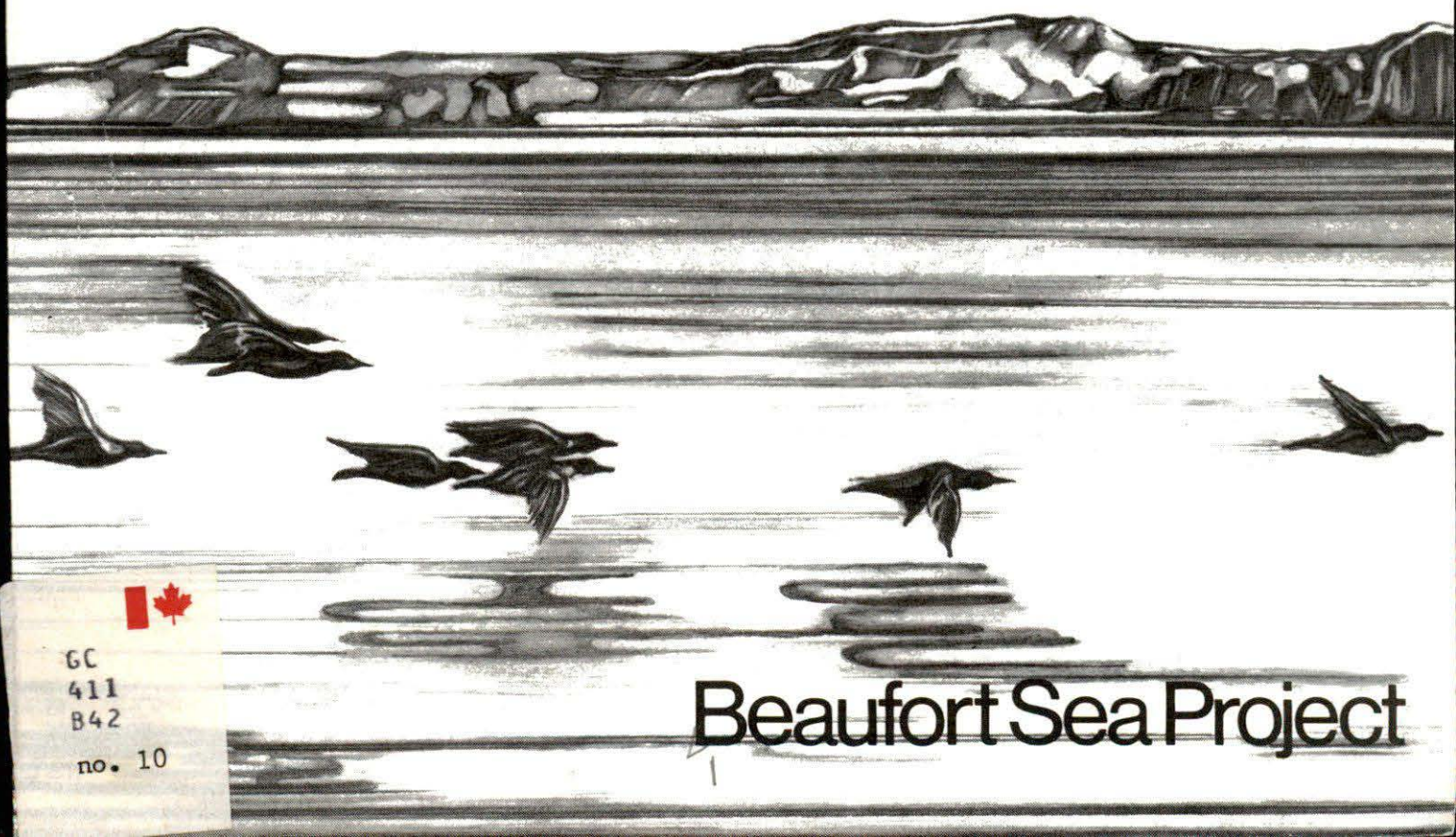
Biodegradation of Crude Petroleum by the Indigenous Microbial Flora of the Beaufort Sea

J.N. BUNCH and R.C. HARLAND

Technical Report No. 10



Beaufort Sea Project

BIODEGRADATION OF CRUDE PETROLEUM BY THE INDIGENOUS MICROBIAL FLORA OF THE BEAUFORT SEA

J. N. Bunch and R. C. Harland

Arctic Biological Station
Fisheries and Marine Service
Environment Canada
P.O. Box 400
Ste. Anne de Bellevue, P. Q.
H9X 3L6

Beaufort Sea Technical Report No. 10

Beaufort Sea Project
Dept. of the Environment
512 Federal Building
1230 Government St.
Victoria, B. C. V8W 1Y4

March 1976

TABLE OF CONTENTS

ACKNOWLEDGEMENTS

The authors would like to extend their deep appreciation to Ms. F. Pilkington and Ms. D. Michaud for their untiring efforts, enthusiasm and excellent work towards the realization of this project within the short time available. Thanks go to Mr. J. Lovrity and Mr. G. Martin for some of the field work. We are especially grateful to the staff of the Inuvik Research Laboratory of the Dept. of Indian and Northern Affairs in Inuvik, N.W.T. for the use of their facilities and, in particular, for their cooperation and invaluable support. The logistical support of the Polar Continental Shelf Project at Tuktoyaktuk, N.W.T. is appreciated.

1. SUMMARY

Despite unusually heavy ice conditions, microbiological samplings were conducted in the south Beaufort Sea during the summers of 1974 and 1975. One metre water from stations occupied during the open-water season produced total viable counts ranging from 1.0×10^6 to 3.0×10^7 CFU/litre of seawater in almost all instances. Incubation of replicate samples at different temperatures demonstrated that this heterotrophic flora was predominately psychrophilic.

Oleoclastic or petroleum-degrading microorganisms appeared to be ubiquitous to the waters and nearshore but not offshore sediments of this region and were relatively abundant when compared to ocean bodies at more southerly latitudes. Mixed cultures produced by an enrichment procedure demonstrated an ability to degrade Norman Wells crude at 0.0^0C. The presence of psychrophilic cell-types was evident within some mixed cultures in that optimum rates of degradation occurred at 20.0^0C or lower. The probability of biodegradation in the south Beaufort Sea is suggested by these data. However, the nutrient requirements for *in situ* biodegradation remain inconclusive as a result of time constraints.

A baseline study of heterotrophic turnover of dissolved organic material by bacteria in the Beaufort Sea has been undertaken by this laboratory. A natural extension of this study was to determine the effect of petroleum upon this activity. Mineralization of glutamic acid by the indigenous heterotrophic flora was generally unaffected, or in some instances, was enhanced by the presence of weathered or unweathered crude. This suggests that *in situ* activity of non-oleoclastic heterotrophs might not be affected by a moderate influx of petroleum in the Beaufort Sea ecosystem.

2. INTRODUCTION

During the summers of 1974 and 1975, microbiological samplings of the south Beaufort Sea were conducted by means of helicopter flights and cruises aboard the M.V. *Theta* and M.V. *Pandora*. During these field seasons an attempt was made to isolate bacterial floras which were capable of degrading crude petroleum as a source of carbon and energy. Previous work by this station established the abundance of heterotrophic bacteria in the south Beaufort Sea but the possibility of an oil-degrading potential within this flora remained to be determined.

Off-shore exploratory drilling for petroleum or the transportation of discovered petroleum pose the problem of accidental spillage into the south Beaufort ecosystem. Although an immediate physical clean-up may remove much of the spilled petroleum, past experience has demonstrated that a proportion will remain in the marine ecosystem or escape from clean-up efforts in an under-ice situation. The question remains as to whether this oil will become a chronic pollutant as has been suggested by some authors, or if a self-cleansing action via biodegradation will take place at the observed environmental temperatures.

The objectives of this study were to:

1. determine if a biodegradation potential exists in the south Beaufort Sea.

2. determine rates of degradation at various temperatures including 0^0C by various cultures isolated.

3. determine optimum temperatures for oil degradation and the requirements for nitrogen and phosphorus at optimal and sub-optimal temperatures.

4. determine, if possible, *in situ* rates of biodegradation.

3. RESUME OF CURRENT STATE OF KNOWLEDGE

With the increasing requirements of society for petroleum resources, emphasis has been focussed on the Canadian and American Arctic as potential sources of petroleum. Belatedly, it was realized that little information existed about the nature of the Arctic marine ecosystem, its interrelationships or its productivity. Problems encountered with oil spills in more temperate waters led to well-founded fears that oil spills in Arctic regions could lead to a major ecological disaster (Boyd, 1970).

The ability of microorganisms to degrade fractions of crude petroleum has been well documented over the last thirty years. The first comprehensive review of the subject was prepared by ZoBell in 1946. Much information has been gained in studies of biodegradation of petroleum in the marine environment, and recent reports have speculated on the possibility of similar biodegrading processes in the Arctic ecosystem.

The world's oceans contain a large indigenous flora of heterotrophic bacteria, that is microorganisms which mineralize organic material formed in the marine ecosystem. Within this heterotrophic flora, there usually exists a population of bacteria which is capable of utilizing hydrocarbons as a source of carbon and energy to form CO_2 and biomass. The size of this population of oleoclastic cells probably depends upon the amount of hydrocarbon substrate available. Therefore, in chronically oil-polluted waters, a much larger population of these cells would be evident than in an ecosystem free of oil contamination. Atlas and Bartha (1973), in fact, have suggested using the population size of oleoclastic bacteria as a sensitive indicator of oil pollution in a body of water. With an influx of hydrocarbons into a marine ecosystem, the population of oleoclastic bacteria increases, and the size of this population is one factor determining the rate of biodegradation of petroleum.

Besides the availability of oleoclastic bacteria, the rate of biodegradation of petroleum is also influenced by such factors as oxygen concentration, nutrient availability and temperature. ZoBell (1969) has calculated that 3 to 4 mg of oxygen are required for the complete oxidation of one mg of hydrocarbon. In a marine ecosystem, however, oxygen may not be a limiting factor except, perhaps,in sediments where petroleum residues might accumulate.

Various reports in the literature have noted that petroleum-enriched bacterial cultures must be supplemented with nitrogen and phosphorus to promote utilization of the petroleum. Bartha and Atlas (1973) found the nitrogen and phosphorus content of seawater to be severely limiting to the process of oil degradation in laboratory experiments. On the other hand, Kinney *et al.* (1969) reported that the concentrations of these nutrients in seawater were not limiting during *in situ* experiments in Cook Inlet, Alaska.

Petroleum biodegradation, like all biological processes, is dependent upon temperature. Ramseier (1973) advanced the possibility of spilled oil remaining in the Arctic Ocean for a long time since the process of biodegradation was not active at the environmental temperatures encountered. Glaeser (1971) reported the isolation of a bacterium, in the waters north of Point Barrow, which was capable of oil dispersion at elevated temperatures, but "no organisms were found which were able to utilize or disperse oils at summertime Arctic temperatures". On the other hand, Kinney *et al.* (1969) observed essentially complete biodegradation of petroleum at $5.0^{0}C$ within two months with water from Cook Inlet, Alaska. Similarly, bacteria obtained from oil-soaked tundra muck and oil-

contaminated waters from the region of Point Barrow, Alaska, were found by ZoBell (1973) to degrade mineral oil at temperatures as low as -1.0°C. After experiments with winter samples of the indigenous marine microflora of the New Jersey coastline, Atlas and Bartha (1972) have suggested that the biodegradation of petroleum in marine waters will be appreciably reduced at low temperatures. In an Arctic marine ecosystem, however, where low temperatures are prevalent throughout the year, the response of an indigenous flora of bacterial heterotrophs to the presence of petroleum would be dependent on the abundance and diversity of this flora and above all its metabolic activity at normally low temperatures. The present study was initiated to determine the petroleum biodegradation potential of the heterotrophic flora of the south Beaufort Sea at low temperatures.

Prior to work undertaken by this laboratory, no information concerning the bacterial flora of the south Beaufort Sea was available. Kriss (1963) described the quantitative distribution of heterotrophic bacteria in the north Beaufort Sea from spatial and seasonal samplings conducted from floating ice stations in 1955-1956. Although a limited number of bacteria were found in water columns during July and September, no bacteria were recovered in the months of April or May and led Kriss to conclude that microbial activity occurred only during the period of intense light in the summer months. The techniques employed to obtain this information, however, did not discern the psychrophilic, or cold-loving populations of cells in these waters and a true estimate of the microbial flora was not obtained. More recently, a report by Robertson *et al.* (1973) of samplings in the waters adjacent to Point Barrow, Alaska, indicated the existence of a heterotrophic flora, but at a low level of activity.

During a cruise conducted by this station in the summer of 1973, the abundance and distribution of heterotrophic bacteria were determined (Bunch, 1974) and were found to be similar to levels observed in more temperate regions. A majority of the cultures characterized from this cruise were found to be psychrophilic. More recently, measurements of the activity of this flora have been undertaken by determining rates of assimilation and/or mineralization of glutamic acid. A comprehensive review of this technique is provided by Wright (1974). As an extension to this study, the effect of petroleum upon glutamate mineralization by the indigenous flora was recorded. These data represent initial observations of the effect of petroleum upon natural microbial processes in a marine ecosystem.

4. METHODS AND SOURCES OF DATA

4.1 Total Viable Counts of Heterotrophs

4.1.1 Plating media

a) ZoBell Marine Broth 2216E (Difco) was prepared

according to the instructions of the manufacturer. The medium was dissolved in deionized water and solidified with 1.2% Bacto-agar (Difco).

b) A modified formulation of the lib X agar medium of Griffiths, Hanus and Morita (1974) was prepared in the following manner: 2.3 g trypticase (Baltimore Biological Laboratories), 1.2 g Bacto-yeast extract (Difco), 7.69 g Tris buffer (Trizma-7.2; Sigma Chemical Co.), 0.3 g sodium citrate, 0.3 g L-glutamic acid, 0.05 g sodium nitrate, 0.001 g ferric chloride, and 12.0 g Bacto-agar (Difco) were dissolved in 1.0 litre of 17$^{o}/_{oo}$ Instant Ocean (Aquarium Systems, Inc.). The medium was autoclaved, cooled and dispensed in petri dishes. Final pH at 5.0^{0}C was 7.8.

c) Plate Count Broth (Difco) was prepared according to the instructions of the manufacturer. The medium was dissolved in deionized water and solidified with 1.2% Bacto-agar (Difco).

4.1.2 Sampling and plating procedure

Water samples from various depths at occupied stations (see Figure 1) were collected aseptically with Niskin SS 1.5 sterile bag-samplers prior to disturbances of the water column by other oceanographic samplings. Water samples collected in this manner were aseptically transferred to cold, sterile, polypropylene bottles (one litre) and were processed within four hours of collection from the water column. A spin-plate technique was employed to dispense an aliquot of water sample with a cold pipette on the surface of a cold agar plate which was then placed in a 5.0^{0}C incubator, unless otherwise stated. Upon absorption of the aliquot of water by the agar medium, the plate was inverted and incubation was continued at 5.0^{0}C for three weeks. Quadruplicate spin-plates were made of each water sample. After incubation, the plates were examined and those with an uneven distribution of colonies were discarded. The colonies of three plates of a replicate set were enumerated, averaged, and the mean value was expressed as a log number of colony-forming units (CFU) per one litre of water sample.

4.2 Chemical Analyses of Seawater

Analyses were performed according to the procedures of Strickland and Parsons (1968). In some instances, data were obtained from Dr. E. H. Grainger.

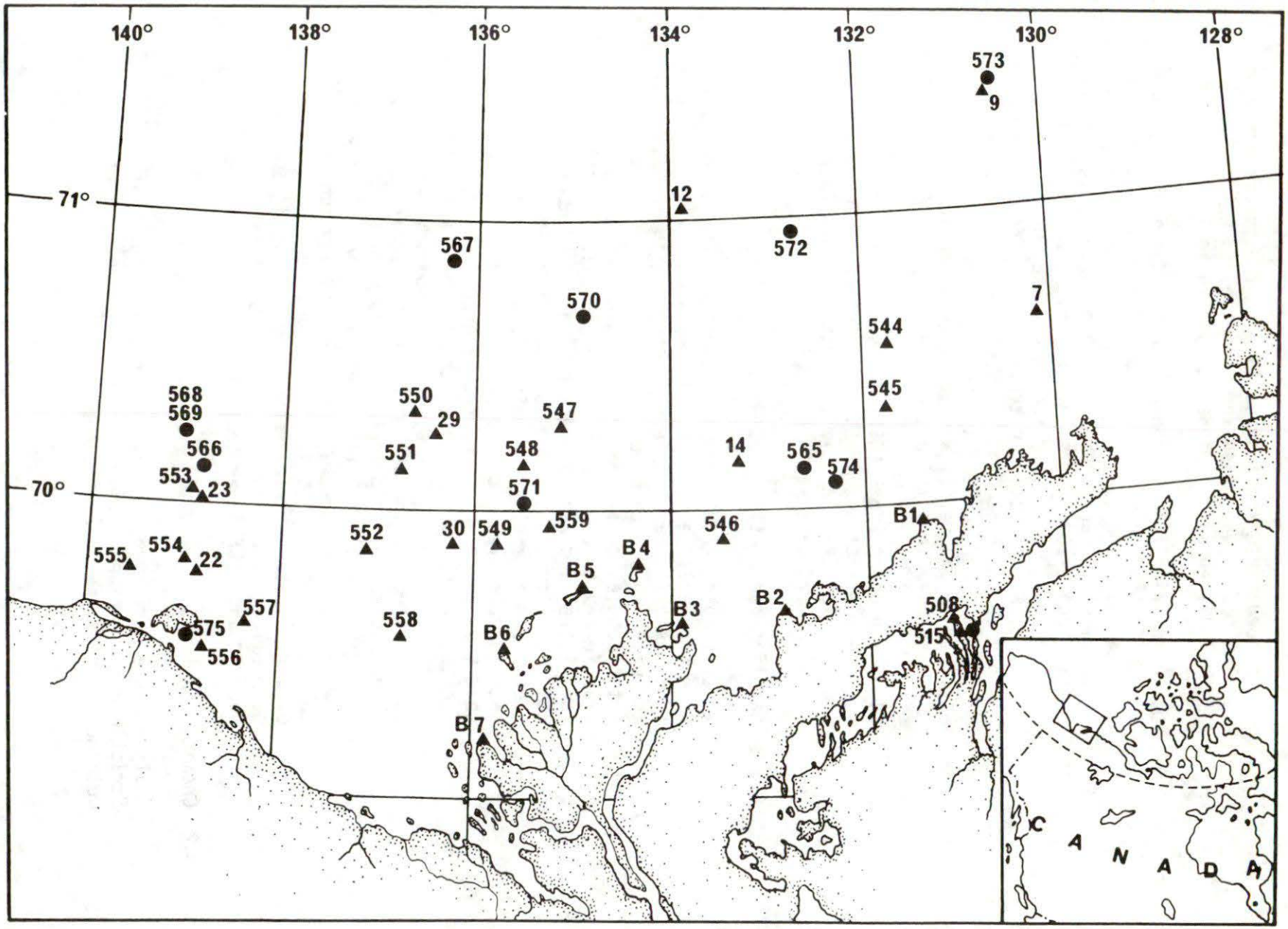


Figure 1. STATION OCCUPATIONS ▲ 1974
● 1975

4.3 Abundance of Oleoclasts

4.3.1 Medium

The defined seawater medium was prepared by adding 5.53 g NaCl, 2.54 g $MgCl_2 \cdot 6H_2O$, 0.1 g KCl, 0.37 g $CaCl_2 \cdot 2H_2O$, 7.69 g Tris buffer (Sigma), 1.0 g NH_4NO_3, 0.1 g K_2HPO_4 and 1.0 ml of chelated metals solution to 1.0 l of glass-distilled water. Final salinity was approximately $9^o/_{oo}$ and pH at 5.0^0C was 7.8. The chelated metals solution was prepared according to the formulation of Carlucci and Strickland (1968) and consisted of 0.004 g $CoCl_2 \cdot 6H_2O$, 0.004 g $CuSO_4 \cdot 5H_2O$, 1.0 g $FeCl_3 \cdot 6H_2O$, 0.3 g $ZnSO_4 \cdot 7H_2O$, 0.6 g $MnSO_4 \cdot H_2O$, 0.15 g $Na_2MoO_4 \cdot 2H_2O$ and 6.0 g EDTA dissolved in 1.0 l of glass-distilled water. The pH was adjusted to 7.5 at room temperature with NaOH.

