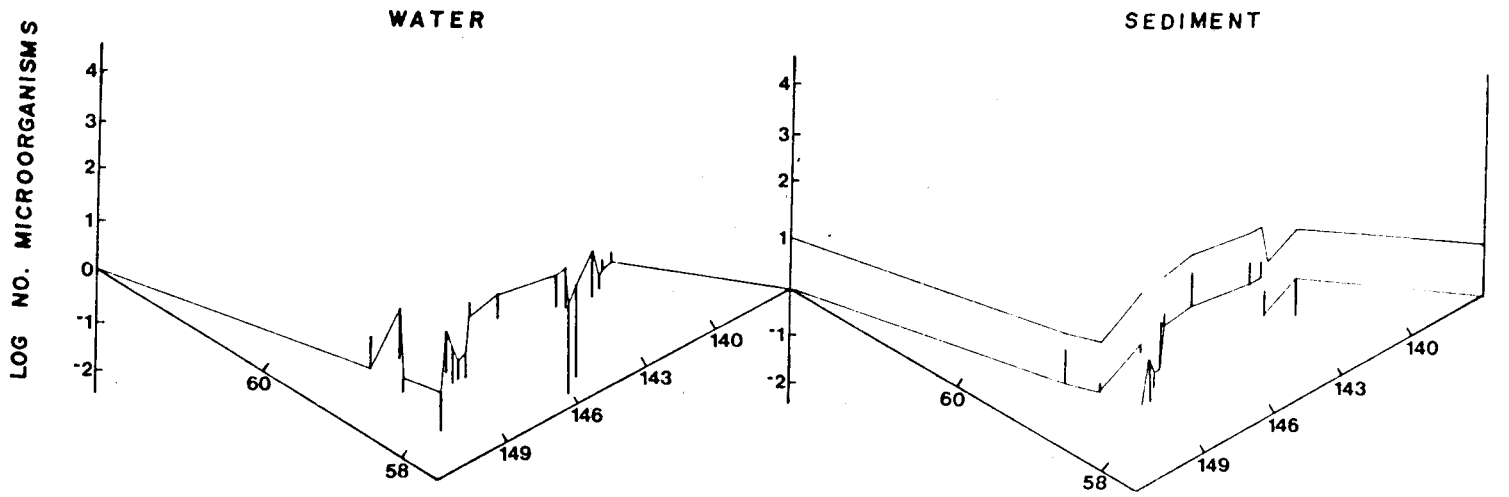

Fig. 12.
Counts of hydrocarbon degraders
in Northeast Gulf of Alaska
during Winter 1976.

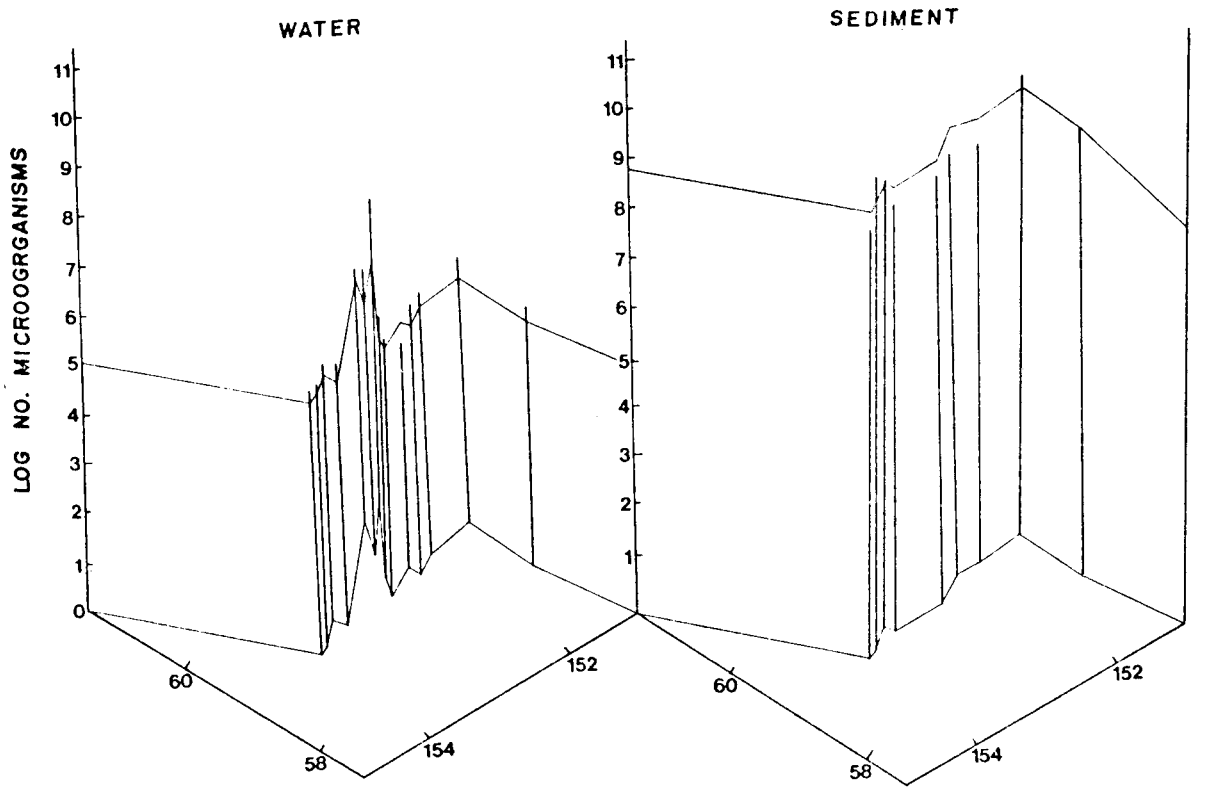

Fig. 13.
Direct counts of bacteria in Cook
Inlet during Summer 1976.

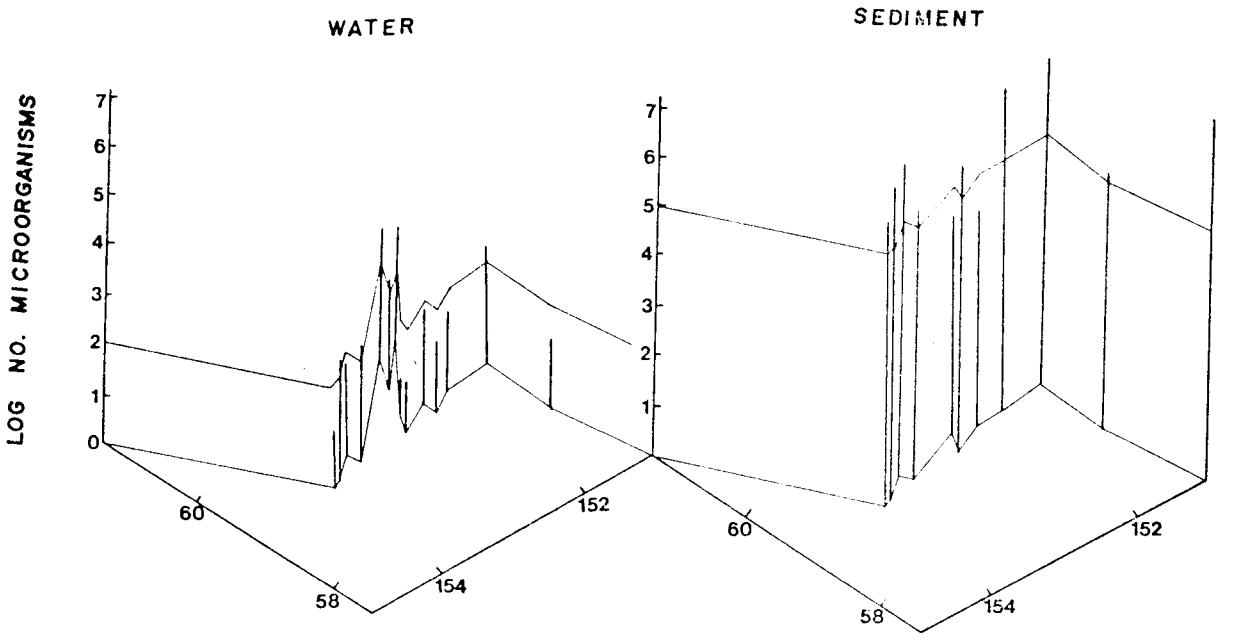

Fig. 14.
Viable bacterial populations enumerated at 4 C from Cook Inlet during Summer 1976.

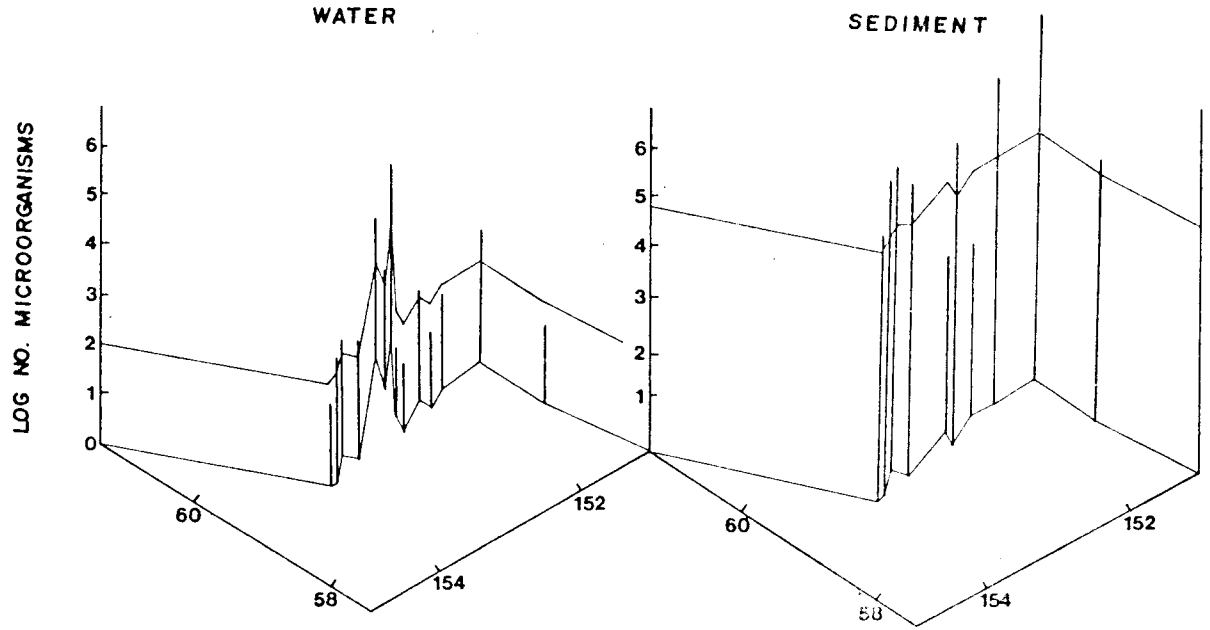

Fig. 15.
Viable bacterial populations enumerated at 20 C from Cook Inlet during Summer 1976.

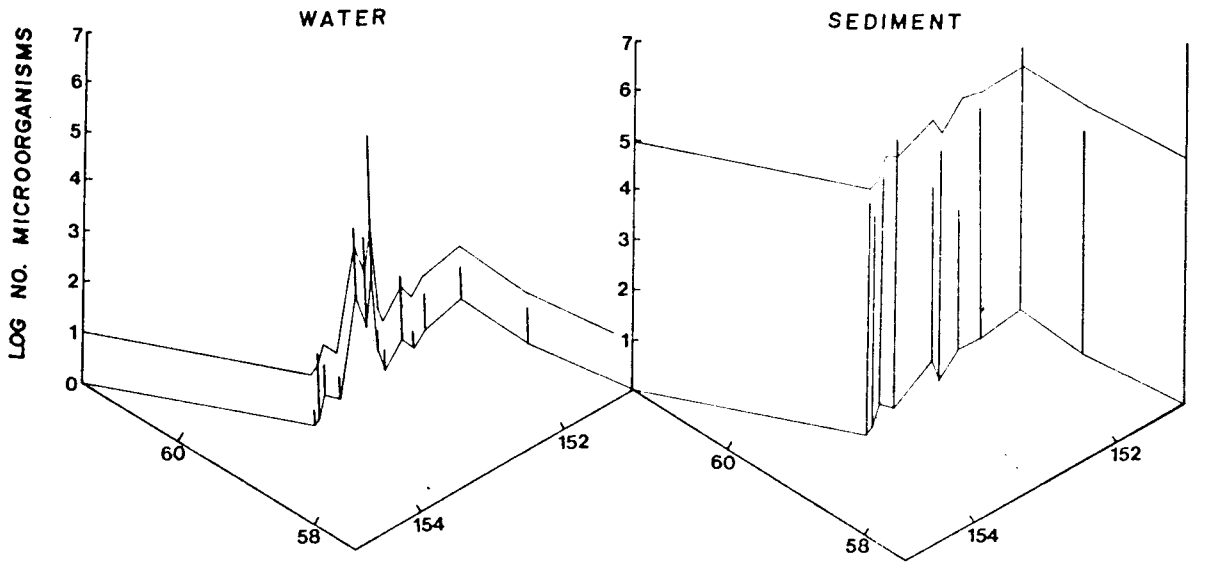

Fig. 16.
Counts of Vibrio spp. in Cook Inlet during Summer 1976 enumerated at 4 C.

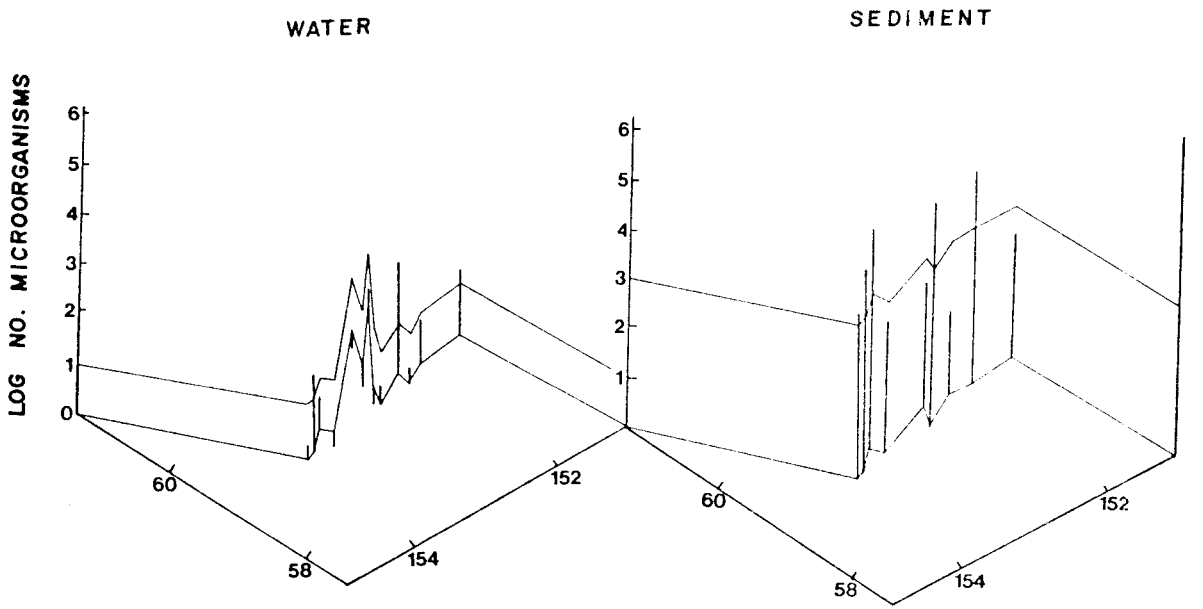

Fig. 17.
Counts of hydrocarbon degraders
in Cook Inlet during Summer 1976.

Spacial and Seasonal Characteristics of Bacterial Populations

The physiological and nutritional characteristics of bacterial populations isolated at 4 and 20 C from water and sediment samples collected during summer 1975 in the Northwest Gulf of Alaska are shown in Figures 18-23. Both the 4 and 20 C populations isolated from water showed a pattern of utilization of fewer compounds and a lower percentage of the population utilizing the compounds that were utilized, in the western stations nearer the Aleutians than in the eastern stations nearer Kodiak (Figs. 18 and 19). For example, at station 156 very few amino acids, carbohydrates and carboxcylic acids were utilized. As examples, at Station 156 glucose was utilized by under 30% of the population and aspartate by under 35% of the population isolated at 4 C compared to 98% for glucose and 98% aspartate at Station 101. The shift in pattern occurs at about the 130 series transect with Stations 137, 145 and 156 showing the lowest substrate utilization. Some hydrocarbons were utilized by isolates from water at all stations, except at Station 106. The physiological tolerance ranges of the water isolates showed some variation between samples, but no clear geographic pattern (Fig. 22). As with the water isolates, fewer substrates were utilized by a smaller percentage of the population in the western stations compared to the eastern stations sampled (Figs. 20 and 21). The 130 transect series was the westernmost transect from which sediment samples were obtained. Hydrocarbons were utilized by a limited percentage of the sediment population at each

Fig. 18.

Patterns of substrate utilization for 4 C water isolates in Northwest Gulf of Alaska during Summer 1975.

Carbohydrates

a: L-arabinose
b: D-ribose
c: D-xylose
d: L-rhamnose
e: D-fructose
f: D-galactose
g: D-glucose
h: D-mannose
i: L-sorbose
j: salicin
k: cellobiose
l: lactose
m: maltose
n: sucrose
o: trehalose
p: raffinose

Alcohols

a: 1-butanol
b: ethanol
c: 1-propanol
d: 2-propanol
e: 1,2-propanedrol
f: glycerol
g: D-arabitol
h: dulcitol
i: D-mannitol
j: D-sorbitol
k: meso-inositol
l: phenol
m: 2-phenylethanol

Fatty Acids

a: acetic acid
b: butyric acid
c: caproic acid
d: caprylic acid

Fatty Acids (cont'd.)

e: lauric acid
f: propionic acid
g: valeric acid
h: glutaric acid
i: malonic acid
j: succinic acid
k: oleic acid
l: fumaric acid
m: itaconic acid
n: DL-glyceric acid
o: β-hydroxybutyric acid
p: DL-lactic acid
q: L(+) tataric acid
r: citric acid
s: 2-ketogluconic acid
t: pyruvic acid
u: α-ketoglutaric acid
v: benzoic acid
w: meta-hydroxybenzoic acid
x: para-hydroxybenzoic acid
y: ascorbic acid
z: galacuronic acid
aa: D-gluconic acid
bb: ortho-hydroxybenzoic acid
cc: stearic acid

Amino Acids

a: L-alanine
b: gamma-aminobutyric acid
c: L-arginine
d: L-asparagine
e: L-aspartic acid
f: L-cysteine
g: L-cystine
h: L-glutamic acid
i: glycine
j: L-leucine
k: L-iso-leucine

Fig. 18 (cont'd.)

Amino Acids (cont'd.)

l: L-lysine
m: L-methionine
n: L-ornithine
o: L-phenylalanine
p: L-proline
q: L-serine
r: L-threonine
s: L-tryptophan
t: L-tyrosine
u: L-valine
v: L-aspartic acid as N source
w: L-glutamic acid as N source
x: L-lysine as N source
y: L-tryptophan as N source

Amines

a: α-amylamine
b: ethanolamine
c: histamine
d: putrescine
e: ethanolamine as N source
f: N-acetylglucoseamine
g: guanine
h: thymine

Hydrocarbons

a: n-hexadecane
b: n-pentadecane
c: 2-methylnaphthalene
d: 1-methylnaphthalene
e: phenyldecane
f: pristane
g: pentadecyclcyclohexane
h: 2,2,4,4,6,8,8-heptamethylnonane

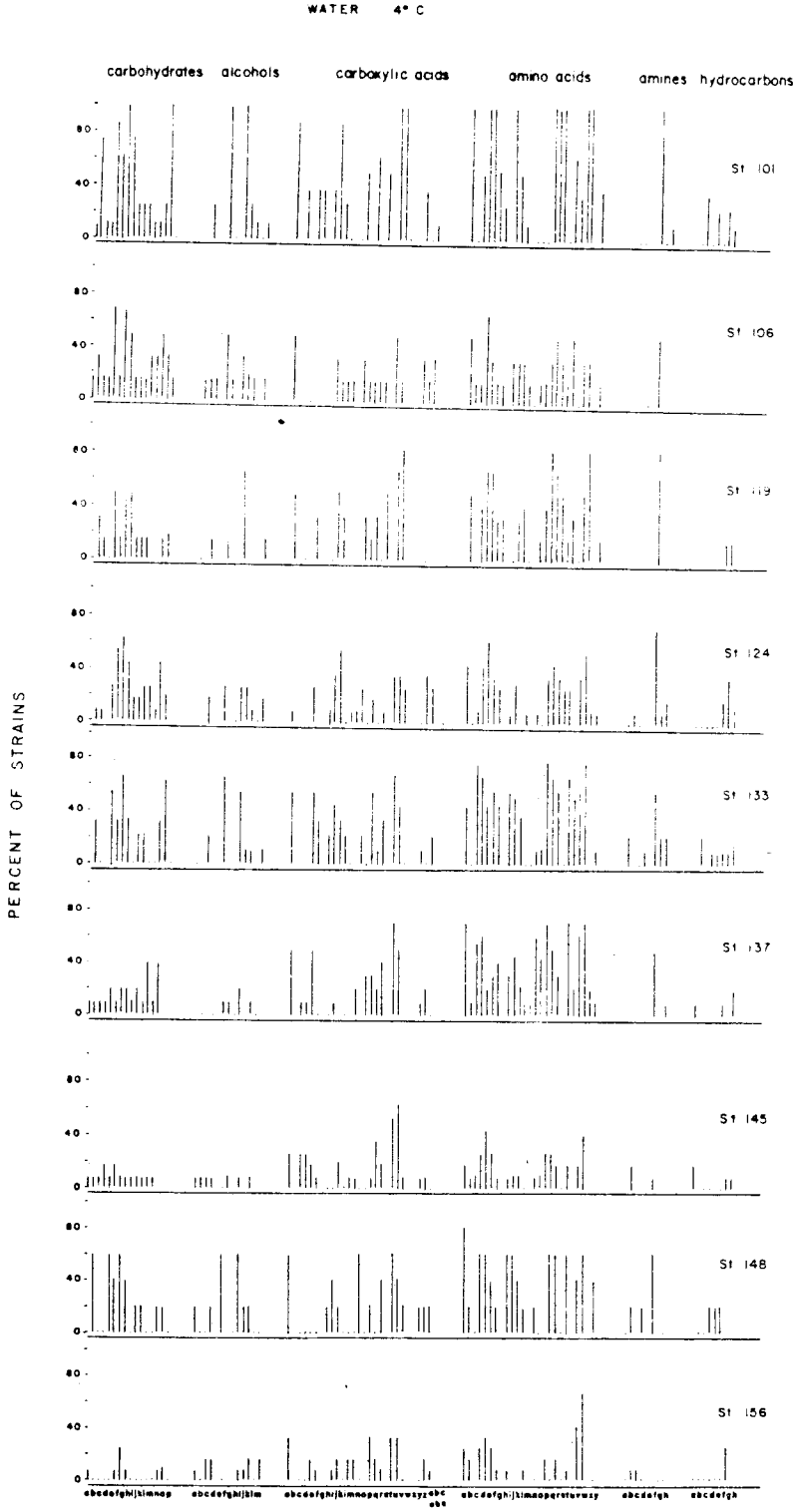
WATER 4° C
carbohydrates
alcohols
carboxylic acids
amino acids
amines
hydrocarbons
PERCENT OF STRAINS
St 101
St 106
St 119
St 124
St 133
St 137
St 145
St 148
St 156
80
40
0

Fig. 18

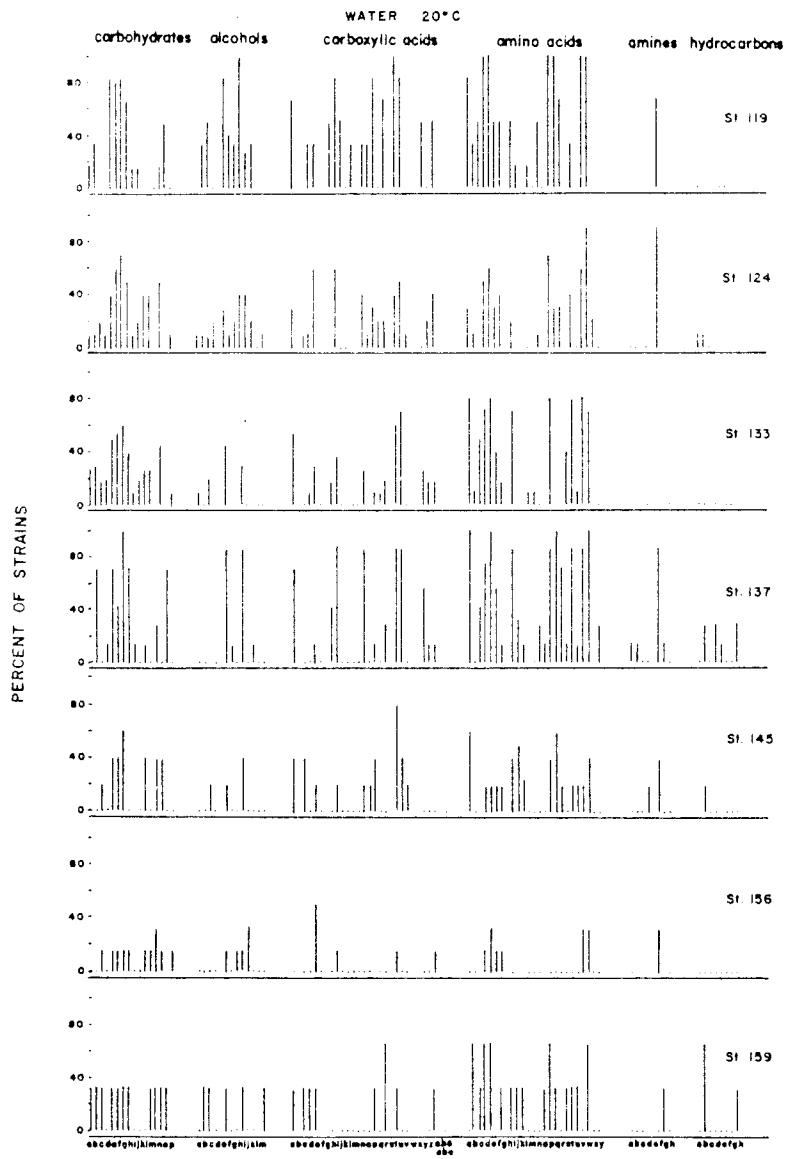

Fig. 19.
Patterns of substrate utilization for 20 C water isolates in Northwest Gulf of Alaska during Summer 1975. (See Fig. 18 for explanation of symbols)

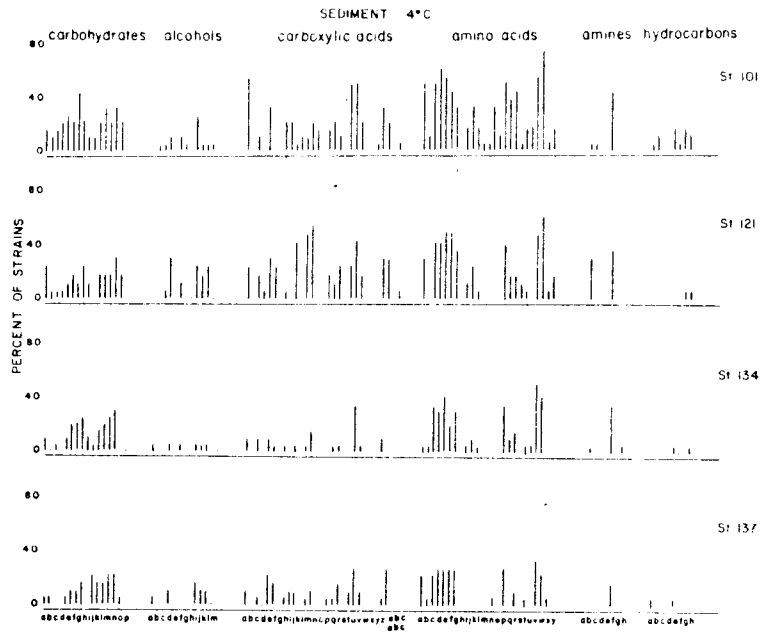

Fig. 20.
Patterns of substrate utilization for 4C sedimentisolates in Northwest Gulf of Alaska during Summer 1975. (See Fig. 18 for explanation of symbols)

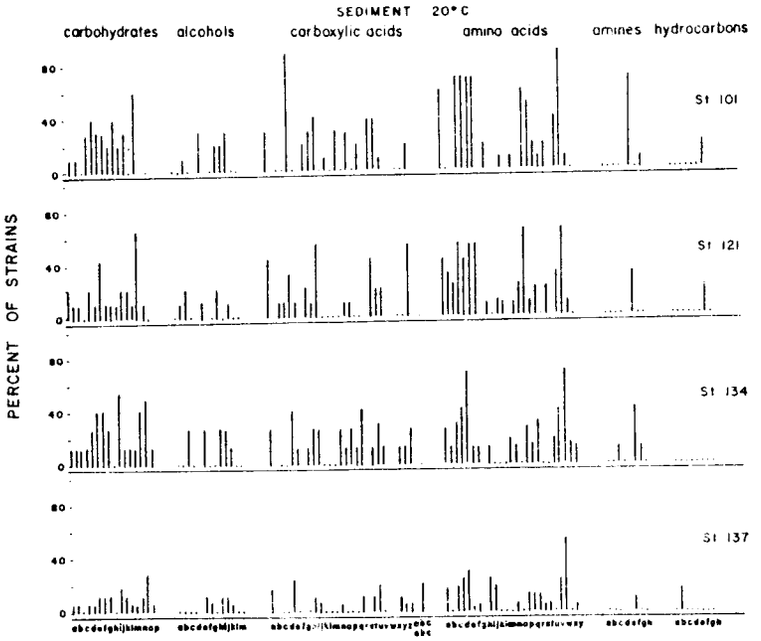

Fig. 21.
Patterns of substrate utilization for 20 C sediment isolates in Northwest Gulf of Alaska during Summer 1975. (See Fig. 18 for explanation of symbols)

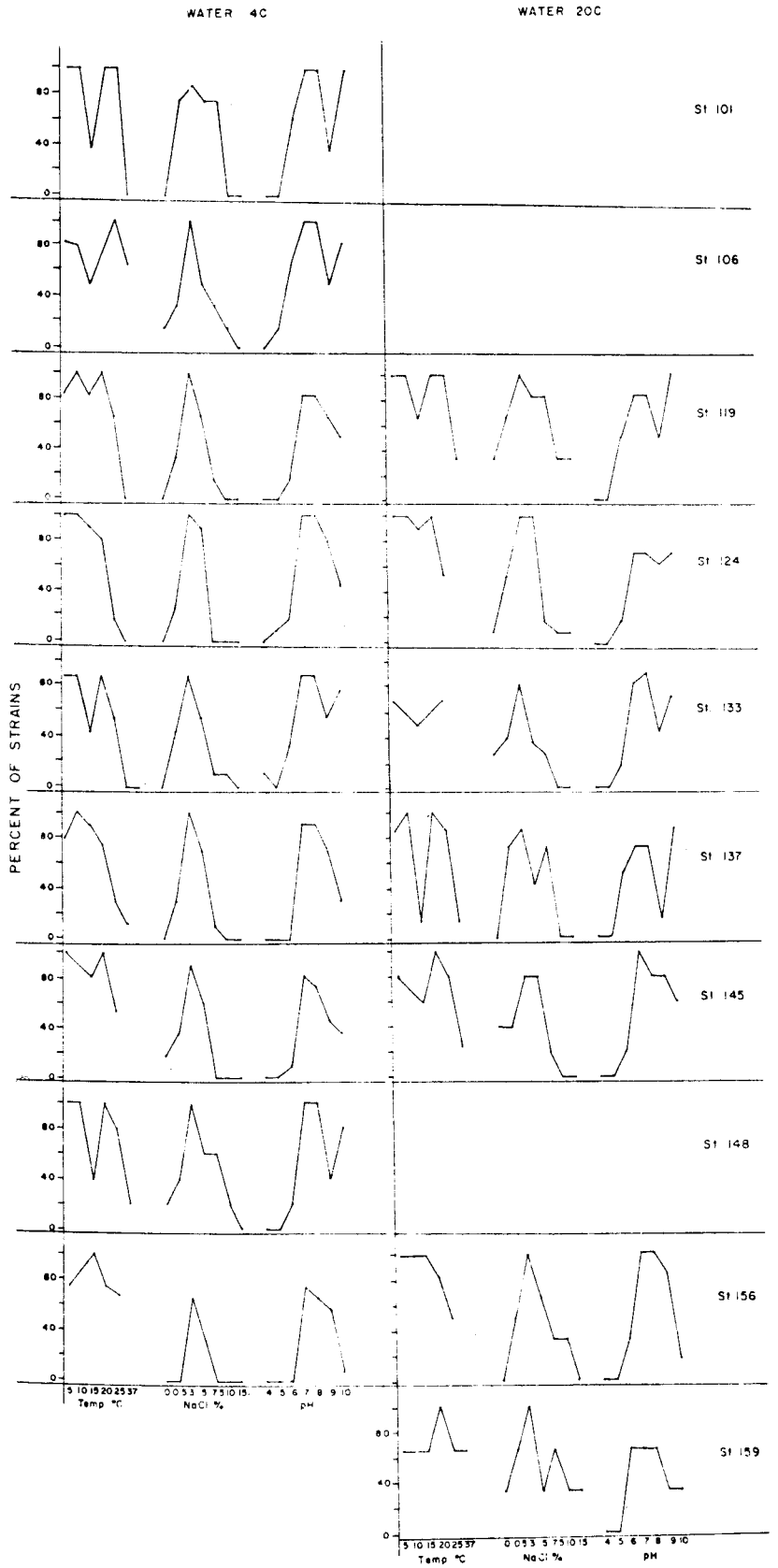

Fig. 22 Physiological tolerance ranges for water isolates from Northwest Gulf of Alaska during Summer 1975

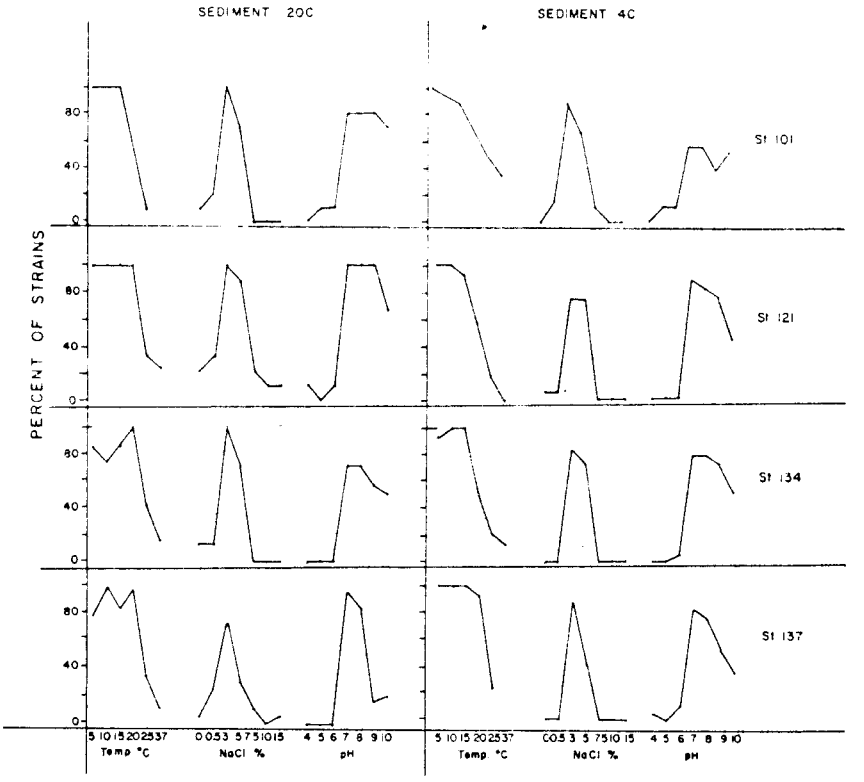

Fig. 23.
Physiological tolerance ranges for sediment isolates from Northwest Gulf of Alaska during Summer 1975.

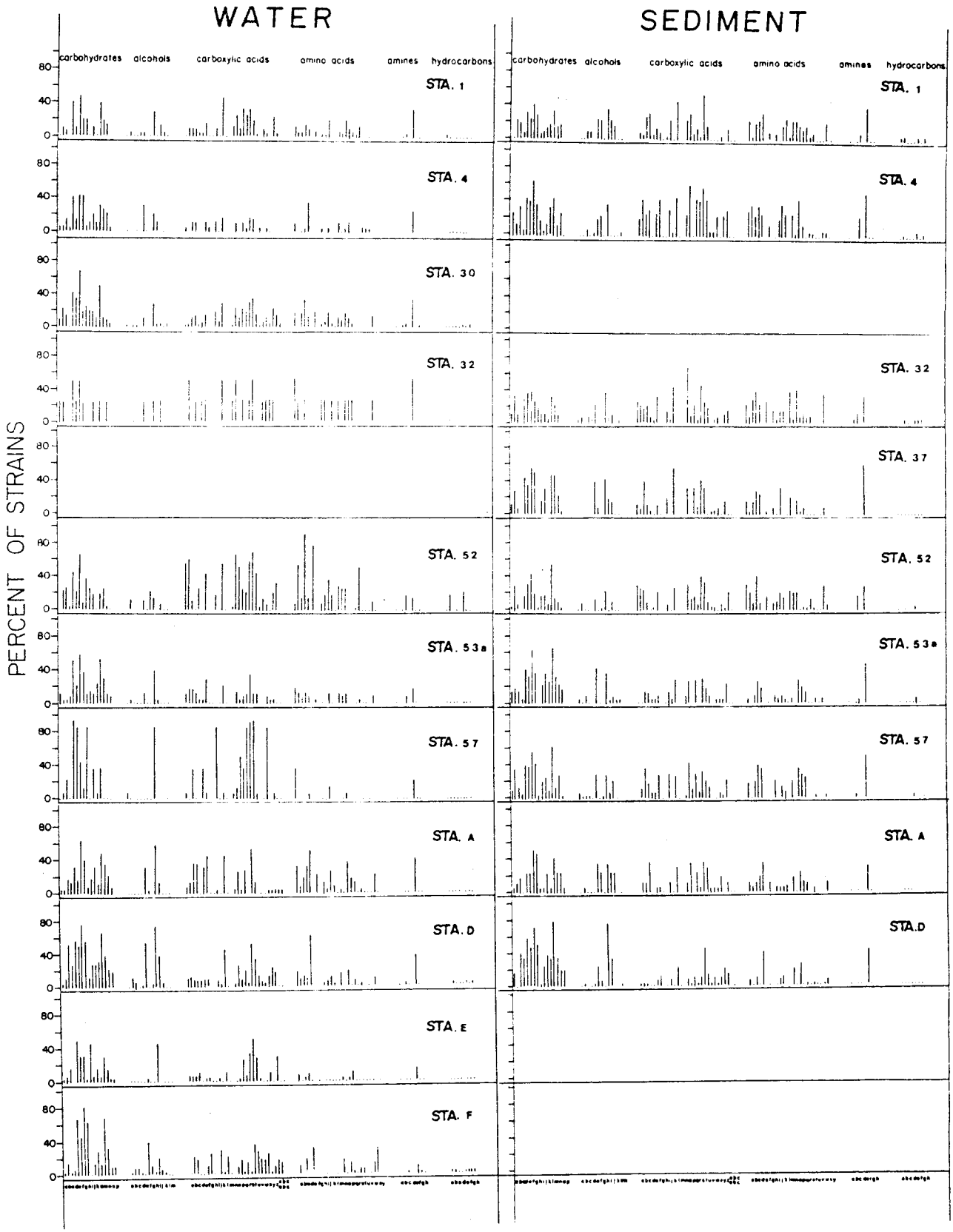

Fig. 24. Patterns of substrate utilization for water and sediment isolates from Northeast Gulf of Alaska during Winter 1976. (See Fig. 18 for explanation of symbols)

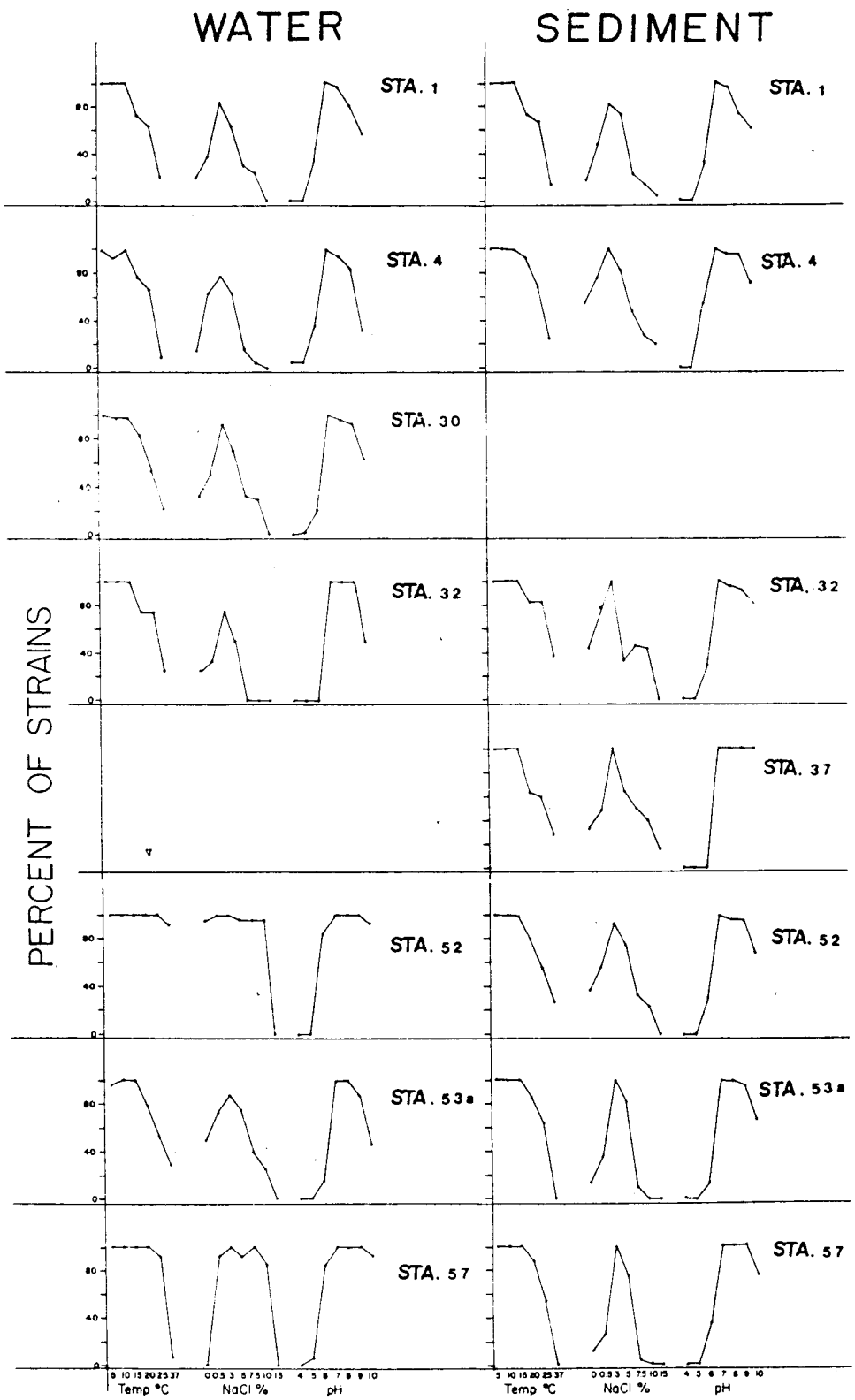

Fig. 25. Physiological tolerance ranges for 4 C water and sediment isolates from Northeast Gulf of Alaska during Winter 1976.

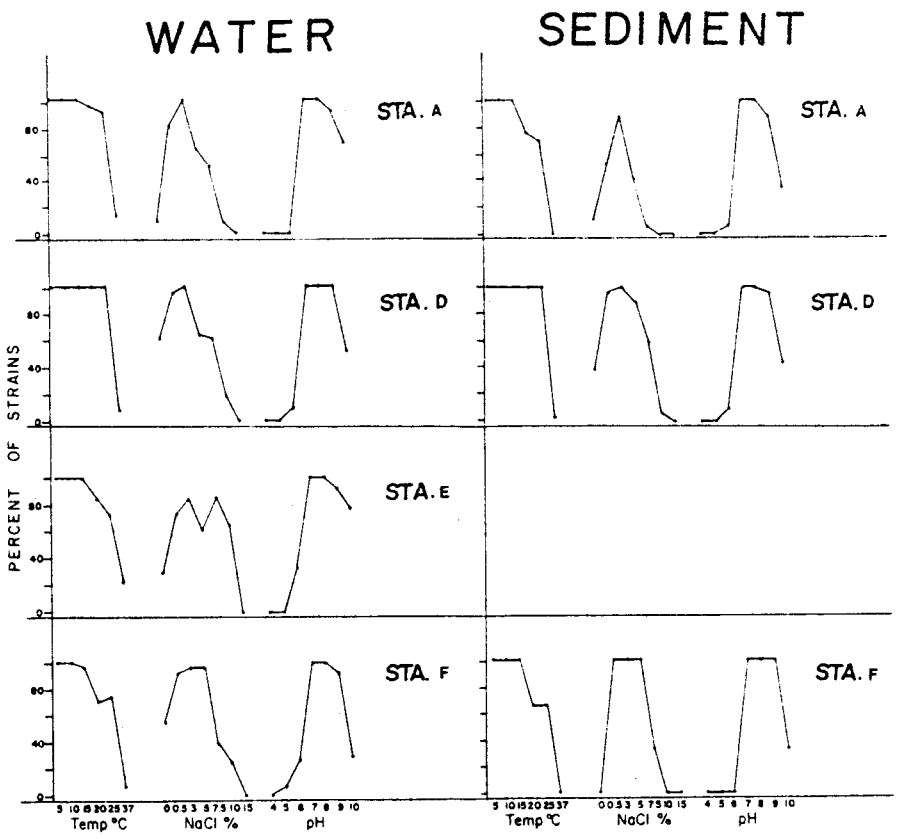

Fig. 26. Physiological tolerance ranges for 4 C intertidal water and beach sediment isolates from Northeast Gulf of Alaska during Winter 1976.

station. As with the isolates from water, there was no geographic pattern to the physiological tolerance ranges of sediment isolates (Fig. 23).

Compared to populations in water, the sediment population generally had a lower maximal upper temperature for growth. Less than 40% of the sediment population could generally grow at 25 C even when the population had been isolated at 20 C. In water greater than 80% of the population isolated at 20 C could generally still grow at 25 C. The salt tolerance range was generally very narrow. The population generally could not tolerate a shift from 3% NaCl to either 0.5% or 5% NaCl. The pH tolerance range was generally between pH 7 and 9 or pH 7-10.

The nutritional utilization capabilities and physiological tolerance ranges for bacterial populations isolated during winter in the northeast Gulf of Alaska during winter 1976 are shown in Figures 24-26. Generally similar patterns of nutritional capability were seen for all populations isolated from benthic sediment (Fig. 24). There was more variability in the substrate utilization capabilities of water populations than sediment populations. Generally a lower substrate utilization pattern was found for intertidal water samples collected in Yakutat Bay than from intertidal waters near Seward or Kodiak.

Except for Station 1 near Seward, the substrate utilization capability was lower in water collected west of longitude 146° (Stations 4, 53a, 57) than east of that longitude (stations 52, 30, 32). The greatest substrate utilization pattern was seen in water at Station 52.

The water isolates at Station 52 also showed a much wider temperature and salt tolerance range than most other Stations (Fig. 25). Organisms from water at Station 57 also showed a wide temperature and salt tolerance range. At other stations few water or sediment isolates could grow above 20 C and the salt tolerance range was very narrow with an optimal salt concentration of about 3%. Most water and sediment isolates could grow in the pH range 7-9. Beach sediment and intertidal water isolates from Kodiak and Hinchinbrook Islands had a wider temperature but narrower salt tolerance range than isolates from beach areas of Seward or Yakutat Bay (Fig. 26). The tolerance ranges were generally similar for populations from intertidal water and sediment at the same station.

Cluster Analyses

The results of cluster analyses are shown as simplified dendrograms in Figures 27-31. Taxonomic groups were defined at approximately the 70% phenotypic similarity level. Some clusters had subgroups of higher similarity.

The cluster analysis of the populations isolated at 4 and 20 C from water and sediment in the Northwest Gulf of Alaska during summer showed 19 taxonomic groups (Fig. 27). Eight of these groups had only two members. Forty-nine percent of the organisms tested fall into one of the taxonomic groups.

The cluster analysis of the populations isolated at 4 C from water in the Northeast Gulf of Alaska during winter showed 30 taxonomic

groups (Fig. 28). Thirteen of these groups contained only 2 members. Fifty-six percent of the organisms tested fell into one of the taxonomic groups.

The cluster analysis of the population isolated at 4 C from sediment in the Northeast Gulf of Alaska showed 26 taxonomic groups (Fig. 29). Sixteen of these groups had only 2 members. Forty-three percent of the organisms fell into one of the taxonomic clusters.

The cluster analysis of the population isolated at 20 C from water in the Northeast Gulf of Alaska showed 18 clusters (Fig. 30). Eight of the clusters had only 2 members. Fifty-two percent of the organisms tested fell into one of the taxonomic clusters.

The cluster analysis of the population isolated at 20 C from sediment in the Northeast Gulf of Alaska showed 12 clusters (Fig. 31). Six of the clusters had only 2 members. Forty-nine percent of the organisms fell into one of the clusters.

Each of the populations examined was characterized by high diversity with only about half of the members of each populations showing significant similarity to other members. There was generally a lack of homogeneous large taxonomic grouping.

Description of Major Taxonomic Groupings

A general summary of the features of the major clusters found for the Northwest Gulf of Alaska populations and the populations isolated at 4 C from the Northeast Gulf of Alaska is described below.

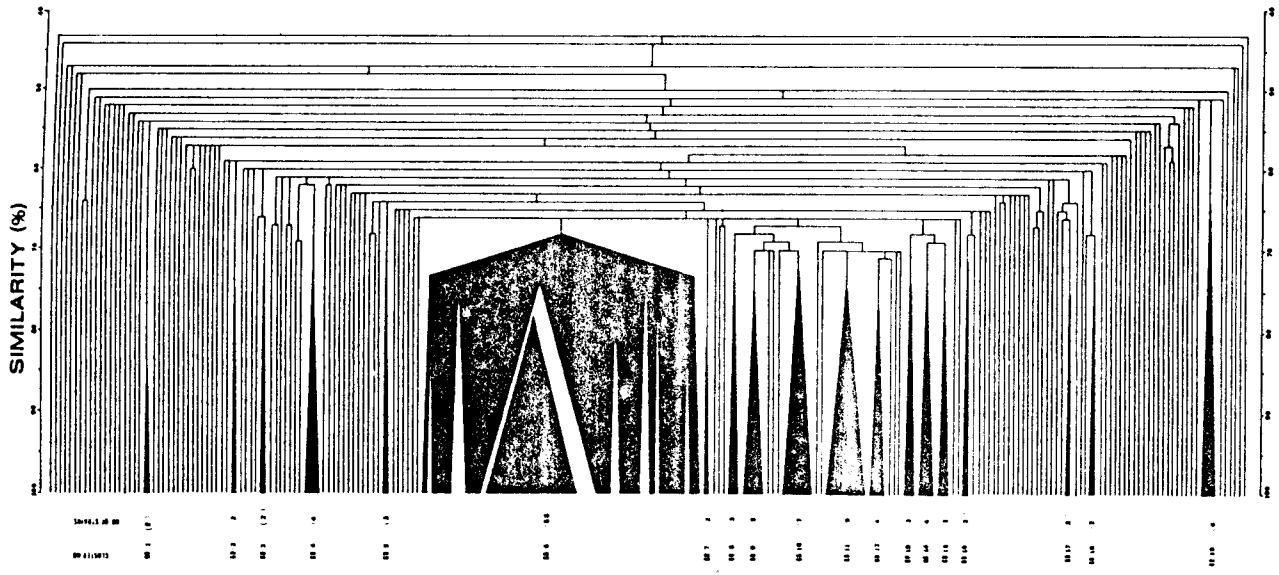

Fig. 27.
Simplified dendrogram for Northwest Gulf of Alaska isolates cluster analysis.

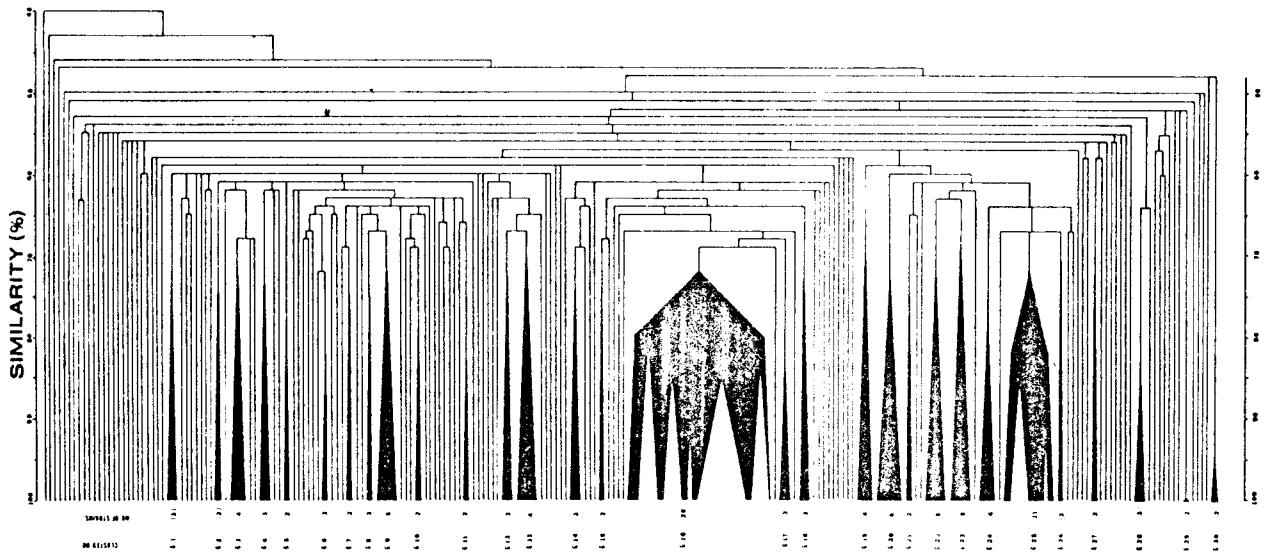

Fig. 28.
Simplified dendrogram for 4 C
Northeast Gulf of Alaska water
isolates cluster analysis.

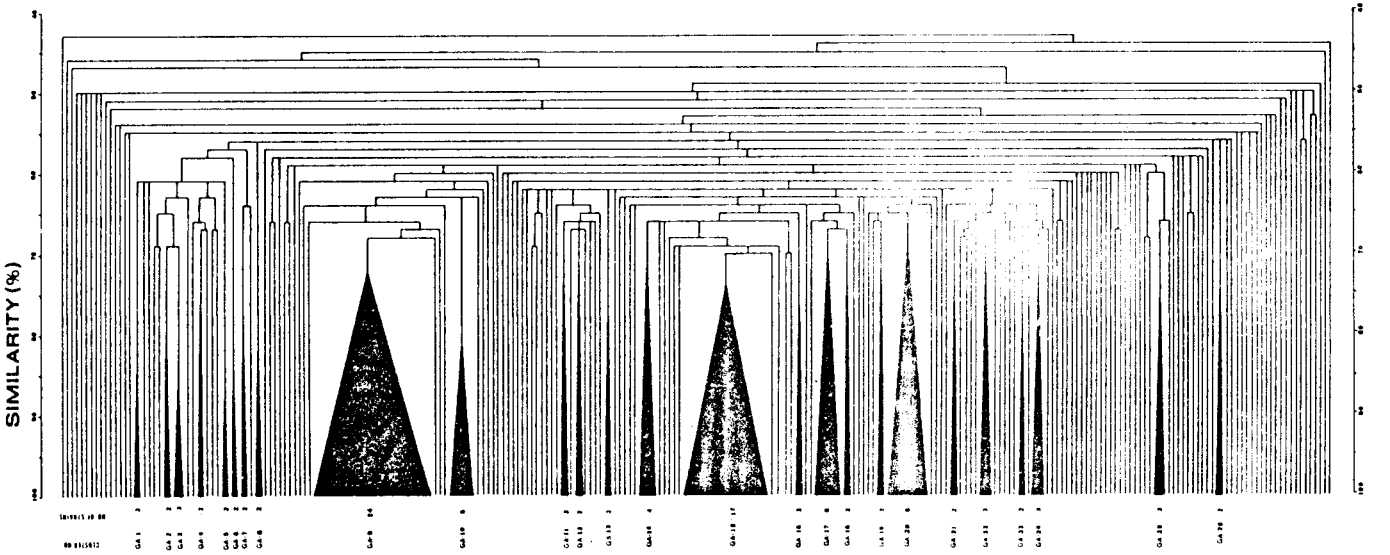

Fig. 29.
Simplified dendrogram for 4 C Northeast Gulf of Alaska sediment isolates cluster analysis.

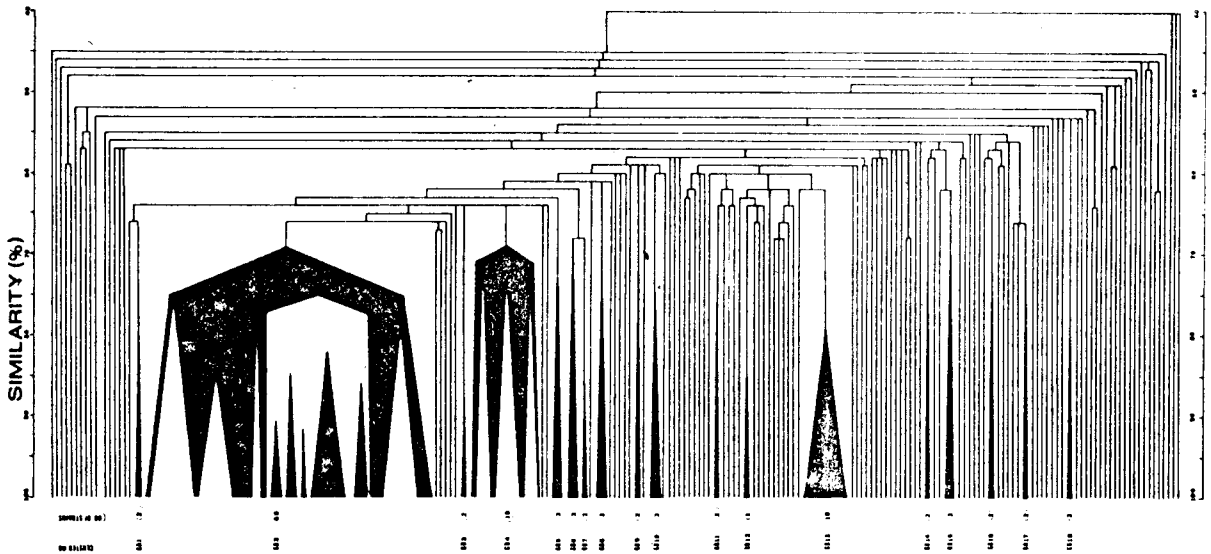

Fig. 30.
Simplified dendrogram for 20 C
Northeast Gulf of Alaska water
isolates cluster analysis.

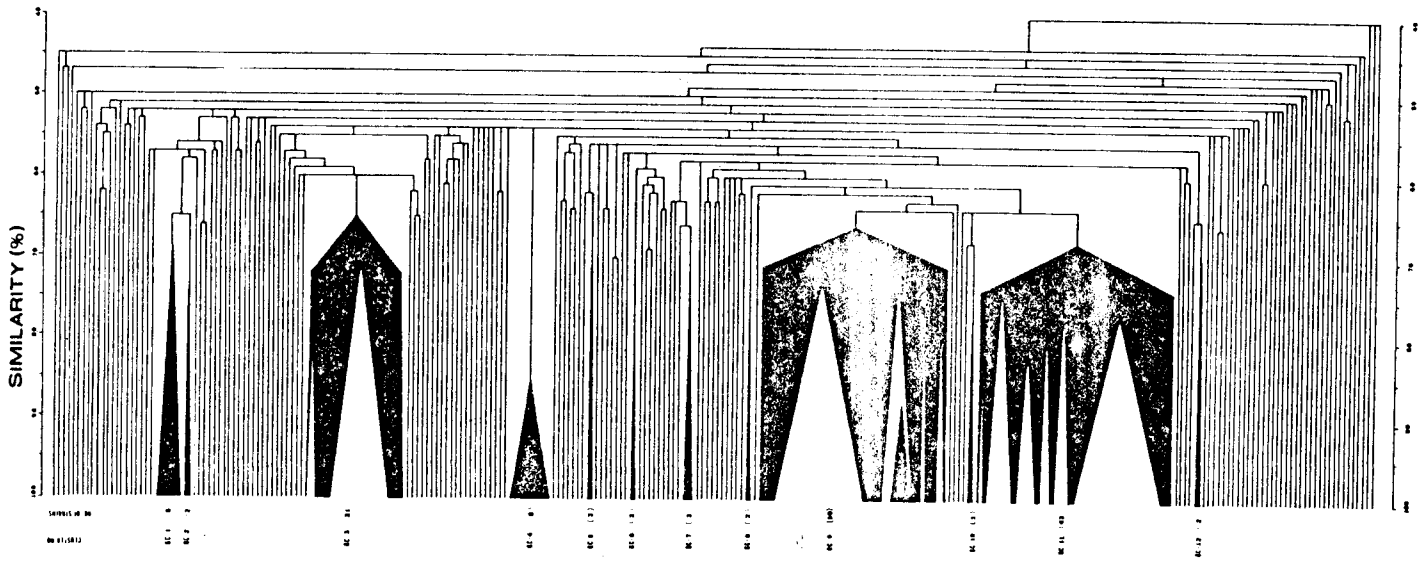

Fig. 31.
Simplified dendrogram for 20 C
Northeast Gulf of Alaska sediment
isolates cluster analysis.

