DISTRIBUTION AND HETEROTROPHIC POTENTIAL OF MICROORGANISMS IN A COLD OCEAN ENVIRONMENT

JAMES CONRAD POWELL


CENTRE FOR NFLD. STUDIES
MAR 23 198
MEMORIAL UNIVERSITY
OF NEWFOUNDLAND

DISTRIBUTION AND HETEROTROPHIC POTENTIAL OF MICROORGANISMS IN A COLD OCEAN ENVIRONMENT

by

James Conrad Powell, B.Sc. (Hons.)

A Thesis submitted in partial fulfillment
of the requirements for the degree of
Master of Science

Department of Biology
Memorial University of Newfoundland

December 1980

St. John's Newfoundland

Abstract

During the period from April 3, 1979 to May 1, 1980, fourteen sets of seawater samples were collected at a single station in Conception Bay, Nfld. Each set, except for a set collected on April 3, 1979, consisted of samples from four depths (0.5, 10, 20 and 40 m). Total viable heterotrophic bacteria were estimated by the standard plate count (SPC) method and by the most probable number (MPN) method. Total bacterial numbers were determined by the acridine orange direct count (AODC) method. Oleoclastic bacteria were enumerated by an MPN method using a buffered basal salts medium to which Venezuelan crude oil had been added. Heterotrophic bacterial activity was determined by the heterotrophic potential method.

Total numbers of bacteria fluctuated over the sampling period but generally were lower in winter. Numbers ranged from 2.5×10^7 to 4.6×10^8 per litre of seawater. Viable bacteria by the MPN method ranged from 3.3×10^3 to 1.6×10^6 per liter and, generally, were highest in the fall. MPN estimates represented 0.003 to 1.750% of the total bacteria determined by the AODC method. The SPC method yielded 6.0×10^3 to 3.3×10^6 viable bacteria per liter during the course of sampling, and the highest numbers by this technique were observed in June, 1979, for all depths tested. Throughout the sampling period, 0.003 to 3.370% of the total bacteria (AODC method) were detected by the SPC method. This method gave higher estimates of viable bacteria in June and

July, while the MPN method gave higher results in October, November, and December. At other times of the year, numbers were approximately the same by the two methods. Oleoclasts ranged in number from 7.7×10^2 to 1.6×10^6 per liter and were higher in the May to September period than at other times of the year. No pattern of distribution with depth was observed for either total, viable, or oleoclastic bacteria.

Heterotrophic activities were determined at *in situ* temperatures and exhibited a seasonal trend. V_{max} increased from spring to summer, decreased during the fall, and remained low during the winter. However, highest V_{max} values at all sampling depths were recorded before the highest water temperature for the year was reached at each depth. Turnover time decreased with increasing temperature and the opposite was also observed. V_{max} ranged from 1.3 to 121.0 ng glutamate $l^{-1}h^{-1}$ over the sampling period, and turnover time varied from 66 to 13,074 h. Neither V_{max} nor turnover showed any pattern with depth.

V_{max} specific activity index (V_{max}/cell) was generally higher in summer than at other times of the year. The highest values at three depths also preceded the maximum water temperature. Values ranged from 0.007 to 1.235 fg glutamate $h^{-1}cell^{-1}$ over the sampling period.

A thermocline was present on four of the fourteen sampling days. The thermocline appeared to have little effect on bacterial numbers but heterotrophic activity was lower below the thermocline than above.

This project has shown that the majority of the bacterial population exists in a dormant state.

This is the first study in which both bacterial numbers and heterotrophic potentials have been determined throughout four consecutive seasons for marine waters of the Canadian Atlantic seaboard.

Acknowledgements

I am grateful to my supervisor, Dr. John Gow, for the advice and encouragement he supplied relating to this project, as well as for the many hours he spent assisting me on sampling trips and in the laboratory. I also thank him for the financial support which enabled me to attend the 1980 meeting of the Canadian Society of Microbiologists in Guelph, Ontario. Here, I was able to discuss aspects of my work with other microbiologists.

I extend my gratitude to Mr. Hugh Jacobs, former skipper of "Elsie G", and to Mr. Dave Butler, the vessel's present skipper, for their cooperation on sampling trips. I am grateful to Mr. Bob Hooper without whose help sampling would not have been possible on several occasions. I also thank Mr. Brendan Hollohan for his assistance on many sampling trips and in the laboratory, as well.

I am grateful to Dr. Pat Dabinett and Dr. Jake Rice for their advice on this project. I thank Dr. George Moskovits for his help and advice, and Dr. Alan Whittick for his assistance regarding the computer aspect of the project. I express my gratitude to Mrs. Debbie McCallum who helped in the drawing of graphs, to Mr. Roy Ficken for photographing and printing the figures, and to Mrs. Carol Hawco who typed the thesis.

TABLE OF CONTENTS

	Page
ABSTRACT	ii
ACKNOWLEDGEMENTS	v
TABLE OF CONTENTS	vi
LIST OF TABLES	viii
LIST OF FIGURES	x
INTRODUCTION	1
MATERIALS AND METHODS	7
Study Area	7
Water Samples	7
Measurement of Heterotrophic Potential	9
Liquid Scintillation Counting	11
Estimation of Bacterial Numbers	11
(A) Direct Count Method	11
(B) Total Viable Heterotrophic Bacteria	12
(C) Total Oleoclastic Bacteria	13
(D) Enumerating Coliform Organisms	14
Sediment Sample	14
Determination of Hydrographic and Nutrient Data	14
RESULTS	16
Part A: Seasonal Changes	16
Part B: Experiments That Were Not Related to Seasonal Changes.	38
DISCUSSION	46
SUMMARY OF RESULTS	68
REFERENCES	70

TABLE OF CONTENTS (CONT'D)

	Page
APPENDIX I: CALCULATION OF KINETIC PARAMETERS	81
APPENDIX II: CALCULATION OF TOTAL BACTERIA	90
APPENDIX III: BACTERIAL NUMBERS	93
APPENDIX IV: KINETIC PARAMETERS	98
APPENDIX V: TEMPERATURE, SALINITY, AND CHEMICAL DATA	103

LIST OF TABLES

TABLE		Page
1	Standard deviations of SPC, MPN, AODC, V_{max} and turnover time.	39
2	Comparison of temperatures, bacterial numbers, V_{max}, V_{max}/cell, and turnover times at 10 m and 100 m.	43
3	Comparison of bacterial numbers, V_{max}, V_{max}/cell, and turnover times in a 40 m water sample and a sediment sample.	45
	APPENDIX I: CALCULATION OF KINETIC PARAMETERS	
1	Sample of type of printout obtained from the Beckman LS3150T liquid scintillation counter.	83
2	Values of t/f obtained from a typical experiment.	88
	APPENDIX III: BACTERIAL NUMBERS	
1	Numbers of total, viable, and oleoclastic bacteria at 0.5 m depth.	94
2	Numbers of total, viable, and oleoclastic bacteria at 10 m depth.	95
3.	Numbers of total, viable, and oleoclastic bacteria at 20 m depth.	96
4	Numbers of total, viable, and oleoclastic bacteria at 40 m depth.	97
	APPENDIX IV: KINETIC PARAMETERS	
1	V_{max}, V_{max} specific activity index, T_t, and	

LIST OF TABLES (CONT'D)