4.3.2 Procedure

The abundance of oleoclasts was determined by the most probable number (MPN) procedure (American Public Health Association, 1971). Ten ml samples of seawater were added to 90 ml of defined seawater medium in screw-cap dilution bottles and ten-fold serial dilutions up to 10^{-8} were prepared in triplicate. The bottles were supplemented with 100 mg of sterile, weathered Norman Wells crude. Bottles were incubated at 15.0^0C for up to four months and scored at intervals for turbidity.

4.4 Determination of Heterotrophic Respiration

Water samples were collected as previously described and processed within four hours. Fifty ml aliquots of sample water were added to four chilled and sterile 125 ml serum bottles containing uniformly-labelled L-glutamic acid - ^{14}C (New England Nuclear). The final concentration of glutamic acid was 0.1 µmol with 10.0 µCi of activity per litre of seawater sample. The fourth bottle in a set was supplemented with 2.0 ml of 5N H_2SO_4 and served as a background control. A second set of four serum bottles prepared from the same sample water was supplemented, in addition, with 50 mg of sterile petroleum crude, weathered or unweathered. Bottles were stoppered with serum caps fitted with a plastic reaction well (Kontes Glass Co.). The wells, suspended above the seawater sample, contained two folded glass filters (Whatman GFA-24mm). The bottles were incubated at 5.0^0C for twelve hours after which the reaction in all vessels was stopped by the addition of 2.0 ml of 5N H_2SO_4 through the rubber cap by means of a syringe. At the same time, 0.2 ml of phenethylamine (New England Nuclear) was added through the cap into the

reaction well where it was completely absorbed by the glass filters. The bottles were further incubated for twelve hours at 40.0°C during which time $^{14}CO_2$ dissolved in the seawater was evolved and absorbed by the phenethylamine-soaked filters. The bottles were then opened and the filters were transferred to 10.0 ml Aquasol (New England Nuclear) in scintillation vials. The vials were transported to Ste. Anne de Bellevue where they were counted in a Nuclear-Chicago Isocap 300 scintillation counter. Quenching was corrected by the channel ratios method.

4.5 Oil Enrichment Broths

4.5.1 Water samples

Sterile, acid-washed one litre media bottles with teflon-lined screw-caps were prepared in the laboratory with 5.0 ml of a stock solution of salts and 500 mg of sterile, weathered Norman Wells crude. Upon dilution with a 500 ml water sample, the salts solution yielded a final concentration of 1.0 g NH_4NO_3, 0.1 g K_2HPO_4 and 7.69 g Trizma (Sigma Chemical Co.) per litre of water sample. Five hundred ml samples of seawater from various stations were aseptically transferred into the chilled media bottles which were incubated at 5.0°C until transferred to the Arctic Biological Station where incubation at 5.0°C was continued for four weeks at 100 rpm on a gyratory shaker.

4.5.2 Sediment samples

Ten ml aliquots of sediment from various stations were removed from grab samples with a modified syringe and transferred to chilled, one litre flasks containing 500 ml of half-strength artificial seawater (Instant Ocean) supplemented with 1.0 g NH_4NO_3, 0.1 g K_2HPO_4, 7.69 g Trizma and 1.0 g sterile, weathered Norman Wells crude per litre of seawater. Culture broths were then treated as above (4.5.1).

In some instances, sediment samples were transferred to a freshwater medium. This medium was prepared by dissolving 0.3 g $MgCl_2 \cdot 6H_2O$, 0.02 g $CaCl_2 \cdot 2H_2O$, 0.001 g $FeCl_3 \cdot 6H_2O$, 0.1 g K_2HPO_4, 1.0 g NH_4NO_3 and 7.69 g Tris buffer in one litre of glass-distilled water.

4.6 Petroleum Degradation Experiments

4.6.1 Media

For viable count determinations, lib X agar was

employed (see before). An oil-seawater broth was used for the maintenance of cultures in all experiments. Quarter-strength artificial seawater (salinity ca. $9^0/oo$), was prepared by dissolving a commercial sea salt mixture (Instant Ocean) in distilled water. The quarter-strength seawater was supplemented with 1.0 g NH_4NO_3, 0.1 g K_2HPO_4 and 7.69 g Trizma per litre unless otherwise indicated, dispensed in 200 ml aliquots in 500 ml Erlenmeyer flasks and autoclaved. After cooling, the medium was supplemented with 200 mg of filter-sterilized, weathered Norman Wells crude. Final pH at 5.0^0C was 7.80. In some experiments the defined seawater medium described previously was employed (4.3.1). The crude petroleum used in these experiments was weathered under a forced air draft for ten days in 150 mm glass petri dishes. After weathering, the residual crude was filter-sterilized with 0.45 µ membrane filters (Millipore) and stored at 5.0^0C in glass vials with teflon-lined caps until required.

4.6.2 Inoculation and protocol

Enriched oil cultures obtained from the Beaufort were used in most experiments. These mixed cultures were maintained by subculturing into fresh oil-seawater broths, and incubating at 5.0^0C on a gyratory shaker at 100 rpm. Two ml of a six-day culture were employed as an inoculum for experiments. In most experiments, experimental media were inoculated and an uninoculated medium served as the oil control. A second flask was inoculated but was not supplemented with petroleum and served as a substrate-free control. Experimental flasks and their respective controls were incubated in an identical fashion and usual experiments consisted of between 40 and 70 flasks. The temperature of incubation varied with experiments but all flasks were agitated during incubation on gyratory shakers at 100 rpm. Experimental flasks were removed at intervals together with control flasks for determination of viable count and subsequent extraction of residual petroleum.

4.6.3 Viable counts

In most experiments, viable counts of all experimental cultures were determined. A one ml aliquot of a culture was serially diluted to 10^{-7} in ten-fold dilutions. A spin-plate technique was employed to inoculate 0.1 ml of each dilution on triplicate plates of lib X. Plates were incubated at 15.0^0C for seven days and the plates prepared from one dilution showing statistically significant growth were chosen for enumeration. Plates of a replicate set were enumerated, averaged, and the

mean value was expressed as the log number of colony-forming units (CFU) per one ml of culture.

4.6.4 Extraction of residual petroleum from cultures

Extraction of residual petroleum from culture and control vessels was accomplished with three 20 ml aliquots of n-pentane in a 500 ml separatory funnel. The n-pentane-oil fraction partitioned above the aqueous layer was removed each time with a pipette. The combined fractions were added to a tared 100 ml beaker and evaporated to dryness at room temperature. When no further weight loss was noted, the weight of oil was recorded and a 40.0 µg/µl solution of the oil was prepared with n-hexane. This solution was stored in teflon-capped glass vials until analysed.

4.6.5 Gas chromatographic analysis of extracted petroleum

A Hewlett-Packard model 5711A gas chromatograph was used for analysis of the oil samples. A 1.0 µl sample of the extract-hexane solution was injected into a 10.0 foot stainless steel column (0.125 in O.D.) packed with 3.0% OV-1 on 100-120 mesh Chromasorb P (Chromatographic Specialties Ltd.). Duel flame ionization detectors were supplied with an air-flow rate of 240 ml/min at 25 psi and a hydrogen-flow rate of 39 ml/min at 16 psi. Nitrogen carrier gas was regulated to 51 ml/min at 70 psi. Oven temperature was programmed for an initial temperature of 100.0^{0}C and increased at a rate of 8.0^0/min to 320.0^{0}C where it was held for four minutes. The readout, attenuated X160, was made on a single-pen Fisher recorder with a chart-speed of 0.5 in/min. Relative losses in the aliphatic fraction were calculated by measuring the area under the GC profile peaks of the 14C, 15C, 16C, 19C, 20C, 21C and 22C saturated hydrocarbons with a planimeter. Peaks were identified by comparison with known standards. These well-defined peaks were considered to be representative of the total profile of the aliphatic fraction after weathering and errors introduced by measuring poorly-defined peaks were avoided. The values of the summed peak areas were expressed as a percentage of the values obtained from the profiles of oil-control vessels incubated in an identical fashion but with inocula deleted. In the preparation of graphs with the values obtained, the dispersion of points on some curves was usually due to the extraction of single experimental vessels. The rapid screening of many cultures did not allow the use of replicate flasks and occasionally single cultures did not respond to culturing conditions.

5. RESULTS

5.1 Total Viable Counts of Heterotrophs

The viable counts of heterotrophs obtained from stations occupied in the Beaufort Sea during 1974 and 1975 are presented in the Appendix. Where available, the corresponding values of salinity, temperature, chlorophyll A, nitrate and phosphate for the same sample waters are also given.

Marine Agar 2216E was employed for the cultivation of marine microorganisms in 1974. This formulation was replaced in the 1975 season by a modified formulation of lib X Agar -- a medium possessing superior physical characteristics for the enumeration of colonies. Both media were found to have similar properties of cultivation in a comparison study in the 1974 season and yielded similar counts from replicate samples of water. Plate Count Agar (PCA) was the medium employed for the cultivation of freshwater microorganisms. The growth on solid media of colony-forming units (CFU) from water samples are an indication of the relative abundance of heterotrophic microorganisms in sampled waters. However, the counts do not reflect absolute numbers of heterotrophic microorganisms but rather that population of cells or clumps of cells capable of growth on a particular cultivation medium at 5.0^{0}C. Surface waters with low salinity values due to melt water from ice or river discharge yielded high counts on both marine and freshwater media. In waters of higher salinity, the predominate marine microbial flora produced counts on marine media which were generally an order of magnitude higher than counts obtained on PCA. The counts obtained on marine media from stations occupied in 1974 and the four stations occupied early in 1975 were generally similar and ranged between approximately 3×10^{6} and 3×10^{7} CFU/litre of seawater from a depth of 1.0 metre. Counts obtained from open-water stations occupied later in 1975 were lower, particularly Stations 572 and 573. The corresponding values for chlorophyll A and nutrients in the same waters were low and suggested a low level of primary productivity.

The low *in situ* temperatures of the Beaufort Sea suggested that the heterotrophic flora of bacteria should be predominately psychrophilic, i.e. requiring lower temperatures for growth and multiplication. This was demonstrated at stations occupied in 1974. Replicate plates of marine agar inoculated with seawater were incubated at 5.0, 15.0, 22.0 and 30.0^{0}C. The results for Station 22 are seen in Figure 2. The counts obtained from one meter water after incubation at 5.0 and 15.0^{0}C were approximately 30 times higher than those obtained at 22.0^{0}C. Similar results were obtained at other stations and suggest that the predominate flora of heterotrophic cells occurring after break-up of the ice-cover are psychrophilic in their response to temperature.

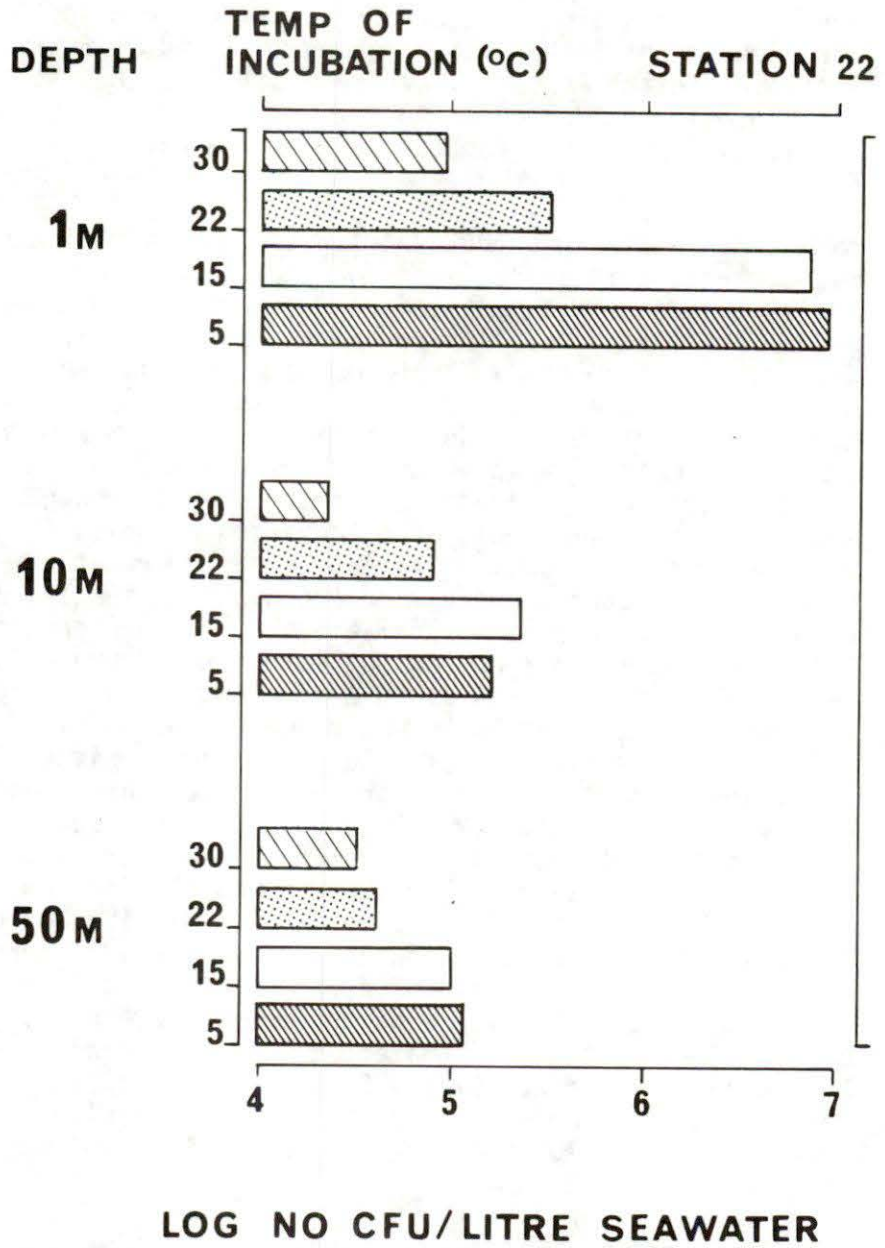


Figure 2. Counts obtained on replicate plates inoculated with seawater and incubated at different temperatures. Seawater samples from 1.0, 10.0 and 50.0 metre depths at station 22 (1974) were inoculated on Marine Agar and incubated at 5.0, 15.0, 22.0, and 30.0°C. The counts obtained are expressed as $\log_{10}$ CFU/litre of seawater sample.

5.2 Abundance of Oleoclasts

The abundance of oleoclasts or oil-degrading bacteria at Stations 565, 566 and 567 was determined by the most probable number (MPN) technique. It is unfortunate that more stations could not be quantitated for oleoclasts but the large number of bottles required for incubation aboard M.V. *Pandora* precluded this possibility. Bottles were observed at intervals across four months of incubation at 15.0°C but no change was noted after six weeks. The results are seen in Table 1 where maximum possible values are given. The total viable counts of heterotrophs cultivated on lib X Agar at 5.0°C from the same waters are listed from the Appendix for comparison. In the waters sampled, oleoclasts ranged from 2.3×10^2 to 9.3×10^4 cells/litre, and represented 0.001% to 1.63% of the total heterotrophic flora.

5.3 Effect of Petroleum on the Respiration of the Indigenous Flora of the Beaufort Sea

As part of a larger study of the heterotrophic potential of the indigenous microbial flora of the Beaufort Sea, the effect of petroleum upon bacterial respiration of uniformly-labelled glutamic acid-^{14}C was determined. Fifty ml aliquots of seawater, containing 0.1 µmol glutamic acid-^{14}C per litre with an activity of 10.0 µCi/l, were incubated at 5.0°C for twelve hours in the presence and absence of fresh and weathered Norman Wells crude. Other experiments (not reported here) have demonstrated that the seawater flora is not altered by this concentration of glutamate. After incubation, respired CO_2 present in the vessels was trapped and its radioactivity was determined. The results are presented in Table 2. Total viable counts of the waters sampled are included from the Appendix for purposes of comparison.

The amount of organic substrate incorporated by the flora in this type of experiment is dependent on the population size. Incorporation is also affected by the suitability of the substrate for a given mixed bacterial population. Other experiments have shown that glutamic acid is readily incorporated by mixed bacterial populations and approximately 30.0 - 42.0% of incorporated glutamate is respired as CO_2.