Northwest Gulf of Alaska - Summer 1975

Details of the nutritional and physiological characteristics of 4 major groups and 1 major subgroup are shown in Figures 32 and 33 respectively.

Group GD6:

(i) Morphological characteristics: Gram-negative rods occurring singly. 85% are motile. Colony gray, glistening and smooth.

(ii) Physiological characteristics: Grow at temperature of 5-25 C. Optimal salt concentration 3%. Grows at pH 7-9.

(iii) Nutritional characteristics: Utilize many carbohydrates, alcohols, carboxylic acids and amino acids. Also a few utilize hydrocarbons. The following substrates were utilized by more than 80% of the members of this group: Pyruvate, alanine, asparagine, glutamate, proline, serine, and N-acetyl-glucosamine.

(iv) Distribution: Found at all stations except the westernmost stations 156 and 159. Found mostly in water but also some found in sediment.

Subgroup GD6a:

(i) Morphological characteristics: Gram-negative rods occurring singly. Motile, gray colony.

(ii) Physiological characteristics: Grow at 5-25 C. Optimal salt concentration 0.5% NaCl. Grow at pH 7-9.

(iii) Nutritional characteristics: Extensively utilizes all substrate classes including hydrocarbons. The following substrates were utilized by more than 80% of the members of this group: ribose, fructose, glucose, mannose, trehalose, arabitol, sorbitol, acetate, alanine, asparagine, aspartate, glutamate, glycine, proline, serine, threonine, tyrosine, ethanolamine, guanine, thymine, n-hexadecane, methylnaphthalene, phenyldecane.

(iv) Distribution: Found only in sediment, mostly at station 134. Isolated only at 4 C.

Group GD10:

(i) Morphological characteristics: Gram-negative rods occurring singly. Nonmotile. Colonies gray, glistening and smooth.

(ii) Physiological characteristics: Grow at temperatures of 5-15 C. Optimal salinity 3% NaCl. Optimum pH 7-8.

(iii) Nutritional characteristics: Do not utilize any of the substrates tested. May require complex growth factors.

(iv) Distribution: Found in water and sediment at several stations. Isolated only at 4 C.

Group GD11:

(i) Morphological characteristics: Encapsulated gram-negative rods occurring singly. Nonmotile. Colonies yellow, smooth and glistening.

(ii) Physiological characteristics: Grow at temperature 5-25 C. Optimal salinity 3% NaCl. Optimal pH 7.

(iii) Nutritional characteristics: Do not utilize any of the substrates tested. May require complex growth factors.

(iv) Distribution: Isolated almost exclusively from sediment at station 137.

Northeast Gulf of Alaska - Winter, 1976
Water 4 C Isolates

Details of the nutritional and physiological characteristics of 8 groups are shown in Figures 34 and 35 respectively.

Group G9:

(i) Morphological characteristics: Gram-negative, nonmotile rods. Yellow pigment produced.

(ii) Physiological characteristics: Growth temperature range 5-25 C. Salinity range 0-7.5% NaCl, pH range 7-9.

(iii) Nutritional characteristics: Did not utilize carboxylic acids, most amino acids, amines, or hydrocarbons. The following substrates were utilized by more than 80% of the members: xylose, glucose, mannose, maltose, mannitol, sorbitol, glycerol.

(iv) Distribution: Isolated only at beach at Kodiak.

Group G13:

(i) Morphological characteristics: Nonmotile gram-negative rods occurring singly, yellow-brown pigments produced.

(ii) Physiological characteristics: Grow at temperatures 5-15 C, salinities 0.5-5% NaCl, pH 7-8.

(iii) Nutritional characteristics: Only utilized some carbohydrates. The following substrates were utilized by 80% of the isolates: fructose, glucose, maltose.

(iv) Distribution: Only found at beach at Yakutat.

Group G16:

(i) Morphological characteristics: Gram-negative motile rods. Poly-β-hydroxybutyrate inclusions found. Gray colony.

(ii) Physiological characteristics: Grow at temperatures 5-25 C, salinities 0.5-7.5%, and pH 7-10.

(iii) Nutritional characteristics: Do not utilize amino acids, hydrocarbons and most alcohols. The following substrates are utilized by 80% of the members: fructose, sorbose, mannitol, 2-ketogluconate, pyruvate, α-ketoglutarate.

(iv) Distribution: Found at several stations.

Group G19:

(i) Morphological characteristics: Gram-negative, nonmotile, cocco-bacilli. Gray colony.

(ii) Physiological characteristics: Grow at temperatures 5-37 C, salinities 0-10% NaCl, pH 7-10.

(iii) Nutritional characteristics: Do not utilize alcohols or most amino acids. The following substrates were utilized by 80% of the members: fructose, glucose, sorbose, α-ketoglutarate, o-hydroxybenzoic acid, cystine.

(iv) Distribution: Only isolated at station 30.

Group G20:

(i) Morphological characteristics: Gram-positive, cocco-bacilli, white colony.

(ii) Physiological characteristics: Grow at temperatures 5-37 C, salinities 0-7.5% NaCl, pH 6-10.

(iii) Nutritional characteristics: The following substrates were utilized by 80% of the members: ribose, xylose, fructose, sorbose, salicin, succinate, lactate, pyruvate, asparagine, leucine.

(iv) Distribution: Only isolated at station 52.

Group G22:

(i) Morphological characteristics: Gram-positive cocco-bacilli occurring singly, white colony.

(ii) Physiological characteristics: Grow at temperatures 5-37 C, salinities 0-7.5% NaCl, pH 6-10.

(iii) Nutritional characteristics: Alcohols were not utilized. The following substrates were utilized by 80% of the members: fructose, glucose, sorbose, fumarate, citrate, 2-ketogluconate α-ketoglutarate, gluconate, asparagine, ornithine.

(iv) Distribution: Only isolated from station 52.

Group G24:

(i) Morphological characteristics: Gram-negative, cocco-bacilli, occurring in pairs, white colony.

(ii) Physiological characteristics: Grow at temperatures 5-37 C, salinities 0-7.5% NaCl, pH 6-10.

(iii) Nutritional characteristics: Only tataric acid was utilized, may require complex growth factors.

(iv) Distribution: Found mainly at beach station E.

Group G25:

(i) Morphological characteristics: Gram-negative, cocco-bacilli, occurring as pairs, white colony.

(ii) Physiological characteristics: Grow at temperatures 5-37 C, salinities 0-7.5% NaCl, pH 6-10.

(iii) Nutritional characteristics: The following substrates were utilized by 80% of the members: glucose, acetate, α-ketoglutarate, benzoate, gamma-amino-butyric acid, asparagine, cystine, tyrosine.

(iv) Distribution: Isolated only at station 52.

Northeast Gulf of Alaska, Winter 1976
4 C Isolates from Sediment

Details of the nutritional and physiological characteristics of 6 major groups are shown in Figures 36 and 37 respectively.

Group GA9:

(i) Morphological characteristics: Gram-negative rods, occurring singly, motile, gray colony.

(ii) Physiological characteristics: Grow at temperatures 5-25 C, salinities 0.5-5% NaCl, pH 7-10.

(iii) Nutritional characteristics: The following substrates were utilized by more than 80% of the members: ribose, fructose, galactose, glucose, maltose, trehalose, glycerol, mannitol, succinate, fumarate, lactate, citrate, aspartate, ornithine, proline, threonine, N-acetylglucosamine.

(iv) Distribution: Found at most stations.

Group GA10:

(i) Morphological characteristics: Gram-negative curved rods occurring singly. Motile. White colony.

(ii) Physiological characteristics: Grow at temperature 5-25 C, salinities 0-5% NaCl, pH 6-10.

(iii) Nutritional characteristics: The following substrates were utilized by more than 80% of the members: arabinose, xylose, fructose, galactose, glucose, mannose, trehalose, glycerol, arabitol, mannitol, butyrate, caproate, caprylate, laurate, propionate, succinate, fumarate, β-hydroxybutyrate, lactate, citrate, 2-ketogluconate, pyruvate, α-ketoglutarate, galacturonate, gluconate, alanine, γ-aminobutyrate, arginine, asparagine, aspartate, leucine, isoleucine, proline, putresine, N-acetylglucosamine.

(iv) Distribution: Isolated only at station 4.

Group GA14:

(i) Morphological characteristics: Gram-negative rods occurring singly, motile, gray colony.

(ii) Physiological characteristics: Grow at temperatures 5-15 C, salinity 3% NaCl, pH7-10 optimum.

(iii) Nutritional characteristics: Did not utilize the substrates tested. May require complex growth factors.

(iv) Distribution: Isolated only at station 37.

Group GA15:

(i) Morphological characteristics: Gram-negative rods occurring singly, nonmotile, gray colony.

(ii) Physiological characteristics: Grow at temperatures 5-20 C, optimum salinity 3%, pH 7-9.

(iii) Nutritional characteristics: The following substrates were utilized by more than 80% of the members: ribose, fructose, galactose, glucose, mannose, maltose, trehalose, glycerol, mannitol, succinate, fumarate, lactate, citrate, proline, threonine, N-acetylglucosamine.

(iv) Distribution: Isolated from most benthic sediment, but no beach sediment stations.

Group GA17:

(i) Morphological characteristics: Gram-negative rods occurring singly. Some motile. Gray colony.

(ii) Physiological characteristics: Grow at temperatures 5-15 C, salinity 3% NaCl, and pH 7-9 optimum.

(iii) Nutritional characteristics: Did not utilize any of the substrates tested. May require complex growth factors.

(iv) Distribution: Isolated at stations 32, 52, 53a and 57.

Group GA20:

(i) Morphological characteristics: Gram-negative rods occurring singly. Nonmotile. Encapsulated. Yellow colony.

(ii) Physiological characteristics: Grow at temperatures 5-20 C, salinity 3% NaCl, and pH 7-9 optimum.

(iii) Nutritional characteristics: Did not utilize any of the substrates tested. May require complex growth factors.

(iv) Distribution: Isolated at stations A, 1, 32 and 37.

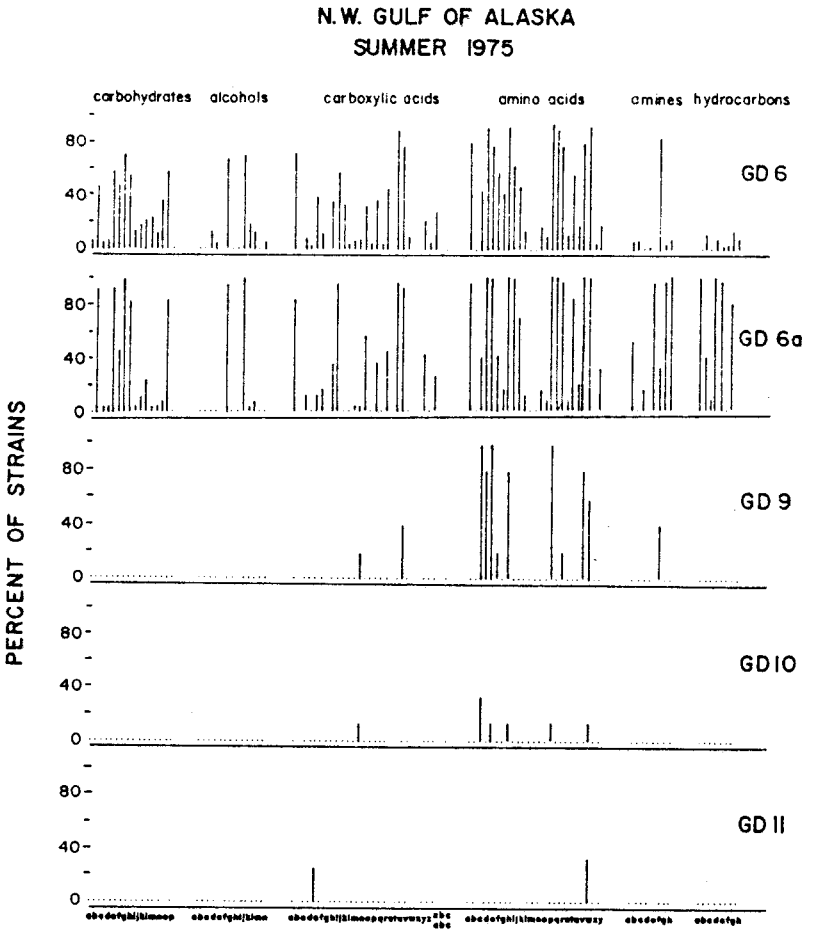

Fig. 32.
Patterns of substrate utilization for clusters from Northwest Gulf of Alaska isolates. (See Fig. 18 for explanation of symbols)

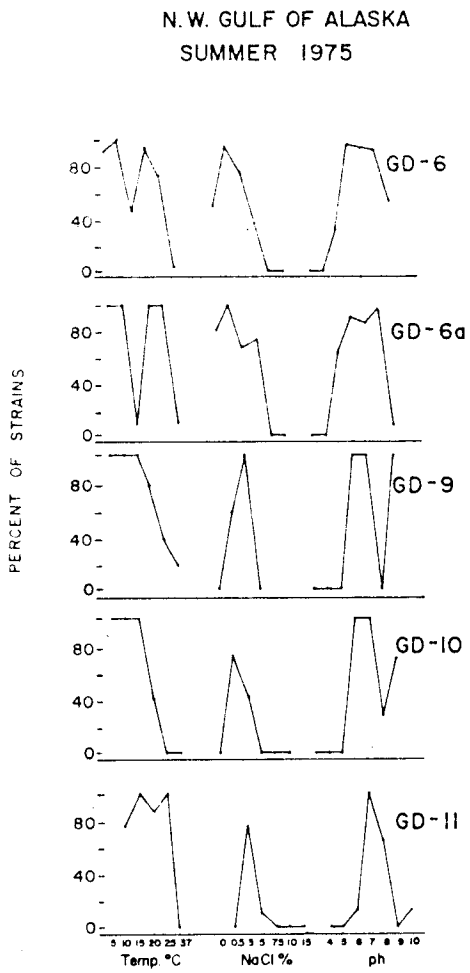

Fig. 33.
Physiological tolerance ranges for clusters from Northwest Gulf of Alaska .

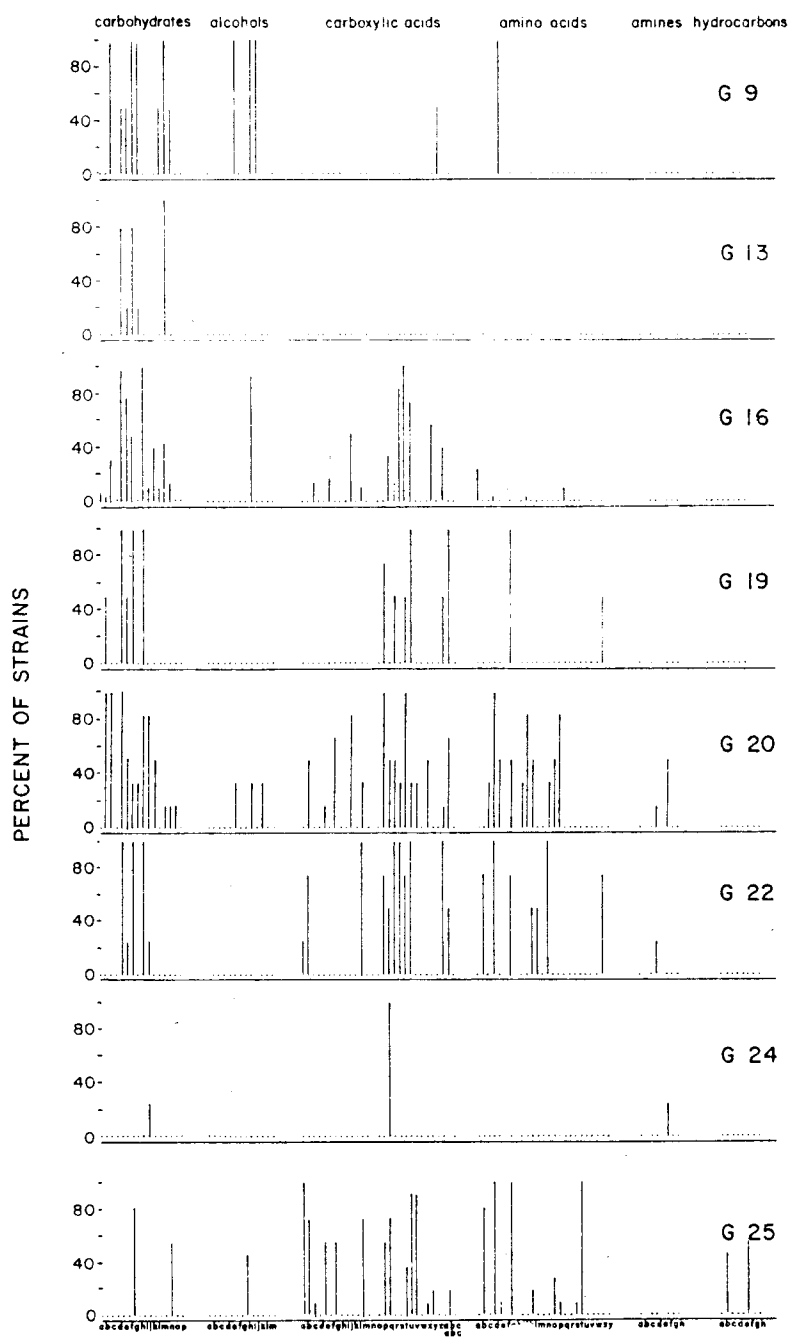

Fig. 34. Patterns of substrate utilization for clusters from 4 C water isolates from the Northeast Gulf of Alaska. (See Fig. 18 for explanation of symbols.)

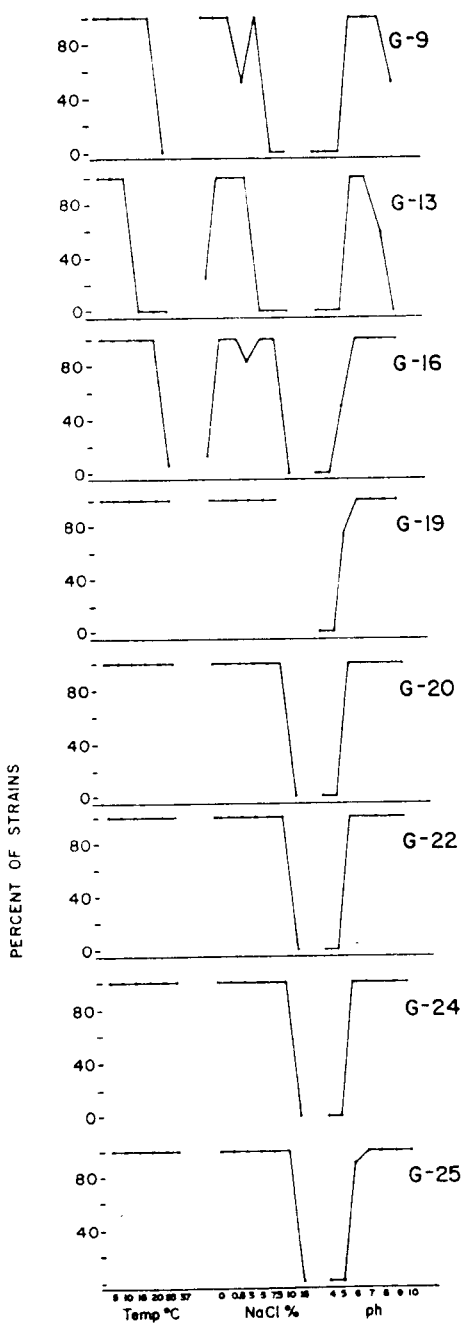

Fig. 35. Physiological tolerance ranges for clusters from 4 C water isolates from Northeast Gulf of Alaska.

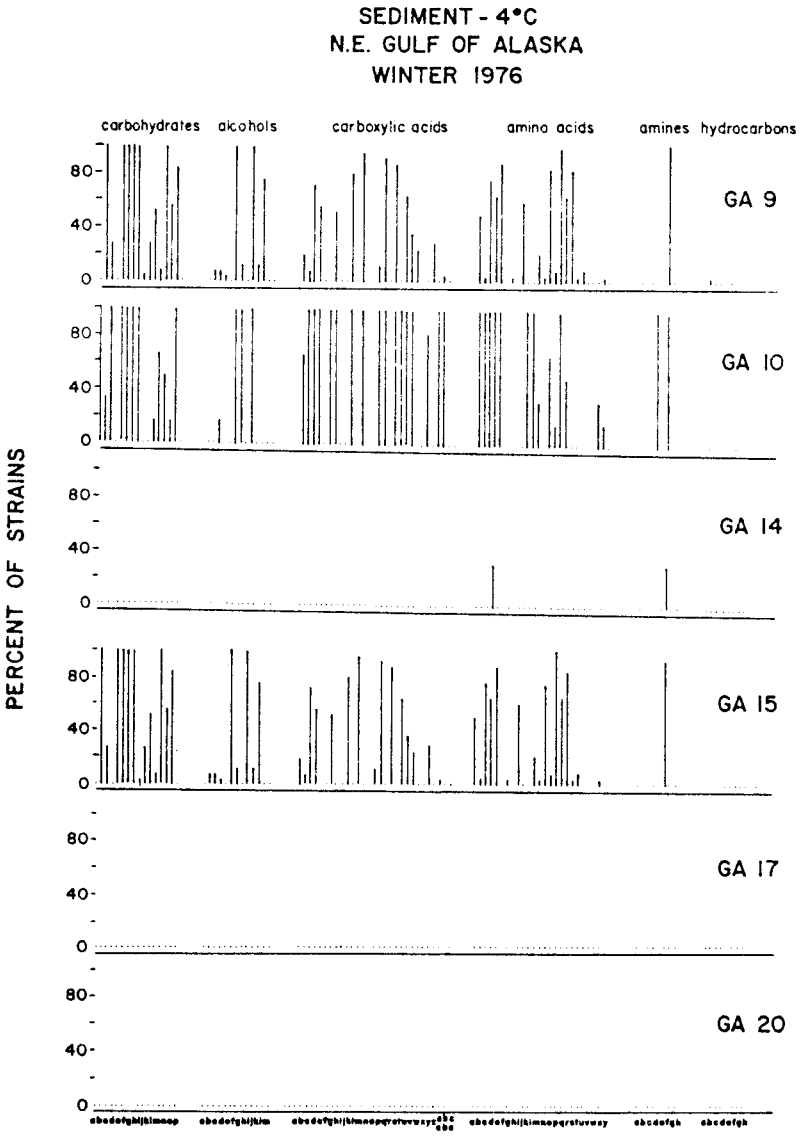

Fig. 36. Patterns of substrate utilization for clusters from 4 C sediment isolates from Northeast Gulf of Alaska. (See Fig. 18 for explanation of symbols)

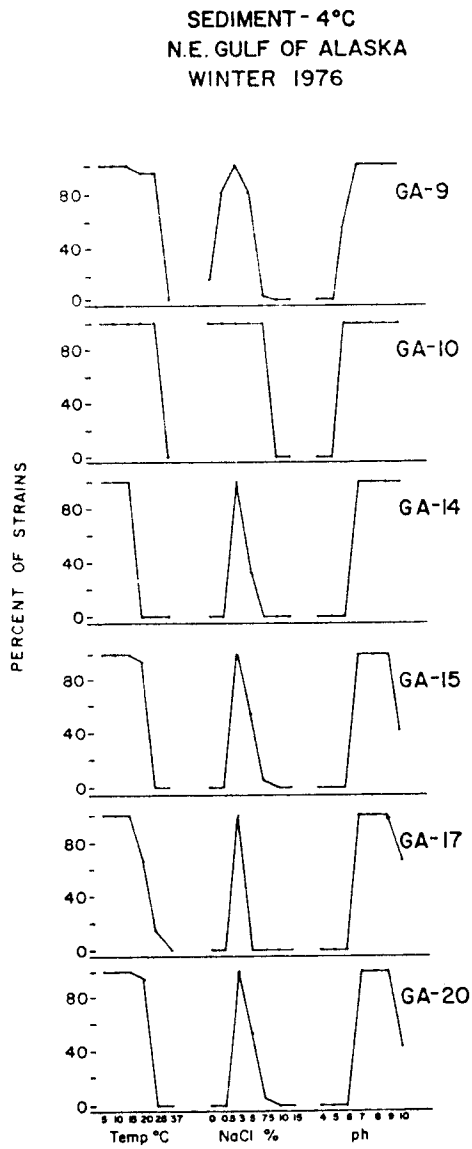

Fig. 37. Physiological tolerance ranges for clusters from 4 C sediment from Northeast Gulf of Alaska isolates.

Hydrocarbon Biodegradation Potential

In addition to the extensive characterization of heterotrophic populations described in the previous sections, the potential of the indigenous populations to degrade petroleum hydrocarbons was examined.

Figures 38 and 39 show the results of the hydrocarbon biodegradation potential measurements as three dimentional histograms. The heights of the bars show the units of activity. Units of activity represent CPM $^{14}CO_2$ produced during 9 weeks of incubation at 5 C. Two reference lines are provided to aid in gaining proper perspective.

During winter higher biodegradation potentials were generally found in water than sediment in the Northeast Gulf of Alaska (Fig. 38). The biodegradation potentials were generally lower in water in the eastern stations sampled. There was wide variation in the biodegradation potentials at different stations in both winter water and sediment samples.

During summer, lower biodegradation potentials were found in Cook Inlet than were found during winter in the NEGOA region (Fig. 39). The biodegradation potential in sediment in Cook Inlet above Kalgin Island was higher than in the rest of Lower Cook Inlet. The biodegradation potentials at 3 stations in summer in Cook Inlet were higher than the mean biodegradation potential in sediment in winter in the NEGOA region. The biodegradation potentials of sediment at the other Cook Inlet stations were lower than the mean NEGOA winter sediment samples. Biodegradation potentials in water during summer were extremely low at all Cook Inlet stations sampled. The biodegradation potentials did not correlate with the number of hydrocarbon degrading microorganisms. Some limiting factor appears to restrict hydrocarbon biodegradation during summer in Cook Inlet.

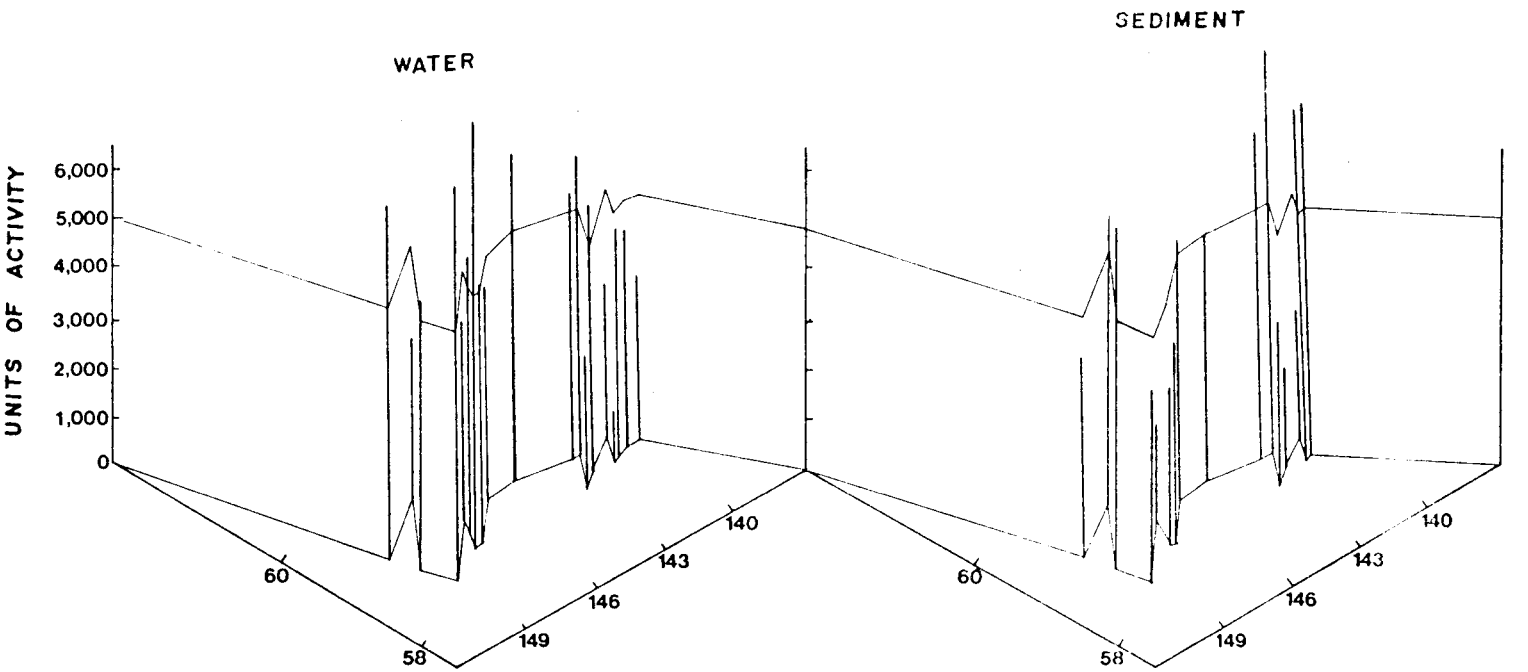

Fig. 38 Hydrocarbon biodegradation potential during winter in Northeast Gulf of Alaska

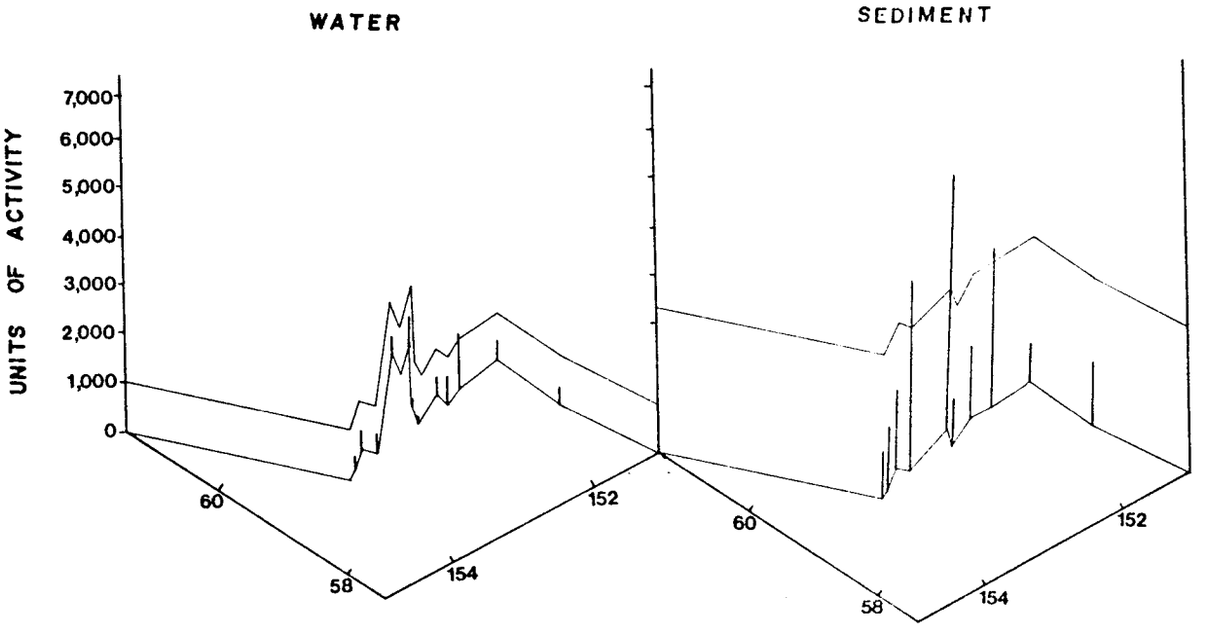

Fig. 39 Hydrocarbon biodegradation potential during summer in Cook Inlet

VII. Discussion and Conclusions

Numbers of viable microorganisms in Gulf of Alaska samples were extremely low for over continental shelf areas. Populations of microorganisms increased west of Kodiak Island toward the Aleutian Islands. Populations were also higher in Upper Cook Inlet than in Lower Cook Inlet. The low numbers of microorganisms could be due to low productivity, perhaps due to competition for available nutrients, or to heavy predation.

Conclusions regarding the seasonal and spacial distribution of dominant groups of microorganisms and regarding diversity of microbial communities cannot yet be made, due in part to the lack of multiple samplings in one geographic area, and in part to the time required to interpret results of computer analyses. It would appear that microorganisms capable of degrading petroleum hydrocarbons are widely distributed in the Gulf of Alaska and Cook Inlet. The biodegradation potential studies in late summer suggest, however, that petroleum biodegradation may be limited at that time of year by abiotic factors.

VIII. Study Needs

The feature frequency and cluster analyses of microorganisms isolated in Lower Cook Inlet during summer 1976 remain to be performed. Also numerous hydrocarbon degrading microorganisms isolated during winter and summer 1976 have been tested but cluster

and feature frequency analyses of the hydrocarbon degrading populations have not yet been completed. The characterization of these microbial populations are important study needs which are presently being carried out.

With respect to future needs, sampling needs to be repeated to assess seasonal variations within a geographic area. A second sampling in Lower Cook Inlet is presently being carried out. It would be wise to extend the sampling area from Lower Cook Inlet to the Kodiak area. With adequate support, both the Kodiak and Lower Cook Inlet lease areas could be sampled at the same times and the microbial communities from both areas characterized. Studies also need to be extended in the future from the Gulf of Alaska into the Bering Sea.

The fate of oil contaminants in these geographic areas also need to be examined. These studies need to develop predictive models that take into account abiotic weathering changes, biodegradation, bioemulsification and bioaccumulation. Some of these studies can best be carried out by examining residual oil following known contamination incidents in these areas. Such studies would require immediate response capability of trained microbiologists.

Effects studies on the impact of oil contaminants, especially at chronic input levels, on microbial communities are needed. These studies should emphasize effects on essential biogeochemical

cycling processes such as nitrogen fixation, denitrification and organic carbon mineralization, as these are critical processes for ecologic balance. Such studies should involve studies with either experimental or accidental oil spills as well as laboratory studies.

IX. Summary of 4th Quarter Activities

No field activities were performed during the 4th quarter. Laboratory activities consisted of testing of several hundred microorganisms isolated from Lower Cook Inlet during October. Both heterotrophic and hydrocarbon degrading microbial populations were tested. Computer analyses of organisms isolated during summer 1975 and winter 1976 in the Northwest and Northeast Gulf of Alaska were completed. The severe cutback of funds during this fiscal year, however, resulted in a decrease in personnel working on this project which has severely decreased the numbers of microorganisms that can be tested and has precluded a thorough interpretation of the computer analyses.

Second Annual Report

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Contract # 03-5-022-68
Research Unit 190-E
Reported Period 1 April, 1976 to
31 March, 1977

Number of pages 74

Study of Microbial Activity in the Beaufort Sea and Gulf of Alaska and Analysis of Hydrocarbon Degradation by Psychrophilic Microorganisms

SUBMITTED BY:

Richard Y. Morita
Principal Investigator
Professor of Microbiology and
Oceanography
Department of Microbiology
Oregon State University
Corvallis, OR 97331

Robert P. Griffiths
Co-Investigator
Research Associate
Department of Microbiology
Oregon State University
Corvallis, OR 97331

Date Submitted

March 22, 1977

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

A. Beaufort Sea

At present it is not known how relative levels of microbial activity or bacterial concentrations relate to the potential of a microbial population to degrade crude oil in situ. The conditions which are known to effect crude oil biodegradation in the marine environment are numerous and it is unlikely that any one parameter can be used to predict potential rates of crude oil degradation. Although there is no data available in the literature correlating relative microbial activity with in situ rates of crude oil biodegradation, it seems reasonable to assume that a positive correlation would exist (all other factors being equal). Should this be the case, then areas which show relatively high microbial activity should have the highest potential for crude oil biodegradation provided that such critical factors such as temperature and the availability of inorganic nutrients are the same.

1. In the Beaufort Sea, dramatic seasonal differences were seen between the levels of activity and cell concentrations observed in water samples taken in the summer and in the winter (Table 19). The relative microbial activity was reduced by a factor of ten during the winter sampling period in both water and sediment samples. The observed reduction in microbial activity can not be entirely explained in terms of decreased water temperature although this may be a contributing factor. The primary cause of this decrease is probably a decrease of the available organic nutrients during the winter months.

This reduced microbial activity and the higher percent respiration seen in the winter populations suggest that the bacteria present during this season are in a different physiological state than normally found in summer. Since the winter populations show signs of an altered physiological state, their interaction with crude oil in the environment may be different from what has been observed in summer populations.

2. The freezing and thawing of seawater and the input of freshwater runoff from the North Slope are factors which we felt might greatly affect microbial activity in the Beaufort Sea. One important aspect of this problem is the effect of diluting seawater with fresh water from ice melt or land runoff on microbial activity. From the data that we have collected, the dilution process itself has very little gross effect on heterotrophic activity. The main effect that sea ice melt might have on potential rates of biodegradation would be indirect. Nutrient analyses of sea ice have shown that ice contains much lower concentrations of dissolved inorganic nutrients than seawater. Presumably, when ice melt water is released into the surrounding seawater, the concentration of available inorganic nutrients would be significantly reduced. Since these nutrients are known to be required for crude oil degradation, it is quite likely that this might be a limiting factor even though bacteria may be present in enough numbers to degrade the oil. This condition would be important since the upper levels of the water column typically are not well mixed. During the August Glacier cruise, we observed a

very shallow lens of relatively fresh surface water which was typically only a few meters deep. Not only would this be an area of potentially low inorganic nutrient concentration but also an area where the water may become saturated with dissolved hydrocarbons from crude oil spills. In the event of an oil spill, this relatively shallow surface layer of unmixed brackish water at the surface with saturation levels of dissolved hydrocarbons, could adversely effect any marine organisms that are in or migrate through this layer.

3. At this point, it is very difficult to assess the fate of crude oil spilled in the Beaufort Sea. The parameters which have been shown to effect rates of biodegradation and emulsification in other marine ecosystems would presumably apply in the Beaufort Sea; however, there are certain unique conditions found in the Beaufort Sea which may present special problems. All of these conditions are ultimately related to the fact that this is a polar region.

Seawater temperature is known to be an important parameter in rates of crude oil degradation. Studies conducted by Dr. Atlas indicate that many of the heterotrophic bacteria in this region have adapted to life at relatively low temperatures. When comparing relative levels of microbial activity in both the water column and sediments of the Beaufort Sea with the same parameters in more temperate waters, we find that despite the lower temperatures, the activities are at least average when compared with activity in warmer waters. Also the bacterial cell concentrations are similar. At this point, it is not known if the hydrocarbon utilizing bacteria in the Beaufort Sea have also adapted to rapid crude oil degradation at low temperatures. There is some evidence that they have not. Dr. D. K. Button (IMS, U. of Alaska) has reported that oxidation rates of spiked crude oil in the Arctic Ocean (Point Barrow) were 1,000 times lower than in the Port Valdez area. After analyzing 200 strains of hydrocarbon utilizing bacteria from the Beaufort Sea for growth-temperature characteristics, we found only three strains that had a maximum growth temperature of 20 C or below (true psychrophilic bacteria). Pilot studies of ^{14}C spiked crude oil degradation at low temperatures by these organisms have produced disappointing results. Co-oxidation studies (hydrocarbons and organic matter) must yet be evaluated in the laboratory.

4. When compared to the water column, the relative microbial activity in the sediments was much greater. This observation has been made in all inshore areas that we have studied. This fact suggests that the sediments may be important in crude oil biodegradation. This supposition has not been tested in Arctic marine sediments and is an area of study that should be pursued.

B. Lower Cook Inlet and the Northeastern Gulf of Alaska (NEGOA)

1. In general, the levels of relative microbial activity found in the NEGOA area sediments were higher than those found in any other area studied. These data suggest that crude oil degradation rates in the NEGOA area marine sediments may be higher than found in the other areas studied. The levels of relative microbial activity found in the water column in this region were relatively low.

2. The average values for relative microbial activity in the offshore water samples were lower than that found in the beach water samples.

The data suggest that the rates of crude oil degradation may be higher along the beach than in open waters. These potentially higher rates of microbial activity would undoubtedly be more than offset by the fact that crude oil floating on the surface would tend to collect along the shoreline.

3. In the Cook Inlet study, the relative levels of microbial activity showed distinct geographical trends. In the northern stations (265, 266, 245, and 246) the microbial activity was the highest observed in the Cook Inlet (Figure 3). The water samples taken in the area near Homer and in Kamishak Bay showed intermediate levels and the lowest levels were seen in stations 105, 106, 205, 206, 207, and 216 (Figure 3). The potential rates of crude oil biodegradation would probably follow the same trends.

4. There appeared to be a correlation between the uptake of glucose by microorganisms found in the waters of the Cook Inlet and crude oil degradation potential observed by Dr. Atlas in the same water samples. The same correlation was not seen in the Beaufort Sea water samples tested during the summer of 1976.

C. Laboratory studies

1. We isolated over 200 bacterial strains from Beaufort Sea sediments plated on crude oil agar plates. Of these, we found only 3 strains that were true psychrophilic bacteria (those organisms with a maximum growth temperature of 20 C or less). These data suggest that if the organisms that were capable of growth on our crude oil agar plates are representitive of all hydrocarbon utilizing bacteria in this region, there are very few organisms that are uniquely adapted to degrading hydrocarbons at very low temperatures.

2. Pilot studies on the effects of chitinase activity have shown that crude oil might significantly inhibit the growth and/or production of chininase in chinoclastic bacteria.

3. A series of experiments were conducted to determine the effects of crude oil on the heterotrophic microorganisms present in seawater samples taken from Yaquina Bay, OR. These studies suggested that crude oil does not significantly reduce the overall rates of heterotrophic potential which are detectable using the methods employed. There was an indication that the level of hydrocarbon utilizing bacteria increased with exposure to crude oil. How these observations apply to natural populations of Arctic marine microorganisms is not known. The data obtained on the physiological state of microorganisms in the winter in the Beaufort Sea indicate that basic seasonal differences do exist in the microbial populations seen in that region. It is also not known if there is any difference between the way summer and winter microbial populations might react to the presence of crude oil.

Even though the presence of crude oil in the above studies did not adversely effect heterotrophic activity, there was evidence that suggests that it might cause basic alterations in the makeup of those populations.

II. Introduction

A. General nature and scope of study

Our main objective has been to study the natural levels of relative microbial heterotrophic activity in natural marine microbial populations

found in the Beaufort Sea, Gulf of Alaska, and Cook Inlet under contrasting seasonal conditions. Other objectives are to evaluate the effects of crude oil on microbial activity and to analyze bacteria isolated from the Beaufort Sea.

B. Specific Objectives

a. Determination of the relative heterotrophic potential in natural marine microbial populations (task number A-27). Studies include representitive samples of both water and sediments in different geological locations and under contrasting seasonal conditions. These studies were designed to give an estimation of the natural variations to be expected in microbial activity in the waters and sediments of the Beaufort Sea, Gulf of Alaska, and Lower Cook Inlet.

b. Isolation and characterization of psychrophilic hydrocarbon utilizing marine bacteria which are capable of degrading and/or emulsifying crude oil at relatively high rates under the conditions found in the Beaufort Sea (task number B-9). Bacteria strains were to be isolated from crude oil enrichment cultures using natural samples taken from the Beaufort Sea as the inoculum. After isolation and purification, the strains were to be subjected to a number of basic physiological studies which would determine the function of these organisms under the conditions found in the Beaufort Sea.

c. Determination of the acute effects of crude oil on the heterotrophic activity of the natural microbial populations found in the Beaufort Sea (task number C-2). These studies were to be supplemented with longer term studies which would be designed to obtain information about how crude oil affects the natural population and how, in turn, the natural population alters the crude oil.

d. Coordination of our studies with the microbial studies of Dr. Atlas and his associates to obtain the most comprehensive data possible on the role of marine bacteria in the marine ecosystems in both the Beaufort Sea and the Gulf of Alaska. To accomplish this end, our studies were to be made using the same samples. In addition, a close liaison was to be established to insure that duplication of effort is minimized. In addition, we were to collect subsamples to be analyzed for inorganic nutrient concentrations by Dr. Alexander.

C. Relevance to problems of petroleum development

Our major area of concern is the interaction between the crude oil that might be accidently spilled during the course of petroleum field development and the microorganisms present in the potential lease areas. Current studies will provide us with the natural levels of microbial activity and the composition of the microbial communities before extensive perturbation occurs. Studies on the effects of crude oil on natural microbial heterotrophic populations will give us some estimation as to how a crude oil spill might effect these organisms. Measurement of relative heterotrophic potential in sediment and water samples should give some indication as to where initial rates of crude oil oxidation might be the highest.

There are many factors that are known to affect the rate of microbial crude oil degradation. The three most predominant are temperature, availability of fixed nitrogen and phosphorus, or organic matter containing nitrogen and phosphorus, and the presence of crude oil degrading microorganisms. Seasonal conditions and geographical location must also be taken into consideration. An educated prediction can be made as to what might happen in the event an oil spill occurs based on baseline data accumulated, the results of various physiological studies on psychrophilic hydrocarbon oxidizers, laboratory studies on perturbing systems with crude oil, studies conducted by Dr. Atlas, and field studies employing crude oil with the water samples when the heterotrophic measurements are made.

III. Current state of knowledge

Most of the data available in the Beaufort Sea area will be found in our first annual report and the annual reports of Dr. Atlas (R.U. 29,30). Studies of hydrocarbon biodegradation in Alaskan marine waters have also been reported by Kinney et al. (1969) and Archelger et al. (1977).

IV. Study areas

During this report period, we conducted four field studies; one in the NEGOA area in March, 1976, one in the Lower Cook Inlet in October, 1976, and two in the Beaufort Sea in April, 1976, and August, 1976. The positions of the stations studied in the NEGOA area are illustrated in Figure 1 and the exact positions are given in Table 1. Two stations that were sampled during this cruise are not shown in Figure 1; one was taken on Kodiak Island and the other was taken at Seward. The stations sampled during the two Beaufort Sea studies are illustrated in Figure 2 and the exact positions are given in Tables 2 and 3. The stations sampled during the Lower Cook Inlet study are illustrated in Figure 3 and the exact positions are listed in Table 4.

V. Methods

A. Sampling procedures

1. Discoverer cruise (March, 1976)

The water samples were taken in sterile Niskin plastic water sample bags fitted on Niskin "butterfly" water samplers. All samples were taken within two meters of the surface and were processes within two hours after they were collected.

All sediment samples except the beach samples taken at stations a, b, d and f were taken with Van Veen grab. Approximately 150 grams of surface sediment were taken as a representitive sample at each station.

2. Beaufort Sea field trip (April, 1976)

Nine inch holes were drilled through the ice using a power head and 9 inch auger. Water samples were taken using the Niskin "butterfly" water samplers in all but four samples (#BW101, 102, 103 and 104). These four samples were taken using a sterile vacuum flask sampler (our own design). The water samples were kept in an ice chest from the time they were taken to the time they were received at NARL for processing. This procedure prevented the samples from freezing while enroute to the laboratory. All Samples were processed within four hours after they were taken.

The sediment samples were taken with a Kahl scientific mud snapper. One or two grab samples were taken at the same time and location and combined with the seawater within the grab. These samples were placed into sterile 250 ml wide mouth glass sample bottles for transport to the laboratory. All samples were maintained at or slightly below the in situ temperature during sampling and transport.

Ice samples were taken from the ice shavings that resulted from drilling the hole through the ice. These samples were placed into sterile sample bags for transport to and storage at the laboratory. The ice samples were melted at 5 C for 24 hours prior to processing.

3. Glacier cruise (August, 1976)

The same sampling methods were used that are described for the Discoverer cruise.

4. Miller Freeman cruise (October, 1976)

Essentially the same methods were used that are described to the Discoverer cruise. The beach water samples were taken with a Niskin "butterfly" sterile bag sampler right at the surf line. The near beach samples were taken in the same manner but at a distance of about 30 meters from the surf line.

B. Heterotrophic potential studies

The techniques used in this study were basically those of Hobbie and Crawford (1969) as further modified by Harrison, Wright, and Morita (1971). This procedure involves the addition of different concentrations of U-^{14}C labeled substrate to identical subsamples. After addition of the 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 H_2SO_4 in order to stop the reaction and release the $^{14}CO_2$. After the addition of the acid, 0.15 ml of the CO_2 absorbent, B-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 CO_2. The filter papers containing the $^{14}CO_2$ were removed from the cup assemblies and added to scintillation vials containing 10 ml of toluene based scintillation fluor (Omniflour, 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 the field laboratory or they were counted on a Nuclear Chicago Mark I liquid scintillation counter located in our laboratory at Oregon State University.

In the sediment samples, a 1.0 ml subsample was diluted with a 32 o/oo (w/v) solution of sterile artificial seawater. After dilution, the one ml subsamples of the sediment slurry were dried and weighed to determine the dry weights. These dry weights were used to calculate the V_{max} values in terms of grams dry weight of sediment. Most of the sediment samples were diluted 1,000 times (w/v).

During the Discoverer cruise, (U-^{14}C) L-glutamic acid with a specific activity of 230 mCi/mmole (New England Nuclear) was used in all water samples. The range of glutamic acid concentrations in the reaction vessels was from 0.6 µg/ liter to 4.6 µg/liter. With the exception of sediment sample number 101GB, all sediment samples were exposed to glutamic acid concentrations in the range of 10.5 µg/liter to 84 µg/liter using (U-^{14}C) L-glutamic acid with a specific activity of 10 mCi/mmole. Duplicate subsamples were used at each of four substrate concentrations. The average incubation temperature used during the cruise

was 4 C which was within 1 C of the in situ surface water temperature. The length of incubation varied from 9 to 12 hours.

During the winter Beaufort Sea study, all sediment and water samples were exposed to glutamic acid with a specific activity of 230 mCi/mmole. The range of glutamic acid concentrations in the reaction vessels was from 0.6 µg/liter to 4.6 µg/liter. The average incubation temperature used was -1.0 C and the length of incubation varied from 8 to 11 hours. All samples were incubated in the dark.

During the Glacier and the Miller Freeman cruises, the heterotrophic potential determinations in water and sediment samples were made using the same concentrations of ^{14}C glutamic acid that were used during the Discoverer cruise. In addition, the uptake and respiration of algal protein hydrolysate and glucose were monitored in both seawater and sediment samples. One concentration of each of these substrates was used in these determinations (38 ng of ^{14}C glucose or 0.9 ng At. carbon of algal protein hydrolysate were added per 10 ml subsample). The average incubation temperature was 0°C and the incubation times ranged from 8 to 12 hours.

C. Calculations

Calculation of the kinetic parameters was made from the relationship:

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

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

The percent respired was calculated by dividing the amount of labeled carbon associated with the CO_2 fraction by the total amount of substrate taken up by the cells (both cell and $^{14}CO_2$ radioactivity) and multiplying this ratio by 100.

D. Microbial activity changes with time in oil enrichment cultures

A series of three experiments were conducted. The first experiment (#1) was designed to measure the variations found between two "identical" subsamples neither of which were exposed to crude oil. This experiment

was designed as a control to evaluate the confidence limits of variations observed in experiments #2 and #3. In this experiment, the uptake of ^{14}C labeled algal protein hydrolysate and D (U-^{14}C) glucose was measured at one substrate concentration. The algal protein hydrolysate had a specific activity of 55 mCi/mAtom carbon/liter. The D (U-^{14}C) glucose had a specific activity of 284 mCi/mmole with a final substrate concentration of 0.33 µg/liter. The other two experiments, (#2 and 3) were designed to measure the effects of crude oil on the natural microbial population found in the seawater samples.

Twenty gallon samples were taken from Yaquina Bay, Oregon, and transported to our laboratory at Oregon State University. Within two hours after the samples were taken, they were split into two equal portions which were placed into 15 gallon aquaria fitted with an aeration system. Forty ml of Prudhoe Bay crude oil was added to one aquarium and nothing was added to the control. The water samples were incubated in the dark with aeration at 8 C (the water sample temperature in situ). At various times, subsamples were removed via a syphon system which allowed crude oil free samples to be collected from the system. These subsamples were used to make measurements of relative microbial activity and percent respiration using ^{14}C-labeled glutamic acid and sodium acetate. They were also used to measure the bacterial concentrations using epifluorescent microscopy, Lib X agar plates (Baross, Hanus, and Morita, 1974) and crude oil agar plates (Atlas and Bartha, 1972).

The agar plates were incubated in the dark at 8 C. Colonies were counted in the Lib X agar plates after incubating one week and the colonies on the crude oil plates were counted after 6 weeks incubation.

The relative microbial activity and percent respiration was determined using the techniques described under "heterotrophic potential studies". The subsamples were incubated in the dark for two hours at 8 C. The same two concentrations of ^{14}C-labeled glutamic acid were used in these experiments as were used in the field studies. The ^{14}C-labeled acetate (New England Nuclear) used had a specific activity of either 54 or 8.7 µCi/µMole. The concentration ranges used were either from 2 to 32 µg/liter or 6 to 100 µg/liter.

E. 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 organe 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 Fl epi-fluorescence condenser microscope fitted with filters KP 500, KP 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.

F. Temperature study

The water sample used in this study was taken from station #3 in Elson Lagoon. Duplicate subsamples were prepared as described above and incubated at each of the temperatures indicated in Figure 3. In cases where there were fluctuations in the incubation temperature, the temperature reported was the mean temperature (in most cases, the temperature range was less than one degree C). In order to reduce the chance of significantly altering the substrate concentrations during the course of the experiment, the incubation time for sample incubated at high temperatures was reduced. Samples at the following temperatures were incubated for the following periods: 20, 15 and 10 C, 6 hours; 4 and -1 C, 8.5 hours.

G. Biochemical studies of arctic bacterial isolates

The methods and taxonomic key used in this study are those of Bain and Shewan (1969).

H. Literature cited

Alexander, V., R. Horner, and R. C. Clasby. 1974. Metabolism of Arctic Sea ice organisms. Univ. Alaska. IMS Rep. R 74-4. 120 pp.

Atlas, R.M. Assessment of potential interactions of microorganisms and pollutants resulting from petroleum development in Cook Inlet/Gulf of Alaska. R.U. 30. Reports to NOAA.

Atlas, R.M. Assessment of potential interactions of microorganisms and pollutants resulting from petroleum developments on the outer continental shelf in the Beaufort Sea. R.U. 29. Reports to NOAA.

Atlas, R. M. and R. Bartha. 1972. Biodegradation of petroleum in seawater at low temperatures. Can J. Microbiol. 18:1851-1855.

Archelger, S.D., B.R. Robertson, and D.K. Button. 1977. Arctic hydrocarbon biodegradation. In Fate and Effects of Petroleum Hydrocarbon in Marine Ecosystems and Organisms. (in press).

Bain, N. and J.M. Shewan. 1969. Identification of Aeromonas, Vibrio, and related organisms. In B.M. Gibbs and D.A. Shapton (ed.), Identification Methods for Microbiologists, Part B. pp 79-84.

Baross, J. A., F. J. Hanus and R. Y. Morita. 1974. The effects of hydrostatic pressure on uracil uptake, ribonucleic acid synthesis and growth of three obligately psychrophilic marine vibrios, Vibrio alginolyticus, and Escherichia coli. p. 180-202. In R. R. Colwell and R. Y. Morita (ed.) Effect of the Ocean Environment on Microbial Activities. Univ. Park Press. Baltimore, Md.

Harrison, M. J., R. T. Wright, and R. Y. Morita. 1971. Method for measuring mineralization in lake sediments. Appl. Microbiol. 21:698-702.

Hobbie, J. E., and C. C. Crawford. 1969. Respiration corrections for bacterial uptake of dissolved organic compounds in natural waters. Limnol. Oceanogr. 14:528-532.

Kinney, P.J., D.K. Button, D.M. Schell. 1969. Kinetics of dissipation and biodegradation of crude oil in Alaska's Cook Inlet. In Prevention and Control of Oil Spills. API-EPA Proceedings. pp 333-340.

Wright, R. T. and J. E. Hobbie. 1966. Use of glucose and acetate by bacteria and algae in aquatic ecosystems. Ecology 47:447-464.

Zimmerman, R., and L.- A. Meyer-Reill. 1974. A New method for fluorescence staining of bacterial populations on membrane filters. Kieler Meeresforschungen 30:24-27.

VI. Results

A. Gulf of Alaska (NEGOA)

During the period March 16 to March 27, 1976, we participated in an oceanographic cruise on board the NOAA ship Discoverer. Twenty-seven water and twenty sediment samples were taken at the stations shown in Figure 1. The date of sampling, station and sample numbers, and station positions are given in Table 1. Measurements of salinity, temperature, the maximum potential glutamic acid uptake (V_{max}) and percent respiration are given for all water samples in Table 5. The average V_{max} value for offshore water samples was 1.4 ng glutamic acid x liter^{-1} x hr^{-1} and the range was from 0.3 to 3.4. The average percent respiration was 72 with a range of 53 to 93. The average water sample temperature was 3.8°C with a range of 2 to 5 and the average salinity was 31.9 o/oo with a range of 30.7 to 32.5. Table 5 also shows the total bacterial concentrations in seawater samples taken from the Gulf of Alaska as determined by direct microscopic counts. The observed values varied from 1.2×10^5 to 2.7×10^5 cells per ml with an average value of 1.9×10^5 cells per ml. This is roughly 1/2 of the average bacterial concentration observed in the Beaufort Sea during our summer 1975 field study period. The bacterial concentrations in seawater observed in the Beaufort Sea during our April, 1976, studies are given in Table 7. The average figure was 1.5×10^5 cells/ml for the seawater samples studied. This is roughly the same as the average concentration of cells found in the Gulf of Alaska and about the same as the average bacterial count observed in the three ice melt samples tested. The average concentration of bacteria in the Beaufort Sea and Gulf of Alaska water samples do not reflect the same trends that were observed in the V_{max} data observed in the same samples. The average V_{max} value was roughly twice as high in the Beaufort Sea as in the Gulf of Alaska even though the average incubation was 5 C lower in the Beaufort Sea samples.

The bacterial concentrations found in twenty Gulf of Alaska sediment samples are given in Table 6. The range of values observed was from 2×10^7 to 3.1×10^9 cells per gram dry weight sediment. The average value of 1.5×10^9 cells per gram was somewhat higher than the average figure of 0.63×10^9 cells per gram observed in the Beaufort Sea during the summer of 1975 and slightly higher than the average figure of 1.0×10^9 cells per gram observed in the Beaufort Sea during the April, 1976, study (Table 8). The maximum potential rate of glutamic acid uptake (V_{max}) values for Gulf of Alaska sediment samples are given in Table 6. These values ranged from 0.1 to 27.5 µg glutamic acid x gram dry weight sediment^{-1} x hr^{-1}. The average value was 4.5 µg x gr^{-1} x hr^{-1}. This value is significantly higher than that observed in any of the other studies (Table 19). Inorganic nutrient concentrations were also measured in all water samples by Dr. Alexander. The following are the average

values obtained from the seawater samples analyzed: PO_4, 1.88 µg-at/liter (range 0.56 to 2.13), NH_3, 0.08 µg-at/liter (range 0 to 0.4); NO_3, 16 µg-at/liter (range 9.4 to 18.1); and SiO_3, 30 µg-at/liter (range 25 to 33).

B. Beaufort Sea (April, 1976)

During our 1976 winter field study period in the Beaufort Sea, we collected and analyzed 23 water, 14 sediment and 3 ice samples. The location of the stations sampled are shown in Figure 2. The date of sampling, the station number, sample number, and position, are given for all samples in Table 2. Measurements of salinity, temperature, V_{max}, percent respiration and cell concentrations are given for all water samples tested (Table 7). The average V_{max} value was 3.1 ng x liter^{-1} x hr^{-1} and the range was from 0.2 to 14. The average percent respiration in the water samples was 85 and the range was from 52 to 100 percent. The average water sample temperature was -1.9°C with a range of -2.0 to -1.5. The average salinity was 24 o/oo with a range of 17 to 29. The average percent respiration in the sediment samples was 45 and the range was from 35 to 87 percent.

Three ice samples were analyzed for heterotrophic activity using the same techniques used in the water samples. The average V_{max} was 2.3 ng glutamic acid x liter^{-1} x hr^{-1}. The average percent respiration for these samples was 98%.

The effect of incubation temperature on the apparent V_{max} in a water sample was tested. The results of this experiment are given in Figure 4.

The following are the average nutrient values observed in the seawater samples analyzed from the Beaufort Sea: PO_4, 1.14 µg-at/liter (range 0.52 to 1.83); NH_3, 2.2 µg-at/liter (range 0 to 32.4); NO_3, 5.1 µg-at/liter (range 11 to 33). During the winter Beaufort Sea field trip, ice samples were taken at each station. The average values for the nutrient analysis of these samples are as follows: PO_4, 0.06 µg-at/liter (range 0 to 0.33); NH_3, 0.9 µg-at/liter (range 0 to 12.3); NO_3, 1.8 µg-at/liter (range 0.7 to 3.0) and SiO_3, 3.6 µg-at/liter (range 1 to 8).

The average bacterial concentration observed in the Beaufort Sea sediments was 1.0 x 10^9 cells/gram dry weight sediment. The range was from 0.5 x 10^8 to 1.7 x 10^{10} cells/gram dry weight (Table 8).

C. Glacier cruise, Beaufort Sea (August, 1976)

During this cruise, 18 water and 13 sediment samples were analyzed for total bacterial concentrations and relative microbial activity as determined by the uptake and respiration of ^{14}C labeled organic substrates. The average concentration of bacteria found in all of the seawater samples tested was 3.7 x 10^5 cells/ml with a range of from 1.6 x 10^5 to 6.1 x 10^5 (Table 11). The average number of bacteria

per gram dry weight of sample sediment taken during the same cruise was 1.06×10^{10} with a range of 0.24×10^{10} to 2.63×10^{10} (Table 12b).