TABLE		Page
	$K_T + S_N$ at 0.5 m depth.	99
2	V_{max}, V_{max} specific activity index, T_t, and $K_T + S_N$ at 10 m depth.	100
3	V_{max}, V_{max} specific activity index, T_t, and $K_T + S_N$ at 20 m depth.	101
4	V_{max}, V_{max} specific activity index, T_t, and $K_T + S_N$ at 40 m depth.	102
	APPENDIX V: TEMPERATURE, SALINITY, AND CHEMICAL DATA	
1	Temperature, salinity, and chemical data at 0.5 m depth.	104
2	Temperature, salinity, and chemical data at 10 m depth.	105
3	Temperature, salinity, and chemical data at 20 m depth.	106
4	Temperature, salinity, and chemical data at 40 m depth.	107

LIST OF FIGURES

Figure		Page
1	Section of the Avalon Peninsula showing the sampling stations.	8
2	Vertical profiles of temperature and total and viable bacteria.	17
3	Total viable heterotrophs by the standard plate count (SPC) method as a percentage of the total direct count (AODC) at all four sampling depths.	20
4	Total viable heterotrophs by the most-probable-number (MPN) method as a percentage of the total count (AODC) at all four sampling depths.	21
5	Total viable heterotrophs by two methods (SPC and MPN) as a percentage of the total direct count. Results are shown for the 10 m depth.	22
6	Relationship between seasonal temperature and numbers of oleoclastic bacteria (MPN method).	24
7	Relationship between seasonal temperature and V_{max}.	26
8.	Relationship between seasonal temperature and V_{max}/cell.	27

Figure		Page
9	Relationship between seasonal temperature, V_{max} and V_{max}/cell at the 10 m sampling depth.	29
10	Relationship between seasonal temperature and turnover time.	30
11	Effect of the thermocline on bacterial numbers, V_{max} and turnover time (August 7, 1979).	31
12	Effect of the thermocline on bacterial numbers, V_{max} and turnover time (September 11, 1979).	32
13	Relationship between amounts of carbon, nitrate, orthophosphate and silicate during the sampling period.	34
14	Relationship, by season, between temperature, turnover time, V_{max} and bacterial numbers at the 10 m depth.	37
15	A comparison of bacterial numbers, V_{max} and turnover time (T_t) at 4 different stations (B, C, X and Y).	40
	Appendix 1: Calculation of kinetic parameters	
1	Quench correction curve showing the relationship between the external standards channel ratio (ESCR) and the counting efficiency (%) for ^{14}C of the Beckman LS3150T liquid scintillation counter.	84

Figure Page

Appendix 1: Calculation of kinetic parameters

2 Sample plot of substrate concentration added (A) versus the reciprocal of incubation time (t) and fraction of substrate taken up (f). 89

INTRODUCTION

Heterotrophic bacteria play an important role in the oceans of the world. They utilize a wide range of substrates and are the major users of dissolved organic matter in marine environments. The recycling of essential nutrients in the ocean is largely due to heterotropic bacteria (Wright and Hobbie, 1965; Williams, 1970).

The total numbers of bacteria in marine waters varies with season and location. Griffiths et al. (1978) reported 1.5×10^8 cells per liter in winter samples from the Beaufort Sea and 4.5×10^8 cells per liter in summer samples. Wright (1978) observed 6.1×10^8 to 1.87×10^9 cells per liter in samples from the Gulf of Maine. Delattre et al. (1979) reported 1.5×10^8 to 5.2×10^8 per liter in samples taken from coastal waters off northern France. Samples from the South Davis Strait contained 7.9×10^6 to 3.2×10^8 cells per liter and 1.8×10^7 to 1.2×10^9 cells per liter in April and August, respectively (Bunch, 1979).

All of the above values for bacterial numbers were obtained by the acridine orange direct count (AODC) method. By using this method it is possible to get a value for the total number of bacteria in a sample rather than a value that reflects whether or not the bacteria will grow on a particular medium. The method requires collecting the bacteria by membrane filtration after staining the bacteria with the fluorescent dye acridine orange. The

cells are counted by using an epifluorescent microscope. The method described by Hobbie et al. (1977) has been the most widely adopted.

The determination of total viable marine bacteria differs from the determination of total bacteria because the former method detects only those bacteria capable of growth. The number of bacteria that grow and can be quantitatively assessed will be dependent upon the testing procedure and the composition of the growth media. The most popular method for determining total viable heterotrophic bacteria (TVHB) has been the standard plate count (SPC) method (A.P.H.A., 1976). However, a statistical estimate of bacterial numbers can be obtained by using the Most Probable Number (MPN) method (A.P.H.A., 1976). The latter method is especially useful for enumerating specific groups of bacteria. Di Geronimo et al. (1978) used the MPN technique to determine the numbers of bacteria utilizing several different aromatic compounds. Lehmicke et al. (1979) used ^{14}C - labeled substrates in an MPN technique to enumerate bacteria capable of utilizing several substrates. Using hydrocarbon substrates as sole carbon sources in media, several authors (Walker and Colwell, 1976; Roubal and Atlas, 1978; Mills et al., 1978) have enumerated petroleum-degrading microorganisms in marine environments.

Wright (1978) reported viable counts ranging from 2.4×10^5 to 6.12×10^6 per liter for Gulf of Maine waters. Counts of 6.3×10^5 to 2.5×10^8 viable cells per liter were

recorded for waters off France (Delattre et al., 1979). Bunch (1979) observed 1.66×10^4 to 4.57×10^5 viable cells per liter in April and 2.51×10^4 to 4.37×10^6 viable cells per liter in August in samples from the South Davis Strait. Noseworthy (1976) reported viable counts of 10 to 4329 per liter of plankton tow for waters off the east coast of Newfoundland. All of these values were obtained by the SPC method.

The determination of either direct counts or total viable counts does not provide the investigator with information about the metabolic activity of the microorganisms. The study of the activity of heterotrophic bacteria was made possible with the introduction by Parsons and Strickland (1962) of a method using ^{14}C-labeled substrates. Different concentrations of labeled substrate were added to various subsamples of a given sample and after a specified incubation period, the subsamples were filtered. The filters, which had trapped the bacteria, and the substrate taken up by the bacteria, were prepared for liquid scintillation counting and the uptake of labeled substrate determined by this method. The authors showed that uptake of glucose and acetate in seawater followed the pattern of Michaelis-Menten enzyme kinetics. Wright and Hobbie (1966) modified the technique so that the following three parameters: turnover time (T_t), V_{max}, and $K_T + S_N$ could be determined. Turnover time was defined as the time required by the natural microbial population to deplete a concentration of substrate equal to the natural concentrat-

ion. V_{max}, the maximum uptake velocity, was defined in units of μg substrate $l^{-1}h^{-1}$ or μg C $l^{-1}h^{-1}$. It indicated the maximum rate at which the bacterial population could utilize a given substrate. $K_T + S_N$ represented the sum of the transport constant (K_T) and the natural substrate concentration (S_N). It defines the upper limits for both constituents and is recorded in μg substrate l^{-1} or μg C l^{-1}. If K_T is very small, $K_T + S_N$ approximates the natural substrate concentration.