In the present experiments, the amount of respired CO_2 (column A) was generally determined by the size of the bacterial population (TVC). Weathered Norman Wells crude had little effect on CO_2 production in water samples from Stations 566, 568 and 575. The results indicate that heterotrophic utilization of glutamate was unaffected. The presence of weathered crude doubled CO_2 production from glutamate in water samples from Station 567 and a 28% increase was noted in water samples from Station 574. Similarly, fresh crude had little effect at Stations 569 and 570 and enhanced glutamate respiration at Station 574 but not to the same degree as weathered crude.

TABLE 1

Abundance of oleoclasts determined by the MPN technique. Bottles were incubated at 15.0°C, and the results, observed after six weeks, are expressed as numbers of oleoclastic cells per litre. Total viable count (TVC), expressed as total colony-forming units per litre, were enumerated on Lib X Agar after three weeks incubation at 5.0°C (see Appendix).

Station	$\frac{\text{Oleoclasts}}{\text{litre}}$	$\frac{\text{TVC}}{\text{litre}}$	$\frac{\text{Oleoclasts}}{\text{TVC}}_{\%}$
565	2.3×10^4	5.4×10^6	0.43
566	2.3×10^2	2.0×10^7	0.001
567	9.3×10^4	5.7×10^6	1.63

TABLE 2

Specific activity of radiolabelled CO_2 produced by the indigenous flora of seawater samples in the presence and absence of fresh and weathered Norman Wells crude. All seawater samples were taken from a depth of one metre and incubated for approximately twelve hours at 5.0^0C with uniformly-labelled glutamic acid-^{14}C. After stopping the reaction with 5N H_2SO_4, the total radiolabelled CO_2 produced by a 50 ml reaction volume of seawater was absorbed on phenethylamine-soaked wicks. Actual disintegrations per minute (DPM) of CO_2 were calculated by the channel ratios method. Total viable count (TVC), expressed as total colony-forming units per litre of seawater sample, were enumerated on lib X Agar after three weeks incubation at 5.0^0C (see Appendix).

Station	Respired CO_2 (DPM) A	Respired CO_2+ fresh crude (DPM) B	$\frac{B}{A}$%	Respired CO_2+ weathered crude (DPM) C	$\frac{C}{A}$%	$\frac{TVC}{litre}$
566	141,138	---	---	133,730	95	2.0×10^7
567	56,692	---	---	116,280	205	5.8×10^6
568	145,621	---	---	141,041	97	1.0×10^7
569	57,806	56,879	98	---	---	2.3×10^6
570	54,170	50,052	92	---	---	4.5×10^6
574	25,341	27,975	110	32,560	128	---
575	48,500	31,168	64	46,398	96	2.7×10^6

Whereas weathered crude did not affect glutamate respiration at Station 575, fresh crude reduced it by 36%, the only significant instance of inhibition of glutamate respiration by crude petroleum.

5.4 Oil Enrichment Broths

5.4.1 Water samples

Oil enrichment broths prepared with water samples from a depth of one metre at Stations 544 to 559 inclusive (see Figure 1) were subcultured twice and each subculturing was incubated at 5.0°C with agitation for four weeks. To obtain initial estimates of degradation, the residual oil from the first subculture of each of the sixteen stations was extracted and analysed by gas chromatography. Relatively complete degradation of the saturated aliphatic fraction was observed in all sixteen extractions. A similar procedure demonstrated relatively complete degradation of the aliphatic fraction in enriched cultures from Stations 567 and 574 (see 5.5.2).

5.4.2 Sediment samples

Sediment samples were submitted to the same procedure as water samples. Sediment samples from offshore stations (Stations 545, 546, 555, 556, 557, 558 and 559) were essentially devoid of oleoclastic cells. Only sediment from Station 546 showed any degree of degradation and this was very limited.

All sediment samples obtained from nearshore stations (Stations B1 to B7 inclusive) demonstrated relatively complete degradation of the saturated aliphatic fraction.

5.5 Parameters Affecting Biodegradation of Petroleum

5.5.1 Temperature

All enriched cultures demonstrated degradation at 5.0°C. However, it was expected that the different enriched cultures, containing a wide variety of cell-types, would demonstrate various temperature optima for petroleum degradation. To ascertain the effect of varying temperature on degradation by enriched cultures, cultures 546, 557, B7 and F1, isolated at one metre from Stations 546 and 557 and from inshore sediment at Stations B7 and B1, respectively, were employed. The results are seen in Figures 3 to 9.

Because of the enrichment procedure at 5.0°C, it was anticipated that psychrophilic oleoclasts would be retained in the mixed cultures and that optimum

degradation would therefore occur at lower temperatures. This was demonstrated in two of the four cultures evaluated.

Four GC profiles used to determine degradation of petroleum crude at 5.0°C by culture 546 are given in Figure 3. It should be noted that all components of the saturated aliphatic fraction, including the branched alkanes pristane and phytane, were essentially absent from the petroleum residue after twenty days. Culture 546 appeared to degrade petroleum faster at 20.0° than at 30.0°C and degradation at 10.0° and 5.0°C was also rapid (Figure 4). Degradation occurred at 0°C but was preceded by a lag. The increase in the population of cells in these cultures indicated that multiplication was taking place at the expense of the petroleum substrate (Figure 5).

The relative maximum cell yields at the various temperatures confirmed the psychrophilic nature of culture 546. At 30.0°C, the viability of the culture began to decrease after three days and greater cell yields were observed at 20.0°, 10.0° and 5.0°C. A lag before multiplication was evident at 0°C and corresponded to the absence of degradation during this time.

In contrast to culture 546, culture 557 demonstrated a continuous increase in the rate of degradation with increasing temperature up to and including 30.0°C (Figure 6). At 0°C, a noticeable lag occurred before degradation commenced but no appreciable lag was observed at the higher temperatures. The effect of temperature upon cell multiplication corresponded to the observed degradation rates and the greatest cell yield was at 30.0°C (Figure 7).

Culture B7 (Figure 8) appeared to be psychrophilic in its response to temperature in that degradation at 20.0° and 30.0°C was similar and degradation rates at 5.0° and 10.0°C, although preceded by lags, were almost as rapid as at 20.0° and 30.0°C. A lag preceded degradation at -1.0°C, similar to those seen with the previous two cultures.

Culture F1 (Figure 9) exhibited the least psychrophilic response to temperature of the four cultures evaluated. Degradation of the petroleum crude was essentially completed at 30.0°C within three days. Degradation at 0°C was preceded by a very long lag of approximately three weeks although degradation was rapid at this temperature once the lag was ended.

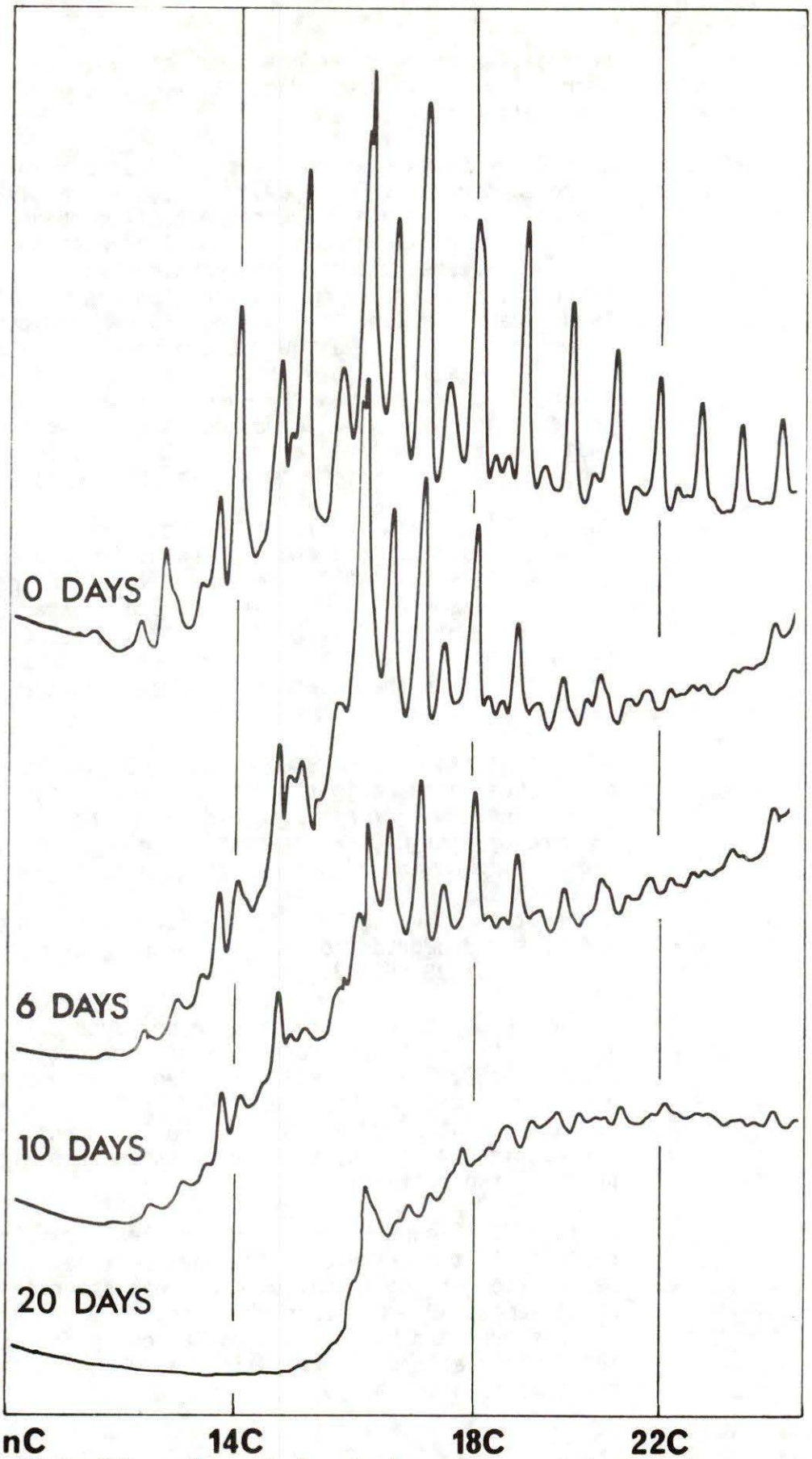


Figure 3. GC Profiles of residual petroleum extracted from four cultures incubated at 5.0°C for various intervals. Carbon numbers (nC) indicate peaks of several saturated hydrocarbons. Values calculated from these profiles were employed in the construction of the 5.0°C curve in Figure 4.

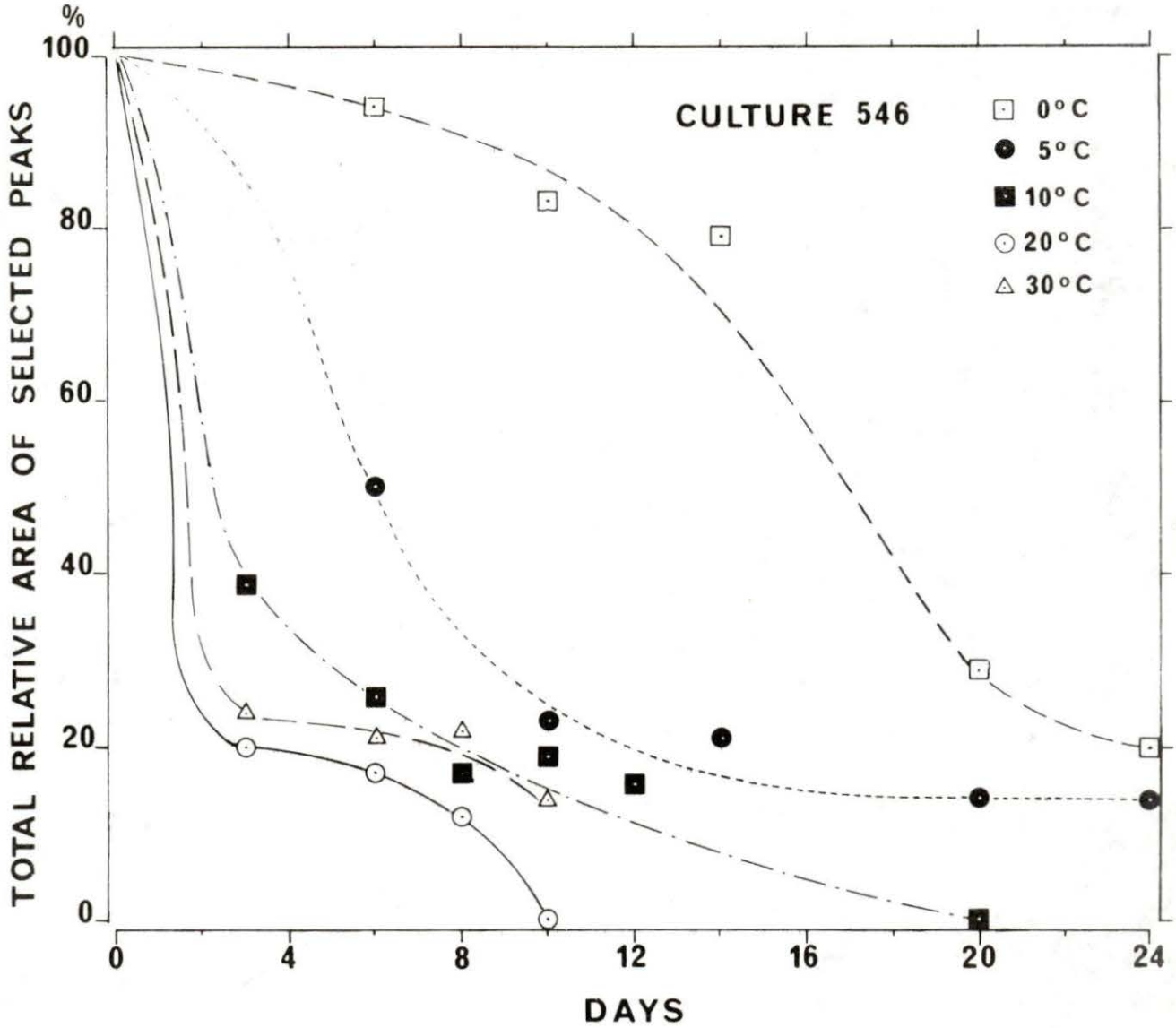


Figure 4. Degradation of weathered Norman Wells crude by culture 546 at defined temperatures. Cultures and oil controls were removed at intervals during incubation and residual petroleum was extracted from each. Summed areas of selected peaks of GC profiles were expressed as percentages of the summed areas of the same peaks in undegraded controls. Percentage values obtained are plotted against incubation interval when extracted.

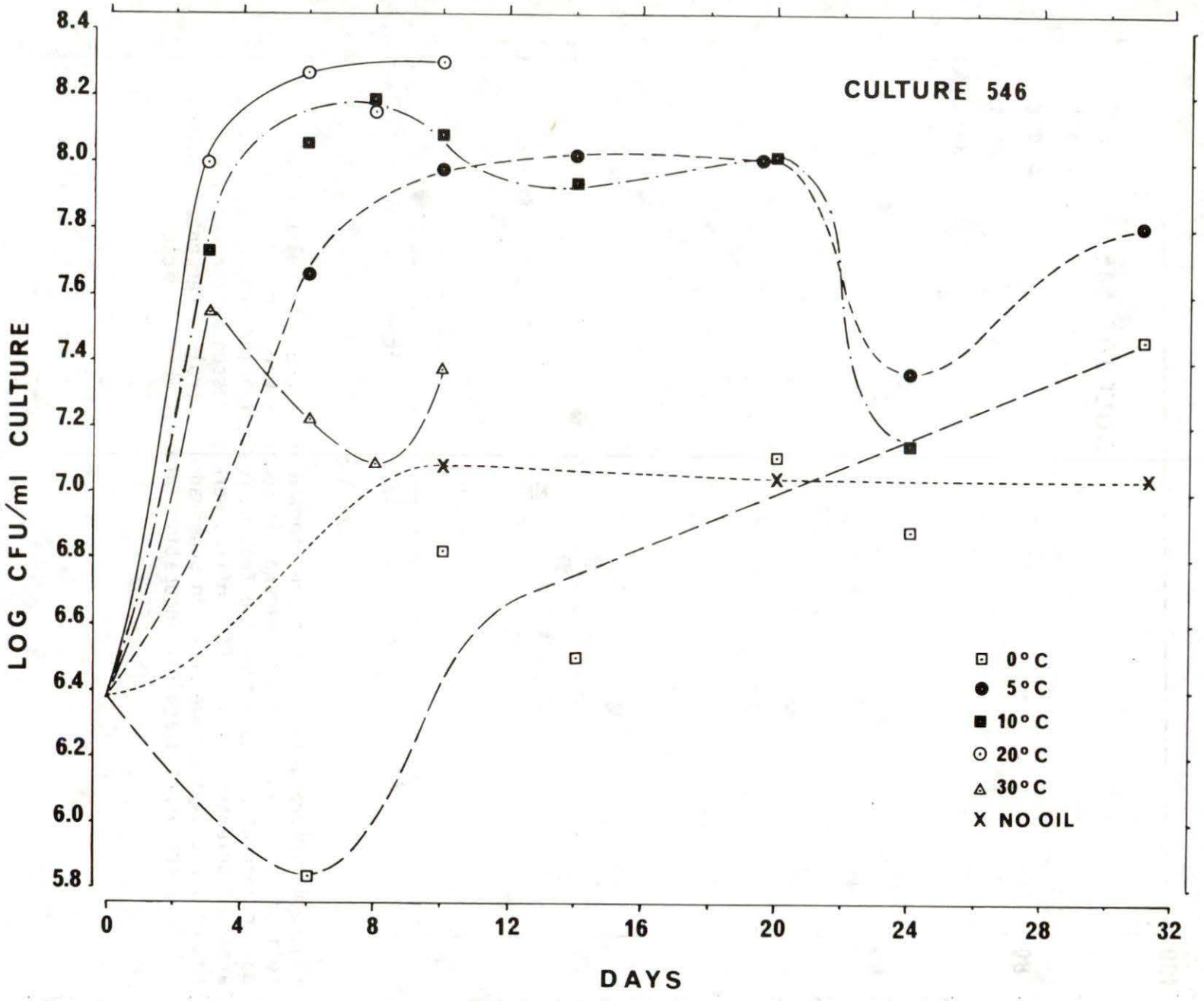


Figure 5. Increase in viable counts of oil and control cultures during degradation of weathered Norman Wells Crude by culture 546 at defined temperatures. Values of extracted petroleum from these cultures are plotted in Figure 4. Viable counts are expressed as log colony-forming units (CFU) per 1.0 ml of culture and are plotted against incubation interval when sampled.

