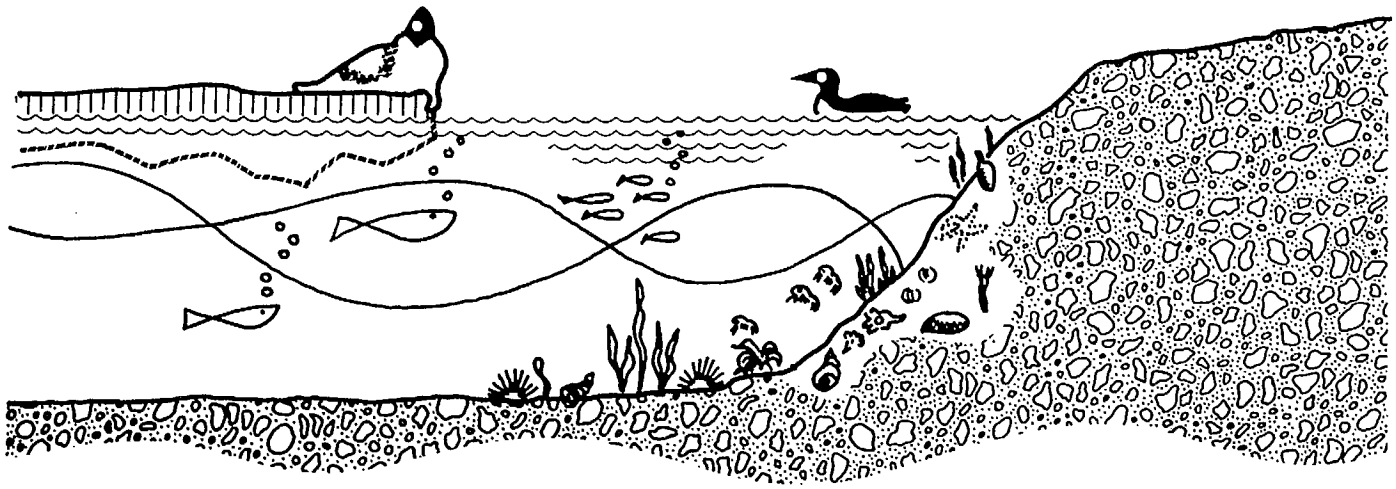
MICROBIOLOGY

1. Effects of Oil on Bacterial Activity



Baffin Island Oil Spill Project

WORKING REPORT SERIES

82-5

1982 STUDY RESULTS

BAFFIN ISLAND OIL SPILL PROJECT
WORKING REPORT SERIES

The Baffin Island Oil Spill (BIOS) Project is a multidisciplinary program of research on arctic marine oilspill fate, effects and countermeasures. The Project commenced in the spring of 1980 and have now completed the fourth and final year of planned field work at an experimental site located on the northern end of Baffin Island, Canada. The results of work performed in each of the various study components under the Project, has been made available on a yearly basis through this working report series. This has been done prior to a complete integration of findings and interpretation with respect to the Project objectives. The working report series should therefore be considered as interim or data reports. The contents do not necessarily reflect the views for policies of the BIOS Project management or funders.

For further information on the BIOS Project write the BIOS Project Manager, c/o Environmental Emergencies Technology Division, Environmental Protection Service, Ottawa, Ontario, Canada K1A 1C8.

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Baffin Island Oil Spill (BIOS) Project
Working Report 82-5

Microbiology

1. Effects of oil on bacterial activity -- 1982 study results

prepared for

Environmental Protection Service
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by

J. N. Bunch and C. Bédard

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Arctic Biological Station
Department of Fisheries and Oceans
555 boul. St-Pierre
Ste-Anne-de-Bellevue, Québec
H9X 3R4

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SUMMARY

Bunch, J. N. and C. Bédard. 1984. Microbiology: 1. Effects of oil on bacterial activity -- 1982 study results. Baffin Island Oil Spill (BIOS) project working report 82-5: viii + 54 p. Environmental Protection Service, Canadian Department of the Environment, Ottawa, Ontario.

Microbiological observations were made in a series of four bays at Cape Hatt, N.W.T. before, during and after experimental releases of petroleum in 1981. Similar observations were made in 1982. As expected, no changes in bacterial numbers or in the uptake of glutamic acid by heterotrophic microorganisms were observed in water columns in 1982 over 1981. In the sediments, analysis of variance indicated that changes in bacterial numbers and the V_{max} of glutamic acid uptake were not consistent across all four bays between 1981 and 1982. Bacterial numbers and V_{max} decreased in bay 7 (the control bay) as well as in bays 10 and 11. In bay 9, where a subsurface release of dispersed petroleum occurred, bacterial numbers increased and V_{max} remained approximately constant. These discrepancies were significant ($p < 0.001$) and related to the dispersed petroleum release in 1981. No changes could be ascribed to the surface release of petroleum in bay 11.

Measurements of hexadecane mineralization in water and sediment samples from the four bays were similar in the two years. This suggested that the activity of oleoclasts, those heterotrophic microorganisms capable of degrading fractions of petroleum, was unaffected by the petroleum releases. Numbers of these microorganisms showed considerable variation in the water and sediment of the bays. Although circumstantial, numbers of oleoclasts appeared to have increased in the sediments of the bays but not the water columns. Such a change in numbers in the sediments, however, could not be ascribed to the petroleum releases.

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1.0 INTRODUCTION

During the third year (1982) of the Baffin Island Oil Spill (BIOS) project at Cape Hatt, N.W.T., several investigations assessed the long term fate and effects of petroleum in this arctic marine environment. We have been conducting a study of the effects of petroleum on the activity and numbers of heterotrophic microorganisms (microheterotrophs) in the nearshore waters and sediments of a series of four bays at Cape Hatt (Bunch et al, 1981; Bunch et al, 1983).

Two controlled releases of petroleum were carried out at the end of August 1981; a surface release in bay 11 and an underwater release in bay 9 in which petroleum had been mixed with a dispersant, Corexit 9527. Bay 10 was chosen as a control in 1980, but was replaced in 1981 by bay 7. Details of the releases are in Dickins (1982).

In 1981, no change in either the numbers or activity of microheterotrophs in the sediment could be related to either of the petroleum releases (Bunch et al, 1983). Activity in the water column was affected for a short period of time by the dispersed petroleum release. Petroleum-degrading microheterotrophs (oleoclasts) were present throughout the sediment and water columns of all bays studied in 1981. Neither numbers nor activity of oleoclasts appeared to have been affected by the petroleum releases.

Our principal objective in the third year of the BIOS project was to determine whether or not the petroleum releases of 1981 led to any changes in numbers and activity of microheterotrophs in the sediment. We also monitored the water column in order to detect natural variations which might affect the sediment, and mask changes due to the petroleum releases. We report here observations made in 1982.

2.0 STUDY AREA

The study area is seen in Figure 1. The schedules of sampling of water and sediment are shown in Tables 1 and 2. At the beginning of the field season, the water columns in each bay were sampled on two separate occasions: 29-30 July and 2 August. Samples collected on these dates were used for determinations of total counts of bacterial cells. A sampling cycle was defined as the period taken to sample all four bays once. The period 6 August - 12 September included four numbered cycles for the water column and seven numbered cycles for the sediment (Tables 1 and 2). Collections of sediment were made by divers. Sediment samples from all stations during the third cycle were shared with a team of Norwegian microbiologists.

3.0 METHODS AND MATERIALS

3.1 Sampling Procedures

3.1.1 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 processed within several hours of collection.

3.1.2 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, a 20-mL wet subsample was measured and suspended in 2 L of filter-sterilized water 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.

3.2 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, weighed and results averaged.

3.3 Bacterial Counts

3.3.1 Total Counts

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 to 15.0 mL, fixed at the time of collection with 0.2% gluteraldehyde, was added to the filter funnel after shaking on a vortex mixer. Sufficient acridine orange (80% dye content), at a concentration of 0.1% in 0.02 mol tris (pH 7.2), was added to the sample to yield a final stain concentration of 0.02%. After two minutes, 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.

3.3.2 Most Probable Number (MPN) of Oleoclasts

The abundance of oleoclastic (petroleum-degrading) heterotrophs was determined by the most probable number (MPN) procedure (American Public Health Association, 1971). Forty-millilitre samples of sea water or sediment suspension, and tenfold serial dilutions up to 10^{-5} in 36.0 mL of filter-sterilized sea water, from a depth of 5m, were prepared in triplicate using 50-mL serum bottles. Each bottle was supplemented with 0.8 mL of nitrate-phosphate nutrient solution, 40.0 µL of sterile Lago Medio petroleum (artificially weathered by 22%), and 125.0 µL of n-(1-^{14}C)-hexadecane (Amersham Corp.). The nitrate-phosphate concentrate yielded a final concentration of 1.0 g NH_4NO_3 and 0.1 g K_2HPO_4 per litre of sample water. Final activity of hexadecane was 6.25 µCi L^{-1} of sample water. The bottles were incubated at 5.0°C for a 60 day period after which time they were acidified by addition of 0.8 mL of 5.0 N H_2SO_4.

In the laboratory at Ste-Anne-de-Bellevue, the bottles were purged of CO_2 in a gas train. The CO_2 was collected in NaOH and precipitated as $BaCO_3$. The precipitates were filtered through two 24.0-mm Whatman GF/C filters and the filters added to scintillation vials containing 7.0 mL of Aquasol (New England Nuclear Corp.). The vials were shaken on a vortex mixer to disperse the precipitate, and 3.0 mL of distilled water

were added to gel the Aquasol and ensure that a uniform distribution of the precipitate was maintained in the vial. Vials were counted in a Nuclear-Chicago Isocap 300 scintillation counter and corrected for quenching by the channel ratios method. Sample counts of more than twice the background value were considered positive. Vials were scored for the highest dilutions yielding positive results; these were used to obtain the MPN of oleoclasts by reference to a standard MPN statistical table.

3.4 Microheterotrophic Activity

3.4.1 Uptake of Glutamic Acid

An extensive modification of the procedure described by Harrison et al (1971) was employed throughout this study. Water samples and sediments 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 were 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 to 60.0 $\mu g\ L^{-1}$ of sample water, and activity of 2.0 or 20.0 $\mu Ci\ L^{-1}$. The specific activity of the L-[^{14}C(U)]-glutamic acid (New England Nuclear Corp.) was approximately 293.0 $mCi\ mmol^{-1}$. A fifteenth vessel containing 1.0 $\mu g\ L^{-1}$ of glutamic acid with 2.0 $\mu Ci\ L^{-1}$ 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 fourteen vessels were incubated at 2.0°C for 9.0 or 18.0 hours, depending on the expected magnitude of activity. Suspended sediment samples were incubated at 2.0°C for four hours. Incubation was stopped by simultaneous filtration of the 14 vessels followed by cold rinsing. Rinsed membranes were transferred to scintillation vials containing one millilitre of 2-ethoxyethanol (BDH Chemicals Ltd.).

To measure substrate respired 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 caps 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 sea water 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. Scintillation vials were transported to Ste-Anne-de-Bellevue. Before counting, seven millilitres of Aquasol (New England Nuclear Corp.) were added to scintillation vials containing membrane filters. Results of membrane and wick counts were combined to yield total uptake of the glutamic acid substrate. Uptake kinetics were generated using computer programs (Section 3.5).

3.4.1.1 Theory

Kinetic parameters from the uptake of the glutamic 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}, y intercept = turnover time in hours (T), and x intercept = (K+S).

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.

The value of (K+S) represents a combined value of the uptake constant and the concentration of the naturally occurring substrate in the water sampled. In a general way, K may be considered as an affinity constant between cell and substrate. Specifically, it is the concentration of substrate required to drive the reaction at

half-maximal velocity. A high value of (K+S) may suggest an unadapted population and/or a high concentration of natural substrate, while a very low value indicates an adapted population and/or a low value of natural substrate. Turnover (T) is the time required for the flora to deplete all the available natural substrate in a litre of the water sample. A very large value of T suggests a high concentration of natural substrate being consumed at a low velocity by an unadapted flora. A very low value of T suggests a highly adapted flora rapidly consuming a low concentration of natural substrate.

In addition to the above kinetic parameters, uptake and assimilation of a radioactive amino acid 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.

3.4.2 Mineralization of Hexadecane

To assess the amount of mineralization of n-(1-^{14}C)-hexadecane in water samples from a depth of 5 m or sediment suspension samples, 40.0-mL aliquots of the samples were added to 50-mL serum bottles and supplemented with 40 µL of sterile Lago Medio petroleum (artificially weathered by 22%) and 40 µL of hexadecane containing ^{14}C-hexadecane to yield a final concentration of 6.25 µCi L^{-1} of sample. To account for the possibility of nutrient limitation, a second set of bottles was also prepared and supplemented with 0.8 mL of a sterile nitrate-phosphate concentrated solution with a final concentration of 1.0 g NH_4NO_3 and 0.1 g K_2HPO_4 per litre of sample. In each set, the control bottle was immediately acidified to pH 2.0 with 0.8 mL of 5.0 N H_2SO_4. All vessels were incubated at 5.0°C and, at 20-, 40- and 60-day intervals, two bottles from each set were acidified. Evolved $^{14}CO_2$ was quantitated as in the MPN procedure. Maximum dpm was generally obtained after 60 days of incubation.