Uptake experiments using ^{14}C-labeled substrates require a method for measuring the $^{14}CO_2$ given off during respiration. Several techniques have been developed for this (Hobbie et al., 1968; Williams and Askew, 1968; Hobbie and Crawford, 1969; Brown, 1979) but the method of Hobbie and Crawford (1969) is the one most widely used. β-phenethylamine - soaked chromatographic paper is used to trap $^{14}CO_2$ in acidified samples. The radiolabeled CO_2 can be quantitated by liquid scintillation counting.

The measurement of heterotrophic potential by the method of Wright and Hobbie (1966), and modifications of that method, has been applied to most aquatic environments: freshwater (Burnison and Morita, 1974; Berman et al., 1979; Cavari and Hadas, 1979); estuarine (Hobbie et al., 1968; Hansen and Snyder, 1979); and marine (Vaccaro and Jannasch, 1967; Williams and Askew, 1968; Takahashi and Ichimura, 1971). The heterotrophic potential of sediments has also been studied (Harrison et al., 1971; Griffiths et al., 1978).

Values for V_{max}, T_t and $K_T + S_N$ vary with season and location, and sometimes with depth. In the northwestern Pacific, Takahashi and Ichimura (1971) recorded V_{max} values ranging from 18.5 to 154 ng glucose $l^{-1}h^{-1}$ at the surface, 18 to 27.7 ng glucose $l^{-1}h^{-1}$ at 100 m depth, and 9.2 to 22ng glucose $l^{-1}h^{-1}$ at 1000 m. Turnover times at these depths were 270 to 420 h, 710 to 1700 h, and 450 to 4300 h, respectively. The values for $K_T + S_N$ were 5 to 50 µg glucose l^{-1} at the surface, 19.8 to 30 µg glucose l^{-1} at 100 m and 10 to 40 µg glucose l^{-1} at 1,000 m. V_{max} for coastal waters off northern France ranged from .0015 to 1.04 µg glucose $l^{-1}h^{-1}$ (Delattre et al., 1979). For glutamic acid, V_{max} in Beaufort Sea samples ranged from 0.2 to 12 ng glutamic acid $l^{-1}h^{-1}$ in winter and from 4 to 113 ng glutamic acid $l^{-1}h^{-1}$ in summer (Griffiths et al., 1978). Crawford et al. (1974) reported V_{max} values as high as 1899 µg glutamic acid $l^{-1}h^{-1}$, turnover times as low as 0.64 h, and $K_T + S_N$ ranging from 6.37 to 53.06 µg glutamate l^{-1} for estuarine water. Values obtained from the South Davis Strait showed V_{max} values ranging from 4.6 to 417.9 ng glutamic acid $l^{-1}h^{-1}$, turnover times of 16 to 2431 h, and $K_T + S_N$ of 0.94 to 27.86 µg glutamic acid $l^{-1}h^{-1}$ (Bunch, 1979).

It has been shown by many authors that bacteria are the main organisms involved in the uptake of dissolved organic compounds in bodies of water (Wright and Hobbie, 1965; Williams, 1970). With this information, Wright (1978) suggested using a specific activity index wherein V_{max} and turnover time are reported on a per bacterium basis. Using AODC

values as denominators and V_{max} or T_t values as numerators, units for V_{max} and T_t specific activity indices are µg substrate $h^{-1}cell^{-1}$ and $h^{-1}cell^{-1}l$, respectively. Wright (1978) observed V_{max} specific activities ranging from 2.8 x 10^{-12} to 28 x 10^{-12} µg glucose h^{-1} $cell^{-1}$ in Gulf of Maine waters.

In this thesis a study of heterotrophic bacteria in Conception Bay, Newfoundland is described. Bacteria were enumerated by the plate count, MPN, and AODC methods. Petroleum-degrading bacteria were estimated by an MPN method. Heterotrophic potential was determined by the method of Wright and Hobbie (1966) incorporating the technique of Hobbie and Crawford (1969) to measure respired $^{14}CO_2$. The substrate used was ^{14}C-glutamic acid. This amino acid is present in marine waters (Andrews and Williams, 1971) and was found to be utilized by 100% of a sampling of 106 bacterial strains isolated from several marine environments including Newfoundland marine waters (Gow, personal communications). Bacterial numbers and activities were monitored over a 13 month period. Environmental factors such as depth, temperature and nutrient concentrations were examined to determine their effect on bacterial numbers and heterotrophic potential.

MATERIALS AND METHODS

Study Area

Water samples were collected routinely at a single station (Lat. 47°34.4'N, Long. 53°1.5'W) in Conception Bay, Newfoundland (Fig. 1). This station is located in the vicinity that is north of Kelly's Island and southwest of Bell Island. The station has been designated Station C by the biologists from Memorial University of Newfoundland, whose research programmes include studies of the flora and fauna of Conception Bay. On several occasions water samples were obtained from other sites in Conception Bay. These sites were given capital letter designations and are shown in Fig. 1. Samples obtained at these sites were used to compare microbial numbers and activities with those of samples from Station C. The depth at Station C was 45 m.

Water Samples

Samples were collected at approximately monthly intervals from April, 1979 until May, 1980. Samples were taken aseptically from depths of 0.5, 10, 20 and 40 m using a Johnson-ZoBell sampling apparatus. Usually two sterile 1 l bottles were filled at each depth and kept at or below ambient temperature until they reached the laboratory. The duplicate samples taken from the same depth were mixed together before subsamples were taken. Experiments were conducted within 5 to 6 hours of the time of collecting the samples.

On each sampling day a bathythermograph (BT) was

Figure 1: Section of Conception Bay showing the sampling stations. Station C was sampled on a regular basis. Stations S, B, X, Y and Z were sampled on occasions specified in the Results section.


Conception Bay
Bell I.
Z.
C.
X.
Y.
Kelly's I.
B.
St. John's
48°30'
53°00'
0
5
10
km

used to measure temperatures throughout the water column. Surface temperature was taken by thermometer in a bucket of surface water and when the BT slide was placed in the slide reader it was adjusted to coincide with the corrected surface temperature.

A portion of each water sample was frozen and later sent to the water analysis laboratory (Chemistry Department, Memorial University of Newfoundland) for the determination of salinity and nutrients. The methods used are described later.

Measurement of Heterotrophic Potential

Heterotrophic potential was measured by the methods of Wright and Hobbie (1966) and Hobbie and Crawford (1969). Twenty-one sterile 100 ml serum bottles were required for each sample. These were divided into 3 sets of bottles. Aliquots of ^{14}C-glutamic acid (New England Nuclear, Canada, Ltd., Cat. no. NEC-290) were pipetted into the bottles in triplicate so that there were six concentrations represented. The final bottle in the set served as a control. It contained one-half the highest concentration of glutamate added to the other bottles. In addition 0.5 ml of 50% phosphoric acid was added to the control bottles. The cells in these bottles were killed by the phosphoric acid and served as background controls for the remaining bottles. During the months in which the water temperature was relatively high, concentrations of glutamate ranging from 1.3 µg/l to 7.8 µg/l were added. During colder months it was necessary to add higher concentrations (5.2

µg/l to 31.2 µg/l) of the substrate. Fifty millilitres seawater was added to each bottle. Two sets of bottles were fitted with ordinary serum caps. The third set was fitted with serum caps that had filter cup assemblies (Kontes Glass Company, Vineland, N.J.) fitted into them. Each filter cup contained a type P-040 25 mm diameter disc prefilter (Bio-Rad Laboratories, Canada, Ltd.). The purpose of this set of bottles was to correct for respired substrate. All serum bottles were incubated within $\pm$ 2°C of the *in situ* water temperature according to the depth at which the sample was taken. The time of incubation varied from 2h during the warmer months to 18-20h during the coldest months.