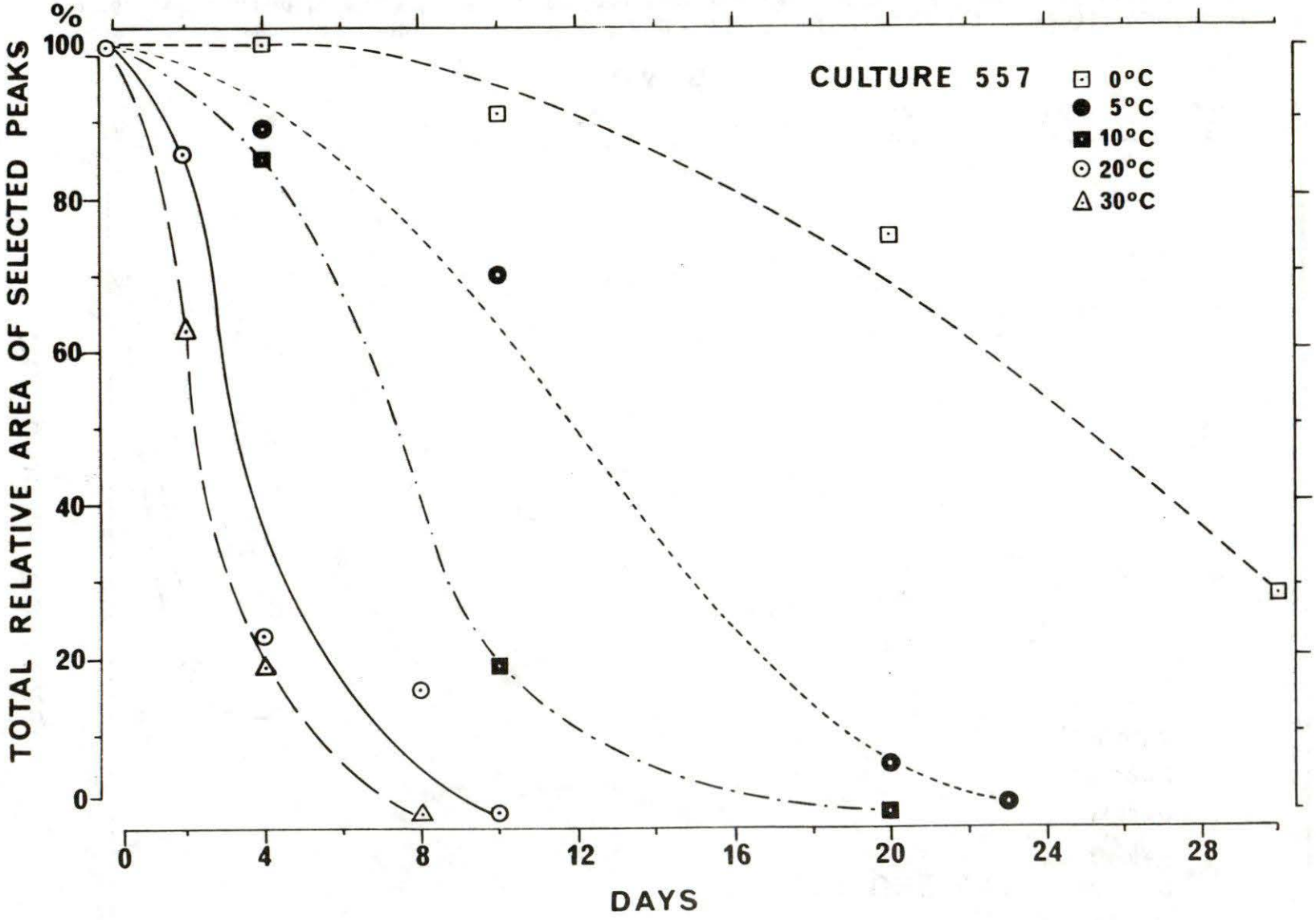


Figure 6. Degradation of weathered Norman Wells crude by culture 557 at defined temperatures. (See legend to Figure 4.)

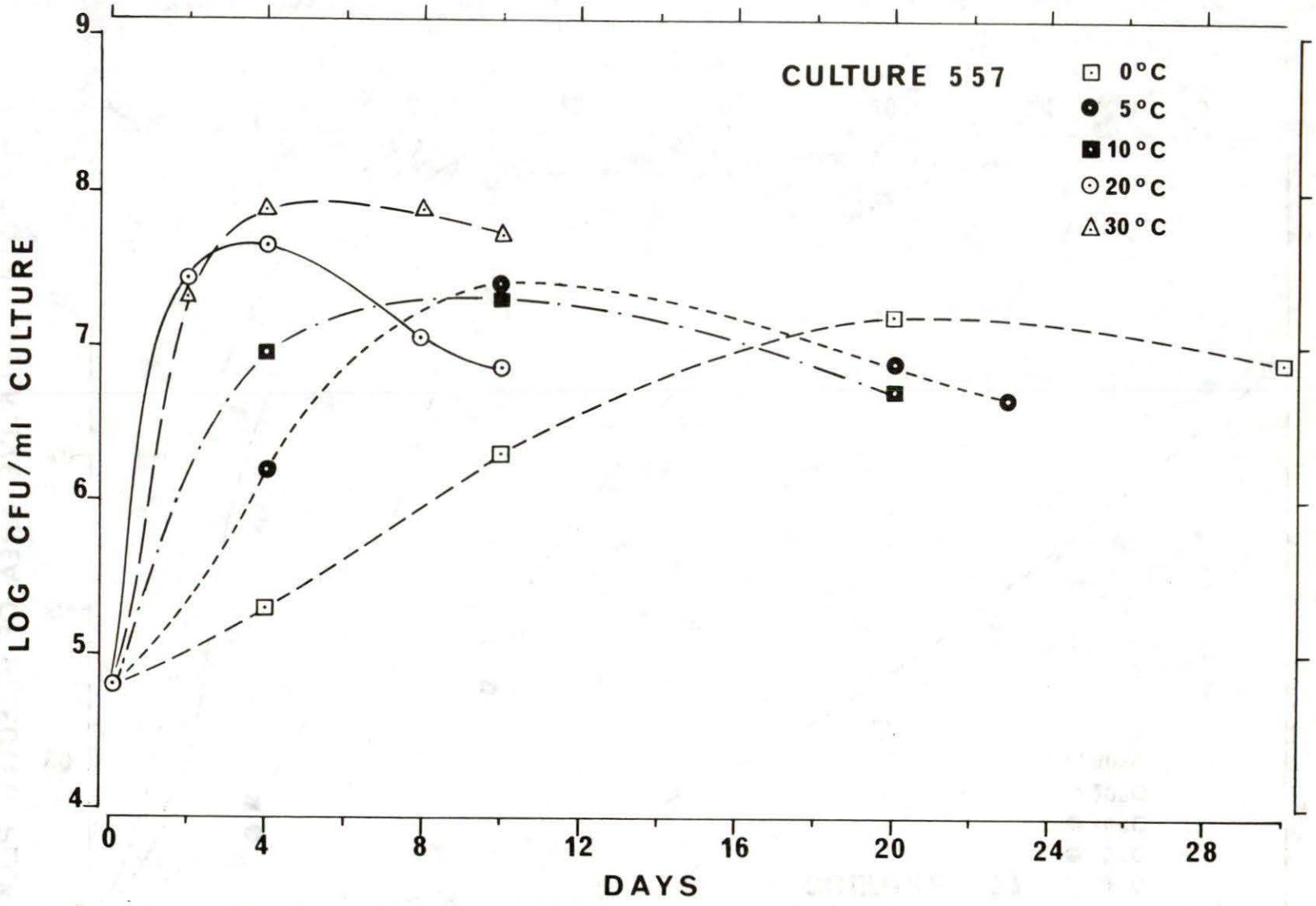


Figure 7. Increase in viable counts of oil and control cultures during degradation of weathered Norman Wells crude by culture 557 at defined temperatures. Values of extracted petroleum from these cultures are plotted in Figure 6. (See legend to Figure 5.)

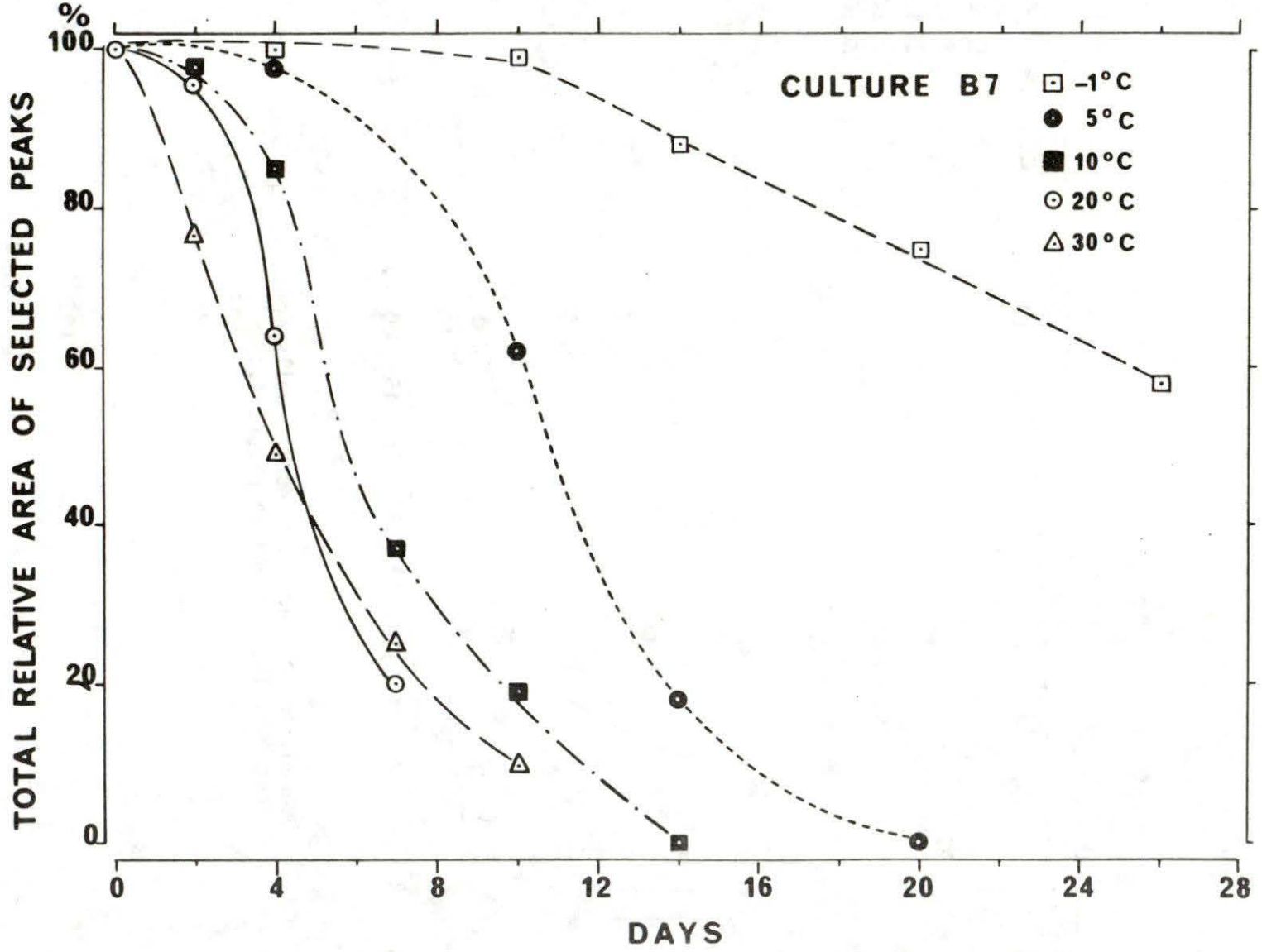


Figure 8. Degradation of weathered Norman Wells crude by culture B7 at defined temperatures. (See legend to Figure 4.)

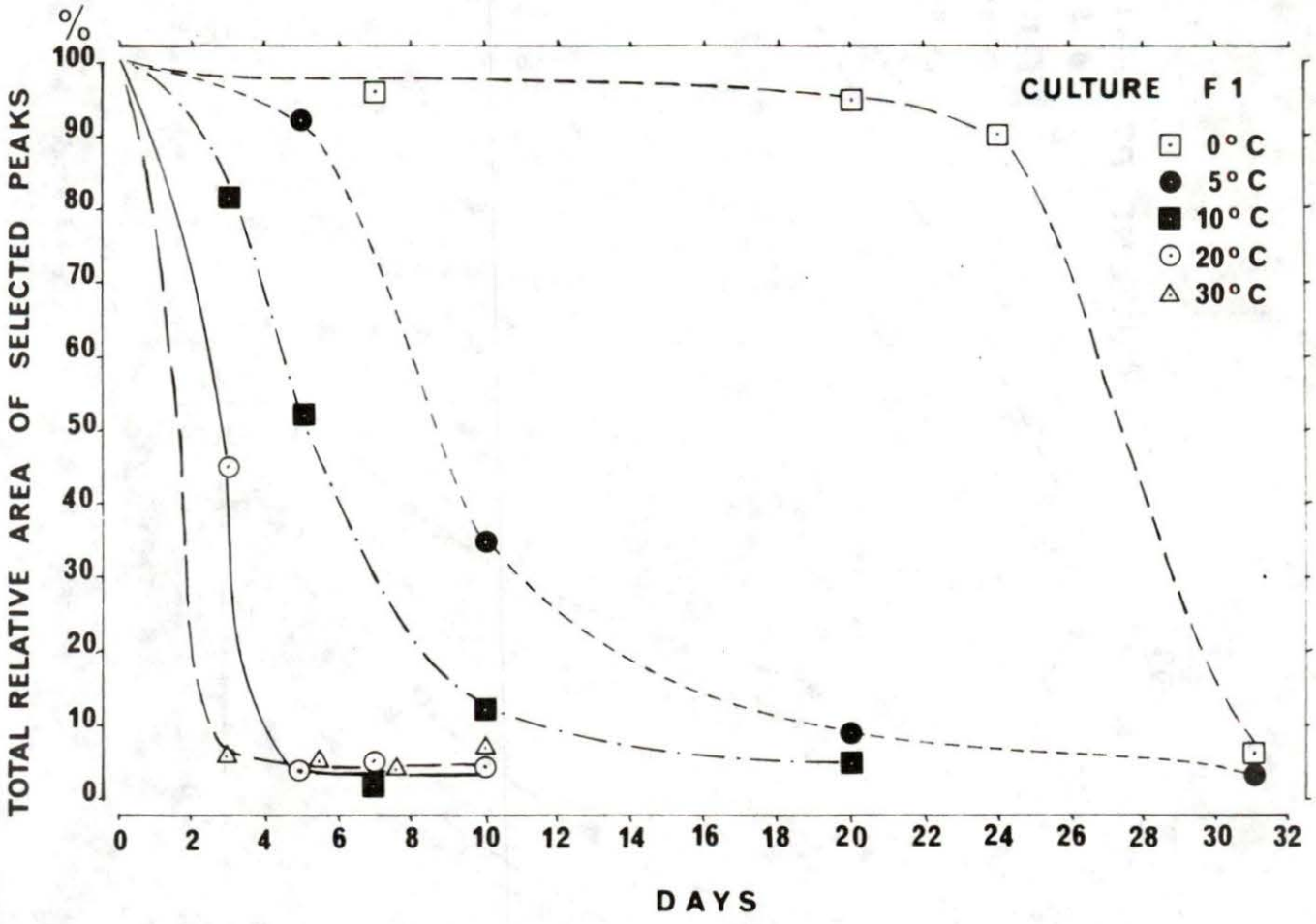


Figure 9. Degradation of weathered Norman Wells crude by culture F1 at defined temperatures. (See legend to Figure 4.)

5.5.2 Availability of oleoclasts in natural waters

The previously described measurements of petroleum degradation were initiated with heavy inocula (approximately 10^5/ml) of oleoclastic cultures obtained by an enrichment procedure. Consequently, the lag before measurable degradation commenced was usually of short duration. Since the number of oleoclasts in natural waters would be expected to be much lower, an experiment was designed to measure the initial lag before active degradation and the subsequent rate of degradation of petroleum by the indigenous flora of freshly-collected seawater samples.

Water was collected from one metre at Stations 567 and 574 and added in 500 ml aliquots to sterile one litre screw-cap bottles. The bottles contained 500 mg weathered Norman Wells crude in addition to 0.5 g NH_4NO_3, 0.05 g K_2HPO_4 and 3.85 g Tris buffer in solution. Control bottles had, in addition, sufficient $HgCl_2$ to form a 1.0% solution in the final volume of seawater added. The bottles were incubated with agitation at 5.0°C for up to 81 days. At intervals, duplicate bottles were removed, the residual oil was extracted, and the averaged value of the aliphatic fraction was expressed as a percent of the value of a control bottle incubated under similar conditions. The results are seen in Figure 10.

In both experiments, a lag of approximately four weeks occurred before a significant loss of the aliphatic fraction of the oil was observed. In previous experiments with large inocula of enriched cultures, measurable degradation was noted within a week at 5.0°C.

The apparent rate of degradation of petroleum in water from Station 567 was rapid once the lag ended and possibly a result of a large indigenous population of oleoclasts at this station. As shown in Table 1, of the stations sampled, the largest population of oleoclasts was found at Station 567. In both experiments, however, degradation of 85-90% of the aliphatic fraction had occurred by 11 to 12 weeks. Incubation at other temperatures was not attempted because of the large number of incubation vessels required.

