Bacterial Populations in the Beaufort Sea

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ABSTRACT. Spacial and seasonal variation patterns were studied for bacterial populations in Beaufort Sea ice, water and sediment. Bacterial populations in the Beaufort Sea were found in concentrations as high as are found in temperate oceans. Bacterial populations, especially viable bacteria, were lower in surface water during winter than summer, but not in sediment. Beaufort Sea bacterial populations are mainly psychrotrophic and are clearly adapted to growth at low temperatures. Ice conditions appear to be important in determining levels of bacterial populations in water.

RÉSUMÉ. Des variations typiques dans l'espace et suivant les saisons, ont été étudiées, dans les sédiments, l'eau et la glace de la Mer de Beaufort, en ce qui concerne les colonies de bactérie. On a trouvé des colonies bactérielles dans la Mer de Beaufort en concentration aussi importante que dans les océans tempérés. Les populations bactérielles, surtout les bactéries, aptes à vivre, étaient moins nombreuses à la surface de l'eau pendant l'hiver qu'en été mais non dans les sédiments. Les populations bactérielles de la Mer de Beaufort sont surtout psychotrophiques et bien adaptées pout grandir avec des températures glaciales. Les conditions de glace paraissent un facteur important du niveau d'importance des populations bactérielles dans l'eau.
Traduit par Alain de Vendegies, Aquitaine Co. of Canada Ltd.

INTRODUCTION

The extreme environmental conditions of polar seas make them interesting ecosystems for studying biological populations that are adapted to survival under such conditions. The Beaufort Sea, like other polar seas, has the unusual feature of being ice covered much of the year. Very few studies of micro-organisms in the Beaufort Sea, however, have been previously conducted. This paper describes a survey of the distribution of bacterial populations in the western Beaufort Sea. This is part of a larger study aimed at characterizing the bacterial populations in this area.

Bunch and Harland (1976) reported that surface waters in the Mackenzie Delta region of the Canadian Beaufort Sea contain 10^3 - 10^4 viable bacteria/ml, which they reported to be mainly psychrophilic. Viable bacterial populations in surface waters of polynias in the central Arctic Ocean have been reported to be less than 1 per ml (Kriss, 1963). Kriss (1963) also reported that viable bacterial populations in the Arctic Ocean near Greenland are less than 1/ml in surface water. Boyd and Boyd (1963) enumerated bacteria from nearshore water samples collected during summer in the Chukchi Sea just west of the Chukchi-Beaufort Sea interface. They reported that bacterial numbers were lower in this area than in temperate region seas and postulated that most of the micro-organisms were of terrestrial origin. Horner and Alexander (1972)

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studying the algal populations and heterotrophic activities in sea ice of the same area of the Chukchi Sea reported that heterotrophic activity in the ice was mainly bacterial, but they did not enumerate the bacterial populations.

In the present study, sampling extended from the Chukchi-Beaufort Sea interface at Barrow studied by Boyd and Boyd (1963) and Horner and Alexander (1972) to a transect west of Prudhoe Bay. Samples were collected within the nearshore barrier island lagoon ecosystems, as well as along offshore transects. Sampling included sea ice, sediment and water samples collected during late winter while the sea was covered by 2 metres of sea ice. This is the first report of a bacteriological study conducted on such samples.

MATERIALS AND METHODS

Sample Collection

Samples were collected at stations in the western Beaufort Sea during late summer (August 20 - September 25) 1975 and 1976 and during late "winter" (April 5 - 18) 1976. The April sampling was considered to be a late winter