3.5 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. The null hypothesis (no effect) was rejected when the probability of it being true was less than 1%. ANOVA was conducted on data from sediment

samples only.

For the study of 1982 variation, data were grouped by bay and cycle 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.

4.0 RESULTS

4.1 The Water Column

The purpose of water analyses was to ensure that no overt biological changes occurred in the water column when compared to 1981. Since the petroleum releases in 1981 were not expected to affect biological activity in the water column in 1982, collections of water from stations in the four bays were reduced from the number of collections taken in 1981.

Values of total counts and uptake of glutamic acid from the three depths sampled at both stations in a bay were averaged for each cycle. The means of each bay are presented in Figures 2 to 5. Data for all stations and depths are presented in Tables 3 and 4. For inter-year comparisons, the means of comparable periods (3 August - 12 September) in 1981 and 1982 were used.

4.1.1 Total Counts of Bacterial Cells

Between the first two sampling cycles, the mean total count of the bays decreased slightly from $3.0 \times 10^8 \pm$ SE 0.1×10^8 cells L^{-1} on 29-30 July to $1.7 \times 10^8 \pm$ SE 0.1×10^8 cells L^{-1} on 2 August. Counts increased after this time to $3.8 \times 10^8 \pm$ SE 0.2×10^8 cells L^{-1} on 14-16 August and remained at this level to the end of the sampling season on 6 September. Counts during the sampling season ranged from 8.8×10^7 to 8.4×10^8 cells L^{-1}. During comparable periods in August and early September, the mean total count of $3.6 \times 10^8 \pm$ SE 0.1×10^8 cells L^{-1} in 1982 was almost identical to a mean of $3.1 \times 10^8 \pm$ SE 0.1×10^8 cells L^{-1} observed in 1981.

4.1.2 Uptake of Glutamic Acid

The mean V_{max} of glutamic acid uptake decreased from 3.7 ± SE 0.4 µg L^{-1} d^{-1} during the first regular sampling cycle on 9-10 August to 2.2 ± SE 0.1 µg L^{-1} d^{-1} on 14-16 August. After this time, the mean value increased to 5.5 ± SE 0.5 µg L^{-1} d^{-1} in the final sampling cycle on 3-6 September. This was due to high values obtained in bays 10 and 11 during this time. Values of V_{max} ranged from 1.2 to 12.0 µg L^{-1} d^{-1} during the sampling season. The seasonal mean of V_{max} of 3.5 ± SE 0.2 µg L^{-1} d^{-1} was comparable to a mean of 3.8 ± SE 0.1 µg L^{-1} d^{-1} in 1981.

Turnover times ranged from 0.4 to 6.6 days across the sampling season. Mean turnover increased across the first three cycles from 1.8 ± SE 0.2 to 3.2 ± SE 0.3 days. The mean turnover in the last cycle was 2.2 ± SE 0.4 days. A mean turnover of 2.4 ± SE 0.1 days was obtained in

1982 compared to 2.0 ± SE 0.1 days in 1981.

The mean (K+S) was approximately 5.0 μg L^{-1} during the first two sampling cycles, after which time the mean per cycle increased, ending at 9.5 ± 0.9 μg L^{-1} during the last cycle. Values of (K+S) during the sampling season ranged from 1.7 to 18.4 μg L^{-1}. The seasonal mean of (K+S) in all bays was 6.8 ± SE 0.4 μg L^{-1} in 1982 and 6.3 ± SE 0.2 μg L^{-1} in 1981.

4.1.3 Most Probable Number of Oleoclasts

Numbers of oleoclastic cells in water samples from the 5 m depth in the four bays were determined by a most probable number (MPN) procedure using n-(1-^{14}C)-hexadecane. The results from three sampling cycles in August are presented in Table 5. Although the number of dilutions in the MPN technique was increased over those of 1981 (see Methods and Materials, 3.1), the increase was insufficient to serially dilute to extinction the oleoclasts in some water samples. Consequently, underestimates of numbers of oleoclasts were derived from some samples although fewer than in 1981. The lack of precise estimates of numbers of oleoclasts precluded an examination of spatial and temporal variation in 1982 or a comparison to 1981.

In 1981, oleoclasts varied from 0.02 x 10^4 to greater than or equal to 6.0 x 10^4 L^{-1}. In 1982, the range was from 0.03 x 10^4 to greater than or equal to 600.0 x 10^4 L^{-1} with considerably fewer underestimates. The sample size in both cases, however, was large. When the most probable numbers in 1982 were recalculated on the basis of the number of dilutions employed in 1981, the proportion of underestimates was found to be about the same in both years (approximately 45%). If it is assumed that the curves of the frequency distribution were similar in both years, we can suggest that the numbers of oleoclasts in the water column were similar in both years.

4.1.4 Mineralization of Hexadecane

The results of ^{14}C-hexadecane mineralization in water samples after various intervals of incubation are presented in Figure 6 and Table 5. Yields of $^{14}CO_2$ resulting from the mineralization of ^{14}C-hexadecane are expressed as disintegrations per minute (dpm) per litre of sample water. Mineralization of ^{14}C-hexadecane was observed in all samples after 60 days of incubation. The seasonal mean for all bays was 36 x 10^4 ± SE 7 x 10^4 dpm L^{-1}. In the absence of nutrient supplementation, replicate samples yielded a seasonal mean of 1.1 x 10^4 ± 0.2 x 10^4 dpm L^{-1}. Only 10 samples supplemented with nutrients showed a positive

response after 20 days. All samples showed a positive response after 40 days of incubation although lower than those incubated for 60 days.

A comparison of $^{14}CO_2$ evolution from water samples incubated with ^{14}C-hexadecane in 1981 and 1982 is given in Figure 12. Essentially no difference was seen in mean values of hexadecane mineralization in the two years.

4.2 The Sediment

4.2.1 Total Counts of Bacterial Cells

Values of total counts in the sediments are listed in Table 6. The mean value of all samples was $103.9 \times 10^7 \pm$ SE 3.8×10^7 cells g^{-1} dry weight of sediment. No significant differences were noted between stations within bays. No significant differences were found between bays.

Significant variation in total counts was observed between sampling cycles. Beginning with a mean of $78.5 \times 10^7 \pm$ SE 7.8×10^7 cells g^{-1} during the first cycle, counts tended to increase across the season, reaching a mean of $124.2 \times 10^7 \pm$ SE 10.9×10^7 cells g^{-1} in the last cycle. The trend was particularly clear in bays 7 and 10 (see Fig. 7).

Seasonal means of total counts for each bay in 1981 and 1982 are presented in Table 7. An analysis of variance combining data from 1981 and 1982 showed that from one year to the next, changes in total counts were not consistent across all bays, as evidenced by a significant bay-year interaction. The interaction no longer appeared once data from bay 9 were removed from the analysis. Contrary to all other bays which showed a moderate decrease in seasonal mean from 1981 to 1982, the mean total count in bay 9 in 1982 increased by 46% over the value of 1981.

The analysis of variance on data from bays 7, 10 and 11 showed that there were no significant changes in total counts between years in these bays. Across the two years there was a significant difference between bays.

4.2.2 Uptake of Glutamic Acid

Values of V_{max}, turnover and (K+S) of glutamic acid uptake are listed in Table 6. The mean values of all samples for each of these parameters were 12.6 ± SE 0.8 $\mu g\ g^{-1}\ d^{-1}$, 1.6 ± SE 0.1 days and 17.9 ± SE 0.9 $\mu g\ g^{-1}$ respectively.

No significant differences were noted between stations within bays or between bays across the season. No significant differences were seen

between cycles when data from all bays were combined. Results from across the season are summarized in Figures 8 to 10.

Seasonal means of V_{max}, turnover and (K+S) for each bay in 1981 and 1982 are presented in Table 7. An analysis of variance combining data from both years showed that at least in the case of V_{max}, changes within bays from 1981 to 1982 were not identical across all bays i.e. a significant bay-year interaction was present. The interaction no longer appeared once data from bay 9 had been removed. In bay 9, V_{max} was essentially the same in both years. Decreases were noted in all other bays, the largest decrease occurring in bay 7.

Analysis of variance on data from bays 7, 10 and 11 showed that there was a significant decrease in V_{max} between years in these bays when taken together. Across the two years there was a significant difference between bays.

It appeared that year to year fluctuations in turnover were not similar in all bays despite the fact that no bay-year interaction was noted. Analysis of variance including data from all bays indicated that there were no significant differences in turnover levels between years. When data from bay 9 were excluded, however, a significant increase was observed. Across the two years, there was a significant difference between bays in turnover.

The mean value of (K+S) in 1982 was significantly lower than in the previous year. Across the two years, there were no significant differences in (K+S) between bays.

4.2.3 Most Probable Number of Oleoclasts

Oleoclasts, in numbers as low as 13 g^{-1} dry weight of sediment, were present in all sediment samples obtained at the microbiology stations during the 1982 season. Estimates of oleoclasts in the sediment are listed in Table 8. Values exhibited a high degree of variability, ranging over at least five orders of magnitude.

Despite the fact that the number of dilutions used in the MPN technique was increased in 1982, underestimates of numbers of oleoclasts were obtained in some cases. This precluded an examination of spatial and temporal variations.

If we had used the same number of dilutions for MPN determinations as in 1981, 76% of the values obtained would have been underestimates compared to 43% obtained in the previous year. Assuming that the shape of the frequency distribution curve of counts was approximately the same in both years, this suggests that numbers of oleoclasts increased from 1981 to 1982.

4.2.4 Mineralization of Hexadecane

Results of hexadecane mineralization are presented in Table 8. Oleoclastic activity, measured by $^{14}CO_2$ evolution from n-(1-^{14}C)-hexadecane, was a widespread characteristic of the sediment as in 1981. All samples amended with nutrients showed activity after 20 days of incubation. Values of disintegrations per minute (dpm) obtained after 60 days of incubation exhibited considerable variability ranging from 0.4 x 10^3 to 101.9 x 10^3 dpm g^{-1} dry weight of sediment. The mean dpm after 60 days for all samples was 19.0 x 10^3 ± SE 3.6 x 10^3 dpm g^{-1}. In samples unamended with nutrients, the mean dpm after 60 days was more than 20 times lower. Essentially no difference in seasonal means was observed between each bay.

Cross-seasonal variation in values of dpm obtained after 60 days is presented for each bay in Figure 11. Bays 7 and 9 tended to show parallel trends across the season, as did bays 10 and 11. Because of the great variability in the data, it is not clear whether these trends are significant.

The mean seasonal value of hexadecane mineralization in bay 11 following 60 days of incubation was lower than the corresponding value in 1981 whereas the mean values in bays 7, 9 and 10 were comparable in both years (see Fig. 13). In bay 11, a threefold decrease was observed.

5.0 DISCUSSION

5.1 The Water Column

Measurements of microbiological parameters from the water columns of the bays were made in 1982 in order to detect any unusual biological events. Such events might alter sediment conditions to the extent of masking the effects due to the petroleum releases in 1981. We did not expect to see any petroleum effects in the water columns in 1982.

Between 1981 and 1982, no major changes were observed in measured microbiological parameters in the water column. Supporting chemical data from the water column, including chlorophyll, also showed little variation. These data will be the subject of a separate report. We therefore concluded that any changes observed in the sediments were unrelated to the water column.

5.2 The Sediment

We demonstrated the importance of spatial (bays) and/or temporal (cycles and years) factors in accounting for significant amounts of variation in the microbiological parameters of the sediments. In addition, the dispersed petroleum release can probably explain a significant amount of variation in two of the parameters measured.

Significant bay-year interactions in the analysis of variance of both V_{max} of glutamic acid uptake and total counts suggest an effect of the dispersed petroleum release. The most likely explanation is that, between 1981 and 1982, the control bay 7 as well as bays 10 and 11 tended towards 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, 1983). Furthermore, the waters overlying the sediments of bays 10 and 11 and particularly bay 7, were never exposed to petroleum concentrations as high as those in bay 9 during the dispersed petroleum release.

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, 1983). 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), we determined a minimum increase in bacterial biomass of 2.0 μg C g^{-1} dry weight of sediment in bay 9. The mean concentration of petroleum in the sediments at the microbiology stations of bay 9 was 4.4 ± SE 0.6 μg g^{-1} in 1982 and 4.9 ± SE 0.9 μg 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 (unpublished data). This increase may be related to petroleum effects on plants and animals, however limited, which occurred during and following the dispersed petroleum release (Cross et al, 1983). Bacterial biomass, uptake of glutamic acid and TOC were not immediately affected following the release (Bunch et al, 1983). 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.

