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Effects of Petroleum Releases on Bacterial Numbers and Microheterotrophic Activity in the Water and Sediment of an Arctic Marine Ecosystem

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ABSTRACT. The effects of a petroleum slick and chemically dispersed petroleum on bacterial numbers and microheterotrophic activity (uptake of glutamic acid by heterotrophic microorganisms) were monitored in the water column and sediments of selected bays at Cape Hatt, Northwest Territories. Observations were made between 1980 and 1983 as a component study of the Baffin Island Oil Spill (BIOS) Project. These data were augmented by measurements of chlorophyll *a*, particulate and dissolved organic carbon and inorganic nutrients in the water column, while total organic carbon (TOC) was measured in the sediments in some years.

Petroleum was released on two occasions in 1981. In the first release, nondispersed petroleum moved across the surface of test Bay 11 and adhered to the intertidal sediments at low tide. No significant effects were seen in chemical or microbiological variables measured in 1981 or 1982.

During the second release in 1981, dispersed petroleum was carried by the current through the water of test Bays 9 and 10 and into the channel beyond. Measurements of V_{max} (maximum velocity) of glutamic acid uptake in water samples taken in these bays during the release showed a transient decrease in V_{max} compared with control Bay 7. Bacterial numbers were unaffected, as were variables measured in the sediment of test Bays 9 and 10 during and after the release. *In vitro* experiments with water samples demonstrated that a combination of petroleum and dispersant or dispersant alone reduced the V_{max} of glutamic acid uptake to a greater extent than petroleum alone.

A bay-year analysis of variance between 1981 and 1982 demonstrated that TOC and bacterial numbers increased in the sediments of test Bay 9 over 1981, while the V_{max} of glutamic acid uptake remained constant. In control Bay 7 and test Bay 11, all variables decreased over 1981 except TOC in Bay 11. In 1983, trends in the sediments of Bay 9 were similar to those of Bay 7.

In test Bay 11, petroleum beached on the intertidal zone in 1981 was observed entering subtidal sediments between 1981 and 1983 and forming a decreasing gradation of petroleum concentrations from nearshore to offshore areas. TOC increased between 1982 and 1983. Microheterotrophic activity remained constant in Bay 11, although it decreased in Bays 9 and 7. Bacterial numbers increased in Bay 11 but decreased in Bays 9 and 7. It was concluded that the changes in Bay 9 in 1982 and in Bay 11 in 1983 were a consequence of perturbations by petroleum. Effects on the benthic macrofauna and flora increased the levels of detritus, and hence TOC, in the sediments. This caused changes in bacterial numbers and microheterotrophic activity.

Key words: Arctic, marine, bacteria, microheterotrophic, petroleum, sediment, oil spill, effects, dispersant

RÉSUMÉ. A Cape Hatt, T.N.-O., certaines baies ont été choisies pour y mesurer les effets produits par une nappe de pétrole ou du pétrole dispersé chimiquement, sur le nombre de bactéries et l'activité microhétérotrophe (incorporation de l'acide glutamique par les microorganismes), dans la colonne d'eau et les sédiments. Les observations ont été faites entre 1980 et 1983 dans le cadre d'une des études constituantes du projet BIOS (Baffin Island Oil Spill). Les données ont été complétées par des mesures de la chlorophylle *a*, du carbone organique dissout et particulaire et d'éléments nutritifs inorganiques dans la colonne d'eau. Certaines années, on a aussi mesuré le carbone organique total (TOC) dans les sédiments.

Deux déversements ont eu lieu en 1981. La première fois, le pétrole sans dispersant s'est déplacé à la surface de la baie expérimentale 11 pour se fixer aux sédiments de la zone intertidale à marée basse. Aucun effet significatif n'a pu être observé au niveau des paramètres chimiques ou microbiologiques mesurés en 1981 ou 1982.

Lors du deuxième déversement en 1981, le courant a transporté le mélange de pétrole et de dispersant à travers les eaux des baies expérimentales 9 et 10 et plus loin dans le chenal. Des mesures du V_{max} (vélocité maximum) d'incorporation de l'acide glutamique, faites à partir d'échantillons d'eau prélevés dans ces baies durant le déversement, ont révélé une diminution temporaire du V_{max} par rapport à celui mesuré dans la baie de contrôle 7. Le nombre de bactéries, ainsi que les paramètres mesurés dans les sédiments des baies expérimentales 9 et 10 pendant et après le déversement, n'ont pas varié. Des expériences effectuées *in vitro* sur des échantillons d'eau, ont montré qu'un mélange de pétrole et de dispersant ou le dispersant seul, réduisaient davantage le V_{max} d'incorporation de l'acide glutamique que le pétrole seul.

Une analyse de variance baie-année effectuée sur les données de 1981 et 1982 a démontré que le TOC et le nombre de bactéries ont augmenté par rapport à l'année 1981 dans les sédiments de la baie expérimentale 9 alors que le V_{max} d'incorporation de l'acide glutamique est demeuré constant. Dans la baie de contrôle 7 et dans la baie expérimentale 11, tous les paramètres, sauf le TOC dans la baie 11, ont diminué comparativement à l'année 1981. En 1983, les tendances dans les sédiments de la baie 9 étaient semblables à celles observées dans la baie 7.

Entre 1981 et 1983, on a pu observer que le pétrole, échoué sur la zone intertidale de la baie expérimentale 11 en 1981, pénétrait les sédiments de la zone infratidale et formait un gradient décroissant de concentrations de pétrole de la côte vers le large. Dans la baie expérimentale 11, le TOC s'est accru entre 1982 et 1983. L'activité microhétérotrophe y est demeurée constante bien qu'elle ait diminué dans les baies 9 et 7. Le nombre de bactéries a augmenté dans la baie 11 mais a diminué dans les baies 9 et 7. Il a été conclu que les variations enregistrées en 1982 dans la baie 9 et en 1983 dans la baie 11 résultaient de perturbations causées par la présence du pétrole. Les effets néfastes pour la macrofaune et la flore benthiques ont accru la quantité de détritus, donc le TOC dans les sédiments, entraînant ainsi des variations dans le nombre de bactéries et l'activité microhétérotrophe.

Mots clés: Arctique, marin, bactérie, microhétérotrophe, pétrole, sédiment, déversement de pétrole, effets, dispersant

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INTRODUCTION

In the marine environment, bacteria and other microheterotrophs play a major role in the breakdown of complex organic material and in the assimilation and utilization of simple organic molecules. The ability of microheterotrophs to degrade components of petroleum is well documented (Atlas, 1981), as is their response to an influx of petroleum. Some studies have addressed the effects of petroleum or its components on the physiological activities of microheterotrophs (Alexander and Schwarz, 1980; Bakke *et al.*, 1982; Bauer and Capone, 1985; Hodson *et al.*, 1977), particularly in an arctic environment (Griffiths *et al.*, 1981a,b). A growing body of literature on effects of petroleum and petroleum fractions on aquatic microorganisms has generated several reviews on the topic (Atlas, 1985; Baker and Griffiths, 1984; Pfaender and Buckley, 1984; Vestal *et al.*, 1984). No studies, however, have monitored the short- and

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long-term effects of experimentally released petroleum on microbiological parameters in an arctic marine environment.

One of the principal objectives of the Baffin Island Oil Spill (BIOS) Project (Sergy and Blackall, 1987) was to compare the fate and effects of a surface release of petroleum with that of chemically dispersed petroleum on a nearshore arctic area. Snow *et al.* (1987) provide background biological information on the site of the project and compare it to other areas. They also discuss in detail the overall strategy of the biological studies of the project and critically review their experimental design. The study reported here was one of the biological components of the BIOS Project and determined the short- and long-term effects of petroleum releases on bacterial numbers and microheterotrophic activity in arctic marine waters and sediments. A report of all chemical data obtained during the years 1981 and 1982 can be found in Bunch *et al.* (1985).

METHODS

Study Area

The study area is seen in Figure 1. It consists of a series of bays along the western coast of the Cape Hatt peninsula on northern Baffin Island. These were chosen as experimental sites because of their similarity, which enabled some to be used as "controls," and because released petroleum could be relatively easily controlled. They were also considered representative of the nearshore Arctic (Snow *et al.*, 1987). More detailed descriptions are available in Sempels (1987), Cretney *et al.* (1987a,b,c) and Dickins (1987).