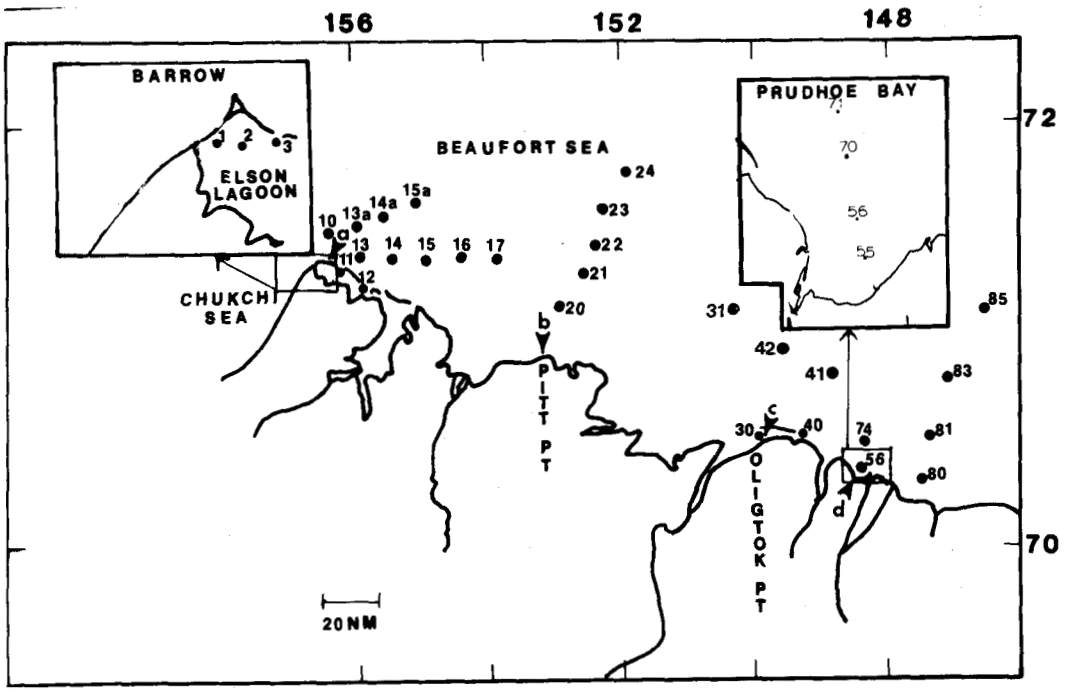

FIG. 1. Map of sampling area. Numbers show sampling sites, letters show shoreline reference points: a. Pt. Barrow; b. Pitt Point; c. Oligtok Point; d. Prudhoe Bay.

sampling as the sea was still covered with maximal thickness ice and the spring phytoplankton bloom had not yet occurred. Sample site locations are shown in Figure 1. Surface water samples (1 m depth) were collected with

Niskin butterfly sterile water sampler (General Oceanics, Miami, Fl.). Surface sediment samples were collected with a mud grabber (Kahl Scientific, San Diego, Ca.). Ice samples were collected by chipping out pieces of surface ice and placing the ice into a sterile container. The ice was allowed to melt and maintained at temperatures below 5°C. Winter water and sediment samples were collected through holes drilled in the ice. All samples were placed in sterile containers using aseptic technique and returned to the laboratory on ice for processing within a few hours (1 - 10 hr.) of collection. The shortest processing times were during summer 1976 when processing was done aboard the sampling vessel; the longest processing times occurred when samples were collected by helicopter or small craft and had to be flown back to the Naval Arctic Research Laboratory at Barrow, Alaska.

Temperature and salinity measurements were made using a Yellow Springs Instruments salinity meter (Yellow Springs, Oh.). For nutrient analyses, samples were immediately filtered through a glass fiber filter (Reeve-Angel HA) and frozen on dry ice in acid washed bottles. Samples were analyzed for ammonium by the method of Head (1971), and nitrate and phosphate by the methods of Strickland and Parsons (1968) using a Technicon auto analyser II system.

Enumeration of Micro-organisms

Enumerations of bacterial populations were performed using both direct count (total count) and plate count (viable count) procedures. For direct counts, samples were preserved with formaldehyde, one part formaldehyde (50%) : one part sample. Samples were filtered through 0.2 μm cellulose nitrate black filters (Sartorius) and stained with acridine orange according to the procedure of Daley and Hobbie (1975). Samples were viewed with an Olympus epifluorescence microscope with a BG-12 exciter filter and 0-530 barrier filter. Ten fields per filter and two filters per sample were viewed and the counts averaged.