1. Water samples

The relative microbial activity in the seawater samples tested was measured using ^{14}C labeled glutamic acid, glucose and agal protein hydrolysate. Only one substrate concentration was used in the glucose and agal protein hydrolysate studies but the usual four substrate concentrations were used for the glutamic acid studies. By using the information from the utilization of glutamic acid by natural populations at these four concentrations, heterotrophic potential data was calculated. Table 10 shows the results of these studies. The values for glucose and glutamic acid uptake are reported in terms of ng substrate utilized by the natural microbial populations per liter of seawater per hr. Since there is no good method for estimating the amount of algal protein utilized by weight, these results are reported in terms of radioactivity incorporated and respired; disintegrations per minute (DPM) per liter of seawater per hr.

As shown in Table 10, the average maximum potential utilization of glutamic acid (V_{max}) was 21 ng per liter per hr with a range of 0.4 to 85. The average amount of glucose taken up at a concentration of 3.8 µg/liter was 5.1 ng per liter per hr with a range of 1.3 to 12.7. The average uptake of glutamic acid at 2.7 µg/liter was 7.9 ng per liter per hr with a range of 0.5 to 15. The average uptake of algal protein hydrolysate at 93 ng-at C/liter (0.05 µCi per sample) was 2.3×10^4 DPM per liter per hr with a range of 0.4×10^4 to 6.2×10^4. In the same seawater samples, the percent of the substrate that was respired relative to that which was taken up by the cells (percent respiration) was calculated for all three substrates tested (Table 11). The average value for glutamic acid was 46% with a range of 20 to 61%. The average value for glucose uptake was 27% with a range of 7 to 41%. The average value for algal protein hydrolysate was 21% with a range of 10 to 32%.

2. Sediment samples

The relative microbial activity was also measured in the sediment samples (Table 10). The average V_{max} for all samples was 0.69 µg glutamic acid taken up per gram dry weight of sediment per hr. A more representative figure would be the average of all samples except BB212 which is atypically high. If BB212 is excluded from the calculation, the average value is 0.12 µg per g dry weight per hr. The range was 0.02 to 6.4 µg per g dry weight per hr. The average uptake of glucose at a concentration of 2.7 µg/liter was 4.2 ng per g dry weight per hr with a range of 1.3 to 15. The average uptake of glutamic acid at 72.8 µg/liter was 0.15 µg glutamic acid per g dry weight sediment per hr with a range of 0.02 to 0.82. The average amount of algal protein hydrolysate taken up at the same concentration used in the water samples was 2.0×10^4 DPM per g dry weight per hr with a range of 0.3 to 10.3×10^4.

The percent respiration (mineralization) was also calculated for all sediment samples tested (Table 12a). The average percent respiration with glutamic acid was 28 with a range from 14 to 35. The average figure for glucose was 20% with a range of 10 to 27% and the agal protein hydrolysate had an average value of 28% with a range of 5 to 43%.

D. Miller freeman cruise, Lower Cook Inlet

During this cruise, 37 water and 12 sediment samples were analyzed for the same parameters that were measured during the Glacier cruise. The average concentration of bacteria found in all seawater samples was 4.2 x 10^5 cells per ml with a range of 0.2 to 16.5 x 10^5 cells per ml (Table 15). In the sediment samples, the average concentration of bacteria was 4.1 cells x 10^9 per g dry weight with a range of 0.4 x 10^9 to 13.0 x 10^9 (Table 17b).

The relative microbial activity in seawater and sediment samples was determined using the same methods and substrate concentrations that were used during the Glacier cruise in the Beaufort Sea. The results of the relative microbial activity determinations for all seawater samples tested are presented in Table 14. There was a significant difference between the average values observed in samples taken off shore and those that were taken on or near the beach (within 30 m of the shoreline). For this reason, these values have been computed separately.

The average value for the offshore V_{max} determinations was 28 ng glutamic acid utilized per liter of seawater per hr with a range of 0.2 to 405 ng per liter per hr. If the V_{max} measured in sample GW322, which is considered to be atypical for this group of samples, is not included in the average value, the average V_{max} for offshore samples becomes 9.3 ng per liter per hr. The average value for shore samples was 84 ng per liter per hr with a range of 0.7 to 405. The uptake of glutamic acid in offshore water samples at 2.7 µg/liter was 4.9 ng per liter per hr with a range of 0.17 to 53. If sample GW322 is not included in the average, a more representative value of 2.3 µg per liter per hr is obtained. At the same glutamic acid concentration, the average shore sample showed uptake of 44 ng per liter per hr with a range of 0.4 to 256. The average uptake of glucose in offshore water samples at a concentration of 3.8 µg/liter was 3 ng per liter pr hr with a range of 0.06 to 33. If sample GW322 is excluded from the average, the value becomes 1.2 ng per liter per hr. The average uptake of glucose at the same substrate concentration in shore samples was 30 ng per liter per hr. The average uptake of algal protein hydrolysate in terms of the amount of radioactivity taken up was 1.9 x 10^4 DPM per liter of seawater per hr with a range of 0.03 to 16.1. If sample GW322 is excluded from the calculations, the value becomes 1.0 x 10^4 DPM per liter per hr. The average value for the shoreline samples was 19.8 x 10^4 DPM per liter per hr with a range of 0.41 to 92.8 x 10^4 DPM.

The following average values were found in the sediment samples taken in the Lower Cook Inlet using the same experimental parameters

used to analyze Beaufort Sea sediments (Table 16): V_{max} with glutamic acid, 0.71 ug per g dry weight per hr (range 0.07 to 6.28); glutamic acid at 72.8 µg/liter, 0.22 ug per g dry weight per hr (range 0.03 to 1.19); glucose at 3.8 µg/liter, 11.5 ng per g dry weight per hr (range 0.07 to 56.3); and algal protein hydrolysate, 5×10^4 DPM per g dry weight per hr (range 0.9 to 17).

The percent respiration for the three substrates studied was also measured in all samples (Tables 15 and 17a). The following are the average values found in the water samples: glutamic acid 58% (range 40 to 78); glucose 28% (range 10 to 84) and algal protein hydrolysate 30% (range 19 to 44). The following are the values found in the sediment samples: glutamic acid, 46% (range 38 to 53): glucose, 28% (range 20 to 43): and algal protein hydrolysate, 42% (range 31 to 51).

The average surface water temperature measured during this cruise was 8.3 C (range -1.5 to 12) and the average salinity in the same waters was 23.8 parts per thousand (range 18.0 to 27.5). The average water temperature just above the sediment was 8.8 C (range 8.0 to 10.0) and the average salinity in the same waters was 25.7 parts per thousand (range 19.8 to 29.0). These data are shown in Table 13.

E. Laboratory studies at Oregon State University

Work has continued on the characterization of selected psychrophilic or psychrotrophic strains of bacteria which were isolated from oil enrichment cultures taken from the Beaufort Sea. The growth temperature profiles for all strains listed in Table 18 except strains G-3 and G-6 were presented in the first annual report. The other characteristics tested to date are presented in Table 18. We do not have enough information at this time to give a positive identification to the genus level on all of the strains listed but the following tentative identifications can be made. Strains 30, 45, and 47 probably belong to the genus Vibrio; strains 52, 53, and 59 belong to the Arthrobacter-Coryneform group; strain G-3 is most probably of the genus Pseudomonas; strain G-6 belongs to the Vibrio-Aeromonas group.

F. Laboratory studies

Two experiments were conducted to determine the effects of crude oil on the potential rates of glutamic acid and acetate uptake and the present respiration of these two substrates in natural microbial populations (experiments 2 and 3). In addition to these factors bacterial cell counts on Lib X agar plates and crude oil plates and total bacterial concentrations were measured using direct microscopic observations. These same factors were also measured in a control experiment designed to determine the variations found in two identical subsamples that had not been perturbed with crude oil (experiment #1).

The results of the glutamic acid uptake studies in the three experiments are given in Figures 5, 6, and 7. The percent respiration of glutamic acid taken up is given in Figures 11, 12, and 13. The results of the acetate uptake studies are given in Figures 8, 9, and 10. The percent

respiration of acetate taken up is given in Figures 14, 15, and 16. The concentration of bacteria per ml was also followed during the course of these three experiments. "Total" bacterial counts were made using Lib X agar plates; these results are reported in Figures 17, 18, and 19. The concentration of crude oil degrading bacteria was determined by plate counts on crude oil agar plates; these results are reported in Figures 20, 21 and 22.

During the course of experiment #1, the uptake of a ^{14}C-labeled algal protein hydrolysate was also followed at one substrate concentration; these results are reported in Figure 23. During this experiment, the uptake of ^{14}C-labeled glucose was also measured at one substrate concentration; these data are reported in Figure 24.

VII. Discussion

A. Relative microbial activity

1. Correlations within geographical areas

In general, the water samples taken in the vicinity of Prudhoe Bay during the summer Beaufort Sea study showed the highest V_{max} values of the samples analyzed during that cruise. This was also the case when the relative activity was measured using algal protein hydrolysate, glucose or glutamic acid at one substrate concentration.

During the Lower Cook Inlet study, definite trends were noted between relative microbial activity and the sample site location within the inlet. The offshore stations can be grouped into three distinct clusters according to the relative microbial activity in seawater samples as determined by V_{max} values. The group of stations showing the lowest activity contained stations number 106, 216, 215, 105, 206, 225, 207, and 206. These stations are all located near the mouth of the inlet. Another group of offshore stations showed intermediate activity and are all located in or near the major bays. This group includes stations 227, 214, 226, 212, and 204. The highest microbial activity seen in the offshore stations was measured in the northernmost stations. These were stations 245, 246, 265, and 266. One station that did not fit this general pattern was station number 229 which was located in Kachemak Bay. Judging from the extremely high levels of activity that were observed at the shore station at Homer (samples GW316 and GW317), it seems reasonable to assume that the relatively high level of activity observed at this station was caused by pollutants dumped into the bay near Homer. The supposition is further supported by the fact that the activity seen in the water sample taken just off the beach at station J (sample number GW314) showed lower activity than that found in the bay at station 229. Station J is located in Halibut Cove which is currently in a relatively natural state.

One station that was of particular interest was station number 266 located near East Foreland. The seawater sample taken at this station showed a level of microbial activity that was roughly one order of magnitude greater than any other offshore station studied. This level of activity is particularly impressive when one compares the values found at this station with the value measured in the seawater sample taken at the control station 246. Station 246 was located on the same side of Lower Cook Inlet as 266 and approximately the same distance off shore but in an area that was less perturbed than 266. The microbial activity at station 266 was roughly 20 times greater than that observed at station 246 as determined by the V_{max} values. It is our understanding that there was a significant spill of JP-5 fuel in this region approximately six weeks before we sampled in this area. At this time we do not know if this high level of activity was related to this spill or not. Only further observations in this region will clarify this point.

When one compares the relative microbial activity in the offshore and beach water samples, there is a marked difference. The same trend is seen here that we observed in the Gulf of Alaska winter field trip (Table 19). The average V_{max} value for offshore water samples was 28

ng per liter per hr. If one excludes the value observed in sample number GW322 (station 266) which we consider to be atypical, the average V_{max} value for the offshore station would be only 9.3 ng per liter per hr. If this higher microbial activity along the beach reflects a generalized phenomenon, then it would appear that when petroleum products do reach the shoreline, the rate of microbial degradation would be greater there than in open waters. The same type of statement can be made for oil spills that might occur in locations within the Lower Cook Inlet where high levels of activity were observed. If the same assumption is made, oil spills which occur in the northern segment of the Lower Cook Inlet should be degraded at higher rates than those which occur near the mouth of the inlet (all other factors being equal).

At each shore station, we took two water samples; one approximately 30 meters from shore and one on the beach. In all cases, the V_{max} value for the sample taken on the beach was higher than that found a short distance offshore (Table 20a). The difference seen between these two values did not appear to be related to either geographical location or to tidal state.

2. Correlations with sample depth (above and below the halocline)

During the Beaufort Sea cruise on the Glacier, a comparison was made between the microbial activity seen in the surface waters and that seen below the halocline (15 m). The salinity at the surface was very low and reflected the effects of ice melt input. It was felt that such a fresh water input might significantly alter the relative microbial activity in these waters when compared to the water just below the halocline. In the four stations where this comparison was made, the surface waters showed as high or higher activity than that observed in the saltier waters at 15 meters (Table 20b). These observations tend to support our previous ice melt data.

3. Comparison of the relative microbial activities observed in all studies made to date

a. Summer data

Table 19 summarizes the data collected during all field studies made to date. The relative microbial activities observed during our three Beaufort Sea sampling periods reflect seasonal differences which were to be expected; namely that microbial activities in both seawater and sediments are higher in the summer than in the winter. Even though the average seawater temperature was significantly lower in the Beaufort Sea water samples, the level of potential microbial activity was very similar to that found in the Lower Cook Inlet. Similarly, the level of activity in the sediments was about the same during both summer sampling seasons in the Beaufort Sea as they were in the Lower Cook Inlet. It will be interesting to see if these trends continue in our future field studies.

b. Winter data

We collected data in both the NEGOA area and the Beaufort Sea within a few weeks of one another during the winter of 1976. The average V_{max} values observed in the offshore water samples analyzed from the NEGOA area was about one half of that observed in the Beaufort Sea. When the data collected from sediments were compared for the two areas, a much

different pattern emerged. The average microbial activity in the sediments was about two orders of magnitude greater in the NEGOA samples than in the Beaufort Sea winter samples.

4. Comparison of relative microbial activity using heterotrophic potential studies and uptake of labeled substrate at one concentration.

During the Beaufort Sea cruise, we attempted to evaluate the relative merits of using the Wright-Hobbie method of determining relative microbial activity which uses four concentrations of the same substrate (labeled glutamic acid in this case) and uptake of glutamic acid, glucose and algal protein hydrolysate at one substrate concentration. We also wanted to compare the relative utility of using more than one substrate in our routine measurements of relative microbial activity in natural microbial populations. The rational behind these studies was twofold; first, to determine which substrates should be used to give the most representative determination of relative microbial activity and secondly, to determine if measurements using one substrate concentration could be substituted for studies using multiple concentrations.

The correlation coefficients between the relative levels of microbial activity using these different substrates and methods have been calculated and are presented in Tables 21a and 21b. These comparisons show that there is no significant advantage of using algal protein hydrolysate instead of glutamic acid for these determinations because the correlation coefficient between these two are very high in all cases (a correlation of 0.90 being the lowest observed). As a whole, the measurements made using glucose correlated the worst when compared with the other substrates and methods used. Since glucose is a much different substrate than either glutamic acid or the algal protein hydrolysate, it is undoubtedly measuring somewhat different functions and possibly different types of microbial populations. Since the uptake of glucose may reflect the relative abundance of a specific group of organisms that might be missed by the glutamic acid studies, we will continue to make measurements using labeled glucose.

At present, the only way that one can measure the potential maximum uptake of a substrate by a mixed natural microbial population is to measure the uptake and respiration of that substrate at several concentrations. This, of course, is very time consuming and tedious work. If similar types of data could be generated using only one substrate concentration, both the field work and the analysis of the data could be greatly simplified. We felt that if, in some samples, only one substrate was used, the errors generated by this approach could be offset by the greater number of samples that could be analyzed with the same work load. We compared the levels of microbial activity observed using one concentration with that obtained from V_{max} determinations using four concentrations of the same substrate. Tables 21a and 21b show the resulting correlation coefficients when these factors are compared. The relatively low correlation that was observed in Beaufort Sea water samples between these methods indicates that under certain conditions,

relative microbial activity should be determined by the multiconcentration method. The high correlations between these factors in all sediments studied and in the water samples taken in the Lower Cook Inlet indicated that the one concentration determination may be useful under other conditions. This method will not allow the determinations of substrate turnover time or substrate transport constents but these have shown to be of very limited use in our past studies.

5. Correlation between relative microbial activity and other factors

When the heterotrophic potential data collected on sediments taken in the NEGOA area are compared with the distribution of benthic detritus utilizing organisms, there was a positive correlation. (Dr. Howard Feder, personal communication). This indicates that the heterotophic potential data are reflecting either levels of bacteria that are being utilized directly by the benthic community or that this indicates that presence of detriuts which is in turn colonized by bacteria. Regardless of the exact relationship, relative microbial activity (V_{max}) values may be used as a rough indicator of areas where higher concentrations of certain benthic organisms might be expected.

A comparision was made between the relative microbial activity in seawater samples taken in the Cook Inlet with crude oil degradation potentials as measured by Dr. Atlas and his associates. In this case the relative microbial activity was determined by the uptake of labeled glucose at one substrate concentration. The correlation coefficient of 0.85 was observed in this study. When a similar comparison was made between the same parameters during the summer Beaufort Sea study, the correlation was much lower. During our next cruise in the Cook Inlet we will compare these parameters again to determine if a correlation exists.

B. Total bacterial concentrations in water and sediment samples

Total bacterial numbers were measured using direct observations through an epilfuorescent microscope. A summary of the direct count data is shown in Table 19. When one compares the counts found in the seawater samples, one is struck by the consistancy of the values obtained. Even though there were large differences in the average V_{max} values obtained in the various studies, the average concentration of bacteria was relatively constant. In general, the bacterial concentrations in both water samples and in sediments were lower during the winter sampling trips than in the summer and fall trips.

C. Percent respiration (mineralization)

In the water samples studied to date, there is a seasonal trend that has been found in the percent respiration (microbial mineralization of substrate to CO_2). In the winter studies, the percent respiration was higher on the average than that found in the summer studies. In contrast to this, the average percent respiration in the sediments was relatively constant regardless of the season.

When the percent respiration in sediment samples is compared with that measured in the water samples, another trend is seen. The average percent respiration was typically higher in water samples than in sediments. It was also noted that the average percent respiration observed in winter ice melt (98%) was higher than in any other type of sample studied. These data suggest to us that the percent respiration may reflect basic different physiological states in the samples analyzed. A higher percent respiration indicates that more of the organic substrate taken up by the microorganisms is being utilized for energy requirements and that less is being channeled into biosynthesis and thus into increasing cell mass. This pattern is typically found in bacteria that are in an unbalanced growth condition. This condition can be brought about by placing bacteria in a stress environment or by exposing them to medium containing an inadequate supply of required growth factors. In the samples we have studied, we feel that the percent respiration probably reflects the presence or absence of an adequately balanced nutritional source in these samples.

D. Laboratory studies (oil enrichment experiments)

Three experiments were conducted; the first was designed to determine the variation that might be expected between two "identical" subsamples neither of which had been perturbed by crude oil. The second and third experiments were designed to show the effects of crude oil on microbial function and cell concentrations in natural seawater samples.

If the V_{max} data for glutamic acid uptake in the three experiments are compared, the variations seen in experiment 1 would account for the differences seen between the oil and nonoil controls in experiments 2 and 3. When the differences in the percent respiration for glutamic acid in the nonoil and control aquaria are compared, there was a significant difference seen in experiment 2 but not in experiment 3 (Figures 11, 12 and 13).

A similar analysis of the acetate uptake date (Figures 8, 9, and 10) reveals that there might be a positive effect of crude oil on the V_{max} of acetate uptake in the early states of incubation before the 10th day; however, the data presented in Figure 8 (experiment #1) represent only rough estimates of the true values, therefore, it is difficult to assess the significance of the trends observed in experiments #2 and #3. It can only be stated at this time that crude oil might have a positive effect on acetate V_{max} in the early stages of exposure to crude oil.

When the effect of crude oil on the percent of acetate respiration was studied (Figures 14, 15, and 16), there was no significant consistent effect observed. The same was true of the effect of crude oil on the relative levels of "total" bacterial numbers as determined by plate count on Lib X agar plates (Figures 17, 18, and 19). In contrast to these data are the results of the effects of crude oil on hydrocarbon utilizing bacteria as determined by plate counts on crude oil plates. The results of both experiments 2 and 3 show that the levels of hydrocarbon utilizing bacteria were higher in the crude oil enrichments than in the control after about two weeks incubation (Figures 20, 21, and 22).

During the course of experiment #1, the uptake of algal protein hydrolysate and glucose at one substrate concentration was also followed

(Figures 23 and 24). These data suggest that it might be profitable to include similar measurements into the routine analysis of sediment and water samples taken in the field. Although these types of data do not generate information about the maximum potential rates of substrate uptake, they do produce percent respiration data and may produce useful information about relative uptake rates of a complex substrate in various natural populations. The glucose data generated patterns of substrate utilization which were different from any of the other substrates tested. It is quite possible that these uptake patterns reflect the metabolism of a different population than that measured using the other substrates. This difference was especially noticeable at time 13 days.

In view of these results, we started measuring glucose uptake at one substrate concentration on all samples taken in the field. These data, along with the uptake of algal protein hydrolysate and glutamic acid uptake kinetics should give us more complete picture of relative heterotrophic potential in seawater and sediment samples taken during field studies.

In summary, this series of experiments showed that of the parameters that we studied, only the concentration of hydrocarbon utilizing bacteria in the seawater samples appeared to be significantly altered in both experiments. It should not be concluded on the basis of these results that crude oil has no effect on the heterotrophic populations found in seawater in general for the following reasons: (1) the number of factors measured were few relative to all functions in heterotrophic bacterial populations (2) the variability between two "identical" subsamples was relatively large; thus there might have been effects that we were not able to measure using the two aquaria system (3) the system that we studied was a highly artifical one in that the seawater samples were contained within a vessel; this is a condition which is known to greatly alter bacterial populations because of surface effects, the accumulation of metabolic byproducts and the depletion of nutrients (4) the water samples were taken from temperate bay waters which may not be typical of colder, less perturbed waters.

VIII. Conclusions

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

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

3. The waters taken from the northern section of Cook Inlet showed much higher levels of activity than those taken near the mouth of the inlet. Here again, we would expect that initial rates of biodegration would be higher in the waters of the Upper Cook Inlet and lower near the mouth.

4. A study of seasonal variations in heterotrophic potential found in water and sediment samples taken in the Beaufort Sea indicate that basic physiological changes do occur during the winter months. How microorganisms might react to crude oil purturbation during this season is not known.

5. The presence of crude oil may effect the growth and/or production of chinase in chinoclastic marine bacteria isolated from the Beaufort Sea.

6. True psychrophilic crude oil oxidizing bacteria appear to be relatively rare in the Beaufort Sea sediments.

7. The decrease of microbial heterotrophic activity seen in the Beaufort Sea during the winter months is probably due to a reduction in the availability of organic nutrients rather than as the result of reduced water temperatures.

8. Studies on the effects of crude oil on natural microbial heterotrophic populations in seawater indicate that crude oil does not suppress heterotrophic activity but it may act to select for certain types of bacteria. There are indications, however, that crude oil may inhibit bacterial growth for a short period after the system is purturbed. These studies were conducted on temperate estuarine water samples and the results may not apply to Arctic marine waters under all conditions. Two pilot studies of this nature were made on summer Beaufort sea water samples with similar results.

9. In the above studies, the concentration of hydrocarbon utilizing bacteria increased with incubation time.

10. The relatively low heterotrophic activity seen in the NEGOA area water samples during the March, 1976, study, indicate that the initial rates of crude oil degradation may be somewhat lower here than in other areas that we have studied.

11. Relative microbial activity rates observed in ice samples taken in the Beaufort Sea indicate that the microbial activity in sea ice fluxuates seasonally the same way that activity in seawater does. It seems quite likely that both bacteria and algae are trapped in brine pockets within the ice and that these populations respond to the absence of presence of light much the way populations in the water column respond.

12. A shallow surface lens of relatively fresh water was observed at most stations studied during the August, 1976, cruise in the Beaufort Sea. This relatively unmixed water mass could become saturated with disolved hydrocarbons during an oil spill.

IX. Needs for future study

1. This study should be continued in potential lease areas on a seasonal basis. More specifically, the same types of studies that have been described in this report should be continued in the region of the barrier islands in the Beaufort Sea, the area surrounding Kayak Island in the NEGOA area, Kamishak and Kachemak Bays in the Lower Cook Inlet, and the potential lease areas near Kodiak Island. These are all areas that have been identified as particularly important geographical areas during the recent synthesis meetings.

2. There is still very little known about how crude oil might effect such specific microbial functions as nitrogen fixation and dentrification. Alterations to those and other critical microbial processes could greatly effect all trophic levels in Arctic marine ecosystems. Studies should be continued in the area of the effects of crude oil on these processes.

3. By far the most important data gap that currently exists is the estimation of potential crude oil degradation rates in the Arctic marine environment. This is an unknown that was continually mentioned at both the Beaufort Sea and the NEGOA synthesis meetings. Of particular concern is how fast will crude oil be degraded in the sediment and waters of the Beaufort Sea? This severe climate may well allow only very slow decomposition of crude oil especially if an oil spill occurred during the winter months. According to the current developmental scenarios, crude oil would most probably be transported to shore from offshore wells via a buried pipeline. If a break did occur, crude oil would be spilled directly into the sediments. There is a possibility that oil introduced into the sediments in this way may be retained for an extended period of time, thus, acting as a chronic source of potentially hazardous hydrocarbon compounds into the surrounding water.

For these reasons, the interaction between crude oil and natural microbial populations in Arctic marine sediments should be studied in detail. This is a study that should be coordinated as closely as possible with those investigators studying other aspects of the benthic ecosystems.

X. Summary of 8th quarter operation.

a. Laboratory work

1. Finished the analysis and reporting of data collected during the October cruise in the Lower Cook Inlet.

2. Continued work on the physiology of the psychrophilic hydrocarbon degrading bacteria isolated from the Beaufort Sea.

3. Initiated studies on the effects of crude oil on nitrogen fixing bacteria and chitinoclastic bacteria isolated from Arctic marine waters.

b. Other activities

Attended the NEGOA and Beaufort-Chukchi Sea synthesis meetings. In our estimation, these meetings were extremely important to all that attended. It was our first opportunity to compare data collected by other investigators in different disciplines. As a result of these meetings,. several correlations came to light that might not have otherwise become evident. It also gave us a better understanding of the state-of-the-knowledge and helped to clarify areas of study which would provide the greatest future benefit.

XI. Projected studies during the next quarter

1. We are scheduled to participate in a cruise in the Lower Cook Inlet during the month of April. We will be studying the same parameters that we have studied in past cruises, and sampling at the same stations that we occupied during the October cruise in the same area.

2. We will continue our studies of crude oil degradation by psychrophilic marine bacteria and our studies of the effects of crude oil on bacterial nitrogen fixation.

3. We will also initiate our analysis of data collected during the above mentioned cruise.

Table 1. Date of sampling, sample number, station number and position information on samples taken in the Gulf of Alaska during the Discoverer cruise.

Station	Water sample number	Sediment sample number	Date	Time of sampling	Position of station Latitude (N)	Longitude (W)
d	GW201	GB201	3/15	1430	57° 39'	152° 30'
1	GW202	GB202	3/17	1600	59 50.3'	149 30.4'
4	GW203	GB203	3/18	0400	59 24.6'	149 04.2'
7	GW204	GB204	3/18	1330	58 58.8'	148 39.5'
68	GW205	---	3/18	2145	59 38.3'	147 48.0'
59a	GW206	GB206	3/19	0530	59 17.2'	147 15.6'
57	GW207	GB207	3/19	1530	59 45.5'	146 31.0'
55	GW208	---	3/19	1940	60 04.7'	146 44.4'
e	GW209	---	3/20	1300	60 06.8'	149 33.2'
53a	GW210	GB210	3/21	0100	60 23.3'	146 54.5'
53b	GW211	GB211	3/21	0540	60 32.5'	147 00.6'
52	GW212	GB212	3/21	1145	60 08.8'	145 02.2'
50	GW213	GB213	3/21	1620	59 47.0'	145 10.2'
42	GW214	GB214	3/22	0645	59 54.3'	143 52.3'
44	GW215	---	3/22	1130	59 38.1'	144 02.0'
37	GW216	GB216	3/22	1800	59 16.7'	142 56.9'
41	GW217	GB217	3/23	0800	59 45.9'	142 44.0'
30	GW218	GB218	3/23	1445	59 44.2'	141 29.0'
32	GW219	GB219	3/23	2100	59 27.1'	141 48.5'
f	GW220	GB220	3/24	1600	59 32.2'	139 47.1'
g	GW221	---	3/24	1400	59 44.7'	140 07.5'
29	GW222	---	3/25	0940	59 34.5'	140 06.6'
28	GW223	GB223	3/25	1130	59 26.7'	140 19.0'
27	GW224	GB224	3/25	1600	59 18.4'	140 29.6'
a	GW225	GB225	3/26	1600	60 20.8'	146 38.4'
b	GW226	GB226	3/26	1630	60 20.6'	146 38.5'
c	GW227	---	3/26	1700	60 21.4'	146 37.4'

Table 2. Sampling date, sample number, station number and position information on samples taken in the Beaufort Sea during April, 1976.

Station	Water sample number	Sediment sample number	Date	Position of station Latitude (N)	Longitude (W)
13a	BW101	---	4/5	71° 30'	156° 06'
14a	BW102	---	4/5	71 34.5'	155 35'
15a	BW103	---	4/5	71 39'	155 04'
3	BW104	---	4/5	71 21.6'	156 21.0'
13	BW105	BB101	4/7	71 23'	155 56'
14	BW106	BB102	4/7	71 23'	155 26'
15	BW107	BB103	4/7	71 23'	154 54'
3	BW107a	---	4/9	71 21.6'	156 21.0'
2	BW108	BB104	4/10	71 21.4'	156 27'
3	BW109	BB105	4/10	71 21.6'	156 21'
81	BW112	BB107	4/12	70 31'	147 24'
80	BW113	BB108	4/12	70 18'	147 30'
74	BB111	BB106	4/12	70 32'	148 27'
85	BB110	---	4/14	71 08'	146 30'
83	BW114	BB109	4/14	70 47'	147 00'
24	BW116	---	4/16	71 46'	151 52'
23	BW117	---	4/16	71 36'	152 12'
22	BW118	BB111	4/16	71 26'	152 22'
21	BW119	BB112	4/16	71 19'	152 33'
20	BW120	BB113	4/18	71 08'	152 55'
17	BW121	BB114	4/18	71 23'	153 50'
16	BW122	BB115	4/18	71 23'	154 22'
14a	BW123	---	4/18	71 34.5'	155 35'

Table 3. Station location and the date and time of sampling for all water samples taken during the August Beaufort Sea cruise on board the Glacier. (*) those water samples that were taken at a depth of 15 meters, all other water samples were taken within one meter of the surface.

Station	Water sample number	Sediment sample number	Date	Time of sampling	Position of station Latitude (N)	Longitude (W)
74	BW201	BB201	8/23	1530	70° 36'	148° 12'
74	BW202	BB202	8/24	1920	70° 36'	148° 12'
80	BW203	BB203	8/25	1930	70° 32'	147° 33'
81	BW204	BB204	8/26	0830	70° 39'	147° 37'
41	BW205	BB205	8/27	1030	70° 50'	149° 00'
42	BW206	BB206	8/27	2245	70° 57'	149° 33'
31	BW207	BB207	8/28	1415	71° 08'	151° 19'
24	BW208	BB208	8/29	1300	71° 43'	151° 47'
24	*BW209	---	8/29	1300	71° 43'	151° 47'
23	BW210	---	8/30	2200	71° 33'	152° 03'
23	*BW211	---	8/30	2200	71° 33'	152° 03'
22	BW212	BB212	8/30	1600	71° 22'	152° 20'
22	*BW213	---	8/30	1600	71° 22'	152° 20'
21	BW214	BB214	8/31	0545	71° 19'	152° 32'
20	BW215	BB215	9/1	0835	71° 08'	152° 57'
16	BW216	BB216	9/2	0315	71° 23'	154° 21'
15a	BW217	---	9/2	1410	71° 36'	155° 32'
15a	BW218	BB218	9/2	1410	71° 36'	155° 32'

Table 4. Summary of station location, water depth at station and the time and date at which the station was occupied during the October cruise in the Lower Cook Inlet. (*) stations taken on shore.

Station number	Surfline water sample	Other water samples	Sediment samples	Date	Time of sampling	Station position Latitude(N)	Longitude(W)
205	---	GW301	GB301	10/18	1545	59° 07.8'	152° 45.5'
216	---	GW302	---	10/19	0400	59° 18.8'	152° 13.8'
215	---	GW303	---	10/19	1330	59° 19.6'	152° 44.3'
212	---	GW304	GB304	10/20	0245	59° 33.4'	153° 25.4'
*R	GW305	GW306	---	10/20	1130	59° 38.8'	153° 25.0'
*M	GW308	GW307	GB308	10/20	1245	59° 44.2'	153° 21.5'
*I	GW310	GW309	---	10/21	1110	59° 22.5'	153° 59.3'
204	---	GW311	GB311	10/21	1600	59° 15.8'	153° 40.8'
225	---	GW312	---	10/22	0300	59° 32.5'	152° 35.9'
229	---	GW313	GB313	10/22	1000	59° 41.9'	151° 10.3'
*J	GW315	GW314	---	10/22	1000	59° 34.7'	151° 11.1'
*K	GW317	GW316	---	10/22	1130	59° 36.4'	151° 24.0'
227	---	GW318	GB318	10/22	1500	59° 33.2'	151° 44.8'
226	---	GW319	---	10/23	0300	59° 32.9'	152° 08.5'
245	---	GW320	---	10/23	0950	59° 60.3'	152° 12.3'
246	---	GW321	---	10/23	1245	60° 02.3'	151° 45.9'
266	---	GW322	---	10/24	0245	60° 41.2'	151° 25.0'
265	---	GW323	---	10/24	0328	60° 33.7'	151° 50.2'
*L	GW325	GW325	GB324	10/24	1420	59° 50.3'	153° 15.0'
*Q	---	GW326	---	10/24	1524	59° 50.4'	152° 59.5'
214	---	GW327	GB327	10/24	2350	59° 20.9'	153° 15.7'
206	---	GW328	GB328	10/25	0130	59° 10.2'	153° 08.5'
204	---	GW329	GB329	10/25	0300	59° 16.1'	153° 40.9'
215	---	GW330	---	10/25	1000	59° 20.8'	152° 43.7'
*P	GW332	GW331	---	20/26	1530	58° 15.95'	154° 16.45'
207	---	GW333	GB333	10/27	2200	58° 59.7'	152° 52.8'
106	---	GW334	---	10/28	0230	59° 02.7'	151° 58.7'
105	---	GW335	GB335	10/28	0620	58° 50.2'	151° 21.3'
*0	GW337	GW336	---	10/28	1030	58° 55.3'	125° 00.1'

Table 5. Summary of physical and heterotrophic potential data collected for all water samples taken from the Gulf of Alaska during the March, 1976, Discoverer cruise. The V_{max} is the maximum potential velocity for the sum of both macromolecular synthesis and respiration reported as ng x liter^{-1} x hr^{-1}. The substrate used in these studies was (U-^{14}C) L glutamic acid. (*) indicates those samples taken from the beach. (low) indicates those samples with activities that were too low to give an accurate estimate of V_{max}. (high) indicates those samples with such high activity that no saturation curve could be established.

Sample number	Station number	Sample temp. °C	Salinity o/oo	Vmax	Percent respiration	Cells/ml x 10^5
*GW201	d	2.0	31.4	113	62	2.0
GW202	1	3.0	31.7	low	57	1.4
GW203	4	3.5	32.2	0.9	58	1.4
GW204	7	3.5	32.3	2.0	65	1.9
GW205	68	3.5	32.1	1.5	77	1.6
GW206	59a	4.0	32.2	low	73	2.0
GW207	57	4.0	32.1	1.0	71	1.2
GW208	55	4.0	31.9	0.9	93	2.6
*GW209	e	3.0	31.1	9.7	76	2.0
GW210	53a	3.0	31.8	1.9	71	1.2
GW211	53b	3.0	31.8	1.1	64	1.6
GW212	52	3.2	31.7	low	80	1.6
GW213	50	3.4	31.8	0.6	81	1.5
GW214	42	4.1	32.1	low	86	2.3
GW215	44	3.0	32.2	1.5	74	2.0
GW216	37	4.5	32.5	3.4	64	2.3
GW217	41	5.0	32.2	2.4	69	1.8
GW218	30	3.8	31.7	0.7	81	2.7
GW219	32	4.5	32.2	0.3	71	2.0
*GW220	f	3.0	32.2	49.4	78	1.5
GW221	g	4.0	30.7	1.3	53	2.2
GW222	29	4.2	31.9	erratic	89	1.6
GW223	28	4.7	32.2	low	89	2.1
GW224	27	4.8	32.2	1.7	69	2.2
*GW225	a	5.0	31.1	89.4	63	2.5
*GW226	b	5.0	30.7	high	55	2.7
*GW227	c	3.2	31.3	53.3	64	2.4
Average values		3.8	31.9	1.4 (offshore) 63.0 (Beach)	72	1.9

Table 6. Summary of data collected for all sediment samples taken from the Gulf of Alaska during the March, 1976, Discoverer cruise. The V_{max} is the maximum potential velocity for the sum of both macromolecular synthesis and respiration reported as μg x gram dry weight^{-1} x hr^{-1}. The substrate used in this study was L- (U-^{14}C) glutamic acid. The bacterial concentrations as determined by direct observation are reported as cells per gram dry weight.

Sample number	Station number	V_{max}	Percent respiration	Cells x 10
GB201	d	0.2	41	0.02
GB202	1	3.6	35	2.2
GB203	4	10.3	34	1.4
GB204	7	5.9	33	1.9
GB206	59a	---	40	1.4
GB207	57	2.0	27	2.1
GB210	53a	4.7	36	---
GB211	53b	2.9	30	1.4
GB212	52	1.0	41	2.6
GB213	50	5.2	42	3.0
GB214	42	1.0	45	1.6
GB216	37	0.5	69	0.1
GB217	41	3.2	40	3.1
GB218	30	0.1	72	0.3
GB219	32	1.3	49	1.3
GB220	f	---	59	0.01
GB223	28	2.0	49	1.2
GB224	27	5.8	39	2.4
GB225	a	---	50	0.9
GB226	b	27.5	46	1.7
Average values		4.5	44	1.5

Table 7. Summary of physical and heterotrophic potential data collected in the beaufort Sea during the April, 1976, field study period. The Vmax is the maximum potential velocity for the sum of both macromolecular synthesis and respiration reported as ng glutamic acid x liter^{-1}x hr^{-1}. (p) indicates that these stations are located near Prudhoe Bay. (pp) indicates that these stations are located in the Pitt Point area. All other stations were located near Point Barrow.

Sample number	Station number	Sample Temp. °C	Salinity o/oo	Vmax	Percent Respiration	Cells/ml x 10^5
BW101	13a	-1.6	28	14	98	1.7
BW102	14a	-2.0	22	12	96	0.8
BW103	15a	-2.0	19	4.2	96	1.5
BW104	3	-2.0	25	5.4	81	1.3
BW105	13	-2.0	25	5.4	96	1.4
BW106	14	-2.0	22	2.0	100	1.5
BW107	15	-2.0	17	0.9	99	1.7
BW107a	3	-2.0	25	4.3	69	1.9
BW108	2	-2.0	31	1.1	68	2.2
BW109	3	-2.0	29	0.7	54	1.0
BW110	85 (p)	-2.0	23	4.2	98	1.2
BW111	74 (p)	-1.5	28	2.8	90	2.6
BW112	81 (p)	-2.0	28	0.5	94	1.4
BW113	80 (p)	-1.5	29	0.7	85	1.3
BW114	83 (p)	-2.0	17	1.0	97	1.1
BW116	24 (pp)	-2.0	24	0.4	78	1.4
BW117	23 (pp)	-2.0	21	0.2	69	1.8
BW118	22 (pp)	-2.0	19	0.2	52	1.7
BW119	21 (pp)	-2.0	20	low	73	1.2
BW120	20 (pp)	-2.0	26	2.1	93	1.2
BW121	17	-2.0	28	1.4	94	1.7
BW122	16	-2.0	29	2.1	88	2.7
BW123	14a	-1.5	28	low	90	1.3
Average values		-1.9	24	3.1	85	1.5

Table 8. Summary of data collected for all sediment samples taken from the Beaufort Sea during the April, 1976, field study period. The V_{max} is the maximum potential velocity for the sum of both macromolecular synthesis and respiration reported as µg x gram dry weight^{-1} x hr.$^{-1}$. The substrate used in this study was L-(U-^{14}C) glutamic acid. The bacterial concentrations as determined by direct observation are reported as cells per gram dry weight.

Sample number	Station number	$V_{max} \times 10^{-2}$	Percent respiration	Cells $\times 10^{8}$
BB101	13	8.7	53	0.5
BB102	14	2.1	56	2.3
BB103	15	0.4	87	19
BB104	2	17.8	36	10
BB105	3	2.7	38	7.7
BB106	74	2.0	39	2.2
BB107	81	7.5	38	17
BB108	80	4.5	41	1.6
BB109	83	6.3	43	17
BB111	22	2.5	47	16
BB112	21	1.8	35	13
BB113	20	5.6	44	13
BB114	17	1.8	37	-
BB115	16	5.6	40	15
Average values		5.0	45	10

Table 9. Temperature and salinity data taken from water samples which were collected during the August, 1976, Beaufort Sea cruise on board the Glacier. In most cases, the bottom water samples were taken within a few meters of the bottom. The average values listed below are measurements made at each station. The sample numbers listed are those for the water samples that we analyzed for microbiological factors and were not the samples used to determine salinity and temperature. These measurements were made at each station by Dr. Horner.

Sample number	Surface		Five meters		Bottom	
	Temperature	Salinity	Temperature	Salinity	Temperature	Salinity
BW201	-0.2	10.0	-0.9	28.5	-1.4	30.3
BW202	"	"	"	"	"	"
BW203	-1.3	6.0	-1.6	28.9	0.7	31.7
BW204	0.3	5.1	-0.8	25.9	-1.6	30.2
BW205	--	10.8	--	--	--	31.9
BW206	0.4	7.6	-1.2	24.6	-1.1	31.6
BW207	--	19.1	0.8	22.8	-0.6	30.7
BW208	0.3	10.2	-1.0	25.6	-0.2	34.9
BW209	"	"	"	"	"	"
BW210	--	--	--	--	--	--
BW211	--	--	--	--	--	--
BW212	0.5	15.7	1.5	25.5	-0.9	32.2
BW213	"	"	"	"	"	"
BW214	0.3	17.4	1.7	25.8	-0.3	31.5
BW216	1.8	20.5	-0.3	26.2	0.3	30.0
BW215	1.2	17.4	0.2	25.3	2.1	29.4
BW217	0.1	8.3	2.3	25.8	0.2	32.0
BW218	"	"	"	"	"	"
Average values	0.3	12.3	0.6	25.9	-0.3	31.4

Table 10. Relative microbial activity observed in seawater and sediment samples taken during the August, 1976, cruise in the Beaufort Sea. The relative microbial activity is expressed in terms of the actual rate of substrate utilization (V_{max}). All of the sediment data is reported in terms of substrate taken up per gram dry weight of sediment per hr.

WATER

Sample number	Station number	Horner chart number	V_{max} (glutamic acid) ng/liter/hr	Algal protein DPM/liter/hr x 10^4	Glucose ng/liter/hr	Glutamic acid ng/liter/hr
BW201	74	15	85	4.5	9.7	15
BW202	74	15	41	6.2	12.7	24
BW203	80	17	34	2.4	6.0	16
BW204	81	18	-	4.4	9.0	22
BW205	41	19A	13	1.5	3.8	6.9
BW206	42	19B	31	4.2	7.8	13
BW207	31	20	14	2.4	4.9	7.3
BW208	24	21	6	1.4	3.7	1.6
BW209	24	21	6	2.3	3.4	2.8
BW210	23	22	27	2.8	3.9	12
BW211	23	22	5	1.3	2.7	4.5
BW212	22	23	6	1.3	4.5	4.7
BW213	22	23	0.4	1.1	2.9	0.5
BW214	21	24	4	0.4	1.3	1.1
BW215	20	25	-	0.6	2.1	1.2
BW216	16	26	56	1.6	6.5	4.7
BW217	15a	27	7	2.4	4.4	4.0
BW218	15a	27	2	1.0	2.7	1.5
Average values			21	2.3	5.1	7.9

SEDIMENTS

			µg/liter/hr	DPM/liter/hr x 10^4	ng/liter/hr	µg/liter/hr
BB201	74	15	0.10	0.9	1.6	0.10
BB203	80	17	0.28	2.9	15	0.18
BB204	81	18	0.18	2.3	5.8	0.16
BB205	41	19A	0.06	0.4	1.8	0.03
BB206	42	19B	0.02	0.3	1.3	0.02
BB207	31	20	0.06	0.8	3.9	0.07
BB208	24	21	0.02	0.5	1.7	0.02
BB212	22	23	6.40	10.3	4.9	0.82
BB214	21	24	0.09	0.5	2.5	0.04
BB215	20	25	0.13	1.7	4.9	0.10
BB216	16	26	0.21	1.5	3.0	0.11
Average values			0.69	2.0	4.2	0.15
Average values without BB212			0.12	1.2	4.1	0.08

Table 11. Percent respiration and bacterial cell concentrations measured in water samples collected during the August, 1976, cruise in the Beaufort Sea. The percent respiration was measured using ^{14}C labeled glutamic acid, glucose, and algal protein hydrolysate.

Sample number	Glutamic acid	Algal protein hyrdolysate	Glucose	Cells/ml seawater x 10^5
BW201	42	23	7	3.4
BW202	42	13	23	3.3
BW203	53	10	11	4.4
BW204	29	15	31	3.7
BW205	45	14	24	5.0
BW206	20	22	18	6.1
BW207	40	17	32	4.7
BW208	31	17	24	3.2
BW209	59	16	24	2.3
BW210	55	24	25	4.6
BW211	61	31	42	4.5
BW212	54	25	35	3.0
BW213	43	30	41	1.9
BW214	56	32	34	1.6
BW215	-	28	29	1.6
BW216	48	22	25	3.5
BW217	54	20	25	3.7
BW218	53	17	41	5.4
Average values	46	21	27	3.7 x 10^5

Table 12a. Percent respiration measured in water samples collected during the August, 1976, cruise in the Beaufort Sea. The percent respiration was measured using ^{14}C labeled glutamic acid, glucose and algal protein hydrolysate. (too low) those samples in which the level of microbial activity was too low to obtain an accurate measurement.

Sample number	Glutamic acid	Algal protein hydrolysate	Glucose
BB201	-	34	27
BB203	27	35	11
BB204	14	5	10
BB205	26	9	27
BB206	26	20	21
BB207	34	43	15
BB208	too low	32	21
BB212	32	31	20
BB214	29	31	25
BB215	35	31	25
BB216	31	27	18
Average values	28	28	20

Table 12b. Total numbers of bacteria present reported as the number of cells per gram dry weight of sediment. Those determinations were made on fixed samples using epifluorescent microscopy.

Sample number	Cells/g dry weight x 10^9
BB201	2.4
BB203	4.8
BB204	26.7
BB205	12.8
BB206	13.5
BB207	11.1
BB208	26.3
BB212	6.7
BB214	7.5
BB215	9.8
BB216	9.9
Average value	10.6 x 10^9

Table 13. Temperature and salinity data for water samples taken at the surface and a few meters off the bottom during the October, 1976, cruise in the Lower Cook Inlet. All depths are in meters and all salinities are in parts per thousand. (*) samples taken at shore stations.

	Surface		Bottom	
Sample number	Temperature	Salinity	Temperature	Salinity
GW301	9.0	27.5	8.5	--
GW302	9.5	27.0	--	--
GW303	9.5	27.0	10.0	27.0
Gw304	8.4	25.0	8.4	26.0
GW305*	8.0	23.3	--	--
GW306*	8.0	23.3	--	--
GW307*	5.5	22.5	--	--
GW308*	6.5	22.0	--	--
GW309*	12.0	21.0	--	--
GW310*	12.0	20.5	--	--
GW311	5.5	28.0	9.1	23.2
GW312	9.5	26.2	10.0	26.0
GW313	8.5	24.0	9.3	26.0
GW314*	10.0	25.0	--	--
GW315*	9.5	24.0	--	--
GW316*	12.0	24.0	--	--
GW317*	12.0	23.0	--	--
GW318	9.5	27.0	9.5	28.0
GW319	9.5	27.0	9.5	28.0
GW320	9.0	26.0	9.0	26.0
GW321	9.0	20.5	9.0	26.5
GW322	8.5	21.0	8.0	22.0
GW323	9.0	19.5	8.5	19.8
GW324*	6.5	16.3	--	--
GW325*	6.0	17.0	--	--
GW326*	8.0	23.0	--	--
GW327	9.0	26.0	9.0	26.0
GW328	9.0	27.5	9.0	27.0
GW329	9.5	25.0	9.8	26.5
GW330	9.5	28.0	--	--
GW331*	2.0	18.0	--	--
GW332*	-1.5	18.0	--	--
GW333	8.0	23.7	7.0	22.5
GW334	7.5	24.0	8.0	29.0
GW335	9.0	25.5	7.5	28.0
GW336*	8.0	27.5	--	--
GW337	5.0	27.5	--	--
Average values	8.3	23.8	8.8	25.7

Table 14. Relative microbial activity observed in seawater samples taken during the October Lower Cook Inlet cruise. The relative microbial activity is expressed in terms of the actual rates of substrate utilization or potential maximum utilization. (*) samples taken at shore stations. (@) samples with activity that was too high for accurate measurement of V_{max}. (¢) samples with activity that was too low for accurate measurement of V_{max}.

Sample number	Station number	V_{max} ng/ liter/hr	Algal protein DPM/liter/hr x 10^4	Glucose ng/ liter/hr	Glutamic acid ng/liter/hr
GW301	205	0.6	0.16	0.31	0.32
GW302	216	0.2	0.09	0.66	0.17
GW303	215	0.2	0.14	0.09	0.20
GW304	212	5.0	1.2	0.97	3.5
GW305*	R*	21	5.4	13	14
GW306*	R*	9.1	1.8	2.8	4.9
GW307*	M*	24	1.1	4.0	5.8
GW308*	M*	@	20.7	34	26
GW309*	I*	83	9.0	14	21
GW310*	I*	104	13.1	27	39
GW311	204	5.7	1.4	1.4	3.5
GW312	225	0.6	0.16	0.03	0.34
GW313	229	12.7	1.8	0.84	3.1
GW314*	J*	6.0	0.63	0.53	0.93
GW315*	J*	55	2.7	3.8	8.3
GW316*	K*	@	92.8	77	239
GW317*	K*	@	91.7	94	256
GW318	227	1.2	0.36	0.28	0.69
GW319	226	3.6	0.45	0.66	1.3
GW320	245	8.7	0.99	1.3	3.0
GW321	246	22	4.3	2.0	11
GW322	266	405	16.1	33	53
GW323	265	53	3.9	8.1	14
GW324*	L*	404	19.6	65	50
GW325*	L*	386	16.9	75	51
GW326*	Q*	23	-	-	11
GW327	214	3.1	0.55	0.97	1.3
GW328	206	0.9	-	-	0.2
GW329	204	5.2	0.47	0.68	1.1
GW330	215	0.5	-	-	0.22
GW331*	P*	9.7	-	-	3.5
GW332*	P*	11	-	-	2.1
GW333	207	0.8	-	-	0.25
GW334	106	¢	0.03	0.06	0.17
GW335	105	0.4	0.36	0.28	0.27
GW336*	0*	0.7	0.41	0.19	0.44
GW337*	0*	38	1.9	2.2	7.3
Average offshore values		27.9	1.9	3.0	4.9
Average offshore values without sample GW266		9.3	1.0	1.2	2.3
Average beach values		84	20	30	44

Table 15. Percent respiration and bacterial cell concentrations measured in water samples collected during the October Lower Cook Inlet cruise. The percent respiration was measured using ^{14}C labeled glutamic acid, glucose and algal protein hydrolysate. (too low) those samples where the activity was too low to accurately estimate the percent respiration.

Sample number	Glutamic acid	Algal protein hydrolysate	Cells/ml seawater Glucose	x 10^5
GW301	66	41	72	2.6
GW302	44	29	84	1.7
GW303	68	44	68	0.8
GW304	59	41	30	2.7
GW305	62	31	28	13.7
GW306	57	28	26	11.0
GW307	56	28	27	1.7
GW308	55	27	27	15.5
GW309	52	25	24	12.5
GW310	57	29	25	12.3
GW311	56	31	22	2.4
GW312	74	19	20	0.8
GW313	63	21	29	3.4
GW314	54	31	24	2.9
GW315	64	34	25	2.7
GW316	68	28	29	3.3
GW317	68	31	31	2.7
GW318	53	25	19	1.4
GW319	59	26	16	0.4
GW320	54	34	15	0.8
GW321	50	29	20	1.3
GW322	40	30	10	11.6
GW323	40	27	12	1.4
GW324	50	28	21	9.7
GW325	51	33	24	16.5
GW326	42	-	-	0.9
GW327	53	22	-	1.3
GW328	59	-	-	1.0
GW329	53	32	16	0.6
GW330	73	-	-	0.5
GW331	55	-	-	6.9
GW332	59	-	-	4.0
GW333	65	-	-	0.5
GW334	too low	24	13	0.5
GW335	68	too low	too low	1.3
GW336	78	31	31	0.2
GW337	68	34	26	1.2
Average values	58	30	28	4.2 x 10^5

Table 16. Relative microbial activity observed in sediment samples taken in the Lower Cook Inlet during the October, 1976, cruise. The relative microbial activity is expressed in terms of the actual rate of substrate utilization or potential maximum utilization. (*) sediment sample taken at a shore station that was essentially a soil sample.

Sample number	Station number	V_{max} µg/g dry weight/hr	Algal protein DPM/g dry weight/hr x 10^4	Glucose ng/g dry weight/hr	Glutamic acid µg/g dry weight/hr
GB301	205	0.11	1.4	2.3	0.07
GB304	212	0.13	3.6	1.9	0.13
*GB308	M	6.28	17.0	56.3	1.19
GB311	204	0.33	5.5	9.7	0.07
GB313	229	0.25	3.1	5.3	0.17
GB318	227	0.22	7.1	12.0	0.12
GB325	L	0.02	--	--	0.03
GB327	214	0.08	1.4	3.5	0.07
GB328	206	0.07	--	--	0.04
GB329	204	0.48	--	--	0.31
GB333	207	0.42	--	--	0.36
GB335	105	0.12	0.9	0.7	0.07
Average values		0.71	5.0	11.5	0.22
Average values without station GB308		0.20	3.3	5.1	0.13

Table 17a. Percent respiration measured in sediment samples collected during the October, 1976, cruise in the Lower Cook Inlet. The percent respiration was measured using ^{14}C labeled glutamic acid, glucose and algal protein hydrolysate.

Sample number	Glutamic acid	Algal protein hydrolysate	Glucose
GB301	41	39	43
GB304	53	51	42
GB308	50	40	23
GB311	49	38	25
GB313	53	48	21
GB318	44	40	20
GB325	44	-	-
GB327	40	31	28
GB328	43	-	-
GB329	49	-	-
GB333	46	-	-
GB335	38	46	25
Average values	46	42	28

Table 17b. Total numbers of bacteria present reported as the number of cells per gram dry weight of sediment. These determinations were made on fixed samples using epifluorescent microscopy.

Sample number	Cells/gram dry weight sediment x 10^9
GB304	5.3
GB308	5.6
GB311	1.4
GB313	10.0
GB318	0.4
GB325	0.4
GB327	3.0
GB328	1.2
GB329	13.0
GB333	1.5
GB335	3.3
Average value	4.1 x 10^9

Table 18. Biochemical and growth characteristics of psychrophilic and psychrotrophic bacterial strains isolated from crude oil agar plates.

Characteristic	30	45	47	52	53	59	G-3	G-6
gram reaction	-	-	-	+	+	+	-	-
growth on broth								
pellicle	-	-	-	-	-	-	+	-
ring	+	+	+	+	+	+	-	+
granular	-	-	-	-	-	-	+	-
even	+	+	+	+	+	+	-	+
slight	-	-	-	+	+	+	-	-
heavy	+	+	+	-	-	-	+	+
growth at various salinities								
0% NaCl	-	-	-	+	+	+	+	+
3% NaCl	+	+	+	-	#	-	+	+
10% NaCl	-	-	-	-	-	-	+	+
3.5% (W/V) Rila Salts	+	+	+	+	+	+	+	+
growth on special media								
Hugh Liefson (aerobic)	@	@	@	@	@	@	@	@
Hugh Liefson (anaerobic)	@	@	@	-	-	-	-	@
Simmon's citrate	+	+	+	-	-	-	#	#
Antibiotic sensitivity								
penicillin	-	-	-	-	-	-	-	-
pteridine O-129	-	-	-	-	-	-	-	-
enzyme production								
oxidase	+	+	+	-	-	-	+	+
catalase	*	*	*	*	*	*	#	+
chitinase	+	+	+	-	-	-	-	-
lipase	+	+	+	-	-	-	+	+
lysine decarboxylase	#	#	#	-	-	-	-	#
arginine "	-	-	-	-	-	-	-	-
ornithine"	-	-	-	-	-	-	-	-
gelatinase	+	+	+	#	#	#	+	+
nitrate reduction	+	+	+	-	-	-	#	+
indole production	+	+	+	-	-	-	-	-

\# marginal or weak reaction
@ growth with acid production
* very strong reaction

Table 19. Data summary of the average values measured during all field studies. (*) Average values calculated with one sample excluded; a value which we consider more typical.

Factor	Units	Beaufort Sea Summer 1975 Ave.	Range	Beaufort Sea Winter 1976 Ave.	Range	Beaufort Sea Summer 1976 Ave.	Range	Lower Cook Inlet Fall 1976 Ave.	Range	Gulf of Alaska Winter 1976 Ave.	Range
V_{max} (Offshore water)	ng x liter^{-1}x hr^{-1}	40	4 to 118	3.1	0.2 to 14	21	0.4 to 85	28 (9.3)*	0.2 to 405	1.4	0.3 to 3.4
V_{max} (Beach water)	ng x liter^{-1}x hr^{-1}	-	- -	-	- -	-	- -	84	0.7 to 404	63.0	9.7 to 113
V_{max} x 10^{-1} (sediments)	µg x gr dry weight^{-1}x hr^{-1}	5.2	0.2 to 17	0.5	0.04 to 1.8	6.9	0.2 to 64	7.1 (2.0)*	0.2 to 63	45	2 to 103
Percent Respiration (water)	%	59	44 to 76	85	52 to 100	46	20 to 59	58	40 to 78	72	53 to 93
Percent Respiration (sediments)	%	43	32 to 71	45	35 to 87	28	14 to 35	46	38 to 53	44	30 to 72
Number of bacteria x 10^5 (seawater)	cells x ml^{-1}	4.5	0.1 to 11.9	1.5	0.8 to 2.7	3.7	1.6 to 2.7	4.2	0.2 to 16.5	1.9	1.2 to 2.7
Number of bacteria x 10^8 (sediments)	cells x dry weight^{-1}	6.3	0.1 to 41.4	10	0.5 to 19	106	24 to 267	41	4 to 130	15	0.1 to 31
Sample Temperature (surface water)	°C	1.2	-0.8 to 3.2	-1.9	-2.0 to -1.5	0.3	-1.3 to 1.8	8.3	-1.5 to 12	3.8	2.0 to 5.0
Sample Salinity (surface water)	o/oo	20.5	9.0 to 26.5	24	17 to 29	12.3	5.1 to 20.5	23.8	20.5 to 27.5	31.9	30.7 to 35.5

Table 20a. A comparison of the V_{max} values measured in samples taken in the surf and those taken approximately 30 meters offshore during work in the Lower Cook Inlet. The V_{max} values are expressed in terms of ng glutamic acid taken utilized per liter of seawater per hr. (E) ebbing tide. (F) flooding tide. (too high) the microbial activity was too high to obtain an accurate measurement of V_{max}. *estimated value.

Station number	Tidal state	V_{max} of sample from beach	V_{max} of sample from offshore
P	E	11	10
R	F	21	09
O	E	38	0.7
J	F	55	06
I	F	104	83
M	F	*203	24
K	F	too high	too high

Table 20b. Comparison of the V_{max} values observed in seawater samples taken above and below the halocline during the Beaufort Sea cruise. The V_{max} values are in ng per liter per hr.

Station number	V_{max} in surface water samples	V_{max} in water samples taken below the halocline
24	6	6
23	27	5
22	6	0.4
15a	7	2

Table 21a. The correlation coefficients between the levels of potential and actual substrate utilization by natural microbial populations studied in the Beaufort Sea.

1. Seawater samples

Factors that were compared	vs.	Correlation coefficient
glutamic acid	Vmax	0.769
glucose	Vmax	0.770
algal protein	glutamic acid	0.902
algal protein	glucose	0.935
glutamic acid	glucose	0.884
algal protein	Vmax	0.648

2. Sediment samples

glutamic acid	Vmax	0.980
glucose	Vmax	0.092
algal protein	glutamic acid	0.962
algal protein	glucose	0.310
glutamic acid	glucose	0.239
algal protein	Vmax	0.967

Table 21b. The correlation coefficients between the levels of potential and actual substrate utilization by natural microbial populations studied in the Lower Cook Inlet.

