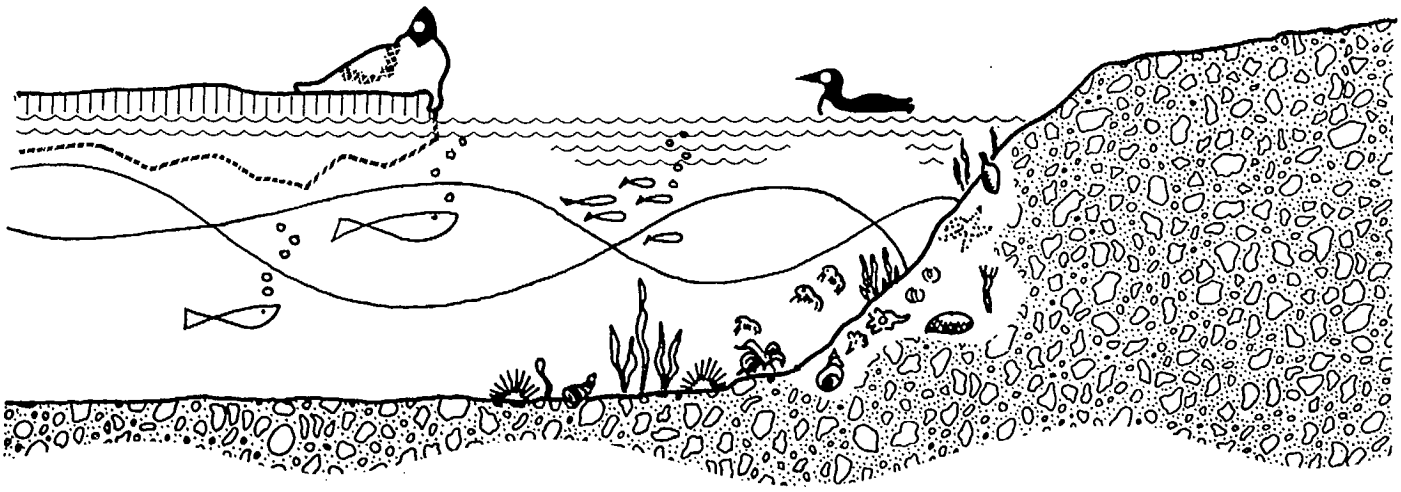
MICROBIOLOGY



Baffin Island Oil Spill Project

WORKING REPORT SERIES
83-5

1983 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.

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Baffin Island Oil Spill (BIOS) Project
Working Report 83-5

Microbiology

1. Effects of petroleum releases on the microheterotrophic flora of arctic sediments -- effects after two years

prepared for

Environmental Protection Service
Canadian Department of the Environment
Edmonton, Alberta

by

J. N. Bunch and T. Cartier

1984

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 T. Cartier. 1984. Microbiology: 1. Effects of petroleum releases on the microheterotrophic flora of arctic sediments -- effects after two years. Baffin Island Oil Spill (BIOS) project working report 83-5: x + 44 p.

The Baffin Island Oil Spill (BIOS) project was initiated to compare the effects of dispersed and nondispersed petroleum in an arctic marine ecosystem. To facilitate this objective, bacterial numbers and microheterotrophic activity (the uptake of glutamic acid by bacteria and other microorganisms such as fungi) were monitored in the water column and sediments of selected bays in Cape Hatt, N.W.T. between 1980 and 1983. 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 sediment of the intertidal zone at low tide. No significant effects were seen in microbiological parameters measured in 1981 or 1982. Petroleum beached on the intertidal zone was observed entering subtidal sediments between 1981 and 1983 and forming a gradation of petroleum concentrations from nearshore to offshore areas.

During the second release in 1981, dispersed petroleum was carried 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} over control bay 7. Bacterial numbers were

unaffected. No changes were measured in the sediment of test bays 9 and 10 during and after the release.

An inter-bay 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 parameters except TOC in bay 11 decreased over 1981. In 1983, trends in the sediments of bay 9 were similar to those of bay 7.

In test bay 11, TOC increased between 1982 and 1983 as it did between 1981 and 1982. Microheterotrophic activity remained constant although it decreased in bays 9 and 7. Bacterial numbers increased in bay 11 but decreased in bays 9 and 7. We concluded that the changes in bay 9 in 1982 and in bay 11 in 1983 were a consequence of perturbations by petroleum. Petroleum effects on the benthic macrofauna and flora increased the levels of detritus and hence TOC in the sediments with a consequent change in bacterial numbers and undiminished microheterotrophic activity. This conclusion is supported by measurements made on a transect of nearshore sediments in test bay 11. Although petroleum concentrations formed a gradient on this transect, numbers of bacteria and uptake of glutamic acid were positively correlated with TOC but were not influenced by petroleum.

Numbers of oleoclasts (petroleum-degrading microorganisms) capable of mineralizing ^{14}C-hexadecane to $^{14}CO_2$ decreased considerably in sediments between 1982 and 1983. Numbers and activity were unrelated to concentrations of petroleum in the sediments.

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1.0 INTRODUCTION

This is the fourth and last report of microbiological observations to be prepared for the Baffin Island Oil Spill (BIOS) project by our laboratory. Our main objective during this four year project was to observe the immediate and long-term effects of petroleum releases in cold marine waters on microheterotrophic organisms. This group consists of microorganisms requiring organic material as a source of carbon and energy and includes many types of bacteria and all fungi (see Bunch et al, 1981). This objective was pursued at Cape Hatt, NWT before, during and after a surface release of relatively unweathered petroleum and a subsurface release of the same stock of petroleum previously mixed with a dispersant. Microbiological observations by ourselves and by a group of Norwegian microbiologists headed by Dr. K. Eimhjellen were conducted at the same time as other chemical, physical and biological studies (see Boehm, 1981; Cross and Thomson, 1981; Eimhjellen et al., 1981).

There were specific objectives in each of the four years of the project. In 1980, the objectives were:

1. to characterize numbers of bacteria and activity of microheterotrophs in the water and sediment of selected bays at Cape Hatt;
2. to assess the above measurements in relation to other biological and chemical cycles.

Baseline data collected in three bays established that no major differences existed between the bays (Bunch et al, 1981). This information allowed us to conclude that two bays could be used for petroleum releases with the third bay serving as a control.

In 1981, the objectives were:

1. to repeat protocols of 1980 before and after releases of petroleum to assess annual

variations and variations due to petroleum releases;

2. to experimentally assess the use of radiolabelled hydrocarbons as substrates for activity by oleoclasts (petroleum-degrading microheterotrophs) in vitro;
3. to experimentally assess the effects of dispersant, petroleum and petroleum-dispersant mixtures on microheterotrophic activity in vitro;
4. to relate microbiological data to hydrocarbon, and physical and chemical oceanographic data and other biological data.

In 1981, oceanographic data concerning the currents in the bays led to uncertainty about the usefulness of bay 10 as a control. This uncertainty was later justified by petroleum contamination of this bay during the release of dispersed petroleum in an adjacent bay (bay 9). To ensure a control for comparative purposes, a fourth bay (bay 7) was selected in 1981 as a control bay. Observations were made in the water columns and sediments of the bays before, during and after a surface release of 8% weathered Lagomedio petroleum in bay 11 and a subsurface release in bay 9 of the same petroleum mixed 10:1 with the dispersant Corexit 9527. Details of the releases can be found in Dickins (1982).

Bunch et al (1983) concluded that the immediate effect of the dispersed petroleum on microheterotrophs in the water column, a decrease in microheterotrophic activity, was transient and minimal, while no effect could be ascribed to the surface release. Activity in the sediments was unaffected in 1981 by either release but it was also noted that the sediment of control bay 7 was dissimilar to the other bays because of the high content of organic carbon and high numbers of bacteria and microheterotrophic activity.

Oleoclasts were ubiquitous throughout the waters and sediments of all bays before and after the releases and were unaffected by the releases.

The objectives in 1982 were:

1. to determine whether the petroleum releases in 1981 had altered the number of bacteria including oleoclasts in the sediments of Cape Hatt or affected activity of microheterotrophs including oleoclasts;
2. to repeat previous protocols in the water column to detect changes which might later affect the sediments.

Between 1981 and 1982, Bunch and Bĕdard (1983) reported that the levels of total organic carbon (TOC) and numbers of bacteria had increased in bay 9 where the dispersed release occurred while uptake of glutamic acid by microheterotrophs remained unchanged. These observations were not seen in the other bays. The sediments of bay 9 had the highest estimated concentrations of petroleum at the offshore microbiology stations compared to other bays (Boehm, 1983). This led Bunch and Bĕdard to conclude that the increased levels of TOC and subsequent microbial changes in the surface sediments of bay 9 had been consequences of the dispersed petroleum release. Acute and long-term effects on benthic plants and animals might have been sufficient to create cellular debris which was quantitated as TOC.

Oleoclasts, measured by their ability to mineralize ^{14}C-hexadecane, appeared to have increased in numbers in the sediments of all bays in 1982 but their ability to mineralize hexadecane remained unchanged from 1981. Observations in the water column were unchanged from the previous year.

In order to sample sediments with a range of concentrations of petroleum, we proposed to set up a transect

in bay 11 in 1983. Petroleum stranded on the intertidal zone of that bay was entering the subtidal sediments (Owens et al., 1983). Upon establishing a gradation of petroleum concentrations along this transect, microbiological observations could be made and compared to similar observations in the control bay 7. Observations of the water column were discontinued. Observations of sediments at the microbiology stations in three bays were reduced to a minimum number which could be used in an inter-year comparison.

Our objectives in 1983, therefore, were:

1. to repeat protocols of 1981 and 1982 with sediments from bays 7, 9 and 11. These protocols included measurements of total organic carbon, numbers of bacteria and oleoclastic microorganisms, and activity of microheterotrophs including oleoclasts;
2. to repeat the same protocols at stations along transect lines in bays 11 and 7 and relate these observations to concentrations of petroleum in the same samples.

The observations made during 1983 are reported here and are related to the observations of previous years.

2.0 STUDY AREA

In 1983, sediments from the same stations as in previous years were collected on three occasions (5, 12 and 19 August) in bays 11 and 9, sites of the petroleum releases in 1981, and bay 7, the control bay (Fig. 1; Table 1).

Sediment samples were also collected on a transect line deployed at the south end of bay 11 (Fig. 2). The transect line ran from a post secured in the sediment at the water edge of the intertidal zone at low tide to station H2 at a depth of 12 m. The positioning of the transect line was based on petroleum analyses of surface sediments in bay 11 from 1982 and varying petroleum concentrations were expected (Boehm, 1983). Eight samples were taken on two occasions (10 and 17 August) at various points along the line (Fig. 3). To ensure a good range of petroleum concentrations in our sediment samples, on-site analyses were carried out with a fluorometer (Turner Designs). Bay 7 was sampled on the same dates at five different depths (2, 4, 6, 8 and 10 m) to serve as a control for the transect work.