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 (1981) 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 (Boehm, 1983). Bakke et al (1982), in an in situ study of marine sediments off the coast of Norway, noted no significant differences 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.

Rates of mineralization of ^{14}C-hexadecane remained constant between 1981 and 1982 and a potential for biodegradation therefore existed in the sediments of the bays between these years. In spite of this potential, Boehm (1983) found no evidence of petroleum biodegradation in petroleum-contaminated sediment samples analysed by gas chromatography.

The two procedures did not employ identical subsets of samples and

Boehm et al (1982) and Boehm (1983) reported patchiness in the distribution of petroleum in the sediments. Samples for both procedures were, however, collected simultaneously in the same location by divers.

Inorganic nutrients have often been cited as a source of limitation of hydrocarbon biodegradation in the marine environment (Atlas, 1981). In the sediments at Cape Hatt, interstitial phosphate and nitrate were almost always detectable and sometimes present in concentrations considerably higher than those in overlying waters (unpublished data). In addition, sediment samples unamended with nutrients demonstrated hexadecane mineralization.

Boehm assessed biodegradation by measuring the loss of n-alkanes relative to recalcitrant branched isoprenoids, a technique which has been successfully employed elsewhere (e.g. Jobson et al, 1972). Horowitz and Atlas (1977), however, noted that most components, including isoprenoids, of petroleum-contaminated water samples from Prudhoe Bay, Alaska, were degraded at similar rates. Similar findings were reported earlier by Atlas (1975). Atlas (1981), however, indicated that the Prudhoe Bay study was in contrast to most studies which showed preferential utilization of n-alkanes over isoprenoids.

If there is a real discrepancy in the interpretation of results from the two techniques, it may be due to the high sensitivity of the ^{14}C-hexadecane procedure. We would conclude, therefore, that the measured potential for biodegradation was not realized in situ.

Of greater concern to us is the apparent increase in the number of oleoclasts in the sediments between 1981 and 1982. Such an increase in the population of oleoclasts should have been reflected in a greater potential for biodegradation in 1982 over 1981. Increases in numbers of oleoclasts such as those seen in sediments at Cape Hatt have often been noted in aquatic environments following a petroleum impact (Atlas, 1981). Such increases in the presence of petroleum should indicate that some fraction of the petroleum is being utilized. As noted above, Boehm (1983) did not find any alteration in the composition of petroleum in the sediments, notably at the 10m depth of the microbiology stations.

In petroleum-impacted environments, increases in the uptake of added radiolabelled hydrocarbons have also been observed (Bakke et al, 1982; Roubal and Atlas, 1978; Saltzmann, 1982). This observation was not made by means of the ^{14}C-hexadecane procedure at Cape Hatt. This would suggest that oleoclasts, although more numerous in 1982, were individually less active than in 1981. Such a possibility, however implausible, would result in the uniformity of hexadecane mineralization observed in the two years. We can only conclude at this time that the

usefulness of measurements of numbers of oleoclasts and their activity in predicting the fate of petroleum in the arctic environment may be marginal.

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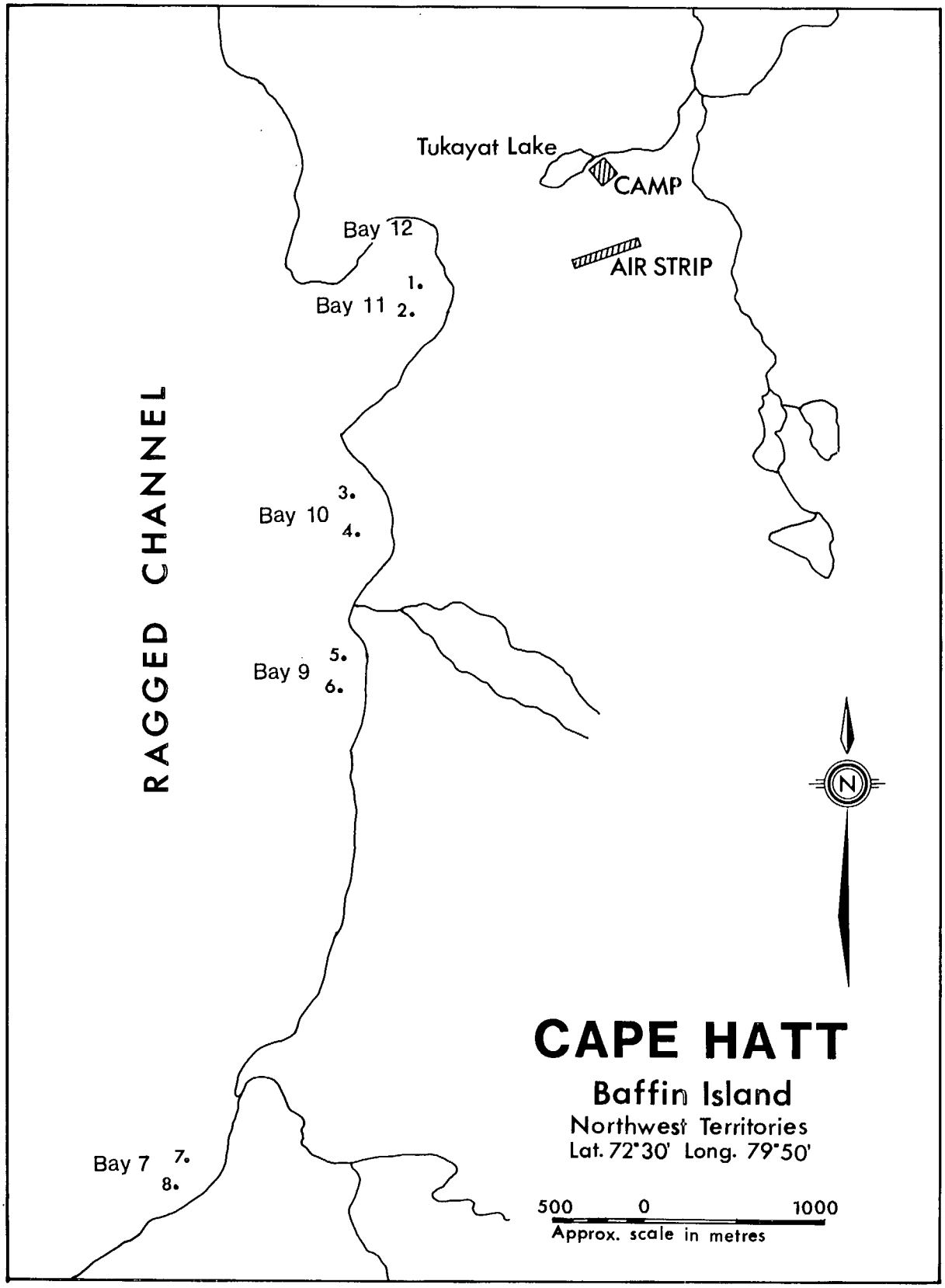

Figure 1. Microbiological stations occupied at Cape Hatt during 1982.

7.0 FIGURES

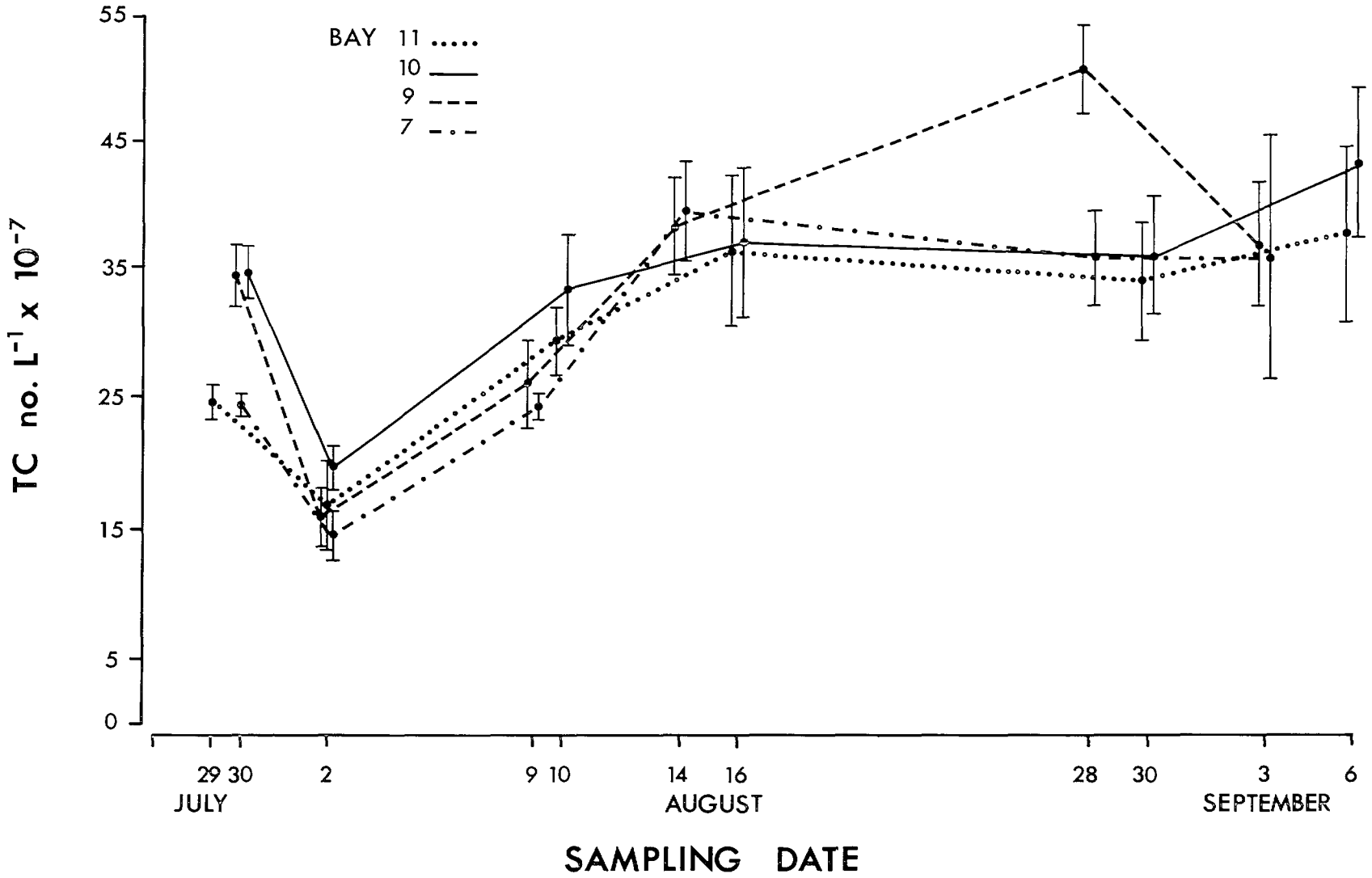

Figure 2. Total counts (TC) of bacterial cells determined in water samples collected at Cape Hatt, 1982. Results are presented as means and standard errors of six values from three depths at two stations in each bay.