1. Seawater samples

glutamic acid	Vmax	0.945
glucose	Vmax	0.757
algal protein	glutamic acid	0.995
algal protein	glucose	0.885
glutamic acid	glucose	0.853
algal protein	Vmax	0.934

2. Sediment samples

glutamic acid	Vmax	0.966
glucose	Vmax	0.983
algal protein	glutamic acid	0.923
algal protein	glucose	0.972
glutamic acid	glucose	0.976
algal protein	Vmax	0.917

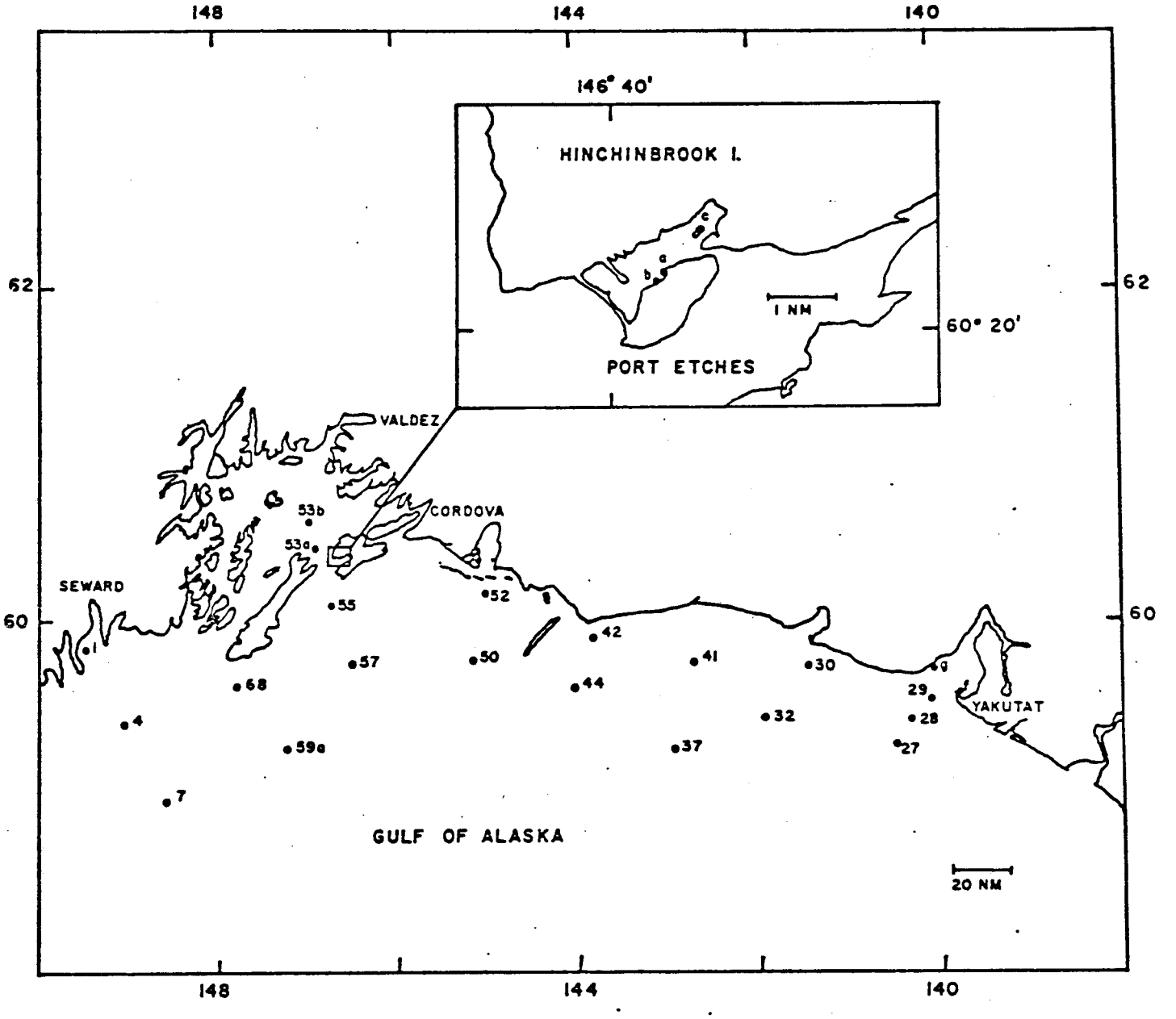

Figure 1. Stations sampled in the Gulf of Alaska during the March, 1976 cruise on the NOAA ship Discoverer.

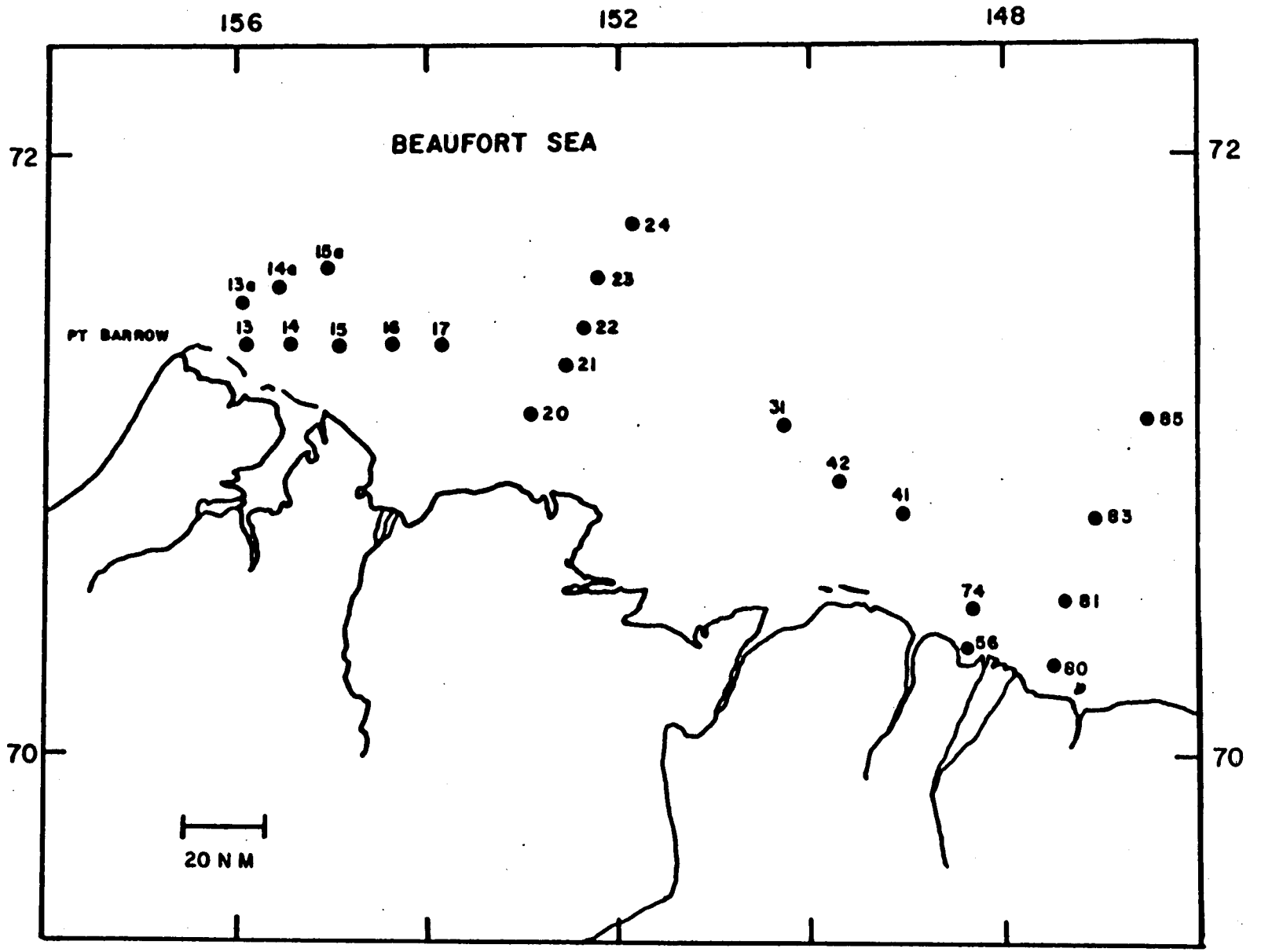

Figure 2. Stations sampled in the Beaufort Sea during April, 1976.

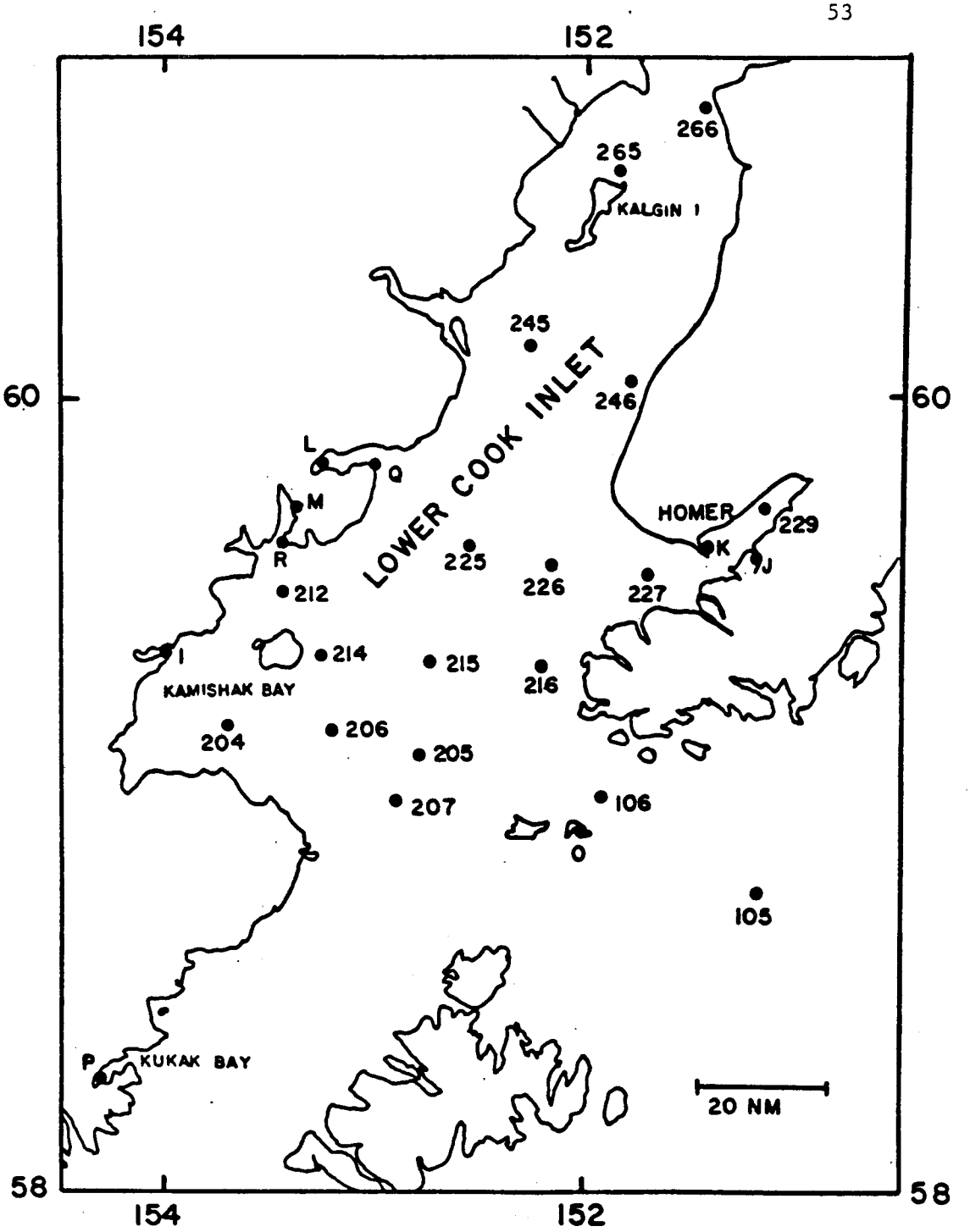

Figure 3. Location of stations sampled during the October, 1976 cruise in the Cook Inlet.

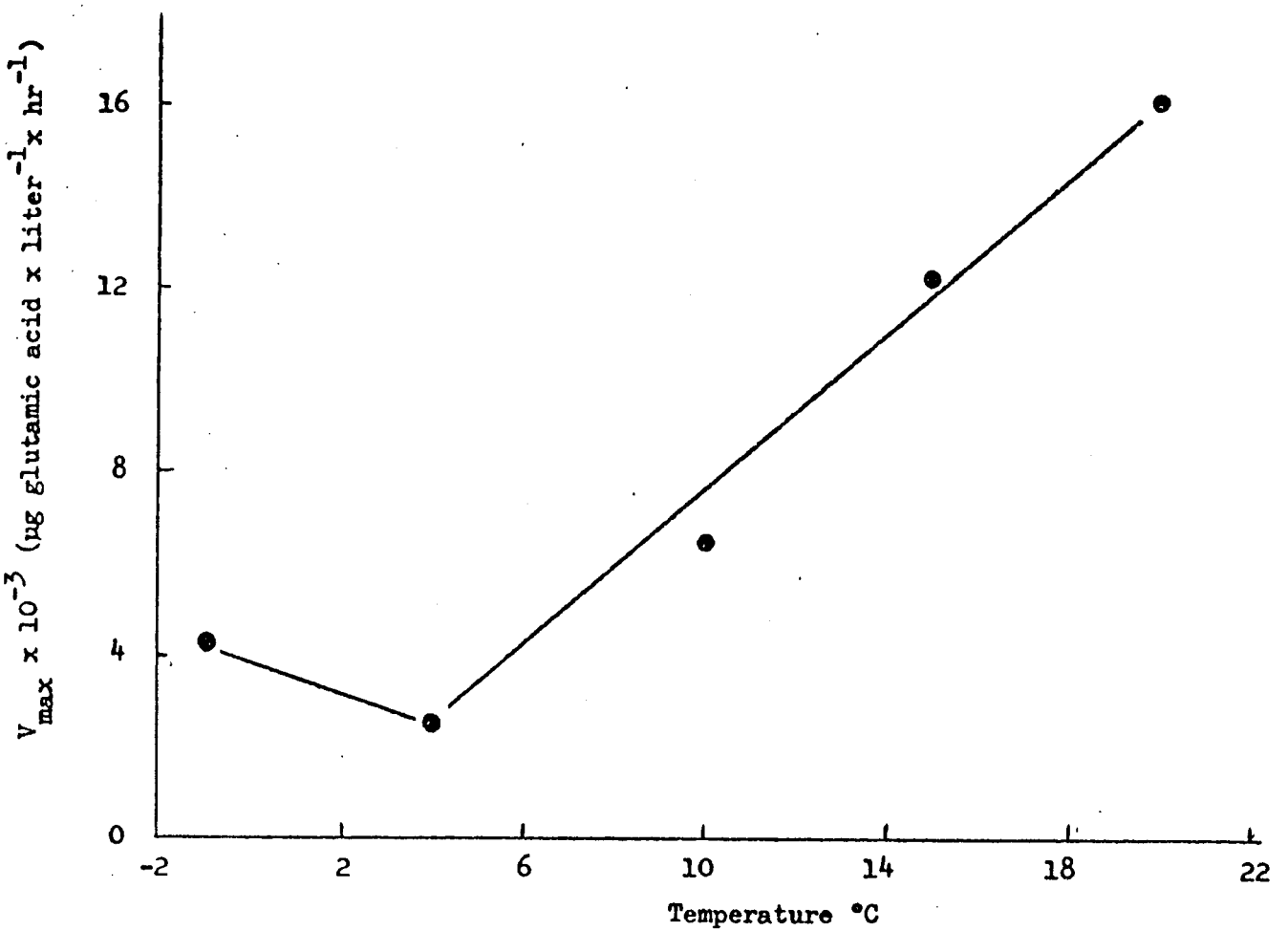

Figure 4. The effects of incubation temperature on apparent V_{max} values.

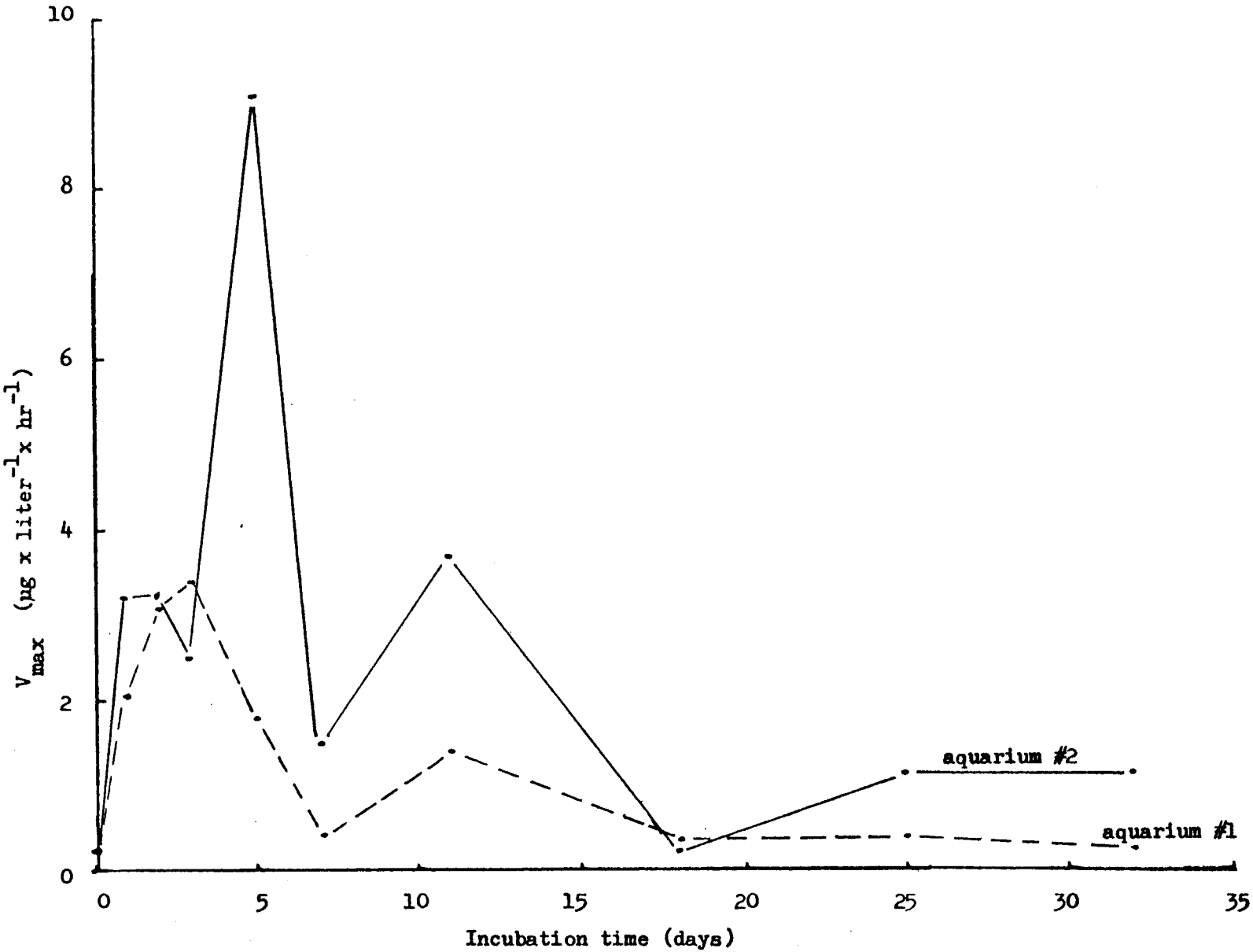

Figure 5. V_{max} of glutamic acid uptake in both seawater samples. Experiment #1.

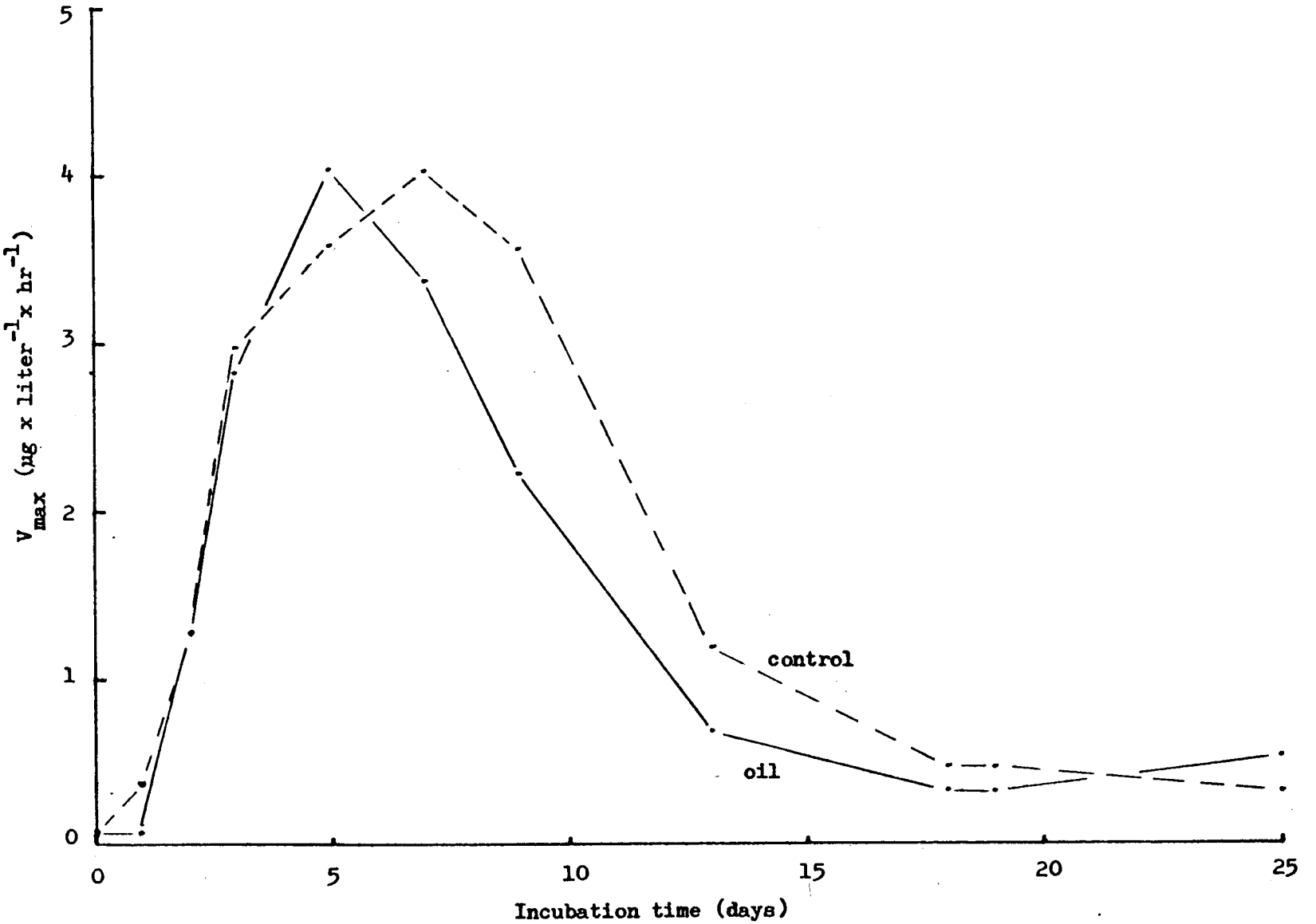

Figure 6. V_{max} of glutamic acid uptake in both the control and oil enrichment cultures. Experiment #2.

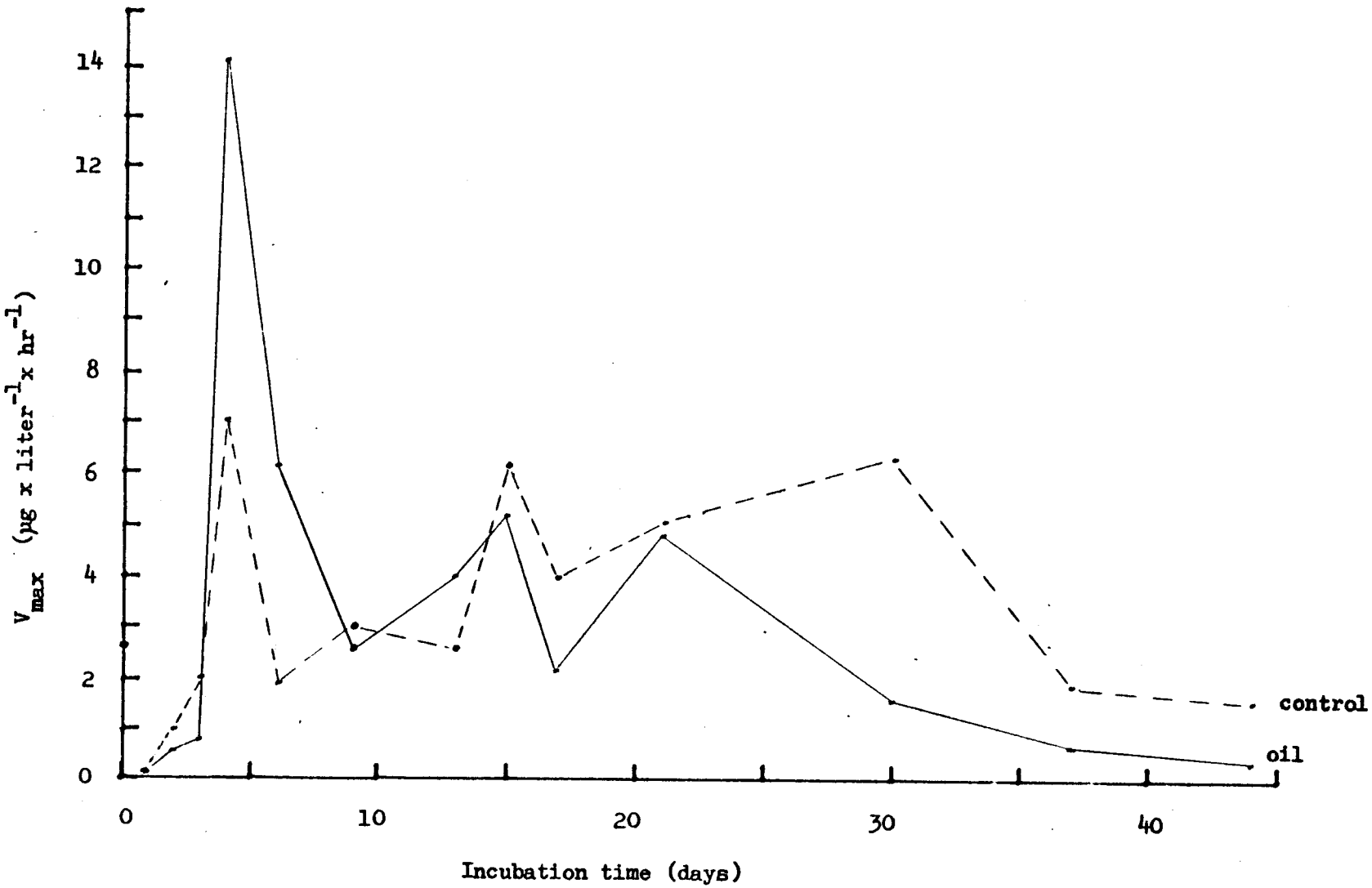

Figure 7. V_{max} of glutamic acid uptake in both the control and oil enrichment cultures. Experiment #3.

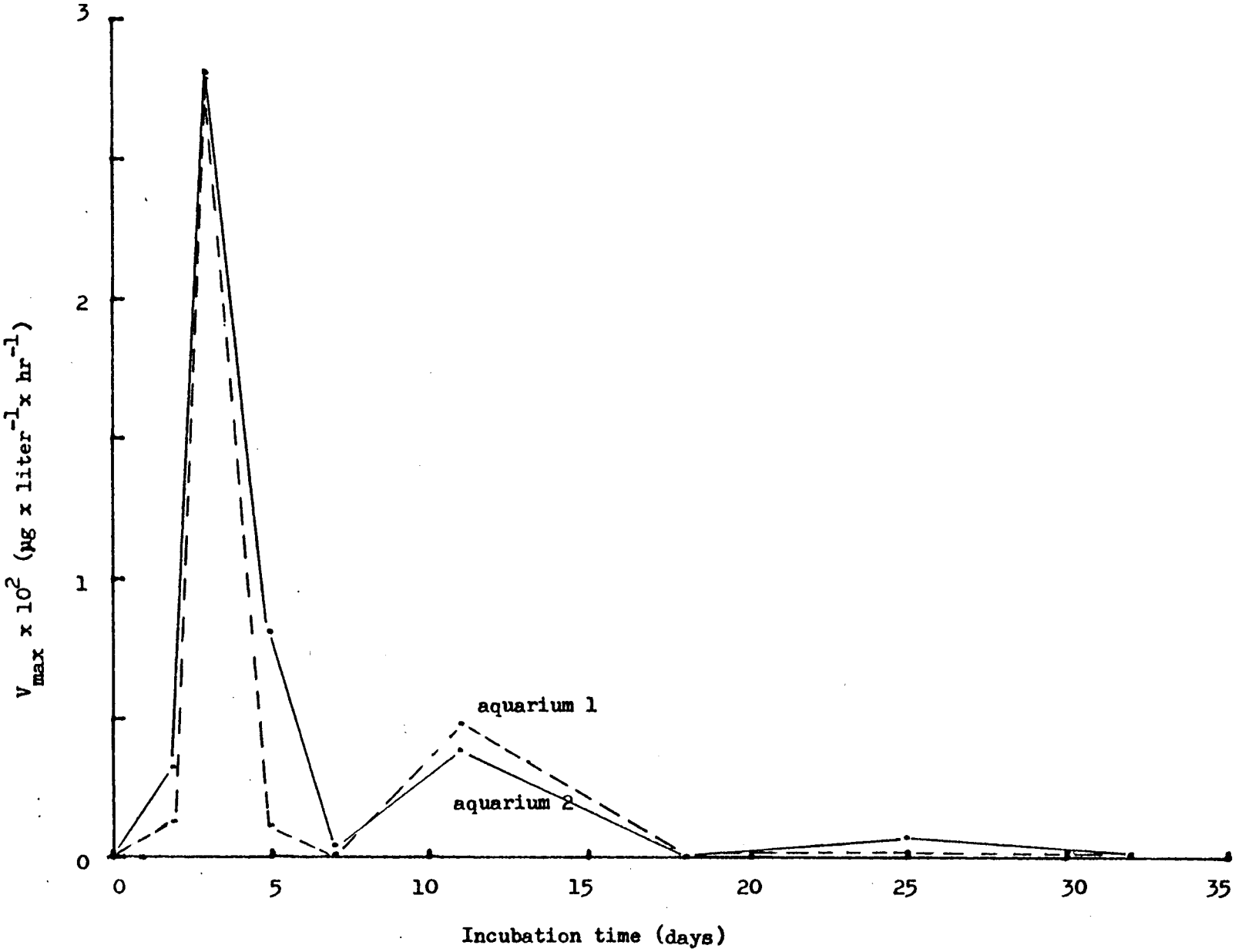

Figure 8. V_{max} of acetate uptake in both the water samples tested. Experiment #1.

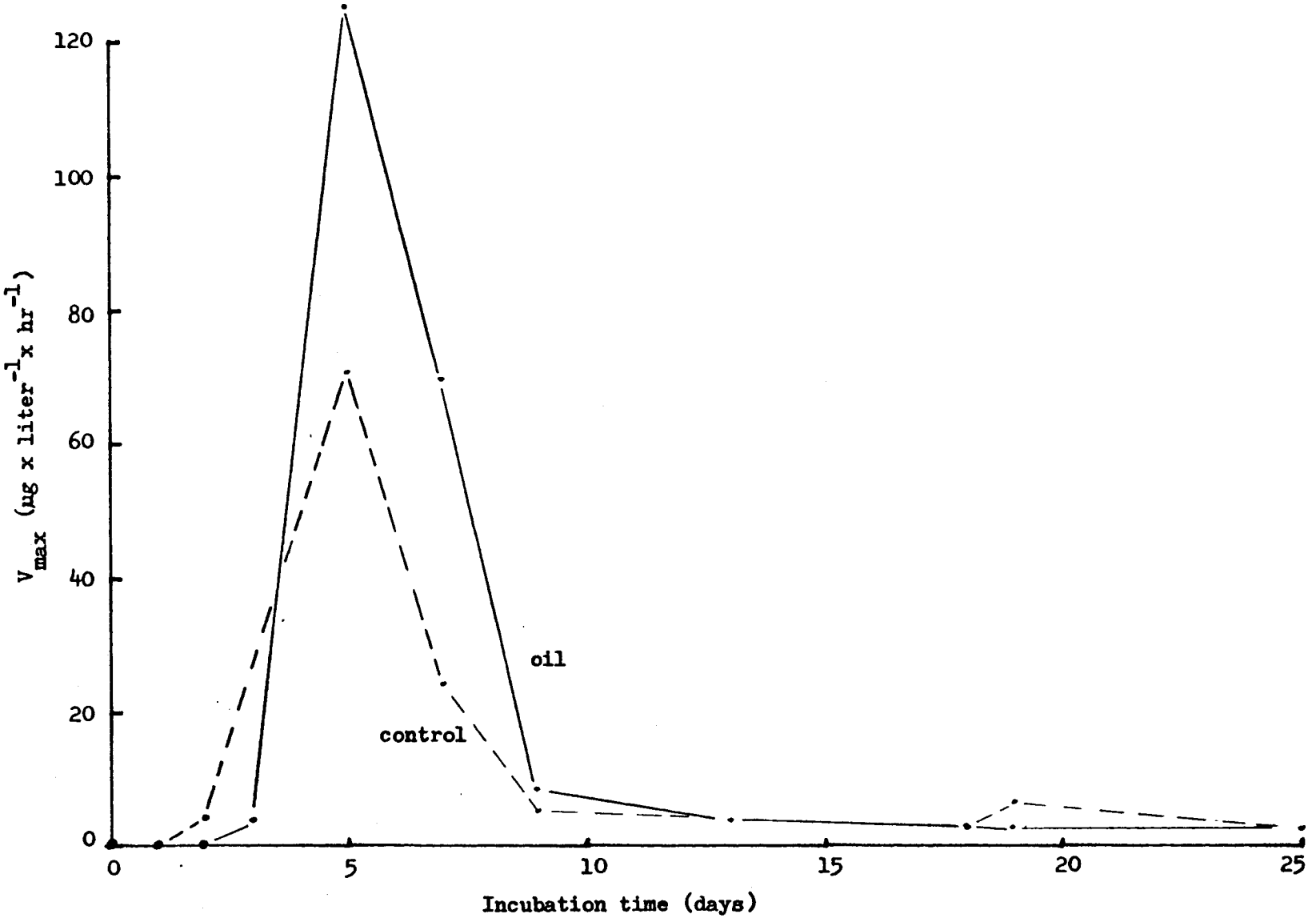

Figure 9. V_{max} of acetate uptake in both the control and oil enrichment cultures. Experiment #2.

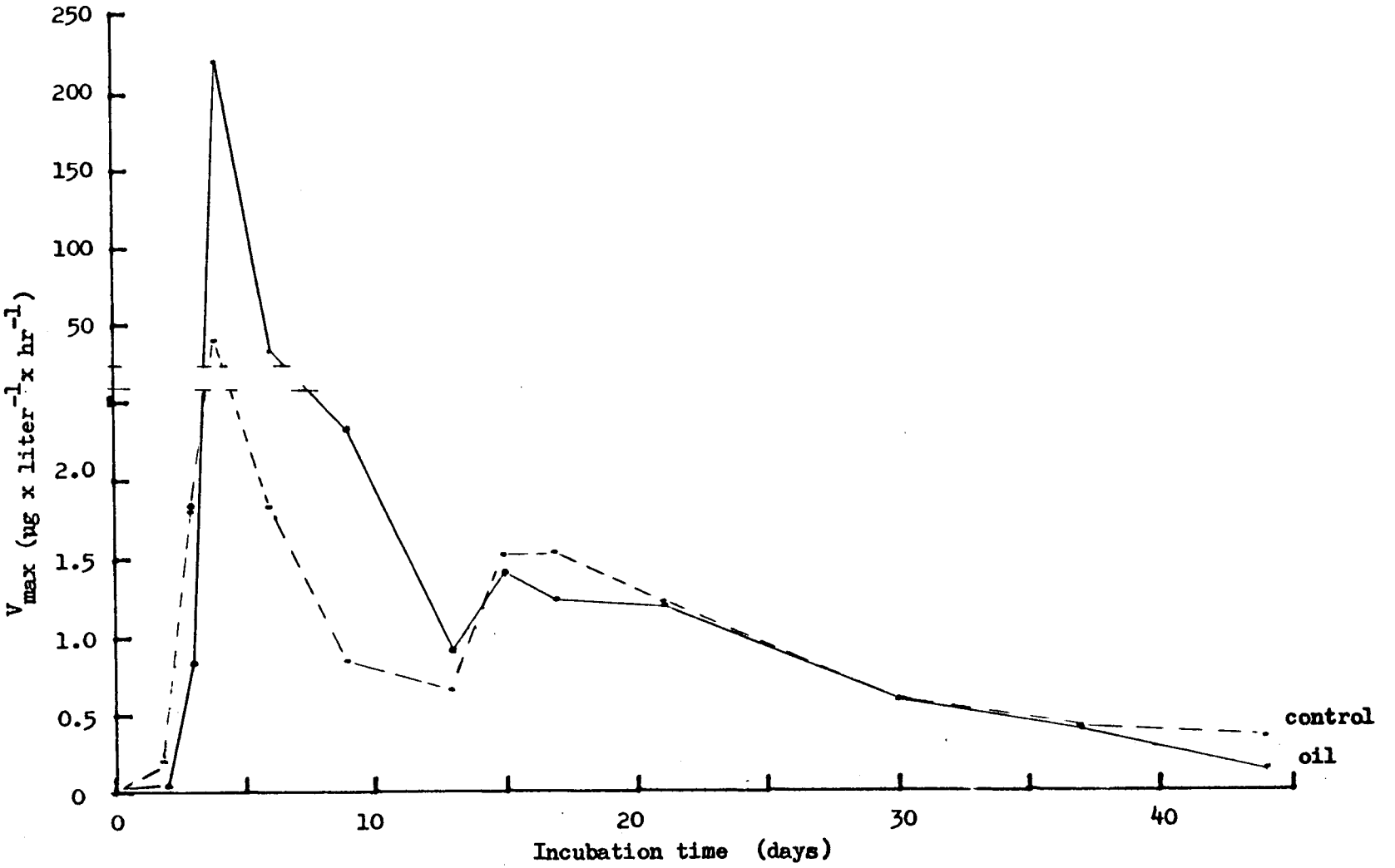

Figure 10. V_{max} of acetate uptake in both the control and oil enrichment cultures. Experiment #3.

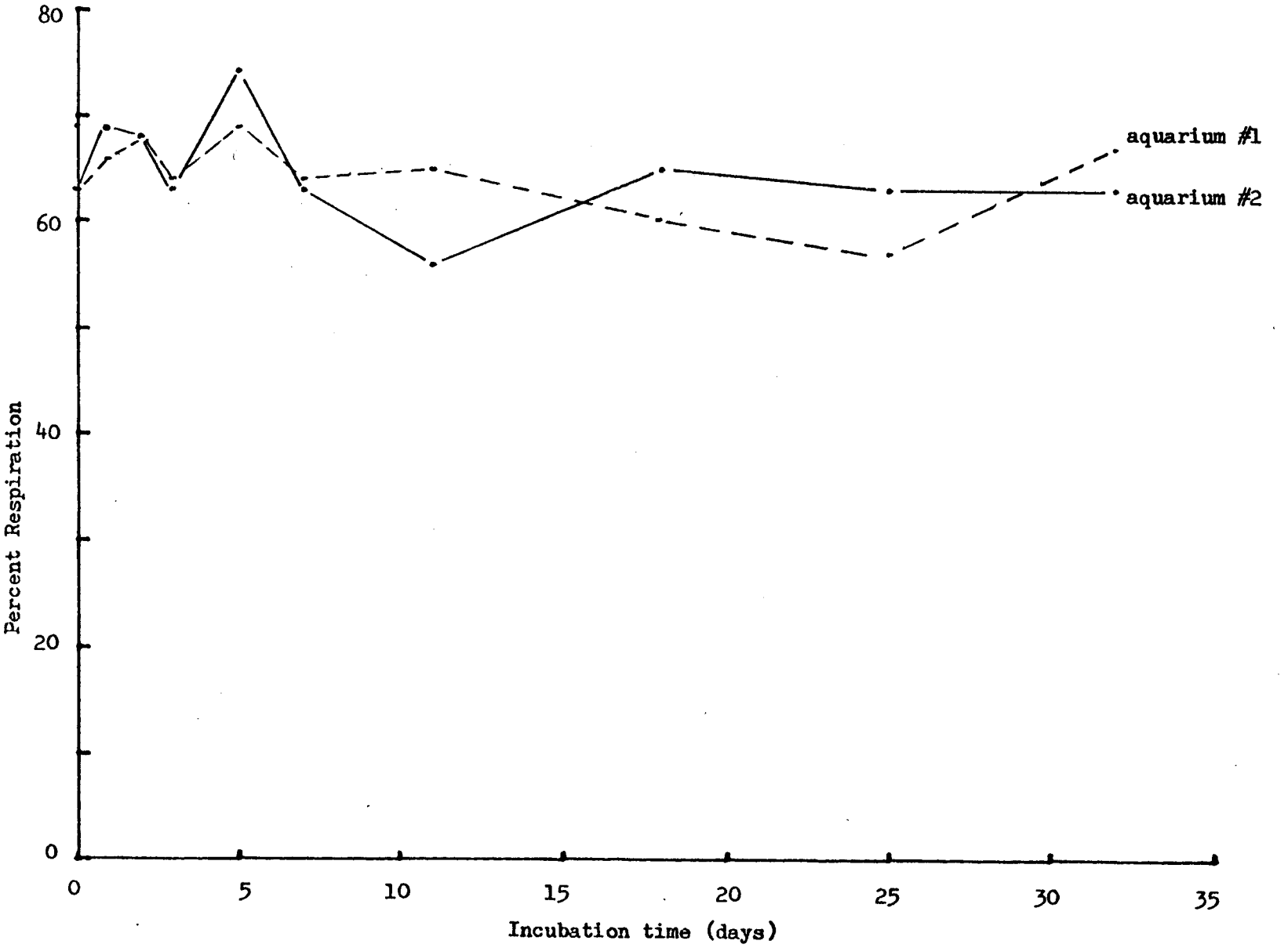

Figure 11. Percent respiration of glutamic acid utilization in both seawater samples. Experiment #1.

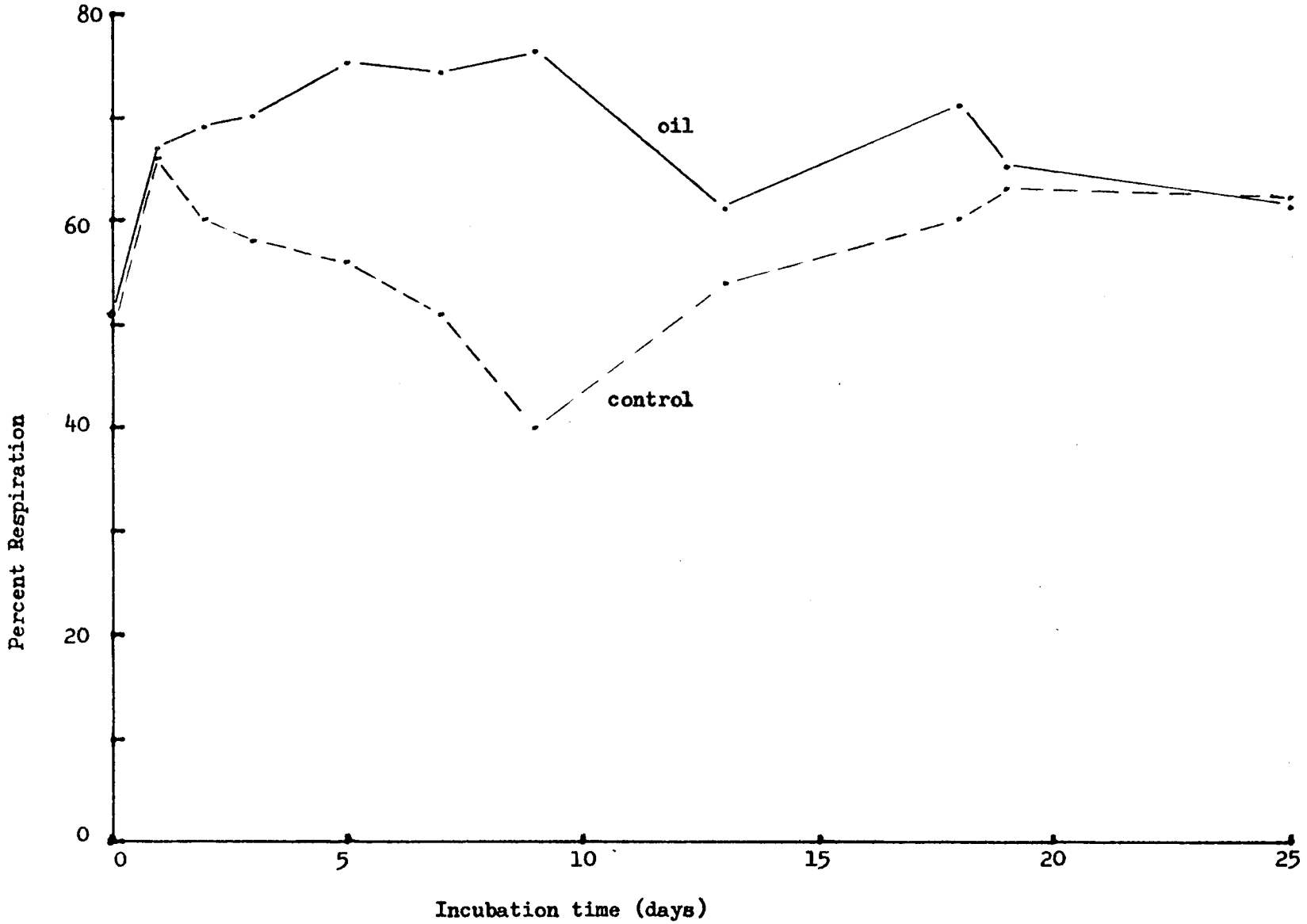

Figure 12. Percent respiration of glutamic acid utilization in both the control and oil enrichment cultures. Experiment #2.

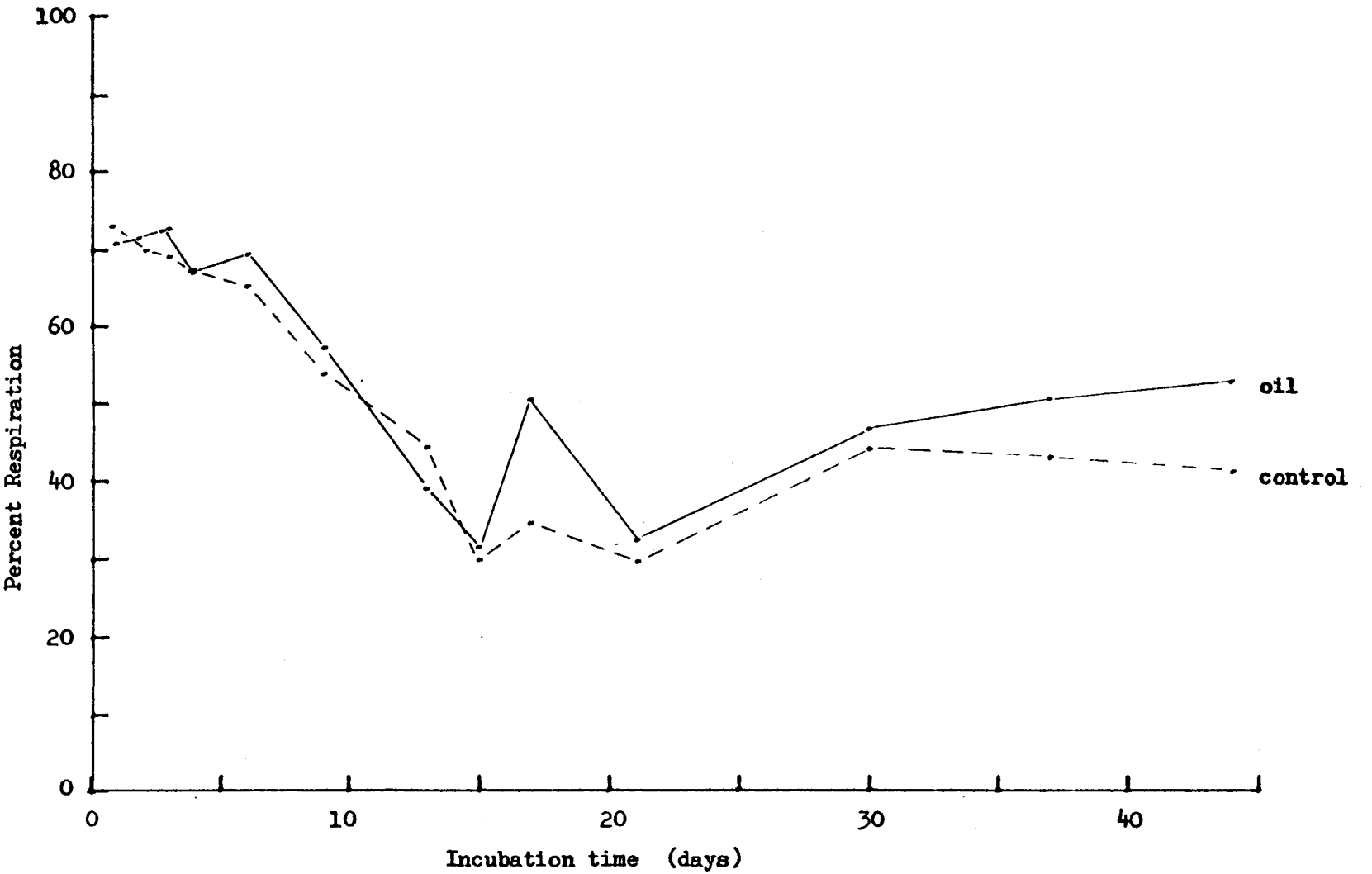

Figure 13. Percent respiration of glutamic acid utilization in both the control and oil enrichment cultures. Experiment #3.

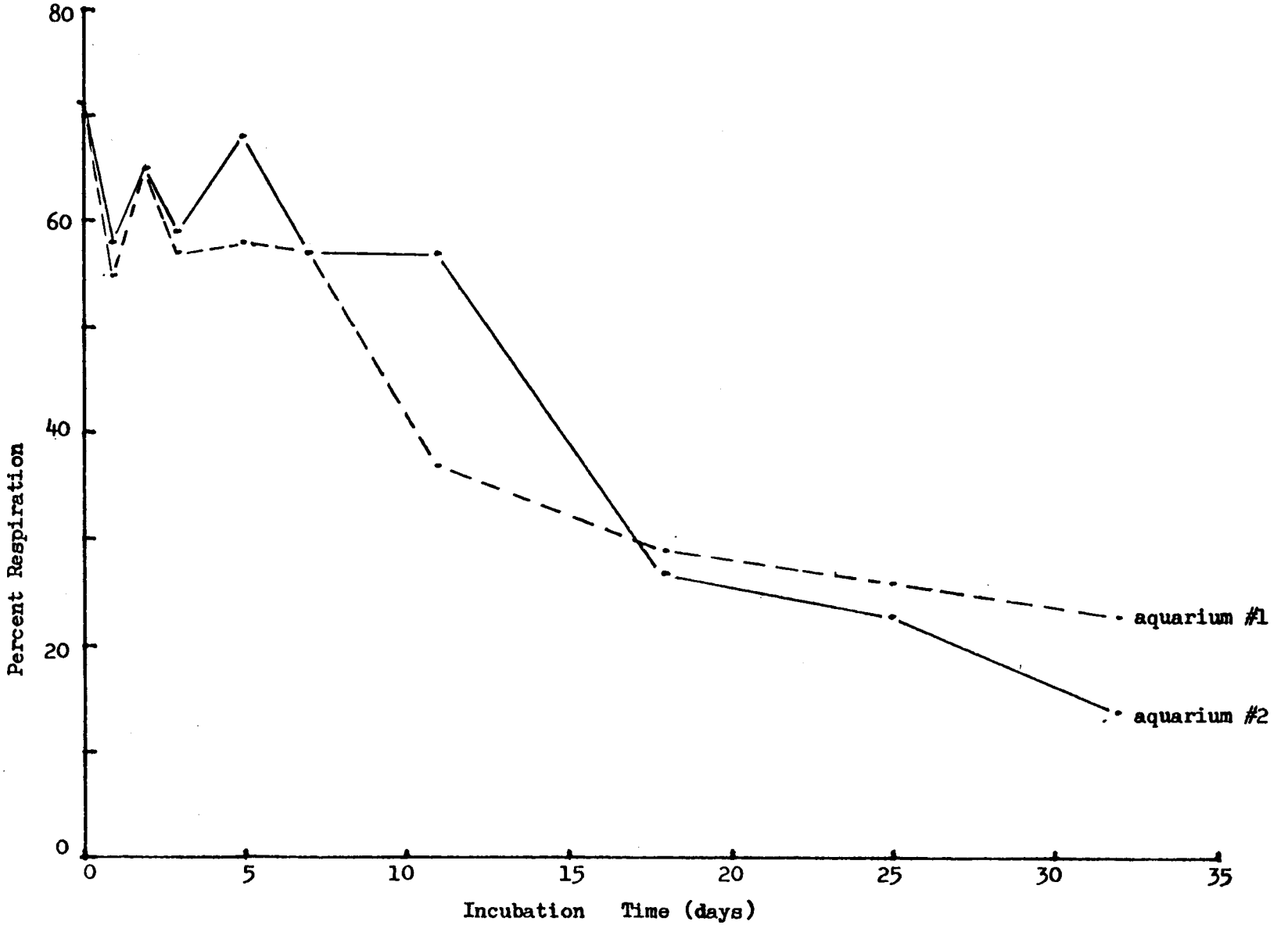

Figure 14. Percent respiration of acetate utilization in both seawater samples. Experiment # 1.

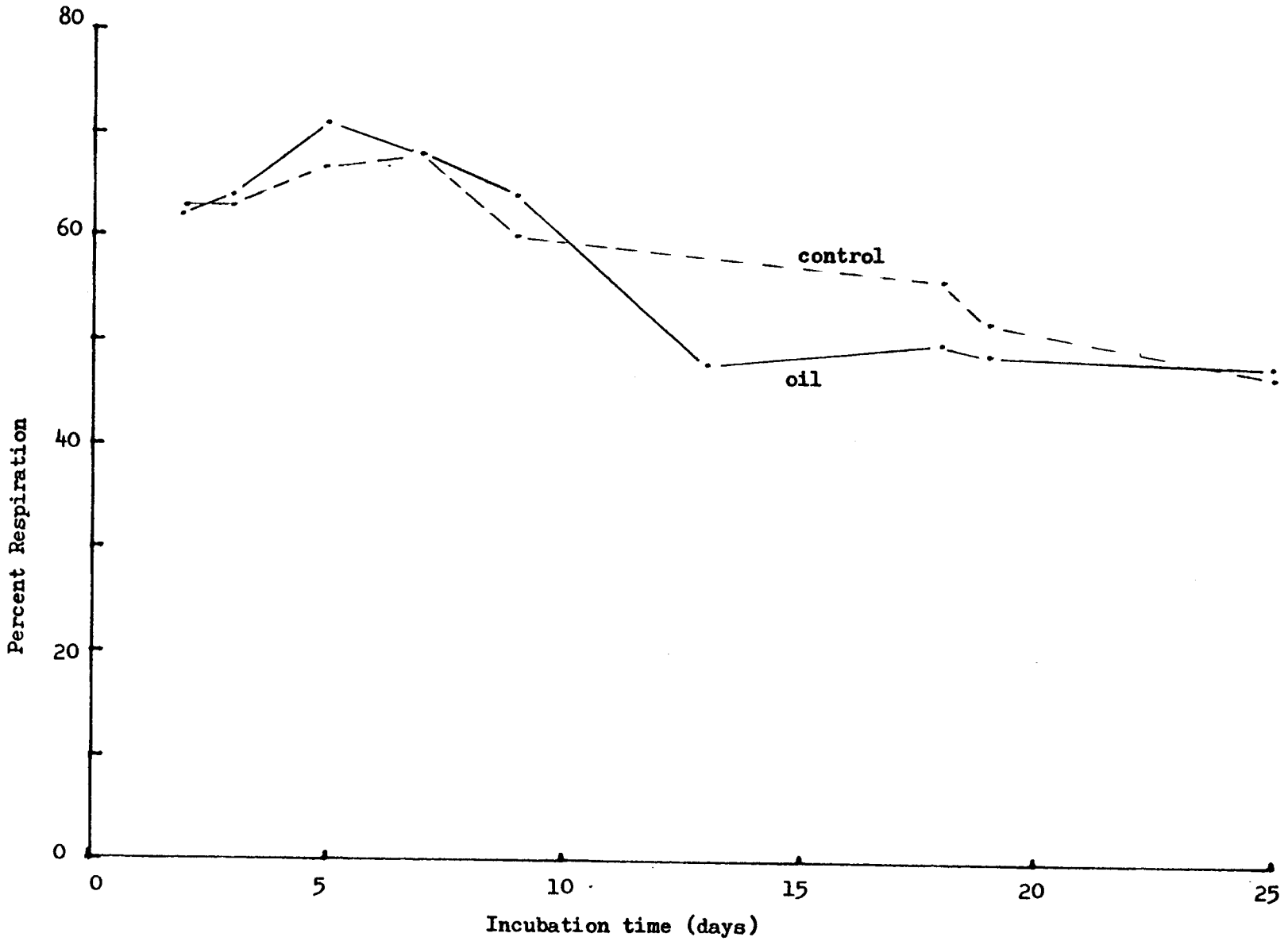

Figure 15. Percent respiration of acetate utilization in both the control and oil enrichment cultures. Experiment #2.

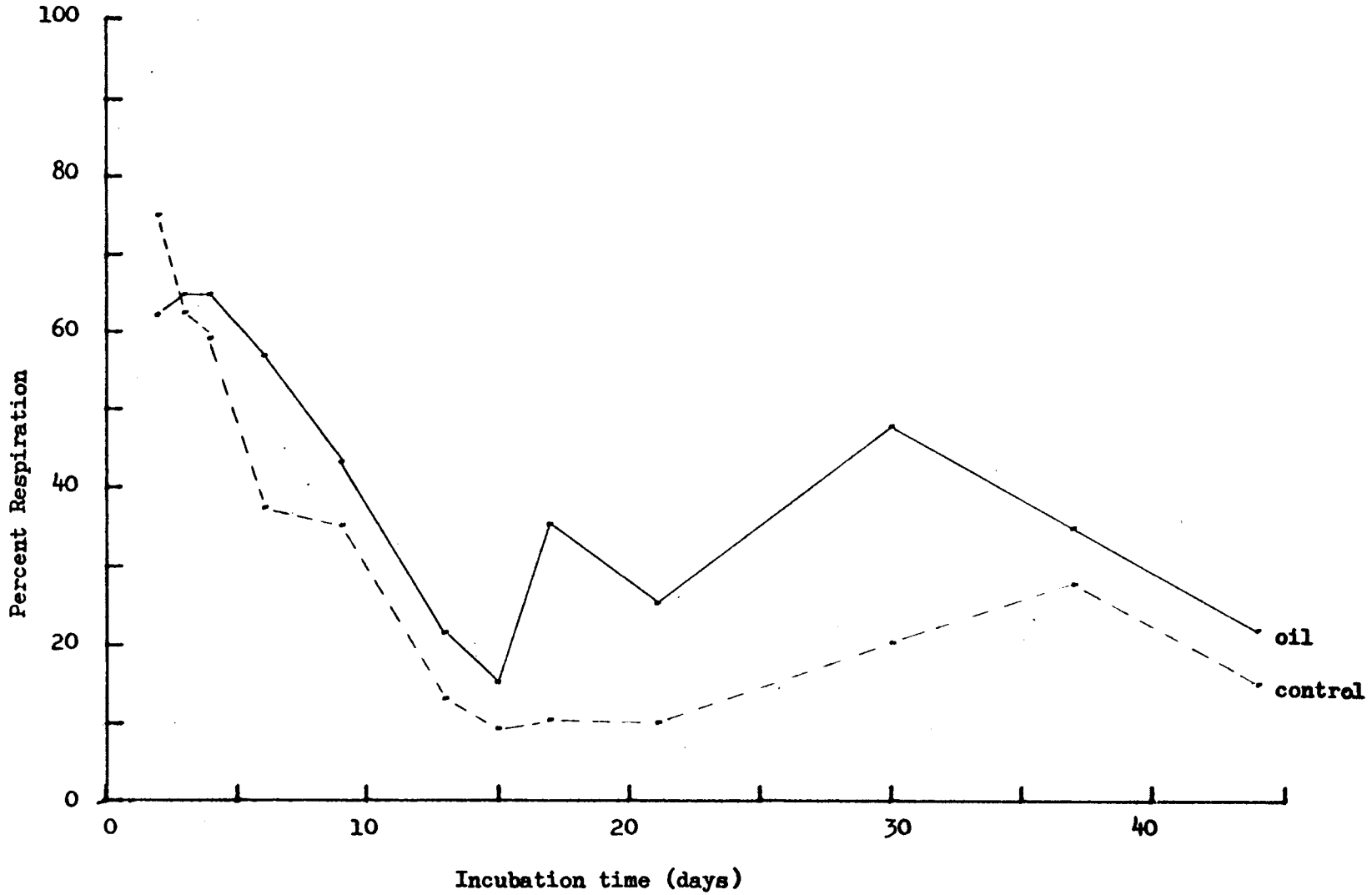

Figure 16. Percent respiration of acetate utilization in both the control and oil enrichment cultures. Experiment # 3.