5.5.3 Nutrient requirements for petroleum biodegradation

Petroleum biodegradation by bacteria, like other forms of biological oxidation, requires an input of

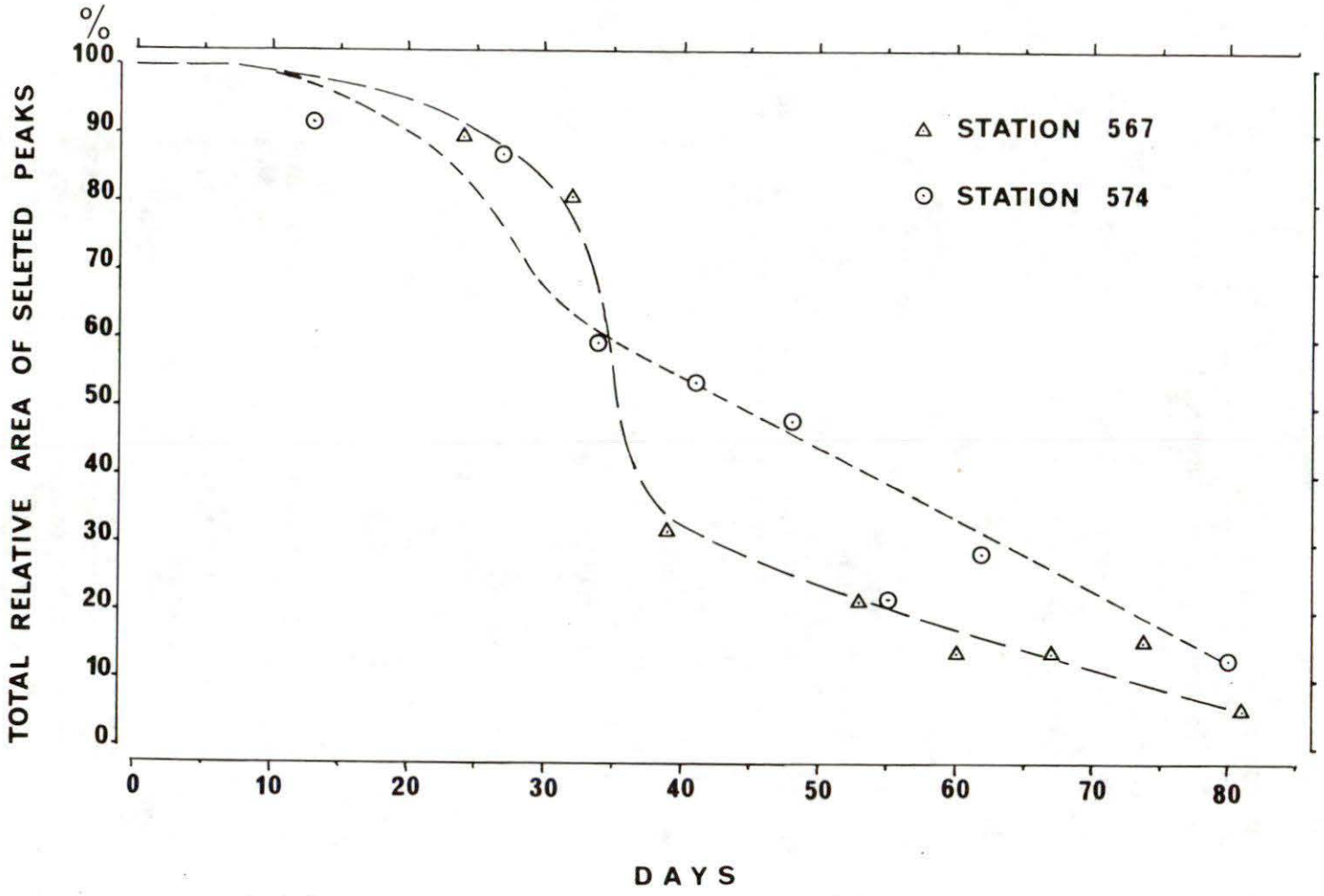


Figure 10. Degradation of weathered Norman Wells crude by the indigenous flora of one metre sea-water samples from Stations 567 and 574 in the Beaufort Sea (see Figure 1). Replicate samples of 500 ml were supplemented with 500 mg of crude and incubated with agitation at 5.0°C. $HgCL_2$ - killed samples served as oil controls. Experimental vessels and oil controls were removed at intervals during incubation and residual petroleum was extracted from each. Summed areas of selected peaks of GC profiles were expressed as percentages of the summed areas of the same peaks in undegraded controls. Percentage values obtained are plotted against incubation interval when extracted.

phosphorus and nitrogen. Large variations in the concentrations of these nutrients were found in the water masses sampled in the Beaufort Sea (see Appendix). In the assessment of *in situ* nutrient requirements for petroleum biodegradation, it would be useful to obtain information on nutrient requirements for degradation by isolated mixed cultures. However, time constraints have prevented anything beyond preliminary experimentation at this time.

The mixed culture 546 was chosen as an inoculum for preliminary experiments because degradation of petroleum by this culture in a defined seawater medium was found to proceed at a rate similar to that obtained in the commercial seawater medium employed in previous experiments.

As a first experiment, the ability of culture 546 to degrade petroleum in the presence or absence of 1.0 g/l NH_4NO_3, 0.1 g/l K_2HPO_4 and 7.69 g/l Tris buffer was determined. Cultures were incubated at 10.0^{0}C. Other experimental conditions and procedures were the same as previously described experiments. The results are seen in Figures 11 and 12.

It can be noted that degradation of Norman Wells crude proceeded in the absence of Tris buffer (Figure 11) and the maximum cell yield was similar to that obtained in the presence of Tris (Figure 12).

The least amount of degradation occurred in the absence of NH_4NO_3. However the multiplication of cells and partial degradation of petroleum in these media was probably due to the introduction of a small amount of NH_4NO_3 together with the inoculum. Similarly, contamination with phosphate ion was sufficient to allow cell multiplication and a degree of petroleum degradation in the absence of K_2HPO_4 in the formulation of the medium. In the absence of petroleum in the medium, only a slight increase in bacterial population was observed (Figure 12).

To determine the minimum concentration of NH_4NO_3 required for degradation of added petroleum, K_2HPO_4 was added to all experimental flasks at a concentration of 0.1 g/l of defined seawater medium and NH_4NO_3 was varied between 0 and 5.0 g/l. The results are seen in Figures 13 and 14. Where NH_4NO_3 was absent from the medium, no petroleum degradation (Figure 13) or cell multiplication (Figure 14) was observed. Somewhat less degradation was observed when NH_4NO_3 was added at a

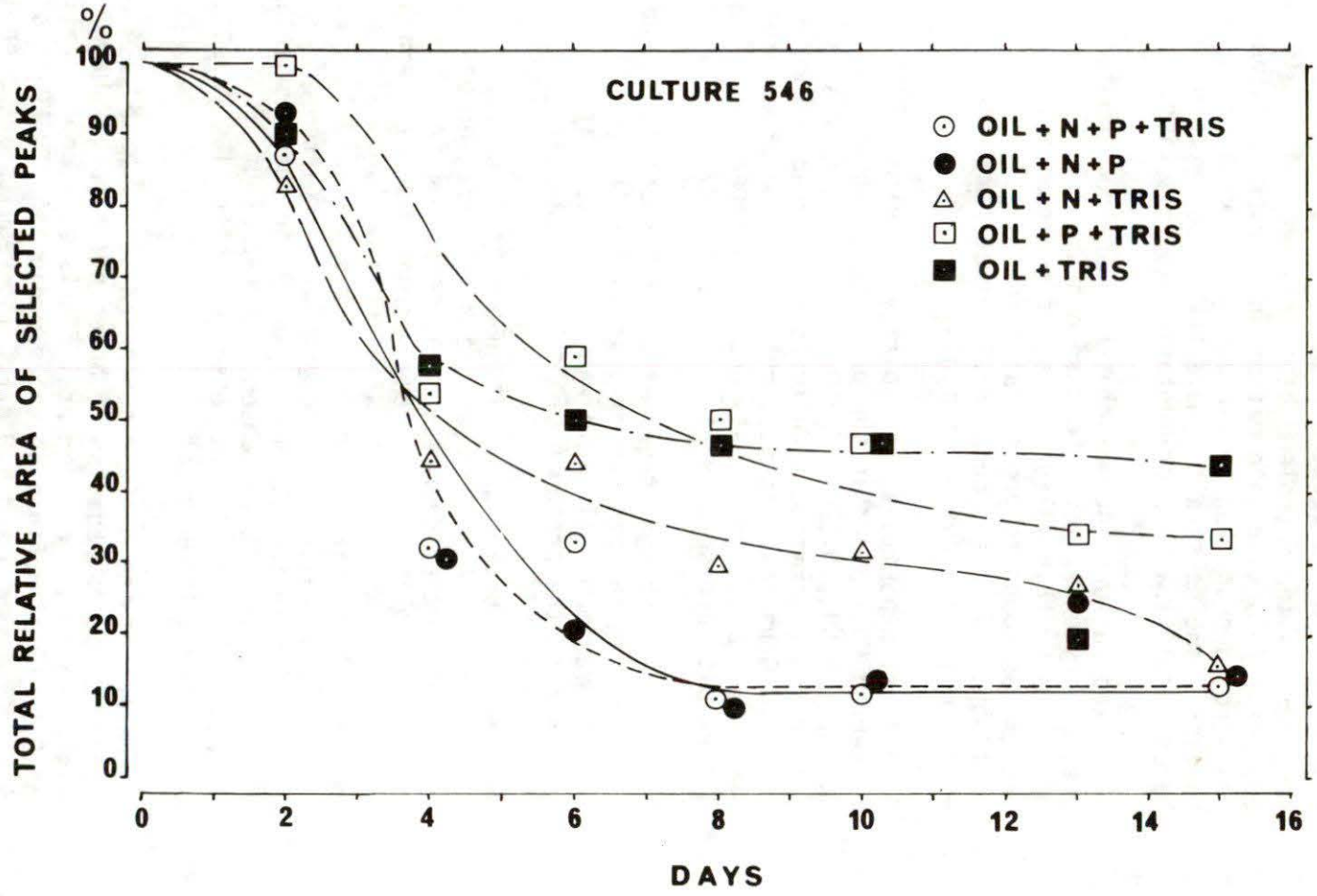


Figure 11. Degradation of weathered Norman Wells crude by culture 546 at 10.0^{0}C in the presence or absence of phosphorus and nitrogen supplements. Cultures were grown in a defined seawater medium. (See legend to Figure 4.)

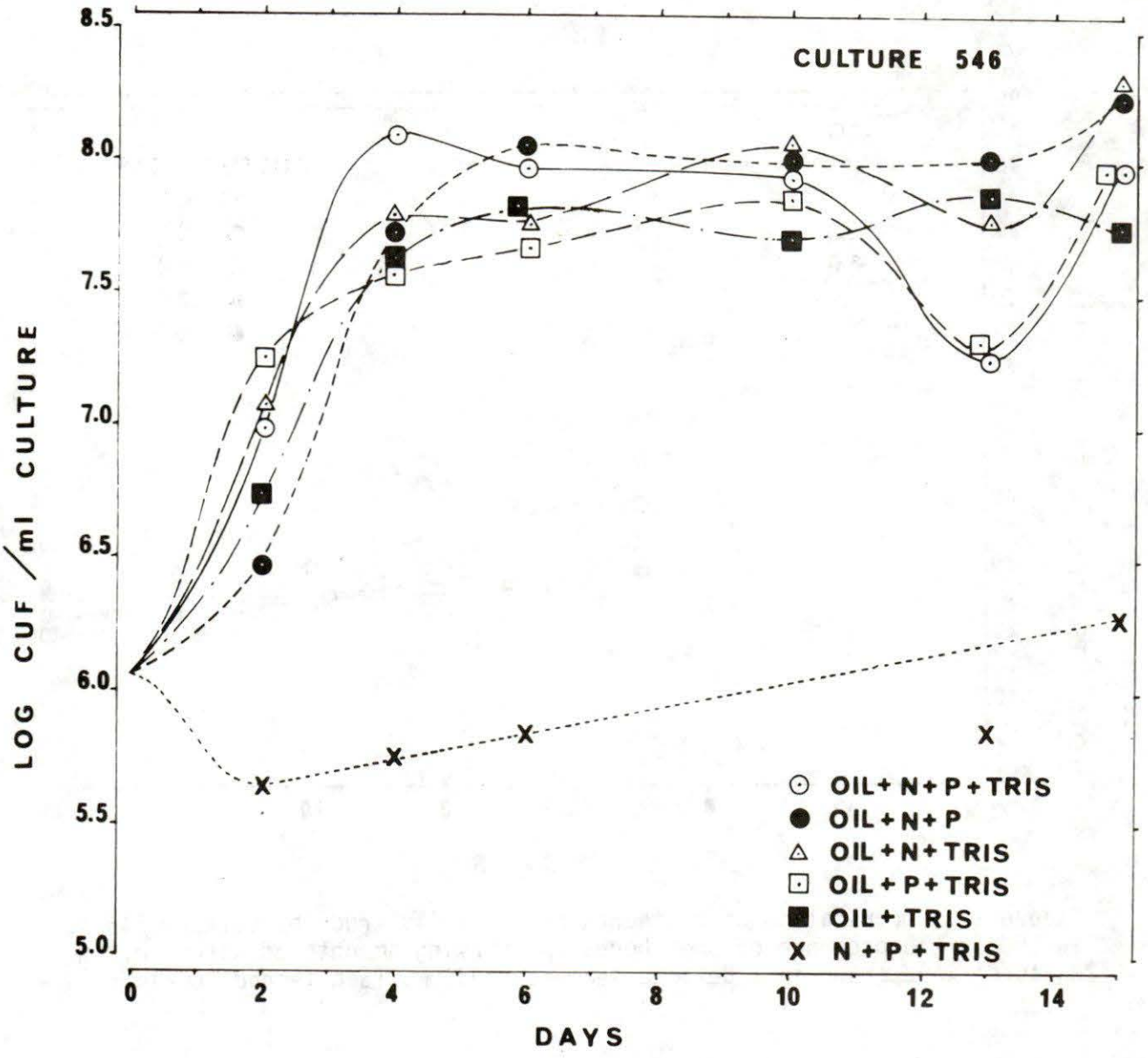


Figure 12. Increase in viable counts of oil and control cultures during degradation of weathered Norman Wells crude by culture 546 at 10.0^{0}C in the presence or absence of phosphorus and nitrogen supplements. Values of extracted petroleum from these cultures are plotted in Figure 11. (See legend to Figure 5.)

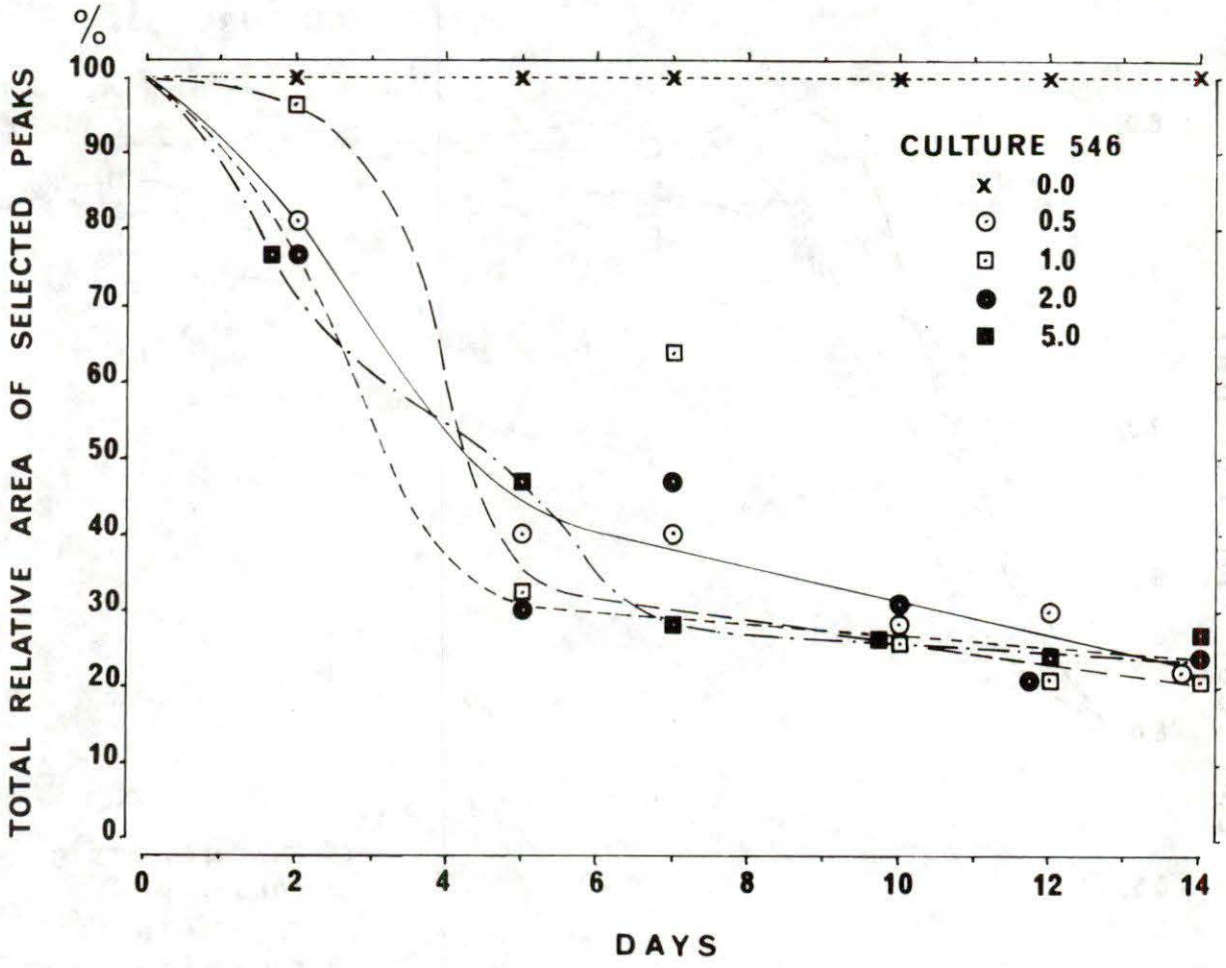


Figure 13. Degradation of weathered Norman Wells crude by culture 546 at 10.0°C in the presence of phosphorus and varying amounts of nitrogen. Cultures were grown in a defined seawater medium. (See legend to Figure 4.)