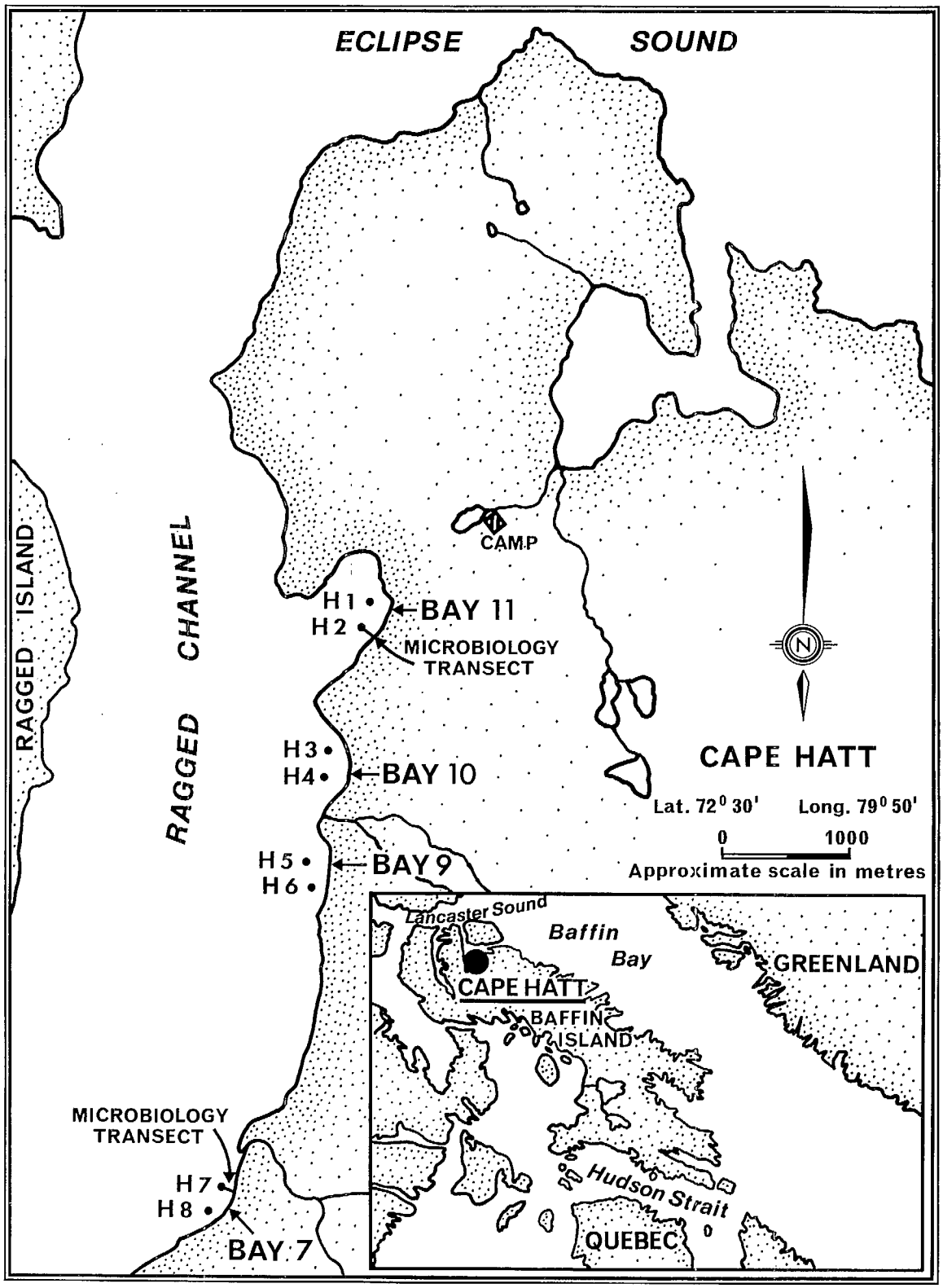

Figure 1. Microbiological stations and transects occupied at Cape Hatt during 1983.

Table 1. Sediment stations occupied at Cape Hatt, 1983.

Cycle	Date	Bay	Station
1	08 05	11	1 2
		9	5 6
		7	7 8
2	08 12	11	1 2
		9	5 6
		7	7 8
3	08 19	11	1 2
		9	5 6
		7	7 8

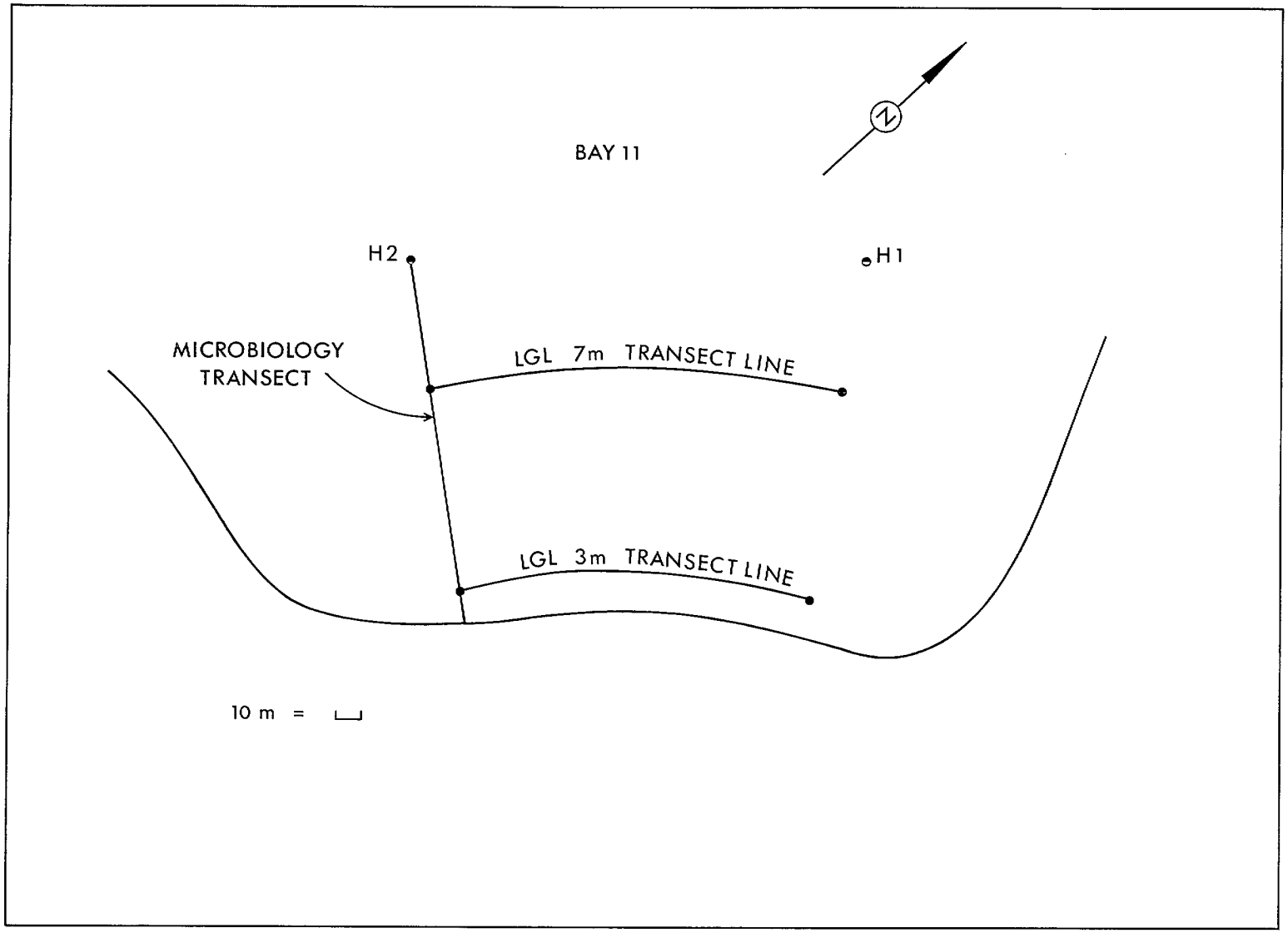

Figure 2. Graphic representation of a transect line located in bay 11 and its relation to microbiology stations H1 and H2, and the 3 and 7 m benthic transect lines of LGL Ltd.

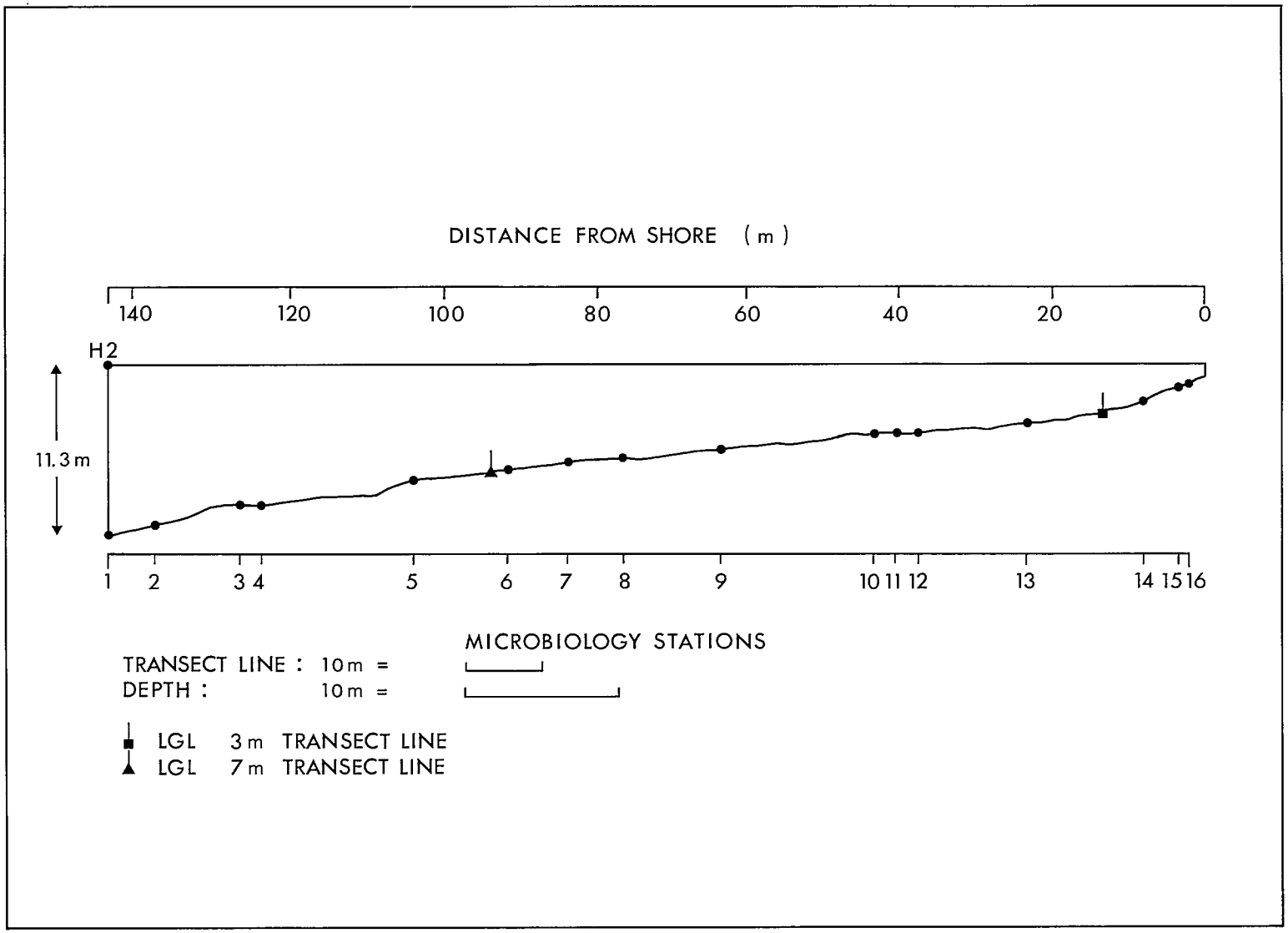

Figure 3. Graphic representation of stations sampled at Cape Hatt in 1983 along a transect line located in bay 11. The line was perpendicular to the shoreline between microbiology station H2 and a post secured at the water's edge of the intertidal zone. The vertical scale is exaggerated by a factor of two.