For viable plate counts, surface spread inoculations from serial dilutions were used. For some sea ice and water samples, concentration by filtration through 0.45μm filters (Millipore Corp.) was also used. Filters were immediately placed onto agar plates. Marine agar 2216E (Difco) was used to enumerate viable heterotrophic micro-organisms. Media and dilution blanks (Rila sea salt solution) were cooled to 4°C before platings were performed. Replicate plates were incubated aerobically at 4°C for 3 weeks and 20°C for 2 weeks to enumerate psychrophilic-psychrotrophic and mesophilic populations respectively (Morita, 1975). Colonies that developed were counted with a binocular microscope. All platings were done in triplicate and the average reported. Counts for water and ice samples are per ml and for sediment per gram dry weight.

Correlation coefficients were calculated using BIMED program P2R (Dixon, 1973) to determine the statistical relationships between population

levels and abiotic parameters. A Pearson correlation coefficient of greater than 0.6 was generally considered necessary to demonstrate a significant correlation. Analyses of variance were performed using BIMED program P2V (Dixon, 1973) to determine the statistical significance of differences in parameters from different regions. A probability of exceeding F at the 0.05 level was considered necessary for establishing a significant relationship.

The abilities of organisms enumerated at 4°C to grow at higher temperatures and of organisms enumerated at 20°C to grow at both lower and higher temperatures were examined. Colonies developing on the countable marine agar plates (ca. 60-100 colonies/plate) were assigned a number. A random number table was used to select organisms for further testing. Approximately 1600 organisms were tested. Selected organisms were inoculated onto replicate marine agar plates which were incubated at 5, 10, 15, 20, 25, 37, and 43°C. Growth was determined during 3 weeks incubation as formation of a visible colony. According to the definitions of Morita (1975), psychrophilic micro-organisms differ from psychrotrophic micro-organisms in their optimal temperatures and temperature growth ranges. We consider organisms capable of growth at both 4°C and 20°C to be psychrotrophs and organisms restricted to growth below 20°C to meet the definition of true psychrophiles.

Organisms selected for temperature growth range testing were also examined for their ability to grow in the absence of NaCl. The basal media for this testing consisted of tryptone, 0.5%; yeast extract, 0.1%; $FeCl_3.6H_2O$, 0.01%; NH_4NO_3, 0.00016%, Na_2HPO_4, 0.0008%; purified agar, 1.5%, pH 8.0. Growth was determined by colony formation during 3 weeks at 4°C for organisms originally selected from 4°C enumeration plates, or during 2 weeks at 20°C for organisms originally selected from 20°C enumeration plates.

RESULTS

Abiotic Parameters

Both surface and bottom temperatures were about 2 - 3°C lower in winter than in summer (Table 1). There were no significant temperature differences between the eastern (Prudhoe Bay) and western (Pt. Barrow) sampling regions during any sampling period. During summer 1975, though, shorefast ice remained all summer in the Pt. Barrow region.

All surface water salinities were lower than "standard sea-water", reflecting the combined influence of freshwater input from rivers and freezing out of salts during ice formation (Table 1). Salinities of sea ice were significantly lower than salinities of underlying water. Bottom salinities were significantly higher for summer 1976 samples than for summer 1975 samples.

Average concentrations of nitrate-nitrogen were higher than average concentrations of ammonium nitrogen (Table 1). Average concentrations of available nitrogen (nitrate + ammonium) were higher in winter than summer.