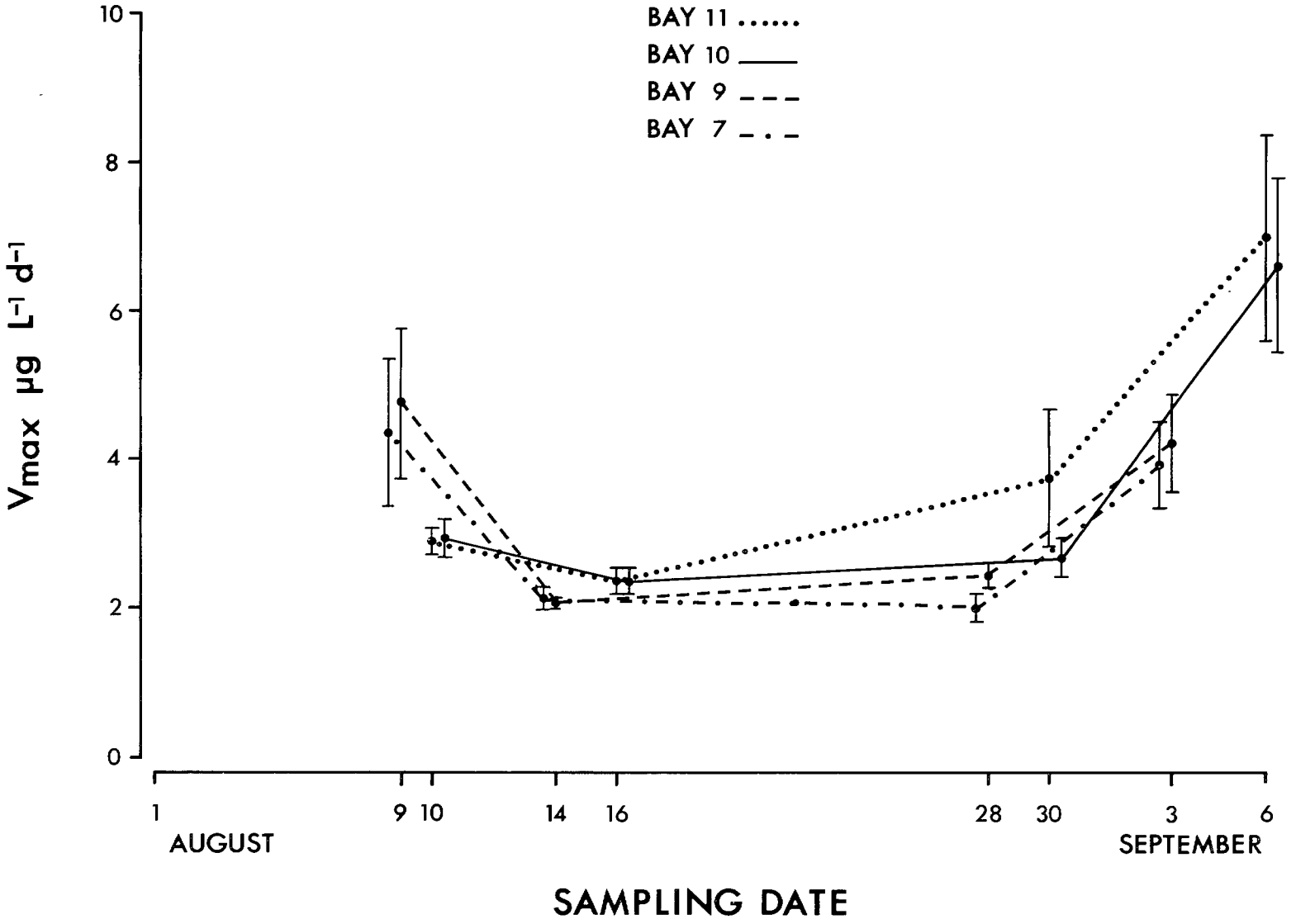

Figure 3. Maximum velocity (V_{max}) of glutamic acid uptake determined in water samples collected at Cape Hatt, 1982. Results are presented as means and standard errors of six values from three depths at two stations in each bay.

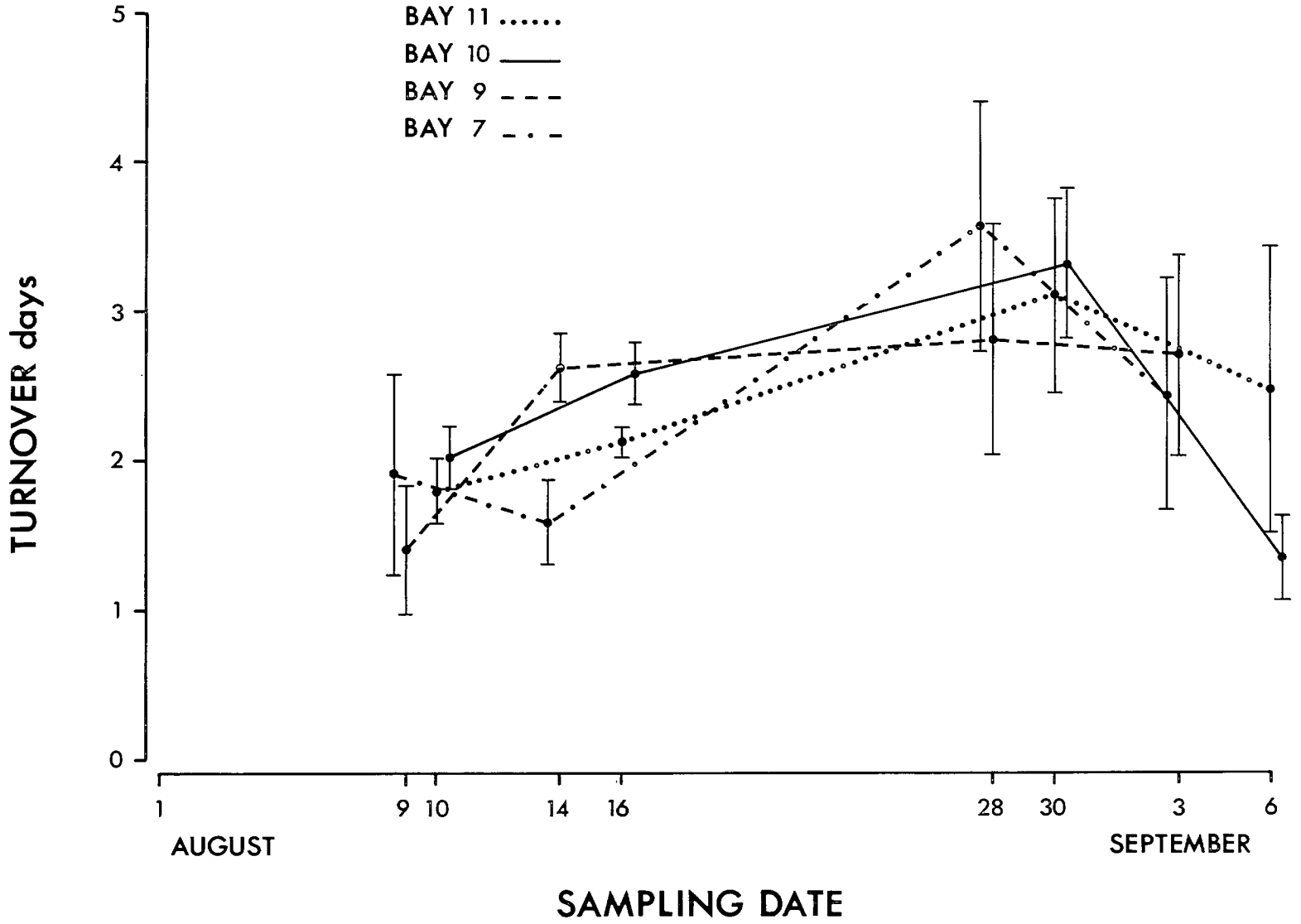

Figure 4. Turnover (days) of glutamic acid uptake determined in water samples collected at Cape Hatt, 1982. Results are presented as means and standard errors of six values from three depths at two stations in each bay.

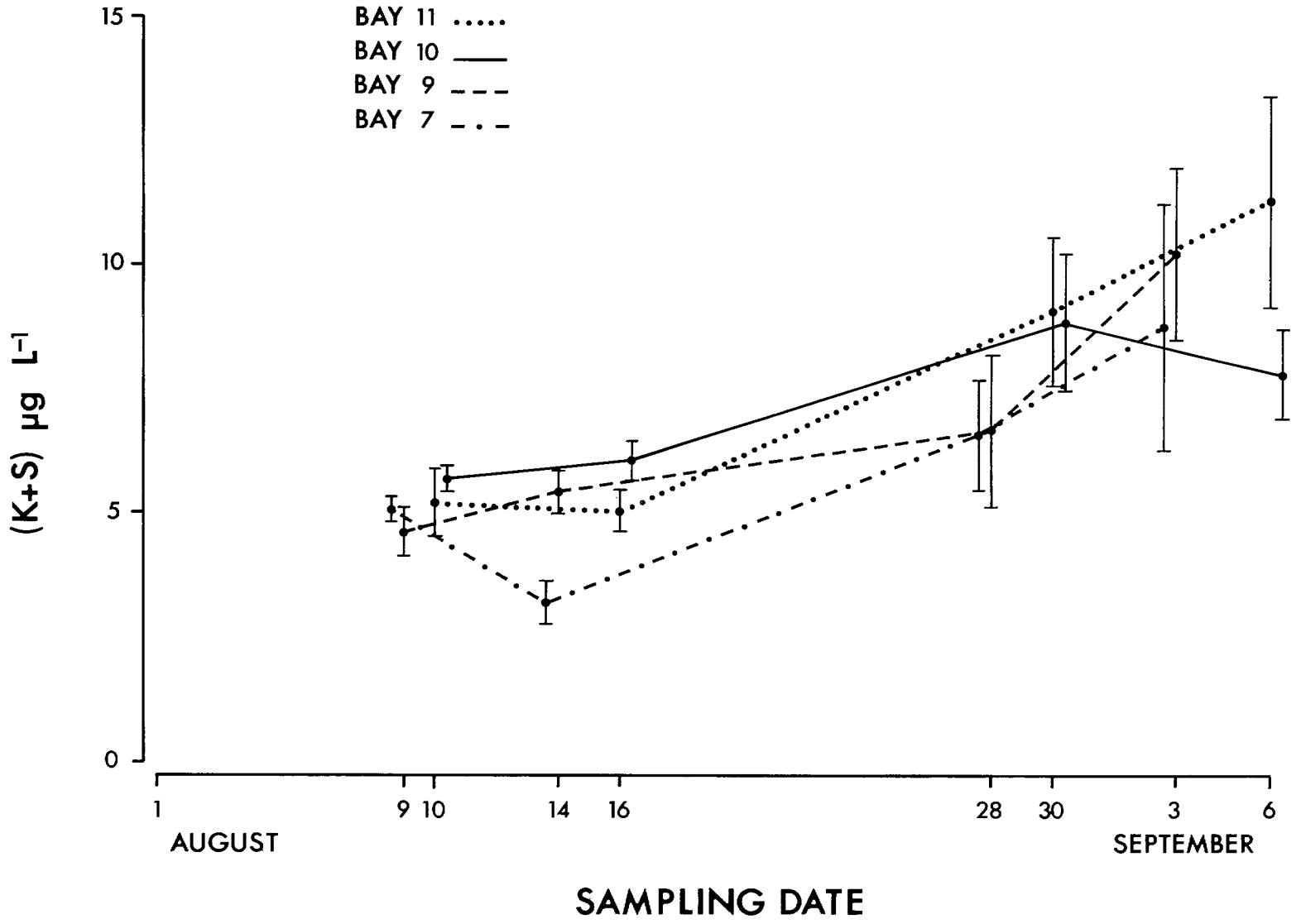

Figure 5. (K+S) of glutamic acid uptake determined in water samples collected at Cape Hatt, 1982. Results are presented as means and standard errors of six values from three depths at two stations in each bay.

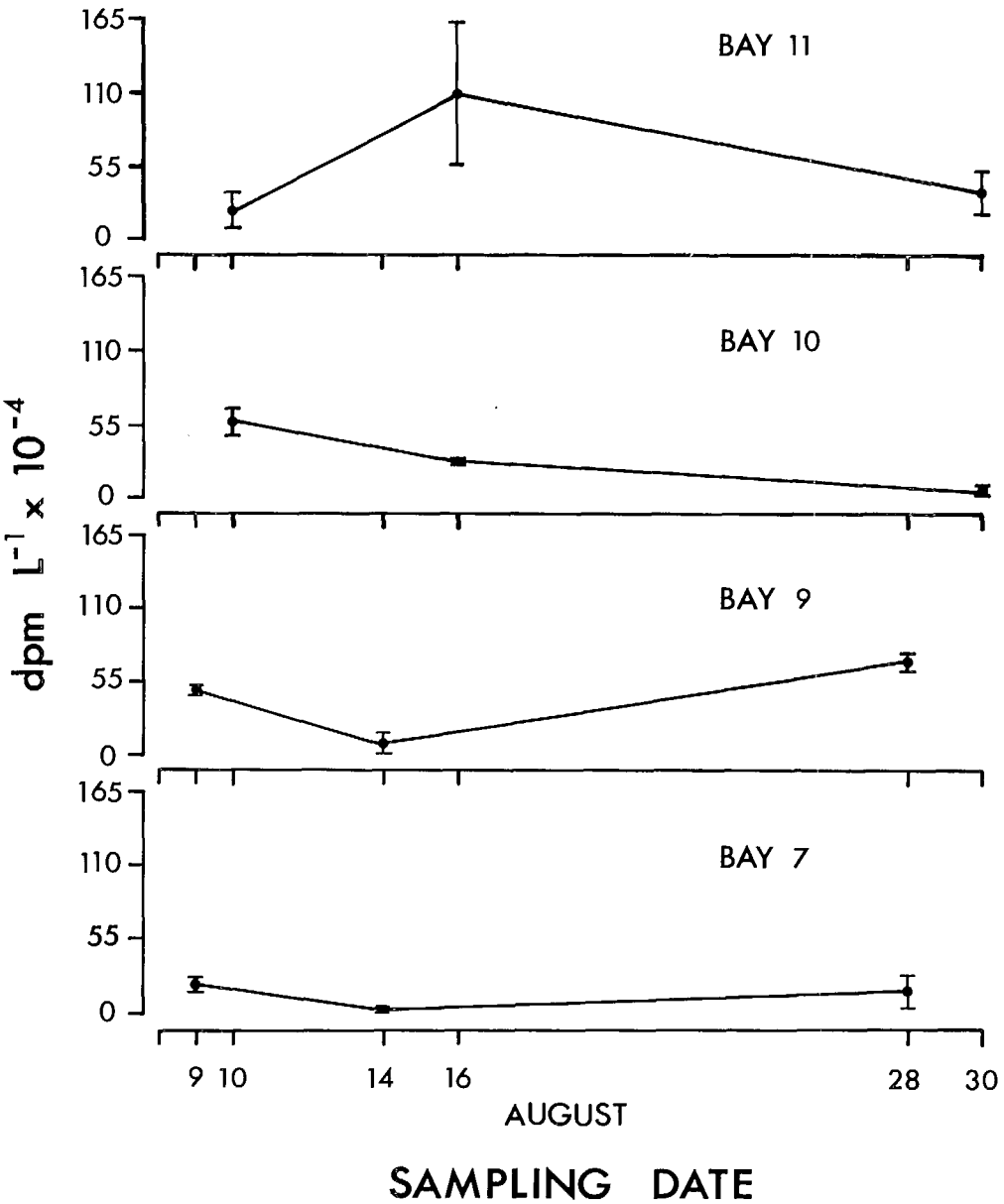

Figure 6. Disintegrations per minute (dpm) obtained from water samples after 60 days of incubation with ^{14}C-hexadecane. Results are the means and standard errors of replicate water samples from a depth of 5 m of two stations in each bay. All incubations were supplemented with 22% weathered Lago Medio petroleum crude and inorganic nutrients.