3.0 METHODS AND MATERIALS

Most of the techniques used in 1983 have been described in detail in previous reports (Bunch et al, 1981; 1983). Following are summaries of these techniques as well as details of new procedures or changes to procedures.

3.1 Sampling Procedure

All sediments were collected by divers. Collections at the microbiology stations were made by taking cores with modified 50-mL plastic syringes. A wide-mouthed 4-oz jar rinsed with freon was employed to collect similar sediment for hydrocarbon analysis.

At transect stations, large volumes of sediment were required, particularly for on-site hydrocarbon analyses by fluorometry. These samples were collected by scraping the surface sediment with wide-mouthed 24-oz jars. Samples from a station were homogenized and subdivided into portions for various analyses.

Samples for hydrocarbon analyses, organic carbon and dry weight determinations were immediately frozen. A large portion was used for immediate hydrocarbon analysis by fluorometry. For other analyses, a 1.0% suspension of sediment in seawater was agitated in a blender for three minutes, allowed to settle for thirty minutes in an ice bath and decanted. The supernatent was employed in analyses.

3.2 Total Organic Carbon

Sediments were thawed in the laboratory, acidified to remove inorganic carbon, and digested in sealed ampules. Evolved CO_2 was quantitated by flame ionization in a gas chromatograph after reduction to CH_4.

3.3 Total Counts of Bacteria

The sediment suspension was preserved with gluteraldehyde. In the laboratory, counts of cells were made under a light microscope using the acridine orange staining procedure and a fluorescent light source.

3.4 Uptake of Glutamic Acid

Parameters of uptake kinetics of glutamic acid were determined by incubating samples with a series of concentrations of ^{14}C-glutamic acid. After 4 h of incubation, reactions were stopped and the samples stored for subsequent counting by liquid scintillation. Parameters of uptake kinetics were generated by computer programs. A complete description of this technique can be found in Bunch et al., (1983). Three kinetic parameters were generated: V_{max} or the maximal velocity at which a given substrate can be transported into the flora of a sample; turnover or the time required for the complete removal of naturally occurring substrate from a given volume of sample; and (K + S), (K) being a transport constant and (S) the concentration of naturally occurring substrate in the sample.

In the case of stations on microbiology transects, only a single concentration of ^{14}C-glutamic acid was employed. Sediment suspensions were incubated in triplicate with 2.0 μCi L^{-1}. Samples were then treated as above. Total uptake was expressed as micrograms glutamic acid assimilated and respired per gram dry weight of sediment during the time of incubation.

3.5 Mineralization of Hexadecane and Most Probable Number (MPN) of Oleoclasts

Sediment suspensions were incubated at 5.0°C with ^{14}C-hexadecane and 0.01% $^{V}/_{V}$ of artificially weathered

Lagomedio petroleum and removed at several intervals to sixty days to determine rates of mineralization of ^{14}C-hexadecane. To determine the MPN of oleoclasts, sediment suspensions were serially diluted to 10^{-9} and incubated as above for sixty days. In all cases, reactions were stopped by acid addition. Samples were purged of $^{14}CO_2$ on a gas train. The $^{14}CO_2$ was trapped in NaOH and precipitated with $BaCl_2$. Samples were then filtered and the filters counted by liquid scintillation. Mineralization of added ^{14}C-hexadecane to $^{14}CO_2$ was expressed as a percentage.

In the case of the MPN procedure, samples were scored positive when $^{14}CO_2$ was detected at three times the level of the control and the MPN was determined by reference to a statistical table.

3.6 Statistical Analyses

Multiway analysis of variance was employed to study the effects of petroleum and spatial and temporal variation on microbiological 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 1.0%. Sigificant bay-year interactions were interpreted as potential indicators of a petroleum effect.

Correlations between variables were calculated by Spearman's coefficient of rank correlation. All statistical analyses were carried out on the McGill University computer using the Statistical Analysis System computer package (Helwig and Council, 1979).

4.0 RESULTS

4.1 Microbiology Stations

4.1.1 Petroleum Concentrations

Concentrations of petroleum in the sediments of the microbiology stations were determined by Dr. P. Boehm (Battelle Corp.) and are presented in Table 2. An analysis of hydrocarbon data from the bays can be found in Boehm et al (1984).

Stations in bays 7 and 9 showed similar values of petroleum concentrations with means of less than 0.63 and 0.92 $\mu g\ g^{-1}$ respectively. In 1982 the mean concentration in bay 7 was 1.21 ± SE 0.22 $\mu g\ g^{-1}$ (n = 6). With a value of 4.37 ± SE 0.62 $\mu g\ g^{-1}$ (n = 6) (Boehm, 1983), 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 which yields comparatively higher estimates than the gas chromatographic procedure employed in 1983. The data in 1983 suggested that petroleum was evenly distributed in the areas of the microbiology stations.

4.1.2 Total Counts of Bacteria

Total counts of bacteria from the sediments of the microbiology stations in bays 7, 9 and 11 are presented in Table 3. Mean total counts in the bays are given in Figure 4A. No significant differences in counts were seen between stations of a bay, between bays or between cycles in 1983. The mean of all values in 1983 was 1.1×10^9 ± SE 0.03×10^9 cells g^{-1} dry weight of sediment.

In 1982, the seasonal mean of total counts was 1.1×10^9 ± SE 0.04×10^9 and in 1981 the mean was 1.1×10^9 ± SE 0.09×10^9 cells g^{-1} dry weight (Bunch and Bédard, 1983). A significant bay-year interaction was seen between 1981 and

Table 2. Estimated concentrations of petroleum in the sediments of the microbiology stations in bays 7, 9 and 11 in 1983. All sediments were collected from a depth of 10 m. Analyses were performed and data provided by Dr. P. Boehm (Battelle Corp.).

Bay	Station	Date	Total hydrocarbon concentration	Phytane	Pristane / phytane	phytane / $n\text{-}C_{18}$	CPI	Estimated petroleum concentration
			$\mu g\ g^{-1}$	$\mu g\ g^{-1}$	(ratio)	(ratio)		$\mu g\ g^{-1}$ (Phy X 156)
7	7	83 08 12	6.15	.005	4.26	2.09	2.16	0.83
7	8	83 08 12	8.06	.002	17.58	4.70	5.05	0.36
9	5	83 08 12	6.65	.009	2.10	1.33	1.73	1.40
9	6	83 08 12	5.91	.003	7.71	2.48	2.50	0.52
11	1	83 08 12	7.76	.004	3.62	2.22	2.01	0.62
11	2	83 08 12	10.93	.018	1.41	1.06	1.49	2.87
11	1	83 08 05	14.74	.027	7.09	1.55	1.85	4.26
11	2	83 08 05	11.32	.011	2.66	1.75	1.58	1.78
9	5	83 08 05	6.71	.006	3.20	1.83	2.37	0.92
9	6	83 08 05	6.23	.001			3.49	0.50
7	7	83 08 05	4.69	.006	6.24	2.92	3.97	0.92
7	8	83 08 05	11.41	.001			6.61	0.50
7	7	83 08 19	6.77	.003	19.56	3.63	3.71	0.42
7	8	83 08 19	8.85	.005	12.96	4.00	4.81	0.73
9	5	83 08 19	5.07	.004	5.81	2.25	2.60	0.56
9	6	83 08 19	10.29	.010	3.19	1.48	3.00	1.61
11	1	83 08 19	13.96	.010	4.45	1.30	1.90	1.61
11	2	83 08 19	8.08	.005	4.58	1.92	1.81	0.73

Table 3. Total counts of bacterial cells, determinations of maximum velocity (V_{max}), turnover (T) and (K+S) of glutamic acid uptake and total organic carbon (TOC) obtained from samples collected from surface sediment at Cape Hatt during August 1983. Quantities are expressed per gram dry weight of sediment.

Date	Station	Total counts of bacterial cells	V_{max}	T	(K+S)	TOC
		no. g^{-1} (10^{-7})	µg g^{-1} d^{-1}	d	µg g^{-1}	% C
08 05	1	115.6	16.6	1.9	31.7	2.3
	2	100.4	14.6	2.3	33.9	2.2
	5	119.0	4.7	2.7	12.7	2.0
	6	87.9	6.4	2.4	15.6	2.3
	7	115.3	11.3	2.1	24.1	3.1
	8	121.2	6.1	2.0	12.4	2.7
08 12	1	88.0	10.0	2.1	21.1	1.8
	2	111.1	10.7	1.7	17.9	1.9
	5	94.9	6.1	2.4	14.4	2.1
	6	97.3	7.1	1.9	13.6	2.1
	7	121.9	12.3	1.9	22.6	2.7
	8	98.9	5.2	3.0	15.4	2.6
08 19	1	101.1	12.2	1.9	23.4	2.2
	2	89.0	10.1	1.9	19.1	2.4
	5	95.1	5.6	2.0	11.0	2.0
	6	125.7	7.7	1.6	12.5	2.1
	7	133.6	6.2	2.0	12.6	2.6
	8	93.3	4.7	1.6	7.6	2.3