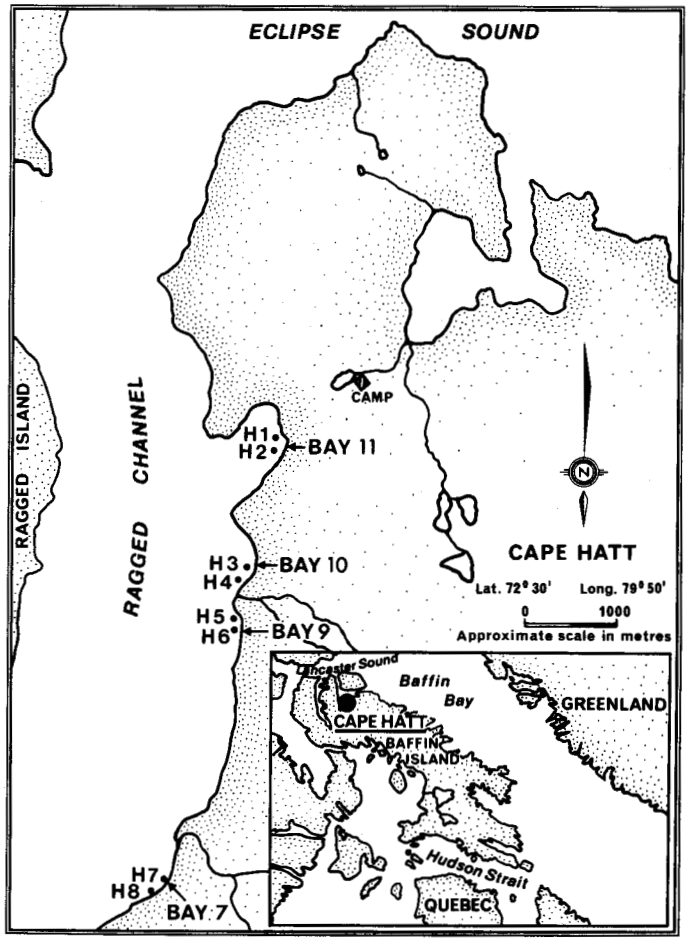

FIG. 1. Microbiological stations occupied between 1980 and 1983 at Cape Hatt, N.W.T. Stations were positioned on the map by W.J. Cretney.

During open water, water samples were collected from the numbered stations in Bays 9, 10 and 11 between 1980 and 1982. Because of concern about possible contamination of Bay 10 (control bay) during the petroleum releases, Bay 7 was chosen as an alternative control bay in 1981. Water samples were collected from numbered stations in Bay 7 in 1981 and 1982. Sediments were collected at all stations between 1981 and 1983 except Bay 10, which was not sampled in 1983.

A Venezuelan petroleum was obtained from Esso Resources Canada Ltd. and artificially weathered 8% by volume by aerating it with an air pump to remove part of the light volatile fraction (Dickins *et al.*, 1987). Fifteen cubic metres of this petroleum crude was pumped onto the surface of Bay 11 on 19 August 1981, near high tide. The release area was contained by booms, and prevailing winds drove the surface slick onto the beach as planned. Petroleum not beached was removed from the water surface by the Canadian Coast Guard. At low tide, the intertidal zone of the beach within the contained area was uniformly coated with petroleum from the release.

On 27 August, 16 m^3 of a mixture (10:1 by volume) of the same petroleum crude and Corexit 9527, a chemical dispersant obtained from Exxon Chemical Co., were discharged from a dispersion pipe located perpendicularly to the shore and suspended 1 m above the sediment at the south end of Bay 9. The petroleum-dispersant mixture moved north through the water column across Bays 9 and 10, including the areas of stations 6, 5, 4 and 3, and out into Ragged Channel. Details of the releases can be found in Dickins *et al.* (1987).

Sampling Protocol

Water Column: Water samples from depths of 1, 5 and 10 m were collected at all microbiology stations using a 5.0 *l* Niskin bottle and hand line. The samples were transferred to 4.0 *l* bottles and transported in a sample box to the Cape Hatt laboratory, where they were refrigerated and processed for analyses within several hours of collection. Water collections from all stations in all bays occurred across a two-day period at intervals of approximately one week. Water samples were collected immediately before, during and after the release of dispersed petroleum. Samples were also collected before and after the surface release of petroleum.

Sediment: Sediments were collected at all stations by divers using modified 50 ml disposable syringes. For each station sampled, seven syringes on average were filled with surface sediments and capped for transportation back to the laboratory. Upon arrival, they were sorted and left to settle in a refrigerated area until processed. The top centimetre of all sediment cores taken from one station were combined and homogenized in a sterile Whirlpak bag (Fisher Scientific). With a 50 ml disposable syringe cut off at the end, a 20 ml wet subsample was measured and suspended in 2 *l* of filter-sterilized water taken from a depth of 10 m. The 1.0% v/v sediment suspension was then manually agitated and the suspension maintained in a crushed-ice bath on a magnetic stirrer while being processed. Remaining homogenized sediments were frozen and later shipped to Ste-Anne-de-Bellevue for total organic carbon and dry weight determinations. Sediments were collected from all stations during one or two days at intervals of approximately one week.

Dry Weight Determinations

For each of the thawed sediment samples, three 1 ml subsamples were measured with a modified 3 ml disposable syringe, put into pre-weighed aluminum boats and dried at 60°C in an oven. After 48 h, the samples were transferred into a desiccator for a period of 30 min and weighed. The results were averaged.

Temperature and Salinity

The temperature of water samples was measured with a laboratory mercury thermometer as soon as they were brought to the surface. Samples for salinity determinations were shipped to the Arctic Biological Station for analysis. Salinities were measured with a Bissett-Berman model 6230 laboratory salinometer. Temperature and salinity of all microbiological water samples were routinely monitored.

Inorganic Nutrients

Duplicate samples of 100 ml for nitrate and phosphate determinations were placed in 125 ml Boston Round bottles. All samples were frozen and shipped to the Arctic Biological Station for analysis.

Immediately before analysis, samples were thawed in a warm water bath. Reactive phosphate and nitrate analyses were carried out in a Technicon Autoanalyzer II. The procedure of Technicon Industrial Systems (1973) was used for phosphate determinations. This procedure is essentially an automated version of the standard technique described by Strickland and Parsons (1972). The limit of detection for phosphate was 0.02 $\mu mol \cdot l^{-1}$. An automated method of the United States Environmental Protection Agency (1979) involving hydrazine reduction was used for determination of reactive nitrate. This procedure was modified to the extent of increasing the concentration of hydrazine phosphate to increase sensitivity. The limit of detection for nitrate was 0.06 $\mu mol \cdot l^{-1}$.

Chlorophyll a

One-litre volumes of sample water were filtered through 0.45μm membrane filters (Millipore HAWPO 4700). Filters were fixed with 1.0 ml of $MgCO_3$ and placed in small Petri dishes, which were wrapped in aluminum foil, frozen and shipped to the Arctic Biological Station for analysis. Chlorophyll *a* was determined spectrophotometrically by the standard method described by Strickland and Parsons (1972). Values of chlorophyll *a* were calculated using the equation of Jeffrey and Humphrey (1975).

Organic Carbon

Water and sediment samples were analyzed for organic carbon by a procedure that modified and combined those of Menzel and Vaccaro (1964) and Stainton (1973). Aliquots of 100 ml of freshly collected water samples were filtered through pre-ashed (500°C) Whatman GF/C 24 mm glass filters contained on acid-washed glass filter holders. Filters and filtrates were immediately frozen for subsequent analysis at the Arctic Biological Station.

Dissolved organic carbon (DOC) was determined by measuring a 20.0 ml aliquot of the thawed filtrate into a pre-ashed glass ampule. Inorganic carbon was removed from the sample by adding 0.05 ml of perchloric acid and purging with a stream of nitrogen for 10 min. Immediately following the gas purge, 0.1 g of potassium persulfate was added and the ampule was flame sealed. The ampules were then heated to 121°C for 1 h in an autoclave to effect the wet oxidation of organic carbon.

For analysis, each ampule was opened as required and the contents transferred to a 50 ml disposable syringe, to which 1.0 ml of 2.0 N H_2SO_4 and 30.0 ml of helium were added. The syringes containing the samples were shaken for at least 5 min. The evolved carbon dioxide-helium mixture was injected into a Hewlett-Packard 5700A gas chromatograph fitted with a high-temperature nickel catalytic oven, where the carbon dioxide was catalytically reduced to methane in a continuous stream of hydrogen. Methane was quantitated by flame ionization and the response recorded on a Hewlett-Packard 3380 recorder-integrator. Values obtained were corrected for sample size and expressed as DOC per litre of sample volume. The analytical procedure was calibrated with prepared glucose standards. The limit of detection for DOC was 54.0 $\mu g\ C \cdot l^{-1}$.