At the end of the incubation period 0.5 ml of 50% phosphoric acid was added via syringe to each respiration bottle. The bottles were periodically shaken for 0.5h after which time 0.15 ml β-phenethylamine (Sigma) was added to the prefilter in each cup. The bottles were incubated for a further 45 min., with periodic shaking. The filters were removed and placed in liquid scintillation vials (New England Nuclear mini-LSC-vial).

The 50 ml seawater in each bottle was filtered through Bio-Rad nucleopore filters (0.2 µm pore size, 25 mm diameter) and washed with 10 ml filtered seawater. The filters were placed in mini-LSC-vials and dissolved with 0.5 ml Protosol (New England Nuclear).

Liquid Scintillation Counting

The liquid scintillation fluid used for counting all samples was toluene based with Omnifluor (New England Nuclear). Five millilitres of scintillation fluid were added to each vial. The vials were stored overnight in the dark to eliminate chemiluminescence and then counted in a Beckman Model LS3150T liquid scintillation counter. Counting was for 20 min per sample or 1.0% efficiency, whichever came first. The resulting counts per minute were recorded and converted to disintegrations per minute. From the corrected counts turnover time, V_{max}, and $K_t + S_n$ were generated by computer programme (Appendix 1).

Estimation of Bacterial Numbers

(A) Direct Count Method.

Total bacteria were enumerated by the acridine orange direct count (AODC) method of Hobbie et al. (1977). For each sample, two 10 ml subsamples were pipetted into bottles containing 0.2 ml formalin. Nuclepore filters (0.2 µm pore size, 25 mm dia.) were stained overnight in a solution of 2g nigrosine (C.I. 50420) in 1 l 2% acetic acid. The filters were dipped in clear water and then used immediately. One millilitre of 0.01% acridine orange (Sigma) was added to each 10 ml subsample. The subsamples were filtered after 10-15 min staining and the filters were mounted on microscope slides. Immersion oil was applied to the filter, a coverslip was placed over the filter and then immersion oil was applied to

the coverslip. The bacteria on each filter were counted at 1,250 x magnification with a Zeiss IV FL epifluorescence condenser microscope fitted with a no. 48-77-09 filter. Representative fields, as delineated by a net micrometer, were counted (See Appendix 2 for an example). Only those cells that fluoresced either orange or green and had a clear outline and bacterial shape were counted as bacterial cells.

(B) Total Viable Heterotrophic Bacteria

Total viable heterotrophic bacteria (TVHB) were estimated by two methods. These were the standard plate count procedure (SPC) (A.P.H.A., 1976) and a most probable number (MPN) method (Alexander, 1965). The medium used for both methods was a peptone, yeast-extract, sea-water medium described by Colwell and Wiebe (1970). This medium contained: 0.1 % Proteose-Peptone no. 3 (Difco); 0.1% Yeast Extract (Difco), and natural aged seawater diluted with distilled water (3:1 v/v). This medium was called PYESW medium. Plating medium (PYESWA) was prepared from PYESW medium with 1.2% Oxoid technical grade agar no. 2. (Oxoid, Can. Ltd.).

The standard plate count technique was modified for surface inoculation of the plates. Ten plates per sample were inoculated with 0.2 ml of sample. The pipette was drawn from the centre of each plate to the edge while the plate was slowly spinning on a Petri dish turntable (Fisher Scientific Co. Ltd.). Plates were incubated at $5^{o}C \pm 1^{o}C$ for three weeks. The colonies on all ten plates were counted and the total multiplied by five hundred to give the TVHB/l.

The MPN procedure of Alexander (1965) was done using 25 tubes per sample. Dilutions of 1:10, 1:100, 1:1000 and 1:10,000 were made using sterile filtered seawater and five tubes per dilution were inoculated each with a 1 ml aliquot of the appropriate dilution. The tubes were incubated at 5°C for three weeks. Turbid tubes were called positive and MPN values were determined from the tables published by Alexander (1965).

(C) Total Oleoclastic Bacteria

Bacteria capable of degrading hydrocarbons were enumerated by the MPN method of Mills et al. (1978) with one modification. The buffered basal salts medium was different. It was prepared by the following method. A double-strength artificial seawater (ASW) was made that contained; 600 mM NaCl, 100 mM $MgSO_4 \cdot 7H_2O$, 20 mM KCl and 2 mM $CaCl_2 \cdot 2H_2O$. A double-strength buffered medium was made that contained; 100 mM tris (hydroxymethyl)aminomethane (Sigma) adjusted to pH 7.5 with HCl, 2.0 mM $(NH_4)_2 HPO_4$, and 52µM $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$. A single-strength buffered artificial seawater (BASW) medium was prepared by mixing equal quantities of BM and ASW. The BASW was dispensed in 5 and 10 ml quantities into test tubes and 25µl and 50µl respectively of Venezuelan crude oil* was added. The medium was sterilized by autoclaving. The tubes were incubated at 5-6°C for three months. Turbid tubes were called growth-positive. Non-turbid tubes were assayed

*The Venezuelan crude oil was obtained from Dr. Douglas Malcolm, Faculty of Engineering and Applied Science, MUN.

for protein by the method of Herbert et al. (1971). Tubes with 30 µg/ml or greater protein were called growth positive also. Most probable number values were calculated from tables in Alexander (1965).

(D) Enumerating Coliform Organisms

Every water sample collected was tested for coliforms by the standard MPN method (A.P.H.A., 1976). Five 10 ml portions of seawater were tested for each sample.

Sediment Sample

On one occasion a sediment sample was taken at a location due east of Kelly's Island. The depth at this location was 45 m. The sample was taken with a ponar grab sampler (Wildco). A portion of the sample was placed in a sterile wide mouth jar and transported to the laboratory. The sediment sample contained about 40% water. Subsamples were diluted and dry weights calculated by the method of Griffiths et al. (1978). Once the dilution was made the sample was treated as described for samples from the water column. The values obtained were calculated in terms of grams dry weight of sediment.

Determination of Hydrographic and Nutrient Data

Hydrographic and nutrient parameters were determined at the Water Analysis Laboratory, Chemistry Department, Memorial University of Newfoundland. The following parameters were measured; silicate, chemical oxygen demand (COD),

reactive nitrate, ortho-phosphate and salinity. Salinity was determined by the low precision titration method (Strickland and Parsons, 1972). The automated system for the determination of silicate was based also on the procedure given in Strickland and Parsons (1972). Phosphate was measured by the ascorbic acid reduction method and carbon was determined by chemical oxygen demand. Both of these methods are standard methods (A.P.H.A., 1976).

The values reported for nitrate were obtained by the method of Armstrong et al. (1967). From April 1979 to August 1979 the Water Analysis Laboratory determined nitrate by the A.P.H.A. (1976) method. The latter method gave nitrate values that were higher than those of the former method and the author was advised to use only those values obtained by the method of Armstrong et al. (1967).