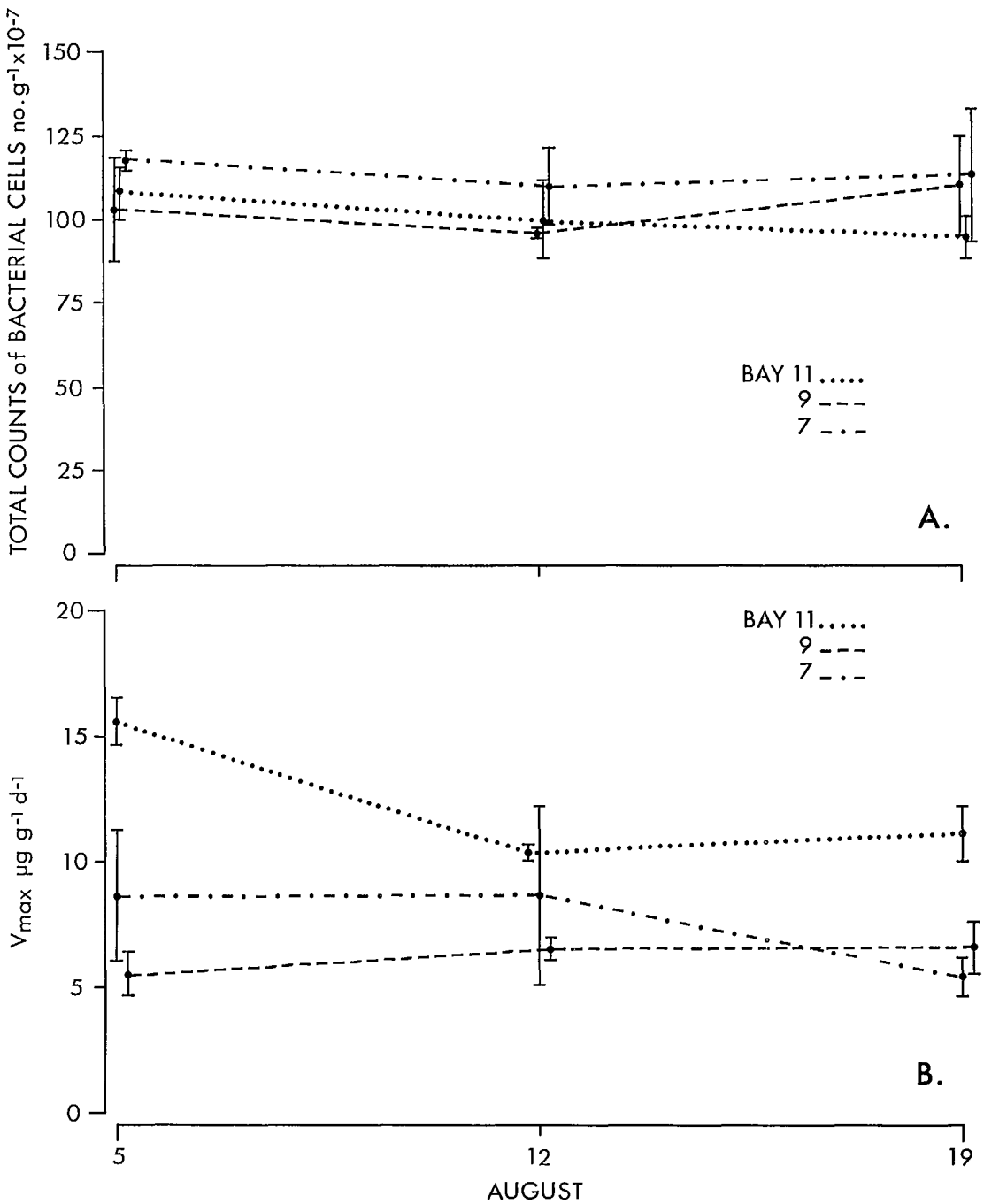

Figure 4. (A) Total counts of bacterial cells and (B) maximum velocity (V_{max}) of glutamic acid uptake determined from samples collected from surface sediment at Cape Hatt, 1983. Results are presented as means and standard errors of values from two stations in each bay.

1982 because the counts in bay 9 increased significantly between the two years while counts in the other two bays decreased (Fig. 5A). The bay-year interaction persisted when the data of 1981 and 1983 were taken together. No bay-year interaction was found when the data of 1982 and 1983 were taken together. The mean value in bay 11, however, increased slightly from 9.5 X 10^8 ± SE 0.7 X 10^8 to 10.1 X 10^8 ± SE 0.5 X 10^8 cells g^{-1} while counts in the other two bays decreased.

4.1.3 Uptake Kinetics of Glutamic Acid

Values of V_{max}, turnover and (K+S) of glutamic acid uptake (see 3.4) are given in Table 3. The means in the three bays are shown in Figures 4B and 6A, B.

Significant differences in V_{max} were seen between the stations in bay 9 during 1983. We attributed this to an inadequate sample size (n = 6). Similarly, the significant change in V_{max} across the season in bay 11 can probably be attributed to the sample size (n = 6). No significant differences in V_{max} between stations or across sampling intervals were seen in bay 7. In 1983, the mean V_{max} of 12.4 ± SE 1.1 µg g^{-1} d^{-1} in bay 11 was significantly higher (two times) than those of the other two bays.

Values of turnover in 1983 were not significantly different between stations in a bay, between cycles or between bays, although the values in bay 11 tended to be lower. The mean turnover of glutamic acid in surface sediment in 1983 was 2.1 ± 0.1 days.

No significant differences in the (K+S) of glutamic acid uptake were seen between stations of any bay in 1983. Only bay 11 showed a significant change across the sampling cycles. The mean value of (K+S) in bay 11 was 24.5 ± SE 2.7 µg g^{-1}, significantly higher by a factor of 1.7 than bays 7 and 9.

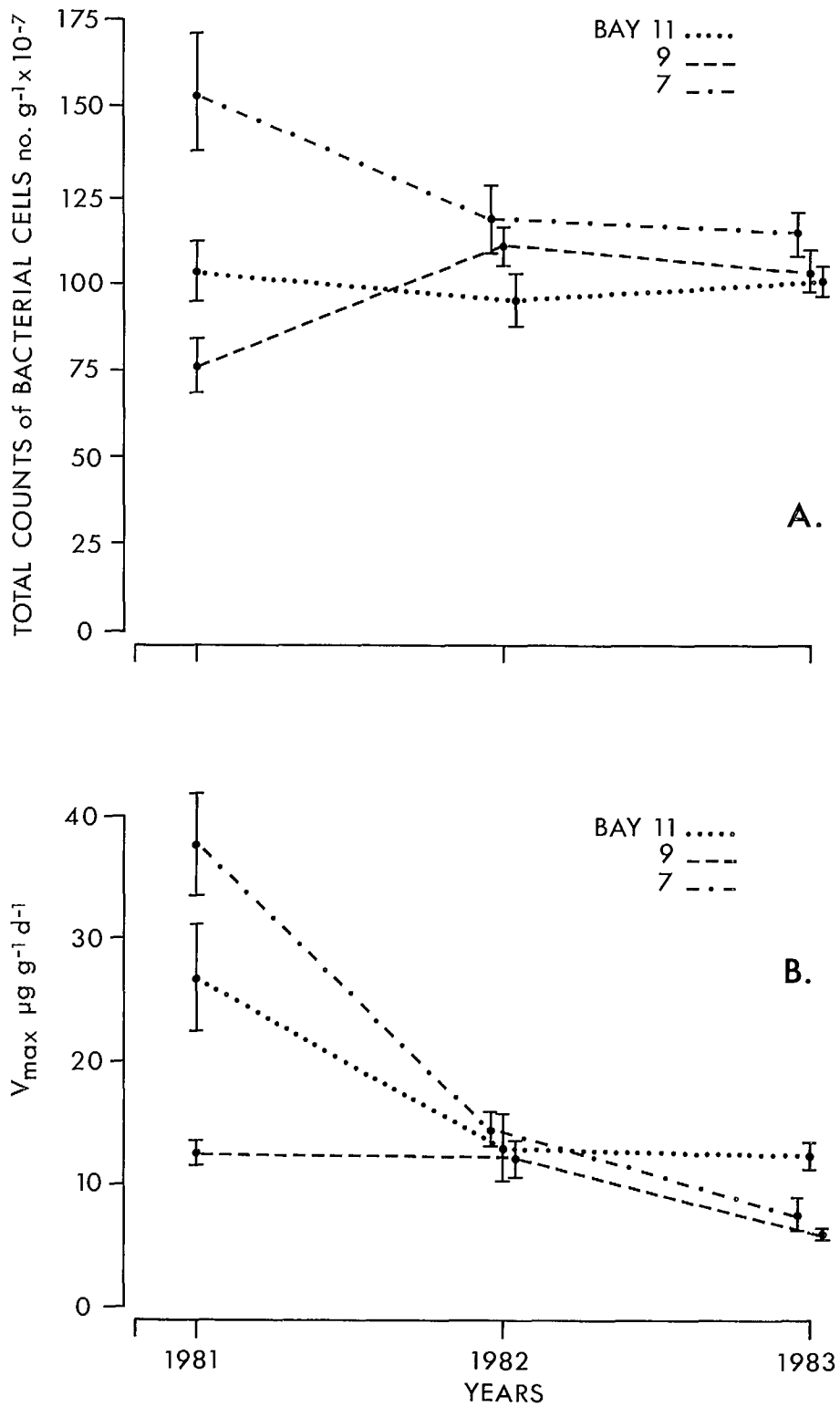

Figure 5. (A) Three year comparison of total counts of bacterial cells and (B) maximum velocity (V_{max}) of glutamic acid uptake in surface sediments at Cape Hatt. Samples were collected in bays 11, 9 and 7 in 1981, 1982 and 1983. Results are presented as seasonal means and standard errors of values from two stations in each bay.