Particulate organic carbon (POC) was determined from the particulate material retained by the filters used for the filtration of DOC samples. Filters containing the particulate samples were placed in pre-ashed glass ampules with 20.0 ml of prepared water containing a known low amount of carbon. The subsequent procedure was identical to that described for DOC determinations. The limit of detection for POC was 20 $\mu g\ C \cdot l^{-1}$.

Total organic carbon (TOC) determinations in the sediments were made following a procedure slightly modified from that used for POC samples. Three 1 ml subsamples of each sediment were dried and reduced to powder by means of a pestle and mortar. Three to 10 mg of the sample powder were added to a pre-ashed and tared ampule and the exact weight determined. After the addition of 20.0 ml of prepared water supplemented with 0.2 ml of perchloric acid, the subsequent procedure was identical to that for POC. Values obtained were corrected for the amount of sediment employed in the analyses, and TOC was expressed as percent carbon.

Total Counts of Bacteria

Total counts of bacteria in sea water and sediments were made using a procedure slightly modified from that described by Watson *et al.* (1977). Polycarbonate membrane filters (Nucleopore Corp.) of pore size 0.2 μm and 25.0 mm diameter were prestained in a 0.2% solution of irgalan black in 2.0% v/v acetic acid. The filter was then rinsed in cell-free distilled water and placed on a 25.0 mm glass filter holder (Millipore Corp.). A sea water or sediment suspension sample of 2.0-15.0 ml, fixed at the time of collection with 0.2% gluteraldehyde, was added to a test tube after shaking on a vortex mixer. Sufficient acridine orange (80% dye content), at a concentration of 0.1% in 0.02 mol tris (tris-hydroxymethylaminomethane, pH 7.2), was added to the sample to yield a final stain concentration of 0.02%. After 2 min, the sample was filtered and rinsed with 5.0 ml of cell-free water. The membrane was then placed on a glass slide, wetted with a drop of Cargille Type A immersion oil and covered with a cover glass. A Zeiss model WL microscope equipped with an epifluorescent condenser, a 50 watt mercury lamp, a BG 12 excitation filter, a No. 50 barrier filter and a No. 500 beam splitter was used to view the fluorescing cells. For counting, a reticule with a 10 mm grid was used. A sufficient amount of sea water or sediment suspension was filtered to yield about 100 cells per grid field. Ten randomly selected grid fields on each sample membrane were counted and the mean value expressed

as the number of cells per litre of sea water or gram dry weight of sediment.

Microheterotrophic Activity

An extensive modification of the procedure described by Harrison *et al.* (1971) was employed throughout this study. Water and sediment samples were collected, as previously described, and processed immediately. To measure substrate assimilated and retained by microheterotrophs, 10.0 ml of water sample or sediment suspension was added to 14 chilled and sterile 55 ml screw-cap bottles containing varying amounts of glutamic acid substrate. Seven varying ratios of labelled to unlabelled glutamic acid were used in these supplements, yielding duplicate vessels containing total glutamic acid concentrations of 1.0-60.0 $\mu g \cdot l^{-1}$ of sample water to excess of saturation and activity of 2.0 or 20.0 $\mu Ci \cdot l^{-1}$. The specific activity of the L-[^{14}C(U)]-glutamic acid (New England Nuclear Corp.) was approximately 293.0 $mCi \cdot mmol^{-1}$. A fifteenth vessel containing 1.0 $\mu g \cdot l^{-1}$ of glutamic acid with 2.0 $\mu Ci \cdot l^{-1}$ of radioactivity served as a background control for the seven concentrations. Upon addition of the sea water or sediment suspension aliquot, the reaction volume of the control vessel was immediately filtered through a 25.0 mm membrane filter (Millipore Corp.) with a pore size of 0.45 μm and rinsed twice with 15.0 ml portions of cold, filtered sea water. The 14 vessels were incubated at 2.0°C for 9.0 or 18.0 h, depending on the expected magnitude of activity. Suspended sediment samples were incubated at 2.0°C for 4 h. Incubation was stopped by simultaneous filtration of the 14 vessels, followed by cold rinsing. Rinsed membranes were transferred to scintillation vials containing 1 ml of 2-ethoxyethanol (BDH Chemicals Ltd.).

To measure substrate mineralized by microheterotrophs, fifteen 50 ml serum bottles were prepared with substrate and sample water or sediment suspension as above. Upon addition of the sample to the control vessel, 0.2 ml of 5.0 N H_2SO_4 was immediately added to reduce the pH of the sample to below 2.0. Bottles were stoppered with serum caps fitted with plastic reaction wells (Kontes Glass Co.). The wells, suspended above the reaction volume, contained a fluted wick of two glass filters (Whatman GF/A-24 mm). After incubation as above, the reaction in the serum vessels was stopped by the addition of 5.0 N H_2SO_4 through the rubber serum cap by means of a syringe. At the same time, 0.2 ml of β-phenethylamine (New England Nuclear Corp.) was added through the cap into the plastic well, where it was completely absorbed by the wick. The bottles were then further incubated for 12 h at 40.0°C, during which time $^{14}CO_2$ was evolved from the sample and absorbed by the phenethylamine-soaked wicks. The bottles were then opened and wicks were transferred to scintillation vials containing 8.0 ml of Aquasol, a scintillation fluor obtained from New England Nuclear Corp. Scintillation vials were transported to Ste-Anne-de-Bellevue. Before counting, 7 ml of Aquasol was added to scintillation vials containing membrane filters. Counts were corrected for quench by the method of channel ratio. Results of membrane and wick counts were combined to yield a total uptake of the glutamic acid substrate. Uptake kinetics were generated using computer programs.

Where uptake incubations were supplemented by petroleum, weathered Norman Wells petroleum crude obtained from Esso Resources Canada Ltd. was employed. An open volume of the petroleum was maintained in a forced draft at room temperature until the volume was reduced by 30%.

Theory: Kinetic parameters from the uptake of the glumatic acid substrate were calculated from a modified Michaelis-Menten equation (Dowd and Riggs, 1965):

$$\frac{D\mu t}{d} = \frac{(K+S)}{V_{max}} + \frac{A}{V_{max}}$$

where $D\mu$ = radioactivity added, d = radioactivity taken up, t = incubation time in hours, K = an uptake constant, S = concentration of the natural substrate, V_{max} = the maximum velocity of uptake and A = concentration of the substrate added. Plotting $\frac{(D\mu t)}{d}$ against A yields a straight line where the reciprocal of the slope = V_{max}. The maximum velocity (V_{max}), or potential of heterotrophic activity, is the velocity of uptake at which the substrate saturates the uptake system such that the velocity can no longer increase. V_{max} is an indication of the physiological state of the microheterotrophic flora in that it demonstrates the potential ability of the flora to use a particular substrate — i.e., its degree of adaptedness to that substrate.

Uptake and assimilation of a radioactive amino acid also denotes conversion of dissolved organic carbon to particulate microheterotrophic biomass (i.e., growth and multiplication). Measurement of released $^{14}CO_2$ provides an estimate of mineralization of an amino acid substrate to CO_2 and ammonia.

Statistical Analyses

Kinetic parameters of glutamic acid uptake and their correlation coefficients were generated using computer programs developed by D. Burrage (Université du Québec à Montréal) and J.N. Bunch. Multiway analysis of variance (Sokal and Rohlf, 1969) was employed to study the effects of petroleum and spatial and temporal variation on bacterial parameters. All variates were transformed to common logarithms. Unless otherwise stated, the null hypothesis (no effect) was rejected when the probability of its being true was less than 1% (p 0.01).

For the study of 1982 variation, data were grouped by bay and sampling interval and a two-way analysis of variance was carried out. Petroleum effects occurring in 1982 were sought by combining data from 1981 and 1982 and conducting a two-way analysis of variance on values grouped by bay and year. A significant bay-year interaction was interpreted as a potential indicator of a petroleum-related effect. All statistical analyses were carried out using either ANOVA or GLM procedures of the SAS computer package (SAS Institute Inc., 1985) available through the McGill University Computing Centre.

RESULTS

The Water Column

Temperature and Salinity: Temperature and salinity were measured in the bays at intervals across the sampling seasons of 1981 and 1982 in conjunction with microbiological sampling (Figs. 2 and 3). Values were similar across all bays, including the intervals after the petroleum releases in 1981. A more extensive treatment of the temperature and salinity regimes during 1980 and 1981 can be found in Buckley *et al.* (1987).