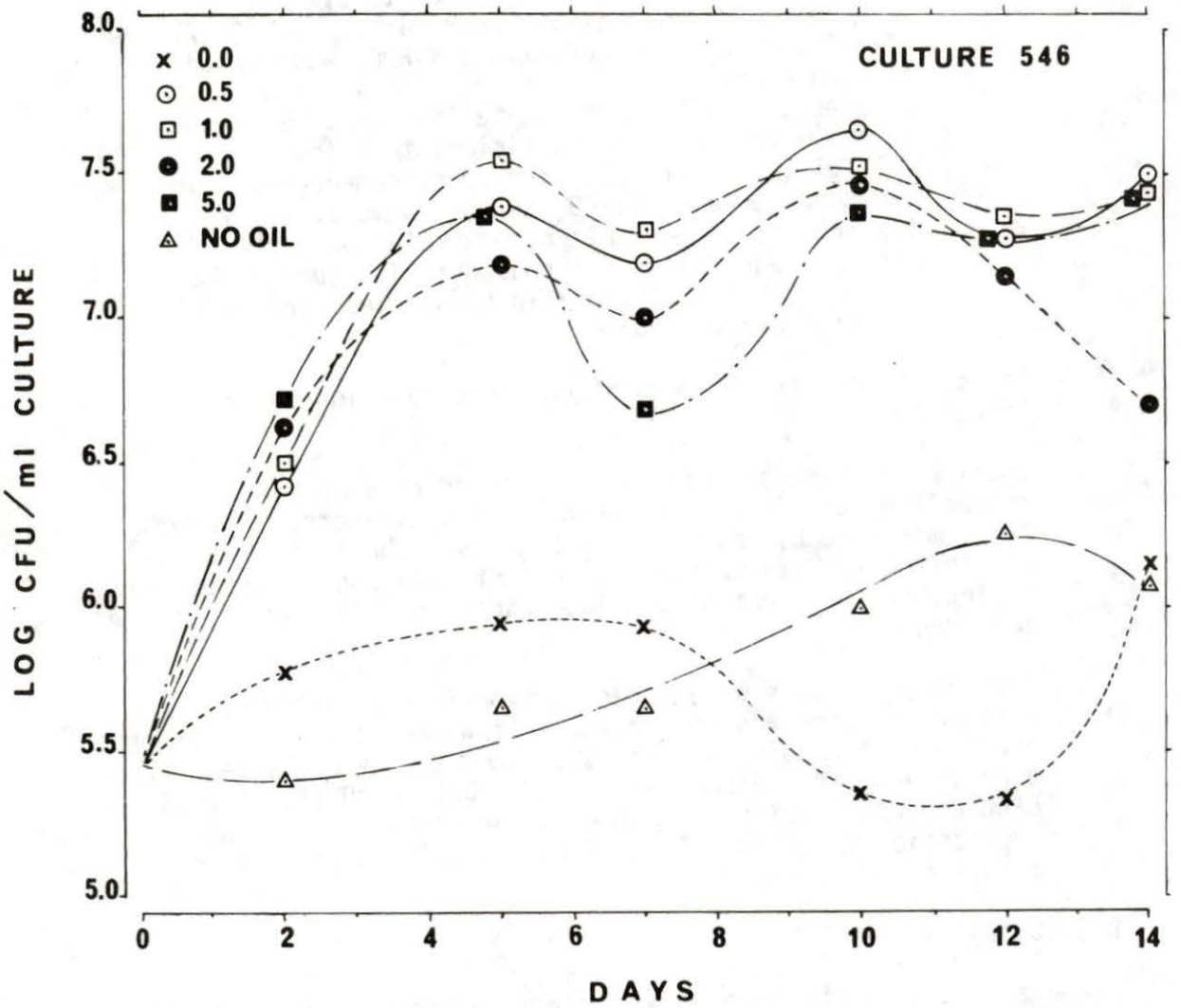


Figure 14. Increase in viable counts of oil and control cultures during degradation of weathered Norman Wells crude by culture 546 at 10.0^{0}C in the presence of phosphorus and varying amounts of nitrogen. Cultures were grown in a defined seawater medium. Values of extracted petroleum from these cultures are plotted in Figure 13. (See legend to Figure 5.)

concentration of 0.5 g/l over that observed when NH_4NO_3 was present at 1.0 g/l.

In a similar experiment to determine the minimum level of phosphate required for degradation of added petroleum, NH_4NO_3 was added to all experimental flasks at a concentration of 1.0 g/l of defined seawater medium and K_2HPO_4 was varied between 0 and 1.0 g/l. The results are seen in Figures 15 and 16. Phosphate introduced with the inoculum proved to be sufficient to allow multiplication of cells and petroleum degradation in the absence of K_2HPO_4 in the formulation of the medium. The result demonstrated only that the minimum requirement for phosphate ion, under the given experimental conditions, was less than 0.05 g/l of seawater medium.

5.6 Effect of Chemical Dispersant on Biodegradation of Crude Petroleum

Chemical dispersants have been proposed as a method of controlling oil spills in northern marine waters. Corexit 8660, marketed by Imperial Oil Ltd. as a chemical dispersant, was tested for its toxicity to culture 546 and its enhancement or inhibition of petroleum biodegradation. The result is seen in Figure 17.

Two concentrations were added to experimental cultures in defined seawater medium and incubation was at 10.0°C. Other experimental conditions were the same as previously described experiments. At concentrations of 0.05% and 0.1%, Corexit 8660 did not significantly inhibit or enhance petroleum degradation by culture 546 and cell multiplication was not affected.

6. DISCUSSION

The viable counts of heterotrophic bacteria obtained from stations occupied in the Beaufort Sea during 1974 and 1975 are in general agreement with values obtained in 1973 (Bunch, 1974). Although sampling techniques and cultivation media vary with investigators, the abundance of heterotrophs in the Beaufort Sea was comparable to values obtained by investigations of estuaries and off-shore areas along the New England coast (Kaneko and Colwell, 1973; Sieburth, 1967; Atlas and Bartha, 1972).

Intensive seasonal sampling of one station in the Eskimo Lakes has demonstrated that the maximum size of the bacterial population in the water column is related to the phytoplankton bloom which occurs soon after the break-up of ice (data not reported). With declining primary productivity, bacterial numbers decrease. Since the occupations of stations in the Beaufort Sea were spatial rather than seasonal, it was difficult to determine the state of the

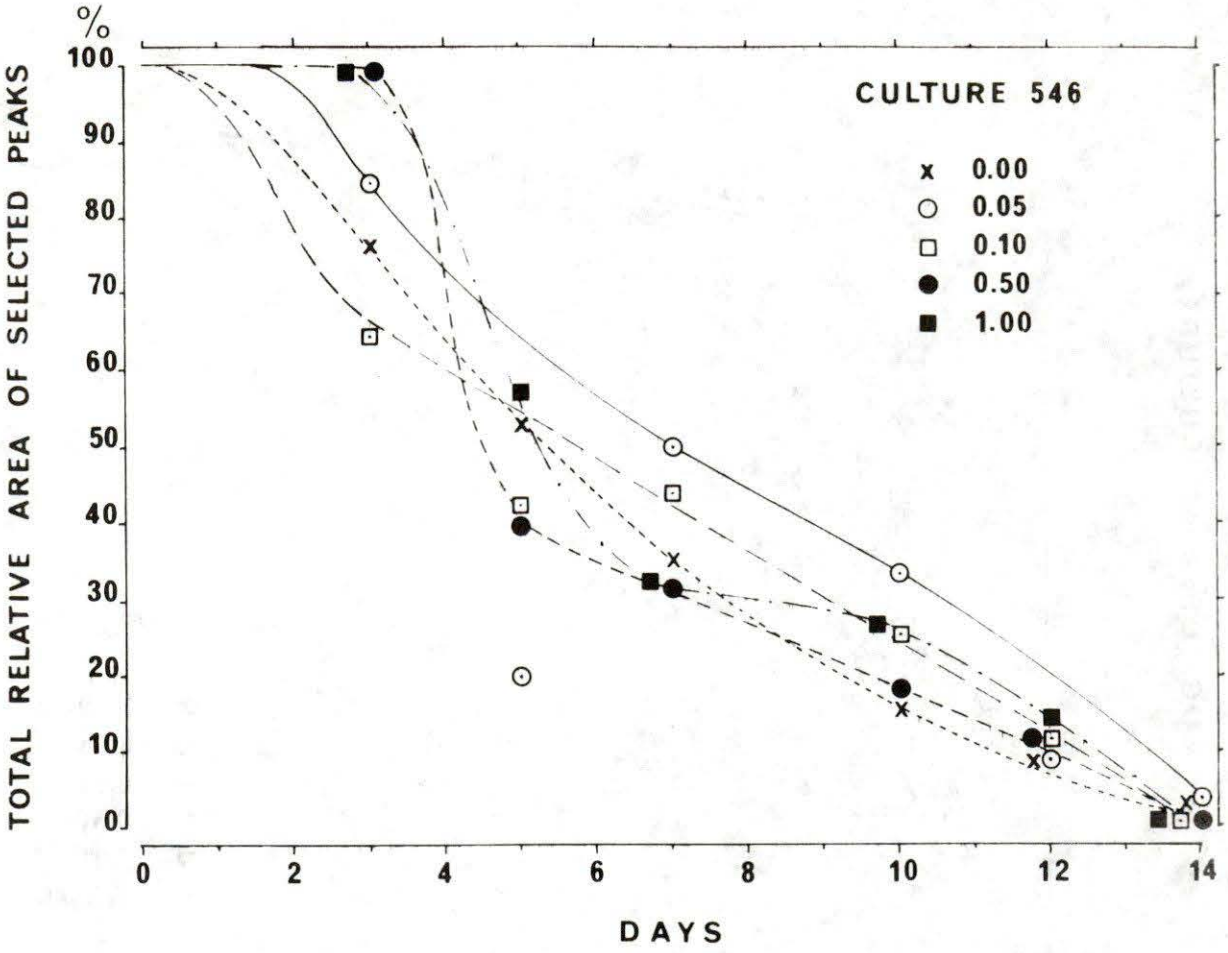


Figure 15. Degradation of weathered Norman Wells crude by culture 546 at 10.0^{0}C in the presence of nitrogen and varying amounts of phosphorus. Cultures were grown in a defined seawater medium. (See legend to Figure 4.)

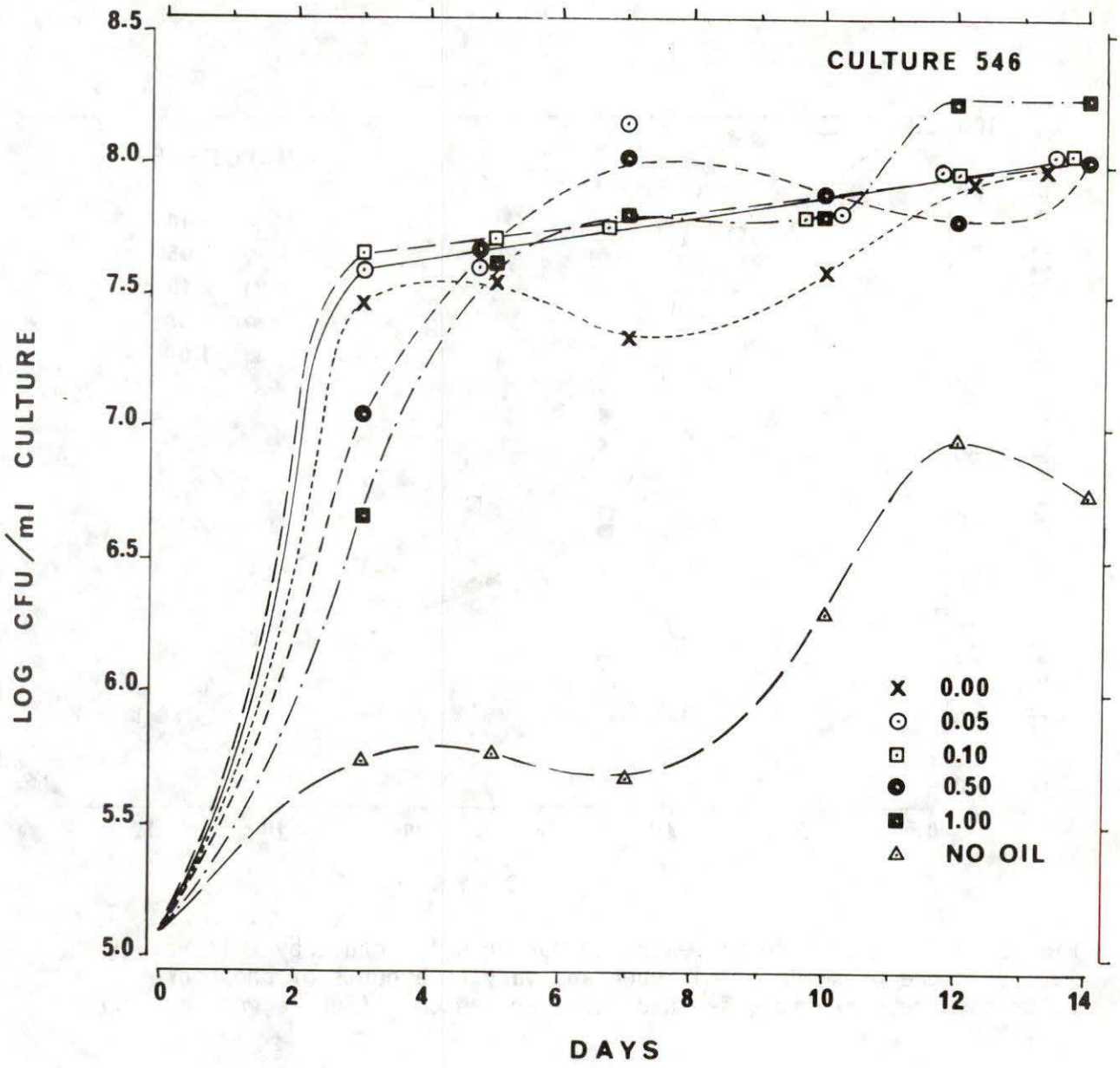


Figure 16. Increase in viable counts of oil and control cultures during degradation of weathered Norman Wells crude by culture 546 at 10.0^{0}C in the presence of nitrogen and varying amounts of phosphorus. Cultures were grown in a defined seawater medium. Values of extracted petroleum from these cultures are plotted in Figure 15. (See legend to Figure 5.)

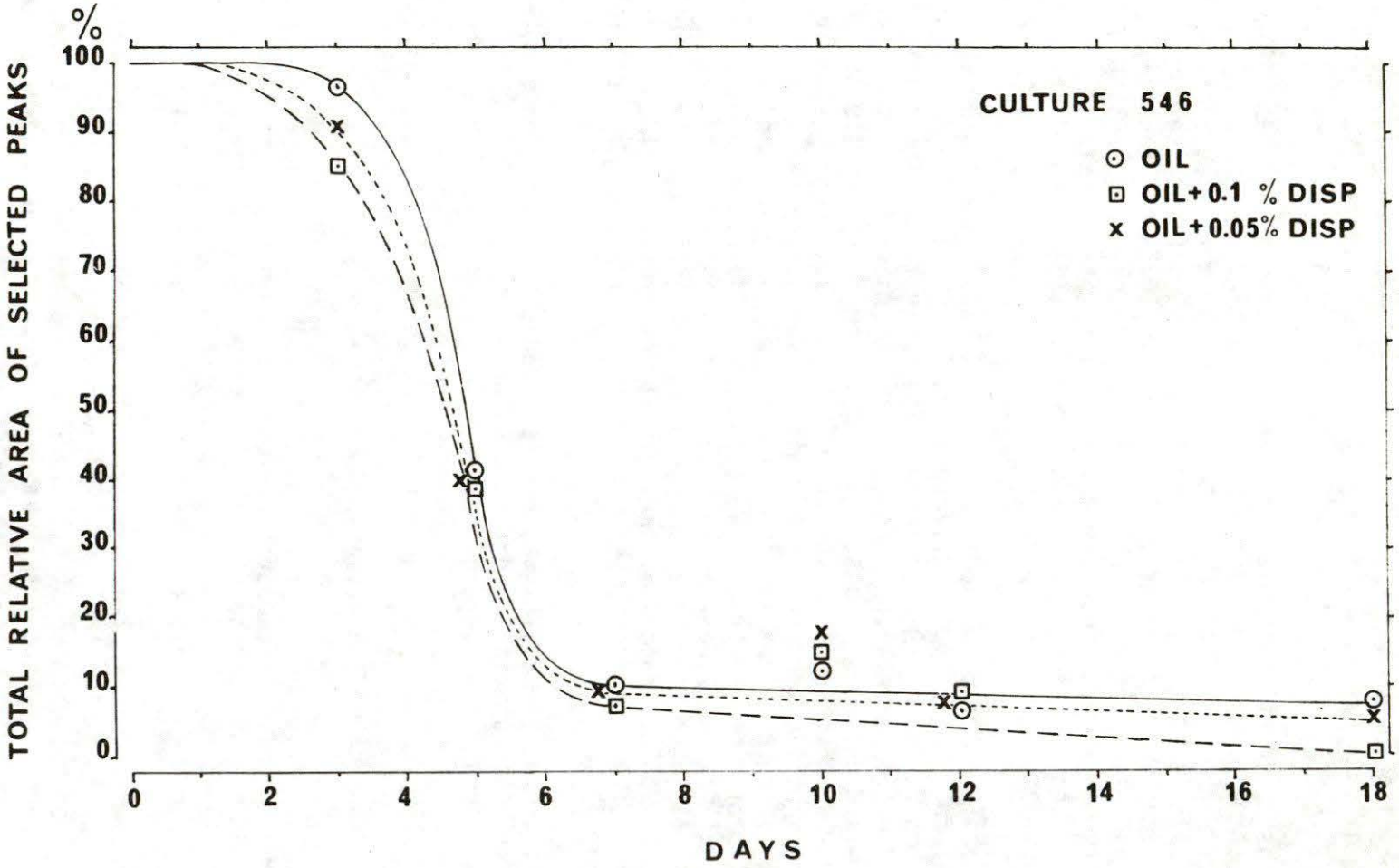


Figure 17. Degradation of weathered Normal Wells crude by culture 546 at 10.0°C in the presence or absence of two concentrations of Corexit 8660. Cultures were grown in a defined seawater medium. (see legend to Figure 4.)

phytoplankton bloom at any particular station. However, low bacterial numbers at Stations 572 and 573 correlated with low chlorophyll A values which indicated low primary productivity. The low nitrate values would suggest that this nutrient had been depleted by a recent bloom of phytoplankton.

As demonstrated at Station 22 and other stations in 1973 and 1974, the great majority of heterotrophic cells isolated from the Beaufort were psychrophilic in their temperature requirements in that replicate plates incubated at 15.0 and 22.0^{0}C resulted in thirty times more growth at 15.0^{0}C. Morita (1975) has recently suggested that those cells which fail to multiply above 20.0^{0}C and have a temperature optimum for multiplication of approximately 15.0^{0}C be considered psychrophilic - a definition which our results would support.