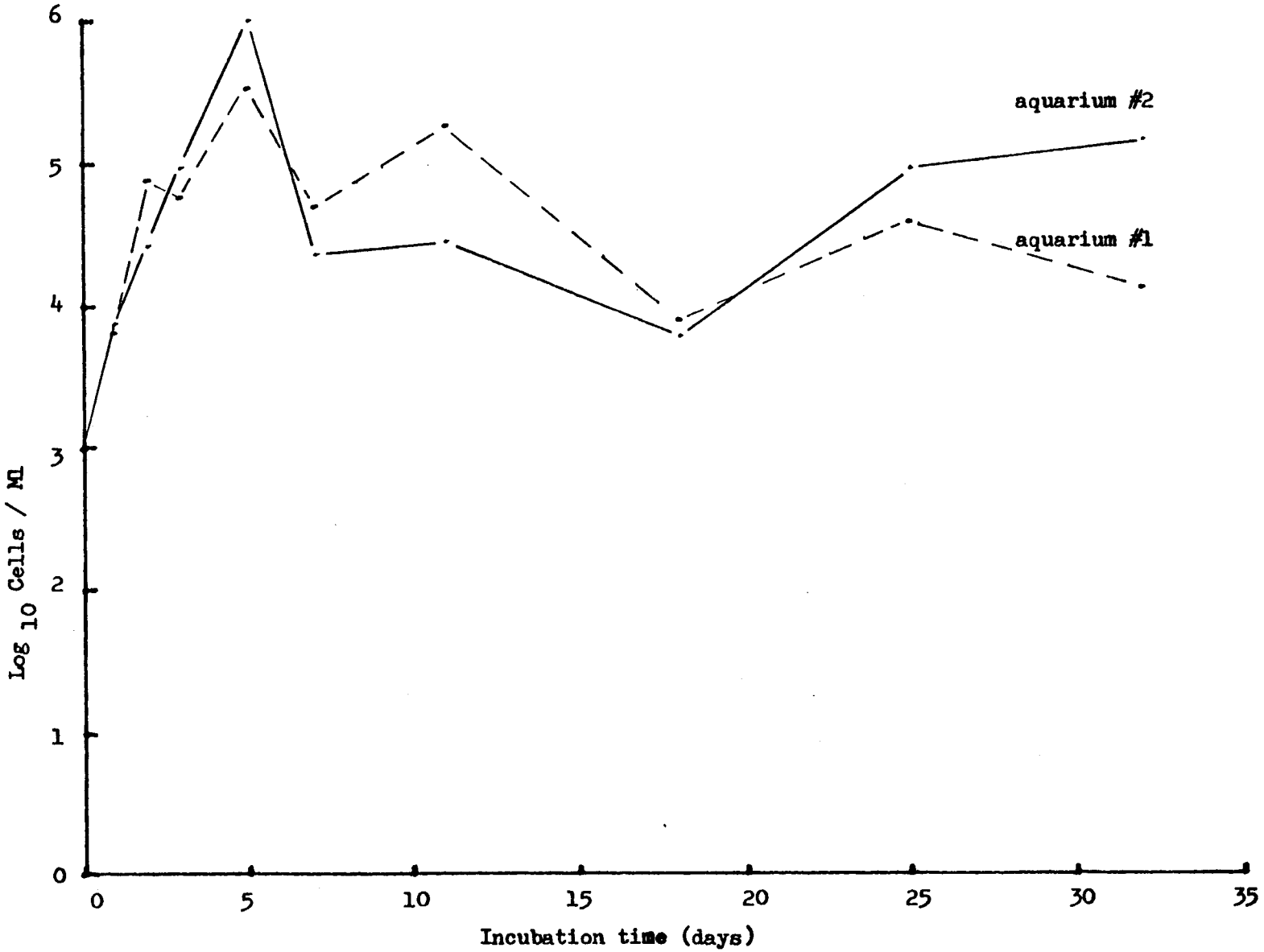

Figure 17. Total number of bacteria per ml as determined by plate counts on Lib X agar plates. Experiment #1

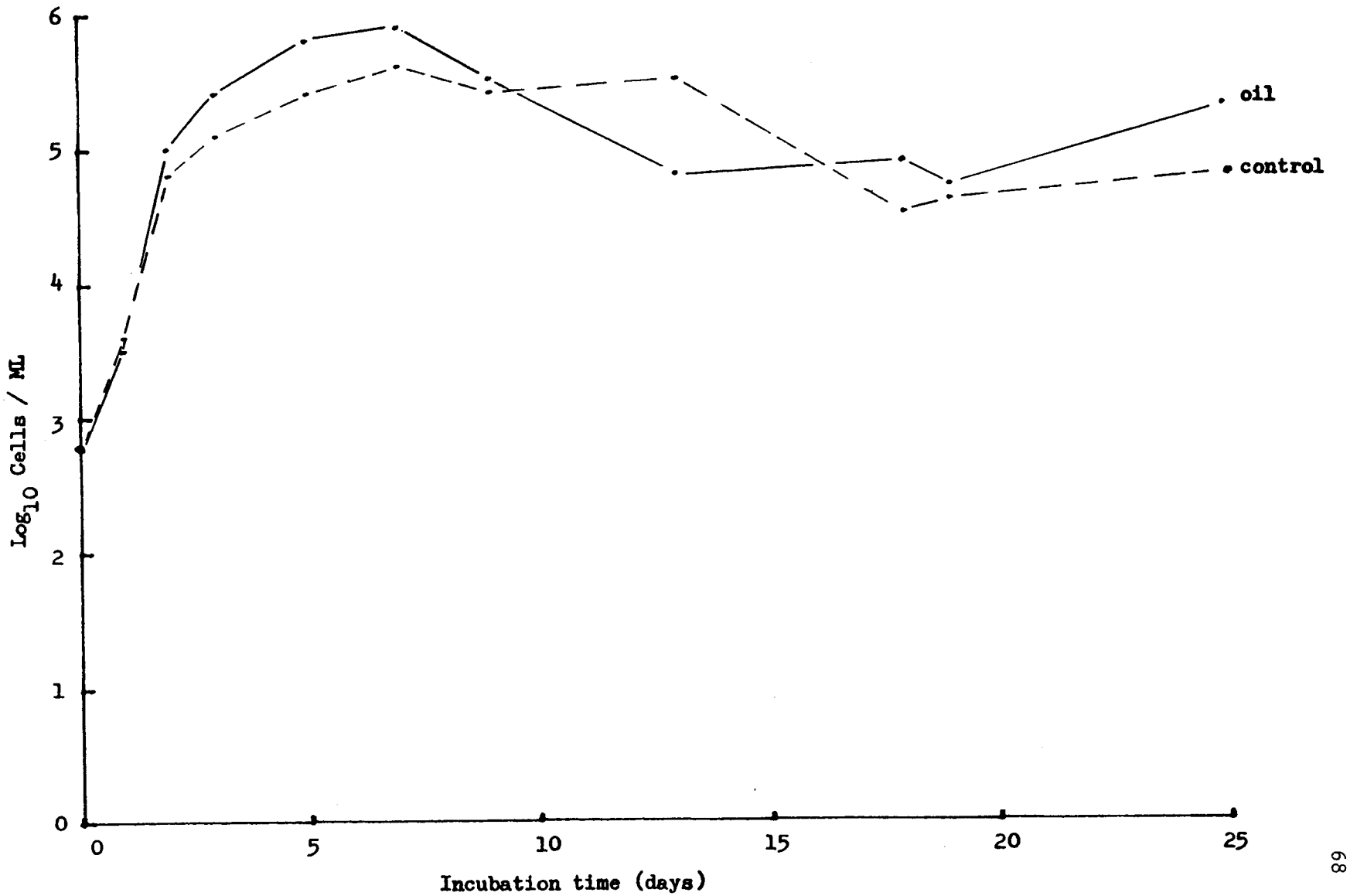

Figure 18. Log of total bacterial cell numbers per ml as measured on "Lib X" agar plates. Experiment # 2.

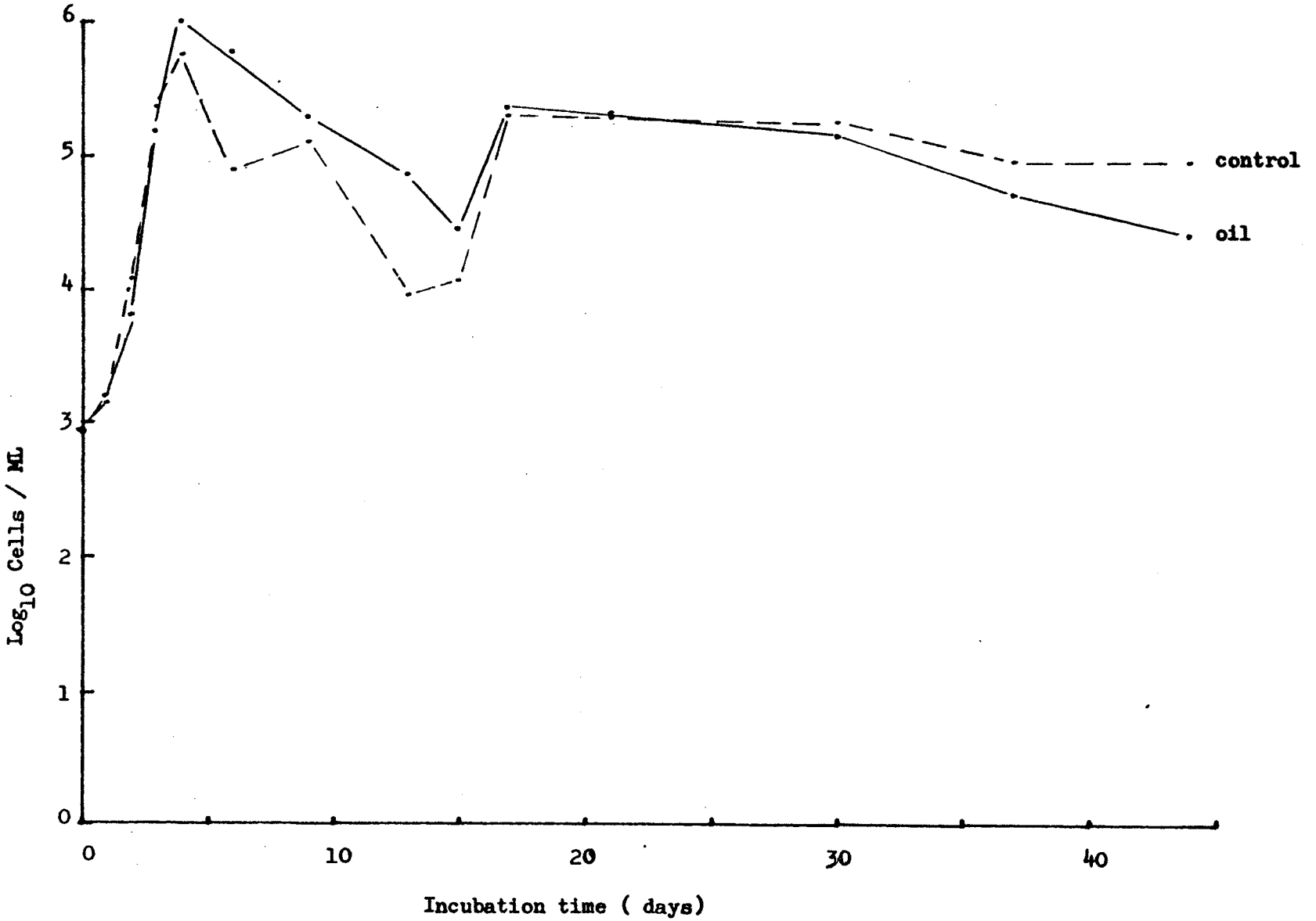

Figure 19. Log of total bacterial cell numbers per ml as measured on "Lib X" agar plates. Experiment # 3.

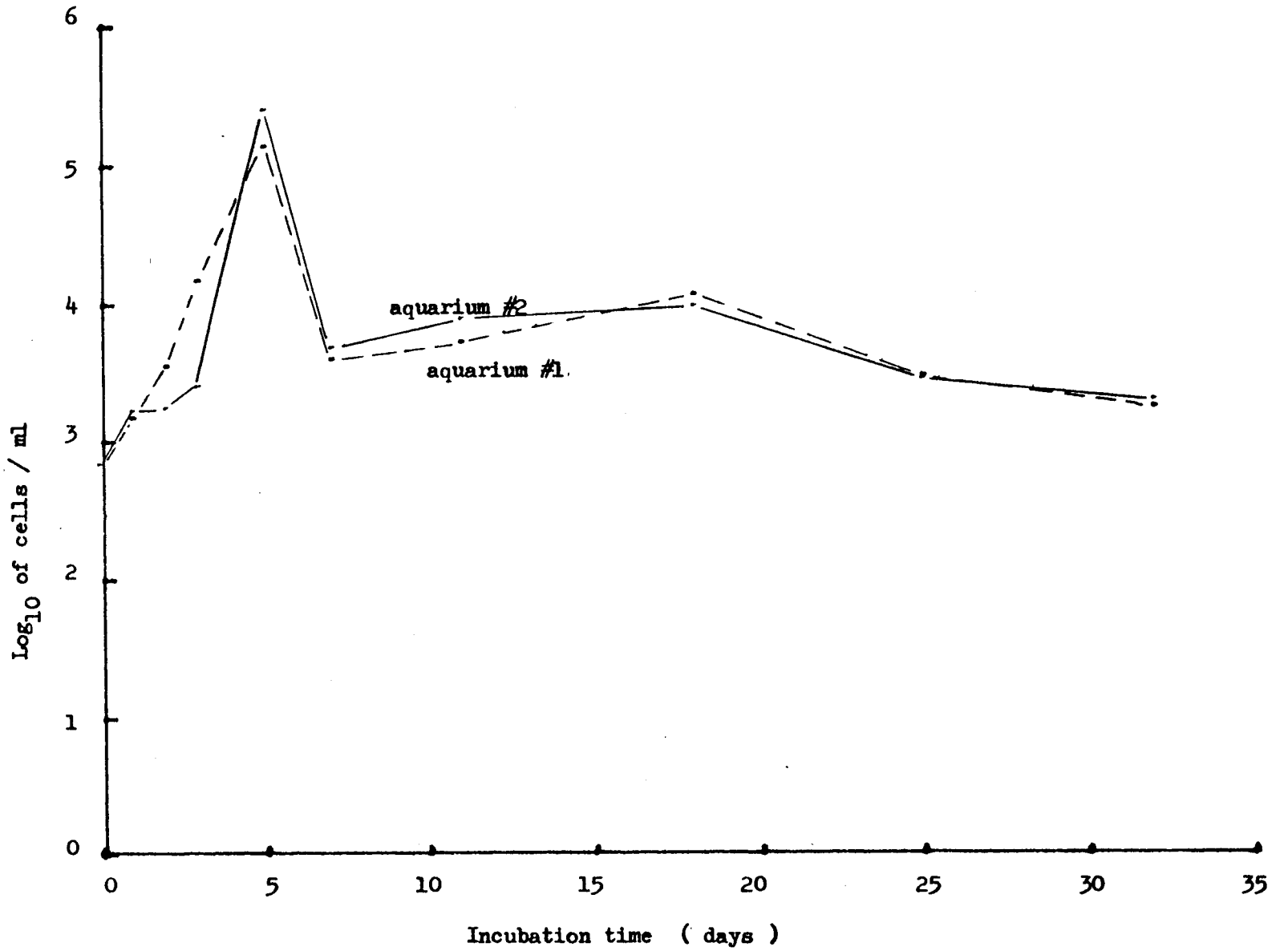

Figure 20. Log of hydrocarbon utilizing bacteria per ml as measured on crude oil agar plates. Experiment # 1.

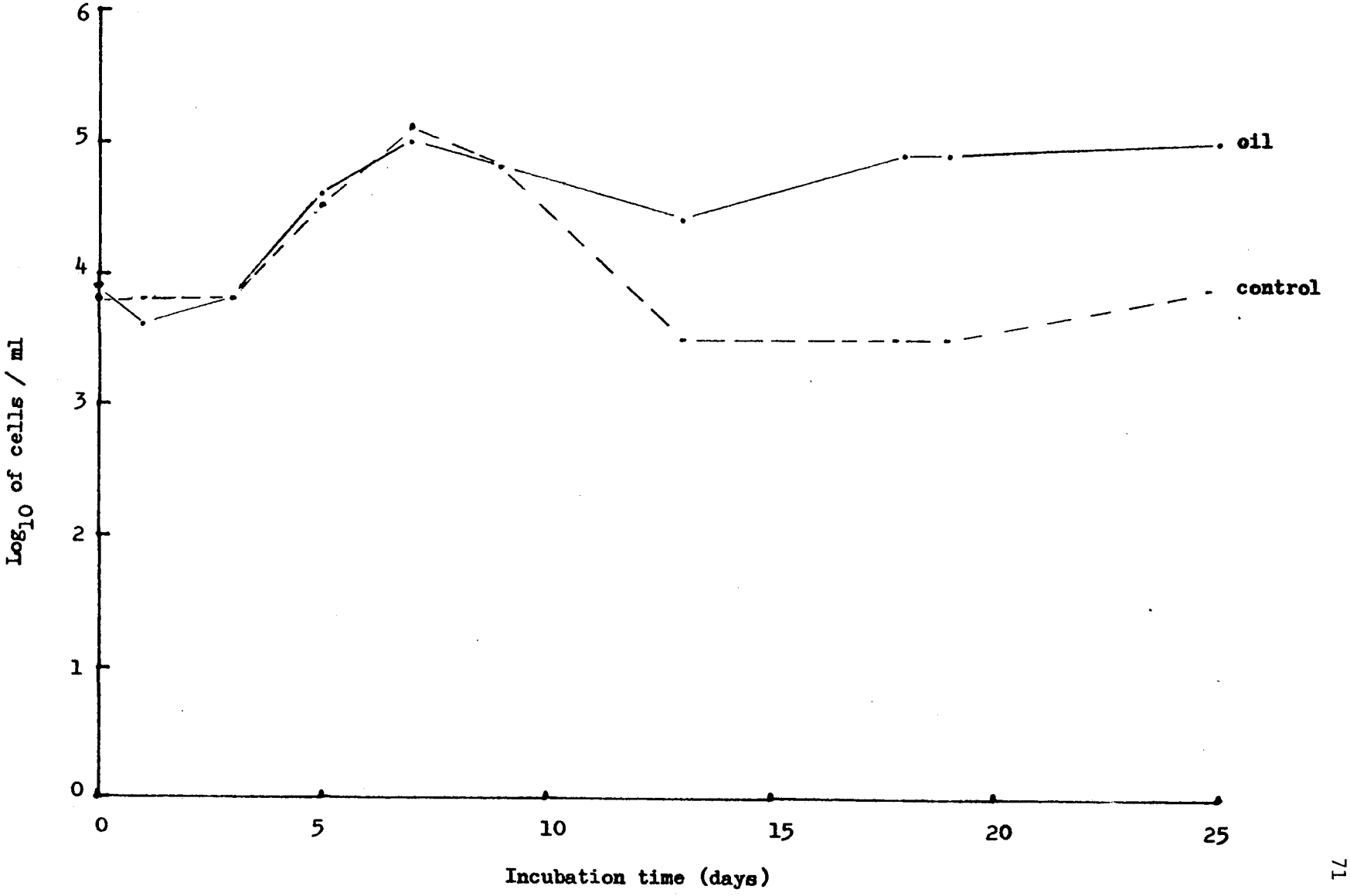

Figure 21. Log of hydrocarbon utilizing bacteria per ml as measured on crude oil agar plates. Experiment # 2.

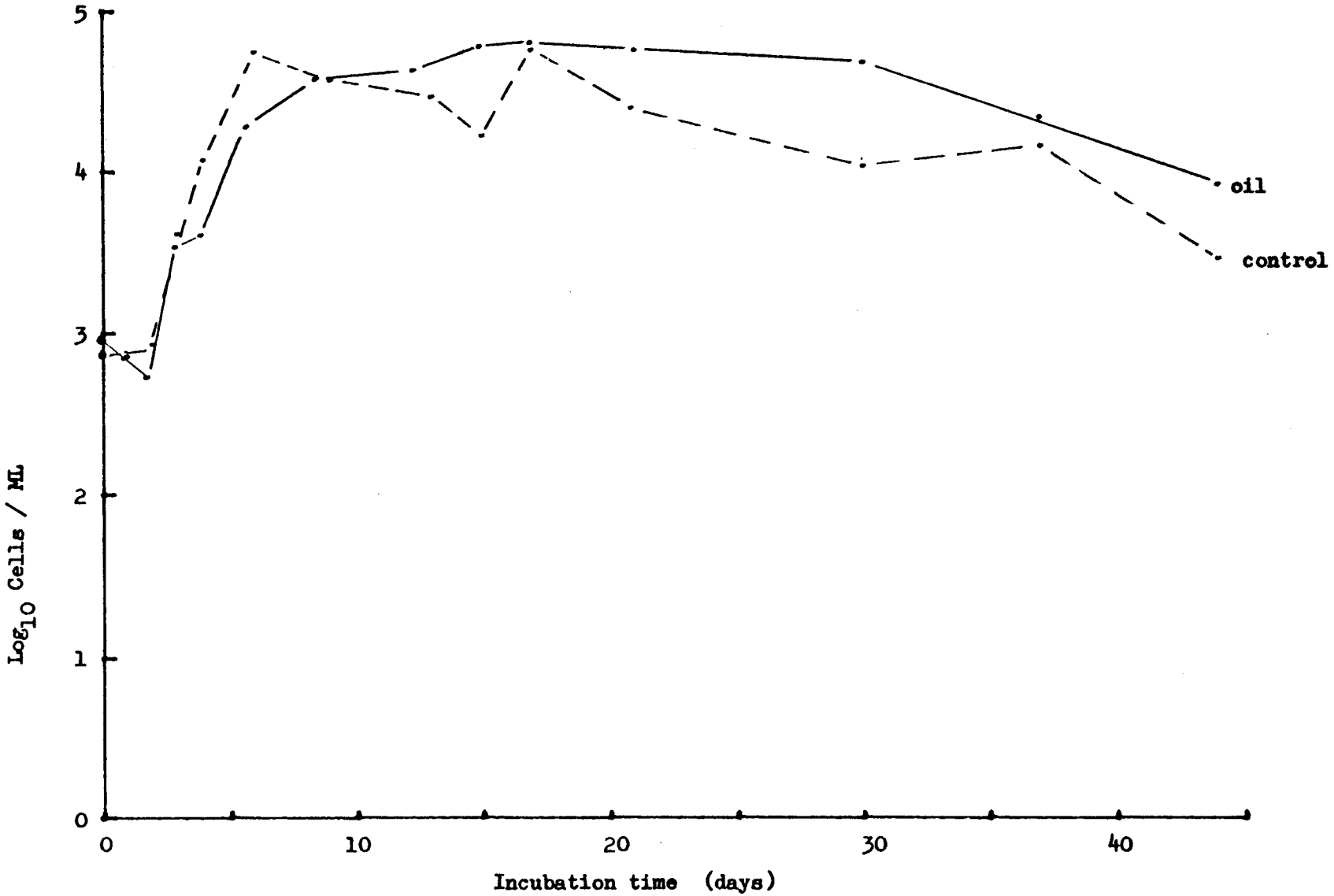

Figure 22. Log of hydrocarbon utilizing bacteria per ml as measured on crude oil plates. Experiment # 3.

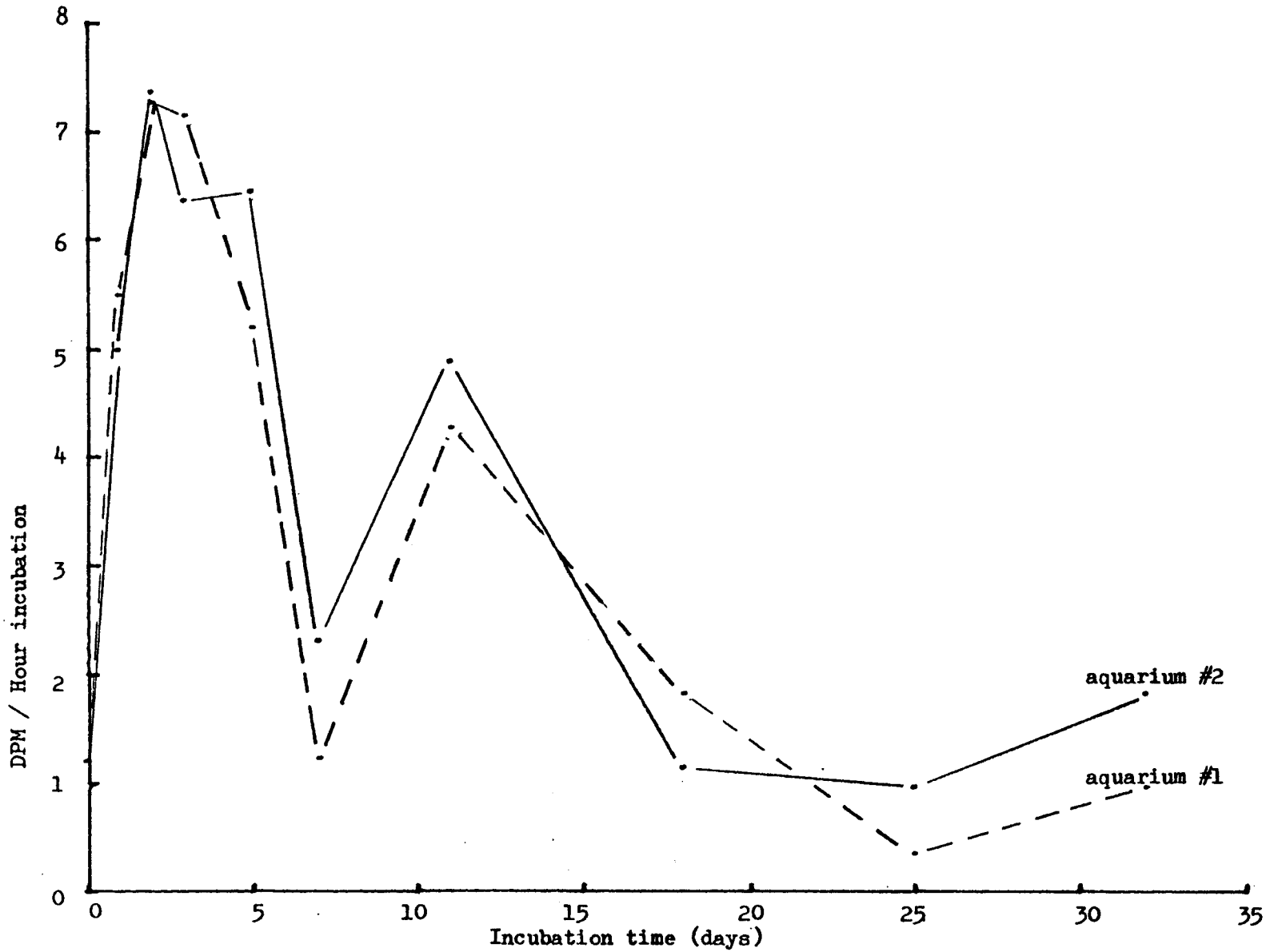

Figure 23. Algal protein hydrolysate uptake in both seawater samples reported as DPM per hour incubation. Experiment #1.

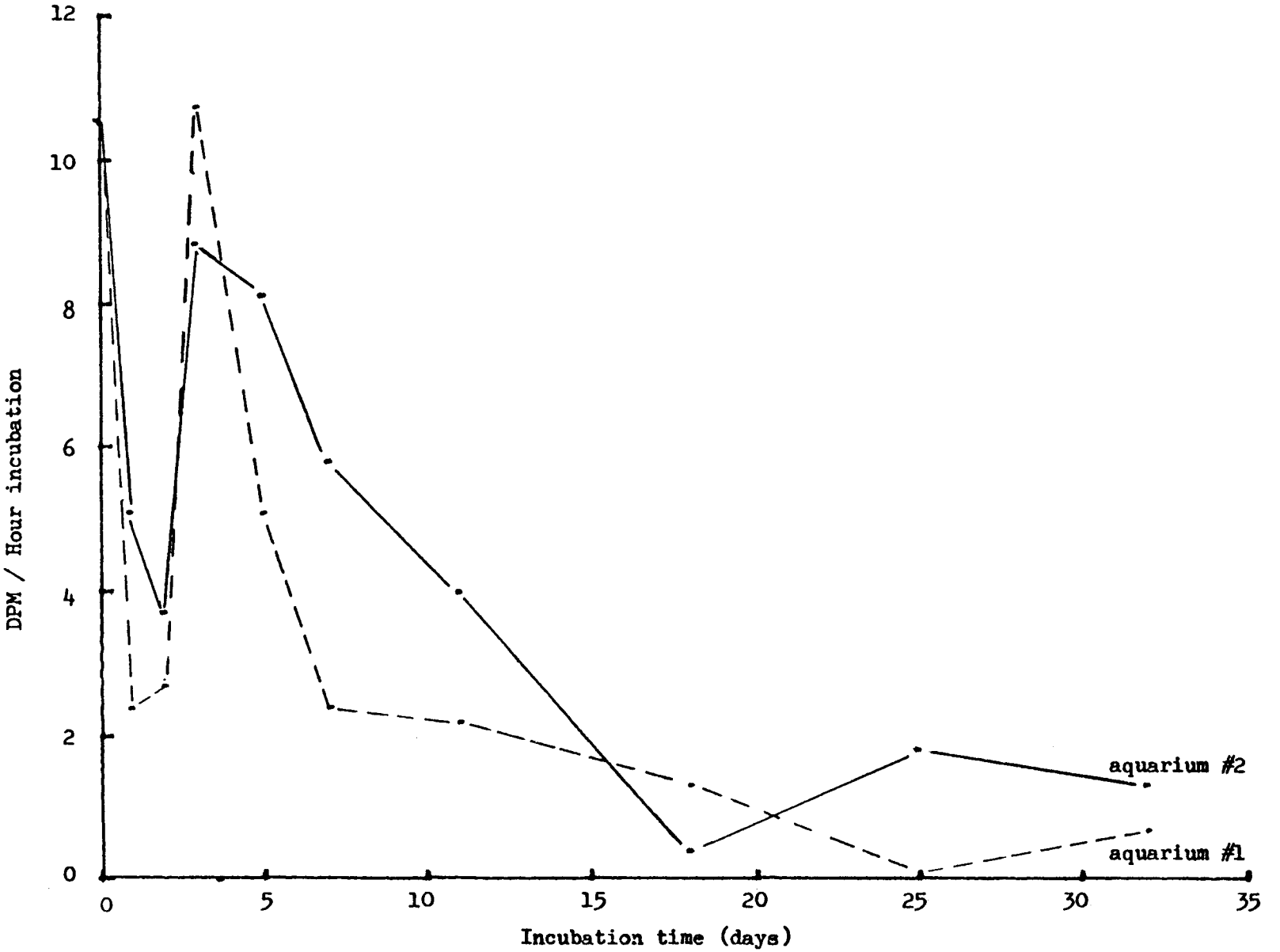

Figure 24. Glucose uptake in both seawater samples reported in DPM / hour incubation . Experiment #1.

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

by

Bruce B. McCain *

Harold O. Hodgins *

William D. Gronlund *

Annual Report
for Research Unit 332, Contract # R7120817
OUTER CONTINENTAL SHELF ENVIRONMENTAL ASSESSMENT PROGRAM
Sponsored by
U.S. Department of the Interior
Bureau of Land Management

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* Principal Investigators, Northwest Fisheries Center, National Marine Fisheries Service, NOAA, 2725 Montlake Boulevard East, Seattle, WA 98112

I. Summary of objectives, conclusions, and implications with respect to Outer Continental Shelf oil and gas development.

A. Objectives

In order to evaluate the impact of oil exploration and production in Alaska's Outer Continental Shelf on marine fish and invertebrates, the baseline health of these animals must be known. During the last year, 99,376 animals representing 49 species of fish and 30 species of invertebrates, captured in the Bering and Chukchi Seas, Norton Sound, and Gulf of Alaska were examined for externally visible pathological conditions. Several such conditions were found in 3,128 animals, representing 10 fish species and six invertebrate species.

B. Conclusions

Six major pathological conditions were found in fish, and they were as follows: epidermal papillomas of rock sole (Lepidopsetta bilineata) and flathead sole (Hippoglossoides elassodon), pseudobranchial tumors of cod (Gadus macrocephalus) and pollock (Theragra chalcogramma), 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 (Eleginas gracilis). The invertebrates had two important pathological conditions, infestation of sea stars (Leptasterias sp.) by parasitic gastropods, and extensive attachment of leech eggs to the appendages of shrimp (Sclerocrangon boreas).

The prevalence, distribution, and biological properties of affected individuals as well as the histopathology of the lesions have been characterized. In addition, efforts are continuing to determine the cause of the abnormalities. Four of the six major fish diseases were more prevalent in certain geographical locations of the sampling areas than in others, such as the waters northwest of Unimak Island.

Some of the fish diseases appeared to have detrimental effects on the hosts; for example, cod and pollock with pseudobranchial tumors were stunted with respect to the total population. The causes of the three non-neoplastic diseases of the fish have been established as viral (lymphocystis of yellowfin sole), bacterial and protozoan (cod skin lesions) and trematode ("black spot" disease of Pacific herring, toothed smelt, and saffron cod). The etiology of the tumors in the remaining five species is not understood; however, viral, protozoan, and chemical causes are being considered.

C. Implications

The baseline study described in this report provides a rough estimate of the prevalence and distribution of the major pathological conditions in marine fish and invertebrates prior to extensive oil drilling in Alaska's OCS. In particular, the preliminary information on the high prevalence of flatfish with skin tumors in waters less than 70 meters deep will be particularly useful because offshore oil drilling at these depths is quite common in other continental shelf areas.

In addition, if laboratory investigations currently underway demonstrate that crude oil and/or petroleum products can cause pathological conditions in marine animals either directly or indirectly by interfering with disease resistance mechanisms, then this investigation has identified (1) the species and their respective pathological conditions which may be affected indirectly by oil pollution, and (2) the species which are presently free of major externally visible abnormalities, but may develop diseases by the direct or indirect effects of oil.

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. This effort requires both field and laboratory activities. Field activities have been 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 were divided into subsamples, and most or all of the individuals in each were examined for externally visible pathological conditions. The biological and pathological characteristics of each affected animal were 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 sampling areas; (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 procedures designed for 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 microorganism 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 baseline research described in this report is relevant in two main ways to understanding the effects of petroleum development on the marine animals in the waters of Alaska's Outer Continental Shelf regions. The most important contribution is to provide baseline data on the health of demersal fish and invertebrates prior to the

time when the environmental impacts of oil drilling occur, so that future effects of oil on marine animals can be assessed. Also, knowledge of the possible causes of pathological abnormalities in demersal animals will provide a clearer understanding of the ways in which exposure of an organism to oil could directly or indirectly affect the frequency and distribution of pathological conditions.

III. Current state of knowledge.

Very little was known concerning the health status of marine fish and invertebrates in Alaskan waters prior to our OCSEAP-supported investigations initiated in 1975. Turner (1886) reported observing skin tumors (probably epidermal papillomas) on starry flounder (Platichthys stellatus) and Arctic flounder (Liopsetta glacialis) in the Aleutian Islands. In the early 1960's, Levings (1967) observed epidermal papillomas on approximately 10% of the rock sole captured in Bristol Bay and the western Gulf of Alaska. In some cases, these skin tumors were seen to cover almost 50% of the body surface.

Published accounts of abnormalities in Alaskan invertebrates include a report by Sparks and Pereyra (1966) which described shrimp of the family Hippolytidae parasitized by an isopod. Van Hyning and Scarborough (1973) isolated fungi from tanner crabs (Chionoecetes sp.) having "black mat" disease, a condition consisting of a tar-like covering on the exoskeleton. Another important Alaskan crab, the King crab (Paralithodes sp.), was reported by Bright et al. (1960) to have a condition known as "rust disease", which causes a darkening and softening of the exoskeleton. Although chitin-destroying bacteria were found associated with "rust disease", the cause is not yet known.

Since our investigation of the health status of fish in the Bering Sea began in 1975 several manuscripts describing our results have been written. Three manuscripts are in press, Wellings et al. (1977), McCain et al. (1977a), and Alpers et al. (1977). One manuscript was recently submitted for publication, McCain et al. (1977b).

For information about pathological conditions in fish and invertebrates in other arctic and subarctic marine waters, a recently completed literature review on this subject has been completed by members of this research unit (Hodgins et al., 1977; and report to OCSEAP by R.U. #75).

IV. Study Area

Four main geographical areas of Alaskan waters were investigated. The latitude and longitude boundaries of these study areas, and the figures in this report in which each area is depicted are as follows:

A. Bering Sea - 54°41' to 58°46' N. Lat.
162°38' to 174°32' W. Long.
(Figure 3)

B. Chukchi Sea - 65°46' to 68°18' N. Lat.
168°48' to 162°2' W. Long.
(Figure 20)

C. Norton Sound - 63°4' to 65°32' N. Lat.
171°51' to 161°15' W. Long.
(Figure 20)

D. Gulf of Alaska - 58°21' to 56°34' N. Lat.
149°4' to 152°32' W. Long.
(Figure 25)

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. Demersal fish and epibenthic invertebrates were sorted according to species, and subsamples were selected by members of OCSEAP R.U.'s #175, #281, and #332. These animals were examined for externally visible pathological conditions and, when feasible, for

readily recognizable internal disorders. The following information was recorded for each haul in the Haul Data Sheet: haul number, date, number of animals examined of each species, sex (this data was available for fish from the length-frequency records of R.U. #175), the type of pathological condition observed, and the number of animals with each type of condition for each species and often each sex.

Animals with apparently abnormal conditions were processed by members of R.U. #332 while still alive or freshly dead. Each animal was assigned a specimen number and the following information was recorded on the Individual Data Sheet: species, sex, dimensions (length for fish), weight, method of age determination (otolith or scale, applicable to fish only), condition, and location and size of the condition(s). Some descriptions of individual invertebrates were kept in a separate notebook to be adjusted to a new format which is presently being formulated and will require approval by OCSEAP. Photographs were taken of representative and unusual conditions. Fish samples were preserved in 10% formalin with phosphate-buffered saline and invertebrates were kept in a 10% formalin and seawater solution. Specimens were also preserved in a special fixative for electron microscopy (Hawkes, 1974). Samples of invertebrates lacking external lesions were also collected to spot-check for internal disorders by routine histology. In some cases, tissue was frozen at -20°C or in liquid nitrogen (-196°C) for later microbiological procedures. The presence of bacteria or fungi inside lesions, tumors, and internal organs was determined by cauterizing the surface of the tissue to be sampled, opening the tissue with a sterile scapel, removing an inoculum with a sterile loop, and streaking the inoculum in petri dishes with Ordal's Seawater Cytophaga Agar (OSCA), brain-heart infusion agar (Difco, dissolved in seawater), or potato dextrose agar (Difco) containing penicillin and streptomycin (for isolation of fungi). In addition to streaking the inoculum onto agar media, a portion of some inocula was spread onto a glass microscope slide and Gram stained. Representative colonies growing

on the culture media were purified by restreaking, stored in tubes containing OSCA, and returned to the laboratory for further tests.

Haemolymph samples from Argis lar and Sclerocrangon boreas from Norton Sound and the Chukchi Sea were taken by sterilizing the cephalothorax/abdomen junction with a drop of alcohol and extracting fluid near the pericardial sinus with a syringe. Smears were made by placing a drop of haemolymph on a clean slide, spreading, and air drying it, and fixing it in absolute methanol for 5 minutes. Microbial cultures were made by placing a drop of haemolymph on OSCA and streaking it as any other inoculum.

Laboratory activities involved processing the specimens and data obtained in the field. Tissue specimens from animals with the main pathological conditions to be examined histologically were matched with the photographic colored slides showing the gross appearance of the lesions.

Bacterial isolates were characterized using standard taxonomic criteria, such as, cell morphology, colony color and morphology, oxidase activity, behavior in oxidation-fermentation media, and motility.

Scales and otoliths from diseased fish were examined and age determinations were made. This data was added to the information contained on the Individual Data Sheet.

Once the information on the Data Sheets is completed, it is 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.

The Haul Data and Individual Data Sheets were analyzed, the data summarized, and in some cases compared with similar data obtained by R.U.#175 for the same hauls. Length frequencies, sex ratios, and length/weight and age/length relationships of diseased and total populations were compared when possible.

VI. Results

The results of our research include the number of fish and invertebrate species with and without pathological conditions, and the distribution, frequency, pathology and biology of affected individuals. This section is divided into three parts according to the geographical area in which the animals were examined.

A. Bering Sea

Of the 26 different species of fish examined in the Bering Sea, 22 were essentially free of recognizable pathological conditions (Table 1). The affected species, associated pathological conditions, and the overall average frequencies of each condition were as follows: Pacific cod, pseudobranchial tumors, 8.7%; Pacific cod, skin ulcers, 1.6%; pollock, pseudobranchial tumors, 1.7%; yellowfin sole, lymphocystis, 2.8%; and rock sole, epidermal papillomas, 1.3% (Table 2).

The gross appearance of the cod tumors was the same as that described by McCain et al. (1977a). Briefly, they ranged in color from yellow, to pink, to brown. They were oval-shaped, smooth-surfaced, extended into the pharyngeal cavity, and ranged in size from just larger than the normal pseudobranch to 50 x 30 x 20 mm (Figure 1). With one possible exception, all of the tumors were bilateral and both were usually the same size. The tumors often had necrotic areas on the surface, and normal-appearing pseudobranchial tissue was located on the surface or in the interior of each tumor.

In one cod with bilateral tumors, another similar tumor was also found attached to a gill filament. This tumor was oval, cream-colored, and about 4 mm in diameter (Figure 2). The tumors had a similar cell organization, composed mainly of X-cells (Alpers et al. 1977), except no pseudobranchial tissue was associated with the smaller gill tumor.

Tumor-bearing cod were distributed throughout the sampling area (Figure 3) as indicated by the fact that 67% of the hauls where cod were captured contained tumor-bearing cod. The range of the disease frequencies in hauls containing cod with tumors was 1.1% to 73.3%.

Analyses of the biological characteristics of tumor-bearing cod showed that about the same number of males had tumors as did females. The relative age composition for cod with tumors was different from that of normal cod ("normal" in this case and for the other species described in this report means those apparently healthy animals captured, speciated, sexed, measured, and when possible aged during 1975 or 1976 by RACE and EC Divisions of the NMFS, NWAFC in the study area). Normal cod ranged in age from one to five years, while no tumor-bearing cod were less than two years (see Figure 15, Oct., 1976, Quarterly Report). In addition, tumor-bearing cod were about 25% shorter than normal cod of the same age.

The histopathological properties of the pseudobranchial tumors have been described previously (Alpers et al. 1977). Briefly, they included the separation of normal-appearing pseudobranchial tissue from the tumor tissue by a connective tissue capsule and the presence of cells known as X-cells. These cells are also found in other marine fish tumors, and they will be discussed later.

The pollock pseudobranchial tumors and the cod tumors were usually grossly similar in color, shape, and texture (Figure 4). However, the pollock tumors were often less protruding, tending to extend up into the roof of the pharynx, and six pollock had unilateral tumors. Also, one pollock was found to have a secondary tumor on the outside of the operculum which had originated from an invasive pseudobranchial tumor. In general, these tumors were smaller than cod tumors with dimensions ranging up to 35 x 20 x 10 mm.

Tumor-bearing pollock were distributed in a pattern very similar to that of cod with tumors (Figure 5). Of the hauls where pollock were captured, 45% contained

pollock with tumors, and the disease frequency in these latter hauls was from 0.6% to 13.2%.

The age and sex composition of tumor-bearing pollock differed sharply from similar data for normal pollock (See Figure 16, Oct., 1976, Quarterly Report). No tumors were detected in fish less than two years of age and only occasionally in those over five years. About twice as many male pollock between the ages of two and five had tumors as did females of the same age. Also, after age six there was a marked decline in the relative abundance of normal males as opposed to females. The growth rate of pollock with tumors was apparently depressed in that they were 15% shorter than their normal cohorts (W.D. Gronlund, B. B. McCain, R. Bakkala, unpublished data).

The histological characteristics of the pollock tumors were very similar to those described for cod and will be described elsewhere (McArn et al. 1977).

The epidermal papillomas of rock sole grossly and histologically resembled similar tumors described for several species of pleuronectids along the western coast of North America (McCain et al. 1977a; Brooks et al. 1969; Wellings et al. 1967). The tumors ranged in size from 3 x 3 x 2 mm to 100 x 70 x 10 mm. They were brown to black and elevated, with a papillary architecture (Figure 6). The tumors were located randomly on the body surface, and frequently a tumor extended to both sides of a fish with both sides being mirror images of each other. No metastases were identified.

There were approximately three times as many male rock sole with tumors as females with tumors. The frequencies of tumor-bearing fish ranged from 0.4% to 58%, but fish with tumors were found in only 14% of the hauls containing rock sole. The distribution of tumor-bearing rock sole appeared to be depth related, with the shallowest stations (47 to 55 meters) having the highest frequencies (Figure 7). The age composition data for normal rock sole showed that the abundance of normal

females was bimodal with peaks at five and ten years (See Figure 17, Oct., 1976, Quarterly Report). The males had a single mode at five years and gradual decrease in abundance up through 13 years. In view of this dramatic difference between males and females, it is of interest that 20 times more males had tumors at ages two and three than did the females. The age compositions of both tumor-bearing males and females were similar after the age of four.

Lymphocystis (a virus-caused disease) of yellowfin sole was characterized by the presence of variously shaped and colored growths on fins and body surfaces (Figure 8). These growths, ranging in size from 1 mm in diameter to 20 x 10 x 5 mm, were of three basic types: (1) single or clusters of translucent, round bodies about 1 mm in diameter, (2) small red sacs on the ends of fin rays, and (3) red to gray amorphous growths. All these types had in common the presence of small round bodies, which, as will be mentioned below, were hypertrophied fish cells. Fin erosion was associated with about 10% of the cases where lymphocystis growths were on fins (Figure 8). Most growths were found on the "blind" side of the fish.

Diseased yellowfin sole were most often observed in the southeastern Bering Sea, just north of Unimak Island (Figure 9). Of the hauls containing this species, 34% had fish with lymphocystis with frequencies ranging from 2.3% to 31.3%. About the same number of males as females had this disease. The age composition of normal yellowfin sole of both sexes was bimodal, having peaks of abundance at six and eight years (See Figure 18, Oct., 1976, Quarterly Report). Diseased fish had an age composition closely paralleling that of the normal population.

The histological properties of lymphocystis growths have been extensively described elsewhere (McCain et al. 1977a; Russell 1974; Templeman 1965). The growths on yellowfin sole contained hypertrophied cells about 0.1 to 1.5 mm in diameter. These cells contained cytoplasmic inclusion bodies which were composed of hexagonal virions about 200 nm in diameter (Figure 10).

Two main types of lesions were observed on Pacific cod: an ulcer (Figure 11) and a ring-shaped lesion (Figure 12). The ulcers were roughly circular and ranged in size from approximately 1 to 50 mm in diameter. The ulcers were either pale white, or red (hemorrhagic) with a dark pigment concentrated in the margin of the surrounding epidermis. Between one and 25 ulcers were observed on each affected fish. The ring-shaped lesions were characterized by a 5 to 20 mm wide, cream-colored strip, sometimes having hemorrhagic foci, surrounding a normal-appearing circular patch of epidermis. These lesions were about 10 to 50 mm in diameter. The number of ring-shaped lesions per diseased fish ranged from one to five. The highest frequencies of skin lesions were found on fish caught northwest of Unimak Island (Figure 13). Both sexes of cod had about the same frequency of skin lesions.

Histological examination of the skin ulcers revealed that in most cases the epidermis was absent from the center of the lesion. Less often, portions of the dermis had also been destroyed. The white covering over some ulcers was composed of residual necrotic epidermis. The periphery of the lesions was hyperemic, hemorrhagic, and contained numerous inflammatory cells (lymphocytes and macrophages) and areas of fibrosis (Figure 14). High concentrations of microorganisms were not observed histologically in the ulcers.

The ring-like lesions had very unusual, but as yet undiagnosed, histological properties. The epidermis and the stratum spongiosum (a component of the dermis directly beneath the epidermis) were the only parts of the skin obviously affected by this condition. The appearance of normal cod skin is shown in Figure 15. Cod epidermis normally contains mucous cells and large cystic structures of unknown function (Bullock and Roberts 1974). In the epidermis of fish affected with the lesion there were large bodies, about four times the size of a normal mucous cell, which contained a very basophilic center surrounded by an eosinophilic margin (Figure 16). Small eosinophilic bodies were observed in this margin and distributed

within the basophilic center. Occasionally the large bodies were seen in the process of releasing their contents through the surface of the epidermis. At this time, the identity of the large bodies or the eosinophilic bodies is not known. In some cases, the stratum spongisum adjacent to the large bodies contained infiltrates of inflammatory cells, suggesting the bodies were infectious organisms.

Attempts were made to isolate bacteria and fungi from all of the above mentioned pathological conditions with the exception of lymphocystis. The only condition from which bacteria were routinely isolated was the skin ulcers of Pacific cod. Ulcers from five different cod yielded pseudomonas-like bacteria, sometimes in pure culture, which so far, have proven to be essentially taxonomically identical.

B. Norton Sound/Chukchi Sea

In the Norton Sound/Chukchi Sea areas, a single pathological condition was found in three of the 28 species of fish examined (Table 3). This condition is called "black spot disease" and is recognizable by the presence of black patches, from 1 to 2 mm in diameter, on the skin. The species involved and the overall frequency of this condition were: Pacific herring (Clupea harengus pallasi), 4.5%; the toothed smelt (Osmerus mordax dentex), 3.9%; and the saffron cod (Eleginus gracilis), 0.8% (Table 4). Fish were considered infected when they had around 20 or more black spots; some heavily infested fish had several hundred such spots (Figure 17).

Microscopic examination of individual black spots revealed the presence of an encysted larval trematode, probably a metacercaria (Figure 18 and 19). The cysts were usually surrounded by melanin-containing cells, such as melanocytes. The trematode is very similar to Cryptocotyle lingua.

The geographic distribution of toothed smelt and saffron cod with "black spot" disease was very similar, with the highest prevalence in Norton Sound (Figure 20).

Pacific herring (Figure 21) with this disease were numerous in both Norton Sound and the Chukchi Sea; however, the condition was much more common in the latter area.

Of the 31 species of epibenthic invertebrates examined, six species with seven potentially pathological conditions were observed (Table 5). Parasitism (in five species) and discoloration (in two species) were the major conditions found during this cruise. The affected species, asociated conditions, and population frequency of each condition were as follows: Sclerocrangon boreas (crangonid shrimp), leech eggs on the host's pleopods, 41.7%; Leptasterias sp. (sea star), parasitic gastropods (snail), 11.3%; Leptasterias polaris (sea star), parasitic gastropods, 4.8%; Pagurus capillatus (hermit crab), rhizocephalan (barnacle) parasite, 5.7%; Argis lar (crangonid shrimp), bopyrid (isopod) parasite, 1.2%; Hyas coarctatus alutaceus (spider crab), darkened exoskeleton suspected to be melanization, 7.1%; Argis lar, pale and enlarged eggs, 2.7% (Table 4).

The leech eggs on S. boreas were found in greatest proportion southeast of St. Lawrence Island (See Figure 6, Jan., 1977, Quarterly Report); the condition was more prevalent, however, in central and eastern Norton Sound where it occurred in 11 of 16 stations. Incidence of this condition within a haul ranged from 9.6% to 100.0%. The leech eggs were circular, about 1 mm in diameter, and peppered the pleopods; but if the surface area was crowded, they were found on the ventral abdomen and/or on egg clutches (Figure 22). Of the affected S. boreas of known sex, 88.3% were female. Almost three-quarters (73.1%) of these were gravid, 14.5% were maturing, 11.2% had spawned, and 1.2% were sexually undeveloped.

The sea stars Leptasterias polaris and Leptasterias sp. carried parasitic gastropods internally in 24 of 49 stations for the former and 17 of 29 stations for the latter, with a range of 0.1% to 75.0% and 1.2% to 37.8% in a haul, respectively. The greatest frequency in Leptasterias polaris occurred near the entrance to

Kotzebue Sound; for Leptasterias sp. the highest proportion was found in central Norton Sound (See Figures 8 & 9, Jan., 1977, Quarterly Report). The adult gastropod, a bright orange, bean-shaped lump, varied in size from approximately 1 mm to 27 mm in length, the larger ones distending the ossicles/epidermis of the sea stars (Figure 23). As many as 25 were found in one host, in the central disc as well as in the rays. Identification as a gastropod was affirmed by the presence of shelled veliger larvae within the parent's body mass (Figure 24).

The parasites seemed to show some preference in location in Leptasterias polaris. A total of 77.3% of the gastropods showed oral orientation; that is, the "proboscis" connecting the parasite inside the sea star with sea water usually passed through the epidermis on the oral side (near the substrate) of the host. Aboral orientation occurred in 15.9% of the cases observed and lateral orientation, along the sides of the rays, was found 6.8% of the time. There seems to be no difference in infestation among the rays selected for parasitism although incidence in the central disc was lowest. Within a single ray the parasites occurred on a gradient, increasing as they approached the central disc; 20.2% were found distally on the rays, 30.6% were midway, and 40.9% occurred proximal to the "body". Markedly fewer (8.3%) were noted at disc/ray junctions.

Rhizocephalan (barnacle) parasites were noted on the abdomens of hermit crabs, primarily Pagurus capillatus in 27 of 43 stations involved. The highest proportion in one haul occurred in the northeastern part of the study area; frequency extended from 2.0% to 50.0% (See Figure 13, Jan., 1977, Quarterly Report).

Argis lar carried bopyrid parasites under the carapace in 23 of 132 stations. The greatest proportion in a haul occurred in southern Norton Sound (See Figure 14, Jan., 1977, Quarterly Report), and frequency ranged from 0.5% to 88.9%. This parasite was most common in stations less than 25 meters in depth (19 of 63) in Norton Sound and Kotzebue Sound.

Darkened and/or eroded exoskeleton was noticed in Hyas coarctatus alutaceus from 12 of 26 stations, with highest frequency in the southeast Chukchi Sea. Of these crabs, 5.3% to 50.0% were affected in a haul (See Figure 18, Jan., 1977, Quarterly Report). Since fouling was usually heavy on these animals, it is assumed that this condition, probably melanization, was a result of prolonged periods between molting or its termination altogether.

Argis lar from 17 of the 132 stations investigated also carried discolored (necrotic) eggs. Shrimp with such eggs ranged in frequency from 1.2% to 90.0% (See Figure 16, Jan., 1977, Quarterly Report). These enlarged, pale eggs were seen only in the Chukchi Sea/Kotzebue areas, with highest incidence in Kotzebue Sound (See Figure 17, Jan., 1977, Quarterly Report).

C. Gulf of Alaska

Five of the 34 species of fish examined in the Gulf of Alaska (GOA) had four significant pathological aberrations (Table 6). Two of these conditions were also observed in the Bering Sea; they are pseudobranchial tumors of Pacific cod and pollock, and epidermal papillomas of rock sole. The mean frequencies of these were 3.23%, 1.80%, and 0.16%, respectively (Table 7). The other two pathological conditions, which have not previously been reported in Alaskan waters, and their mean frequencies were epidermal papillomas of flathead sole (Hippoglossoides elassodon), 0.8%, and epitheliod tumors of Pacific Ocean perch (Sebastes alutus), 21.05% (Table 7).

The gross appearance and histopathology of the pseudobranchial tumors of cod and pollock from the GOA were very similar to what has been described for this disease in the Bering Sea. In addition, about the same number of male cod had tumors as did females in both areas. The age and sex composition of tumor-bearing pollock in the two areas differed slightly. Pollock with tumors between the ages of two and four from the Bering Sea had the highest tumor frequency, while all the tumor-

bearing pollock from the GOA were two years old. Also, twice as many male pollock in the Bering Sea had tumors than did females, but in the GOA, three times more males than females had tumors.

The frequencies of tumor-bearing cod and pollock in hauls in the GOA where pseudobranchial tumors were observed in these species ranged from 1.0% to 50% for cod and 0.32% to 14% for pollock. The distribution of the two diseases are shown in Figure 25 and Figure 26, respectively.

Although the gross appearance and histopathology of the epidermal papillomas on rock sole in the GOA were essentially identical to what was found in the Bering Sea, the prevalence of tumor-bearing rock sole was very much lower in the GOA (0.17% vs 1.3%). Another similarity between the GOA and the Bering Sea, was the strong relationship between shallowness of the sampling station and a high frequency of tumor-bearing rock sole.

The epidermal papillomas of flathead sole were very similar in appearance to the rock sole skin tumors (Figure 27). The ages of tumor-bearing flathead sole ranged from 4 to 11 years, with an average age of 6.5 years. The distribution of tumor-bearing flathead sole followed an inshore pattern (Figure 28) similar in nature to that reported for tumor-bearing rock sole in the Bering Sea.

The epitheliod tumors in Pacific Ocean perch were located primarily on the underside of each operculum (Figure 29) but in some cases they had spread onto the gill arches and body surface. No gross tumors were observed in internal organs. The ages of the affected fish ranged from 9 to 16 years.

Examination of histological preparations of the perch tumors revealed that they have a cellular structure very similar to the pseudobranchial tumors of cod and pollock and that they contain tumor-specific cells closely resembling the X-cells found in the cod and pollock tumors as well as the epidermal papillomas of flatfish (Figure 30).

VII. Discussion

Bering Sea

Both the prevalence and distribution of three diseases: pseudobranchial tumors of cod, lymphocystis of yellowfin sole, and skin tumors of rock sole found by us in the Bering Sea in 1975 (McCain et al. 1977a) were very similar to those we found in 1976. The 1976 incidences for each of the above diseases were slightly higher by 1.3%, 0.7%, and 0.3%, respectively. In both years, the distributions of tumor-bearing cod and yellowfin sole with lymphocystis were almost identical. Rock sole with tumors in 1975 appeared to be distributed in significantly different patterns from those found in 1976; however, this deviation may be explained by differences in the locations of sampling stations. Also, as will be discussed below, the stations sampled in 1975 and 1976 which had the highest frequencies of tumor-bearing rock sole were also the shallowest.

The causes of all but possibly two of the pathological conditions of demersal fishes found near the Outer Continental Shelf of the Bering Sea 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) and epidermal papillomas of rock sole, are neoplasms of unknown cause(s).

An interesting observation in our study was the uneven distribution of three of the five diseases. Yellowfin sole with lymphocystis, rock sole with epidermal papillomas and Pacific cod with skin lesions were most prevalent in the southeastern Bering Sea near Unimak Island.

Diseased yellowfin sole were often part of massive schools which are frequently found in this area of the Bering Sea during the spring (Fandeev 1970). The inevitable close contact between fishes in such schools may facilitate virus transmission.

As mentioned earlier, the two stations near Unimak Island with the highest frequencies of rock sole with epidermal papillomas were also the two shallowest stations of the cruise. Previous studies of pleuronectids with epidermal papillomas have shown that young flatfish between six months and two years of age most often have tumors (Miller and Wellings 1971; Angell et al. 1975). Young rock sole are initially found near the beaches and move into deeper water as they grow older (Clemens and Wilby 1961). Therefore, it is not surprising that the shallow stations would have yielded the most tumor-bearing rock sole.

The frequency data for pollock and rock sole with and without tumors may partially explain some of their population fluctuations. For normal pollock between the ages of one and six, the age compositions for males and females were about the same; however, after age six there were about twice as many females as males. This difference in the proportion of males and females could be explained by differences in life history, such as migration patterns, life expectancies, and by sampling artifacts. However, a critical factor may be the detrimental effects of pseudobranchial tumors. The rapid decline in the abundance of tumor-bearing pollock after age four suggests that affected fish are removed from the population by tumor-related deaths or by preferential migration. The likelihood of the former possibility is strengthened by the reduced growth rate of tumor-bearing pollock. In view of the apparent adverse effects of these tumors, the differential survival of older female pollock may be largely attributable to their low tumor frequency (one-half that of males) at ages two to four.

About equal numbers of young male and female rock sole up to about age seven were captured; however, fish over seven years old were predominately females. Various factors in the life history of rock sole and the sampling technique may explain this observation. Nevertheless, the fact that 20 times more males than females between the ages of two to three years had tumors must also be given

consideration. For example, seven to eight years ago, the males of the present 10-year-old class may have had high tumor frequencies. Although only 1.3% of the total number of rock sole were found with epidermal papillomas, most of the fish examined were captured in deep-water trawls and were over four years old. When younger fish were examined, tumor frequencies of 35% and 59% were found. Our observations and those of Levings (1967) demonstrated that tumors on older fish can spread over as much as 50% of the body surface, including the head region. Extensive tumors and other possible tumor-related factors very likely cause affected fish to be removed from the population. Thus, the numbers of male rock sole may decline due to tumor-related deaths.

As was mentioned above, the apparent predisposition of male rock sole and pollock to tumor development may be caused by life history differences between the sexes. In addition, physiological and other genetic factors may account for the differences. If specific agents cause the tumors, then males may not have the degree of disease resistance that females do, possibly due to hormonal differences. The possibility that these tumors are transmitted vertically from parent to offspring has not been eliminated.

Norton Sound/Chukchi Sea

The most striking aspect of our investigations of the baseline health status of marine animals in Norton Sound and Chukchi Sea has been the low frequency of pathological conditions in these areas. Tumors and tumor-like lesions observed in fish of the eastern Bering Sea have been totally absent in this study. Parasitic conditions seem to be the most prevalent and severe conditions encountered. Parasitism may be an indicator of general health in that an animal that has been weakened by other factors may be more susceptible to parasitic infestation. Conversely, parasitism may lower an animal's resistance to environmental insult.

The reasons for the lack of recognizable pathological conditions on fish in the Norton/Chukchi area are not clear. Conditions found in the nearby Bering Sea included pseudobranchial tumors of cod (Gadus macrocephalus) and pollock, skin tumors of rock sole (Lepidopsetta bilineata), and the virus-caused lymphocystis of yellowfin sole. The total absence of cod and rock sole and the small numbers of pollock in the study area are some of the reasons for the differences between the two areas. However, since yellowfin sole were present in sufficient numbers to detect lymphocystis, it appears likely that in this case, the virus or vector responsible for virus transmission is not present in the Norton/Chukchi area.

Other disease-causing factors may be in the Norton/Chukchi area at reduced levels compared to the Bering Sea. For example, pseudobranchial tumors are presently known to be in three species of gadids: Pacific cod, pollock, and Atlantic cod (Gadus morhua). It is possible that all gadids are susceptible to this disease; nevertheless, over 10,000 saffron cod and 2,952 Arctic cod (Boreogadus saida) were examined in the Norton/Chukchi area and no tumors were found. Either the hypothesis concerning the universal susceptibility of gadids to these tumors is not valid, or the saffron and Arctic cod we examined were not exposed to the tumor-inducing factor. Marine animals capable of transmitting an infectious agent which may enter this area may not come in close enough contact with other fish for transmission to occur.

It is difficult to assess at this time the pathological effects of the "black spot" disease on the Pacific herring, toothed smelt, and saffron cod. Cryptocotyle lingua, a trematode that has metacercariae very similar in appearance to the trematode we found in the above species, has been reported to cause "black spot" conditions in cod (G. callarias), plaice (Pleuronectes platessa), and herring (Clupea harengus) in the Atlantic Ocean (Sinderman, 1970). Cercariae of C. lingua have been experimentally shown to blind and kill immature herring. Nevertheless, the species examined in the Norton/Chukchi area with the "black spot" condition did not appear to be adversely affected.