In 1981, temperatures varied over a very narrow range from –0.5 to 5.0°C. A maximum mean difference of 2.3°C was observed between 0 and 10 m during 3-5 August 1981. In 1982, the maximum mean difference was 3.2°C during 9-10 August. Stratification of the water column by temperature was, therefore, not significant from a biological point of view.

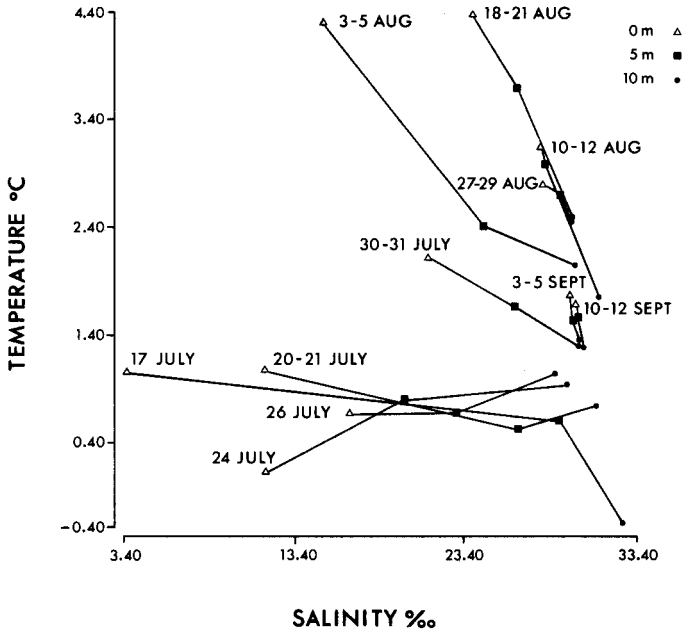

FIG. 2. Temperature-salinity (TS) diagram of the 1981 field season. Results are means for all bays.

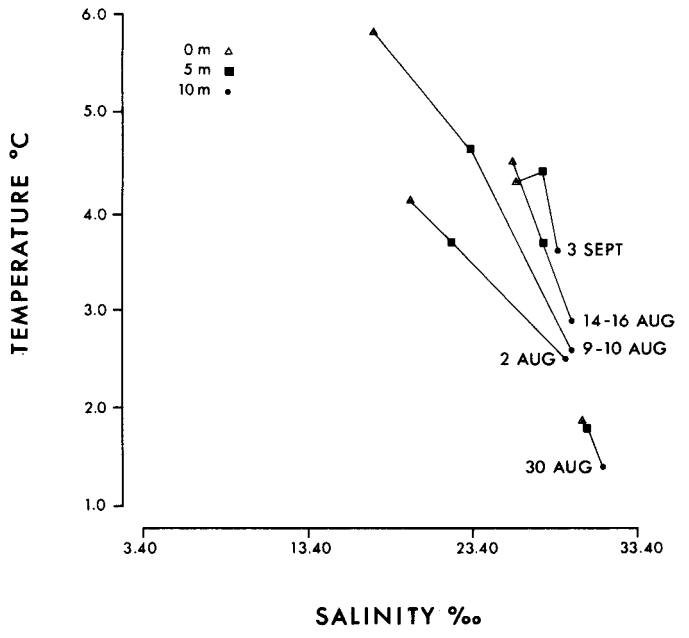

FIG. 3. Temperature-salinity (TS) diagram of the 1982 field season. Results are means for all bays.

Fresh water from melting sea ice and land run-off reduced salinity in surface waters. All bays were essentially clear of ice by late July in both years. In 1981, a maximum mean difference of 29.05‰ between 0 and 10 m was observed on 17 July. Water at 10 m showed little fluctuation in salinity throughout the sampling season (seasonal mean of 30.08 ± SE 0.13‰; n = 77). Observations began later in 1982, and the maximum mean difference between 0 and 10 m was smaller (11.97‰) than in 1981. The homogeneous water columns observed on 29-30 July were attributed to a windstorm on 29 July (Fig. 3).

The effect of a storm surge on temperature and salinity is seen in Figure 4. Bays 7 and 9 were sampled on 10 August 1981 and a windstorm followed. The cold, saline water observed in Bays 10 and 11 on 12 August was probably present throughout the entire study area at this time. A similar event occurred on 4 September. This displacement of water was also evident in other parameters measured.

Chlorophyll a: In 1981, values of chlorophyll *a* varied between 0.32 and 10.16 $\mu g \cdot l^{-1}$. The high values were obtained during the first sampling interval in mid-July during ice breakup (Fig. 5). In 1982, ice had cleared from the bays when the sampling season began, and low chlorophyll levels suggested that the bloom had already occurred. During August and into September in both years, the level of chlorophyll was relatively low and constant across all bays, although higher in 1981.

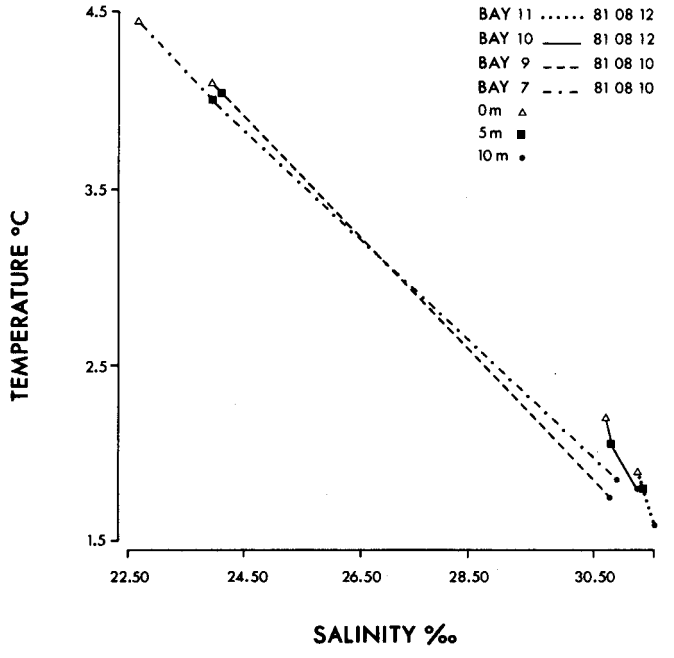

FIG. 4. Temperature-salinity (TS) diagrams for each bay during the period 10-12 August 1981. Results are presented as means of two stations in each bay.

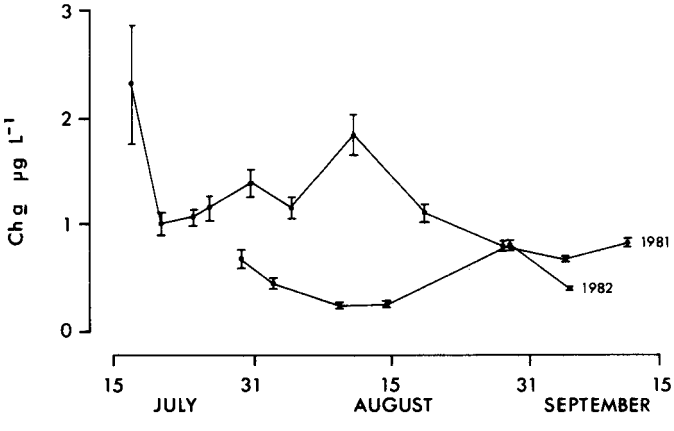

FIG. 5. Two-year comparison of chlorophyll *a* (Ch *a*) determined from water samples collected in 1981 and 1982. Results are presented as means and standard errors of values from all bays for each sampling interval.

Exceptions to this occurred during the storm surges, where displacement of the water masses in the bays by offshore water elevated the level of chlorophyll (see Fig. 5, 10-12 August 1981). Where a salinity gradient existed during July and August of either year, the greatest concentration of chlorophyll was at 10 m at the bottom of the water column.