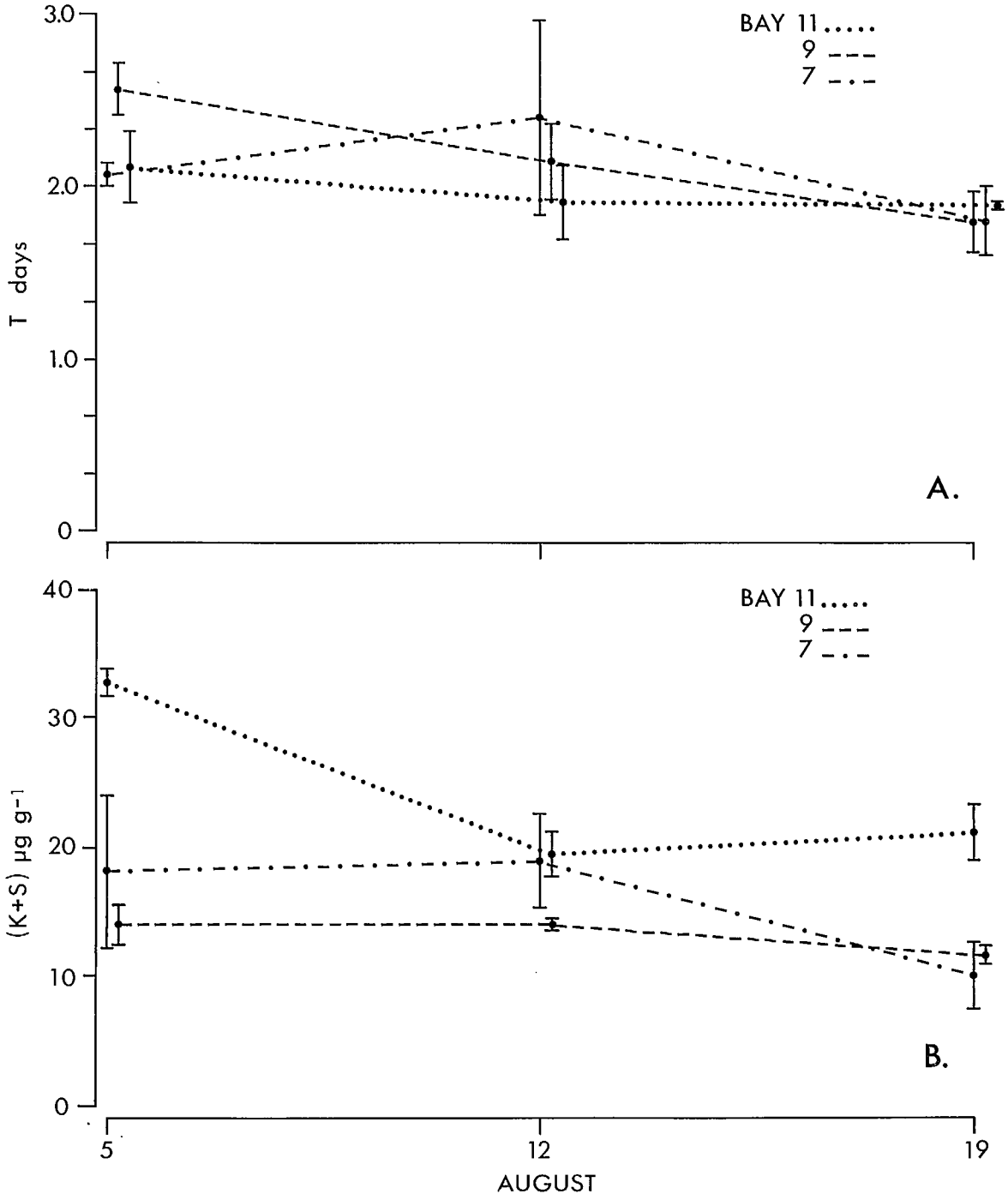

Figure 6. (A) Turnover (T) and (B) (K+S) of glutamic acid uptake determined from samples collected from surface sediment at Cape Hatt, 1983. Results are presented as means and standard errors of values from two stations in each bay.

Data of kinetics from 1981, 1982 and 1983 are summarized in Figures 5B and 7A, B. Between 1981 and 1982, the V_{max} of glutamic acid uptake 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, showed a decrease between 1981 and 1982, but remained constant between 1982 and 1983. The difference in changes among the bays across the two years was seen in the significant bay-year interaction when data for the three bays were combined.

Although no significant differences in turnover of glutamic acid were seen between bays in 1983, values of turnover increased significantly in bays 7 and 9 in 1983 over 1982 but not in bay 11. The difference in changes in the bays was not reflected in a significant bay-year interaction when data for the three bays were taken together.

Between 1981 and 1982, the (K+S) of glutamic acid uptake decreased significantly in all three bays, but within each year no bay was significantly different from any other. Between 1982 and 1983, a significant increase occurred in bay 11, whereas in bays 7 and 9 (K+S) decreased but not significantly. This difference in changes in the bays was seen in a significant bay-year interaction.

4.1.4 Total Organic Carbon

In 1983, no significant differences in total organic carbon (TOC) were seen between stations within a bay or in each bay across the sampling season (Table 3). With a seasonal mean of 2.6 ± SE 0.1% C, the carbon content in the surface sediment of bay 7 was significantly higher than the other two bays (Fig. 8). The content of petroleum in these sediments was insignificant when compared to the amount of TOC.

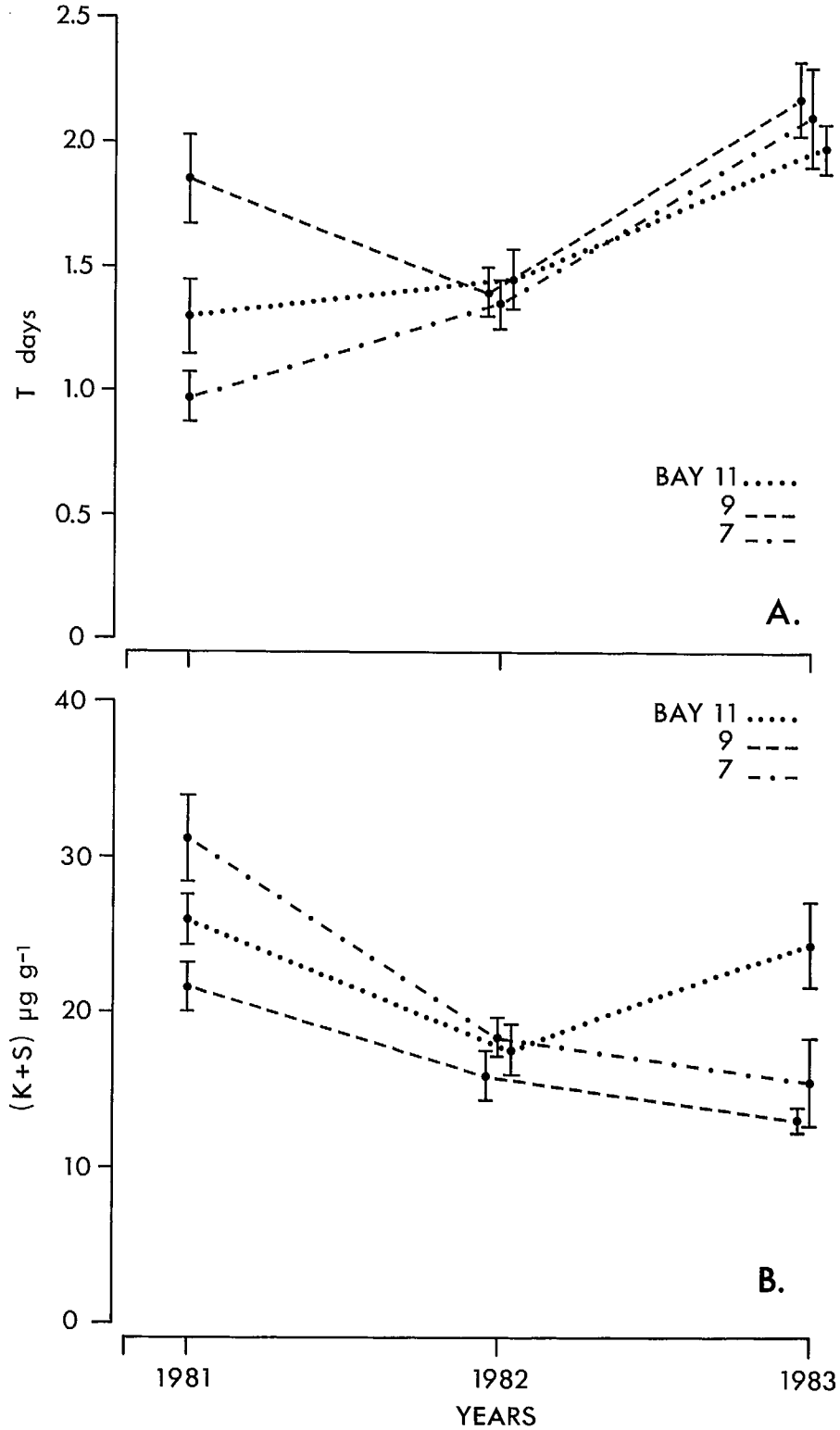

Figure 7. (A) Three year comparison of turnover (T) and (B) (K+S) of glutamic acid uptake in surface sediments at Cape Hatt. Samples were collected in bays 11, 9 and 7 in 1981, 1982 and 1983. Results are presented as seasonal means and standard errors of values from two stations in each bay.

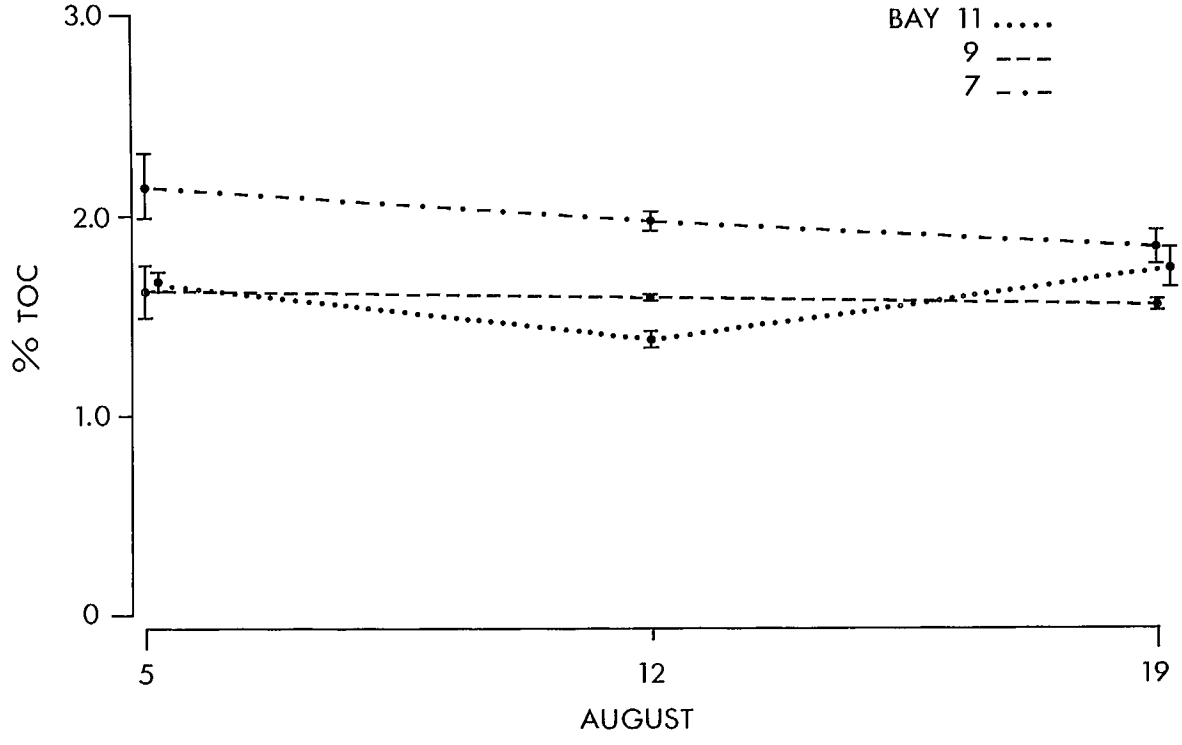

Figure 8. Percent total organic carbon (TOC) determined from samples collected from surface sediment at Cape Hatt, 1983. Results are presented as means and standard errors of values from two stations in each bay.

The high organic carbon content in bay 7 was also seen in 1981 and 1982 and was not significantly different in the three years, although it decreased slightly between 1982 and 1983 (Fig. 9). The organic carbon content of surface sediment in bay 9 increased 35% between 1981 and 1982 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.