TABLE 1. Average Abiotic Parameters

Source		Mean	Standard Deviation	Range	No. of Samples
			Temperature (°C)		
Summer 1975	Water	+1.1	0.9	-1.2 - +3.2	43
Summer 1975	Sediment	+1.5	1.0	-2.0 - -1.5	18
Winter 1976	Ice	-2.0	0.3	-2.2 - -1.5	11
Winter 1976	Water	-1.9	0.2	-2.0 - -1.5	20
Winter 1976	Sediment	-1.8	0.2	-2.0 - -1.5	15
Summer 1976	Water	+0.5	1.0	-1.3 - +2.7	20
Summer 1976	Sediment	+0.1	1.2	-1.6 - +2.1	13
			Salinity (°/oo)		
Summer 1975	Water	17.4	7.0	11.1 - 27.0	43
Summer 1975	Sediment	19.8	1.6	17.0 - 22.8	18
Winter 1976	Ice	6.7	6.0	1.0 - 11.0	11
Winter 1976	Water	24.3	4.3	17.0 - 31.0	20
Winter 1976	Sediment	-	-	-	0
Summer 1976	Water	16.5	7.8	5.1 - 29.5	20
Summer 1976	Sediment	31.0	3.5	24.5 - 39.2	13
			$PO_4^{\equiv}$ (µg at./l)		
Summer 1975	Water	2.7	2.4	0.3 - 9.6	37
Summer 1975	Sediment	2.8	1.0	1.9 - 3.8	2
Winter 1976	Ice	0.1	0.1	0.0 - 0.3	18
Winter 1976	Water	1.1	0.2	0.5 - 1.4	17
			NH_4^+ (µg at./l)		
Summer 1975	Water	0.8	0.9	0.1 - 5.2	33
Summer 1975	Sediment	0.7	0.2	0.5 - 0.8	2
Winter 1976	Ice	0.9	2.7	0.0 - 10.0	18
Winter 1976	Water	2.3	7.4	0.0 - 32.0	17
			NO_3^- (µg at./l)		
Summer 1975	Water	1.6	1.6	0.6 - 10.0	37
Summer 1975	Sediment	1.3	0.3	1.1 - 1.5	2
Winter 1976	Ice	1.9	0.9	0.7 - 3.1	18
Winter 1976	Water	5.0	2.0	2.0 - 8.5	17

There were large variations between available nitrogen concentrations in the samples, especially for winter ammonium concentrations.

Average phosphate concentrations were 5 times higher during summer 1975 in water samples from the Pt. Barrow region (range 1.0 - 9.2, mean 4.6 µg at./l), than from the Prudhoe Bay region (range 0.3 - 2.8, mean 0.9 µg at./l). There was no significant difference in concentrations of phosphate in water samples collected during winter or summer 1976 between the Pt. Barrow and Prudhoe Bay regions.

Enumerations of Microbial Populations

The enumerations of microbial populations are shown as three dimensional histograms (Figs. 2-4). The geographic location of each sample is shown by latitude and longitude in the XY plane. The numbers of micro-organisms

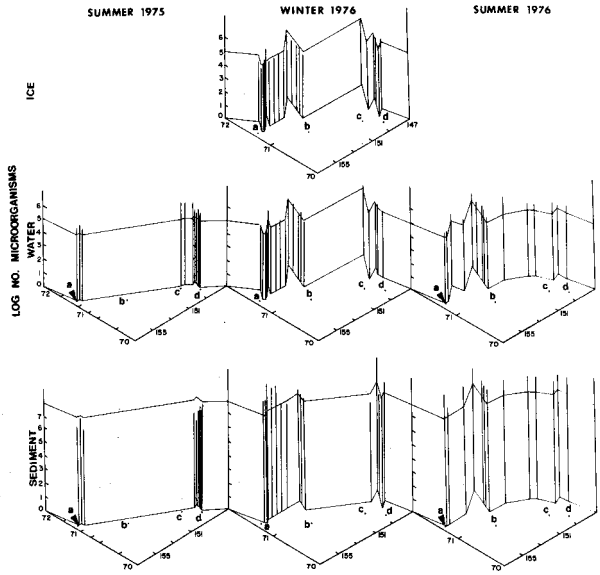

FIG. 2. Enumeration of total microbial populations by direct count procedures. Geographic location of sampling site is shown by latitude and longitude in the XY plane at the base of the vertical bars. The heights of the bars represent the numbers of micro-organisms enumerated. Lines at base and near tops of bars are reference lines for gaining perspective. Letters (a-d) are shoreline reference points shown in Figure 1.