The probability of psychrophilic oleoclasts was suggested by the observation of a predominately psychrophilic flora of heterotrophs in the Beaufort Sea. All enriched cultures from occupied stations degraded petroleum at 5.0^{0}C and temperature experiments with four of the mixed cultures demonstrated degradation at 0 or -1.0^{0}C. The mixed cultures contained a variety of cell-types, each presumably having a specific temperature optimum but two of the four mixed cultures appeared to prefer low temperatures for growth and multiplication on a petroleum substrate. The detection of psychrophilic cell-types in these cultures demonstrated that the process of biodegradation in the Beaufort Sea, although temperature-dependent, would result from the activity of a portion of the indigenous flora degrading petroleum preferentially at temperatures below 20.0^{0}C.

The presence of oleoclasts was demonstrated at a depth of one metre at all selected stations and this population was quantitated at three stations. The range of values from 2.3 X 10^2 to 9.3 X 10^4 cells/litre of seawater corresponds closely to a range of 9.3 X 10^2 to 4.6 X 10^5 cells/l determined in the North Sea (Gunkel, 1973) and a range of 6.6 X 10^3 to 7.3 X 10^4 cells/l taken along a Halifax-Bermuda transect in the Atlantic Ocean (Mulkins-Phillips and Stewart, 1974a). Low winter values of 7.9 X 10^2 and 0.6 X 10^2 cells/l were reported by Atlas and Bartha (1973) off the coast of New Jersey when water temperatures were 4.7 to 4.9^{0}C. The low numbers of oleoclasts, however, may have been a result of the quantitation procedure involving an incubation temperature of 28.0^{0}C and consequent loss of activity of psychrophilic cells.

The percent ratios of oleoclasts to total heterotrophs ranged from 0.001 to 1.63% at the three stations which were quantitated and the range of values demonstrated that the populations of oleoclasts were independent of the total numbers of heterotrophs. However, the numbers of oleoclasts may be an indication of the levels of hydrocarbon substrate available in the sampled waters. As suggested by Atlas and Bartha (1973) the relative abundance of oleoclastic cells would increase in response to increasing levels of hydrocarbons. Although data of hydrocarbon concentrations in the Beaufort Sea are not available, it is interesting to note that

the surface waters of Stations 565 and 567 contained the largest numbers of oleoclasts. These locations (see Figure 1) are directly influenced by the discharge of the Mackenzie River which probably contains varying levels of petroleum and petroleum by-products from natural seepage at Norman Wells, N.W.T. and from commercial traffic on the river.

The lack of oleoclastic cells in offshore sediments demonstrated that a hydrocarbon substrate is not present in these sediments or is unavailable due to sedimentation. The presence of oleoclasts in shoreline sediments would probably result from the discharge of petroleum from the Mackenzie River.

The availability of oleoclasts in the Beaufort Sea together with their demonstrated ability to degrade petroleum crude at *in situ* temperatures assures a degree of petroleum biodegradation in open waters of the Beaufort Sea. An initial lag of at least four weeks, as suggested in Figure 10, would be expected before degradation could be detected.

The nutrient requirements for *in situ* degradation remain inconclusive. Preliminary experiments suggested minimum requirements of 0.5 to 1.0 g/l (12.5 - 25.0 mg-at) of nitrogen and less than 0.05 g/l (2.9 mg-at) of phosphorus to degrade the aliphatic fraction of 200 mg of Norman Wells crude in batch culture. These values are considerably in excess of those determined at 1.0 metre depths of occupied stations in the Beaufort Sea (0 - 2.3 µg-at/l of nitrate; 0 - 0.36 µg-at/l of phosphate). However, the incubation of oleoclasts with petroleum in a closed system requires an initially high level of nutrients. In the open ocean, where nutrients are constantly recycled, degradation should proceed with low concentrations of nutrients which will be replenished. Kinney *et al.* (1969) reported that the concentrations of nutrients in seawater were not limiting to *in situ* degradation in Cook Inlet, Alaska.

The addition of Corexit 8660 to Norman Wells crude did not appear to enhance or retard degradation of the crude by a mixed culture of oleoclasts. This result is in general agreement with those observed by Mulkins-Phillips and Stewart (1974b) who evaluated four different dispersants. Dispersants should increase emulsification of petroleum and therefore enhance the ability of bacteria to come into contact with and degrade the petroleum. This did not appear to be the case in this initial experiment and experiments with higher concentrations of dispersant are indicated. In addition, such experiments would also determine the possible toxicity of higher concentrations of dispersant.

Although the purpose of the present study was to evaluate the petroleum biodegradation potential of the Beaufort Sea, it was of interest to determine the effect of petroleum crude upon the heterotrophic activity of the predominate flora of non-oleoclastic bacteria. A baseline study of heterotrophic turnover of dissolved organic material by bacteria in the Beaufort Sea has been

undertaken by this laboratory and a natural extension of the study was to determine the effect of petroleum upon this activity.

In all cases but one, weathered or unweathered crude did not appear to significantly inhibit the rate of mineralization of glutamic acid by the predominate flora. Where glutamate mineralization was enhanced, the large amount of hydrocarbon substrate (0.1%) in the reaction vessel may have increased the rate of utilization of glutamate by oleoclastic heterotrophs present in the sample. Alternatively, the population of oleoclastic cells may have increased across the twelve hour incubation period, resulting in greater mineralization of glutamate. It is noteworthy that the highest level of oleoclasts was found at Station 567 where glutamate mineralization increased twofold in the presence of weathered crude.

The results suggest that the *in situ* activity of heterotrophs in the cycling of organic material would not be adversely affected by an influx of petroleum. Indeed, added petroleum would contribute to the natural carbon cycle over a period of time.

7. CONCLUSIONS

(1) One metre water from stations occupied during the open-water season of 1974 and 1975 produced total viable bacterial counts ranging from 1.0×10^6 to 3.0×10^7 CFU/litre of seawater in almost all instances. These results confirmed those obtained in 1973. Incubation of replicate samples at different temperatures demonstrated that this heterotrophic flora was predominately psychrophilic.

(2) Mineralization of glutamic acid by the indigenous heterotrophic flora was generally unaffected, or in some instances, was enhanced by the presence of weathered or unweathered crude. This suggests that *in situ* activity of non-oleoclastic heterotrophs might not be affected by a moderate influx of petroleum in the Beaufort Sea ecosystem.

(3) Oleoclastic heterotrophs appeared to be ubiquitous in the surface waters and shoreline sediments of the south Beaufort Sea but were not evident in offshore sediments. Quantitation of these cells in one metre water of three stations gave values ranging from 2.3×10^2 to 9.3×10^4 cells/litre of seawater and these values corresponded closely to values in ocean bodies at more southerly latitudes. That their abundance was independent of the total heterotrophic count suggests the existence of a hydrocarbon substrate in these waters. The source of this substrate must, to a large extent, be the effluent of the Mackenzie River. The presence of numerous oleoclastic bacteria in the Mackenzie plume areas of the Beaufort Sea strongly suggests this.

(4) Nitrogen, but probably not phosphorus, may be limiting to biodegradation in the Beaufort Sea. It might be assumed that the natural cycling of water masses would replenish exhausted inorganic

nutrients in an area of active degradation of petroleum. However, further experimentation is required before any conclusions can be drawn.

(5) Enriched cultures were found to degrade petroleum at 0 or -1.0°C and a psychrophilic flora was present in these mixed cultures. The availability of oleoclasts in the Beaufort Sea together with their demonstrated ability to degrade petroleum at *in situ* temperatures assures a degree of petroleum biodegradation in the Beaufort Sea, albeit at a rate yet to be determined. Incubation of seawater samples with Norman Wells crude resulted in a lag of approximately four weeks before measurable degradation commenced at 5.0°C and complete degradation of the aliphatic fraction had occurred within 11 to 12 weeks. This would necessarily be a minimum length of time before a degree of biodegradation of an oil spill would occur in open waters of the south Beaufort Sea.

(6) Degradation of Norman Wells crude by a mixed culture of oleoclastic cells was unaffected by the presence of Corexit 8660.

8. IMPLICATIONS AND RECOMMENDATIONS

An estimate of the time required for the complete degradation of a given quantity of oil in the Beaufort Sea cannot be made on the basis of the data presented in this report. Nor can such an estimate be made on the basis of information available in the relevant literature. The complexity of the natural parameters involved in biodegradation, both physical and biological, together with the technical problems inherent in quantitative *in situ* experimentation, have frustrated attempts to determine quantitatively the ultimate fate of petroleum in the marine ecosystem.

The presence of numerous oleoclastic bacteria, and their ability to degrade Norman Wells crude at *in situ* temperatures, have been established. It can be concluded, therefore, that degradation will proceed in open water and will not be limited by the availability of oleoclasts. As a clean-up technique, the "seeding" of oil spills with a heavy inoculum of bacteria should not be considered in view of the sizable indigenous flora. Moreover, the effectiveness of this technique in the marine ecosystem has yet to be documented.

It cannot be ascertained at this time whether temperature or nutrient supply would be the limiting factor to *in situ* biodegradation at the observed environmental temperatures. The nutrient supply in surface waters appears to be rapidly depleted by the summer bloom of phytoplankton (Grainger, 1975). A spill immediately after this time might result in degradation being limited by nutrients until these nutrients could be replenished in the yearly cycle. In the event of a spill or the release of ice-entrained oil before the annual bloom of phytoplankton, oleoclastic cells would presumably compete with the phytoplankton bloom for

nutrients if, in fact, the bloom occurred under a layer of oil on the surface waters.

It seems reasonable to assume that degradation will not occur in one open-water period in the Beaufort Sea. With excess nutrients, the flora of seawater samples required 11 to 12 weeks to degrade the aliphatic fraction of Norman Wells crude at 5.0°C. Oil remaining overwinter would be entrained in ice and therefore be unavailable to microbial action. Further degradation would occur with the release of the oil into open water the following spring. The possibility of complete degradation, i.e. the disappearance of the heavy asphaltene fraction, remains in the realm of conjecture at this time. Asphaltenes may ultimately reside in sediments where further degradation would be minimal or nonexistent.

Concern over the toxicity of low-boiling fractions of a spill may be overstated. Scott (1973) observed that within 24 hours, 27% (by weight) of Norman Wells crude was weathered from a spill on a freshwater pond where the water temperature was 0°C and the air temperature was near freezing. This loss included the fractions usually considered acutely toxic to organisms. Oil entrained in ice overwinter would, therefore, be less toxic if initially exposed to the atmosphere. In the event of a persistent blowout under formed ice, however, weathering would presumably not occur with the consequent dangers of exposure to organisms and the possibility of toxic fractions entering the food chain after solubilization in the water. With ice-breakup, however, weathering would be rapid, and degradation would proceed.

The many uncertainties regarding the persistence of a spill in the Beaufort Sea demonstrate the impossibility of assessing the parameters involved within the time constraints of a short-term project. Thusfar, only a superficial treatment of the problem has been possible. The technical difficulties inherent in the problem together with the extraordinary logistics required to pursue experimentation in the field emphasize the need for a much longer period of study.

9. NEEDS FOR FURTHER STUDY

Many uncertainties about the persistence of petroleum in the Beaufort Sea ecosystem can best be resolved by means of a small, controlled oil spill. This would be similar to the spill undertaken by the Beaufort Sea Project at Cape Perry, N.W.T. but with continuous biological and chemical monitoring of the affected area over a period of two years to ensure two open-water seasons. One initial year would be required to obtain base-line data. For the duration of the spill, a number of quantitative techniques would be employed at frequent intervals to measure activities and changes in the ecosystem and to determine the fate of the residual oil. Such a spill would also provide information on the possibility of petroleum degradation under sea ice. This information is urgently

required in view of the ice conditions in the Beaufort Sea during 8 to 9 months of the year.

A small spill would also aid in the development of a novel technique to assess hydrocarbon degradation rates by the use of ^{14}C-labelled hydrocarbons. Initial experiments during the 1975 field season were very encouraging. The technique may well prove to be a rapid method of determining the biodegradation potential of relatively inaccessible Arctic marine waters.

Much more information is required on the nutrient requirements of biodegradation at low temperatures. Laboratory experimentation is required to determine the effect of decreasing temperature on nutrient requirements.

10. REFERENCES

American Public Health Association. 1971. Standard methods for the examination of water and waste water. 13th ed. APHA, N.Y., N.Y.

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

Atlas, R. M., and R. Bartha. 1973. Abundance, distribution and oil biodegradation potential of microorganisms in Raritan Bay. Environ. Pollut. 4: 291-300.

Bartha, R., and R. M. Atlas. 1973. Biodegradation of oil in seawater: limiting factors and artificial stimulation, p. 147-152. In D. G. Ahearn and S. P. Meyers (ed.). The microbial degradation of oil pollutants. Center for Wetland Resources. Publication No. LSU-SG-73-01. Louisiana State U., Baton Rouge, La.

Boyd, H. 1970. Oil poses urgent problems in Canada. Mar. Pollut. Bull. 1: 69-71.

Bunch, J. N. 1974. Distribution and abundance of heterotrophic bacteria in the South Beaufort Sea, p. 175-195. In The marine ecology of the Mackenzie delta and Tuktoyaktuk Peninsula region. Environmental-Social Committee, Northern Pipelines, Task Force on Northern Oil Development, Report No. 74-22, part 111b.

Carlucci, A. F., and J. D. H. Strickland. 1968. The isolation, purification and some kinetic studies of marine nitrifying bacteria. J. exp. mar. Biol. Ecol. 2: 156-166.

Glaeser, J. L. 1971. A discussion of the future oil spill problem in the Arctic, p. 479-484. In Proc. API/FWPCA Joint Conf. on Prevention and Control of Oil Spills. American Petroleum Institute, Washington, D.C.

Grainger, E. H. 1975. Biological productivity in the southern Beaufort Sea: the physical-chemical environment and the plankton. Beaufort Sea Project Technical Report No. 12. Beaufort Sea Project, Victoria, B.C.

Griffiths, R. P., F. J. Hanus, and R. Y. Morita. 1974. The effects of various water-sample treatments on the apparent uptake of glutamic acid by natural marine microbial populations. Can. J. Microbiol. 20: 1261-1266.

Gunkel, W. 1973. Distribution and abundance of oil-oxidizing bacteria in the North Sea, p. 127-139. In D. G. Ahearn and S. P. Meyers (ed.). The microbial degradation of oil pollutants. Center for Wetland Resources. Publication No. LSU-SG-73-01. Louisiana State U., Baton Rouge, La.

Jones, G. E. 1971. The fate of freshwater bacteria in the sea. Developments in Industrial Microbiology. 12: 141-151.

Kaneko, T., and R. R. Colwell. 1973. Ecology of *Vibrio parahaemolyticus* in Chesapeake Bay. J.Bacteriol. 113: 24-32.

Kinney, P. J., D. K. Button, and D. M. Schell. 1969. Kinetics of dissipation and biodegradation of crude oil in Alaska's Cook Inlet, p. 333-340. In Proc. API/FWPCA Joint Conf. on Prevention and Control of Oil Spills. American Petroleum Institute, Washington, D.C.

Kriss, A. E. 1963. Marine Microbiology: 31-34. Oliver and Boyd. London, U.K.

Morita, R. Y. 1975. Psychrophilic bacteria. Bact. Rev. 39: 144-167.

Mulkins-Phillips, G. J., and J. E. Stewart. 1974a. Distribution of hydrocarbon-utilizing bacteria in Northwestern Atlantic waters and coastal sediments. Can. J. Microbiol. 20: 955-962.

Mulkins-Phillips, G. J., and J. E. Stewart. 1974b. Effect of four dispersants on biodegradation and growth of bacteria on crude oil. Appl. Microbiol. 28: 547-552.

Ramseier, R. O. 1973. Possible fate of oil in the Arctic Basin. Paper presented at First World Congress on Water Resources. Chicago, Illinois. 24-28 September.

Robertson, B., S. Arhelger, P. J. Kinney, and D. K. Button. 1973. Hydrocarbon biodegradation in Alaskan waters, p. 171-184. In D. G. Ahearn and S. P. Meyers (ed.). The microbial degradation of oil pollutants. Center for Wetland Resources. Publication No. LSU-SG-73-01. Louisiana State U., Baton Rouge, La.

Scott, B. F. 1975. Investigation of the weathering of a selected crude oil in a cold environment, p. 514-525. In Water Quality Parameters. ASTM STP 573. American Society for Testing and Materials, Philadelphia, Pa.

Sieburth, J. McN. 1967. Seasonal selection of estuarine bacteria by water temperature. J. exp. mar. Biol. Ecol. 1: 98-121.

Strickland, J. D. H., and T. R. Parsons. 1968. A practical handbook of seawater analysis. Fish. Res. Bd. Canada Bulletin 167: 311 p. Ottawa, Ont.

Wright, R. T. 1974. Mineralization of organic solutes by heterotrophic bacteria, P. 546-565. In R. R. Colwell and R. Y. Morita (ed.). Effect of the Ocean Environment on Microbial Activities. University Park Press, Baltimore, Md.

ZoBell, C. E. 1946. Action of microorganisms on hydrocarbons. Bact. Rev. 10: 1-49.

ZoBell, C. E. 1969. Microbial modification of crude oil in the sea, p. 317-326. In Proc. API/FWPCA Joint Conf. on Prevention and Control of Oil Spills. American Petroleum Institute, Washington, D.C.

ZoBell, C. E. 1973. Bacterial degradation of mineral oils at low temperatures, p. 153-161. In D. G. Ahearn and S. P. Meyers (ed.). The microbial degradation of oil pollutants. Center for Wetland Resources. Publication No. LSU-SG-73-01. Louisiana State U., Baton Rouge, La.

11. APPENDIX

Stations occupied during 1974 and 1975. Viable counts of heterotrophic bacteria obtained on Marine Agar 2216E or Lib X and Plate Count Agar (PCA), after incubation at 5.0°C, are expressed as Log_{10} colony-forming units (CFU)/litre of seawater sample.