Gulf of Alaska

Although the frequencies of cod and pollock with pseudobranchial tumors and of rock sole with epidermal papillomas were somewhat lower in the GOA than in the Bering Sea, the existence of these pathological conditions over a wide geographical area of Alaskan marine waters has been established. Also, many of the characteristics of diseased fish in the two areas were similar; for example, more male pollock had tumors than did females by a factor of three in the GOA and a factor of two in the Bering Sea. Also, young pollock between the ages of two and five in the Bering Sea had the highest tumor frequencies while the two-year old pollock had tumors in the GOA. Most Bering Sea and GOA flathead sole and rock sole with tumors were captured at the shallowest sampling stations. With the finding of flathead sole with epidermal papillomas and Pacific Ocean perch with epitheliod tumors in the GOA, the number of Alaskan species with tumors containing the tumor-specific X-cells has been increased to five. As yet, it has not been determined if these cells are chemically or virally transformed host cells or an as yet unidentified group of single-celled parasites.

VIII. Conclusions

Of the six major diseases of fish characterized during the last year, four had a restricted range of geographical distribution. Lymphocystis of yellowfin sole and skin lesions of Pacific cod were essentially found in the Bering Sea; "black spot" disease of Pacific herring, toothed smelt, and saffron cod was found only in the Norton/Chukchi area; and the epitheliod opercular tumors of Pacific Ocean perch were only observed in the GOA.

Pseudobranchial tumors of cod and pollock and epidermal papillomas of rock sole and/or flathead sole, the remaining two diseases, were found in both the Bering Sea and GOA. Although tumor-bearing Pacific cod and pollock were evenly distributed throughout both areas, rock sole with tumors were most often captured in the shallower sampling stations. The depth-related aspect of the rock sole tumors most likely

occurs because young fish probably have the highest tumor frequency, and they tend to be found in shallower water.

Tumor-bearing Pacific cod and pollock were found to have reduced growth rates. This observation may explain why tumor-bearing pollock between the ages of two and four were found in over 10% of the population, but after that age they became very rare. Pollock with tumors may be removed from the population because the size of the tumors prevents the ingestion of food, the tumors serve as an initiation site for infection by microorganisms, or because the tumors have a direct toxic affect on the host.

Male rock sole and pollock appear to be more susceptible to tumors than do females. Rock sole males between the ages of two to three had a 20 times higher tumor frequency than did females, although, for all age classes three times more male rock sole had tumors than females. Male pollock had tumors two and three times more frequently than did females in the Bering Sea and GOA, respectively.

The most outstanding aspect of the data on the baseline health status of the fish and invertebrates in the Norton Sound and Chukchi Sea was the paucity of pathological conditions. Parasitic infestations with questionable pathological consequences were the most common abnormalities.

Because the same general area of the Bering Sea was sampled in both 1975 and 1976, an opportunity was provided to test the accuracy and reliability of our sampling procedures. Both the geographical distribution and the prevalence of the three main fish diseases, pseudobranchial tumors of Pacific cod, lymphocystis of yellowfin sole, and skin tumors of rock sole, were very similar. The major difference between the two years was the findings of pseudobranchial tumors in pollock and skin lesions on cod in 1976.

IX. Needs for further study

A. The near-shore coastal waters of the Gulf of Alaska have not yet been investigated for the baseline health status of demersal fishes and invertebrates. Several types of evidence indicate that it is extremely critical that the information on the distribution and prevalence of pathological conditions in these areas be obtained. During our investigation in the Bering Sea and the GOA, the frequency of skin tumors in rock and flathead sole was very much higher in shallower waters; for example, in the Bering Sea during 1976 the mean frequency of skin tumors in rock sole was 1.3%, but at the two shallowest stations (around 50 meters) the frequency of tumor-bearing sole was 21% and 59%. Also, the only sampling station at which tumor-bearing rock sole were captured in the GOA was the shallowest (63 meters).

Environmental stresses are known to make fish and invertebrates more susceptible to disease agents. Coastal and estuarine waters have greater extremes of these types of stress than do deeper waters, including higher and lower temperatures, more suspended sediments, lower salinity, and closer proximity to urban pollution.

Another justification for baseline research in coastal waters is that certain age classes and species are found there that have not been encountered in our deep-water work. The starry flounder is a good example, so far we have only examined adult starry flounders over 3 years old and none had tumors. Nevertheless, one of the first reports of skin tumors in Alaskan waters was a report by Turner (1886) describing starry flounder with such tumors captured near an Aleutian Island. The main reason we have not yet seen tumor-bearing starry flounder is probably that the young flounder which would have the highest tumor frequency (Wellings et al. 1976) are found almost exclusively in estuaries and shallow waters (Clemens and Wilby 1961).

B. Baseline data on fish diseases in the Beaufort Sea has not been obtained because of the following factors: (1) due to ice conditions, it is feasible to sample this area for only a short period each year; (2) the techniques used by the

Alaska Dept. of Fish and Game to sample fish in this area largely employ fish traps and gill nets, procedures which are not satisfactory for pathological studies; and (3) the remoteness of the area has made transportation of samples and direct participation by members of our research unit very difficult.

We propose to take a more direct role in the collection of fish and invertebrates in the Beaufort Sea. A small boat could be bought, borrowed, or leased which would tow a small otter trawl. Two or more members of our research unit would go to the area when the maximum amount of ice is gone, and spend two to three weeks collecting specimens with this boat. The specimens would be examined for pathological conditions in the same manner as described for our other sampling areas.

In addition, Dr. Albert Sparks, a well known invertebrate pathologist recently arrived at the NWAFC, Seattle, is very much interested in participating as a co-principal investigator with our research unit. Invertebrate specimens collected for or by him in any of the above mentioned sampling areas would be returned to the NWAFC for thorough histopathological examination. Dr. Sparks' expertise in marine invertebrates and fish pathology will surely enhance the effectiveness of our research unit.

X. Summary of 4th Quarter Operations

A. Ship and Laboratory Activities

1. Ship schedule

Vessel: NOAA Ship Miller Freeman

Cruise: MF-77-1, Leg I

Dates : January 20 to February 10, 1977

2. Scientific Party

a. Ship activities

Mark S. Myers
NMFS, NOAA, NWAFC

Role: Party Chief. Examined fish and invertebrates for pathological conditions, collected tissue specimens, processed biological data, and prepared cruise reports.

b. Laboratory Activities

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.

Mark S. Myers
NMFS, NOAA, NWAFC

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

William D. Gronlund
NMFS, NOAA, NWAFC

Role: Participates in data processing and analyses of biological data.

Katherine King
NMFS, NOAA, NWAFC

Role: Invertebrate pathologist, participates in data processing, analyses of biological data, and histopathological examination of invertebrate tissue specimens.

S. R. Wellings, M.D., Ph. D.
Department of Pathology, School of Medicine
University of California (Davis)

Role: Coordinates histopathological analyses of tissue specimens.

3. Methods

a. Field Activities

Fish were sorted according to species, and subsamples were selected by members of OCSEAP R.U.'s #175. Fish were examined for externally visible pathological conditions and, when feasible, for readily recognizable internal disorders. The following information was recorded for each haul in the Haul Data Sheet: haul number, date, number of animals examined of each species, sex (this data was available for fish from the length-frequency records of R.U. #175), the type of pathological condition observed, and the number of animals with each type of condition for each species and each sex.

Animals with abnormal conditions were processed while still alive or freshly dead. Each animal was assigned a specimen number and the following information was recorded on the Individual Data Sheet: species, sex, length, weight, method of age determination (otolith or scale), condition, and location and size of the condition(s). Photographs were taken of representative and unusual conditions. Tissue samples were preserved in 10% formalin with phosphate-buffered saline. Specimens were also preserved in a special fixative for electron microscopy.

b. Laboratory Activities

Laboratory activities were mainly concerned with processing the specimens and data obtained during Legs I and II of Cruise MF-76-B and from Leg I of Cruise MF-77-1. Tissue specimens from animals with the main pathological conditions to be examined histologically were matched with the photographic colored slides showing the gross appearance of the lesions. Histological procedures are being performed. Specimens from normal-appearing invertebrates were trimmed and blocked, and selected pieces of tissue were carefully described, placed in small vials containing fixative, and were sent to the Department of Pathology, School of Medicine, University of California (Davis) for histological processing under the supervision of Dr. S. R. Wellings.

Scales and otoliths from abnormal fish were examined for age determination.

The Haul Data and Individual Data Sheets for fish were key punched onto computer cards and analyzed, and the invertebrate data was summarized as much as possible. However, since fish parameters do not apply to invertebrates, a new lesion location code and computer format have been developed by PMEL (Mr. Dean Dale). After approval by OCSEAP personnel, the data will be transcribed to new data sheets and then transferred to computer cards for further analysis. Information on the Haul Data Sheets was also used to construct distribution maps of the major abnormalities.

4. Sample localities

The location of the sampling area in the GOA is described in the attached annual report.

5. Data collected

a. Number and types of samples.

In the GOA, 10,779 fish, representing 34 species were examined and 91 animals had pathological conditions.

b. Number and types of analyses.

From the GOA, 37 tissue specimens preserved for light microscopy and 19 for electron microscopy were returned to the NWAFC and are being processed.

Nine fish and 30 invertebrate specimens from the Norton Sound/Chukchi Sea cruise have been examined histologically.

6. Milestone chart and data submission schedules.

a. Data submission schedules.

Data collected in the GOA and Norton/Chukchi area concerned with fish pathology are presently being transferred from data sheets to computer cards, and these cards will be submitted to OCSEAP by April 30, 1977.

Table 1

BERING SEA FISH SPECIES EXAMINED EXHIBITING NO SIGNIFICANT PATHOLOGY

Species	Number Examined	Number of Sampling Stations Where Examined
Flathead sole *Hippoglossoides elassodon*	2977	47
Arrowtooth flounder *Atheresthes stomias*	2416	35
Halibut *Hippoglossus stenolepsis*	513	28
Greenland turbot *Reinhandtius hippoglossoides*	979	26
Alaska plaice *Pleuronectes quadrituberculatus*	728	21
Longhead dab *Limanda proboscidea*	169	5
Pacific Ocean perch *Sebastes alutus*	149	9
Pacific sandfish *Trichodon trichodon*	10	1
Black cod *Anoplopoma fimbria*	18	3
Herring *Clupea harengus pallasi*	175	4
Rainbow smelt *Osmerus mordax dentex*	20	1
Capelin *Mallotus villosus*	100	1
Wattled eelpout *Lycodes palearis*	2	1
Dogfish *Squalus acanthius*	1	1
Snailfish *Careproctus sp.*	3	1
Starry flounder *Platichthys stellatus*	41	9
Dover sole *Microstomus pacificus*	1	1
Sturgeon poacher *Agonus acipenseris*	1	1
Gymnocanthus sp.	1	1
Myoxocephalus sp.	9	1
Myoxocephalus jaok	1	1
Myoxocephalus polyacanthocephalus	1	1
	8,417	

TABLE 2

THE AVERAGE DISEASE FREQUENCY FISH FOR EACH OF THE FIVE PATHOLOGICAL CONDITIONS FOUND IN THE BERING SEA.

Species and Disease	No. of Fish Examined	No. of Fish Affected	Average Disease Frequency (%)
Pacific cod Pseudobranchial tumors	4,654	403	8.7
Pacific Cod Skin Ulcers	4,654	73	1.6
Pollock Pseudobranchial tumors	9,173	156	1.7
Yellowfin Sole Lymphocystis	8,036	228	2.8
Rock Sole Epidermal papillomas	6,440	87	1.3

Table 3

Major fish species exhibiting no significant pathology in Norton Sound and the Chukchi Sea.

Species	No. examined	No. of stations where examined
Pleuronectes quadrituberculatus (Alaska plaice)	1,423	104
Limanda aspera (Yellowfin sole)	3,279	88
Platichthys stellatus (Starry flounder)	739	82
Boreogadus saida (Arctic cod)	2,952	135
Agonus acipenserinus (Sturgeon poacher)	53	12
Theragra chalcogramma (Walleye pollock)	270	26
Mallotus vellosus (Capelin)	215	10
Limanda probiscidea (Longhead dab)	322	19
Hippoglossoides robustus (Bering flounder)	144	42
Liparid sp. (5 species) (Snail fish)	267	13
Myoxocephalus sp. (3 species) (Sculpin)	480	28
Liopsetta glacialis (Arctic flounder)	63	15
Other (7 species)	69	19

Table 4

The average disease frequency for fish and invertebrates with major pathological conditions found in the Norton Sound and Chukchi Sea.

Species and condition	No. examined	No. affected	Average disease frequency (%)
Clupea harengus pallasi (Pacific herring) "black spot"	2,027	92	4.5
Osmerus mordax dentax (Toothed smelt) "black spot"	3,772	146	3.9
Eleginus gracilis (Saffron cod) "black spot"	10,826	91	0.8
Sclerocrangon boreas (Shrimp) Leech eggs	659	278	42.2
Leptasterias sp. (Sea star) Parasitic gastropod	769	87	11.3
Hyas coarctatus alutaceus (Spider crab) Dark/eroded exoskeleton	645	46	7.1
Pagurus capillatus (Hermit crab) Rhizocephalan parasite	1,529	87	5.7
Leptasterias polaris (Sea star) Parasitic gastropod	4,951	238	4.8
Argis lar (Shrimp) Pale, enlarged eggs	6,704	183	2.7
Bopyridean parasite	6,704	84	1.3

Table 5

Major invertebrate species exhibiting no significant pathology in Norton Sound and the Chukchi Sea.

Species	No. examined	No. of stations where examined
Chionoecetes opilio (Tanner crab)	5,036	66
Pagurus trigonocheirus (Hermit crab)	1,692	39
Pandalus goniurus (Shrimp)	636	21
Strongylocentrotus drobachiensis (Sea urchin)	617	25
Crangon dalli (Shrimp)	598	27
Labidochirus splendescens (Hermit crab)	447	18
Paralithodes camtschatica (King crab)	400	36
Telmessus cheiragonus (Crab)	269	22
Other (16 species)	330	48

Table 6

Fish species with no detectable pathological abnormalities in the Gulf of Alaska

Species	#Examined
Hippoglossus stenolepsis (Pacific Halibut)	370
Atheresthes stomias (Arrowtooth flounder)	2,397
Dasycottus setiger (Spinyhead sculpin)	56
Anoplopoma fimbria (Black cod)	192
Hexagrammos decagrammus (Kelp greenling)	2
Glyptocephalus zachirus (Rex sole)	651
Microstomus pacificus (Dover sole)	5
Pleuronectes guadritubercularus (Alaska plaice)	1
Gymnocanthus galeatus (Armorhead sculpin)	50
Hemilepidotus jordani (Yellow Irish Lord)	271
Myoxocephalus polyacanthocephalus (Great Sculpin)	100
Microgadus proximus (Tom cod)	4
Hexagrammos stelleri (White spotted greenling)	6
Triglops pingeli (Ribbed sculpin)	41
Triglops macellus (Roughspine sculpin)	3

Table 6 (Cont'd)

Species	# Examined
Triglops forcacata (Sissortail sculpin)	3
Parophrys vetulus (English sole)	3
Platichthys stellatus (Starry flounder)	2
Osmerus mordax dentex (Rainbow smelt)	9
Raja kincaidi (Black skate)	4
Sebastes ciliatus (Dusky rock fish)	31
Sebastes polyspinus (Northern rockfish)	55
Sebastes aleutianus (Rougheye rock fish)	91
Malacacottus kincaidi (Blackfin sculpin)	10
Bathymaster signatus (Searcher)	21
Pleurogrammas monopterygius (Atka mackerel)	20
Zaprora silenus (Prowfish)	1
Total	10,779

Table 7.

The average Disease Frequency of fish with significant pathological conditions in the Gulf of Alaska

Species & Disease	Number affected per number examined	Average Frequency (%)
Cod - pseudobranchial tumors	38/1175	3.23%
Pollock - pseudobranchial tumors	34/1879	1.80%
Flathead - epidermal papilloma	10/1250	0.80%
Rock sole - epidermal papilloma	3/1853	0.16%
Pacific Ocean Perch - epitheliod tumors	4/19	21.05%

Figure 1. Bilateral pseudobranchial tumors (T) in the pharynx of a Pacific Cod.

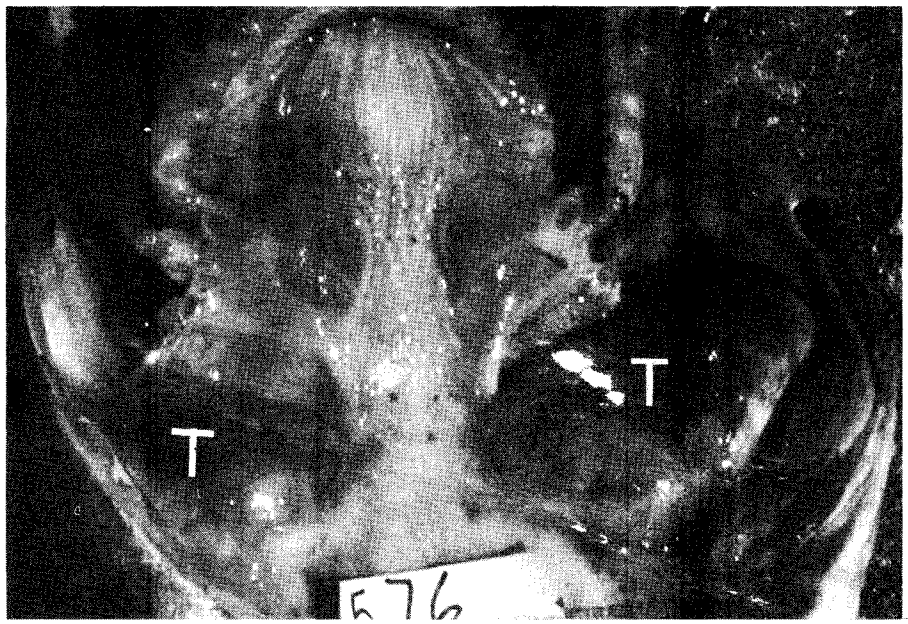
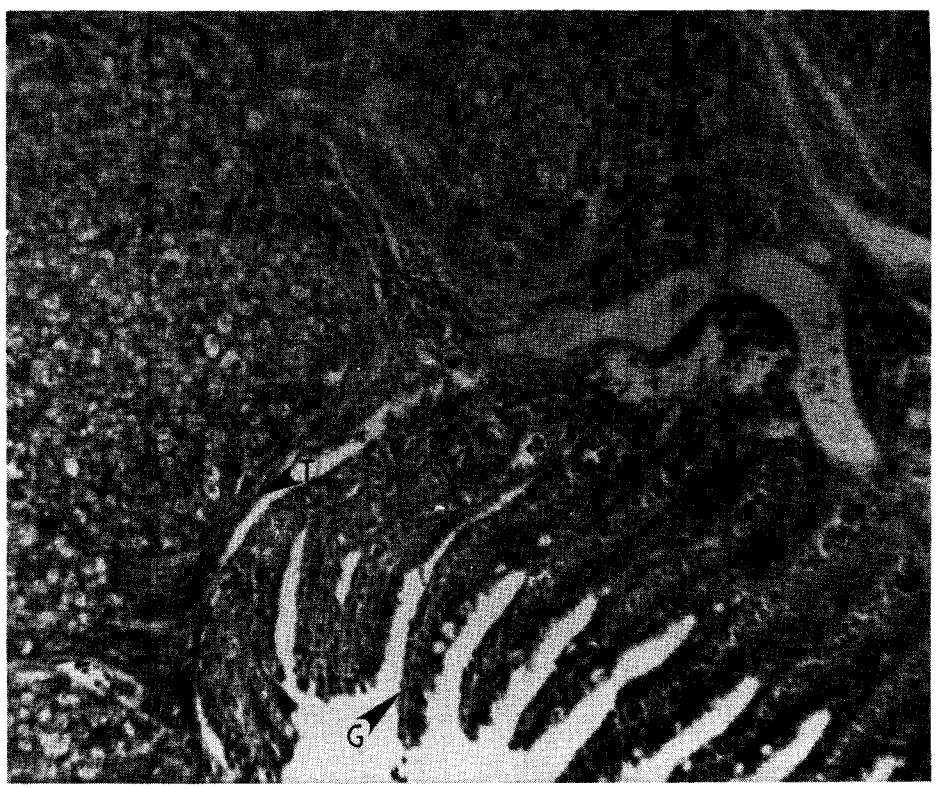


Figure 2. Section of a secondary pseudobranchial tumor (T) attached to the gill filament (G) of a Pacific Cod. (Hematoxylin and Eosin, X450)



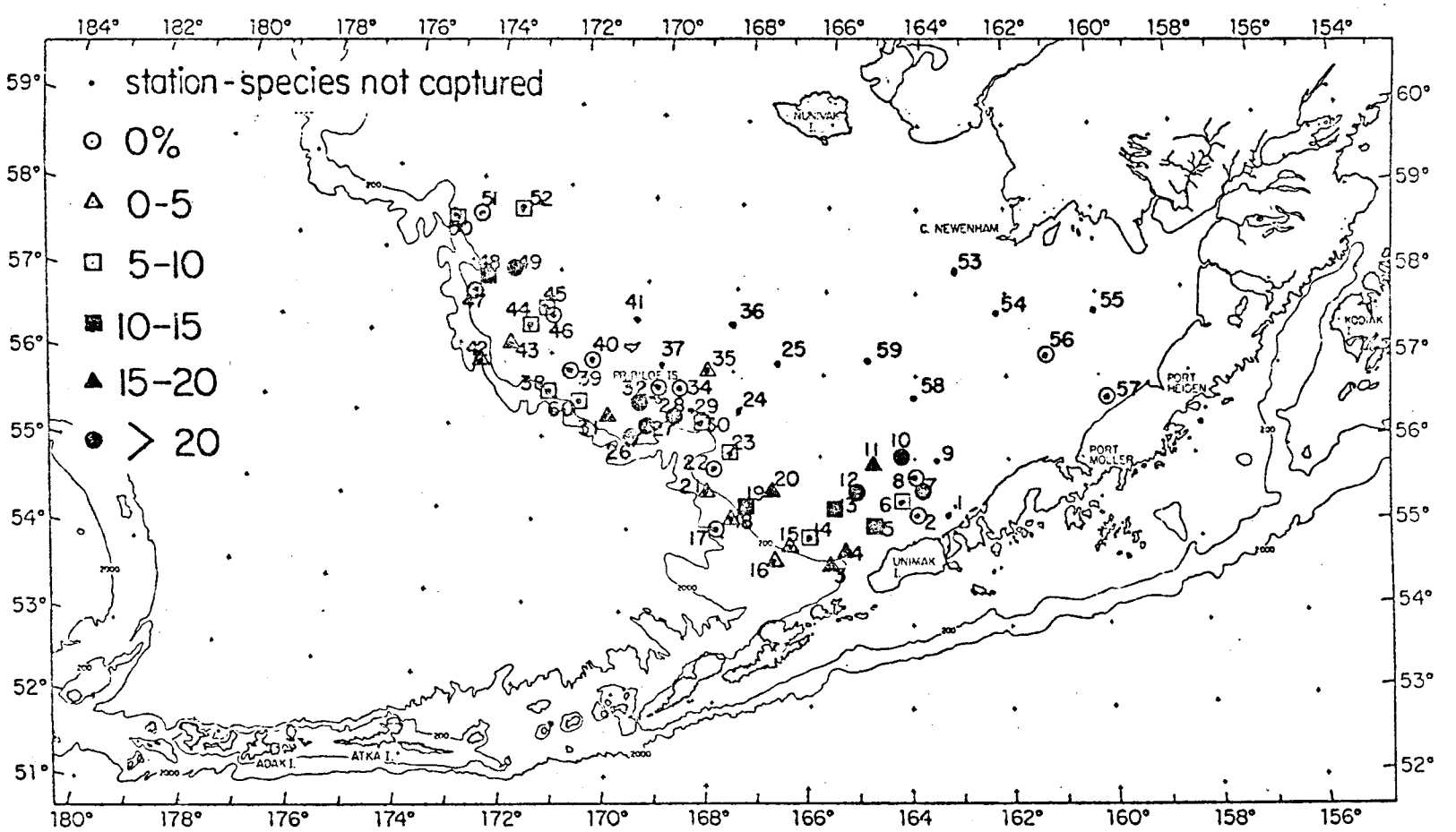


Figure 3. The disbribution and frequencies of cod with pseudobranchial tumors in the Bering Sea.

Figure 4. Bilateral pseudobranchial tumors (arrows) in the pharynx of a pollock.

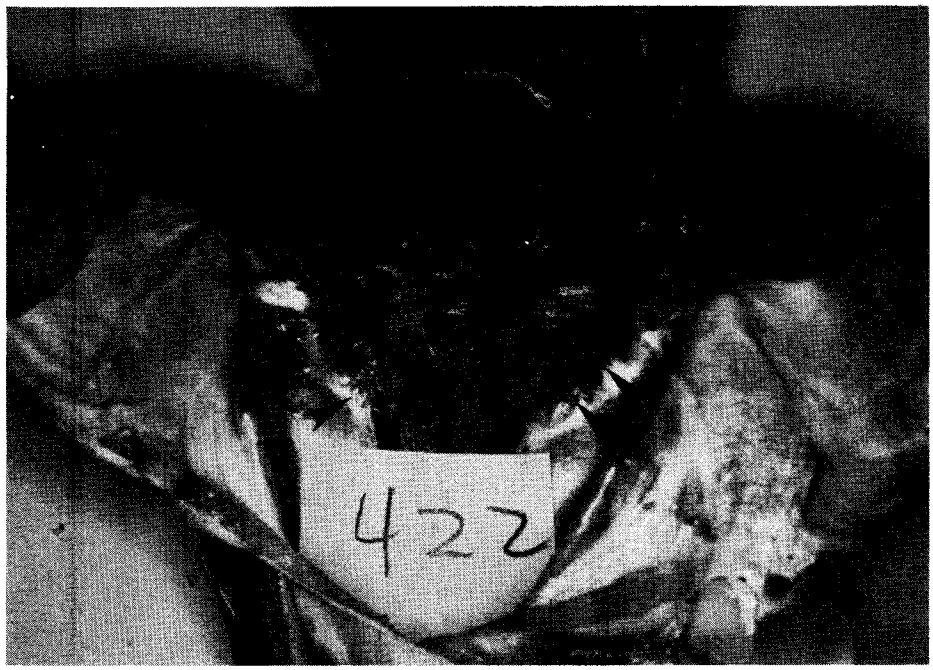
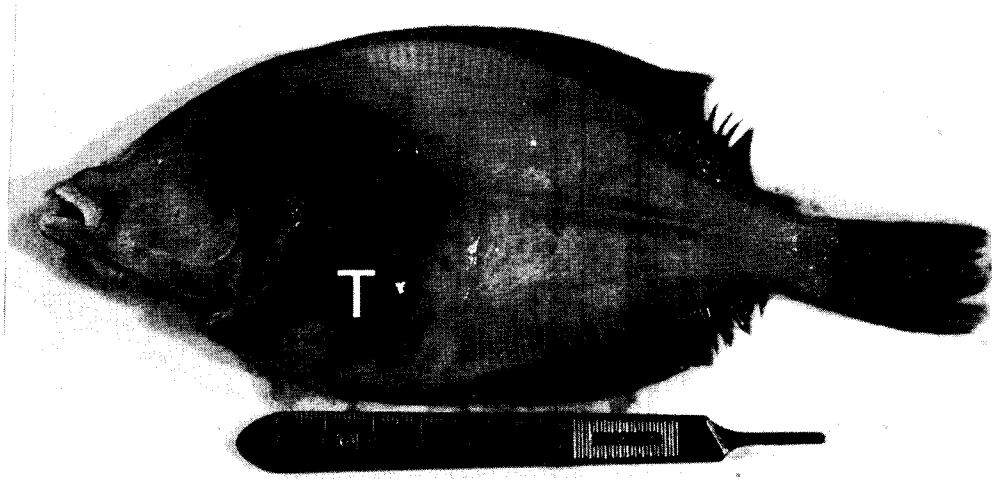


Figure 6. Rock sole with an epidermal papilloma (T) on the "blind" side.



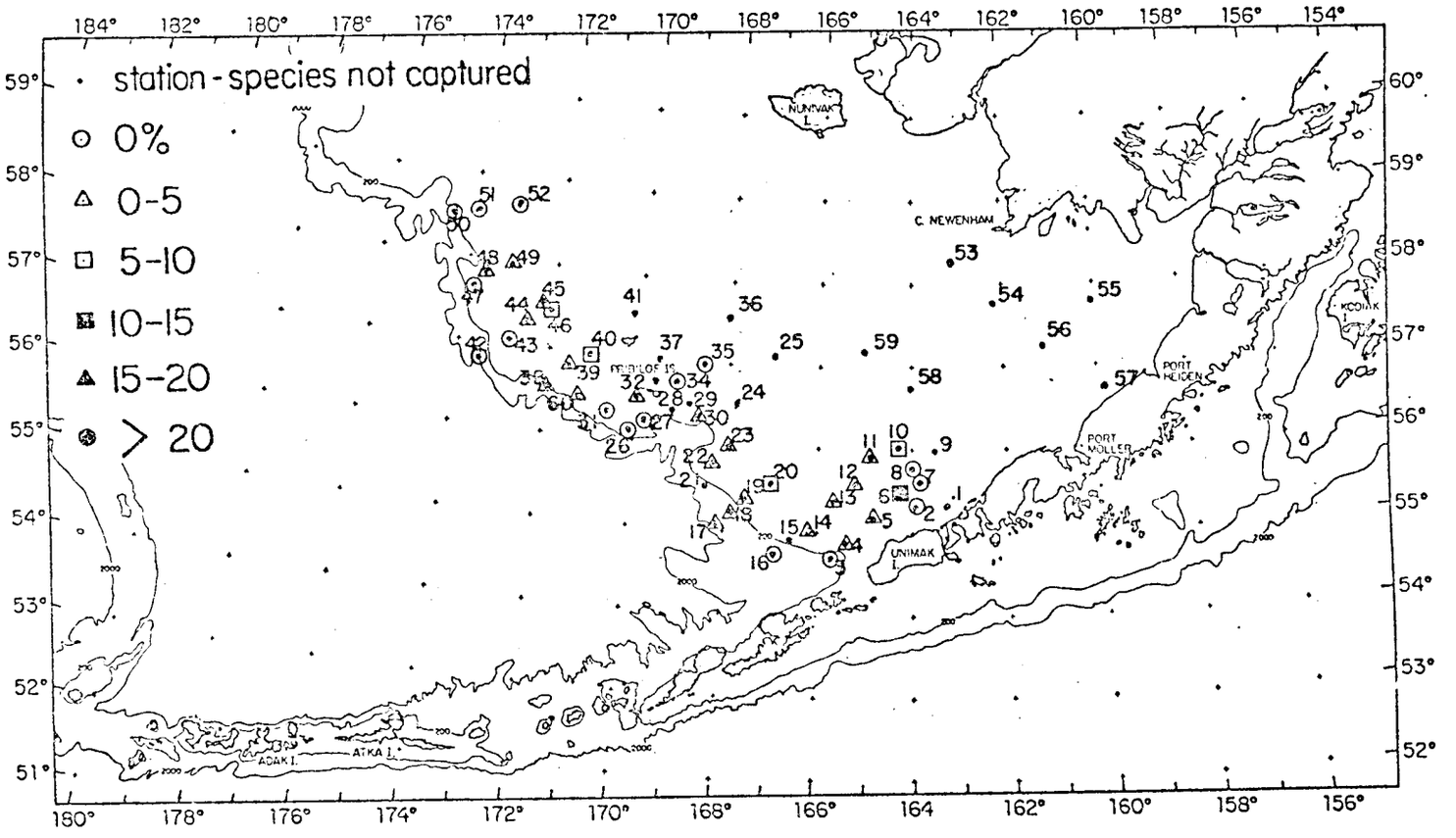


Figure 5. The distribution and frequencies of pollock with pseudobranchial tumors in the Bering Sea.

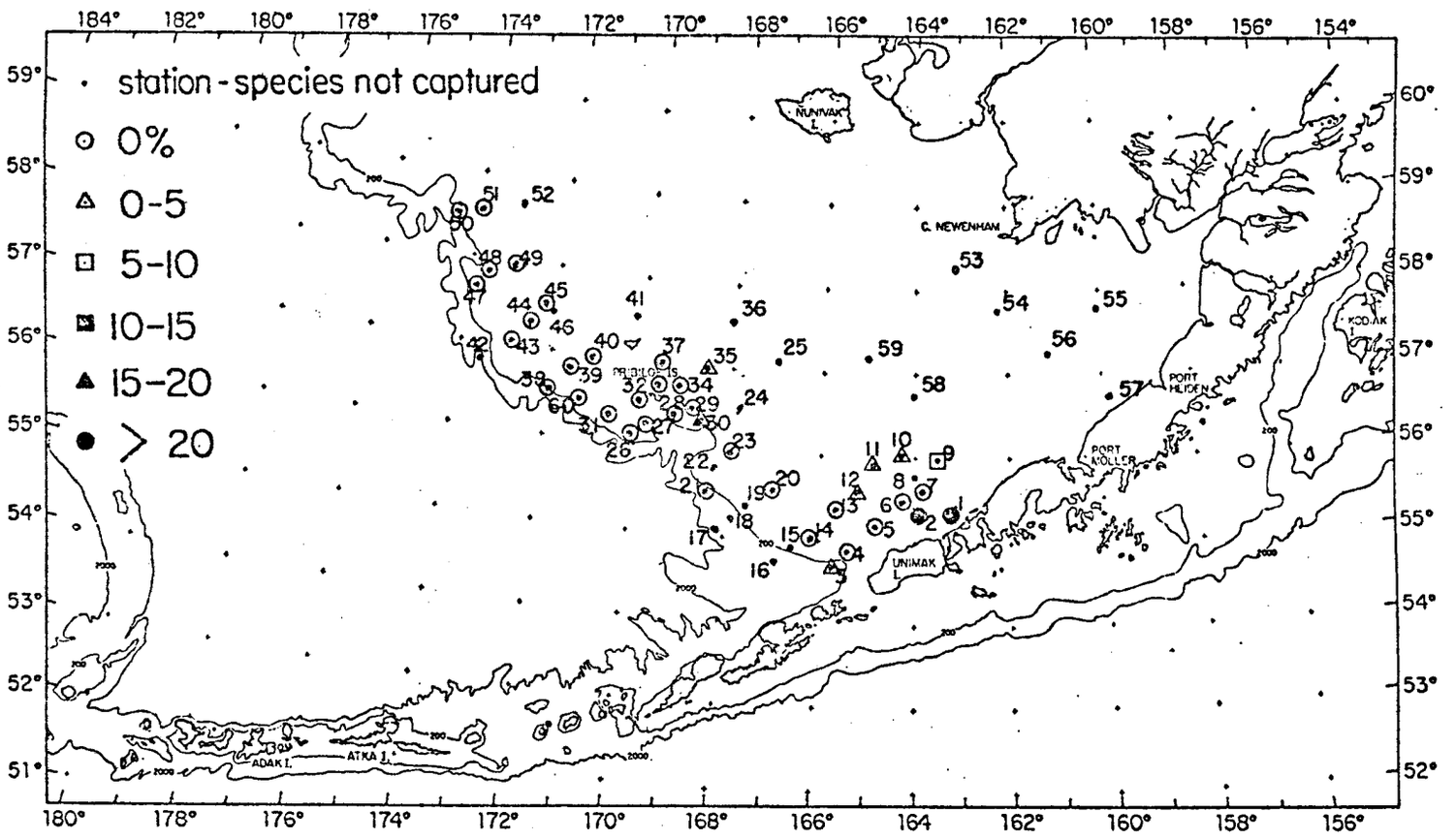


Figure 7. The distribution and frequencies of rock sole with epidermal papillomas in the Bering Sea.

Figure 8. Lymphocystis growth on the [redacted] side pectoral fin of a yellowfin sole.

Figure 10. Electron micrograph of hexagonally-shaped lymphocystis virions (200 nm in diameter) in a lymphocystis cell from a yellowfin sole.

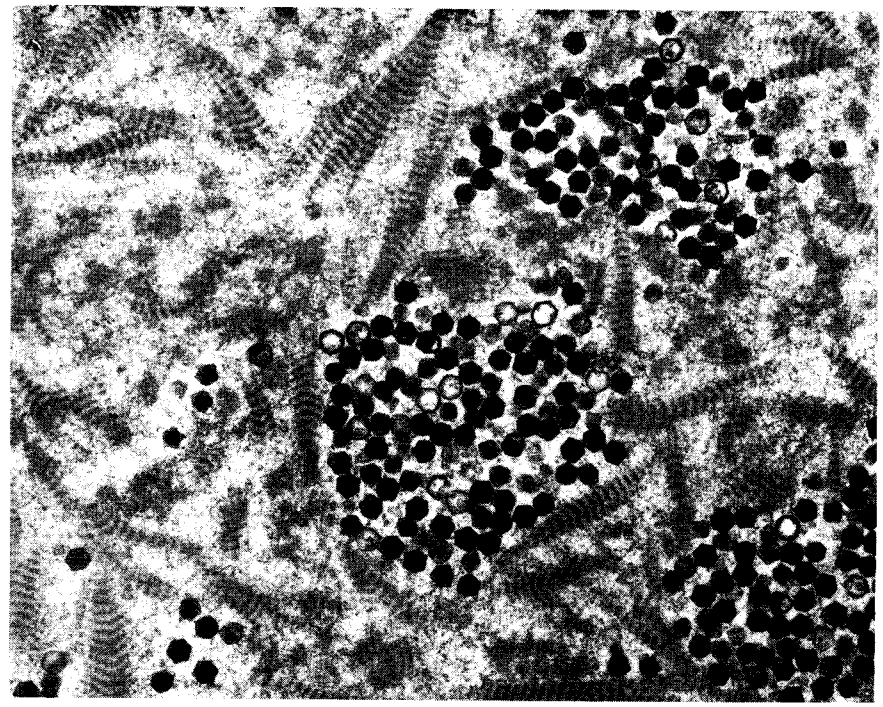


Figure 9. The distribution and frequencies of yellowfin sole with lymphocystis in the Bering Sea.

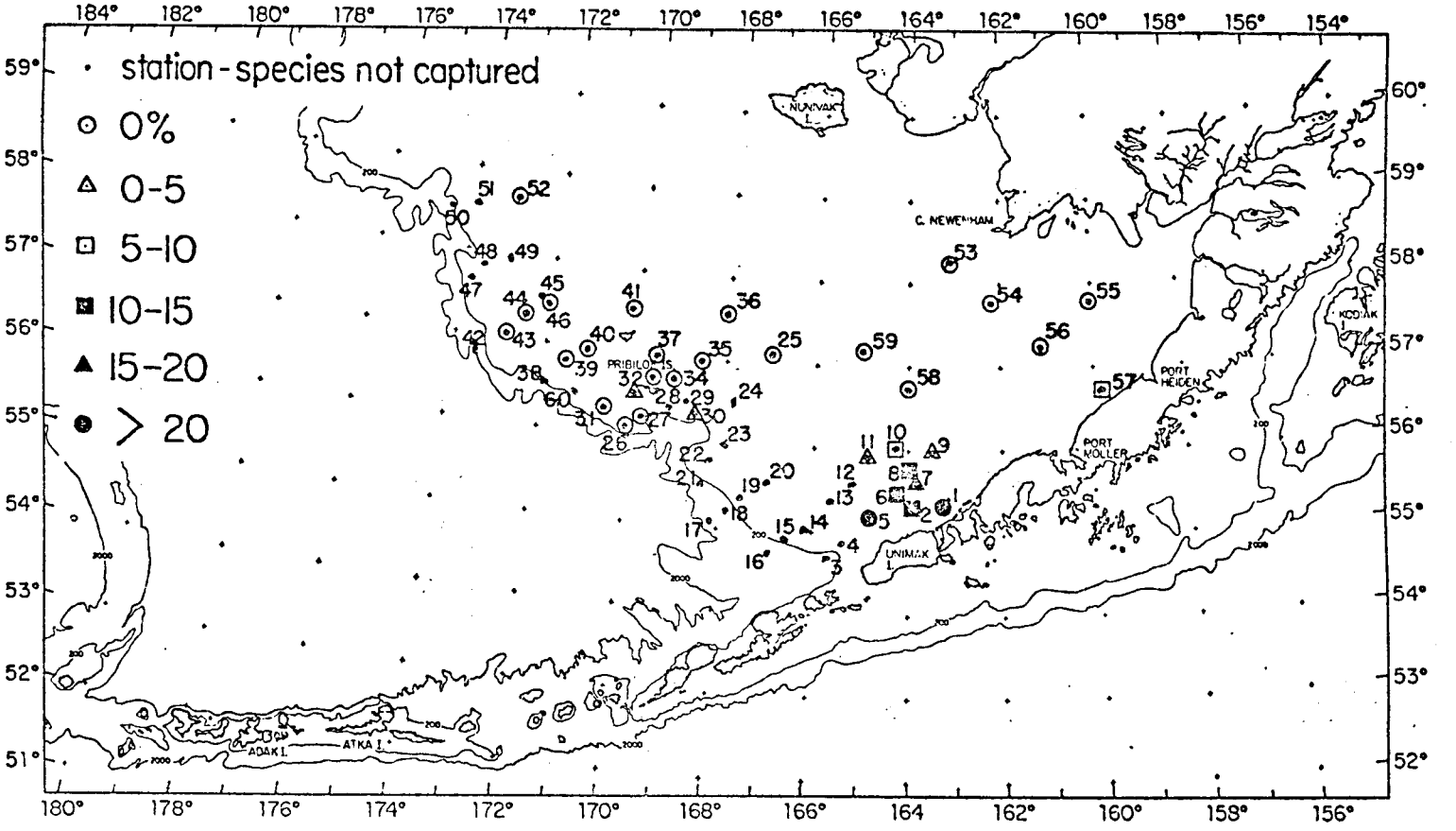


Figure 11. Skin ulcers on the ventrolateral surface of a Pacific cod.

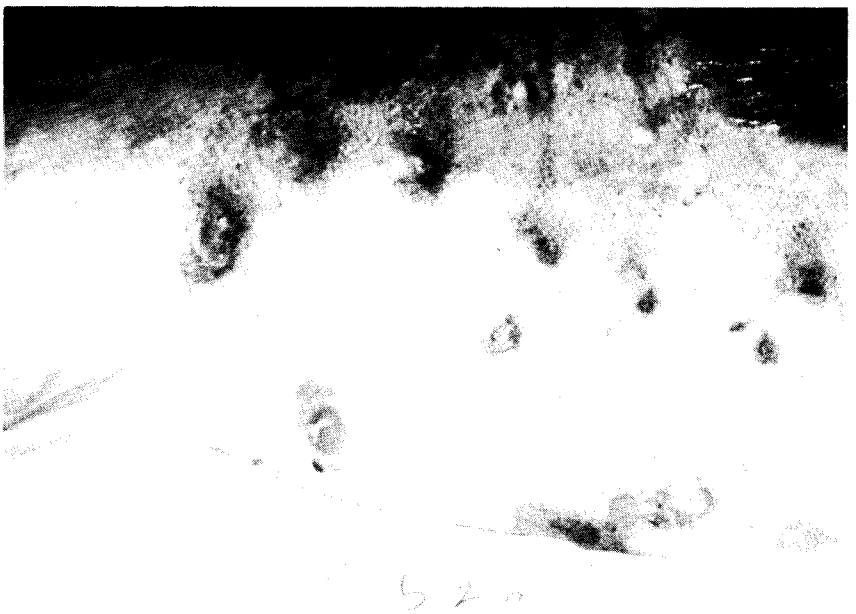


Figure 12. Two ring-like lesions near the caudal region of a Pacific cod.

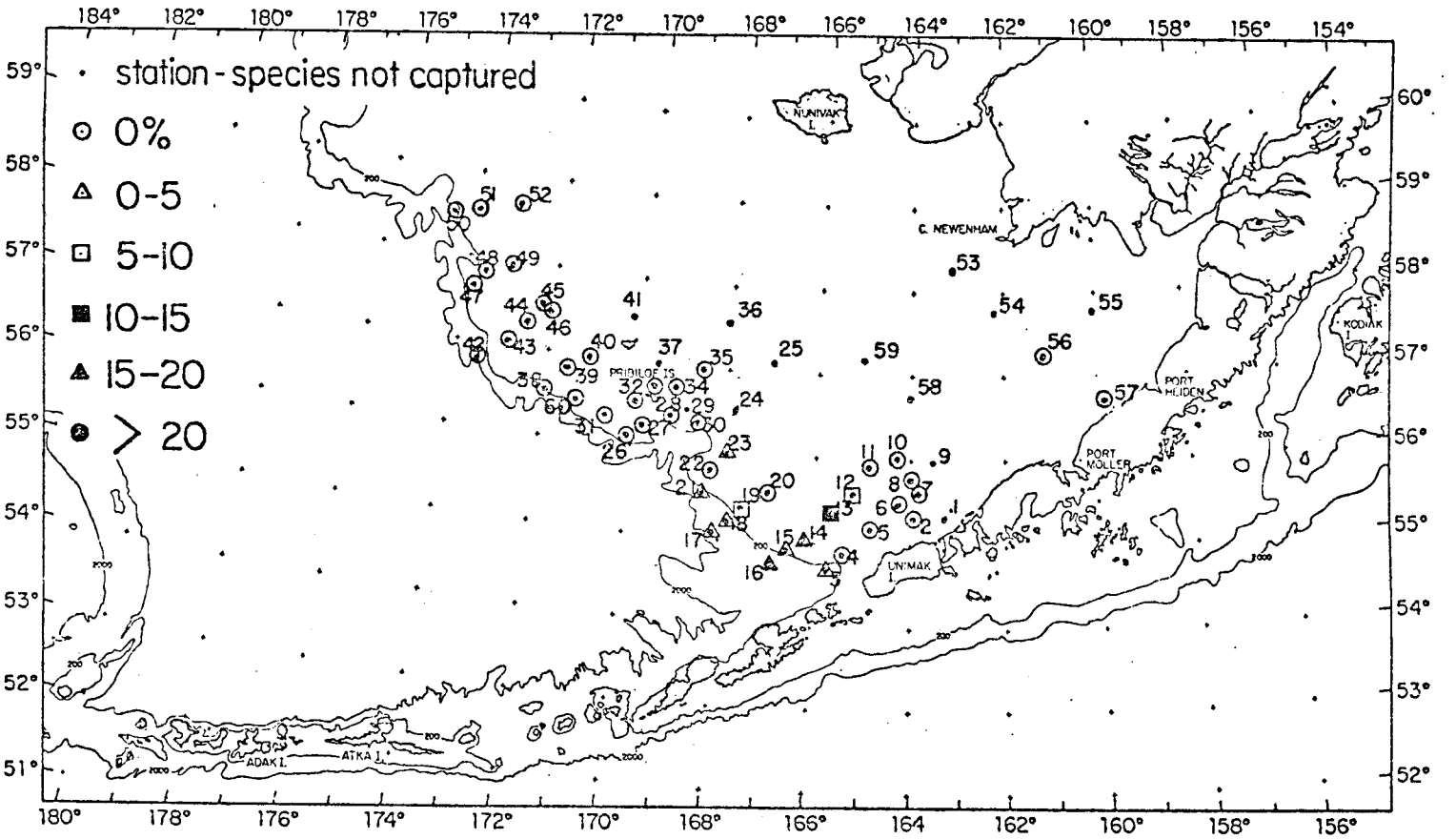


Figure 13. The distribution and frequencies of Pacific cod with skin ulcers in the Bering Sea.

Figure 14. Section of a skin ulcer on a Pacific cod. Normal appearing epidermis (E) is to the left, the ulcerated area (U) is to the bottom, and the dermis surrounding the ulcer has extensive hyperemia (h). (Hematoxylin and Eosin, X250)

Figure 15. Section of normal skin from a Pacific cod showing epidermis (E) containing mucous cells (M) and large cystic structures (CS). The normal-appearing dermis (D) is also present. (Hematoxylin and Eosin, X250)

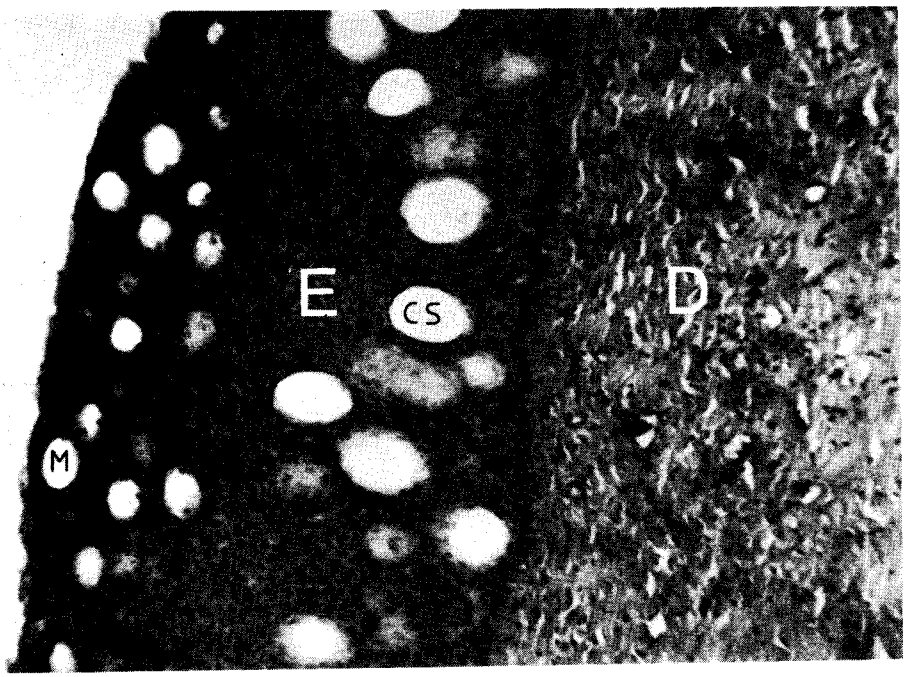


Figure 16. Section of a ring-shaped skin lesion from a Pacific cod demonstrating the basophilic bodies (b) in the epidermis (E). The dermis (D) associated with this lesion has extensive infiltration by erythrocytes and leucocytes. (Hematoxylin and Eosin, 300X)

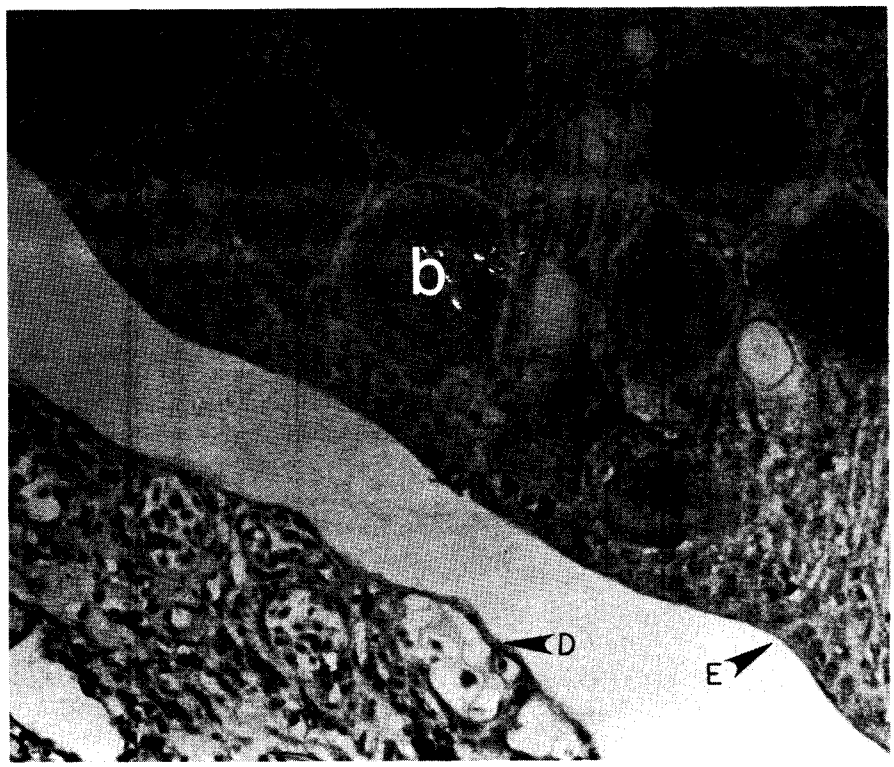


Figure 17. Saffron cod from Norton Sound / Chukchi Sea area with "black spots" on its skin.

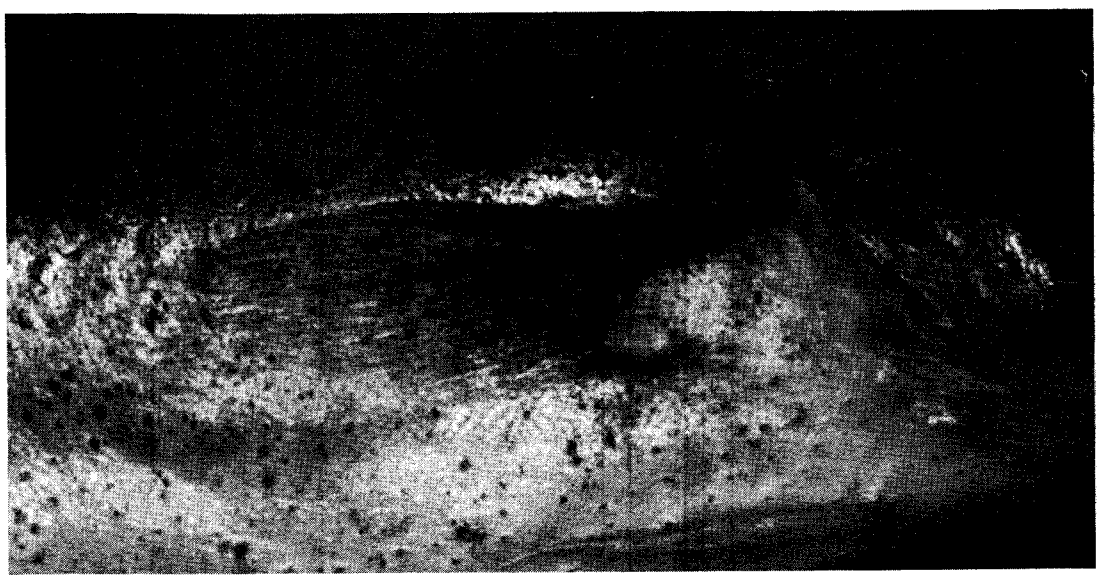


Figure 18. Metacercaria (m) of unidentified trematode from a toothed smelt (Osmerus mordax dentex) in Norton Sound / Chukchi Sea. (Hematoxylin - Eosin, X450)

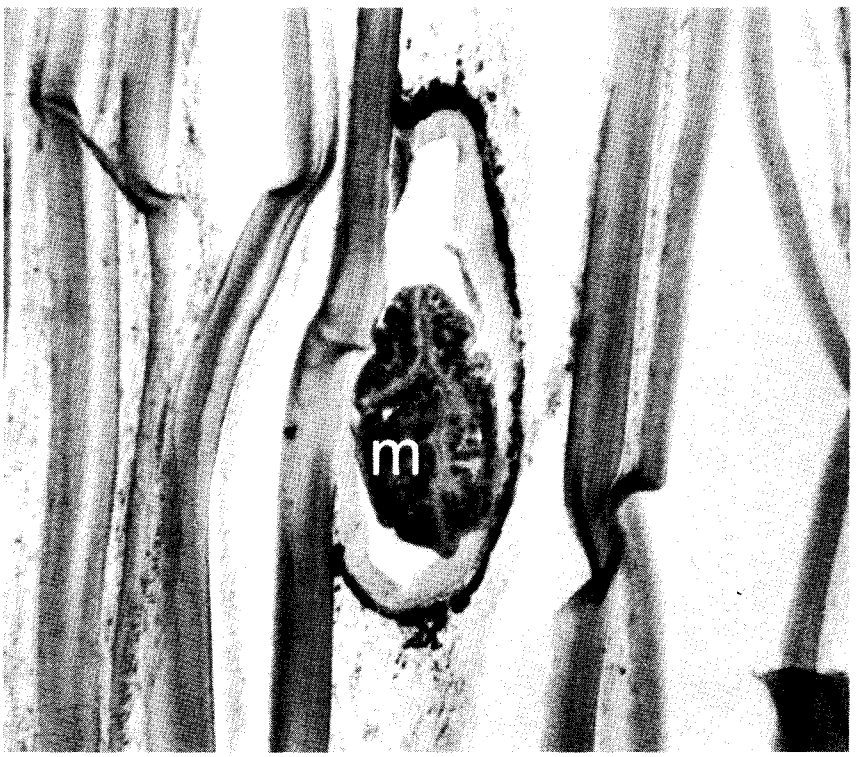


Figure 19. A photomicrograph of a "black spot" in the pectoral fin of a saffron cod in Norton Sound / Chukchi Sea area. Encysted area contains a larval helminth. X450.

Figure 20. Frequencies of "black spot" in smelt at the haul stations where this species was caught in the Norton Sound / Chukchi Sea areas.

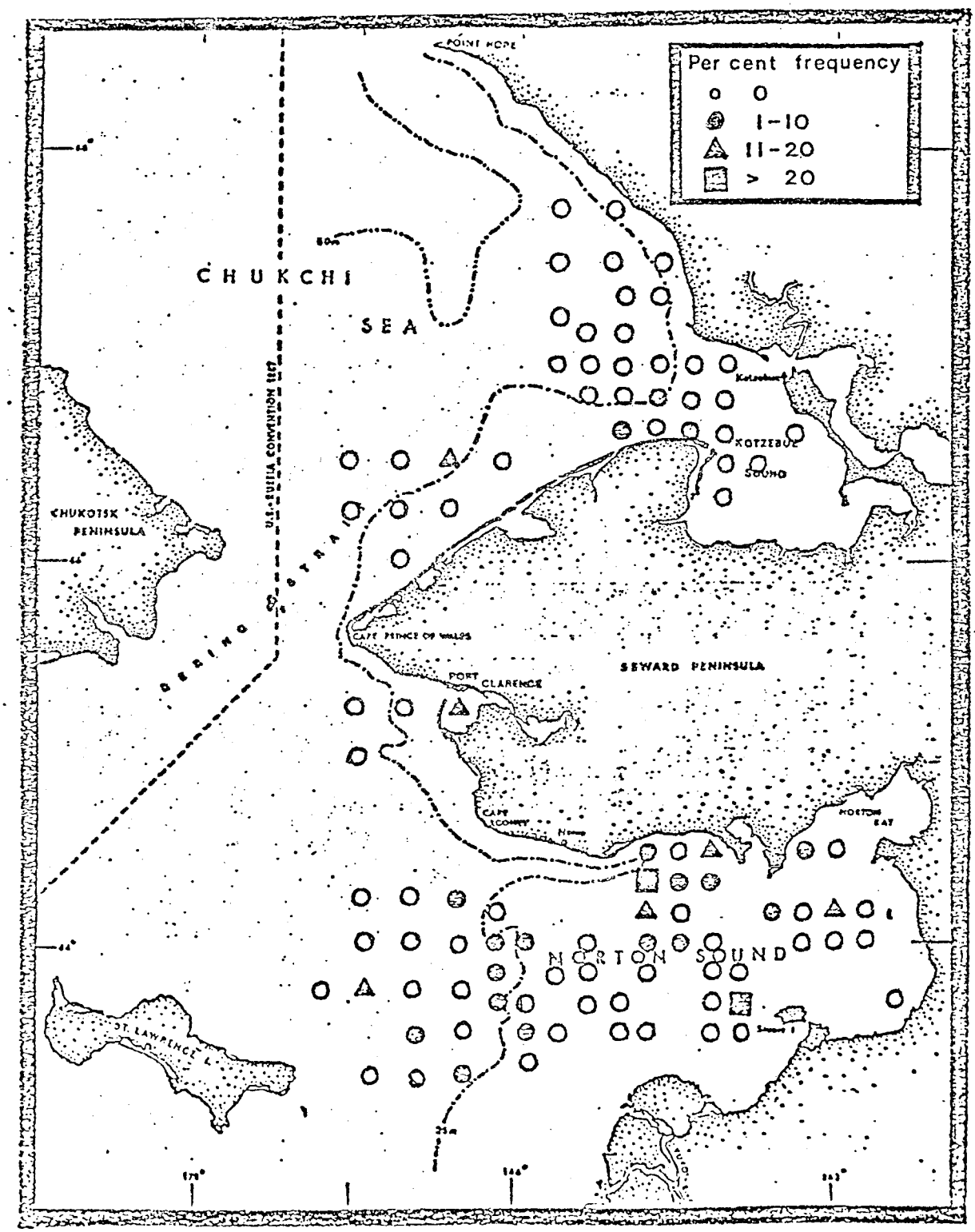


Figure 21. Frequencies of "black spot" in Herring at haul stations where this species was caught in the Norton Sound / Chukchi Sea areas.