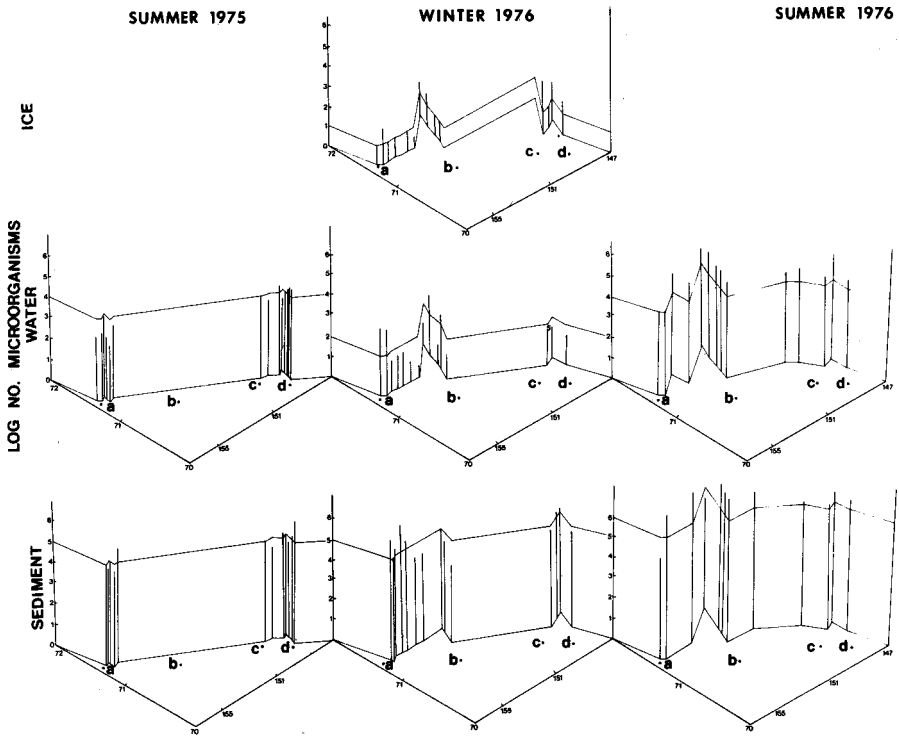

FIG. 3. Enumeration of heterotrophic microbial population at 4°C. (Psychrophilic and psychrotrophic populations). Refer to legend Figure 2 and text for interpreting figure.

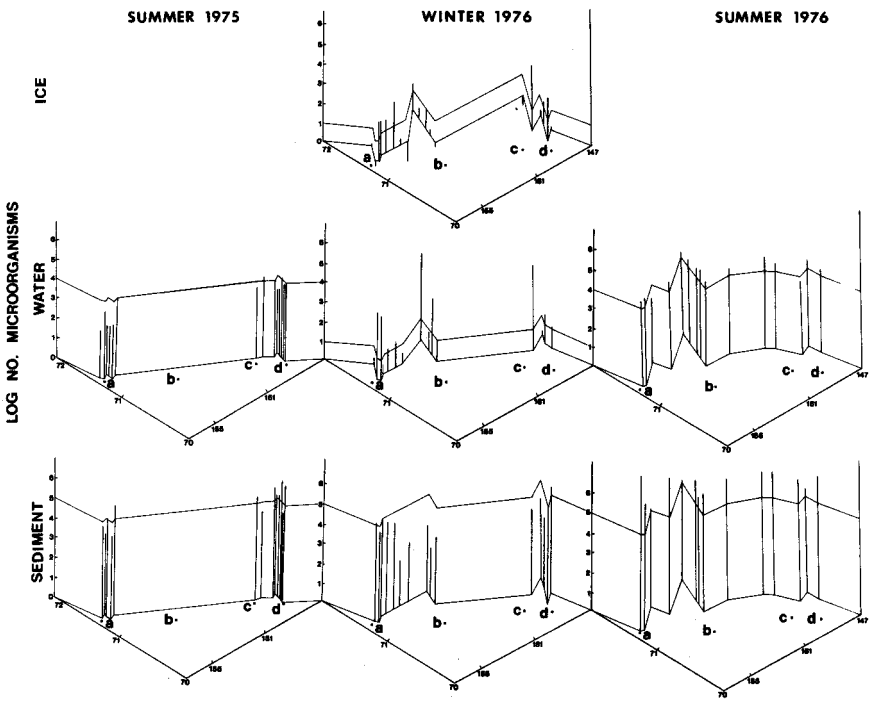

FIG. 4. Enumeration of heterotrophic microbial population at 20°C. (Mesophilic, including psychrotrophic populations). Refer to legend Figure 2 and text for interpreting figure.