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

4.1.5 Numbers and Activity of Oleoclasts

As in 1981 and 1982, no consistent pattern in the numbers or activity of oleoclasts was seen in the surface sediments of the microbiology stations in the three bays in 1983. Numbers of oleoclasts per gram dry weight as determined by the MPN procedure were at or near zero (Table 4).

Activity was measured by the percent mineralization of ^{14}C-hexadecane added to sediment samples incubated for sixty days. The results are seen in Table 5 and compared to the results of 1981 and 1982. The mean percent mineralization at all stations was 4.31 ± SE 0.74% and 2.34 ± SE 0.54% in 1981 and 1982 respectively. In 1983, hexadecane mineralization was considerably reduced with a mean value of 0.43 ± SE 0.39%.

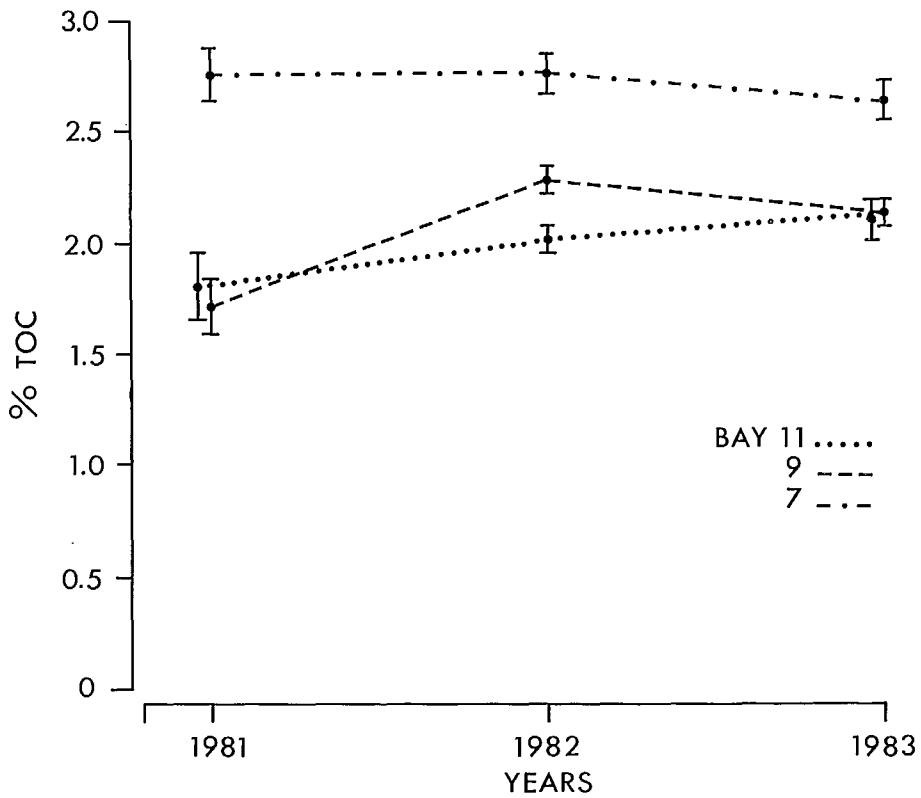

Figure 9. Three year comparison of percent total organic carbon (TOC) in surface sediments at Cape Hatt. Samples were collected in bays 11, 9 and 7 in 1981, 1982 and 1983. Results are presented as seasonal means and standard errors of values from two stations in each bay.

Table 4. Most probable number (MPN) of oleoclastic cells in sediment samples collected from microbiology stations at Cape Hatt during 1981, 1982 and 1983. A single collection was made in 1983.

Station	(n)*	1981	(n)*	1982	1983
1	7	2400 ± 1000	6	114200 ± 62600	1.4
2	7	2100 ± 800	5	59400 ± 31700	1.0
5	7	1800 ± 600	5	216400 ± 75100	3.1
6	7	1200 ± 500	6	169000 ± 69600	0.6
7	8	2800 ± 800	6	106800 ± 63000	3.6
8	8	3400 ± 900	6	142800 ± 83400	0.4

*number of samples.

Table 5. Percent mineralization of ^{14}C-hexadecane in sediment samples collected from microbiology stations at Cape Hatt during 1981, 1982 and 1983. A single collection was made in 1983.

Station	(n)*	1981	(n)*	1982	1983
1	7	2.71 ± 0.88	5	1.44 ± 0.58	0.00
2	7	7.40 ± 2.56	5	2.84 ± 1.43	2.35
5	7	3.25 ± 1.18	5	4.42 ± 2.51	0.18
6	7	6.73 ± 1.90	6	1.28 ± 0.88	0.01
7	8	1.55 ± 0.63	6	2.19 ± 1.24	0.02
8	8	4.57 ± 2.36	6	2.16 ± 0.97	0.00

*number of samples

4.2 Microbiology Transects

4.2.1 Petroleum Concentrations

Estimates of petroleum concentrations in sediments collected at stations along the microbiology transects in bays 11 and 7 were provided by Dr. P. Boehm (Battelle Corp.). These data are given in Tables 6 and 7. The data are presented graphically in Figures 10A, B and 11A, B (see Figure 3 for a profile of the transect in bay 11).

In bay 11, concentrations of petroleum along the transect decreased with increasing distance from shore, ranging from 410.0 to 0.8 μg g^{-1}. Boehm (1983) concluded from data obtained in 1982 that petroleum stranded on the intertidal zone during the surface release in 1981 was gradually moving into the subtidal sediments. Owens et al (1983) estimated that 75% of the stranded petroleum remained in the intertidal zone in 1982. It was expected that this petroleum would continue to enter the subtidal sediments in 1983 (Dr. E. H. Owens - personal communication). The data presented here confirm this suggestion. The estimated petroleum concentrations showed a strong negative correlation ($p < 0.001$) with both depth of water and distance from shore.

The estimated concentrations of petroleum along the transect in bay 7 were uniformly low. Four of the five determinations were less than 0.5 μg petroleum g^{-1} while the station furthest from shore, at a depth of approximately 10 m, showed a concentration of 1.8 μg g^{-1}.

4.2.2 Total Organic Carbon

No particular trend was evident in the concentrations of total organic carbon found along the transects in bays 11 and 7 (Tables 6 and 7; Fig. 10B and 11B). The mean values determined for the transects were 1.6 ± SE 0.09 and 2.6 ± SE 0.05 % C in bays 11 and 7 respectively. The higher content

Table 6. Determinations of uptake of ^{14}C-glutamic acid, total counts of bacterial cells, estimates of petroleum concentrations, phytane/n-C_{18} ratios and total organic carbon (TOC) obtained from samples collected from surface sediment along a transect located in bay 11 at Cape Hatt, on 10 and 17 August 1983. Samples were incubated with ^{14}C-glutamic acid for four hours.

	Transect station	Approximate depth	Distance from shore	Uptake of ^{14}C-glutamic acid	Total counts of bacterial cells	Est. petroleum* concentrations	Phytane* / n-C_{18}	TOC
		m	m	μg g^{-1} (10^3)	no. g^{-1} (10^{-7})	μg g^{-1}	(ratio)	% C
Bay 11	1	11	143	7.2	39.6	1.7	1.6	2.0
	2	11	137	5.8	97.9	0.8	0.90	1.9
	3	9	126	4.6	71.0	0.8	0.71	1.7
	4	9	123	10.8	57.8	1.2	0.89	1.7
	5	8	103	5.6	47.3	1.7	1.2	1.6
	6	7	91	8.4	64.6	0.9	0.75	1.6
	7	6	83	10.9	85.5	4.4	1.7	1.8
	8	6	76	14.2	82.8	4.5	1.7	1.6
	9	6	63	59.9	152.3	29.0	2.7	2.2
	10	5	43	39.6	117.3	40.7 ± 3.0	2.7 ± 0.1	1.2
	11	5	40	115.3	243.7	42.0	1.8	1.9
	12	5	37	36.9	87.5	36.0	2.2	1.5
	13	4	23	32.3	117.8	120.0	2.6	1.6
	14	2	8	39.7	142.8	410.0	1.1	1.8
	15	2	3	23.6	28.4	44.0	3.6	0.8
	16	1	2	29.8	62.2	87.0	2.0	1.0

*Data obtained from Boehm (1984).

Table 7. Determinations of uptake of ^{14}C-glutamic acid, total counts of bacterial cells, estimates of petroleum concentrations, phytane/n-C_{18} ratios and total organic carbon (TOC) obtained from samples collected from surface sediment along a transect located in bay 7 at Cape Hatt, on 10 and 17 August 1983. Samples were incubated with ^{14}C-glutamic acid for four hours.

	Transect station	Approximate depth	Distance from shore	Uptake of ^{14}C-glutamic acid	Total counts of bacterial cells	Est. petroleum* concentrations	Phytane* / n-C_{18}	TOC
		m	m	µg g^{-1} (10^3)	no. g^{-1} (10^{-7})	µg g^{-1}	(ratio)	% C
Bay 7	1	10	--	5.7	36.4	1.8	2.2	2.6
	2	8	--	16.3	41.8	0.5	--	2.6
	3	6	--	12.8	69.0	0.5	--	2.7
	4	4	--	10.3	42.3	0.5	0.25	3.0
	5	2	--	15.1	83.1	0.5	0.58	2.6
	6	10	--	13.9	98.5	--	--	2.7
	7	8	--	8.6	80.8	--	--	2.4
	8	6	--	11.0	72.8	--	--	2.5
	9	4	--	9.2	34.5	--	--	2.5
	10	2	--	6.6	22.9	--	--	2.5

*Data obtained from Boehm (1984).