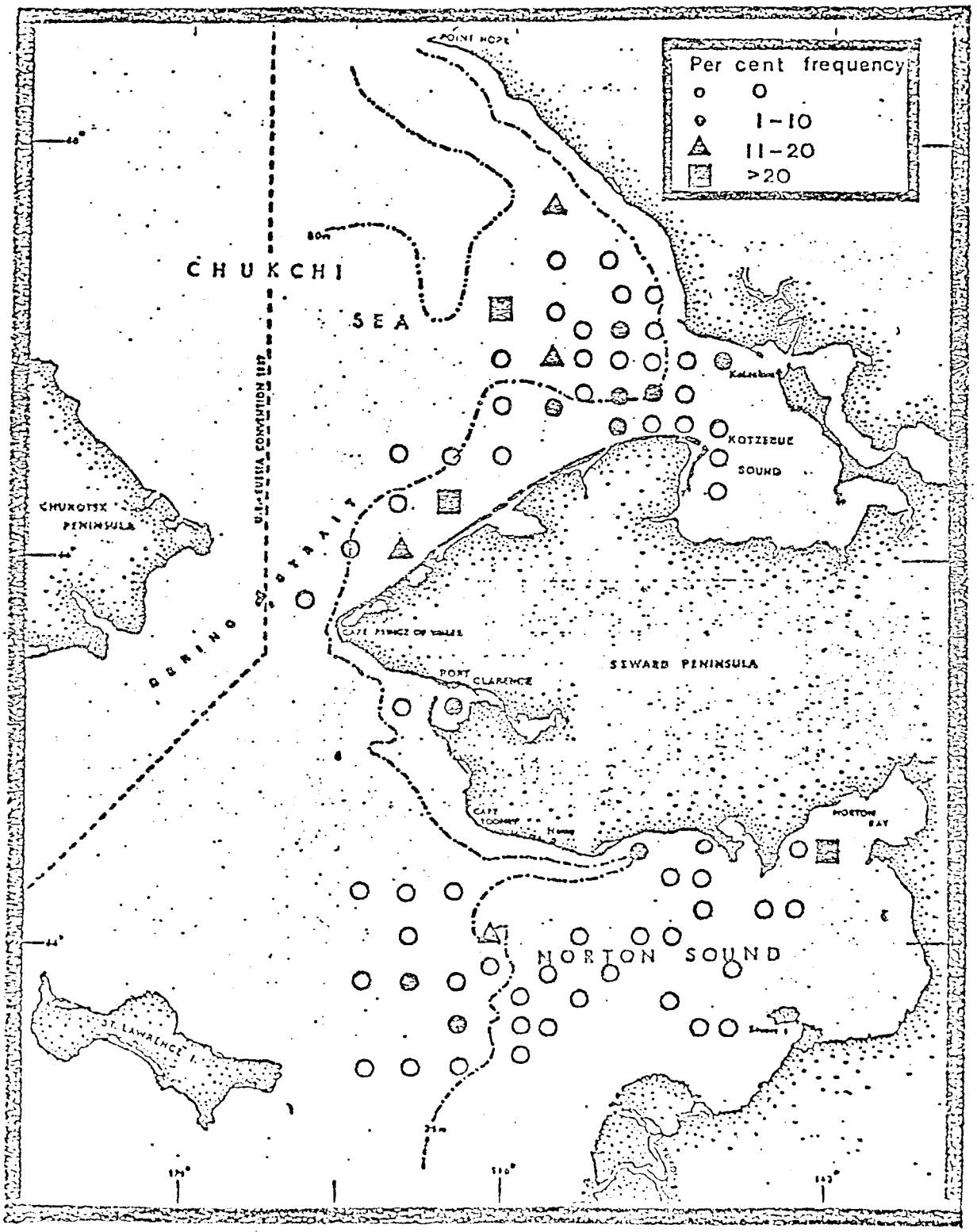


Figure 22. Leech eggs (le) on pleopods of gravid Sclerocrangon boreas in Norton Sound / Chukchi Sea areas.

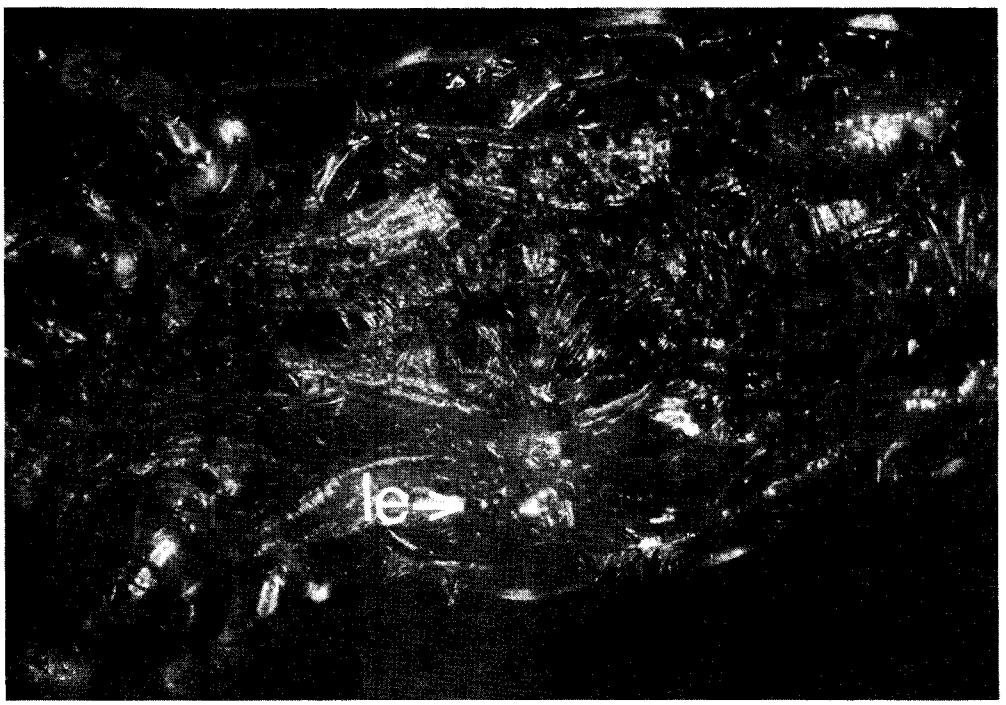


Figure 23. Parasitic gastropod distending the surface of Leptasterias polaris in Norton Sound / Chukchi Sea areas.

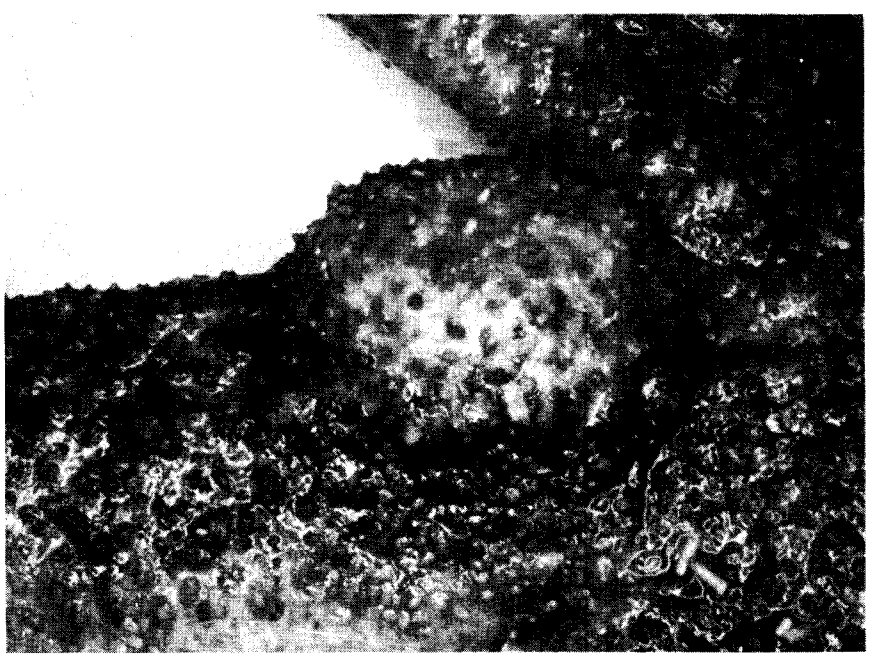


Figure 24. Veliger larvae found in body mass of adult parasitic gastropod in Leptasterias polaris in Norton Sound / Chukchi Sea areas. X150.

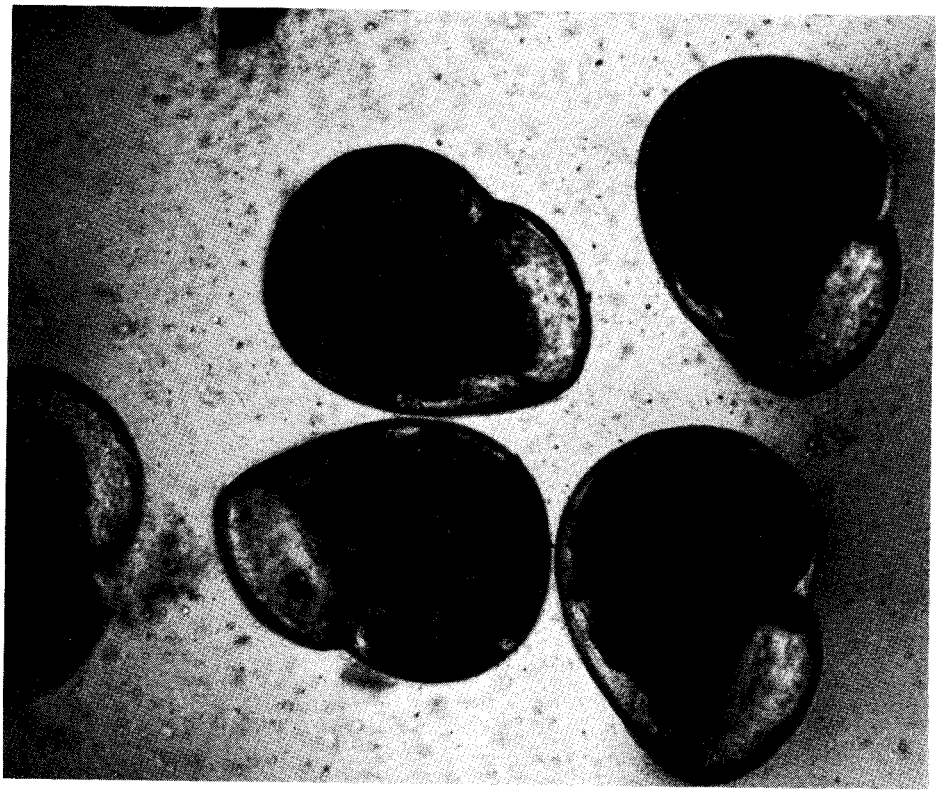


Figure 27. Flathead sole with an epidermal papilloma (T) on the "blind side" of the caudal region.

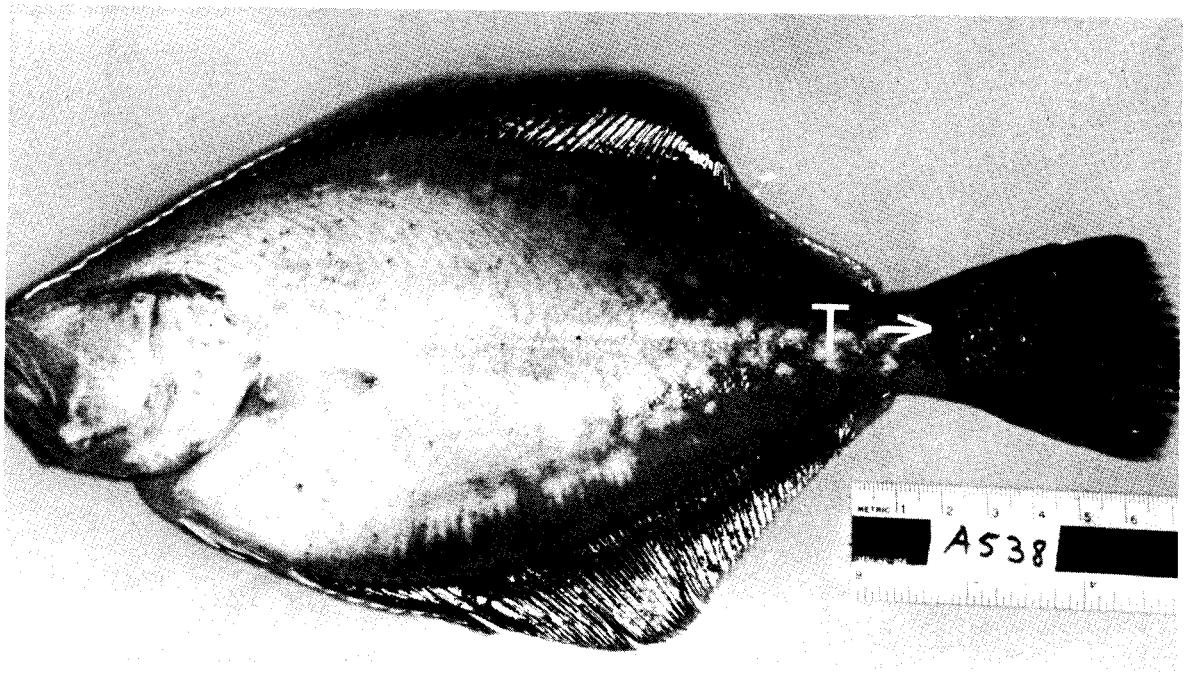


Figure 25. Distribution of frequencies (%) of Pacific cod (Gadus macrocephalus) with pseudobranchial tumors in the GOA.

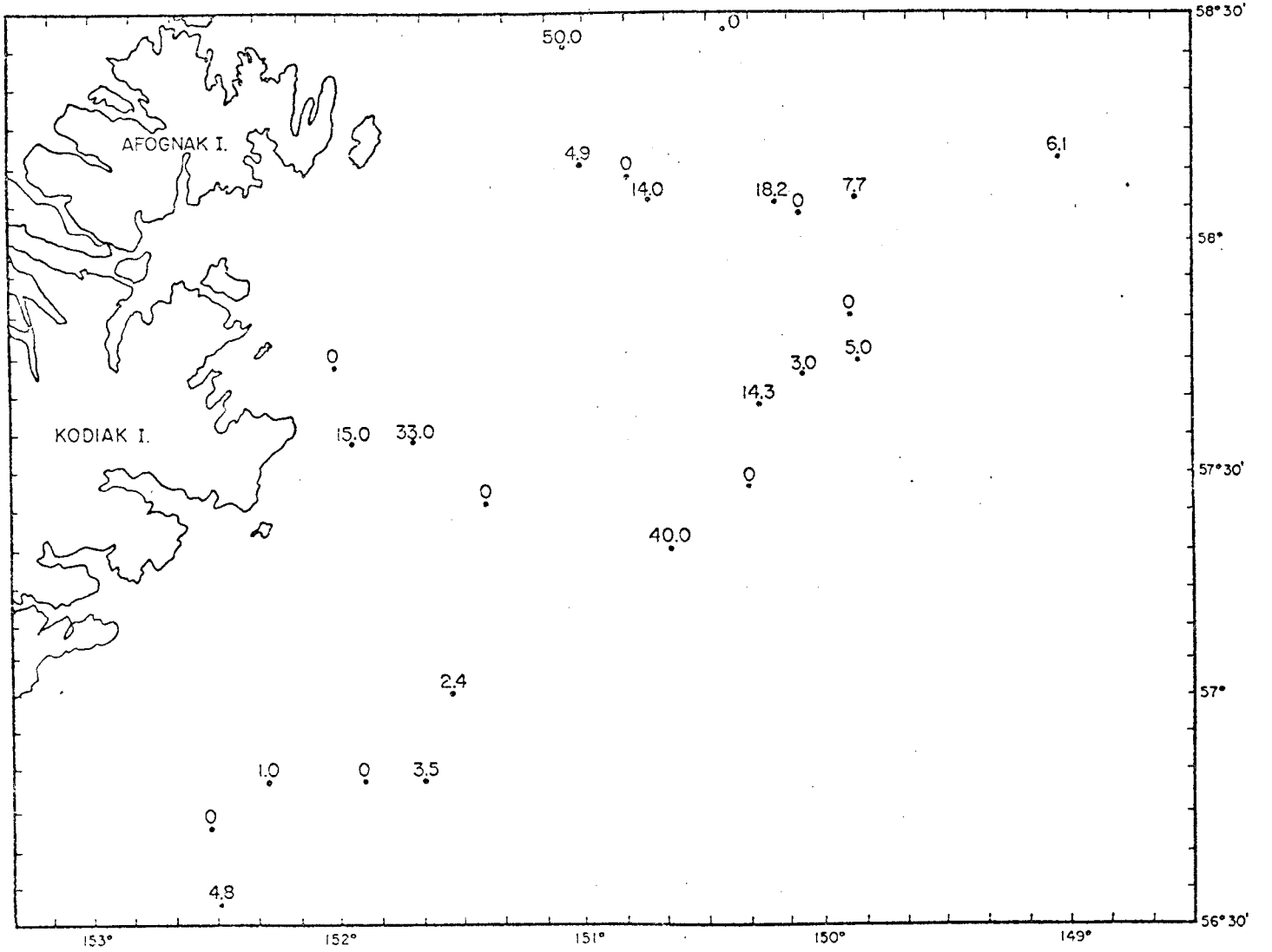


Figure 26. Distribution of frequencies (%) of pollock (Theragra chalcogramma) with pseudobranchial tumors in the GOA.

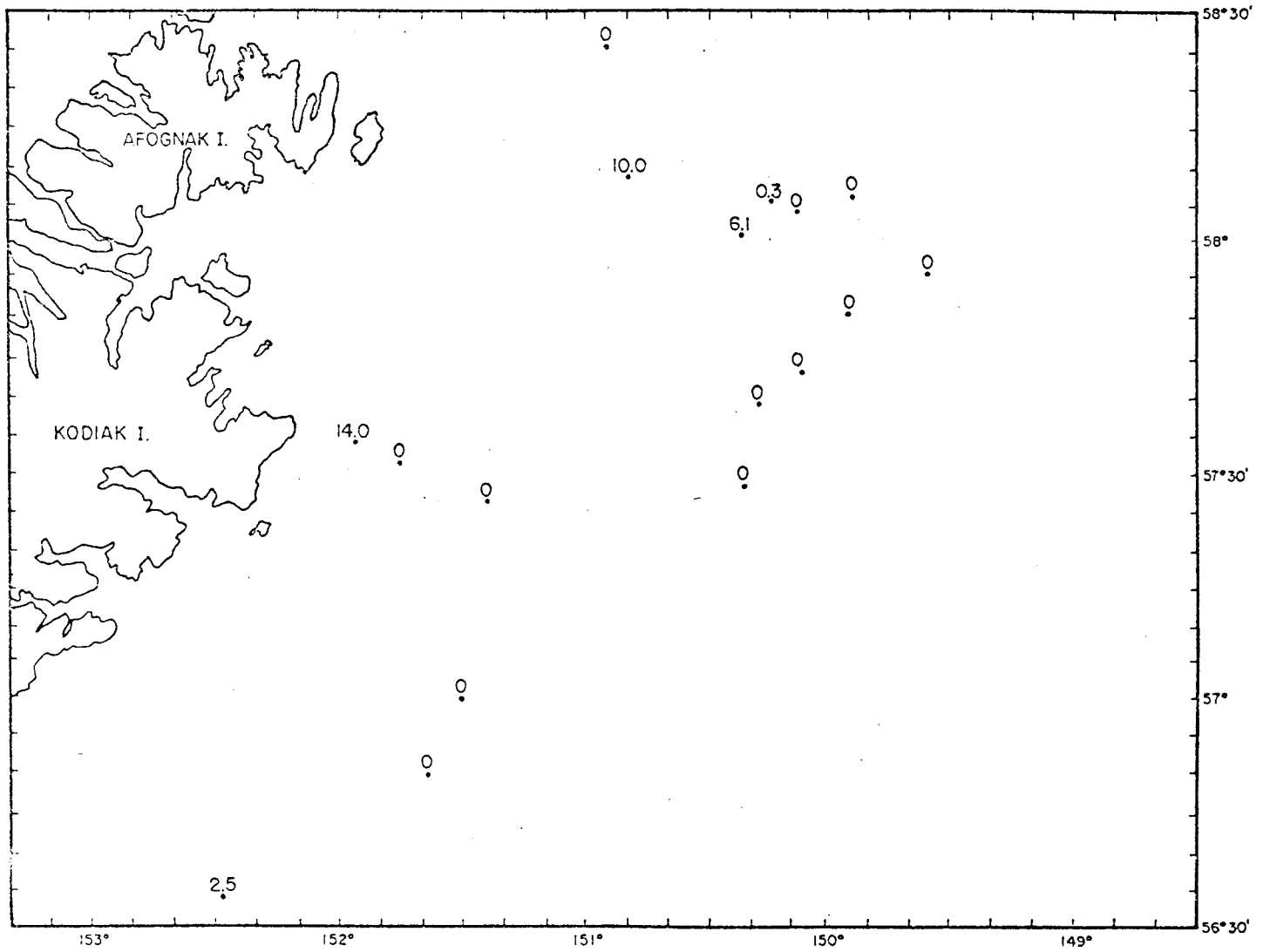


Figure 28. Distribution of frequencies (%) of Flathead sole (Hippoglossoides elassodon) with epidermal papilloma in the GOA.

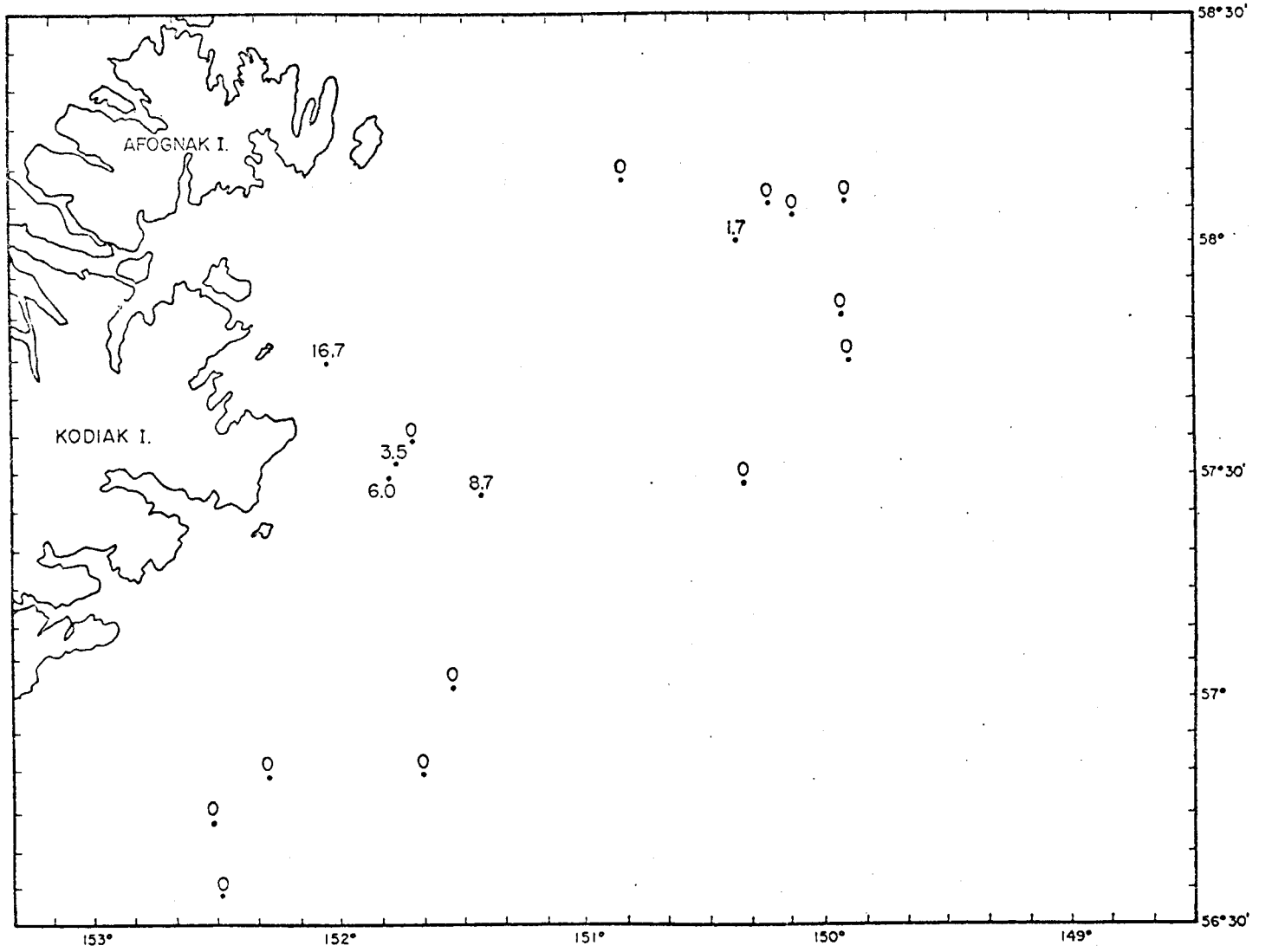


Figure 29. Underside of the operculum of a Pacific Ocean perch with multiple epidermoid tumors.

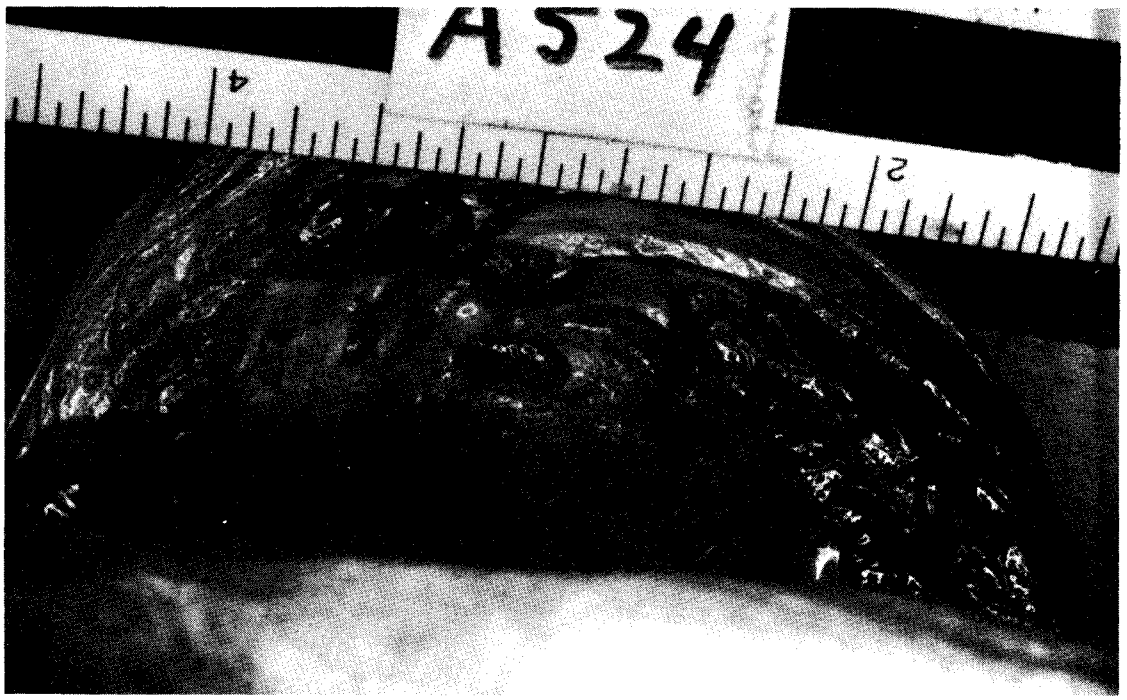


Figure 30. Section of a gill-associated epidermoid tumor (T), from a Pacific Ocean perch. (Hematoxylin and Eosin, X450)

References

Alpers, C. E., B. B. McCain, M. S. Myers, and S. R. Wellings.

1977. Pathology of pharyngeal tumors in Pacific cod, Gadus macrocephalus, of the Bering Sea. J. Natl. Cancer Inst. (In press).

Angell, C. L., B. S. Miller, and S. R. Wellings.

1975. Epizoology of tumors in a population of juvenile English sole (Parophrys vetulus) from Puget Sound, Washington. J. Fish. Res. Board Can. 32: 1723-1732.

Bright, D. B., F. E. Durham, and J. W. Knudsen (1960). King crab investigations of Cook Inlet, Alaska. Unpublished report.

Brooks, R. E., G. E. McArn, and S. R. Wellings.

1969. Ultrastructural observations on an unidentified cell type found in epidermal tumors of flounders. J. Natl. Cancer Inst. 43: 97-100.

Bullock, A. M. and R. J. Roberts.

1974. The dermatology of marine teleost fish. I. The normal integument. Oceangr. Mr. Biol. Ann. Rev. 13: 383-411.

Clemens, W. A. and G. U. Wilby.

1961. Fishes of the Pacific coast of Canada. Fish. Res. Board Can. Bull. 68, 443 p.

Fandeev, N. S.

1970. The fishery and biological characteristics of yellowfin sole in the eastern part of the Bering Sea. Tr. Vses. Nanchno-issled. Inst. Morsk, Rzbu. Oceanogr. 58: 121-138.

Hawkes, Joyce, The structure of fish skin. 1974. I. General organization, Cell Tissue Res. 149, 147-158.

Levings, C. D.

1967. A comparison of the growth rates of the rock sole, Lepidopsetta bilineata Ayres, in Northeast Pacific waters. Fish. Res. Board Can. Tech. Rep. No. 36, 43 p.

McArn, G. E., B. B. McCain, and S. R. Wellings.

1977. The histopathology of pseudobranchial tumors in the walleye pollock (Theragra chalcogramma) from the Bering Sea. (Manuscript in preparation.)

McCain, B. B., S. R. Wellings, C. E. Alpers, M. S. Myers and W. D. Gronlund.

1977a. The frequency, distribution, and pathology of three diseases of demersal fishes in the Bering Sea. Fish. Bull. (In Press.)

McCain, B. B., M. S. Myers, W. D. Gronlund, S. R. Wellings.

1977b. Baseline data on diseases of fishes from the Bering Sea for 1976. U. S. Natl. Marine Fish. Serv., NWAFC, Seattle, Wash. Submitted for publication.

Miller, B. S. and S. R. Wellings.

1971. Epizootiology of tumors on flathead sole (Hippoglossoides elassodon) in East Sound, Orcas Island, Washington. Trans. Am. Fish. Soc. 100: 247-266.

Russell, P. H.

1974. Lymphocystis in wild plaice Pleuronectes platessa (L.), and flounder, Platichthys flesus (L.), in British coastal waters: A histopathological and serological study, J. Fish Biol. 6, 771-778.

Sindermann, C. J.

1970. Principal diseases of marine fish and shellfish. Academic Press, New York, 369 p.

Sparks, A. K., and W. T. Pereyra.

1966. Benthic invertebrates of the southeastern Chukchi Sea. In: Environment of the Cape Thompson Region, Alaska. Ch. 29, p. 811-38 (N.J. Wilimovsky, ed.), U.S. Atomic Energy Commission.

Templeman, W.

1965. Lymphocystis disease in American plaice of the Eastern Grand Bank, J. Fish. Res. Board Can. 22, 1345-1356.

Turner, L. M.

1886. Results of investigations made chiefly in the Yukon District and the Aleutian Islands. In: Contributions to the Natural History of Alaska, U.S. Army.

Van Hyning, J. M., and A. M. Scarborough.

1973. Identification of fungal encrustation on the shell of the snow crab (Chionoecetes bairdi). J. Fish. Res. Board Can. 30: 1738-9.

Wellings, S. R., R. G. Chuinard, and R. A. Cooper.

1967. Ultrastructure studies of normal skin and epidermal papillomas of the flathead sole, Z. zellforsch. 78: 370-387.

Wellings, S. R. B. B. McCain, and B. S. Miller.

1976. Epidermal papillomas in Pleuronectidae of Puget Sound, Washington. Review of the current status of the problem. Prog. in Experimental Tumor Res. 20, 55-74.

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PHYTOPLANKTON STUDIES IN THE BERING SEA

Dr. Vera Alexander - Principal Investigator

Institute of Marine Science
University of Alaska
Fairbanks, Alaska 99701

31 March 1977

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

I & II. SUMMARY OF OBJECTIVES AND INTRODUCTION

The principal task involved in this program is to study the dynamics of phytoplankton populations, particularly along the edge of the seasonal ice-pack in the Bering Sea, and with emphasis on describing the probable effects of petroleum development impact. The southeastern Bering Sea is a highly productive area in terms of fisheries, and as such is very important to the Alaskan economy. In addition, the Bering Sea shelf as well as the southeast shelf-break area supports large populations of birds and mammals. This suggests a rather dynamic system in terms of phytoplankton productivity. The problem is, however, that the phytoplankton primary productivity which supports all the activity higher in the food chain is highly seasonal in this area, and its timing and the mechanisms of food chain transfer are probably extremely important to maintaining the integrity of the system. Prior to determining safe human development, it is absolutely essential that the exact nature of the highly seasonal phytoplankton production peaks is known, and the major pathways and rates of transfer of this food chain base to higher levels is understood. This is a very large and difficult task, certainly beyond any short-term project capability. However, by obtaining certain minimum data on seasonal productivity of phytoplankton populations, understanding in particular the unique role of ice in this system, and by carrying out a program coordinated with zooplankton studies, a significant beginning can be made which will provide sufficient understanding for a first cut opinion and estimate of impact. This, then, describes two of the tasks originally laid out in the proposal for this program: to study the seasonal dynamics of production with emphasis on the ice-edge and to cooperate with the zooplankton program in designing and executing experiments

to measure organic matter transfer between primary producers and zooplankton. The third aspect is to be a review of the foreign and domestic literature dealing with phytoplankton in the eastern Bering Sea south of St. Matthew Island, and obtain and incorporate currently unpublished material dealing with the region.

III. CURRENT STATE OF KNOWLEDGE

Prior to the initiation of the present Bering Sea project, only patchy data were available on primary productivity and the role of sea ice in this area. Estimates based on these data appear to be rather high, and the extreme seasonality of the major plant growth period was not adequately appreciated. With respect to OCS development, the potential of causing severe perturbations during specific times of year should result in careful seasonal and spatial timing of activities to minimize such impact.

The previous Bering Sea work has been summarized in several documents and books prepared during the planning phase of the NSF PROBES program. These are readily available. In connection with our work, we now have rather good seasonal coverage, with emphasis on the spring period of ice recession, and have the data base to calculate a reasonable estimate of annual primary production and its seasonal components. Data is still needed on the duration and extent of epontic algal growth (growth within sea ice at the water interface). Hopefully, this will in part be obtained during the present season.

IV. STUDY AREA

Stations sampled on this project during the two *Surveyor* cruises are shown in Figure 1. The major sampling effort occurred in and around the ice edge and sample locations were naturally determined by ice conditions and location of the ice edge. An effort was made to sample stations on either side of the shelf break and in deeper waters but since these were areas of lesser interest to other projects using the ship's facilities stations in these areas were frequently omitted in deference to ice edge stations of interest to all scientific parties. The ice edge stations fell into general groupings labeled in Figure 1 from west to east as groups A, B, C, D and E. Groups B, C and D were sampled during Leg I while groups A and E were part of Leg II sampling effort.

The timing of the two cruises was especially important for this ice edge study since we were able on Leg I to measure the characteristics of the water column prior to the initiation of the ice edge bloom phenomena. By Leg II the bloom had begun and was well defined. Combined with data collected from Cruise 808, *Discoverer*, Leg I, May 15-May 30, 1975 and *Discoverer*, Leg II, June 2-June 20, 1975 we have been able to study the phytoplankton dynamics associated with the receeding ice edge from mid-March prior to the initiation of the ice edge bloom through mid-June when the ice edge phenomena is complete in the southern areas over the shelf.

V. SOURCES, METHODS AND RATIONALE OF DATA COLLECTION

Ship Schedules

Two ice edge cruises have been undertaken in the past year.

1. Cruise SU 001. *Surveyor*, Leg II. March 14-April 2, 1976, NOAA.
2. Cruise SU 002. *Surveyor*, Leg II. April 12-April 30, 1976, NOAA.

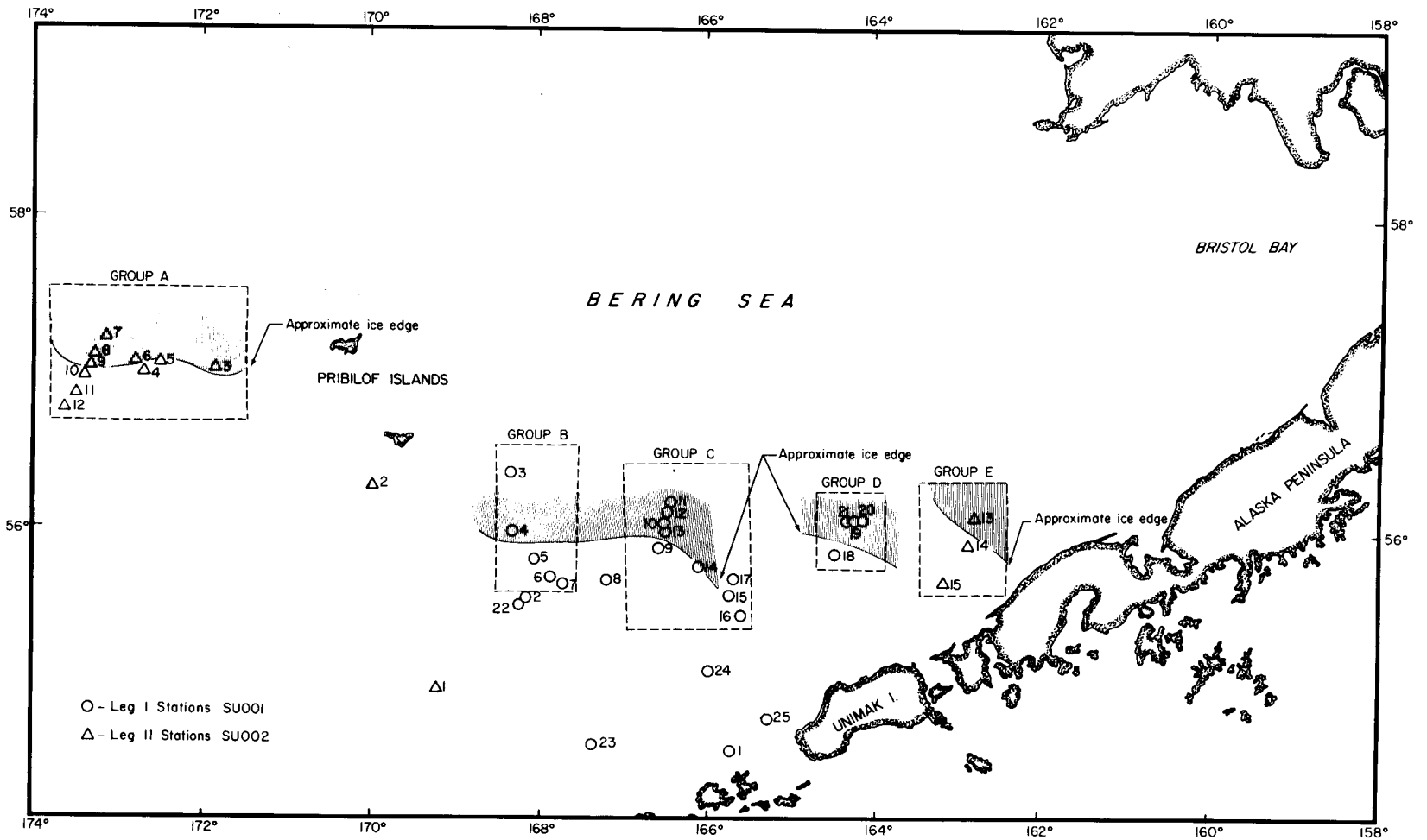

Figure 1. Stations sampled during the two *Surveyor* cruises.

Scientific Party

Cruise SU 001, Leg I:

David Brickell, Technician, University of Alaska
Carl Tobin, Technician, University of Alaska
Marilyn Sigmund, Graduate assistant, University of Alaska

Cruise SU 002, Leg II:

Robert Barsdate, Professor of Marine Science, University of Alaska
David Brickell, Technician, University of Alaska
Margie Young, Technician, University of Alaska
Lewis Molot, Graduate assistant, University of Alaska

Methods

Field sampling and laboratory analyses

The following analyses were carried out on both cruises:

Chlorophyll *a* - One liter of water taken from the Niskin bottle/Rosette hydrocast at various depths was filtered through glass micropore filters and the filters were extracted in 10 ml of 90% spectral grade acetone in a cool dark place for 24 hr, centrifuged and fluorescence of the extract of chlorophyll measured with a Turner Model III fluorometer. At representative stations and depths a duplicate sample of 1 ℓ was filtered and the filter frozen immediately. These filters were returned to the laboratory for spectrophotometric analysis of the pigments using a Perkin-Elmer Model 202 Ultraviolet-Visible Spectrophotometer. Chlorophyll *a* concentrations were calculated from the Strickland-Parsons equation and the results used to calibrate the fluorometer and to calculate chlorophyll concentrations from the fluorometric readings.

Nutrients - Water samples for nutrient analysis were filtered through glass micropore filters into aged polyethylene bottles (125 ml) and immediately frozen. Analyses for nitrate, nitrite, ammonia, silicate and

phosphate were carried out in our Fairbanks facilities 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. Counting and identification was performed upon return to the laboratory using sedimentation and an inverted microscope technique.

Primary productivity - Samples from five depths were placed into light and dark bottles 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 temperatures. Following 24 hr incubation the samples were filtered through an HA Millipore filter, rinsed twice with 10 ml of filtered seawater, the filter dried and counted on a Picker low background β counter. pH and alkalinity were measured to determine the inorganic carbon available. pH was measured with a Coleman portable pH meter. Alkalinity was measured by adding a standard equivalent of HCl and back titrating with standardized NaOH.

Ice cores and ice sampling was done in the vicinity of the ship using small boat operations and at greater distances from the ship with the aid of helicopter flights. Visual observations from the helicopter were used to search for productive areas as represented by ice coloration or bloom conditions within the water column around the ice edge and within leads. A SIPRE corer was used in an attempt to obtain algal samples from within the ice. On Leg II ships' divers aided in the collection of samples from beneath and around the edges of floating ice chunks using a

50 ml hypodermic syringe to collect algae from pockets and depressions within the under-ice surface.

In vivo *experiments*

On 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 µm Nitex netting to remove zooplankters and enriched with a nutrient stock solution to yield a final enrichment of 0.3 µg-at PO_4-P/ℓ and 4.5 µg-at NO_3-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 µm orifice allowing counts of particles from 4-80 µm 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 marshallae*)
2. Copper ($CuSO_4$) concentrations of 2, 4, or 8 µg Cu^{++}/ℓ, or
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 sub-samples were again preserved with modified Lugol's solution for phytoplankton identification and counting to be compared with the initial population, and 24 hr primary productivities using $H^{14}CO_3^-$ 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):

$$(1) \qquad F = \frac{V \cdot \ln (C_o/C_t)}{t \cdot N}$$

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

V = volume mls

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

C_t = particle concentration after time t

t = time interval in hrs

N = number of grazers

and assuming that the detrital fraction is insignificant.

Growth rates were calculated from:

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

where r is the growth rate/hr.

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

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

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

Data Collected or Analyzed

SU 001, Leg I

1. Number and types of samples:

Phytoplankton	102 from 22 stations
Chlorophyll *a*	204 from 25 stations
Nutrients	202 from 22 stations
Primary Productivity	60 from 12 stations
pH	60 from 12 stations
Alkalinity	60 from 12 stations
Ice cores	24 from 3 stations

2. Number and types of analyses

   All phytoplankton counts, chlorophyll determinations, nutrient analyses for phosphate, silicate, nitrate, nitrite and ammonia, alkalinity, pH and primary productivity analyses are completed and calculated. Data submission will be completed this spring.

3. Miles of trackline not known and not significant.

SU 002, Leg II

1. Number and types of samples:

Phytoplankton	102 from 15 stations
Chlorophyll *a*	114 from 15 stations
Nutrients	145 from 15 stations

Primary Productivity	60 from 12 stations
pH	60 from 12 stations
Alkalinity	60 from 12 stations
Ice cores	6 from 2 stations

Grazing, oil and copper toxicity experiments

2. Number and types of analyses:

   All analyses are complete and results calculated. Interpretation of data is in progress and data submission will be complete this spring.

3. Miles of trackline:

   Not known and insignificant.

VI, VII & VIII. DISCUSSION, CONCLUSIONS

Leg I

When this project was first initiated in May 1975, Cruise 808, *Discoverer*, we arrived at the ice edge to find an extensive band of highly productive water near the receeding ice edge which at that time had receeded a considerable distance from the shelf break area which is normally the limit of winter ice cover. The ice front was somewhat north of St. Paul Island at approximately 58°N latitude. Here we observed very high rates of carbon fixation (38 µg/ℓ/hr) and chlorophyll *a* concentrations of 17-31 µg/ℓ in the upper 10 m of water combined with very low concentrations of nitrate. The Secchi disc readings were frequently less than 2 m suggesting that the bloom was occurring with such intensity that the deeper waters were probably light limited by the concentrated phytoplankton population in the upper waters.

By August, Cruise 810, *Discoverer*, the area over the shelf showed

uniformly low nutrient concentrations with depth and low chlorophyll concentrations ranging from 0.03-2.4 μg/ℓ.

Likewise during the November Cruise 815, *Miller Freeman*, just as the first sea ice was forming, chlorophyll concentrations and primary productivity were low with uniformly low nutrient concentrations in the water column over the shelf.

These observations lead us to the hypothesis that the primary productivity over the Bering Sea Shelf occurs as a normal but intense spring bloom which develops in response to the renewal of light and removal of the ice cover. This bloom appears to strip the water column of nutrients which remain low until darkness and ice once more return to the Bering Sea. Then advective processes associated with cooling of the surface water or transport currents result in the replenishment of nutrients within the water column. This hypothesis does not apply to the area in the vicinity of the Aleutian Passes nor parallel to the Alaska Peninsula in Bristol Bay where there is an influx of North Pacific water. It does appear from our summer cruise data that this North Pacific water is depleted of nutrients during its east-northeasterly sojourn along the Peninsula before turning west to supply the major area of the continental shelf west of Bristol Bay. Circulation data is from Arsen'ev, 1967.

Recognizing that the primary productivity of the Bering Sea shelf area is restricted by nutrient supply when adequate light exists we were most anxious to be able to measure the nutrient levels existing over the shelf area prior to the initiation of the bloom. Since production appears to be nutrient limited, particularly with respect to nitrogen, and since we have not observed any major influx of nutrients into the area during

summer and fall, nor much regeneration of spring nitrate into ammonia, it is reasonable to consider the possibility that the potential production of the area is determined by the post-winter nitrogen supply, and that a realistic production budget may be estimated from nitrogen cycle dynamics.

Results for each leg will be presented separately since Leg I data characterizes the oceanographic situation prior to the initiation of the bloom while Leg II data concerns the early bloom situation. Data will be considered in groups as indicated in Figure 1. Groups B, C and D represent the three major ice stations occupied on Leg I and will be discussed first.

Leg I - Ice Edge Studies

Group B includes 2 stations within the ice pack, stations 3 and 4, and 3 stations on a transect at 10, 20 and 30 miles from the ice edge, stations 5, 6 and 7 respectively. These stations all lie close to the shelf break. Nutrient concentrations were relatively uniform and high throughout the water column while standing stock of phytoplankton represented by chlorophyll concentrations were low. Carbon fixation rates were low (·36 mg/m^3/day at the surface at ice station 4). The data for group B stations are shown in Table I.

The ice cover consisted of large unbroken plates with few leads or open water. Ice cores taken at this station showed no pigment layer and chlorophyll analyses of the melted core showed undetectable chlorophyll concentrations.

Furthermore, while sampling the ice, a southerly swell developed and caused a very large fresh lead to occur exposing the ice for observation - far more effectively than the coring effort. Visual observation of the ice stratigraphy showed that no pigment layer existed.

TABLE I

DATA FROM GROUP B STATIONS

Station	3	4	5	6	7
Position	56°16.7'N 168°36.2'W (ice)	55°59.2'N 168°32.0'W (ice edge)	55°51.4'N 168°18.3'W (10 mi)	55°40.0'N 168°00.0'W (20 mi)	55°37.0'N 167°52.7'W (30 mi)
Chlorophyll (µg/ℓ)/ carbon fixation rate µg/ℓ/day					
Depth (m)					
0	0.48	0.32/0.36	0.29	0.41	0.13
5	0.51	0.35/0.30	0.29	-	0.25
10	0.51	0.25/0.34	0.32	-	0.32
15	-	0.25/0.40	0.29	0.38	0.29
20	0.48	0.22/0.08	0.32	0.38	0.25
25	0.48	-	0.29	0.38	0.29
30	0.48	-	-	-	-
50	0.48	-	-	-	-
75	0.10	-	-	-	-

Station	3			4			5			6	7		
Nutrients	P	N	Si	P	N	Si	P	N	Si		P	N	Si
Phosphate/Nitrate/ Silicate (µg-atoms/ℓ)													
0	1.48	16.2	46	1.54	19.5	38	1.66	19.4	42	-	1.84	22.3	49
10	1.47	16.7	35	1.66	19.1	40	1.74	20.4	41	-	1.84	18.1	40
20	1.43	14.9	34	1.74	19.0	40	1.52	16.7	36	-	1.59	15.7	36
30	1.61	18.1	34	1.61	18.6	40	1.55	16.0	37	-	-		
50	1.85	18.9	38	1.63	17.4	38	1.70	17.6	38	-	1.79	19.7	42
75	1.86	20.8	41	-			-			-	1.95	22.7	48
100	1.93	24.8	58	1.79	20.6	47	1.89	18.5	47	-	2.06	23.5	50

Group C stations consisted of 5 ice stations from innermost to ice edge being stations 11, 12, 10, 13 and 9, covering a distance of approximately 24 mi. Proceeding from station 13, surface chlorophyll samples were collected at 4 mi intervals on the 24 mi transect to the ice edge at station 14 from the ships sea chest water supply system. Because color was observed on the chlorophyll filter pads from the deeper samples (90-120 m) at these group C ice stations a series of ^{14}C uptake incubations was done on deep water samples to find whether low light adapted photosynthesis was occurring near the bottom. In addition stations 14, 15 and 16 represent a transect (approximately 30 mi) away from the ice edge in this group. All group C stations were located well up on the shelf. The ice station data is summarized in Table II. The 4 mi interval surface chlorophyll concentrations and data from the transect away from the ice are given in Table III. Presentation of phytoplankton identification data is beyond the scope of this report but Table IV presents a listing of the species composition and cell numbers present in the surface waters (0 and 5 m) at ice station 10 of group C stations. Relatively few species are present as compared to the ice station samples later in the spring on Leg II.

Group D stations 18, 19, 20 and 21 were the easternmost ice and ice edge stations sampled on Leg I and once again reflect the high nutrient levels and low chlorophyll concentrations observed at the other ice station groups. Ice cores taken in this area had no pigment layer and chlorophyll values were undetectable.

The data for group D stations is given in Table V.

TABLE II

DATA FROM GROUP C ICE STATIONS

Station	11	12	10	13	9
Position	56°12.0'N 166°30.2'W	56°07.2'N 166°37.4'W	56°03.7'N 166°35.2'W	56°01.3'N 166°38.7'W	55°53.0'N 166°41.5'W
Total depth	113 m	121 m	136 m	126 m	133 m
Conditions	ice	ice	ice	ice	ice edge

Chlorophyll (µg/ℓ)/ carbon fixation rate (µg C/ℓ/hr)

Depth (m)					
0	0.38/2.16	1.05/2.12	0.38/0.37	0.67/	0.22/
5	0.41/2.14	-	0.38/0.97	-	0.25/
10	0.48/1.73	1.02/2.01	0.48/0.80	0.60/	0.22/
15	0.54/1.67	-	0.41/0.52	-	0.25/
20	0.48/0.93	0.86/1.32	0.32/0.11	-	0.29/
30	0.38/	0.38/0.12	-	0.44/	-
50	0.19/	0.19/0.11	-	0.19/	-
70	-	-	-	0.25/	-
80	-	-	-	0.16/0.12	-
90	-	-	-	0.10/0.03	-
100	-	-	-	0.06/0.05	-
bottom (10 m above total depth)	-	-	-	0.10/0.02	-

Phosphate/Nitrate Silicate (µg-atoms/ℓ)

Station	11			12			10			13			9		
Nutrients	P	N	Si	P	N	Si	P	N	Si	P	N	Si	P	N	Si
0	1.44/17.1/39			1.40/17.0/40			1.34/16.1/30			0.94/17.1/32			1.63/17.6/34		
10	1.58/16.9/39			1.48/16.7/33			1.46/17.7/33			1.41/17.5/32			1.59/17.1/33		
20	1.42/16.7/41			1.44/16.9/34			1.46/17.5/32			-			1.61/17.2/32		
30	1.38/16.9/33			1.44/17.5/34			1.50/17.9/37			1.47/17.5/32			-		
50	1.44/17.7/35			1.29/17.7/33			1.58/18.1/33			1.25/16.1/29			-		
70	-			-			-			1.41/17.5/32			-		
80	-			1.60/18.5/36			-			1.39/18.5/35			-		
90	-			2.06/25.6/57			-			2.08/25.7/58			-		
100	-			2.08/25.7/56			2.14/27.4/55			2.10/25.1/56			-		
bottom (10 m above total depth)	-	/23.0/42		-			2.10/26.5/52			2.16/25.7/58			1.74/26.1/57		

TABLE III

DATA FROM GROUP C SURFACE CHLOROPHYLL TRANSECT
AND TRANSECT AWAY FROM ICE EDGE

A. Surface chlorophyll concentrations at 4 mi intervals from Ice station 13 to Ice edge station 14.

Distance from station 13 (mi)	0	4	8	12	16	20	24
µg Chlor *a*/ℓ	0.67	0.60	0.48	0.29	0.25	0.19	0.41

B. Transect away from ice edge.

Station		14			15			16	
Position		55°42.3'N 166°11.0'W			55°33.8'N 165°51.5'W			55°24.2'N 165°42.3'W	
Total depth		124 m			120 m			115 m	
Conditions		ice edge			ice free			ice free	
Chlorophyll (µg/ℓ)	Depth (m)								
	0	0.41			0.35			0.25	
	10	0.41			0.35			0.32	
	20	0.41			0.38			0.35	
	30	0.32			0.38			0.35	
	50	0.29			0.29			0.25	
	70	-			0.13			0.16	
	80	0.10			0.13			0.06	
	90	0.10			0.10			0.13	
	100	0.10			0.10			0.13	
	110	0.10			0.10			0.13	
Station		14			15			16	
Nutrients		P	N	Si	P	N	Si	P	N Si
Phosphate/Nitrate/ Silicate (µg-atoms/ℓ)	0	1.43/17.1/32			-			1.45/17.9/34	
	10	1.49/17.1/32			-			1.45/17.7/33	
	20	1.63/17.5/32			-			1.39/17.5/32	
	30	1.59/18.1/33			-			1.49/17.4/34	
	50	1.49/17.7/33			-			1.37/16.3/30	
	70	-			-			1.51/18.5/35	
	80	2.14/25.3/56			-			2.02/25.1/55	
	90	2.06/25.5/57			-			1.73/21.0/55	
	100	2.18/33.1/85			-			1.94/23.4/53	
	110	2.06/25.3/56			-			2.04/27.4/54	

TABLE IV

PHYTOPLANKTON SPECIES COMPOSITION AND CELL COUNTS
IN SURFACE WATERS AT ICE STATION 10, GROUP C

0 m	cells/liter
Diatoms	
Chaetoceros sp.	1200
Chaetoceros sp.	800
Chaetoceros sp.	400
Cylindrotheca closterium	1200
Nitzschia sp.	400
Skeletonema costatum	1600
Thalassiosira decipiens	1200
Unidentified pennates	1200
Unidentified centric	1600
Coccolithiphorid sp.	400
Flagellates	
4 μ	18400
8 μ	2400
12 μ	800
Total cell count	3.2×10^4 cells/liter

5 m	
Diatoms	
Cylindrotheca closterium	2400
Thalassionema nitzochioides	3200
Thalassiosira sp.	3200
Unidentified pennates	1600
Flagellates	
4 μ	36000
8 μ	4800
8 μ	2400
Unidentified	3600
Total cell count	5.7×10^4 cells/liter

TABLE V

DATA FOR GROUP D ICE AND ICE EDGE STATIONS

Station		18	19	20	21
Position		55°51.6'N 164°32.7'W	56°05.4'N 164°15.0'W	56°05.8'N 164°10.5'W	56°05.8'N 164°23.1'W
Total depth		100 m	87 m	87 m	87 m
Conditions		ice edge	ice	ice	ice
Chlorophyll (µg/ℓ)/ carbon fixation rate (µg C/ℓ/day)	Depth (m)				
	0	0.32/0.29	0.51/0.59	0.41/0.89	0.35/ -
	5	-	-	-	0.32/ -
	10	0.53/0.57	0.67/0.72	0.48/0.74	-
	15	-	-	-	0.32/ -
	20	0.35/0.43	0.41/0.43	0.32/0.57	-
	25	-	-	-	0.51/ -
	30	0.29/0.51	0.38/0.48	0.25/0.54	-
	35	-	-	-	0.25/ -
	40	0.25/ -	0.32/ -	0.29/ -	-
	45	-	-	-	0.38/ -
	50	0.22/0.31	0.35/0.05	0.29/0.29	-
	55	-	-	-	0.19/ -
	60	-	0.32/ -	0.32/ -	-
	70	-	0.32/ -	0.29/ -	-
	80	-	0.35/ -	0.22/ -	0.25/ -

Station		18	19	20	21
Nutrients		P N Si	P N Si	P N Si	P N Si
Phosphate/Nitrate Silicate (µg-atoms/ℓ)	0	1.53/17.3/32	1.44/16.8/32	1.44/16.1/32	1.50/16.3/33
	5	-	-	-	1.53/16.3/32
	10	1.46/17.3/32	1.41/16.1/32	1.41/16.6/34	-
	15	-	-	-	1.56/16.3/32
	20	1.48/17.3/32	1.30/14.2/26	1.48/17.0/33	-
	25	-	-	-	1.53/16.1/32

TABLE V

CONTINUED

Station		18			19			20			21		
Position		55°51.6'N 164°32.7'W			56°05.4'N 164°15.0'W			56°05.8'N 164°10.5'W			56°05.8'N 164°23.1'W		
Total Depth		100 m			87 m			87 m			87 m		
Conditions		ice edge			ice			ice			ice		
Nutrients		P	N	Si	P	N	Si	P	N	Si	P	N	Si
Phosphate/Nitrate/Silicate (µg-atoms/ℓ)	Depth (m)												
	30	1.50	17.7	32	1.44	17.0	32	1.48	17.0	34	-		
	35	-			-			-			1.55	16.8	33
	40	1.53	17.7	33	1.46	17.0	32	1.46	17/0	33	-		
	45	-			-			-			1.57	16.6	32
	50	1.50	18.0	33	1.44	18.0	34	1.53	17.0	32	-		
	55	-			-			-			1.51	16.8	34
	60	1.50	18.0	33	1.44	17.0	32	1.44	17.3	34	-		
	70	1.46	17.7	33	1.46	16.8	34	1.53	16.8	34	-		
	80	1.42	17.0	33	1.42	17.0	33	1.53	17.0	32	1.57	16.8	33

Deep Water Stations

Station 23 was the only deep water station for which we have data on Leg I. Although low chlorophyll concentrations are recorded, the nutrient concentrations are considerably greater than anywhere over the shelf. The data for deep water station 23 are given in Table VI.

Leg II

Upon returning to the ice edge on Leg II on April 17, the primary productivity regime was greatly changed with the bloom in progress. The ice front consisted of broken ice chunks with obvious algal growth visible on the edges. On Leg II we sampled the ice edge extensively in two locations as shown in Figure 1. Again stations have been grouped for presentation as groups A and E. In general, chlorophyll concentrations and primary productivity rates were more than 20 times greater than observed on Leg I and nutrient levels were beginning to decline, although not yet limiting. In addition to continuing our routine sampling effort experiments were done with zooplankton grazers and with oil and copper additions to determine their effects on the phytoplankton population growth rate and population composition.

Leg II - Ice Edge Studies

Group A stations consisted of a series of stations parallel to the ice edge, stations 3, 4, 5 and 6; a series of stations within the ice pack, stations 7, 8 and 9; and a series of stations away from the ice edge, stations 10, 11 and 12.

TABLE VI

DATA FOR DEEP WATER STATION 23

Station 23 Position 54°29.0'N 167°31.8'W Total Depth 740 m

Depth	Chlor *a* µg/ℓ	PO_4-P	NO_3-N	SiO_3-Si
		µg atoms/liter		
0	0.29	2.18	29.1	56
10	0.25	2.21	29.1	56
20	0.22	1.90	29.9	52
50	0.25	2.16	29.1	60
75	0.10	2.29	31.0	60
100		2.27	30.1	60
200		2.53	34.3	72
300		2.67	30.0	63
500		2.63	32.6	56
725		3.22	33.3	93

The data for stations 3, 4, 5 and 6 are presented in Table VII, that for stations 7, 8 and 9 in Table VIII and that for stations 10, 11 and 12 in Table IX.

Group E stations represent a transect from within the ice to approximately 20 mi outside the ice front. The data are presented in Table X. A listing of phytoplankton species and species numbers/liter found in the ice core sample at station 13 are listed in Table XI and a listing for the water column at ice station 13 is given in Table XII.

Again, only one deep water station was sampled on Leg II. Nutrient concentrations at the surface were greater than any measured over the shelf although productivity was low, reflecting the combined effect of the shelf and the sea ice on the productivity regime. The data are given in Table XIII.