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 near the 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 concentrations of total numbers of micro-organisms were several orders of magnitude higher in sediment than water. In sediment, direct counts were significantly higher during winter 1976 (avg. 3.2×10^8), than the previous summer (avg. 6.2×10^7) and increased further during summer 1976 (avg. 2.1×10^9). In contrast, total numbers of micro-organisms were lower in surface waters during winter 1976 (avg. 1.8×10^5) than the previous summer (avg. 8.2×10^5), and rose again during summer 1976 (avg. 5.2×10^5). Total numbers of micro-organisms were not significantly lower in sea ice than in underlying water. The direct counts did not show significant patterns of spacial variation.

In contrast to the total counts, viable heterotrophs in sediment did not increase during winter 1976 compared to the previous summer; populations enumerated at 4°C were not significantly different between summer 1975 and winter 1976 (Fig. 2), and populations enumerated at 20°C were significantly lower during winter (Fig. 3). Viable counts in both water and sediment were significantly higher in summer 1976 than the previous winter or summer. In surface waters, viable counts were lower during winter than summer. Viable

counts were lower in sea ice than in underlying water. The only pattern of geographic variation was found in water during summer 1975 when viable counts near Prudhoe Bay were significantly higher than near Pt. Barrow.

Relation Between Abiotic Parameters and Microbial Populations

There was no significant correlation in any season between numbers of viable or total micro-organisms and temperature nor between numbers of viable or total micro-organisms and concentrations of inorganic nitrogen. During summer, however, in surface water there was an inverse relationship between numbers of viable micro-organisms enumerated at 4°C and salinity (Fig. 5) and also between numbers of viable micro-organisms enumerated at

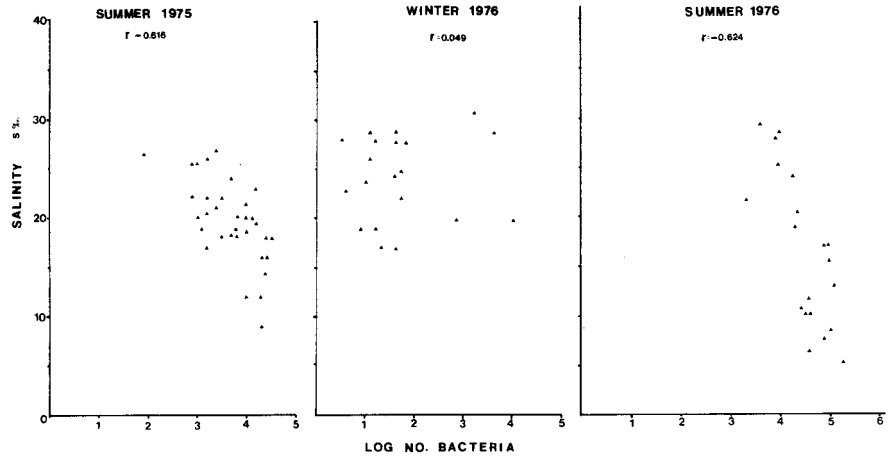

FIG. 5. Relationship of salinity to number of viable micro-organisms enumerated at 4°C.

4°C and phosphate concentrations (data not presented). During winter, there was no correlation between either salinity or phosphate concentration and numbers of viable micro-organisms. Enumerations by direct counts did not show a significant correlation with any of the abiotic parameters measured.

Almost all organisms isolated from the 20°C enumeration plates were psychrotrophs also capable of growth at 4°C (Table 2). A larger percentage of the populations enumerated at 4°C. from water and sediment were true psychrophiles during winter than during either summer. The percentages of psychrophiles in sediment were almost identical during both summers, but in surface water, a significantly higher percentage of the microbial populations was psychrophilic during summer 1976 than summer 1975. Average water temperatures were only 0.6°C lower in summer 1976 than summer 1975 and ice conditions were actually less severe in summer 1976. Surprisingly, the microbial populations of winter sea ice were mainly psychrotrophic with a lower percentage of true psychrophiles in the sea ice microbial populations than in the underlying water.