Results

Part A: Seasonal Changes

Temperatures and bacterial numbers at the depths sampled are shown in Figure 2A-2M. Generally, temperature was indirectly proportional to depth except on days when the temperature was constant throughout the entire water column or portions of it (Figure 2F to 2L). A thermocline was observed on May 5 (Figure 2A), June 22 (Figure 2B), August 7 (Figure 2D), and September 11 (Figure 2E) during 1979.

On a seasonal basis, temperatures were low in the winter and early spring, they increased throughout the late spring and summer, and then decreased throughout the fall. The maximum temperature at the 0.5 m and 10 m depths was observed in August. At 20 m and 40 m, the maximum temperature occurred in September. The range of temperatures was -0.5°C to 16.5°C at 0.5 m, -0.5°C to 15.5°C at 10 m, -1.0°C to 13.0°C at 20m, and -1.0 to 8.0°C at 40 m. The seasonal change in temperature was 17.0°C at 0.5 m, 16.0°C at 10 m, 14.0°C at 20m, and 9.0°C at 40 m.

Figure 2A-2M also shows the vertical and seasonal variations in total bacteria by direct count and total viable heterotrophic bacteria. The figures obtained are given in Appendix 3. Total bacteria, as determined by AODC, were relatively constant throughout the water column on any given sampling day. The only seasonal trend that was observed for total bacterial numbers was that they were lower in winter

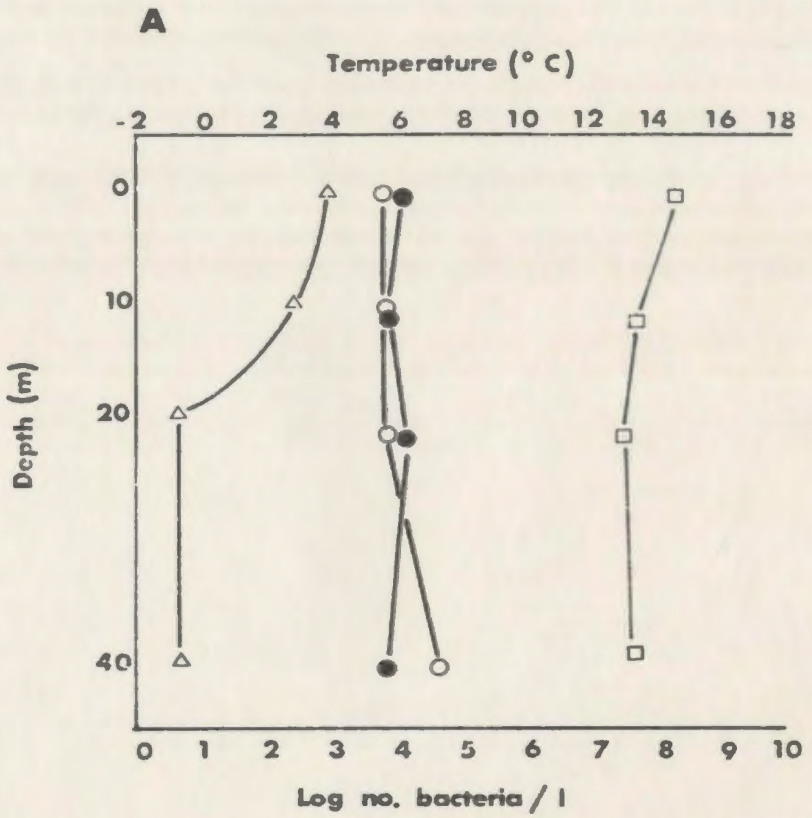
Figure 2 Vertical profiles of temperature and total bacteria. The sampling date is given for each day sampled (parts A to M).