Station	Date Location	Depth	Log_{10} CFU/litre Marine Agar	PCA	Salinity (‰)	Chlorophyll A (mg/m^3)	Nitrate (µg at/l)	Phosphate (µg at/l)	Temp (°C)
7BS	19.7.74	1	7.38	6.42	<3.0	0.34	0.0	0.00	1.0
	70°39'N	5	6.11	4.89	29.85	0.24	3.7	0.81	-1.6
	130°06'W	10	6.23	5.01	29.82	0.29	3.4	0.65	-1.6
		20	6.47	5.78	29.82	0.11	3.7	0.59	-1.6
9BS	25.7.74	1	7.40	6.90	7.87	0.17	0.1	0.20	0.9
	71°22'N	5	6.27	5.37	30.23	0.00	0.0	0.76	-1.3
	130°24'W	10	4.87	5.35	30.16	0.10	0.0	0.83	-1.3
		20	5.73	4.81	30.71	0.10	0.0	0.83	-1.4
		50	5.41	3.22	31.60	0.06	3.9	1.04	-1.5
		75	5.21	3.22	32.03	0.06	8.2	1.46	-1.4
		110	5.21	3.22	32.53	0.06	12.4	1.67	-1.4
12BS	29.7.74	1	7.00	6.70	3.40	0.05	0.1	0.02	0.3
	71°02'N	5	5.26	4.30	29.91	0.0	0.0	0.77	-1.3
	133°56'W	10	4.99	4.07	29.98	0.09	0.1	0.80	----
		20	4.30	3.22	30.53	0.16	0.1	0.73	-1.3
		50	4.92	----	31.19	0.17	4.5	1.03	-1.5
		75	4.80	3.52	31.75	0.22	7.4	1.33	-1.3
		100	5.22	3.92	32.51	0.11	13.5	1.68	-1.4
		200	5.35	3.70	33.70	0.0	15.6	1.64	-1.0
		260	5.30	3.52	34.32	0.06	15.4	1.18	-0.1
14BS	14.7.74	1	6.28	6.38	<3.0	0.78	2.3	0.13	1.4
	70°09'N	5	6.17	6.43	28.99	0.78	2.2	0.15	0.8
	133°24'W	10	4.78	4.13	29.16	0.55	1.8	0.79	-1.5
		20	5.20	4.18	30.05	0.33	2.3	0.79	-1.5
		32	5.56	3.92	31.28	0.22	3.5	1.23	-1.6

Station	Date Location	Depth	Log_{10} CFU/litre Marine Agar	PCA	Salinity (‰)	Chlorophyll A (mg/m^3)	Nitrate (µg at/l)	Phosphate (µg at/l)	Temp (^0C)
22BS	12.8.74	1	6.95	6.42	5.02	2.94	0.2	0.00	0.8
	69°46'N	5	5.26	6.37	5.39	0.58	0.6	0.03	0.9
	138°52'W	10	5.20	5.63	28.42	0.11	2.2	0.60	-1.5
		20	4.89	5.64	30.36	0.18	2.2	0.70	-1.6
		50	5.04	4.84	31.99	0.0	9.1	0.60	-1.5
		75	5.14	4.75	32.04	0.0	7.7	1.12	-1.5
23BS	16.8.74	1	6.89	6.19	4.30	1.75	0.2	0.00	0.6
	70°01'N	5	6.79	6.29	4.40	1.13	0.2	0.00	0.5
	138°56'W	10	5.58	5.55	25.75	0.06	3.8	0.48	-1.3
		20	4.52	4.77	30.48	0.06	4.1	0.81	-1.6
		50	4.70	4.54	32.06	0.06	10.6	1.21	----
		75	4.48	4.34	32.15	0.12	11.5	1.42	-1.5
		100	4.70	4.50	32.17	0.06	9.6	1.33	-1.5
		220	4.92	4.13	33.34	0.06	14.0	1.54	----
29BS	2.8.74	1	----	6.38	5.91	3.67	0.5	0.09	----
	70°17'N	5	----	6.18	7.21	0.9	1.1	0.09	----
	136°26'W	10	7.01	5.25	29.23	0.39	2.8	0.83	----
		20	5.01	4.58	30.66	0.21	2.7	0.85	----
		30	4.52	0.0	31.16	0.32	4.2	1.02	----
		50	5.22	0.0	31.95	0.30	8.4	1.30	----
30BS	2.8.74	1	6.72	----	9.49	1.63	1.2	0.02	1.7
	69°55'N	5	6.70	----	9.22	0.11	0.0	0.02	1.3
	136°12'W	10	5.48	----	28.13	0.07	4.5	0.87	-1.4
		24	5.17	----	30.39	0.35	4.0	0.98	-1.6
544	27.8.74	1	6.89	6.22	-----	----	---	----	----
	70°33'N	5	6.89	6.18	17.43	----	0.2	0.00	-0.8
	131°42'W	10	5.82	4.78	28.14	----	0.8	0.44	-1.3
		20	5.56	4.22	30.24	----	0.2	0.77	-1.4
		35	5.39	4.07	31.10	----	2.9	0.93	-1.5

Station	Date Location	Depth	Log_{10} CFU/litre Marine Agar	PCA	Salinity (‰)	Chlorophyll A (mg/m^3)	Nitrate (µg at/l)	Phosphate (µg at/l)	Temp (^0C)
545	27.8.74	1	7.00	6.08	-----	----	---	----	----
	70°23'N	5	7.00	6.11	14.64	----	0.8	0.00	-0.1
	131°42'W	10	6.05	4.79	28.18	----	0.2	0.38	-1.3
		20	5.60	4.26	30.33	----	0.1	0.40	-1.4
		35	5.54	4.85	-----	----	4.6	0.93	----
546	27.8.74	1	6.63	5.72	-----	----	---	----	----
	69°56'N	5	6.73	5.73	<5.0	----	1.6	0.00	2.5
	133°27'W	10	6.15	5.53	27.42	----	2.9	0.46	-1.3
		18	5.45	4.79	30.12	----	3.5	0.71	-1.5
547	28.8.74	1	7.09	6.25	-----	----	---	----	----
	70°18'N	5	6.01	6.12	7.62	1.46	0.6	0.29	-0.5
	135°10'W	10	5.79	5.13	27.81	0.14	0.7	0.66	-1.4
		20	5.03	4.67	30.48	0.04	1.3	0.31	-1.5
		30	5.08	4.34	30.76	0.04	1.2	0.57	-1.6
		50	5.55	----	31.94	0.08	5.6	1.04	-1.4
548	29.8.74	1	6.87	6.17	-----	----	---	----	----
	70°08'N	5	5.93	6.35	<6.0	----	0.5	0.00	2.1
	135°34'W	10	6.23	5.47	27.22	----	2.5	0.44	-1.3
		20	5.62	5.01	30.07	----	2.2	0.46	-1.6
		30	4.64	4.22	30.91	----	2.1	0.55	-1.6
		40	5.41	4.90	-----	----	6.2	0.95	----
549	30.8.74	1	6.37	5.72	-----	----	---	----	----
	69°56'N	5	6.70	5.81	6.66	----	1.1	0.00	2.0
	135°48'W	10	6.30	5.62	27.57	----	2.8	0.42	-1.3
		20	5.58	5.01	29.77	----	3.2	0.53	----
550	30.8.74	1	6.87	6.38	-----	2.37	---	----	----
	70°21'N	5	5.59	5.73	19.59	0.37	1.4	0.31	-1.0
	136°36'W	10	6.17	5.41	27.39	0.70	1.2	0.42	-1.4
		20	5.42	4.96	29.98	0.14	2.0	0.49	-1.6
		30	5.14	4.74	30.76	0.10	2.6	0.66	-1.6
		55	5.09	4.26	32.12	0.13	7.2	1.08	-1.4

Station	Date Location	Depth	Log_{10} CFU/litre Marine Agar	PCA	Salinity (‰)	Chlorophyll A (mg/m^3)	Nitrate (µg at/l)	Phosphate (µg at/l)	Temp (^0C)
551	30.8.74	1	6.65	5.99	-----	----	---	----	----
	70°07'N	5	6.68	5.76	16.66	----	1.5	0.24	-0.8
	136°50'W	10	6.18	5.54	28.20	----	3.0	0.64	-1.4
		20	5.28	4.99	30.40	----	3.2	0.60	-1.6
		35	5.16	4.58	31.63	----	5.5	0.75	-1.5
552	31.8.74	1	7.00	6.16	-----	----	---	----	----
	69°56'N	5	6.95	6.31	10.59	----	0.5	0.00	-0.6
	137°05'W	10	6.44	5.51	26.26	----	2.6	0.40	-1.3
		20	5.57	5.06	30.25	----	3.2	0.46	-1.6
		30	4.97	4.65	31.44	----	4.7	0.55	-1.6
		40	5.46	4.95	-----	----	8.0	0.64	----
553	31.8.74	1	6.53	5.65	-----	0.28	---	----	----
	70°05'N	5	6.81	5.71	16.26	0.50	2.4	0.00	0.7
	139°08'W	10	6.14	5.33	28.16	0.11	2.6	0.29	-1.4
		20	5.44	5.03	30.22	0.03	2.4	0.18	-1.6
		50	4.80	4.48	31.75	0.11	5.4	0.90	-1.5
		70	4.80	3.82	32.15	0.08	7.7	0.97	-1.5
		100	4.82	4.07	32.38	0.11	12.4	1.26	-1.5
		190	4.82	3.22	33.98	0.02	12.9	0.84	-0.5
554	1.9.74	1	6.66	5.82	-----	----	---	----	----
	69°47'N	5	6.66	5.84	6.99	----	1.0	0.00	1.9
	138°56'W	10	6.08	5.37	29.04	----	3.8	0.24	-1.4
		20	5.62	4.86	31.06	----	4.9	0.49	-1.6
		50	4.75	4.43	32.42	----	9.1	0.93	-1.5
		70	5.07	4.40	32.44	----	9.9	1.10	-1.5
		90	5.07	4.56	32.45	----	9.7	0.95	-1.5
555	1.9.74	1	6.79	5.73	-----	----	---	----	----
	69°45'N	5	6.38	5.53	23.26	----	3.5	0.22	-0.5
	139°37'W	10	6.20	5.33	29.56	----	3.9	0.38	-1.5
		26	4.99	4.65	31.77	----	6.7	0.71	-1.6

Station	Date Location	Depth	Log_{10} CFU/litre Marine Agar	PCA	Salinity (‰)	Chlorophyll A (mg/m^3)	Nitrate (µg at/l)	Phosphate (µg at/l)	Temp (^0C)
556	1.9.74	1	6.79	5.69	-----	----	---	----	----
	69°27'N	5	6.88	5.76	7.81	----	1.3	0.00	2.5
	138°49'W	10	6.09	5.35	29.56	----	4.6	0.44	-1.3
		20	5.64	4.99	31.49	----	6.1	0.64	-1.6
		30	5.26	4.78	32.27	----	7.7	0.66	-1.6
		50	5.23	4.86	32.39	----	8.3	0.53	-1.6
557	2.9.74	1	6.09	5.71	-----	0.93	---	----	----
	69°36'N	5	6.69	5.61	2.75	1.00	1.2	0.15	4.6
	138°21'W	10	6.11	5.29	27.92	0.06	3.4	0.31	-1.3
		20	5.20	4.85	31.12	0.0	4.5	0.46	-1.6
		50	4.85	4.73	32.21	0.10	9.3	0.93	-1.5
		70	4.78	4.60	32.32	0.16	9.1	0.88	-1.5
		120	4.67	4.54	32.48	0.05	11.9	1.06	-1.5
558	2.9.74	1	6.19	5.72	-----	----	---	----	----
	69°33'N	5	5.85	5.63	<5.0	----	1.4	0.00	2.9
	136°58'W	10	6.12	5.42	26.98	----	4.8	0.33	-1.2
		18	5.65	5.08	-----	----	5.0	0.38	----
559	2.9.74	1	----	5.48	-----	----	---	----	----
	69°60'N	5	6.42	5.46	<2.0	----	1.6	0.00	3.6
	135°21'W	10	5.53	5.36	26.58	----	3.0	0.29	-1.2
		20	5.49	5.13	29.79	----	3.1	0.44	-1.5
		30	5.51	5.15	-----	----	4.1	0.42	----

Station	Date Location	Depth	Log_{10} CFU/litre Lib X	PCA	Salinity (‰)	Chlorophyll A (mg/m^3)	Nitrate (µg at/l)	Phosphate (µg at/l)	Temp (^0C)
565	17.6.75	1	6.73	6.72	13.36	1.46	2.3	0.04	2.3
	70°08'N	5	6.51	6.70	29.70	0.93	0.0	0.43	----
	132°37'W	10	----	4.67	30.86	0.75	0.0	0.56	-0.1
		20	5.78	4.52	31.47	2.94	0.0	1.00	-1.3
		30	5.56	4.00	31.60	9.20	3.6	1.26	-1.6
566	5.7.75	1	7.30	7.30	<2.8	0.00	0.2	0.09	0.1
	70°06'N	5	6.42	6.28	21.85	1.69	0.1	0.36	-1.3
	138°56'W	10	6.22	5.58	29.86	2.84	0.2	0.58	-1.5
		20	6.50	----	30.11	2.72	0.9	0.70	-1.6
		50	4.50	4.54	32.41	0.11	10.0+	1.64	-1.4
		75	3.92	----	32.91	0.09	10.0+	1.51	-1.4
		100	4.07	----	33.06	0.09	10.0+	1.49	-1.4
		200	4.50	----	33.55	0.06	10.0+	1.64	-1.1
		250	4.76	----	34.26	0.08	10.0+	1.26	-0.3
		315	4.78	----	34.61	0.07	10.0+	1.17	0.0
567	12.7.75	1	6.76	7.00	9.58	1.94	0.0	0.04	0.1
	70°49'N	5	6.15	5.69	26.78	0.16	0.6	0.46	-1.2
	136°22'W	10	5.12	5.20	29.25	0.20	0.5	0.50	-1.4
		20	4.67	4.99	30.62	0.26	1.9	0.79	----
		30	4.26	4.40	31.08	0.26	4.8	0.94	-1.6
		50	4.12	----	32.07	0.09	9.4	1.27	-1.5
		100	4.58	----	32.61	0.07	10.4+	1.35	-1.4
		300	4.30	----	34.76	0.02	10.4+	0.87	0.3
		500	4.22	----	34.84	0.00	10.4+	0.79	0.4
		720	4.07	----	34.85	0.07	10.2	0.81	0.1
568	18.7.75	1	7.00	----	5.42	1.03	0.1	0.02	0.8
	70°14'N	5	6.14	6.54	10.68	2.63	0.0	0.00	----
	139°04'W	10	6.39	6.28	29.23	1.22	0.1	0.55	-0.9
		20	5.23	4.95	30.73	0.52	0.2	0.61	----
		30	5.12	4.65	31.78	2.43	7.7	1.14	----
		50	4.99	4.87	32.23	0.22	10.4+	1.36	----
		75	4.73	----	32.90	0.11	10.4+	1.47	----
		100	4.22	----	33.14	0.06	10.4+	1.55	----

Station	Date Location	Depth	Log_{10} CFU/litre Lib X	PCA	Salinity (‰)	Chlorophyll A (mg/m^3)	Nitrate (µg at/l)	Phosphate (µg at/l)	Temp (^0C)
569	4.8.75	1	6.37	5.51	-----	----	---	----	----
	70°14'N	5	6.41	5.80	22.24	0.38	14.7	1.30	0.7
	139°04'W	10	5.78	5.45	28.60	0.54	0.1	0.15	-0.8
		20	4.70	4.40	-----	0.25	---	----	----
		50	4.80	4.70	32.16	0.26	12.1	1.83	-1.5
		70	4.90	----	-----	----	---	----	----
		100	4.86	----	32.85	0.10	16.0	2.24	-1.5
		300	5.22	----	34.64	0.18	12.9	1.38	0.0
		430	4.75	----	-----	0.12	---	----	----
570	5.8.75	1	6.65	5.74	11.60	0.37	0.0	0.00	5.0
	70°42'N	5	6.18	5.40	20.63	0.37	0.0	0.14	5.2
	134°45'W	10	5.41	4.88	25.46	0.58	0.0	0.04	2.0
		20	5.35	4.68	30.52	0.08	0.4	0.55	0.9
		50	5.11	4.26	31.55	0.03	0.9	0.76	-1.4
571	6.8.75	1	6.16	5.41	12.40	0.33	0.3	0.04	7.8
	70°02'N	5	6.24	5.29	12.52	0.49	0.1	0.03	7.6
	135°34'W	10	5.80	4.67	21.62	0.43	0.0	0.22	6.5
		20	4.70	4.43	30.24	0.19	0.3	0.88	-0.8
		30	5.43	4.50	31.68	0.15	5.8	1.22	-1.5
572	7.8.75	1	5.34	4.12	26.85	0.17	0.0	0.59	5.1
	70°56'N	5	5.52	3.82	26.86	0.27	0.0	0.48	5.1
	132°33'W	10	4.93	3.70	30.51	0.24	0.0	0.83	5.1
		20	4.73	4.00	31.42	0.28	0.0	0.54	3.4
		50	4.37	----	32.00	0.08	0.7	1.08	-1.5
		60	4.87	----	32.27	0.31	8.9	2.21	-1.5
573	7.8.75	1	5.15	4.00	-----	0.16	---	----	----
	71°22'N	5	4.60	----	30.36	0.13	0.0	0.63	5.4
	130°24'W	10	4.18	4.00	30.71	0.14	0.0	0.63	6.0
		20	4.37	3.70	31.45	0.19	0.0	0.87	1.1
		50	4.37	3.92	32.37	0.23	6.6	1.80	-1.5
		66	5.14	----	-----	----	---	----	----

Station	Date Location	Depth	Log_{10} CFU/litre Lib X	PCA	Salinity (‰)	Chlorophyll A (mg/m^3)	Nitrate (µg at/l)	Phosphate (µg at/l)	Temp (^0C)
574	8.8.75	1	----	4.00	18.72	0.32	0.0	0.24	7.8
	70°04'N	5	5.81	4.00	18.73	0.51	0.0	0.31	7.8
	132°17'W	10	4.82	3.52	25.89	0.31	0.0	0.56	7.2
		20	4.90	4.00	29.15	0.16	0.0	0.53	5.1
		27	5.45	4.00	-----	0.09	---	----	----
575	9.8.75	1	6.43	6.07	18.01	0.94	0.1	0.36	4.4
	69°56'N	5	6.29	6.06	21.63	2.06	0.2	0.46	3.7
	138°56'W	7	6.29	5.58	21.82	2.40	0.2	0.58	3.7