Testing of the micro-organisms isolated from summer samples showed that approximately 90% of water and 80% of sediment isolates required NaCl for growth in both summer and winter. During winter, 60% of the water and ice isolates required added NaCl, but almost 100% of the sediment isolates required added NaCl for growth. Due to the isolation procedures on marine agar 2216, these organisms are also capable of growth at salinity 30°/oo and pH 8.

TABLE 2. Growth Temperature Characteristics of Bacterial Populations

		Population Enumerated at 4°C			Population Enumerated at 20°C	
Season		No. of Isolates Tested	Psychrotrophs Growth at 4° & 20°C	Psychrophiles Growth at 4° But Not 20°C	No. of Isolates Tested	Psychrotrophs Growth at 4° & 20°C
Summer 1975	Water	121	93%	7%	132	98%
Summer 1975	Sediment	155	54%	46%	145	98%
Winter 1976	Ice	80	71%	29%	101	100%
Winter 1976	Water	151	44%	56%	100	99%
Winter 1976	Sediment	140	40%	60%	120	100%
Summer 1976	Water	225	63%	37%	0	-
Summer 1976	Sediment	150	56%	44%	0	-

DISCUSSION

Unlike the conclusion of Boyd and Boyd (1963), we did not find bacterial populations to be lower in the Beaufort Sea than in temperate oceans. In fact, viable bacterial populations in surface waters were several orders of magnitude higher than we find in the Gulf of Alaska (Hauxhurst, Kaneko and Atlas, unpublished data). Viable bacterial counts in surface waters were several orders of magnitude higher in the Beaufort Sea than have been reported for the Antarctic Ocean (Wiebe and Hendricks 1974). Viable bacterial populations in Beaufort Sea water and sediment were within the ranges reported by ZoBell (1946) for nearshore areas and higher than offshore areas. Direct counts in surface waters were also higher than we find in the Gulf of Alaska and were as high as have been reported for Atlantic Ocean water (Daley and Hobbie, 1975).

The inverse correlation between viable bacterial numbers and salinities in summer surface waters may suggest that these bacteria are of terrestrial origin as was concluded by Boyd and Boyd (1963). The fact that most of the isolates we tested, though, actually required sodium chloride for growth, suggests that most of the enumerated viable bacterial were probably not from terrestrial sources. A wide salinity tolerance range would be of adaptive advantage for bacteria in the Beaufort Sea since wide fluctuations in salinity occur.

The lower numbers of bacteria, especially viable bacteria, in surface waters during winter could be due to several factors including reduced growth rates at the lower temperature found in water, reduced growth rates or starvation during winter when nutrients from phytoplankton productivity are not available, or damage or exclusion from the water column by freezing out

during ice formation. Parts of our data could support each of these possibilities, but it is not possible from our data to make any definite conclusions.

It is noteworthy that most of the bacteria isolated from water during summer were psychrotrophs and not obligate psychrophiles. The ability to grow over a wide range of temperatures would be of adaptive advantage since summer water temperatures can reach 15°C in these regions (Atlas and Schofield, 1975). A higher percentage of the bacterial populations was psychrophilic in winter than in summer. Clearly the bacteria of the Beaufort Sea are adapted to growth at low temperature.

Sea ice appears to play an important role in determining bacterial population levels in the Beaufort Sea. During summer 1975, when ice conditions were severe, bacterial populations were lower than during summer 1976 when there was substantial movement of sea ice away from shore. Also during summer 1975, viable populations were higher near Prudhoe Bay where there was more open water than near Pt. Barrow where ice conditions were more severe. Morita *et al.* (1976) also reported similar spatial patterns for heterotrophic metabolic activities measured on the same samples.

Differences between total and viable counts may be due to the selectivity of plate count procedures. Some bacteria may require lower temperatures or lower concentrations of nutrients than used in the plate count procedures. It is also possible that many bacteria that are metabolically inactive or are incapable of dividing, are preserved for prolonged periods of time in cold Arctic ecosystems.

ACKNOWLEDGEMENTS

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