Total direct counts (AODC) □—□

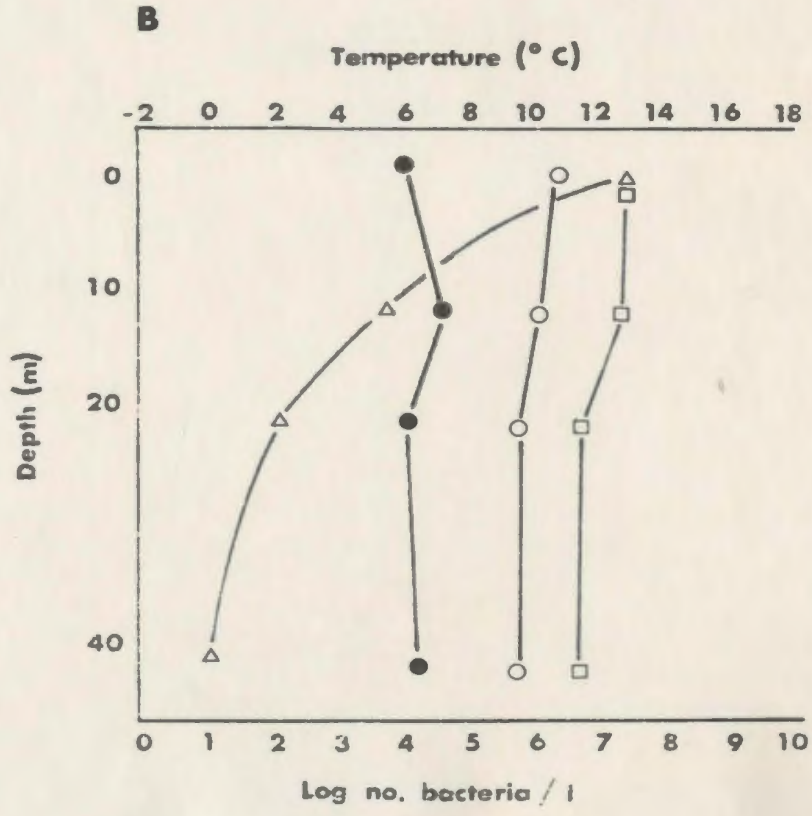
Total viable counts (MPN) method ●—●

Total viable counts (SPC) ○—○

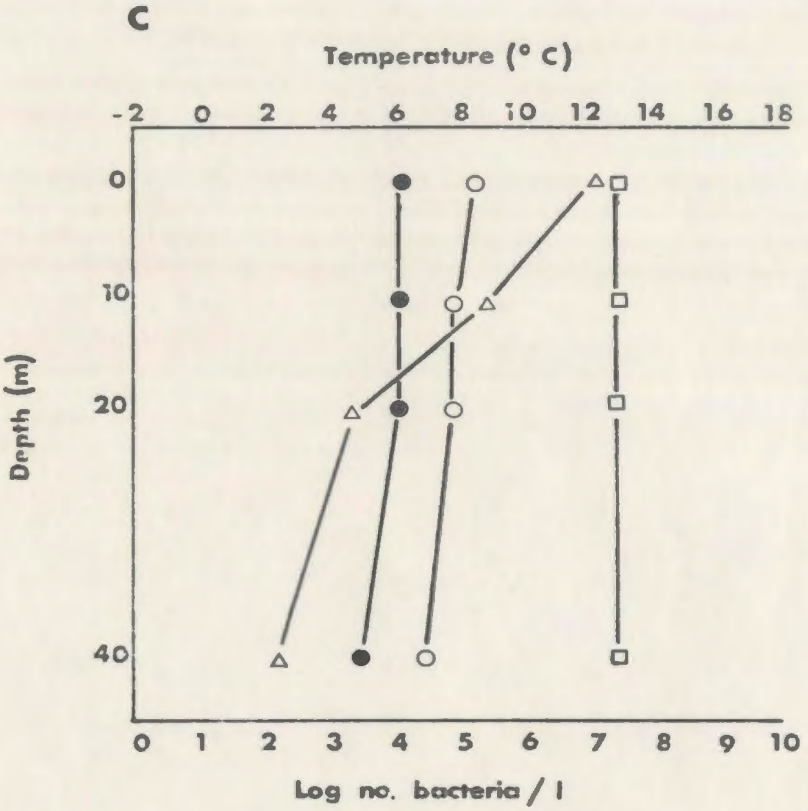
Temperature △—△



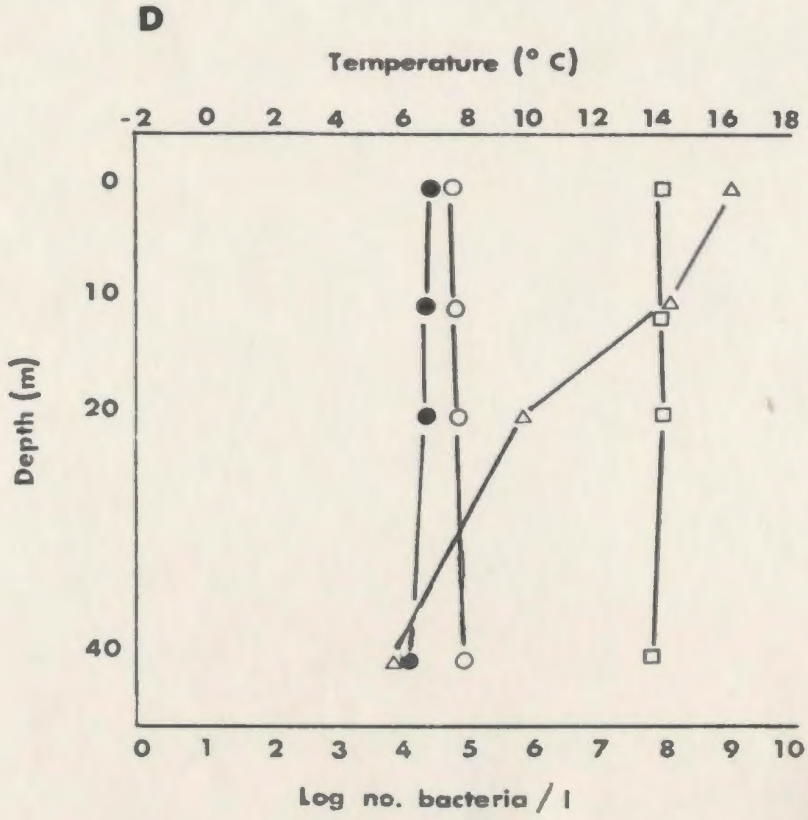