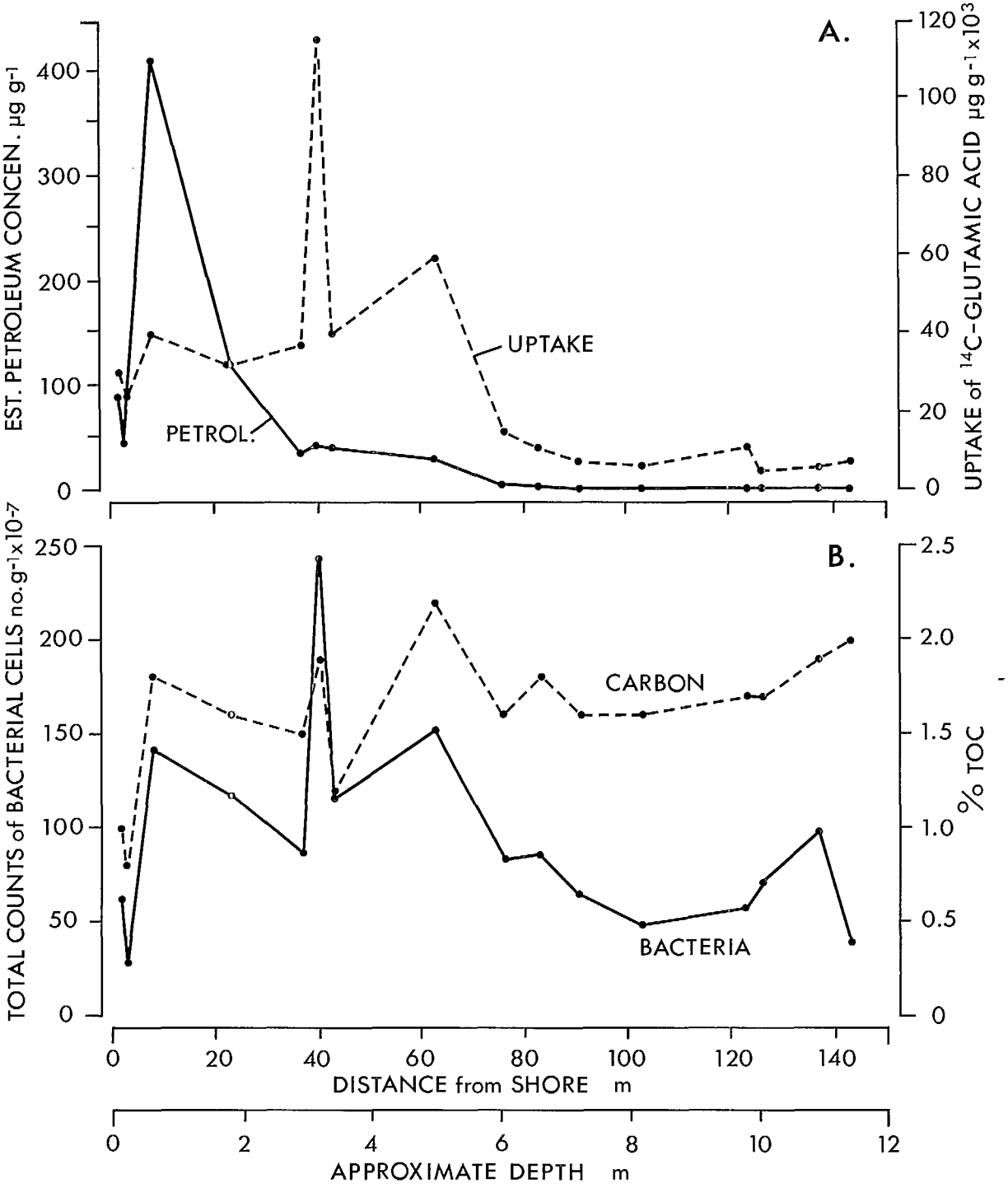

Figure 10. (A) Comparison of estimates of petroleum concentrations and uptake of ^{14}C-glutamic acid and (B) total counts of bacterial cells and total organic carbon (TOC) at stations on the bay 11 transect in 1983. Samples were collected from surface sediment on 10 and 17 August.

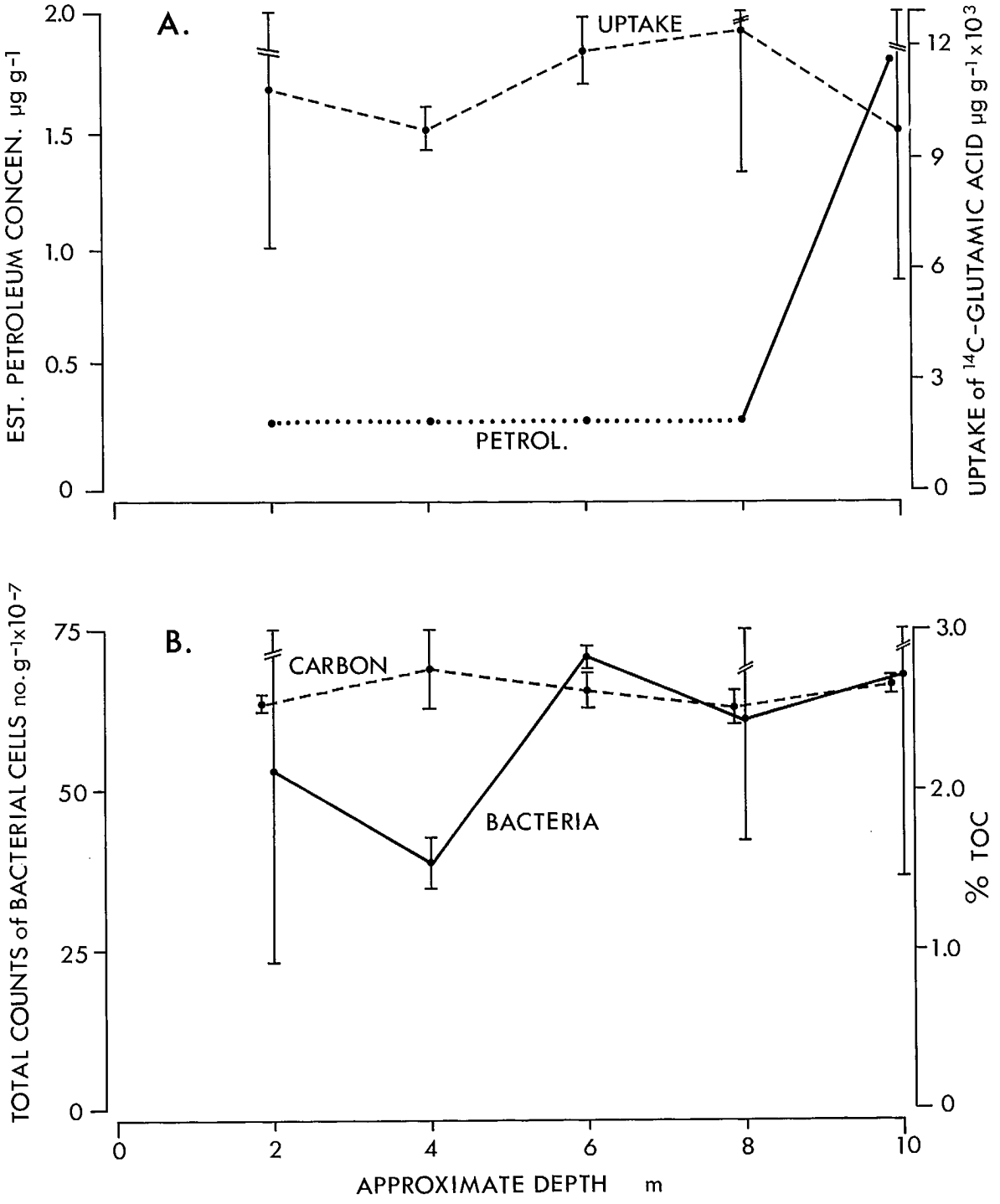

Figure 11. (A) Comparison of estimates of petroleum concentrations and uptake of ^{14}C-glutamic acid and (B) total counts of bacterial cells and total organic carbon (TOC) at stations on the bay 7 transect in 1983. Estimates of petroleum concentrations that were less than 0.5 are represented by a dotted line. Samples were collected from surface sediment on 10 and 17 August. Results of uptake of ^{14}C-glutamic acid, total counts of bacterial cells and total organic carbon are presented as means and standard errors of values from two sampling dates.

of organic carbon in bay 7 over bay 11 was previously noted in the three years of data from the microbiology stations (Fig. 9).

Three stations along the transect in bay 11 demonstrated somewhat elevated levels of organic carbon. The transect in bay 11 had an extended gradual slope from the beach to station H2 and an irregular relief was noted by the divers (M. Fabijan - personal communication). Stations with elevated levels of organic carbon were probably located in depressions on the transect. The transect in bay 7 was shorter and the slope much steeper when compared to bay 11, and stations on this transect, where the substrate dropped uniformly without depressions, showed remarkably uniform concentrations of organic carbon.

Organic carbon correlated positively ($0.05 > p > 0.01$) with distance from shore and depth of the water column on the bay 11 transect. This was primarily due to the low concentrations of organic carbon found in the inshore sediments. When stations T15 and T16, the two stations nearest to shore, were dropped from the correlation analysis, the correlation disappeared. These stations, being in a shallow high energy area, were not comparable to other subtidal stations on the transect and were dropped from subsequent statistical analyses. No correlation was found between concentrations of petroleum and organic carbon in bay 11. No correlations were found with concentrations of organic carbon in bay 7.

4.2.3 Bacterial Numbers and Uptake of Glutamic Acid

Values of bacterial numbers and uptake of glutamic acid from sediments on the transects are given in Tables 6 and 7. The same data are graphically presented in Figures 10A, B and 11A, B. The mean value of bacterial numbers at stations

along the transect in bay 11 was 93.6 X 10^7 ± SE 13.3 X 10^7 cells g^{-1}. When the offshore half of the transect was compared to the nearshore half, the mean value on the nearshore half was 119.0 X 10^7 ± SE 23.0 X 10^7 cells g^{-1}, about twice as high as the offshore half of the transect. This high value occurred in spite of the very low values at shoreward stations T15 and T16. The mean number of bacteria on the transect in bay 7 was 58.2 X 10^7 ± SE 8.1 X 10^7 cells g^{-1}, similar to the offshore half of the transect in bay 11.

Numbers of bacteria in the surface sediment of bay 11 correlated negatively with distance from shore and positively with concentrations of petroleum ($0.01 > p > 0.001$), two parameters which were themselves strongly correlated ($p < 0.001$). When the nearshore half of the transect was analysed, the correlation with petroleum disappeared and numbers of bacteria correlated with percent organic carbon in the surface sediment ($0.05 > p > 0.01$). This correlation was not found with percent organic carbon in bay 7.

Uptake of glutamic acid was determined as the amount of ^{14}C-glutamic acid removed from the incubation medium across a four hour period. The kinetics of uptake were not determined as at the microbiology stations (see 3.4). The mean value of uptake of glutamic acid from sediments at stations on the transect in bay 11 was 27.8 X 10^{-3} ± SE 7.1 X 10^{-3} µg of glutamic acid g^{-1}. This compared to a mean of 10.9 X 10^{-3} ± SE 1.1 X 10^{-3} µg g^{-1} on the transect in bay 7. The uptake of glutamic acid on the bay 11 transect correlated positively with the concentration of petroleum and counts of bacteria ($p < 0.001$). No correlation was observed with organic carbon.

When the nearshore and offshore halves of the transect in bay 11 were compared, the means of uptake were 47.1 X 10^{-3} ± SE 10.4 X 10^{-3} and 8.4 X 10^{-3} ± SE 1.1 X 10^{-3} µg glutamic

acid g^{-1} respectively. Correlations between parameters were calculated for each half of the transect. Nearshore, the uptake of glutamic acid and organic carbon concentrations correlated with numbers of bacteria ($0.05 > p > 0.01$). The uptake of glutamic acid did not correlate with petroleum concentrations in the nearshore or offshore halves of the transect.

In bay 7, the uptake of glutamic acid did not correlate with any parameter.

4.2.4 Numbers and Activity of Oleoclasts

Numbers and activity of oleoclasts from the transects in bays 7 and 11 are presented in Table 8. Oleoclasts were low on the transects of both bays and comparable to the results obtained at the microbiology stations in 1983 (Table 4). Values in 1983 were approximately five orders of magnitude less than in 1982.