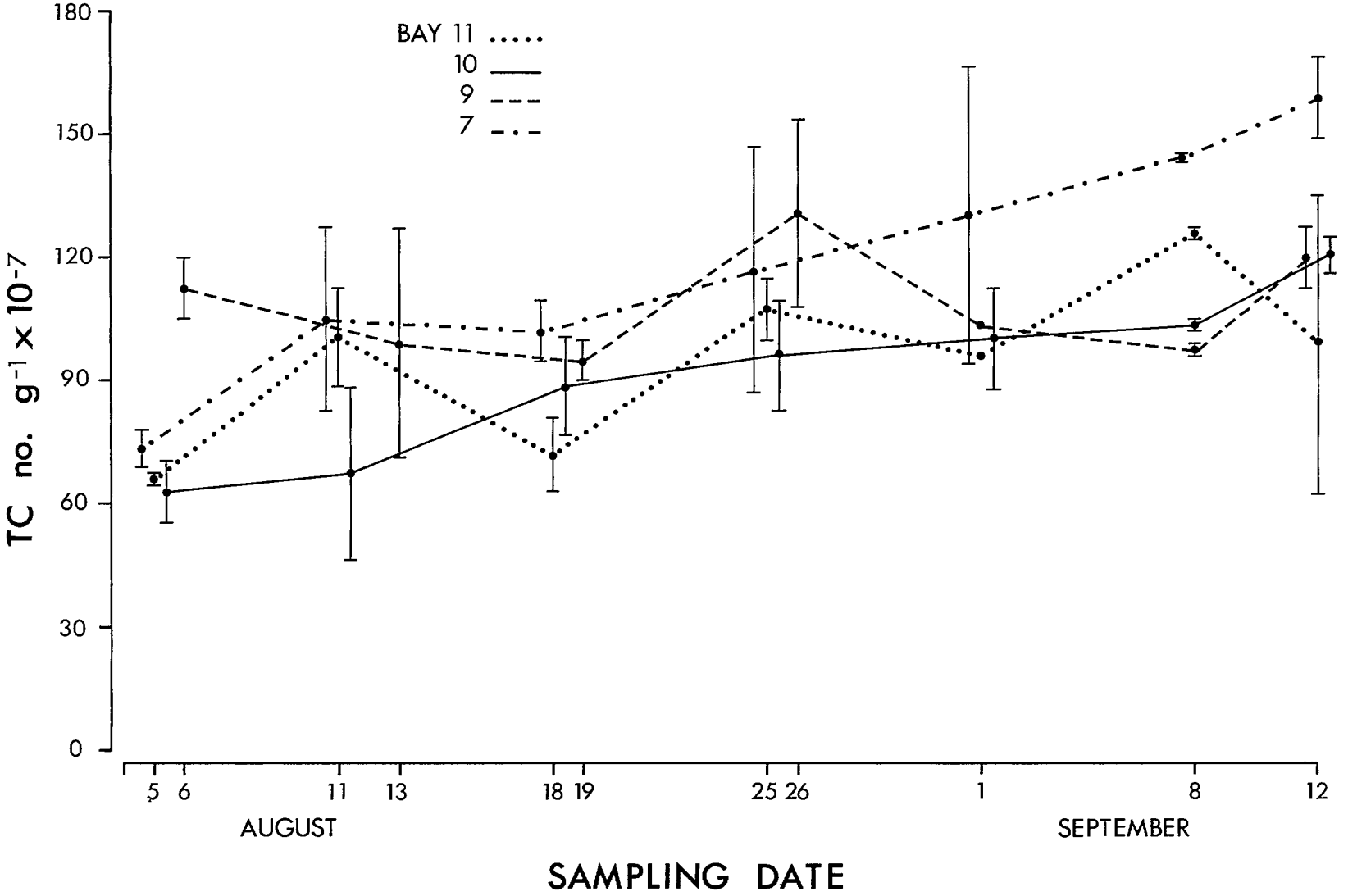

Figure 7. Total counts (TC) of bacterial cells determined from sediment suspension samples collected at Cape Hatt, 1982. Results are presented as means and standard errors from two stations in each bay.

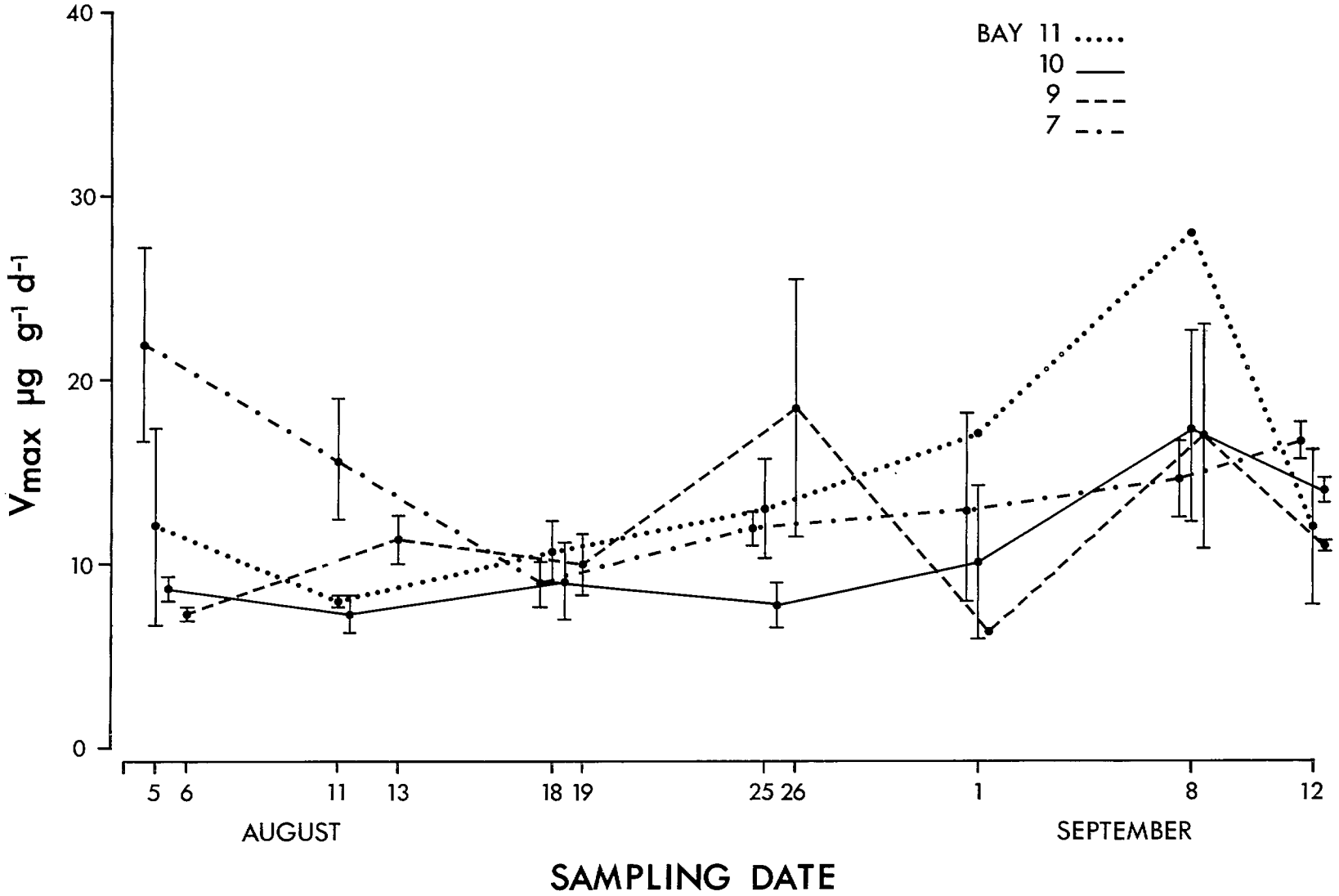

Figure 8. Maximum velocity (V_{max}) of glutamic acid uptake determined from sediment suspension samples collected at Cape Hatt, 1982. Results are presented as means and standard errors of values from two stations in each bay.

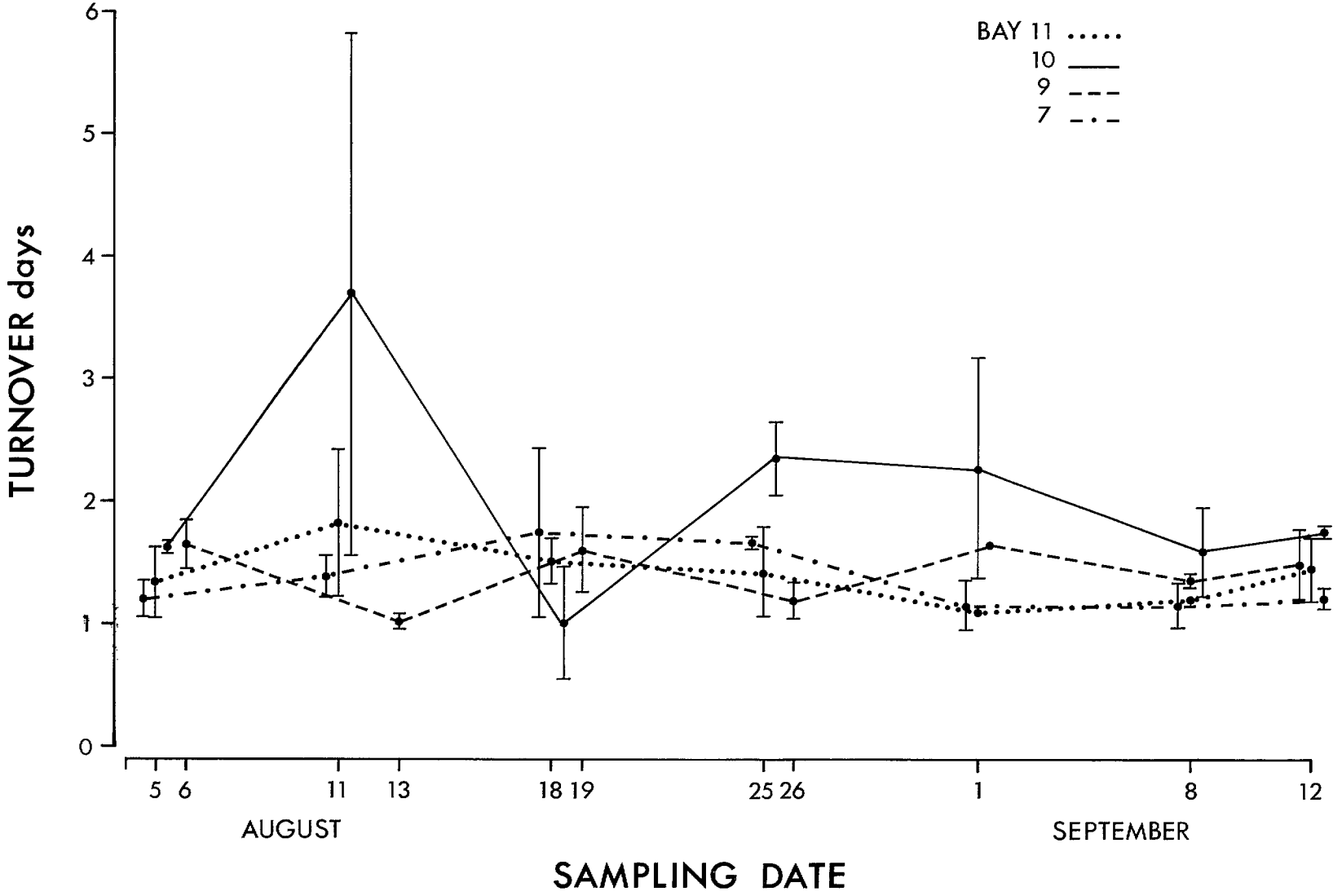

Figure 9. Turnover of glutamic acid uptake determined from sediment suspension samples collected at Cape Hatt, 1982. Results are presented as means and standard errors of values from two stations in each bay.