Inorganic Nutrients: During open water, levels of inorganic nutrients, particularly nitrate, were uniformly low as a consequence of their depletion by phytoplankton growth (Fig. 6). Values were similar across all bays, including the sampling intervals after the petroleum releases in 1981. The seasonal means of nitrate in 1981 and 1982 were similar at 0.16 ± SE 0.02 $\mu mol \cdot l^{-1}$ (n = 468) and 0.15 ± SE 0.01 $\mu mol \cdot l^{-1}$ (n = 288), while phosphate at 0.28 ± SE 0.01 $\mu mol \cdot l^{-1}$ (n = 466) in 1981 compared to 0.50 ± SE 0.02 $\mu mol \cdot l^{-1}$ (n = 288) in 1982. By comparison, in May 1982, prior to a bloom of phytoplankton, the mean concentrations of nitrate and phosphate were 9.04 ± SE 0.43 $\mu mol \cdot l^{-1}$ (n = 8) and 1.69 ± SE 0.12 $\mu mol \cdot l^{-1}$ (n = 5) respectively (unpublished data). Increased levels of both phosphate and nitrate in the latter part of both years were probably due to vertical mixing. Storm surges previously noted also increased nutrient levels.

Dissolved and Particulate Organic Carbon: The level of dissolved organic carbon (DOC) was uniform across the bays during the sampling seasons of 1981 and 1982 (Fig. 7). With a seasonal mean of 2.15 ± SE 0.08 $mg \cdot l^{-1}$ (n = 90) in 1980, DOC was approximately twice as high as in the subsequent years.

Values of particulate organic carbon (POC) varied considerably during the three years, in part due to material resuspended in the water column following wind storms (Fig. 8). Seasonal means in the three consecutive years were very similar, at 162 ± SE 4 (n = 89), 183 ± SE 5 (n = 468) and 162 ± SE 15 $\mu g \cdot l^{-1}$ (n = 288). Neither variable was affected by residual petroleum in the sampling intervals after the petroleum releases.

Effect of Petroleum on Bacterial Numbers and V_{max} of Heterotrophic Uptake: Concentrations of petroleum in the water column following the two releases are detailed by Humphrey *et al.* (1987). During the surface release, petroleum did not penetrate the water column beyond 1 m, and concentrations observed under the slick were less than 2.0 $mg \cdot l^{-1}$ (Humphrey *et al.*, 1987). During the release of dispersed petroleum, concentrations measured in the microbiological samples averaged about 5.0 $mg \cdot l^{-1}$ away from the dispersion pipe in Bays 9 and 10. Humphrey *et al.* (1987) obtained an average of over 50.0 $mg \cdot l^{-1}$ of petroleum hydrocarbons from a large number of observations in the water column of Bay 9 during the release. By the next day, concentrations of petroleum had dropped to an average of 0.8 $mg \cdot l^{-1}$ and subsequently fell several orders of magnitude as it became dispersed throughout the area.

Numbers of bacteria in the waters of the bays before, during and after the petroleum releases in 1981 are seen in Figure 9. When sampling commenced in July, total counts of bacterial

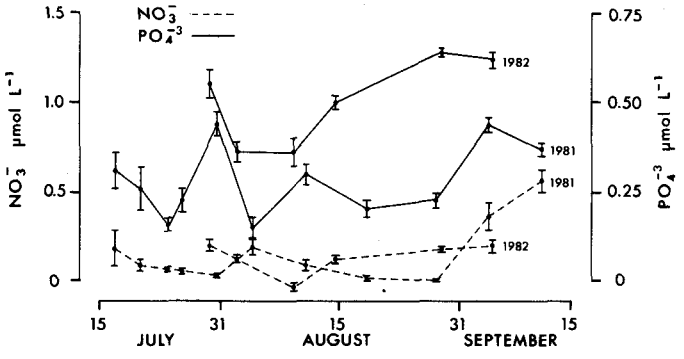

FIG. 6. Two-year comparison of nitrate (NO_3^{-1}) and phosphate (PO_4^{-2}) determined in water samples collected in 1981 and 1982. Results are presented as means and standard errors of values from all bays for each sampling interval.

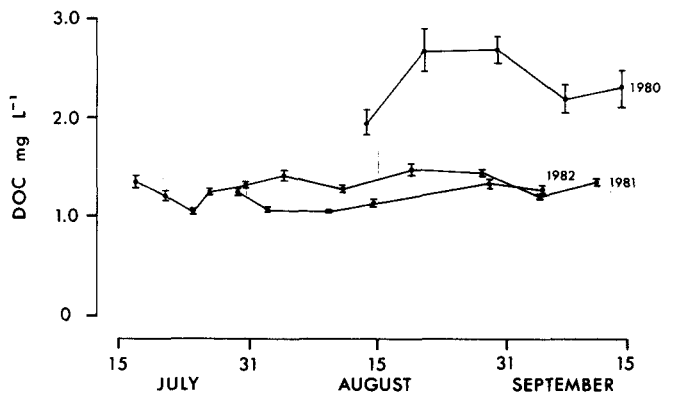

FIG. 7. Three-year comparison of dissolved organic carbon (DOC) determined in water samples collected between 1980 and 1982. Results are presented as means and standard errors of values from all bays for each sampling interval.

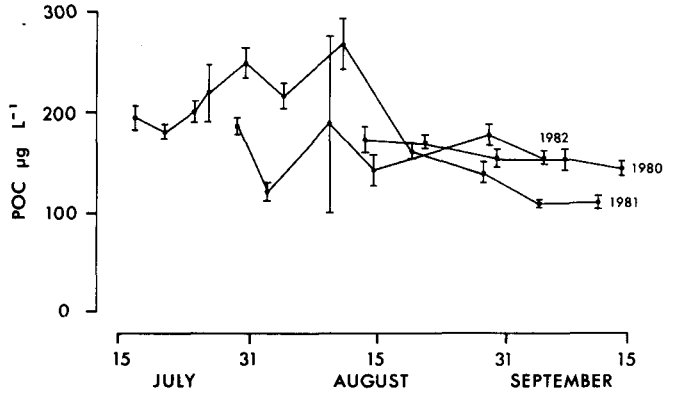

FIG. 8. Three-year comparison of particulate organic carbon (POC) determined in water samples collected between 1980 and 1982. Results are presented as means and standard errors of values from all bays for each sampling interval.

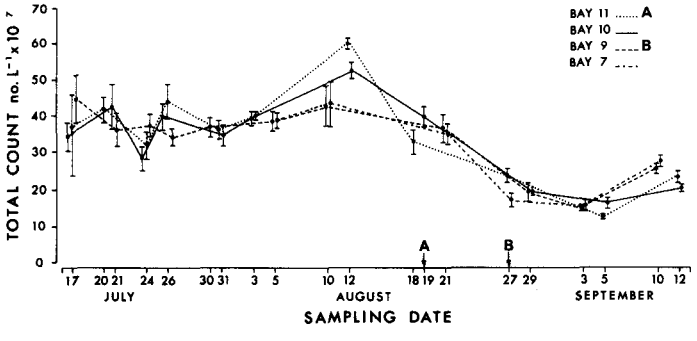

FIG. 9. Total counts of bacterial cells determined in water samples collected in 1981. Results are presented as means and standard errors of six values from three depths at two stations in each bay. Surface and dispersed releases are represented by A and B respectively.

cells were high relative to values found in late August and September, with a mean of $3.9 \times 10^8 \pm$ SE 0.4×10^8 cells·l^{-1} (n = 90) recorded in three bays. A maximum was reached in Bay 11 with $6.0 \times 10^8 \pm$ SE 0.1×10^8 cells·l^{-1} (n = 6) observed on 12 August. The high values in Bays 10 and 11 on that date were probably due to the storm surge previously mentioned. Subsequently, numbers decreased steadily in all bays during the last half of August to a mean of $1.5 \times 10^8 \pm$ SE 0.1×10^8 cells·l^{-1} (n = 24) recorded between 3 and 5 September. Samples taken during the last interval indicated a slight increase in all bays, although cell numbers did not reach levels observed earlier in the season when sampling started. No significant differences were seen between the bays when allowance was made for the storm surge on 12 August, and no significant bay-interval interactions were found.