The activity of oleoclasts expressed as percent mineralization of ^{14}C-hexadecane was also low on the two transects and comparable to the results obtained at the microbiology stations. The single exception was station T1 on the transect in bay 11.

Dr. K. Eimhjellen independently determined numbers of oil-degrading bacteria (ODB) and total viable heterotrophs (TVH) in subsamples of some transect sediments collected for our procedures (Table 9; extracted from Eimhjellen and Josefsen, 1984). The data were obtained by MPN procedures, details of which can be found in Eimhjellen et al (1981). At $34.9 \times 10^5 \pm$ SE 12.0×10^5 cells mL^{-1}, the mean of ODB on the bay 11 transect was about 2.5 times that obtained on the bay 7 transect. Numbers of ODB correlated negatively ($0.05 > p > 0.01$) with depth and distance from shore on the bay 11 transect. No correlations were found with TVH or

Table 8. Percent mineralization of ^{14}C-hexadecane and most probable number (MPN) of oleoclastic cells in sediment samples collected from stations located along transect lines in bays 11 and 7 at Cape Hatt on 10 and 17 August 1983.

	Transect station	% mineralization of ^{14}C-hexadecane	MPN cells g^{-1}
Bay 11	1	14.4	3.6
	2	0.0	0.5
	3	0.0	0.4
	4	3.7	4.1
	5	0.0	0.5
	6	0.0	0.4
	7	0.5	7.8
	8	0.0	0.5
	9	1.8	5.6
	10	0.0	0.4
	11	0.0	0.7
	12	0.0	0.5
	13	0.0	31.1
	14	0.0	0.6
	15	0.0	0.5
	16	0.0	0.4
Bay 7*	1	0.4 ± 0.4	0.4 ± 0.0
	2	0.8 ± 0.4	2.4 ± 1.3
	3	0.3 ± 0.3	12.3 ± 11.8
	4	0.6 ± 0.4	23.7 ± 20.4
	5	0.0 ± 0.0	2.0 ± 1.5

*(n = 2)

Table 9. Determinations of total viable heterotrophs (TVH) and oil-degrading bacteria (ODB) in surface sediment on the microbiology transects in 1983 (extracted from Eimhjellen and Josefsen, 1984). Sediments were subsamples of material collected 10 August and employed for other determinations (Tables 6 and 7). Growth in a most probable number procedure was denoted by acid production from the organic substrate (see Eimhjellen et al, 1981).

	Station	Approximate depth	TVH	ODB
		m	cells mL^{-1} (10^{-5})*	cells mL^{-1} (10^{-5})*
Bay 11	1	11	120	4.5
	4	9	170	25.0
	5	8	46	9.6
	7	6	130	25.0
	9	6	490	15.0
	10	5	110	45.0
	13	4	800	110.0
	15	2	330	45.0
Bay 7	1	10	170	2.5
	2	8	110	9.5
	3	6	800	7.5
	4	4	490	4.5
	5	2	1100	45.0

*mL of wet sediment.

concentrations of petroleum. In bay 7, TVH correlated positively with total counts of bacteria ($0.05 > p > 0.01$).

Eimhjellen et al (1981) reported a mean of $0.3 \times 10^5 \pm$ SE 0.2×10^5 ODB mL^{-1} in all sediment samples taken from microbiology stations in 1980 ($n = 5$). The various methods of sediment collection during that year, however, together with the lack of homogeneity of the samples, reduced the usefulness of sediment observations (Bunch et al, 1981).

A yearly mean of $1.1 \times 10^5 \pm$ SE 0.2×10^5 cells mL^{-1} was reported for all bays in 1981 ($n = 29$), from sediments collected before and after the petroleum releases (Eimhjellen et al, 1982). A similar mean was obtained in 1982 (Eimhjellen et al, 1983). In each of the three years, the range of counts exceeded tenfold. In 1983, transect stations T1 in bay 11 and T1 in bay 7 were occupied at the locations of microbiology stations H2 and H7 from previous years. Single determinations at these transect stations (see Table 9) yielded counts of 4.5×10^5 cells mL^{-1} (bay 11) and 2.5×10^5 cells mL^{-1} (bay 7).

The estimates provided by Dr. P. Boehm (Battelle Corp.) of petroleum concentrations on the bay 11 transect also included calculated ratios of phytane to n-C_{18} (Table 4). On the assumption that undegraded petroleum has a phytane to n-C_{18} ratio of 0.61, the degree of biodegradation of petroleum correlated positively with the concentration of petroleum ($0.01 > p > 0.001$) and negatively ($0.05 > p > 0.01$) with distance from shore. This suggests that biodegraded petroleum moved offshore from the intertidal zone.

5.0 DISCUSSION

5.1 Effects on the Microheterotrophic Flora

The existing literature concerning the effects of petroleum on the abundance of bacteria and microheterotrophic activity is limited (see Bunch et al, 1983; Bunch and Bĕdard, 1983). Frequently, investigators employ unrealistically high concentrations of petroleum which cannot be related to an actual impact of petroleum in the marine environment.

The microbiology component of the BIOS project has provided information on the effects of petroleum releases in an arctic marine environment. The objective of the project was to produce realistic spills of petroleum with and without dispersants and compare the effects produced by these spills. Bunch et al (1981) noted a decrease in the uptake of glutamic acid by microheterotrophs in the water column during the dispersed release. The decrease, however, was both transient and minimal. Bacterial numbers were not affected. Subsequent observations of the sediments in the dispersed petroleum test bay over a three week period did not reveal any changes in microheterotrophic activity or bacterial numbers. This was not unexpected since very little of the dispersed petroleum actually entered the sediment. The vast majority of the dispersed petroleum was rapidly flushed out and diffused into the waters of the area within a few days of the release. Clearly, the immediate effect of the dispersed petroleum on measured microbiological parameters was inconsequential.

Although petroleum stranded on the intertidal zone during the 1981 surface release was entering the subtidal sediment in 1982, Bunch and Bĕdard (1983) did not observe any microbiological effects. The sediments of bay 9, which received the dispersed petroleum, however, showed significant bay-year interactions when compared to the other three bays.

Bacterial numbers in the sediment had increased over 1981 while the V_{max} of glutamic acid uptake remained constant. In all other bays these parameters had decreased. The activity in the sediment of bay 9 could be readily ascribed to the increased level of total organic carbon (TOC). The increased level of TOC, Bunch and Bĕdard (1983) suggested, arose through the detritus produced from benthic algae and macrofauna after their disturbance by the dispersed petroleum release in 1981.

The observations in 1983 tend to support the conclusion of Bunch and Bĕdard. The trends seen in bay 9 in 1982 were reversed in 1983 while the sediments in bay 11 showed significant bay-year interactions in the V_{max} and (K+S) 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 therefore greater than in the other two bays and did not decrease in 1983 over 1982 as did the other bays. Although a significant interaction was not found, the level of organic carbon in the sediment of bay 11 increased over 1982 as did numbers of bacteria. These data tend to suggest that a petroleum effect is occurring in bay 11 as a consequence of stranded petroleum entering the sediments.

Lacking at this time is sufficient evidence that benthic algae and possibly the macrofauna of bays 11 and 9 were 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. Variation in the biomass of the flora and fauna may be due to other causes. We nevertheless speculate 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. This speculation is supported

by the correlation between TOC and bacterial numbers and the strong correspondance between bacterial numbers, TOC and the uptake of glutamic acid on the nearshore, heavily-oiled subtidal sediment of the transect in bay 11.

5.2 Biodegradation

Biodegradation of petroleum in the environment can be detected in three ways:

1. by measuring in vitro a relative increase in the numbers of oleoclasts;
2. by measuring in vitro a relative increase in the rate of degradation of a hydrocarbon substrate;
3. by measuring in vitro a relative loss of petroleum fractions by chemical analysis.

Of the three methods above, only the chemical procedure provides semi-quantitative information of actual losses in situ. This information was not obtained from the subtidal sediments at Cape Hatt (see Boehm, 1982; 1983; Boehm et al, 1984) for several reasons. The amount of petroleum deposited subtidally by the dispersed release was so low as to make the quantitation of biodegradation an equivocal procedure. Secondly, although a large portion of the petroleum stranded on the intertidal zone after the surface release entered the subtidal sediments of bay 11 by 1983, it consisted of both highly degraded and recently exposed, undegraded petroleum. As a consequence, it was impossible to ascribe biodegraded petroleum in bay 11 sediments to subtidal processes.

The microbiological procedures provide information of relative change in numbers or activity of oleoclasts by measuring these parameters in vitro. No microbiological

procedures at this time quantitate biodegradation in situ in subtidal sediments. With any one of several substrates, the ability of oleoclasts in a sample to degrade the substrate is measured by observing their effect on the substrate. A positive result suggests the "potential" for such activity in situ and succeeding temporal measurements may indicate a change in the "potential".

Oleoclasts and oil-degrading bacteria (ODB) are two terms for microorganisms which degrade components of petroleum. At Cape Hatt, several procedures and a variety of treated petroleums and petroleum components were employed to determine degradation by observing the products resulting from this process (Bunch et al, 1981; 1983; Eimhjellen et al, 1981).

Eimhjellen and Josefsen (1984) determined numbers of ODB from samples of subtidal sediments on the microbiology transects. Their procedure quantitated these microorganisms by detecting a non-specific response (acid production) to Lagomedio petroleum artificially weathered to a high degree. The mean of observations from the bay 11 transect was approximately 30 times higher than the mean of observations of the microbiology stations of all bays in the preceding two years. In the bay 7 control transect, where petroleum concentrations were low, the mean of ODB exceeded the two-year mean by a factor of 12. No correlation between ODB and concentrations of petroleum was found on the transect of bay 11 although a trend of increasing numbers of ODB from offshore to the shoreline was suggested. This result may have been confounded by the dispersion of ODB with biodegraded petroleum from intertidal to subtidal sediments. Taken together with the inherent variation in the technique of Eimhjellen et al (1981), the results do not suggest a large increase in the number of ODB in the subtidal sediments

of bay 11 in response to increased concentrations of petroleum.

In contrast to Eimhjellen and Josefsen, we found very few oleoclasts capable of mineralizing ^{14}C-hexadecane to $^{14}CO_2$ in samples from the sediments of the microbiology stations. Counts from the transects of the two bays were similarly low. Compared to our observations from the microbiology stations in 1982, the counts in 1983 decreased by five orders of magnitude.

Little or no significant change in cell numbers over the two-year period was interpreted from acid production from undefined petroleum components. There is no reason to develop a different interpretation of change in the same population during the same period on the basis of CO_2 production from hexadecane although an inexplicable decrease in the population was observed by this method. Hexadecane and other alkane components of petroleum are usually degraded before other components, particularly with inorganic nutrient supplementation (Bunch and Harland, 1976; Fedorak and Westlake, 1981; Kator et al, 1971). If the oleoclastic flora of the subtidal sediments of bay 11 increased as a consequence of petroleum entering these sediments, we can only conclude that the techniques discussed did not describe the increase.

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