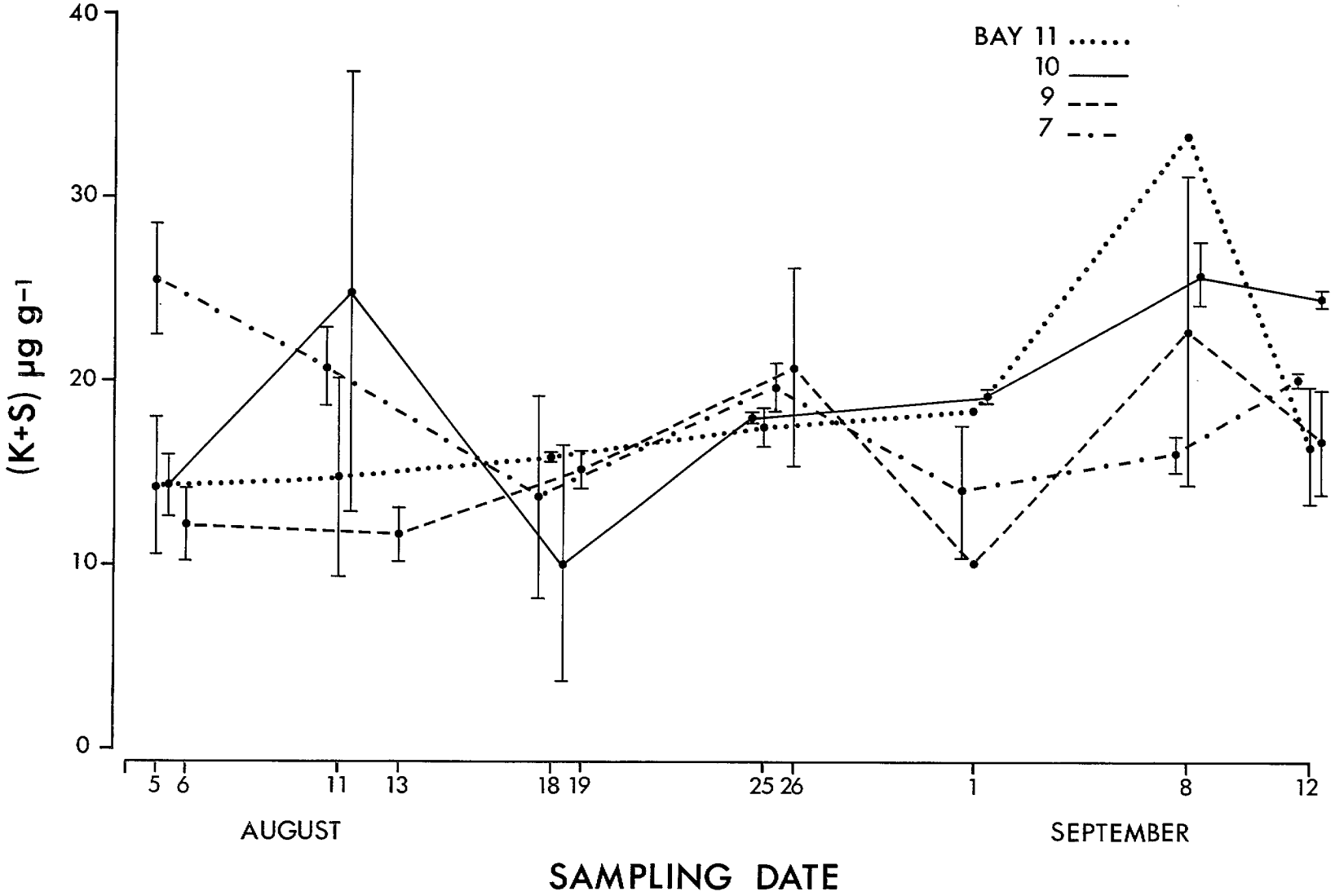

Figure 10. (K+S) of glutamic acid uptake determined from sediment suspension samples collected at Cape Hatt, 1982. Results are presented as means and standard errors of values from two stations in each bay.

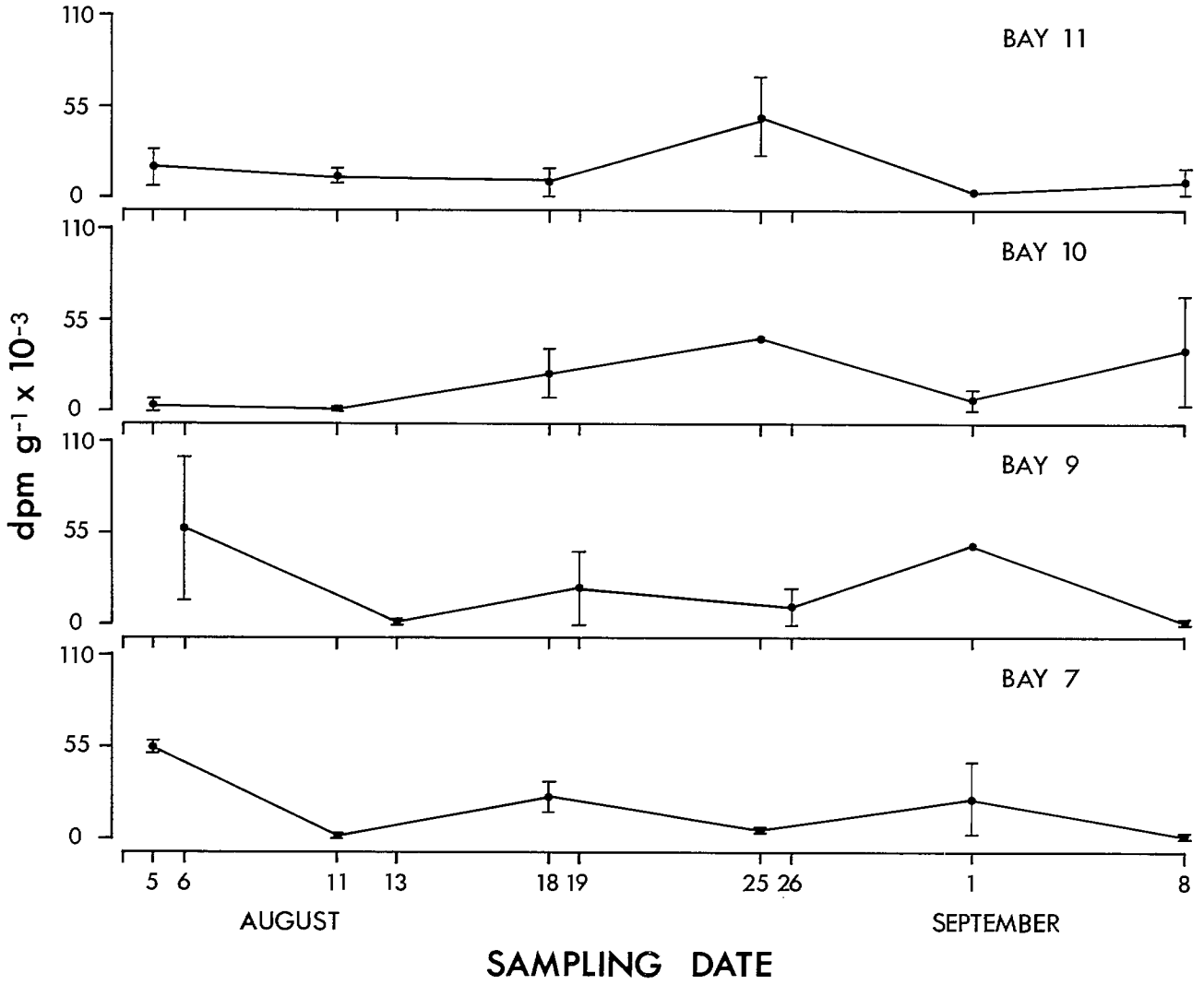

Figure 11. Disintegrations per minute (dpm) obtained from sediment suspensions after 60 days of incubation with ^{14}C-hexadecane. Results are the means and standard errors of replicate samples from two stations in each bay and are expressed as dpm per gram dry weight of sediment. All incubations were supplemented with 22% weathered Lago Medio petroleum crude and inorganic nutrients.

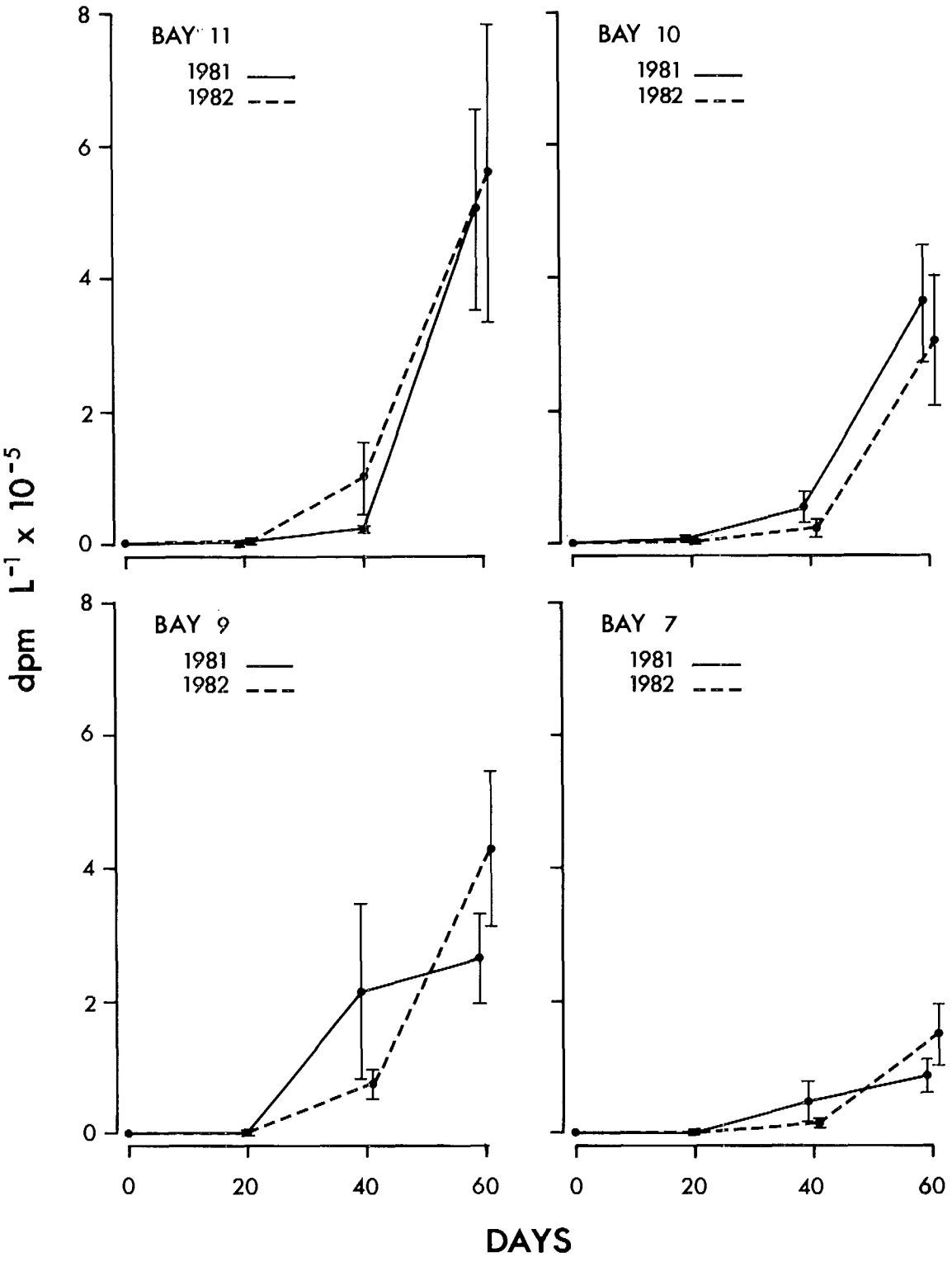

Figure 12. Comparison of disintegrations per minute (dpm) obtained from water samples after 20, 40 and 60 days of incubation with ^{14}C-hexadecane. Samples were collected at a depth of 5 m in four bays at Cape Hatt in 1981-1982. Results are expressed as seasonal means and standard errors of 12 samples supplemented with 22% weathered Lago Medio petroleum crude and inorganic nutrients.