The maximum velocity (V_{max}) of glutamic acid uptake by microheterotrophs in the waters of the bays during the year of the petroleum releases is seen in Figure 10. During the first sampling interval, values of V_{max} for each of the bays were near their respective recorded maximum, which was reached on 10-12 August for the four bays. At that time, V_{max} ranged between $4.2 \pm$ SE 0.4 (n = 6) and $6.5 \pm$ SE $0.2\ \mu g \cdot l^{-1} \cdot d^{-1}$ (n = 6). Following a subsequent drop in activity in all four bays, V_{max} increased to levels similar to those observed earlier in August. September values were characterized by a slow decline, with

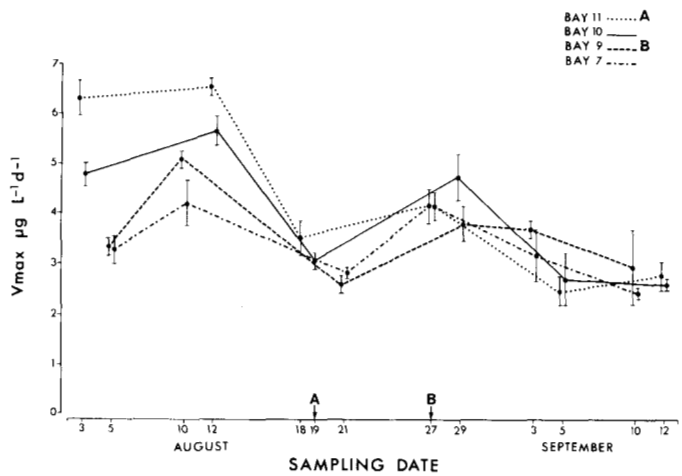

FIG. 10. Maximum velocity (V_{max}) of glutamic acid uptake determined in water samples collected in 1981. Results are presented as means and standard errors of six values from three depths at two stations in each bay. Surface and dispersed releases are represented by A and B respectively.

measurements equal to about half the seasonal maximum. With the first interval of sampling (3-5 August) included in an analysis of variance, significant differences in V_{max} were seen in the bays. In the absence of the first sampling interval from the analysis, no differences or interactions were observed.

Water collections were made in Bay 11 on 18 August as part of the second sampling interval. After the surface release of petroleum in Bay 11 on 19 August, a supplementary collection of water samples was made from that bay on 21 August. The uptake of glutamic acid was determined in these samples and results compared to those obtained on 18 August (Table 1). On 21 August the mean of V_{max} determined from six depths decreased by one-fourth when compared to the same depths on 18 August. When the data from the second, third and fourth

TABLE 1. Comparison of V_{max} of glutamic acid uptake before and after the surface release of petroleum[1]

Date	Station no.	Depth (m)	V_{max} ($\mu g \cdot l^{-1} \cdot d^{-1}$)
08 18	1	0	4.05
		5	3.83
		10	2.74
	2	0	4.15
		5	4.13
		10	2.15
08 21	1	0	2.03
		5	1.97
		10	3.04
	2	0	3.04
		5	3.43
		10	2.35

[1]Water samples were collected at Stations 1 and 2 in Bay 11 on 18 and 21 August 1981.

sampling intervals (including 18 August; see Fig. 10) from Bays 7 and 11 were compared by a three-way analysis of variance, no significant interactions were observed for V_{max}.

Water samples were collected from various depths in the water mass containing dispersed petroleum on the day of the dispersed petroleum release (27 August) and the day after. Replicate samples were supplemented with ^{14}C-glutamic acid and the V_{max} of uptake determined. These values and concentrations of hydrocarbons in the replicate samples are given in Table 2. In a three-way analysis of variance between Bays 7 and 9 for the three sampling intervals of 21 August, 29 August and 3 September, no interactions were observed for V_{max}. When data from 27 August were substituted for Bay 9 data of 29 August, a significant bay-interval interaction was determined when the high value of 14.45 from station 6 on 27 August was deleted

TABLE 2. Determinations of maximum velocity (V_{max}) of glutamic acid uptake from water samples collected at Cape Hatt on 27 and 28 August 1981[1]

Date	Sampling location*	Depth (m)	V_{max} ($\mu g \cdot l^{-1} \cdot d^{-1}$)	Field fluorometry ($mg \cdot l^{-1}$)	Fluorometry calibration ($mg \cdot l^{-1}$)
08 27	6τ	5	3.13	ND	ND
	6	4	14.45	2.10	0.53
	5	10	4.45	0.70	—
	Discharge pipe	10	1.38	—**	193, 238***
	4	10	3.05	0.80	0.64
	Centre Bay 9	4	1.09	17.00	—
08 28	5	11	3.82	0.45	—
	5	10	2.19	1.10	—
	6	9	1.92	0.41	0.69
	4	10	4.49	0.40	—
	4	8	3.51	0.95	0.77, 1.16***
	3	7	4.73	0.50	—

[1]Samples were collected at various points in Bays 9 and 10 before, during and after the dispersed petroleum release. Subsamples were analyzed for hydrocarbons by Seakem Oceanography Ltd.

τControl.

*Numbers refer to microbiology station numbers.

**Not reliable (saturated).

***Two determinations.

ND (no data)

from the analysis. No interactions were observed when the data from 28 August were similarly treated.

Across the sampling season of 1981, the mineralization of glutamic acid was measured in replicate sets of samples in the presence or absence of 0.1% v/v Norman Wells petroleum (artificially weathered approximately 30% by volume), 0.01% v/v Corexit 9527 or a mixture of both. In each instance, the V_{max} of mineralization of glutamic acid to CO_2 was derived (Table 3). In a one-way analysis of variance, no significant difference was seen in the presence or absence of petroleum, although changes were observed. In the presence or absence of Corexit 9527, significant differences were seen in V_{max} ($0.05>p>0.01$). In the presence or absence of petroleum and Corexit, a highly significant difference ($p<0.01$) was seen. No significant difference was seen between treatments of dispersant-petroleum mixture or dispersant alone.

TABLE 3. Mineralization of glutamic acid in the presence of 0.1% v/v weathered Norman Wells petroleum crude with and without Corexit 9527 supplementation[1]

	V_{max}* $\mu g \cdot l^{-1} \cdot d^{-1}$
Control	1.26 ± 0.20
Petroleum	0.79 ± 0.24
Corexit	0.52 ± 0.23
Petroleum + Corexit	0.35 ± 0.07

[1]Samples were collected from a depth of 5 m at Stations 3 and 7 at Cape Hatt during August and September 1981. Subsamples without supplementation served as controls.
*Means (and standard errors) of 5 samples.

The Sediment

Petroleum Concentrations: Concentrations of petroleum in the sediments at the microbiology stations before and after the releases are summarized by Boehm *et al.* (1987) and a detailed analysis is presented there. Stations in Bays 7 and 9 showed similar petroleum concentrations, with means of less than 0.32 and 4.85 $\mu g \cdot g^{-1}$ respectively. In 1982 the mean concentration in Bay 7 was 1.47 ± SE 0.18 $\mu g \cdot g^{-1}$ ($n=6$). With a value of 4.37 ± SE 0.62 $\mu g \cdot g^{-1}$ ($n=6$), the mean concentration of petroleum in Bay 9 was approximately four times higher in 1982 than in 1983. Values in 1982, however, were determined by ultraviolet fluorescence, a procedure that yields comparatively higher estimates than the gas chromatograph procedure employed in 1983. The data in 1983 (P. Boehm, pers. comm. 1984) suggested that petroleum was evenly distributed in the areas of the microbiology stations.

Total Organic Carbon: In each of the three years of sampling between 1981 and 1983, no significant differences in total organic carbon (TOC) were seen between stations within a bay or in each bay across the sampling season. The seasonal means of Bays 7, 9 and 11 for each of the three years are seen in Figure 11. In each of the years, the seasonal mean in Bay 7 was consistently higher than in either of the other two bays but was not significantly different between the years, although it decreased slightly. The TOC content of surface sediment in Bay 9 increased 34% between 1981 ($n=14$) and 1982 ($n=13$), and this resulted in a significant bay-year interaction when the data for the bays in the two years were combined. Between 1982 and 1983, organic carbon declined in the sediment of Bay 9 and the change was not significantly different from the change in Bay 7.

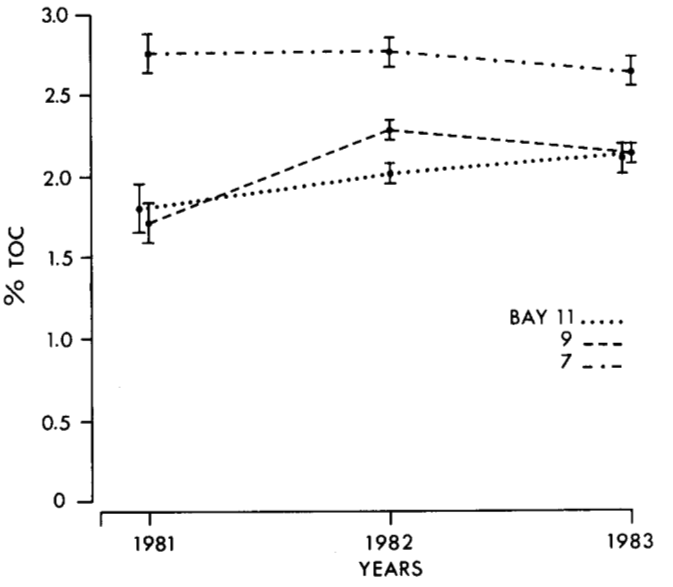

FIG. 11. Three-year comparison of percent total organic carbon (TOC) in surface sediments. Samples were collected in Bays 11, 9 and 7 in 1981, 1982 and 1983. Results are presented as seasonal means and standard errors of values from two stations in each bay.