Grazing and Toxicity Experiments

Mean size and concentration of algal blooms at various stations with associated ice conditions are shown in Table XIV. The bloom occurring among the broken ice consisted of larger organisms and was more concentrated than the bloom in ice-free water. Ice algae (station 13B) sampled from the underside of an ice flow 1 m thick were smaller (4.6 and 23 μm diameter) than ice-free algae but were extremely concentrated. The core sample, however, is semi-quantitative at best. These data are in agreement with the findings of McRoy and Goering (1974). They found that the standing stock and productivity of phytoplankton under the ice pack and in the

TABLE VII

DATA FROM GROUP A ICE EDGE STATIONS

Station		3	4	5	6
Position		57°04.4'N 172°06.0'W	57°03.8'N 172°59.6'W	57°07.1'N 172°47.3'W	57°07.1'N 173°07.4'W
Total depth		112 m	119 m	115 m	120 m
Conditions		ice	ice	ice	ice
Chlorophyll (µg/ℓ)/ Carbon fixation rate (µg C/ℓ/day)	Depth (m)				
	0	12.63/16.91	12.63/19.51	13.99/20.23	12.29/17.03
	10	11.95/16.73	12.63/11.28	14.68/ -	10.24/18.40
	20	9.90/9.853	12.63/5.037	15.02/15.13	14.33/ -
	30	-	9.56/3.90	15.36/14.98	7.85/7.46
	40	10.92/5.32	-	8.87/ -	-
	50	5.74/0.45	1.46/0.08	1.03/0.43	1.20/1.28
	60	1.11/ -	1.43/ -	0.25/ -	0.25/ -
	70	0.41/ -	0.32/ -	0.19/ -	0.25/ -
	80	-	-	-	-
	90	-	-	-	-
	100	0.25/ -	-	-	-
	110	-	-	-	0.22/ -
Station		3	4	5	6
Nutrients		P N Si	P N Si	P N Si	P N Si
Phosphate/Nitrate/ Silicate (µg-atoms/ℓ)	0	0.96/10.2/34	1.05/18.8/53	1.06/09.6/36	1.27/15.9/55
	10	0.92/09.9/32	1.17/14.5/40	1.03/10.8/37	1.15/10.7/39
	20	0.88/12.2/32	1.01/11.8/31	1.13/09.3/34	1.43/15.1/41
	30	-	1.50/18.3/45	1.31/10.8/37	2.02/18.9/46
	40	1.05/13.7/32	1.52/20.7/49	1.86/18.6/46	1.86/20.3/50
	50	1.54/19.1/40	1.58/19.0/40	2.12/22.8/50	2.05/24.2/53
	60	1.97/23.8/45	1.91/23.0/47	2.11/25.3/54	2.19/25.5/56
	70	2.01/25.3/51	2.07/25.5/ -	2.15/25.0/53	2.31/25.8/56
	80	-	-	-	-
	90	-	2.09/26.8/ -	2.10/25.3/53	2.32/25.9/57
	100	1.70/25.3/51	1.56/19.0/ -	2.21/25.5/53	2.39/25.7/56
	bottom (10 m above)	-	1.17/1.74/ -	2.23/25.3/55	2.52/25.4/56

TABLE VII

CONTINUED

Station		3	4	5	6
Position		57°04.4'N 172°06.0'W	57°03.8'N 172°59.6'W	57°07.1'N 172°47.3'W	57°07.1'N 173°07.4'W
Total depth		112 m	119 m	115 m	120 m
Conditions		ice	ice	ice	ice
Phytoplankton Cells/liter		($\times 10^5$)	($\times 10^5$)	($\times 10^5$)	($\times 10^5$)
	Depth (m)				
	0	2.7	-	2.8	4.0
	10	3.6	2.6	3.5	-
	20	4.5	2.5	3.1	-
	30	-	2.1	3.2	-
	40	2.1	1.9	-	-
	50	0.92	0.77	0.20	-
	60	-	0.32	0.73	-
	70	0.13	0.15	0.32	-
	90	-	0.27	-	
	100	0.076	0.17	-	-
	Bottom (10 m above)	-	0.13	0.38	-

TABLE VIII

DATA FROM GROUP A ICE STATIONS

Station		7	8	9
Position		57°17.0'N 173°26.9'W	57°09.1'N 173°37.2'W	57°06.7'N 173°39.5'W
Total depth		124 m	137 m	159 m
Conditions		ice	ice	ice
Chlorophyll (µg/ℓ)/ Carbon fixation rates (µg C/ℓ/day)	Depth (m)			
	0	19.45/29.59	17.41/ -	15.70/ -
	10	20.14/27.16	18.09/ -	16.38/ -
	20	20.48/18.40	18.09/ -	16.72/ -
	30	18.77/ 7.92	11.26/ -	14.33/ -
	40	10.24/ -	4.28/ -	12.29/ -
	50	7.51/ 0.51	4.78/ -	3.86/ -
	60	-	2.23/ -	-
	70	0.77/ -	0.94/ -	1.37/ -
	80	-	-	-
	90	-	-	-
	100	-	-	0.25/ -
	bottom (10 m above)	0.35/ -	0.57/ -	-

Station		7	8	9
Nutrients		P N Si	P N Si	P N Si
Phosphate/Nitrate/ Silicate (µg-atoms/ℓ)	0	0.96/ 8.1/43	1.09/10.7/49	1.21/11.8/38
	10	1.29/ 6.1/36	1.09/10.1/48	1.24/10.1/39
	20	1.40/12.4/56	1.03/ 8.8/39	1.16/13.7/44
	30	1.37/11.0/37	1.39/16.0/42	1.31/13.2/39
	40	2.15/24.6/66	1.80/20.3/46	1.64/16.9/43
	50	2.24/20.3/48	2.11/26.7/67	1.89.21.4/48
	60	-	1.85/22.8/48	-
	70	2.22/26.3/56	2.25/24.3/50	2.01/24.3/52
	80	-	-	-
	90	2.28/27.3/55	-	-
	100	2.37/26.0/56	2.35/25.6/53	2.27/28.5/62
	bottom (10 m above)	2.43/25.7/55	2.48/31.9/74	2.74/33.6/75

TABLE VIII

CONTINUED

Station		7	8	9
Position		57°17.0'N 173°26.9'W	57°09.1'N 173°37.2'W	57°06.7'N 173°39.5'W
Total Depth		124 m	137 m	159 m
Conditions		ice	ice	ice
Phyto-plankton (cells/ℓ)	Depth (m)	(x 10^5)	(x 10^5)	(x 10^5)
	0	6.2	7.3	4.7

TABLE IX

DATA FROM GROUP A
TRANSECT AWAY FROM ICE EDGE

Station		10	11	12
Position		57°02.1'N 173°42.2'W	56°54.9'N 173°49.8'W	56°47.6'N 173°57.4'W
Total depth		338 m	745 m	1145 m
Conditions		ice free	ice free	ice free
Chlorophyll (µg/ℓ)/ Carbon fixation rates (µg C/ℓ/day)	Depth (m)			
	0	6.48/8.21	3.00/ -	1.46/ -
	10	6.48/7.93	2.91/ -	1.14/ -
	20	6.14/5.50	2.23/ -	1.17/ -
	30	5.80/3.21	3.00/ -	1.14/ -
	40	5.12/ -	1.71/ -	-
	50	0.76/ .03	3.60/ -	0.25/ -
	75	0.29/ -	1.37/ -	0.16/ -
	100	0.19/ -	0.51/ -	0.13/ -
	bottom (10 m above)	-	-	-
Station		10	11	12
Nutrients		P N Si	P N Si	P N Si
Phosphate/Nitrate/ Silicate (µg-atoms/ℓ)	0	1.75/18.1/45	1.54/22.2/48	1.70/20.8.45
	10	1.66/18.8/45	1.52/20.7/47	1.79/27.2/69
	20	1.69/18.9/45	1.56/20.7/47	1.68/19.5/45
	30	1.51/20.4/45	1.56/20.7/47	1.69/19.3/44
	40	1.76/20.0/47	1.91/22.6/49	-
	50	1.99/22.2/49	1.93/22.6/50	2.05/23.5/52
	75	2.57/24.8/48	1.99/23.4/51	2.14/25.3/55
	100	2.46/26.8/56	2.10/24.8/53	2.21/26.4/59
	bottom (10 m above)	2.99/37.8/83	4.04/43.7/67	3.46/40.4/85

TABLE IX

CONTINUED

Station		10	11	12
Position		57°02.1'N 173°42.4'W	56°54.0'N 173°49.8'W	56°47.6'N 173°57.4'W
Total depth		338 m	745 m	1145 m
Conditions		ice free	ice free	ice free
Phytoplankton (Cells/liter)	Depth (m)	($\times 10^5$)	($\times 10^5$)	($\times 10^5$)
	0	1.1	0.95	0.99
	10	1.1	1.2	1.0
	20	0.86	1.2	1.0
	30	0.93	0.86	0.48
	40	1.1	-	-
	50	0.42	1.26	0.068
	75	0.14	-	-
	100	0.064		

TABLE X

DATA FROM GROUP E TRANSECT

Station		13	14	15
Position		56°05.0'N 162°48.2'W	55°54.2'N 162°53.4'W	55°52.0'N 162°56.8'W
Total depth		78 m	82 m	82 m
Conditions		ice	ice edge	ice free
Chlorophyll (µg/ℓ)/ Carbon fixation rates (µg C/ℓ/day)	Depth (m)			
	0	7.03/21.30	1.11/3.28	0.76/1.87
	10	6.34/18.99	2.19/3.46	0.63/2.34
	20	5.57/11.76	2.54/3.55	0.95/1.52
	30	1.03/ 0.76	1.30/1.09	0.76/0.55
	40	0.94/ -	0.54/ -	-
	50	0.86/ 0.12	0.44/ .07	0.41/ .09
	60	0.94/ -	-	0.25/ -
	75	1.03/ -	0.38/ -	-
Station Nutrients		13 P N Si	14 P N Si	15 P N Si
Phosphate/Nitrate/ Silicate (µg-atoms/ℓ)	0	1.06/7.0/27	1.42/13.6/31	1.57/13.7/30
	10	1.08/ 7.9/26	1.38/13.0/32	1.53/13.7/30
	20	1.23/ 9.3/28	1.32/11.2/30	1.53/14.4/31
	30	1.53/13.4/32	1.55/12.9/31	1.55/15.1/34
	40	1.68/13.4/33	1.59/14.0.32	1.57/15.1/34
	50	1.60/13.4/34	1.59/14.0/33	1.60/14.9/33
	60	1.59/14.1/33	1.75/14.0/32	1.57/15.1/34
	75	1.59/13.4/33	1.63/14.5/34	-
Station		13 ($\times 10^5$)	14 ($\times 10^5$)	15 ($\times 10^5$)
Phytoplankton (cells/liter)	0	4.8	1.94	-
	10	2.9	3.75	15.1
	20	3.7	2.42	2.1
	30	0.4	0.37	-
	50	0.52	0.38	-
	75	0.86	-	-

TABLE XI

PHYTOPLANKTON SPECIES COMPOSITION AND CELL COUNTS
FROM ICE CORE SAMPLE AT STATION 13, GROUP E

	Cells/liter
Diatoms	
Achnanthes sp.	3,232,000
Actinoptychus undulatus	3,200
Amphiprora sp.	57,600
Asterionella kariana	33,600
Biddulphia aurita	88,000
Chaetoceros debilis	4,800
Chaetoceros sp.	33,600
Chaetoceros sp.	4,800
Coscinodiscus centralis	1,600
Coscinodiscus excentricus	8,000
Coscinodiscus oculus iridis	1,600
Coscinodiscus radiatus	8,600
Coscinodiscus	30,400
Cylindrotheca closterium	32,000
Detonula confervacea	360,000
Eucampia zoodiacus	1,600
Nitzschia sp. (*Fragilariopsis*)	241,600
Nitzschia sp. (*Fragilariopsis*)	1,100,800
Nitzschia sp. (*Fragilariopsis*)	332,800
Nitzschia sp. (*Fragilariopsis*)	107,200
Nitzschia sp. (*Fragilariopsis*)	1,654,400
Fragilariopsis oceanica f. circularis	217,600
Gyrosigma or *Pleurosigma* sp.	4,800
Melosira sulcata	230,400
Navicula sp.	14,400
Nitzschia frigida	17,600
Nitzschia sp.	115,200

TABLE XI

CONTINUED

	Cells/liter
Diatoms (cont.)	
Pleurosigma sp. 1	49,600
Pleurosigma sp. 2	100,800
Skeletonema costatum	8,000
Thalassionema nitzschioides	86,400
Thalassiosira aestivalis	16,000
Thalassiosira decipiens	40,000
Thalassiosira gravida	9,600
Thalassiosira norderskioldii	28,800
Thalassiosira spp.	232,000
Thalassiothrix sp.	1,600
Unidentified pennates	
10-20 µm	131,200
20-40 µm	635,200
40-60 µm	110,400
60-80 µm	12,800
>100 µm	11,200
Unidentified cells	9,600
Dinoflagellates	
Peridinium sp.	6,400
Silicoflagellates	
Dictyocha fibula	1,600

TABLE XII

PHYTOPLANKTON SPECIES COMPOSITION AND CELL COUNTS FOR THE WATER COLUMN AT STATION 13, GROUP E

	Cells/liter
0 meters	
Diatoms	
Biddulphia aurita	800
Chaetoceros compressus	4,000
Chaetoceros socialis	9,600
Chaetoceros sp.	0
Corethron criphilum	800
Cylindrotheca closterium	1,600
Detonula confervacea	122,400
Nitzschia sp. (*Fragilariopsis*)	16,000
Nitzschia sp. (*Fragilariopsis*)	116,800
Nitzschia sp. (*Fragilariopsis*)	51,200
Navicula vanhöffeni	10,400
Porosira glacialis	800
Thalassiosira decipiens	3,200
Thalassionema nitzschioides	3,200
Thalassiosira gravida	61,600
Thalassiosira nordenskioldii	3,200
Thalassiosira rotula	13,600
Thalassiosira sp.	4,000
Thalassiosira spp.	34,400
Unidentified pennates	1,600
Dinoflagellates dinophyta	
cf. *Gymnodinium* sp.	800
Flagellates	
3 µm	800
4 µm	4,000
8 µm	5,600
Prasinophyta	800
TOTAL CELL COUNT	4.8×10^5 cells/liter

TABLE XII

CONTINUED

	Cells/liter
10 meters	
Diatoms	
Asterionella kariana	1,600
Biddulphia aurita	800
Chaetoceros compressus	11,200
Chaetoceros concavicornis	800
Chaetoceros debilis	10,400
Chaetoceros septentrionalis	1,600
Chaetoceros socialis	8,800
Chaetoceros sp.	8,800
Cylindrotheca closterium	800
Nitzschia sp. (*Fragilariopsis*)	20,000
Nitzschia sp. (*Fragilariopsis*)	96,800
Navicula pelagica	9,600
Thalassiosira aestivalis	9,600
Thalassiosira gravida	24,800
Thalassiosira nordenskioldii	18,400
Thalassiosira rotula	12,000
Thalassiosira sp.	29,600
Unidentified pennates	
20 µm	2,400
25 µm	800
Flagellates	
3 µm	1,600
4 µm	12,800
8 µm	7,200
TOTAL CELL COUNT	2.9×10^5 cells/liter

TABLE XII

CONTINUED

20 meters	Cells/liter
Diatoms	
Achnanthes sp.	2,400
Biddulphia aurita	800
Chaetoceros debilis	4,800
Chaetoceros septentrionalis	1,600
Chaetoceros socialis	6,400
Cylindrotheca closterium	1,600
Detonula confervacea	64,800
Navicula pelagica	9,600
Nitzschia sp. (*Fragilariopsis*)	52,000
Nitzschia sp. (*Fragilariopsis*)	26,400
Nitzschia sp. (*Fragilariopsis*)	93,600
Nitzschia sp.	800
Nitzschia frigida	16,000
Porosira glacialis	800
Thalassionema nitzschioides	1,600
Thalassiosira gravida	29,600
Thalassiosira nordenskioldii	4,800
Thalassiosira rotula	2,400
Thalassiosira sp.	11,200
Thalassiosira spp.	14,400
Unidentified pennates	14,400
20 µm	800
Dinoflagellates	
cf. *Gymnodinium* sp.	3,200
Peridinium minisculum	800
Flagellates	
3 µm	1,600
4 µm	8,800
8 µm	6,400
TOTAL CELL COUNT	3.7×10^5 cells/liter

TABLE XII

CONTINUED

	Cells/liter
30 meters	
Diatoms	
Achnanthes sp.	1,200
Biddulphia aurita	1,200
Chaetoceros socialis	14,000
Chaetoceros sp.	1,600
Nitzschia sp. (*Fragilariopsis*)	10,000
Thalassiosira decipiens	800
Thalassiosira nordenskioldii	1,600
Thalassiosira spp.	5,200
Unidentified pennate	
20 µm	800
Flagellates	
4 µm	3,200
8 µm	800
TOTAL CELL COUNT	4.0×10^4
50 meters	
Diatoms	
Bacteriosira fragilis	1,200
Chaetoceros debilis	1,600
Chaetoceros socialis	800
Cylindrotheca closterium	800
Navicula sp.	400
Nitzschia sp. (*Fragilariopsis*)	4,000
Nitzschia sp. (*Fragilariopsis*)	4,800
Nitzschia sp. (*Fragilariopsis*)	14,000
Thalassionema nitzschioides	800
Thalassiosira gravida	2,400
Thalassiosira nordenskioldii	1,600

TABLE XII

CONTINUED

	Cells/liter
50 meters (contd.)	
Thalassiosira sp.	6,400
Silicoflagellates	
Dictyocha fibula	800
Flagellates	
3 µm	400
4 µm	8,400
8 µm	2,800
TOTAL CELL COUNT	5.2×10^4 cells/liter
75 meters	
Diatoms	
Chaetoceros atlanticus	8,800
Chaetoceros septentrionalis	800
Corethron criphilum	800
Cylindrotheca closterium	800
Fragilariopsis oceanicaf circularis	15,200
Nitzschia sp. (*Fragilariopsis*)	26,400
Nitzschia sp. (*Fragilariopsis*)	15,200
Nitzschia sp.	800
Thalassiosira aestivalis	2,400
Thalassiosira nordenskioldii	1,600
Thalassiosira rotula	1,600
Thalassiosira sp.	2,400
Unidentified pennate	
3 µm	3,200
Flagellates	
4 µm	2,400
8 µm	3,200
TOTAL CELL COUNT	8.56×10^4 cells/liter

TABLE XIII

DEEP WATER STATION 1 FOR LEG II

:ation 1 Position 54°53.7'N 169°22.3'W Total depth 2688 m

:epth	chlor *a* µg/ℓ	C µg/ℓ/day	PO_4-P	NO_3-N	SiO_3-Si	cells/ℓ
			µg-atoms/ℓ			
0	0.60	2.46	1.64	27.2	53	8.56×10^4
10	0.54	3.11	1.68	20.7	40	2.2×10^5
20	0.44	2.20	2.03	26.9	53	-
30	0.48	1.28	1.87	26.7	52	3.8×10^4
50	0.44	0.56	2.05	26.9	53	-
75	0.32	-	2.97	27.4	54	-
100	0.25	-	2.11	28.3	56	-
200	-	-	2.28	29.6	58	-
500	-	-	2.97	37.5	62	-
1000	-	-	3.47	45.0	153	-
1500	-	-	3.40	44.0	174	-

Phytoplankton composition and cell counts from Station 1

0 meters	cells/liter
Diatoms	
Chaetoceros compressus	3,200
Chaetoceros debilis	3,200
Chaetoceros decipiens	1,600
Chaetoceros socialis	3,200
Cylindrotheca closterium	1,600
Leptocylindrus danicus	3,200
Nitzschia sp. (*Fragilariopsis*)	6,400
Nitzschia sp.	800
Thalassiosira gravida	1,600
Thalassiosira spp.	5,600
Unidentified pennates	
6 µm	3,200
10 µm	1,600
Unidentified cell	4,000
Flagellates	
4 µm	25,600

TABLE XIII

CONTINUED

	Cells/liter
0 meters (contd.)	
Flagellates (contd.)	
4 µm	16,800
20 µm	2,400
Dinoflagellates	
cf. *Gymnodinium* sp.	1,600
TOTAL CELLS/LITER	8.56×10^4
10 meters	
Diatoms	
Bacteriosira fragilis	2,400
Chaetoceros septentrionalis	1,600
Chaetoceros socialis	7,200
Chaetoceros sp.	800
Chaetoceros debilis	1,600
Corethon criphilum	1,600
Cylindrotheca closterium	2,400
Navicula pelagica	3,200
Navicula sp.	800
Nitzschia sp. (*Fragilariopsis*)	58,400
Nitzschia sp. (*Fragilariopsis*)	15,200
Thalassiosira decipiens	13,600
Thalassiosira gravida	14,400
Thalassiosira nordenskioldii	800
Thalassiosira sp.	3,200
Thalassiosira spp.	53,600

TABLE XIII

CONTINUED

	Cells/liter	
10 meters (contd.)		
Unidentified pennates		
3 µm	2,400	
89 µm	1,600	
50 µm	800	
Dinoflagellates		
cf. *Gymnodinium* sp.	3,200	
Flagellates		
4 µm	17,600	
4 µm	8,800	26,400
8 µm	4,000	
Prasinophyta	4,800	
TOTAL CELLS PER LITER	2.2×10^5	
20 meters		
Diatoms		
Chaetoceros socialis	2,400	
Cylindrotheca closterium	1,200	
Thalassiosira sp.	3,200	
Unidentified pennates		
5 µm	2,000	
17 µm	1,200	
Dinoflagellates		
cf. *Gymnodinium* sp.	2,800	
Unidentified dinoflagellate	2,800	
Flagellates		
4 µm	4,400	
8 µm	16,400	
Prasinophyta	1,600	
TOTAL CELLS PER LITER	3.8×10^4	

TABLE XIV

SIZE AND ABUNDANCE OF ALGAL BLOOMS WITH ASSOCIATED ICE CONDITIONS AT NINE STATIONS IN THE BERING SEA, APRIL 1976

Station	Date	Depth (n)	Ice Conditions	Mean size of bloom		Abundance (mg/ℓ)
				Volume (μm^3)	Diameter (μm)	
1	4/15/76	0	No ice	51200	46.1	0.3
2	4/16/75	0	No ice	51200	46.1	0.4
4	4/18/76	0	Ice floes	150000	65.9	4.5
6	4/20/76	0	Ice floes	150000	65.9	4.8
8	4/21/76	0	Ice floes	150000	65.9	6.3
13	4/24/76	0	Ice floes	150000	65.9	2.9
13B	4/24/76	0	Sample taken from ice core	50 + 6400	4.6 + 23.0	-;16.43
14	4/25/76	0	Ice edge	400 + 51200	9.1 + 46.1	.21 + .26
14	4/25/76	20	Ice edge	400 + 25600	9.1 + 36.6	.58 + .21
14	4/25/76	50	Ice edge	50; 25600+102400	4.6; 36.6 + 58.1	-.07 + .07
15	4/26/76	0	No ice	50; 400+51200	4.6; 9.1 + 46.1	-.02 + .02

open water were low in contrast to the waters of the ice-floe zone.

Three depths were sampled at station 14 (0, 20 and 50 m). The largest bloom (0.58 mg/ℓ) was found at 20 m with a peak diameter of 9.1 μm.

Table XV lists the total number of cells per liter, percentage diatoms and flagellates and major diatom species. The most common species of diatoms are *Thalassiosira* spp. and *Nitzschia* sp. (*Fragilariopsis*). It is interesting to note that *Detonula confervacea* is present both in the water column (station 13) and under the ice (station 13B). Both stations 13 and 13B were collected from the same area.

The distribution of flagellates was fairly uniform although they are somewhat more abundant in ice-free water. Abundance does not vary much down to a depth of 50 m in ice-free and ice-floe areas. The flagellates were small, being 4 to 10 μm in diameter.

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 fibre filtered sea water. Counts were obtained after 1 and 5 hr. After 1 hr there existed 0.21 mg/ℓ total particulate matter with a peak at 58 μm. After 5 hr, 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

TABLE XV

SPECIES COMPOSITION AT NINE STATIONS IN THE BERING SEA, APRIL 1976

Station	No. of Cells x10^4/ℓ	% Diatoms	% Flagellates	Major Diatom Species
1 (0 m)	8.6	45.8	52.3	*Thalassiosira* sp. *Nitzschia* sp. (*Fragilariopsis*)
2 (0 m)	12.0	14.0	83.4	Unidentified species 20 µm pennate 6 µm pennate *Thalassiosira* spp. *T. rotula* *T. gravida*
4 (0 m)	27.0	95.9	4.1	*Thalassiosira* spp. *Thalassiosira* sp. *T. gravida* *T. nordenskioldii* *Nitzschia* sp. (*Fragilariopsis*) *Nitzschia* sp. (*Fragilariopsis*) *Nitzschia* sp. (*Fragilariopsis*)
6 (0 m)	40.4	84.7	15.0	*Nitzschia* sp. (*Fragilariopsis*) *Nitzschia* sp. (*Fragilariopsis*) *T. gravida* *Thalassiosira* spp.
8 (0 m)	73.0	94.5	4.6	*Nitzschia* sp. (*Fragilariopsis*) *T. nordenskioldii*
13 (0 m)	48.0	97.9	1.9	*Detonula confervacea* *Nitzschia* sp. (*Fragilariopsis*)

TABLE XV

CONTINUED

Station	No. of Cells x10^4/ℓ	% Diatoms	% Flagellates	Major Diatom Species
13 (20 m)	37	94.6	4.5	*Nitzschia* sp. (*Fragilariopsis*) *Nitzschia* sp. (*Fragilariopsis*) *Nitzschia* sp. (*Fragilariopsis*) *Melosira* sp. *Nitzschia frigida* *T. gravida* *Thalassiosira* sp. *Thalassiosira* spp.
13 (50 m)	5.2	76.9	22.3	*Nitzschia* sp. (*Fragilariopsis*) *Nitzschia* sp. (*Fragilariopsis*) *Nitzschia* sp. (*Fragilariopsis*) *T. gravida* *T. nordenskioldii* *Thalassiosira* spp.
13B (ice)	94.3	99.9	.02	*Achnanthes* sp. *Detonula confervacea* *Nitzschia* sp. (*Fragilariopsis*)
14 (0 m)	19.4	38.1	57	*Nitzschia* sp. (*Fragilariopsis*) *Fragilariopsis oceanica f. circularis* *Nitzschia frigida*
14 (20 m)	24.2	22.6	54.9	*Achnanthes* sp. *Nitzschia* sp. (*Fragilariopsis*) *Thalassiosira* sp.
14 (50 m)	3.76	27.7	68.1	*Melosira sulcata* *Thalassiosira* spp. *T. gravida*
15 (0 m)	16.3	18.4	70.2	*Chaetoceros wighami* *Navicula pelagica* *Thalassiosira* sp. 4.6 µm unidentified pennate

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 XVI. 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 2 shows the results for station 13 after 26 hr 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 x 10^3 CPM/mg of particles for the control and 8.65 x 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 XVII and Figures 3 and 4. A concentration Of 2 µg/ℓ had no observable effect on large phytoplankton (10-80 µm) in ice-free and ice-flow areas. Above 2 µg/ℓ 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 2 parallel each other.

Mean carbon uptake rates per unit biomass for total phytoplankton from ice-floe areas do not appear to be influenced by copper concentrations.

TABLE XVI

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)	mls/hr/Individual		mls/hr/mg dry wgt.	
					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	-	.43	-
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 hr^{-1}

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

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

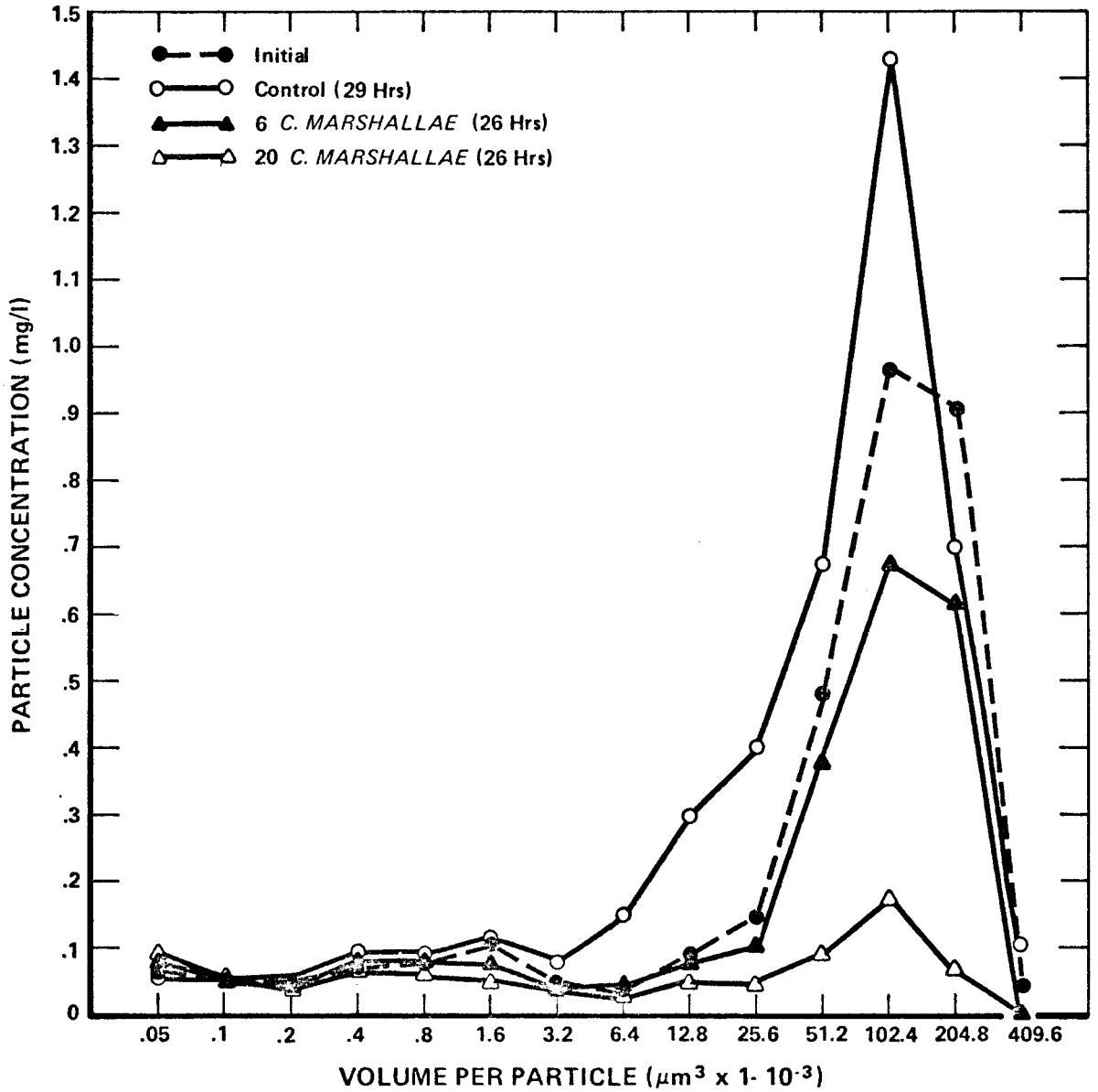

Figure 2. Particle concentration vs. particle size for station 13 grazing experiment.

TABLE XVII

PHYTOPLANKTON GROWTH RATES (HR^{-1}) AT FOUR COPPER CONCENTRATIONS FOR SIX STATIONS IN THE BERING SEA[1]

Station	Copper Concentration (µg/ℓ) 0	2	4	8	Length of Expt (hrs)
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, 8, 13 (ice floe)	.0067	.0069	.0065	.0050	-
Mean all stations	.0059	.0060	.0056	.0034	-

(1) Phytoplankton were 10-80 µm in diameter

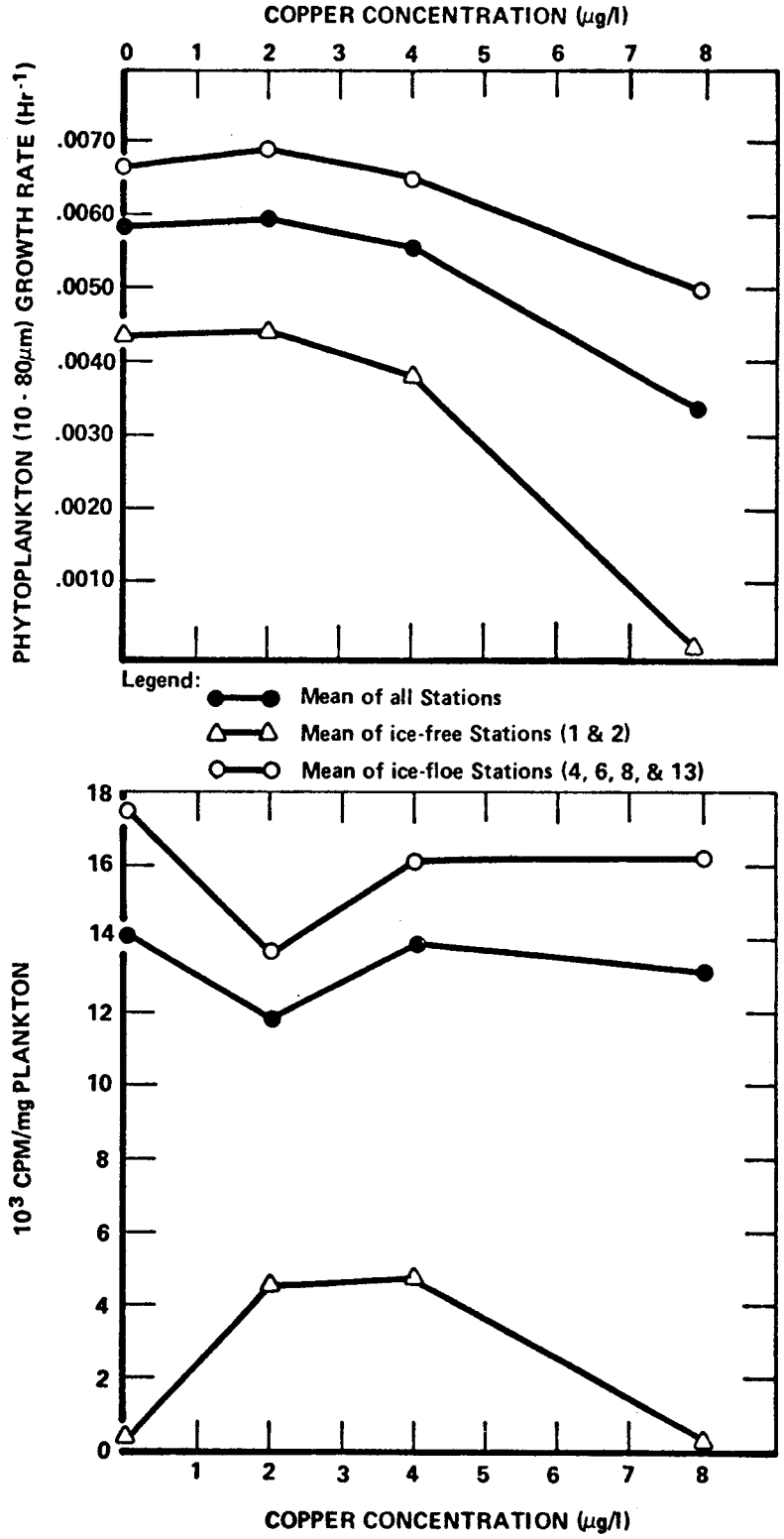

Figure 3. Phytoplankton growth rate vs. copper concentration for phytoplankton 10-80 µ diameter.

Figure 4. 24 hour primary productivity for total phytoplankton at four concentrations of copper. The plankton populations were first subjected to copper for periods ranging from 105 to 151 hours.

This suggests that enzyme and/or transport systems other than the 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 5. Particle size at peak concentration does not decrease with increasing copper concentration.

Oil Toxicity

Results of the oil toxicity experiments are shown in Table XVIII and Figures 6 and 7. Concentrations of 10 and 30 PPM oil inhibited the growth rates of large phytoplankton (10-80 μm) from ice-floe 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 8. Particle size at peak concentration decreased with increasing oil concentration.

Figure 6 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.

IX. DISCUSSION

Undoubtedly one of the most important aspects of this cruise series was obtaining data immediately prior to the bloom. On all former cruises the ice edge bloom phenomena had already begun or had finished thus we have had no data on initial nutrient concentration. Since the bloom appears to be nutrient limited, knowledge of the initial supply available to the

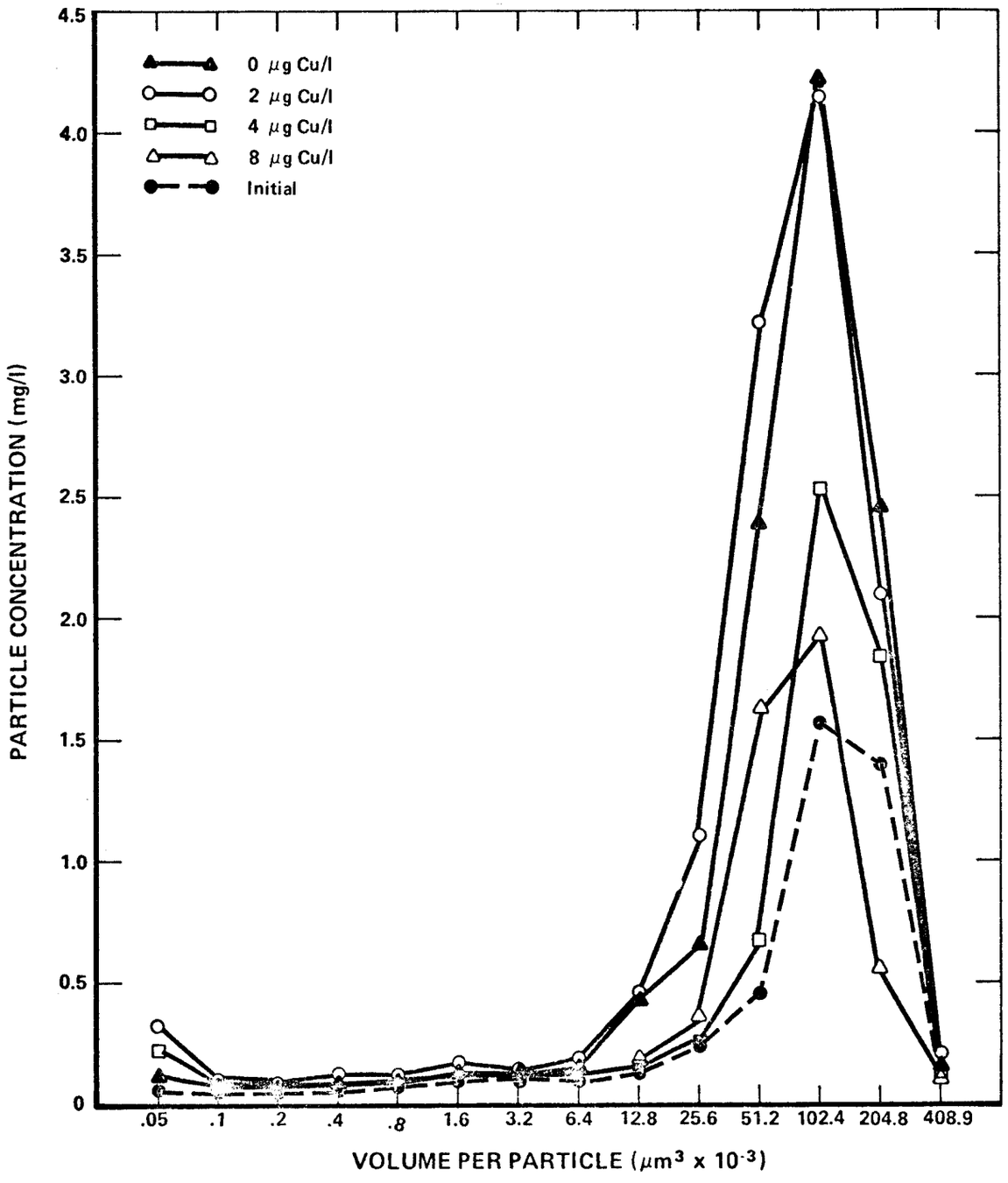

Figure 5. Particle concentration vs. particle size at station 4 at four copper concentrations after 126.5 hrs.

TABLE XVIII

PHYTOPLANKTON GROWTH RATES (HR^{-1}) AT THREE CONCENTRATIONS OF CRUDE OIL FOR SIX STATIONS IN THE BERING SEA[1]

Station	Oil concentration (ppm) 0	10	30	Length of Expt (Hrs)
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 floe)	.0067	.0014	-.0047	-
Mean all stations	.0059	.0028	-.0034	-

[1] Phytoplankton were 10-80 μm in diameter

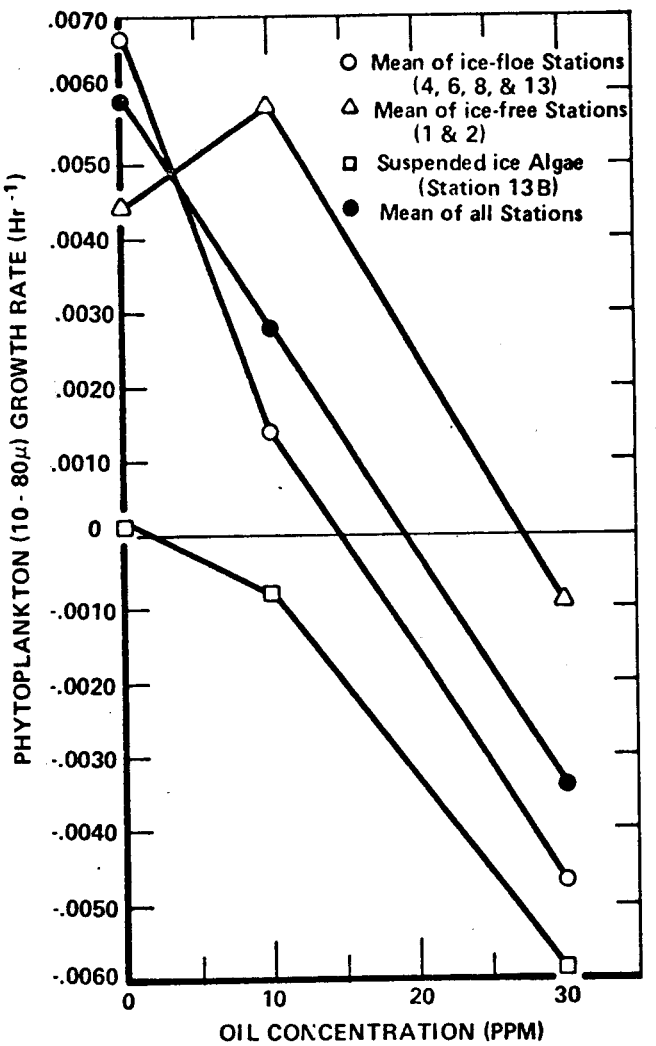

Figure 6. Phytoplankton growth rate vs. oil concentration for plankton 10-80 μm diameter.

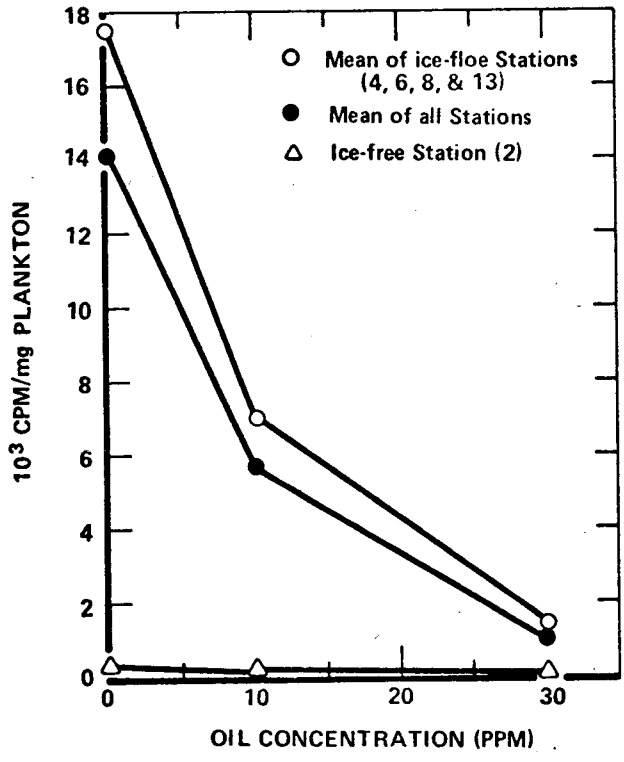

Figure 7. 24 hour primary productivity for total phytoplankton at three concentrations of Prudhoe crude oil. The plankton populations were first subjected to oil for periods ranging from 105 to 151 hrs.

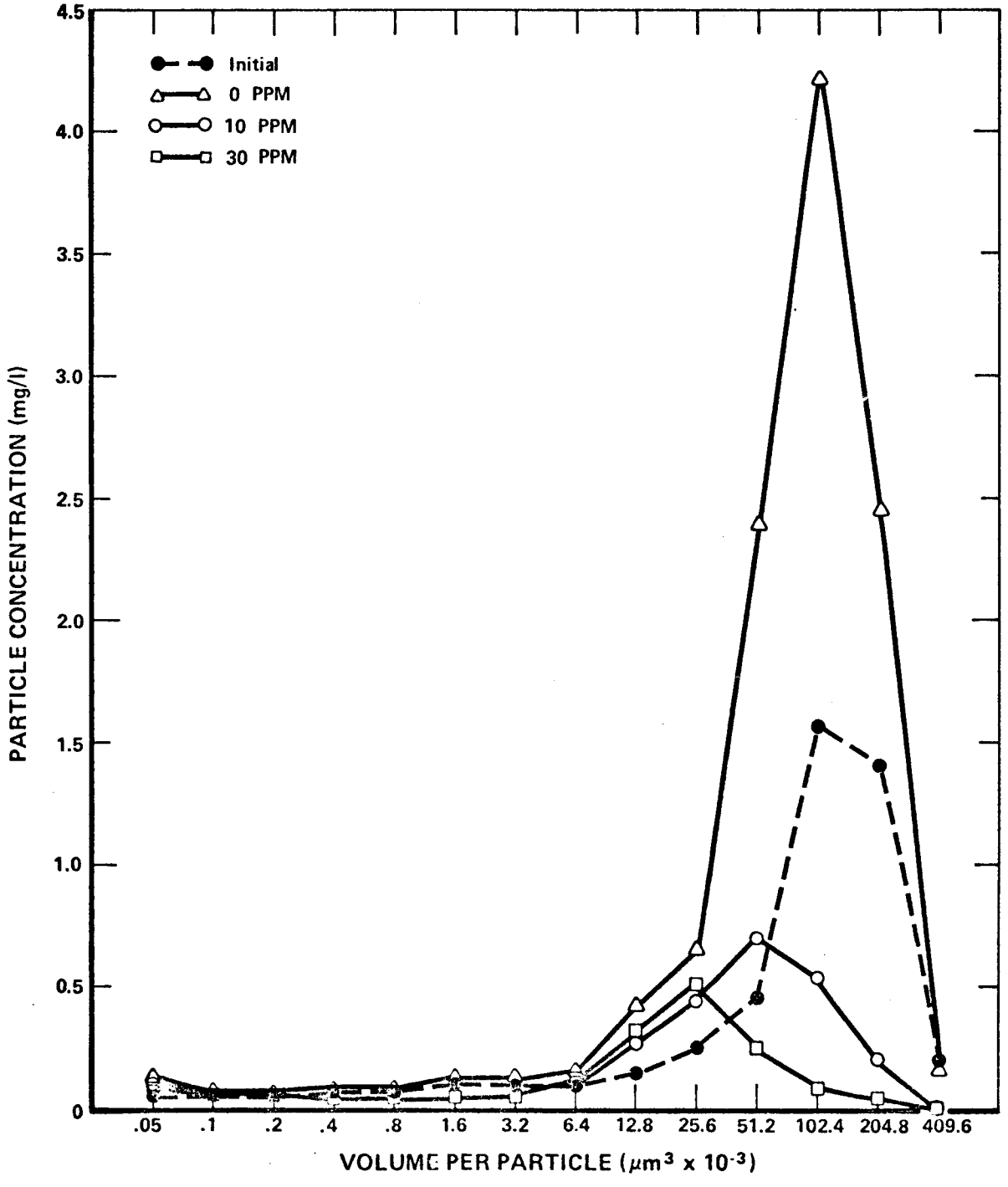

Figure 8. Particle concentration vs. particle size at station 4 for three concentrations of crude oil after 126.5 hrs.

phytoplankton is essential. Leg I of the *Surveyor* cruise supplied this information. In general nutrient conditions everywhere over the shelf were relatively constant for the euphotic zone. Nitrate concentrations were approximately 17.5 µg-at/ℓ , phosphate approximately 1.5 µg-at/ℓ and silicate approximately 34 µg-at/ℓ over the shelf in contrast to distinctly higher concentrations observed in the surface waters of the deep water station - approximately 30, 2.2 and 56 µg-at/ℓ respectively. This suggests that nutrient cycling and regeneration over the shelf involves processes unique to that area and are not strongly affected by the open ocean situation.

On earlier cruises we have observed nutrient depletion of the surface waters by the intense bloom occurring there with nutrients found only at deeper depths. It appears that the initial surface bloom occurs with such intensity as to severely reduce the amount of light penetrating to deeper depths and that production occurs in progressively deeper water with time (or distance from the ice edge). The waters above the bloom layer depth are stripped of essential nutrients while production continues at depths until light limitation occurs. Our summer and fall data indicate continued low nutrient concentrations and little productivity within the waters over the shelf. A June 1974 curise aboard the *Alpha Helix* showed significant chlorophyll *a* only at depths Of 30-40 m. By the August *Discoverer* cruise (1975) no deep chlorophyll layer was seen. Routinely we observe coloration on the samples collected within 10-20 m from the bottom throughout the year but this coloration on the filter pads does not respond as chlorophyll upon spectrophotometric analysis nor does it fluoresce when excited by chlorophyll excitation wavelengths. Repeated efforts to measure productivity at these depths with ^{14}C have shown negative results. We suggest that

this deep layer is detrital organic matter having settled from the upper waters following the bloom. The concommitant warming of the surface waters with the spring bloom would encourage sinking of the cells and inhibit any upward movement of regenerated nutrients until advective processes associated with winter cooling allow for vertical transport.

Since nitrogen appears to be the limiting nutrient it is possible that a realistic annual production estimate could be made based on nitrogen cycling dynamics. Figures 9 and 10 demonstrate the inverse relationship between nitrate concentration and standing crop of phytoplankton as measured by chlorophyll *a* concentration. Certainly we must attempt to understand the mechanisms of nutrient supply to the shelf area.

Upon arrival at the ice edge in May on the *Discoverer* cruise when extremely high productivity was observed the ice consisted of large (ca. 10 m diameter) chunks of ice with visible algal growth at the edges and on the undersurface in pockets and depressions. The open water between the chunks provided a window for light penetration. On the early *Surveyor* cruise when barren conditions existed the ice cover was much more extensive occurring in large unbroken plates with few open spaces between. While sampling the ice on one occasion a southerly swell developed breaking up the large plates producing conditions similar to those described above except lacking any obvious algal growth on the edges and sides. Since nutrient conditions were able to support a bloom condition it appears that the initial triggering of the bloom is light dependent, and is aided by a permanent breaking up of the ice, for by the time we returned to the ice edge two weeks later the ice chunks were laden with algae as observed before. On the forthcoming cruise we will be making extensive light measurements.

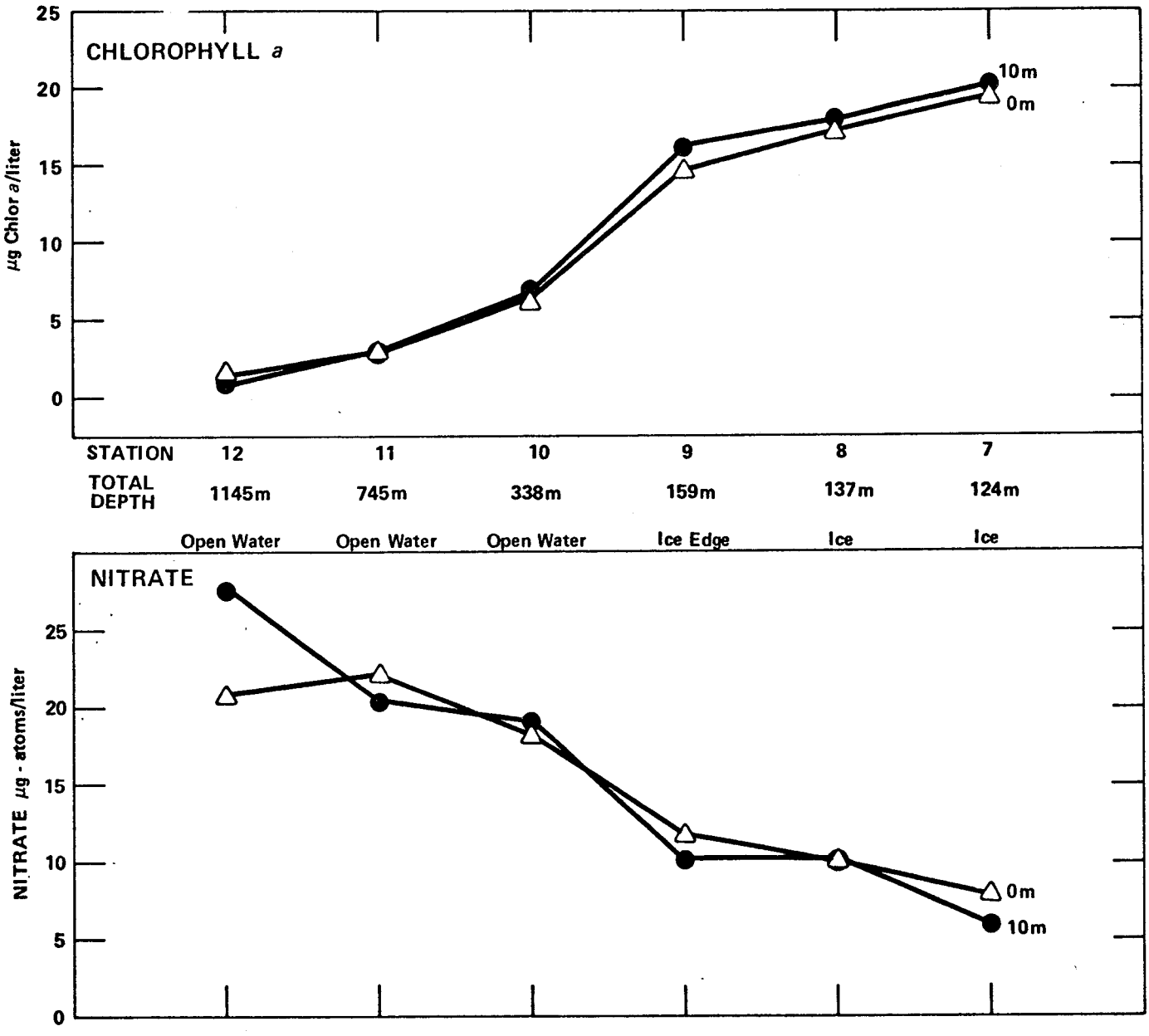

Figure 9. Group A, 0 and 10 m chlorophyll and nitrate concentrations for transect beginning within the ice and proceeding over the shelf break into open water (stations 7-12).

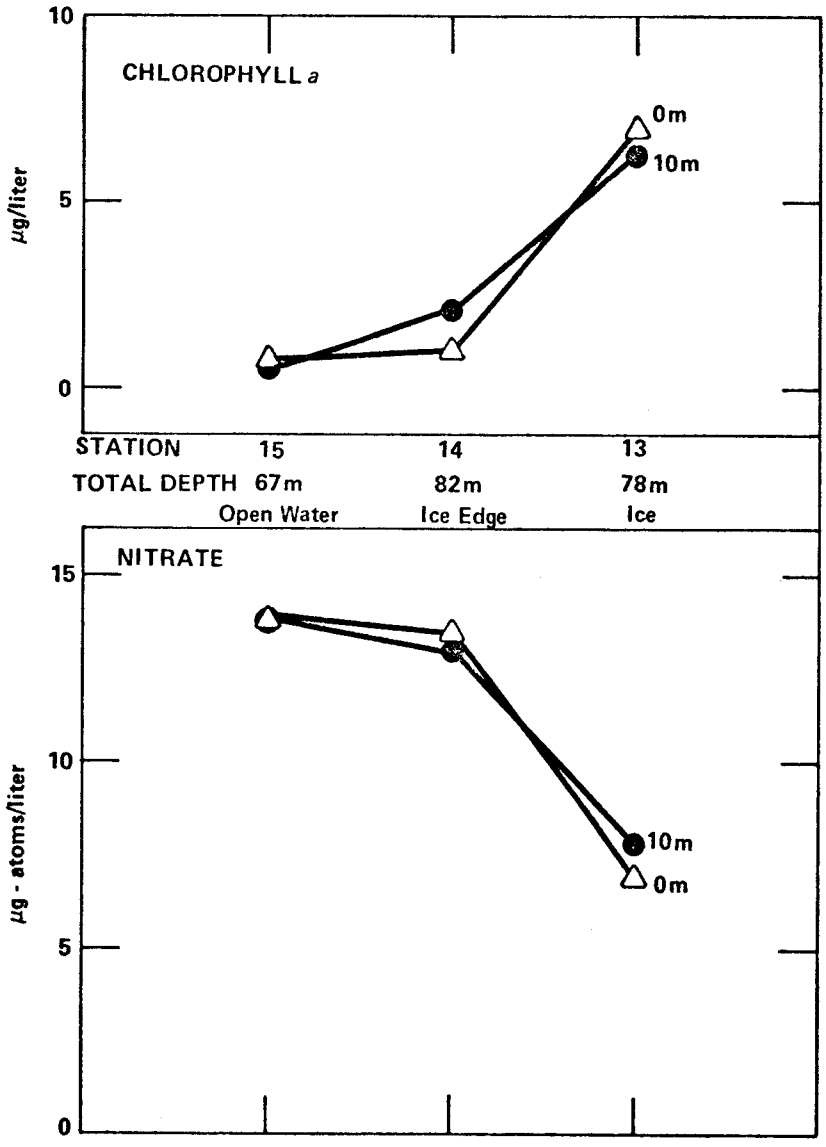

Figure 10. Group E, 0 and 10 m chlorophyll and nitrate concentrations for transect beginning in ice and proceeding to open water (stations 13-15).

The ice edge once the bloom had begun was an area of intense biological activity as witnessed on previous cruises. Chlorophyll concentrations of 20 µg/ℓ and carbon fixation rates of 30 mg C/m^3/day were observed at the innermost ice station in group A. The effect of the ice cover on productivity is shown in both Figures 9 and 10 representing the two ice transects during the bloom period. Both figures show the greatest production at the innermost ice station although the group A transect is also affected by the transition over the shelf break. On the other hand the group D stations occurred entirely over the shelf and yet show maximum production closest to the ice edge even though nutrient conditions are higher at the other stations further from the ice front. Both of these transects represent the initiation of the bloom, but it is curious to note the enhancement of production by ice cover. Since most shelf stations sampled on this cruise were close to the shelf break it will be interesting to observe this forthcoming cruise data since the ice edge is predicted to be considerably further up on the shelf. This will give us a better idea of whether the intense initial productivity is correlated more with the shelf itself or the ice edge influence.

Over one hundred different phytoplankton organisms have been identified from the samples collected and have been catalogued according to the Alaska Marine Taxonomic Code (University of Alaska, Institute of Marine Science, 1975). Line drawings have been made of unidentified species and photographs are being taken using an American Optical Differential Contrast Microscope. We plan to use cluster analysis techniques to delineate changes in community structure between stations, depths and seasons and to correlate these changes with other parameters such as light intensity, nutrient

concentration, salinity, etc. Naturally the differences between the ice communities and planktonic communities will be studied, but we have not progressed sufficiently with this data analysis to report it here. The data shown in Tables XI and XII show a greater number of phytoplankton species and more cells/liter to occur in the ice core sample than in the water column beneath.

X. PROBLEMS/CHANGES

Hereafter we will be making extensive light measurements at sea, both atmospheric and underwater, to be applied to our productivity work. We intend to simultaneously measure both solar radiation as is usually reported in watts m^{-2} and the photosynthetically active radiation in microeinsteins (or photons) i.e. that energy falling in the 400-700 nm waveband. At high latitudes particularly with the intense cloud cover common to the Bering Sea and especially in the water beneath ice floes we expect the two measurements to differ and will deal with the quantum measurements for our work but compare the two.

Also we will have aboard a prototype *in situ* fluorometer compatible with the Plessy "fish", and will be able to obtain a trace of the chlorophyll concentration with depth. This will enable us to select sample depths with some foreknowledge.

Further work should now emphasize the interrelationships between the ice production, ice edge production and the low summer activity with the grazer populations.

REFERENCES

Arsen'ev, V. S. 1967. Currents and water masses of the Bering Sea [in Russian, English summary]. Izd. Nauka, Moscow. (Translated, 1968, National Marine Fisheries Service, Northwest Fisheries Center, Seattle, Wash.), 135 pp.

Lovegrove, T. 1966. The determination of the dry weight of plankton and the effect of various factors on the values obtained. *In* Some contemporary studies in marine science. H. Barnes (ed.), Hafner Publishing Co., New York.

McRoy, C. P. and J. J. Goering. 1974. The influence of ice on the primary productivity of the Bering Sea. *In* Oceanography of the Bering Sea. D. W. Hood and E. J. Kelley (eds.). Institute of Marine Science, University of Alaska, Fairbanks, Alaska 99701.

Rigler, F. H. 1971. Feeding rates. *In* A manual on methods for the assessment of secondary productivity in fresh waters. IBP Handbook No. 17, W. T. Edmonson and G. G. Winberg (eds.). F. A. Davis Co., Philadelphia.

XI. SUMMARY OF FOURTH QUARTER ACTIVITIES

A. Ship or Laboratory Activities

1. Ship or field trip schedule

   None

2. Scientific party

   None

3. Laboratory analysis on samples collected during the previous quarter have been carried out using methods described in Section V.

4. None

5. None

6. See data submission schedule

B. Improved methods on handling the light input will be incorporated into the study. This is especially important for the modeling which has been initiated during this winter.

C. To be appended.

OCS COORDINATION OFFICE

University of Alaska

ENVIRONMENTAL DATA SUBMISSION SCHEDULE

DATE: March 31, 1977

CONTRACT NUMBER: 03-5-022-56 T/O NUMBER: 1 R.U. NUMBER: 159/164/427

PRINCIPAL INVESTIGATOR: Dr. Vera Alexander and Dr. Ted Cooney

Submission dates are estimated only and will be updated, if necessary, each quarter. Data batches refer to data as identified in the data management plan.

Cruise/Field Operation	Collection Dates		Estimated Submission Dates[1]			
	From	To	Batch 1	2	3	4
Discoverer Leg I #808	5/15/75	5/30/75	submitted	submitted	None	None
Discoverer Leg II #808	6/2/75	6/19/75	submitted	submitted	None	None
Discoverer Leg I #810	8/9/75	8/28/75	submitted	submitted	None	None
Miller Freeman #815	11/10/75	11/26/75	submitted	submitted	None	None
Surveyor Su/001/2	3/76	4/76	5/15/77	5/15/77	None	None

Note: [1] Data Management Plan and data Formats have been approved and are considered contractual. An update of data management plan, reflecting FY '77 Work Statement will be forthcoming shortly.

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