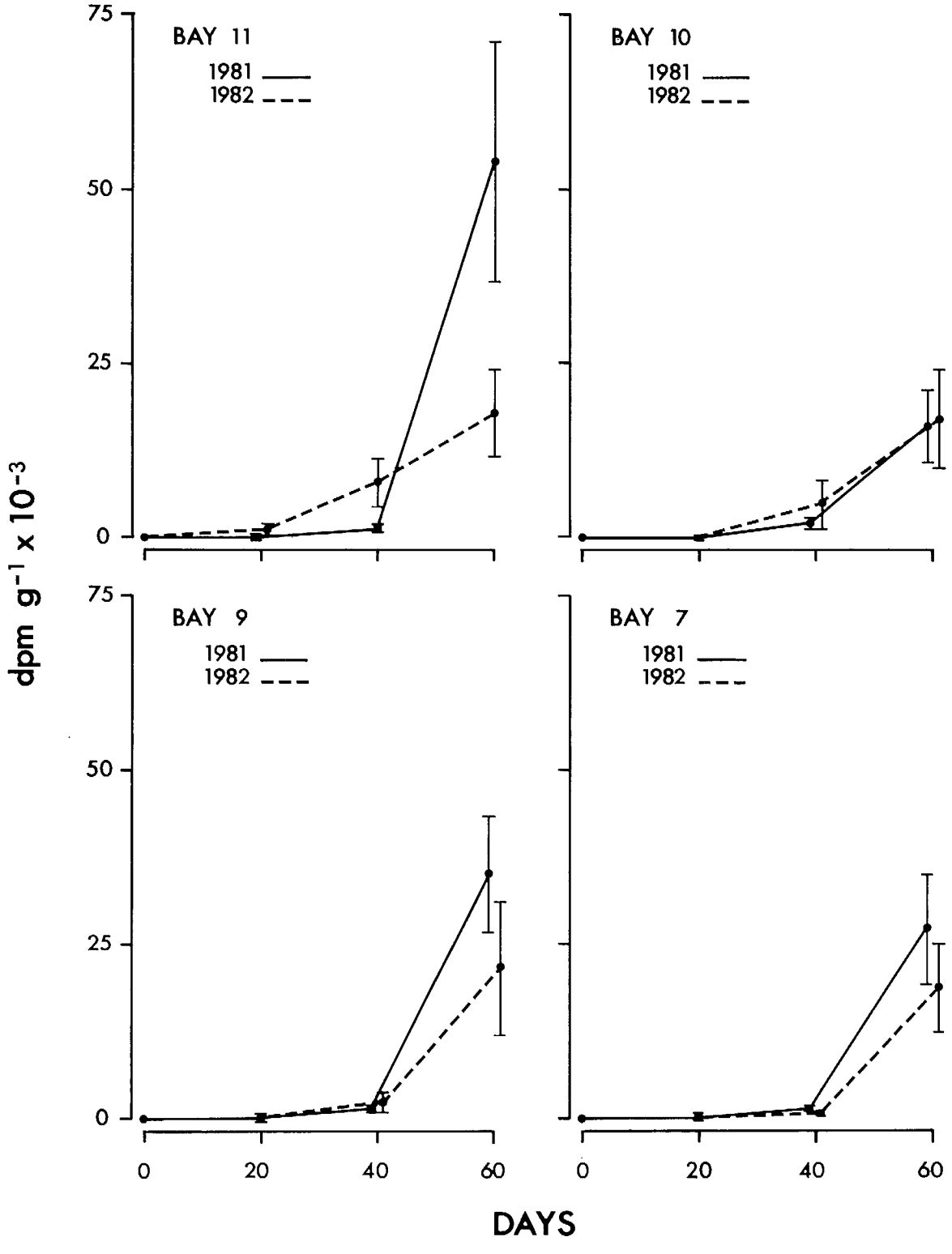

Figure 13. Comparison of disintegrations per minute (dpm) obtained from sediment suspension samples after 20, 40 and 60 days of incubation with ^{14}C-hexadecane. Samples were collected in four bays at Cape Hatt in 1981-1982. Results are expressed as seasonal means and standard errors of 12 samples supplemented with 22% weathered Lago Medio petroleum crude and inorganic nutrients.

Table 1. Water column stations occupied at Cape Hatt, 1982.

Cycle	Date	Bay	Stations	
1	08 10	11	1	2
		10	3	4
	08 09	9	5	6
		7	7	8
2	08 16	11	1	2
		10	3	4
	08 14	9	5	6
		7	7	8
3	08 30	11	1	2
		10	3	4
	08 28	9	5	6
		7	7	8
4	09 06	11	1	2
		10	3	4
	09 03	9	5	6
		7	7	8

8.0 TABLES

Table 2. Sediment stations occupied at Cape Hatt, 1982.

Cycle	Date	Bay	Stations	
1	08 05	11	1	2
		10	3	4
		7	7	8
	08 06	9	5	6
2	08 11	11	1	2
		10	3	4
		7	7	8
	08 13	9	5	6
3	08 18	11	1	2
		10	3	4
		7	7	8
	08 19	9	5	6
4	08 25	11	1	2
		10	3	4
		7	7	8
	08 26	9	5	6
5	09 01	11	1	-
		10	3	4
		9	-	6
		7	7	8
6	09 08	11	1	2
		10	3	4
		9	5	6
		7	7	8
7	09 12	11	1	2
		10	3	4
		9	5	6
		7	7	8

Table 3. Determinations of total counts (TC) of bacterial cells from water samples collected at Cape Hatt during July and early August 1982.

Station	Date	Depth	TC
		m	no. L^{-1} (10^{-7})
1	07 29	0	28.89
		5	24.32
		10	25.40
2	07 29	0	27.06
		5	19.12
		10	24.82
3	07 30	0	38.01
		5	40.43
		10	40.07
4	07 30	0	29.45
		5	29.60
		10	32.81
5	07 30	0	43.72
		5	33.67
		10	39.57
6	07 30	0	27.36
		5	30.90
		10	33.64
7	07 30	0	25.53
		5	22.71
		10	22.34

Table 3. (Cont'd)

Station	Date	Depth	TC
		m	no. L^{-1} (10^{-7})
8	07 30	0	23.84
		5	25.18
		10	29.30
1	08 02	0	18.27
		5	9.20
		10	9.70
2	08 02	0	11.42
		5	24.74
		10	28.58
3	08 02	0	17.91
		5	26.81
		10	16.68
4	08 02	0	19.20
		5	15.45
		10	23.14
5	08 02	0	11.33
		5	18.12
		10	22.54
6	08 02	0	11.68
		5	10.35
		10	22.06

Table 3. (Cont'd)

Station	Date	Depth	TC
		m	no. L^{-1} (10^{-7})
7	08 02	0	8.82
		5	13.07
		10	22.19
8	08 02	0	12.71
		5	13.57
		10	17.29

Table 4. Determinations of total counts (TC) of bacterial cells, maximum velocity (V_{max}), turnover (T) and (K+S) (see text) of glutamic acid uptake from water samples collected at Cape Hatt during August and September 1982.

Station	Date	Depth	TC	V_{max}	T	(K+S)
		m	no. L^{-1} (10^{-7})	µg L^{-1} d^{-1}	d	µg L^{-1}
1	08 10	0	20.15	2.75	2.31	6.36
		5	27.34	2.35	1.40	3.29
		10	29.75	3.55	0.99	3.52
2	08 10	0	28.78	2.92	2.26	6.58
		5	32.50	2.58	1.60	4.14
		10	39.71	3.30	2.21	7.29
3	08 10	0	33.25	2.63	2.12	5.57
		5	22.60	2.89	2.06	5.95
		10	43.65	2.82	2.29	6.46
4	08 10	0	34.51	2.37	2.60	6.17
		5	21.59	2.67	2.04	5.44
		10	47.12	4.25	1.07	4.55
5	08 09	0	21.41	6.02	0.70	4.23
		5	26.71	6.01	1.00	6.00
		10	29.32	1.86	2.63	4.91
6	08 09	0	24.67	5.61	0.49	2.75
		5	15.95	7.59	0.74	5.61
		10	41.05	1.46	2.89	4.22

Table 4. (Cont'd)

Station	Date	Depth	TC	V_{max}	T	(K+S)
		m	no. L^{-1} (10^{-7})	µg L^{-1} d^{-1}	d	µg L^{-1}
7	08 09	0	26.91	4.84	1.11	5.37
		5	25.23	5.64	1.01	5.67
		10	25.13	1.19	4.70	5.61
8	08 09	0	22.49	6.63	0.64	4.22
		5	27.01	6.49	0.79	5.10
		10	20.95	1.43	3.18	4.55
1	08 16	0	34.55	1.92	2.13	4.10
		5	42.18	1.94	2.21	4.28
		10	38.11	2.85	2.06	5.87
2	08 16	0	10.10	2.28	2.38	5.42
		5	45.12	2.47	1.68	4.15
		10	50.48	2.81	2.31	6.51
3	08 16	0	31.26	1.83	3.36	6.14
		5	41.18	2.85	1.97	5.61
		10	51.93	2.79	2.74	7.63
4	08 16	0	22.17	2.09	2.87	6.00
		5	23.00	2.59	2.43	6.32
		10	55.12	2.16	2.14	4.63
5	08 14	0	30.49	1.80	2.48	4.47
		5	43.25	2.01	3.38	6.80
		10	52.07	2.15	2.80	6.01

Table 4. (Cont'd)

Station	Date	Depth	TC	V_{max}	T	(K+S)
		m	no. L^{-1} (10^{-7})	µg L^{-1} d^{-1}	d	µg L^{-1}
6	08 14	0	33.84	2.03	3.01	6.09
		5	28.29	2.21	1.94	4.28
		10	44.40	2.31	2.08	4.82
7	08 14	0	33.32	1.81	1.80	3.26
		5	45.17	2.12	2.12	4.51
		10	56.73	2.44	0.68	1.65
8	08 14	0	31.76	1.68	2.59	4.36
		5	35.75	2.21	1.34	2.96
		10	36.73	2.64	0.98	2.57
1	08 30	0	45.43	1.99	5.27	10.51
		5	21.29	2.14	2.89	6.20
		9	31.12	3.44	2.72	9.36
2	08 30	0	45.66	2.97	3.87	11.50
		5	21.77	3.96	3.40	13.44
		10	41.42	8.12	0.42	3.41
3	08 30	0	19.89	2.40	4.13	9.90
		5	45.79	3.64	2.06	7.50
		10	36.59	1.80	2.10	3.78
4	08 30	0	36.82	2.42	4.36	10.56
		5	28.60	3.06	2.45	7.50
		10	50.77	2.88	4.82	13.88

Table 4. (Cont'd)

Station	Date	Depth	TC	V_{max}	T	(K+S)
		m	no. L^{-1} (10^{-7})	µg L^{-1} d^{-1}	d	µg L^{-1}
5	08 28	0	49.80	2.32	2.08	4.82
		5	41.58	3.14	2.32	7.29
		10	56.53	2.77	0.90	2.49
6	08 28	0	45.35	2.12	1.81	3.85
		5	64.60	2.44	3.48	8.48
		10	47.39	2.07	6.27	12.96
7	08 28	0	37.69	2.64	1.14	3.02
		5	44.80	2.36	1.91	4.52
		8	48.49	1.81	5.47	9.90
8	08 28	0	26.98	1.74	2.87	4.98
		5	31.00	2.17	3.62	7.86
		9	27.99	1.41	6.43	9.06
1	09 06	0	16.27	2.76	6.58	18.12
		5	60.29	8.29	0.94	7.82
		9	33.58	6.07	2.41	14.65
2	09 06	0	49.92	4.01	3.69	14.82
		5	23.79	8.87	0.65	5.76
		8	44.58	11.98	0.55	6.55
3	09 06	0	63.27	8.25	0.86	7.12
		5	56.65	11.33	0.87	9.82
		8	34.14	6.94	1.07	7.43

Table 4. (Cont'd)

Station	Date	Depth	TC	V_{max}	T	(K+S)
		m	no. L^{-1} (10^{-7})	µg L^{-1} d^{-1}	d	µg L^{-1}
4	09 06	0	25.13	5.03	0.84	4.23
		5	44.94	4.48	2.37	10.61
		8	37.51	3.71	2.05	7.61
5	09 03	0	30.94	3.85	3.21	12.39
		5	47.88	3.70	2.47	9.13
		8	24.03	3.98	0.61	2.43
6	09 03	0	34.38	3.78	2.89	10.91
		5	30.92	2.74	5.45	14.91
		8	55.36	7.40	1.56	11.56
7	09 03	0	26.47	2.70	5.10	13.77
		5	83.70	5.59	1.22	6.85
		8	27.60	4.03	4.56	18.40
8	09 03	0	25.46	3.17	1.98	6.27
		5	25.71	2.57	0.98	2.52
		8	28.47	5.64	0.82	4.63

Table 5. Determinations of most probable number (MPN) of oleoclastic cells and maximum disintegrations per minute (dpm) obtained from water samples incubated with ^{14}C-hexadecane. Samples were collected from 5 m at Cape Hatt during August and September 1982 and replicates were incubated for 20, 40 or 60 days with ^{14}C-hexadecane, 22% weathered Lago Medio (L.M.) petroleum crude and inorganic nutrients. Similar samples were incubated for 60 days without inorganic nutrients. Results were corrected for aliquot volume and expressed per litre of sample volume.