The surface sediment of Bay 11 gave mean values of organic carbon that were not significantly different in any of the three years, although the mean increased slightly between 1981 and 1983. The difference in year-to-year changes among the bays did not result in a significant bay-year interaction in the combined data between 1982 and 1983.

In all samples collected after the petroleum releases in 1981, the levels of hydrocarbons determined by Boehm *et al.* (1987) were too low to affect the observed concentration of TOC. In most instances, the observed concentration of hydrocarbon components was below the limit of detection by the total organic carbon procedure.

Effect of Petroleum on Bacterial Numbers and V_{max} of Heterotrophic Uptake: No significant differences in bacterial numbers were seen between stations within a bay or in each bay across each sampling season from 1981 to 1983. The seasonal means of Bays 7, 9 and 11 in each of the three years is presented in Figure 12. A significant bay-year interaction was seen between 1981 and 1982 because the counts in Bay 9 increased significantly between the two years, while counts in the other two bays decreased. The bay-year interaction persisted ($0.05>p>0.01$) when the data of 1981 and 1983 were taken together. No bay-year interaction was found when the data in 1982 and 1983 were taken together. The mean value in Bay 11, however, increased slightly, from 95.1×10^7 ± SE 7.3×10^7 ($n=13$) to 100.9×10^7 ± SE 4.6×10^7 cells$\cdot g^{-1}$ ($n=6$) dry weight of sediment, while counts in the other two bays decreased.

Between 1981 and 1983, no significant differences in the maximum velocity (V_{max}) of glutamic acid uptake were seen between stations within a bay or in each bay across each sampling season. The seasonal means of V_{max} for the three years are summarized in Figure 13. Between 1981 and 1982, V_{max} was seen to decrease in Bays 7 and 11 but remained constant in Bay 9. Between 1982 and 1983, Bays 7 and 9 showed similar decreases in V_{max} and were not significantly different from each other in the two years. Bay 11, however,

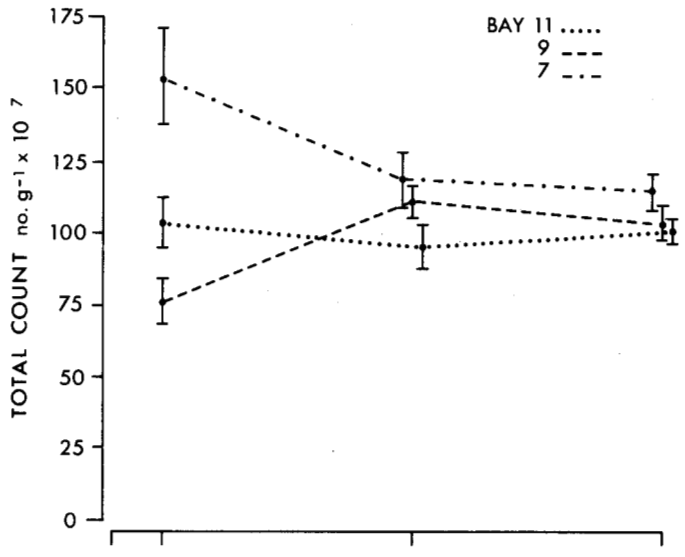

FIG. 12. Three-year comparison of total counts of bacterial cells in surface sediments. Samples were collected in Bays 11, 9 and 7 in 1981, 1982 and 1983. Results are presented as seasonal means and standard errors of values from two stations in each bay.

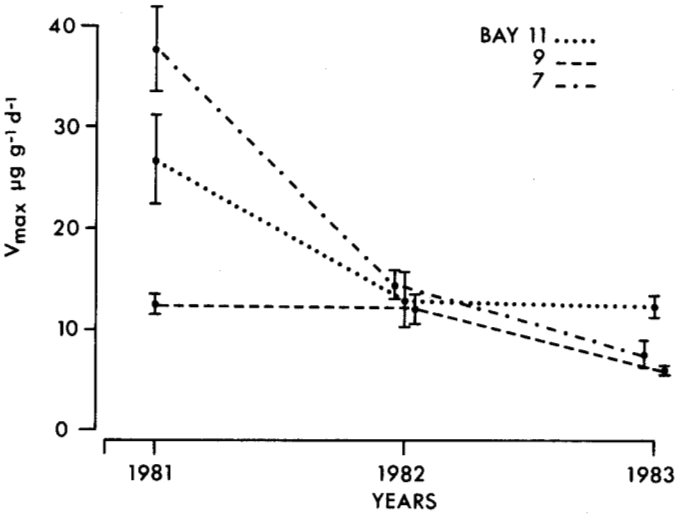

FIG. 13. Three-year comparison of maximum velocity (V_{max}) of glutamic acid uptake in surface sediments. Samples were collected in Bays 11, 9 and 7 in 1981, 1982 and 1983. Results are presented as seasonal means and standard errors of values from two stations in each bay.

showed a decrease between 1981 and 1982 but remained constant between 1982 and 1983. The difference in changes among the bays between 1982 and 1983 was seen in the significant ($0.05>p>0.01$) bay-year interaction when data for the three bays were combined.

DISCUSSION

The baseline oceanographic data provide a useful description of the Cape Hatt site and demonstrate the typicality of the site as an arctic location. Typicality is further discussed by Snow *et al.* (1987), Buckley *et al.* (1987), Cretney *et al.* (1987a,b,c) and Sempels (1987). Snow *et al.* (1987) included background microbiological data of the region and comparisons to other areas. The nearshore location of the microbiology stations in the bays contributed to the variability of oceanographic data, particularly during storm surges. Observations across three years, however, suggested a pattern of biological events found in other arctic locations. The sampling season began late in 1980, when sea ice was cleared from the bays and outer Ragged Channel. In 1981, this was rectified by some sample collections during breakup in mid-July. The prolonged season of 1981 tended to confirm observations of 1980 and 1982 and certainly suggested that the petroleum releases did not affect seasonal oceanographic data, including microbiological data. The releases were considered representative of real spills and expectant water column hydrocarbon contamination (Dickins *et al.*, 1987; Humphrey *et al.*, 1987).

That no effects of the petroleum releases were noted in the seasonal data of bacterial numbers and the V_{max} of glutamic acid uptake in the water column was not unexpected since the waters of the bays were rapidly flushed of petroleum after both releases. The slight, transient perturbation of V_{max} in the water column during the day of the dispersed release can probably be attributed to light, toxic fractions of the petroleum that were held in the water column by dispersant. Considerable adverse effects were seen in the benthic community at the same time (Cross and Thomson, 1987; Cross *et al.*, 1987a,b). Griffiths *et al.* (1981b) experimentally supplemented arctic water samples with 0.1% V/v fresh crude and noted significant decreases in the uptake of glucose and glutamic acid. They suggested that their study approximated an actual spill where similar concentrations of water-soluble (i.e., toxic) components of the fresh petroleum would be in the water column directly adjacent to the spill. Hodson *et al.* (1977) found 15% inhibition of glucose assimilation in the presence of 0.8 $mg \cdot l^{-1}$ of aqueous extracts of petroleum. These studies are in agreement with the result seen during the dispersed release, where presumably similar toxic fractions were in the water column due to the dispersant and the method of discharge. No perturbation of V_{max} was observed after the surface release, but water-soluble fractions of the petroleum may have been too low after two days of evaporation and some flushing of the bay.

Alexander and Schwarz (1980) supplemented coastal water samples from off the coast of Louisiana with 0.1% V/v fresh petroleum and found little effect on glucose mineralization. They suggested that their results differed from those of Hodson *et al.* (1977) because of the different petroleums used in the two studies. Griffiths *et al.* (1981b), on the other hand, suggested that Alexander and Schwarz had sampled in chronically hydrocarbon-polluted waters and the heterotrophic flora they challenged was adapted to the presence of petroleum.