May 5 / 79



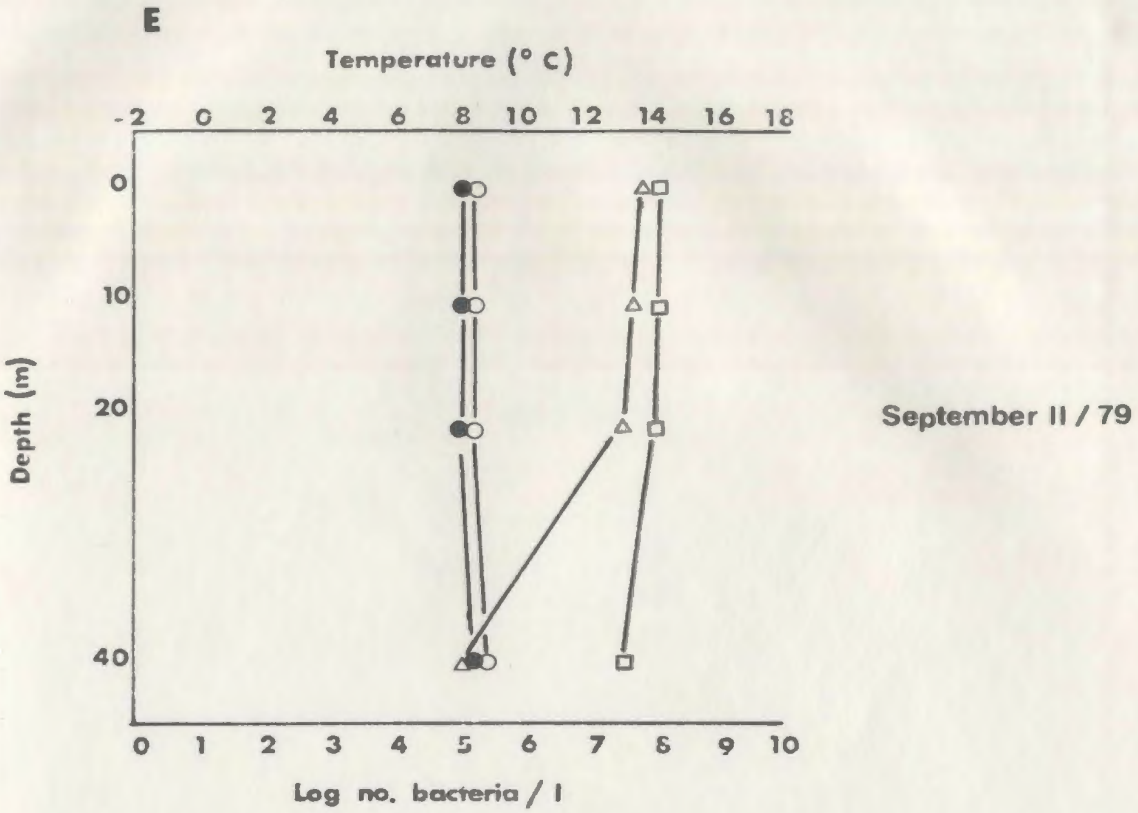
June 22/79



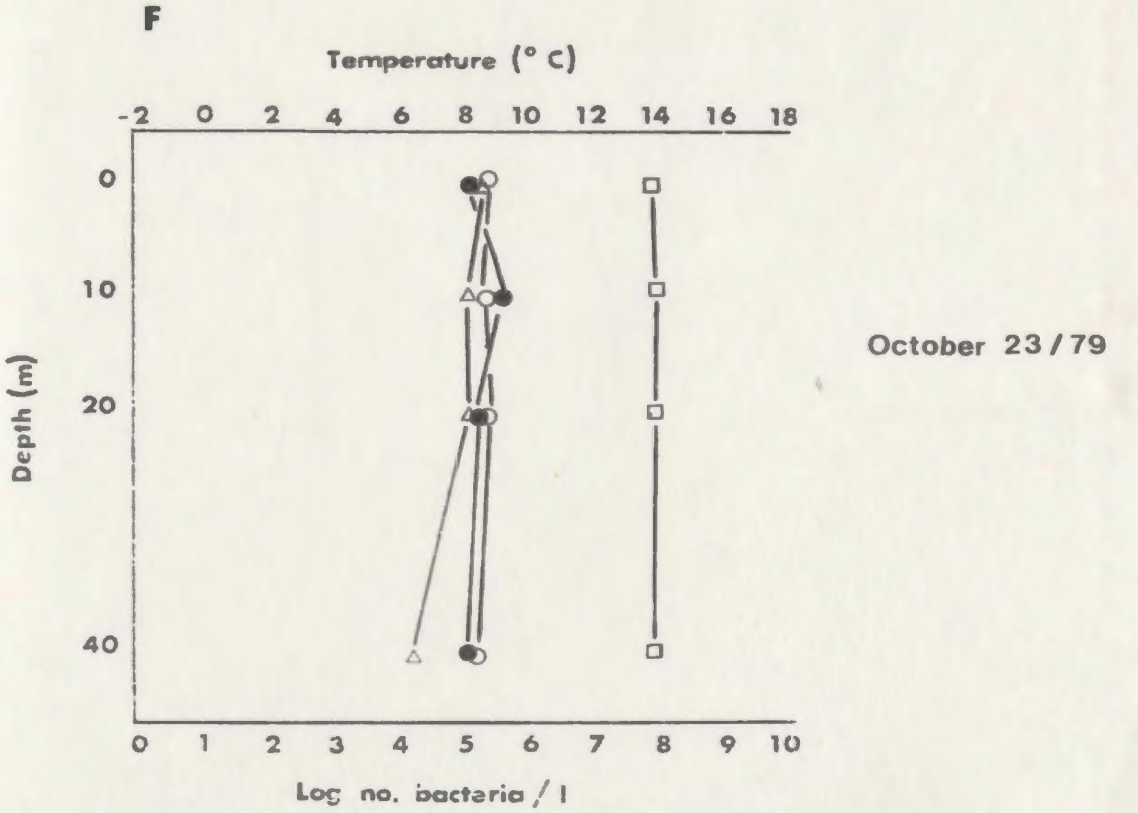
July 5 / 79



August 7 / 79

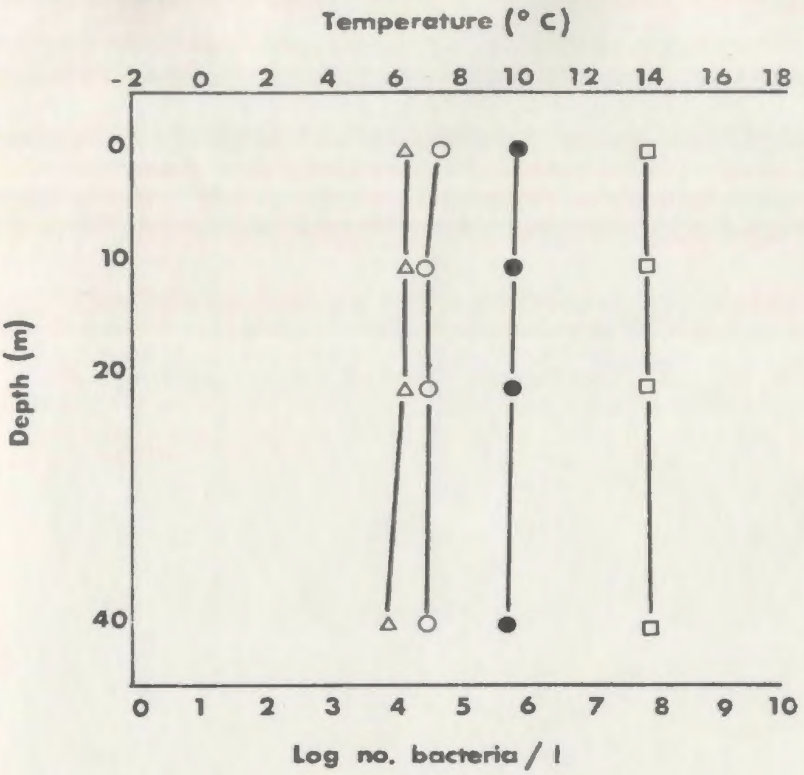


September 11 / 79



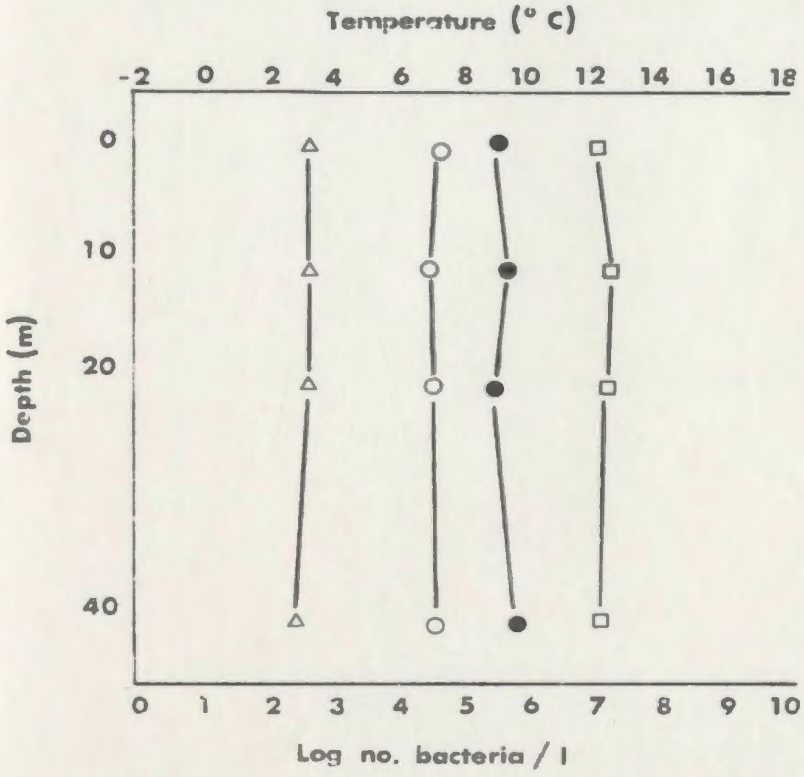