	Station	Date	20 days	40 days	60 days	60 days (no nutrients)	Oleoclasts
			dpm L^{-1} (10^{-4})	dpm L^{-1} (10^{-4})	dpm L^{-1} (10^{-4})	dpm L^{-1} (10^{-4})	no. L^{-1} (10^{-4})
Bay 7	7	08 09	0	3.86	27.98	1.04	0.60
	8		0	2.38	16.17	0.95	0.10
	7	08 14	0	0.60	5.37	0.49	0.11
	8		0	0.37	3.27	0.59	0.03
	7	08 28	0	1.88	30.11	1.16	$\geqq$600.00
	8		0.12	0.65	7.10	1.02	$\geqq$600.00
Bay 9	5	08 09	0	11.47	52.37	1.70	0.53
	6		0	8.42	45.78	0.97	1.60
	5	08 14	0	0.49	1.35	0.67	0.11
	6		0	0.80	18.03	0.52	0.05

Table 5. (Cont'd)

	Station	Date	20 days	40 days	60 days	60 days (no nutrients)	Oleoclasts
			dpm L^{-1} (10^{-4})	dpm L^{-1} (10^{-4})	dpm L^{-1} (10^{-4})	dpm L^{-1} (10^{-4})	no. L^{-1} (10^{-4})
Bay 9	5	08 28	0.35	10.28	64.37	1.14	≧600.00
	6		0.50	13.56	76.05	1.37	275.00
Bay 10	3	08 10	0	9.27	67.63	0.84	1.88
	4		0	1.69	47.51	1.36	0.11
	3	08 16	0.19	1.90	29.47	4.80	275.00
	4		0.11	0.50	25.99	0.72	≧600.00
	3	08 30	0.19	0.70	10.39	1.03	≧600.00
	4		0	0.21	2.22	0.50	≧600.00
Bay 11	1	08 10	0	1.78	8.75	0.53	1.88
	2		0	17.05	36.65	1.12	0.53
	1	08 16	0.25	1.83	161.73	0.72	≧600.00
	2		2.20	35.00	56.57	1.18	≧600.00
	1	08 30	0.03	1.78	19.83	0.76	9.75
	2		0.57	2.33	51.59	1.78	3.75

Table 6. Determinations of total counts (TC) of bacterial cells, maximum velocity (V_{max}), turnover (T) and (K+S) (see text) of glutamic acid uptake from sediment suspension samples collected from surface sediment at Cape Hatt during August and September 1982. Quantities are expressed per gram dry weight of sediment.

Station	Date	TC	V_{max}	T	(K+S)
		no. g^{-1} (10^{-7})	µg g^{-1} d^{-1}	d	µg g^{-1}
1	08 05	67.69	17.28	1.05	18.03
2	08 05	63.57	6.45	1.63	10.50
3	08 05	70.76	8.02	1.59	12.71
4	08 05	55.64	9.54	1.67	15.89
5	08 06	105.10	7.67	1.86	14.21
6	08 06	119.39	6.98	1.46	10.14
7	08 05	77.11	16.75	1.36	22.50
8	08 05	68.43	27.05	1.06	28.57
1	08 11	88.94	7.73	1.22	9.38
2	08 11	112.52	8.33	2.42	20.23
3	08 11	46.78	6.33	5.82	36.78
4	08 11	88.37	8.29	1.55	12.89
5	08 13	127.12	10.10	1.00	10.16
6	08 13	71.66	12.65	1.04	13.10
7	08 11	82.20	12.41	1.55	18.62
8	08 11	128.00	19.03	1.21	22.88
1	08 18	62.41	12.19	1.32	16.08
2	08 18	81.45	9.18	1.70	15.59
3	08 18	99.75	11.17	1.47	16.51
4	08 18	76.35	6.89	0.55	3.74
5	08 19	89.79	8.29	1.94	16.11
6	08 19	99.94	11.38	1.25	14.19
7	08 18	94.07	10.11	2.42	19.25
8	08 18	109.68	7.60	1.06	8.11

Table 6. (Cont'd)

Station	Date	TC	V_{max}	T	(K+S)
		no. g^{-1} (10^{-7})	µg g^{-1} d^{-1}	d	µg g^{-1}
1	08 25	114.71	15.61	1.06	16.49
2	08 25	99.85	10.22	1.80	18.45
3	08 25	82.87	6.60	2.65	17.45
4	08 25	109.49	9.08	2.03	18.40
5	08 26	153.50	25.46	1.02	26.10
6	08 26	108.32	11.47	1.34	15.30
7	08 25	146.78	10.99	1.68	18.11
8	08 25	86.77	12.78	1.65	21.02
1	09 01	95.41	16.88	1.08	18.27
3	09 01	112.17	14.26	1.37	19.43
4	09 01	87.63	5.98	3.16	18.88
6	09 01	103.25	6.18	1.64	10.12
7	09 01	93.60	7.89	1.35	10.35
8	09 01	166.03	18.20	0.96	17.61
1	09 08	124.91	--	--	--
2	09 08	127.77	28.06	1.19	33.37
3	09 08	103.85	22.64	1.22	27.61
4	09 08	102.72	12.28	1.96	24.05
5	09 08	112.36	10.84	1.33	14.42
6	09 08	111.39	22.84	1.37	31.13
7	09 08	143.99	16.62	0.97	14.92
8	09 08	143.50	12.51	1.35	16.89

Table 6. (Cont'd)

Station	Date	TC	V_{max}	T	(K+S)
		no. g^{-1} (10^{-7})	µg g^{-1} d^{-1}	d	µg g^{-1}
1	09 12	62.13	7.88	1.69	13.23
2	09 12	134.95	16.18	1.21	19.55
3	09 12	116.22	13.44	1.79	24.09
4	09 12	125.27	14.77	1.69	24.84
5	09 12	126.42	10.88	1.78	19.40
6	09 12	112.14	11.40	1.20	13.74
7	09 12	148.56	17.64	1.14	19.86
8	09 12	168.10	15.63	1.30	20.16

Table 7. Seasonal means and standard errors of total counts (TC) of bacterial cells, maximum velocity (V_{max}), turnover (T) and (K+S) (see text) of glutamic acid uptake obtained from sediment samples in 1981 and 1982.

Year	Bay	TC	V_{max}	T	(K+S)
		no. g^{-1} (10^{-7})	µg g^{-1} d^{-1}	d	µg g^{-1}
1981	7	153.65 ± 16.57	37.72 ± 4.52	0.98 ± 0.10	31.33 ± 2.61
1982	7	118.34 ± 9.27	14.66 ± 1.38	1.36 ± 0.10	18.49 ± 1.36
1981	9	75.69 ± 7.74	12.47 ± 1.02	1.84 ± 0.18	21.68 ± 1.74
1982	9	110.80 ± 5.43	12.01 ± 1.60	1.40 ± 0.09	16.01 ± 1.73
1981	10	97.64 ± 9.80	14.10 ± 1.13	1.64 ± 0.20	20.65 ± 1.28
1982	10	91.28 ± 6.15	10.66 ± 1.22	2.04 ± 0.33	19.52 ± 2.10
1981	11	103.53 ± 9.00	26.83 ± 4.39	1.31 ± 0.16	25.82 ± 1.77
1982	11	95.10 ± 7.33	13.00 ± 1.78	1.45 ± 0.12	17.43 ± 1.75

Table 8. Determinations of most probable number (MPN) of oleoclastic cells and maximum disintegrations per minute (dpm) obtained from sediment suspension samples incubated with ^{14}C-hexadecane. Samples were collected from surface sediment at Cape Hatt during August and September 1982 and replicates were incubated for 20, 40 or 60 days with ^{14}C-hexadecane, 22% weathered Lago Medio (L.M.) petroleum crude and inorganic nutrients. Similar samples were incubated for 60 days without inorganic nutrients. Results were corrected for dilution and expressed per gram dry weight of sediment.

	Station	Date	20 days	40 days	60 days	60 days (no nutrients)	Oleoclasts
			dpm g^{-1} (10^{-3})	dpm g^{-1} (10^{-3})	dpm g^{-1} (10^{-3})	dpm g^{-1} (10^{-3})	no. g^{-1} (10^{-3})
Bay 7	7	08 05	0.26	0.75	58.90	0.42	67.32
	8		0.15	1.90	50.00	0.78	≧401.90
	7	08 11	0.06	0.27	0.80	0.75	5.46
	8		0.09	0.49	1.45	0.66	2.48
	7	08 18	0.34	0.97	34.65	0.99	5.79
	8		0.27	1.83	15.29	0.81	6.87
	7	08 25	0.06	0.71	6.00	0.27	1.14
	8		0.19	1.01	6.36	0.74	25.26
	7	09 01	0.03	0.86	2.68	0.36	167.58
	8		0.04	0.74	46.22	0.30	≧410.79

Table 8. (Cont'd)

	Station	Date	20 days dpm g^{-1} (10^{-3})	40 days dpm g^{-1} (10^{-3})	60 days dpm g^{-1} (10^{-3})	60 days (no nutrients) dpm g^{-1} (10^{-3})	Oleoclasts no. g^{-1} (10^{-3})
Bay 7	7	09 08	0.14	0.68	1.04	0.55	≧393.39
	8		0.16	0.41	1.31	0.63	9.51
Bay 9	5	08 06	0.25	18.50	101.89	1.13	≧322.93
	6		0.33	1.24	15.85	0.58	22.87
	5	08 13	0.08	0.31	0.76	--	≧342.49
	6		0.08	1.41	0.57	0.25	171.41
	5	08 19	0.27	1.09	43.32	0.18	0.33
	6		0.11	0.23	0.37	0.28	0.01
	5	08 26	0.16	0.53	21.90	0.79	68.88
	6		0.31	0.69	0.71	0.77	68.88
	6	09 01	0.11	0.58	48.52	0.31	≧387.67

Table 8. (Cont'd)

	Station	Date	20 days dpm g^{-1} (10^{-3})	40 days dpm g^{-1} (10^{-3})	60 days dpm g^{-1} (10^{-3})	60 days (no nutrients) dpm g^{-1} (10^{-3})	Oleoclasts no. g^{-1} (10^{-3})
Bay 9	5	09 08	0.10	0.37	1.63	0.55	≧347.38
	6		0.10	0.43	1.52	0.43	≧363.28
Bay 10	3	08 05	0.21	0.51	0.70	0.76	0.21
	4		0.20	4.12	7.02	0.64	5.61
	3	08 11	0.24	0.59	0.53	1.28	≧351.70
	4		0.09	0.37	0.76	0.62	34.89
	3	08 18	0.21	43.06	38.94	0.79	3.32
	4		0.21	0.51	8.08	0.54	0.63
	3	08 25	0.31	0.53	--	0.61	8.86
	4		0.40	3.20	45.13	0.63	≧346.24
	3	09 01	0	0.64	0.70	0.31	≧363.26
	4		0.16	1.03	13.25	1.68	≧337.86

Table 8. (Cont'd)

	Station	Date	20 days dpm g^{-1} (10^{-3})	40 days dpm g^{-1} (10^{-3})	60 days dpm g^{-1} (10^{-3})	60 days (no nutrients) dpm g^{-1} (10^{-3})	Oleoclasts no. g^{-1} (10^{-3})
Bay 10	3	09 08	0.12	0.74	3.01	--	≧367.51
	4		0.14	0.45	70.32	1.01	156.06
Bay 11	1	08 05	0.22	23.58	29.01	0.63	199.38
	2		0.22	2.83	8.39	0.56	32.34
	1	08 11	0.10	0.49	8.18	0.48	3.02
	2		0.09	0.21	17.24	0.46	0.54
	1	08 18	10.79	0.66	1.38	0.58	3.18
	2		0.21	19.56	18.96	0.94	10.71
	1	08 25	0.83	33.48	25.18	0.89	77.10
	2		0.46	1.13	72.93	5.39	174.08
	1	09 01	0.05	0.75	3.02	0.36	≧387.35
	1	09 08	0.08	4.16	17.74	0.41	15.21
	2		0.07	0.46	1.12	0.63	79.55

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