At Cape Hatt, the mineralization of glutamic acid in water samples was not significantly affected in *in vitro* experiments by the addition of 0.1% V/v weathered petroleum, although a decrease was observed. The concentration of soluble hydrocarbons in the water samples in these experiments was probably less than concentrations produced during the dispersed release at the BIOS Project site. Replicate samples, however, were significantly affected by the presence of 0.01% Corexit, as were samples amended with a dispersant-petroleum mixture. No significant difference was seen between treatments of dispersant-petroleum mixture and dispersant alone. Whether the decrease in V_{max} in the samples supplemented with the mixture was due to Corexit or to Corexit dispersing the petroleum into the water sample is not clear. The petroleum, however, was

highly weathered and presumably contained few toxic components. A similar effect of Corexit was noted by Griffiths *et al.* (1981c). Lee *et al.* (1985), on the other hand, employing initial concentrations of 2.0 $mg \cdot l^{-1}$ of Corexit in Controlled Ecosystem Enclosures, measured increased uptake of thymidine and glucose by microheterotrophs. This response, which occurred after several days, may have been due to the release of organic compounds from phytoplankton exposed to the Corexit. The heterotrophic use of these compounds as substrates could not be distinguished from the use of Corexit itself as a substrate.

The effect of Corexit was further evaluated by examining other kinetic parameters of glutamic acid uptake in the same experiments. V_{max} significantly decreased approximately twofold, while turnover (the time taken for all the natural substrate in a sample to be consumed) and K + S (an affinity constant + the concentration of natural substrate) significantly increased by tenfold and twofold respectively over the control in samples supplemented with Corexit alone. Had V_{max} remained unchanged, this could be interpreted as a competitive inhibition of the uptake of the glutamic acid substrate by a component of the dispersant. This unknown component would necessarily be taken up by cells through the same mechanism that transports glutamic acid across the cell membrane. Non-competitive inhibition or the loss of the transport mechanism was not suggested, since (K + S) increased significantly over the control.

The presumed multiplicity of unknown components in Corexit 9527 does not facilitate the interpretation of the data. One possibility is that Corexit contains a component, perhaps the carrier, that is readily taken up and respired by microheterotrophs. This would conceivably reduce the energy requirements of the assemblage of cells in the sample and therefore decrease the mineralization of ^{14}C-glutamic acid to measurable $^{14}CO_2$.

The observed effect on V_{max} during the dispersed release might have been due to the Corexit in the water column. Corexit could not be quantitated in water samples taken on the day of the dispersed release but presumably was present at one-tenth or less of the concentrations of hydrocarbons (this being the ratio of dispersant to petroleum released). A maximum concentration of Corexit in the water column, therefore, would have been approximately three orders of magnitude less than the supplements used in the experimental studies, and this concentration can probably be discounted. Nevertheless, future studies involving controlled releases should consider dispersant alone as a control.

The releases of petroleum at Cape Hatt demonstrated that a surface slick of petroleum or the dispersion of a slick into the water column would be inconsequential or marginally deleterious to bacterial numbers or the microheterotrophic uptake of glutamic acid. After two years of observations of the sediment, the effect of petroleum on the sediment would appear to be indirect and long term. No immediate effects were observed. Very little petroleum entered the sediment of Bay 9 from the dispersed release. Petroleum beached on the intertidal zone after the surface release was transported by physical processes into subtidal sediments of Bay 11 during the open-water seasons (Owens *et al.*, 1987). Concentrations of petroleum at the microbiology stations (1 and 2) in this bay, however, remained low, even two years later in 1983 (Boehm *et al.*, 1987).

Significant bay-year interactions in the analysis of variance of both V_{max} of glutamic acid uptake and total counts in the sediments suggest a possible long-term effect of the dispersed petroleum release. Between 1981 and 1982, the control Bay 7 as well as Bays 10 and 11 tended toward decreased total counts and V_{max}, while in Bay 9, as a result of the dispersed petroleum release, total counts increased and V_{max} remained at levels comparable to 1981. It is unlikely that the changes observed in Bays 7, 10 and 11 were due to the petroleum releases. Although the sediments of Bay 7 were slightly contaminated with petroleum, the level was the lowest of all four bays (Boehm *et al.*, 1987). Furthermore, the waters overlying the sediments of Bays 10 and 11 and particularly Bay 7 were exposed to petroleum concentrations orders of magnitude lower than those in Bay 9.

Bacterial multiplication resulting from petroleum utilization was probably not responsible for the increase in total counts seen in Bay 9. Petroleum did not appear to have been biodegraded in the sediment (Boehm *et al.*, 1987). Nevertheless, a low level of biodegradation might not be detected by the analytical techniques employed. Using a conservative estimate of the carbon content of individual bacterial cells (Fuhrman *et al.*, 1980), a minimum increase in bacterial biomass of 2.0 $\mu g\ C \cdot g^{-1}$ dry weight of sediment in Bay 9 was determined. The mean concentration of petroleum in the sediments at the microbiology stations of Bay 9 was 4.4 ± SE 0.6 $\mu g \cdot g^{-1}$ in 1982 and 4.9 ± SE 0.9 $\mu g \cdot g^{-1}$ in 1981 following the dispersed petroleum release. A large and measurable amount of petroleum would have to be removed from the sediments in order to account for the increase in bacterial biomass. If an undetectable amount of petroleum was utilized, it can only account for a very small portion of the increase in bacterial carbon.

Analysis of variance of values of organic carbon from 1981 and 1982 showed that total organic carbon (TOC) in Bay 9 increased significantly between the two years from a mean of 1.7% to a mean of 2.3% of sediment dry weight. This increase may be related to petroleum effects on plants and animals, however limited, that occurred during and following the dispersed petroleum release (Cross and Thomson, 1987; Cross *et al.*, 1987a,b). Bacterial biomass, uptake of glutamic acid and TOC were not immediately affected following the release. After a period of time, however, organic carbon produced by scavenging and decomposition would enter the sediment. Bacterial activity and production would tend to respond to this increase in organic carbon, yielding the results observed in Bay 9.

The trends in Bay 9 in 1982 were not seen in 1983. A significant bay-year interaction was seen in the V_{max} of glutamic acid uptake when the 1982 and 1983 data for Bays 7, 9 and 11 were taken together. The activity of microheterotrophs in Bay 11 was greater than in the other two bays and did not decrease in 1983 over 1982, as did the other bays. Although significant interactions were not found, the mean levels of organic carbon in the sediment of Bay 11 increased over 1982, as did mean numbers of bacteria. These data tend to suggest that a petroleum effect may be occurring in Bay 11 as a consequence of stranded petroleum entering the sediments.

There have been few *in situ* studies of the long-term effects of petroleum on bacterial biomass and activity in marine sediments. Griffiths *et al.* (1981a) noted a depression of glutamic acid uptake and a decrease in bacterial biomass in petroleum-contaminated sediments, although depression of glutamic acid uptake was less drastic and sometimes insignificant at low concentrations of petroleum or when the petroleum had been artificially weathered. The concentrations of petroleum used, 0.1 to 50 ppt (V/v), were much higher than those encountered at Cape Hatt. Bakke *et al.* (1982), in an *in situ* study of marine sediments off the coast of Norway, noted no significant differ-

ences in total counts or glucose uptake between sediments contaminated with diesel oil and a control. Although we cannot make direct comparisons, concentrations of petroleum in the study of Bakke *et al.* were probably similar to those encountered at Cape Hatt. While measuring glucose uptake in sediment material from coastal New York, Bauer and Capone (1985) found little or no inhibition by aromatics.

Baker and Griffiths (1984) concluded that conflicting evidence concerning petroleum effects on microheterotrophic uptake in marine sediments might involve the recent history of the sediment in question. Petroleum-impacted sediments would show fewer effects upon further contamination, whereas "pristine" sediments would be affected to a greater degree. The present study does not support this, but it can be suggested that variable results of studies might be due to the concentration and degree of weathering of petroleum at the time of impact on the sediment. Contrary to the conclusion of Atlas (1985), the adverse effects of petroleum on microheterotrophic activity, at least in a realistic scenario where weathering of the petroleum occurs, are probably marginal.

Lacking at this time from the Cape Hatt site is sufficient evidence that the macrobenthos of Bays 11 and 9 was disturbed enough by the releases to cause a large increase of TOC in Bay 9 in 1982 and a small increase in Bay 11 in 1983. Observed variations in the biomass of the flora and fauna are discussed by Cross and Thomson (1987) and Cross *et al.* (1987a,b). Evidence linking these variations to the petroleum releases is scant. It can nevertheless be speculated that in subsequent years, as stranded petroleum continues to enter subtidal sediments, the sediments of Bay 11 will show high levels of TOC and concomitant high numbers of bacteria and microheterotrophic activity.

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