October 23 / 79

G

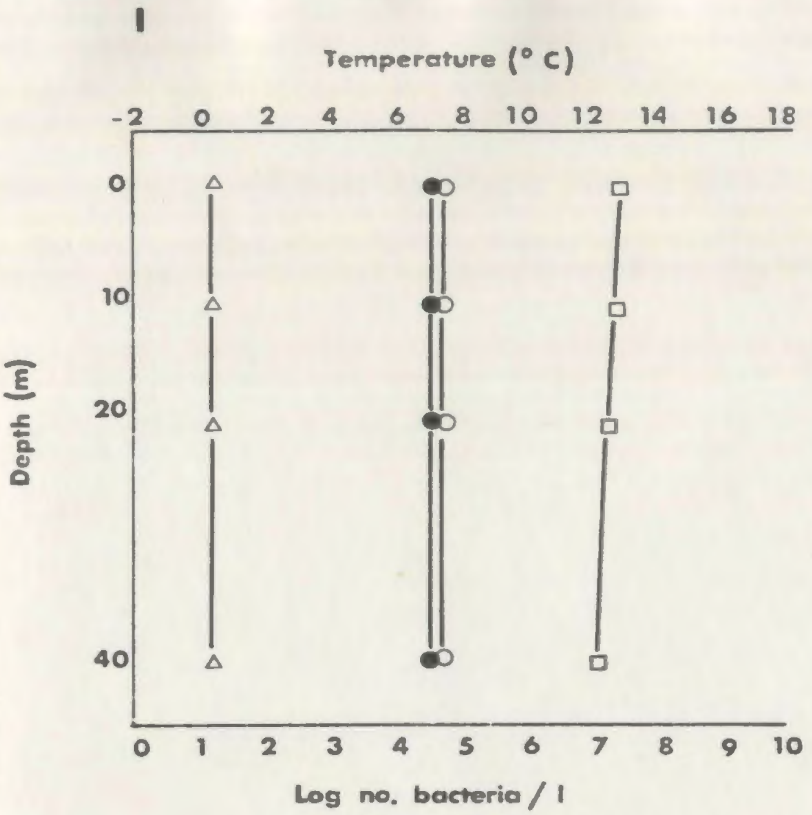


November 21 / 79

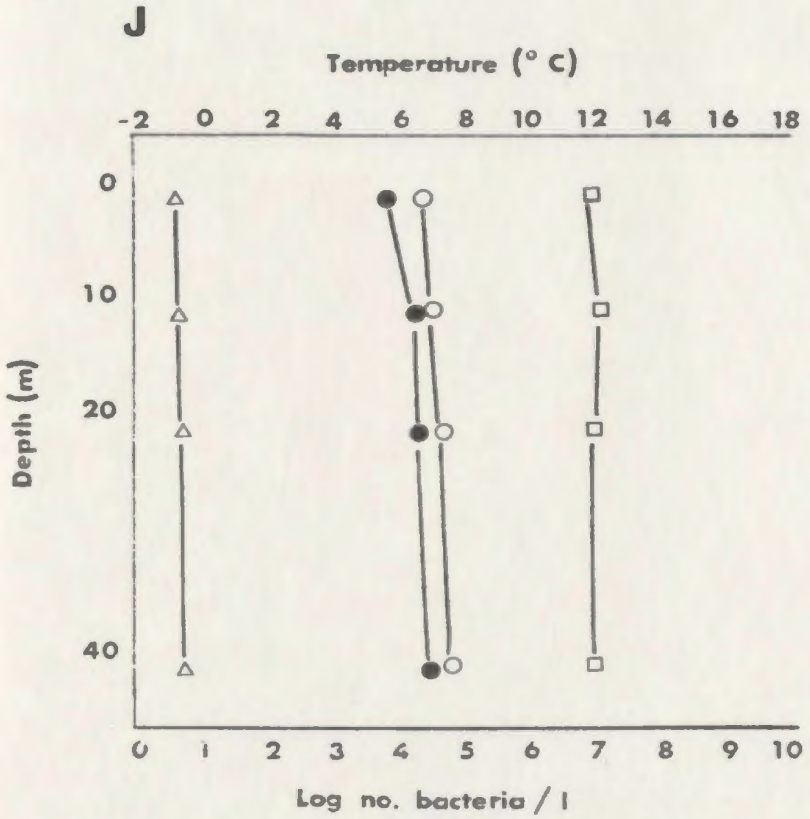
H



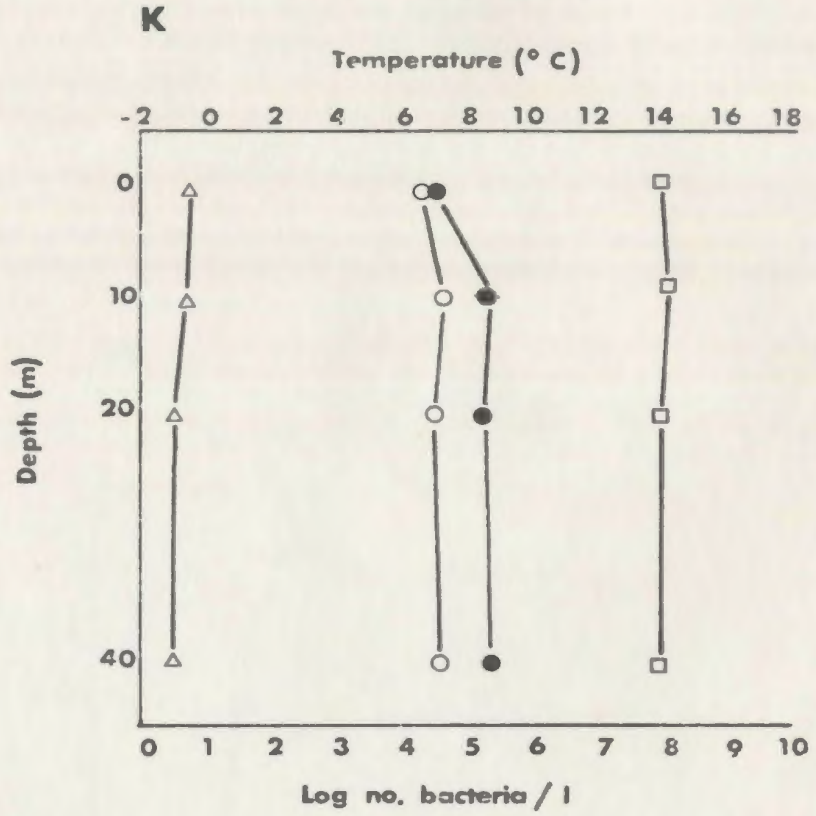
December 12/79



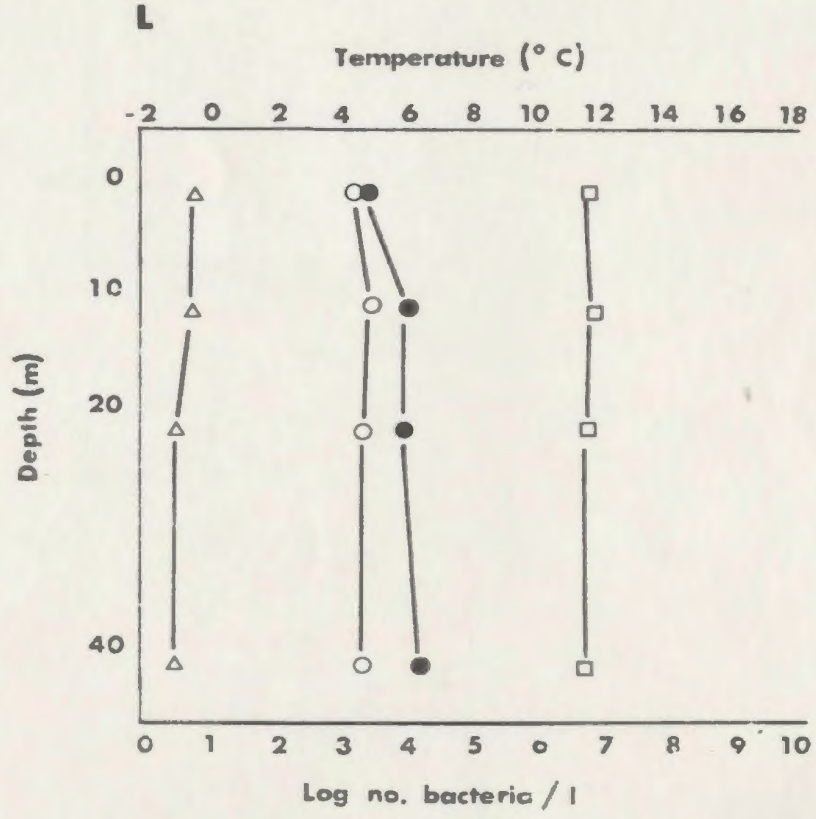
January 15 / 80



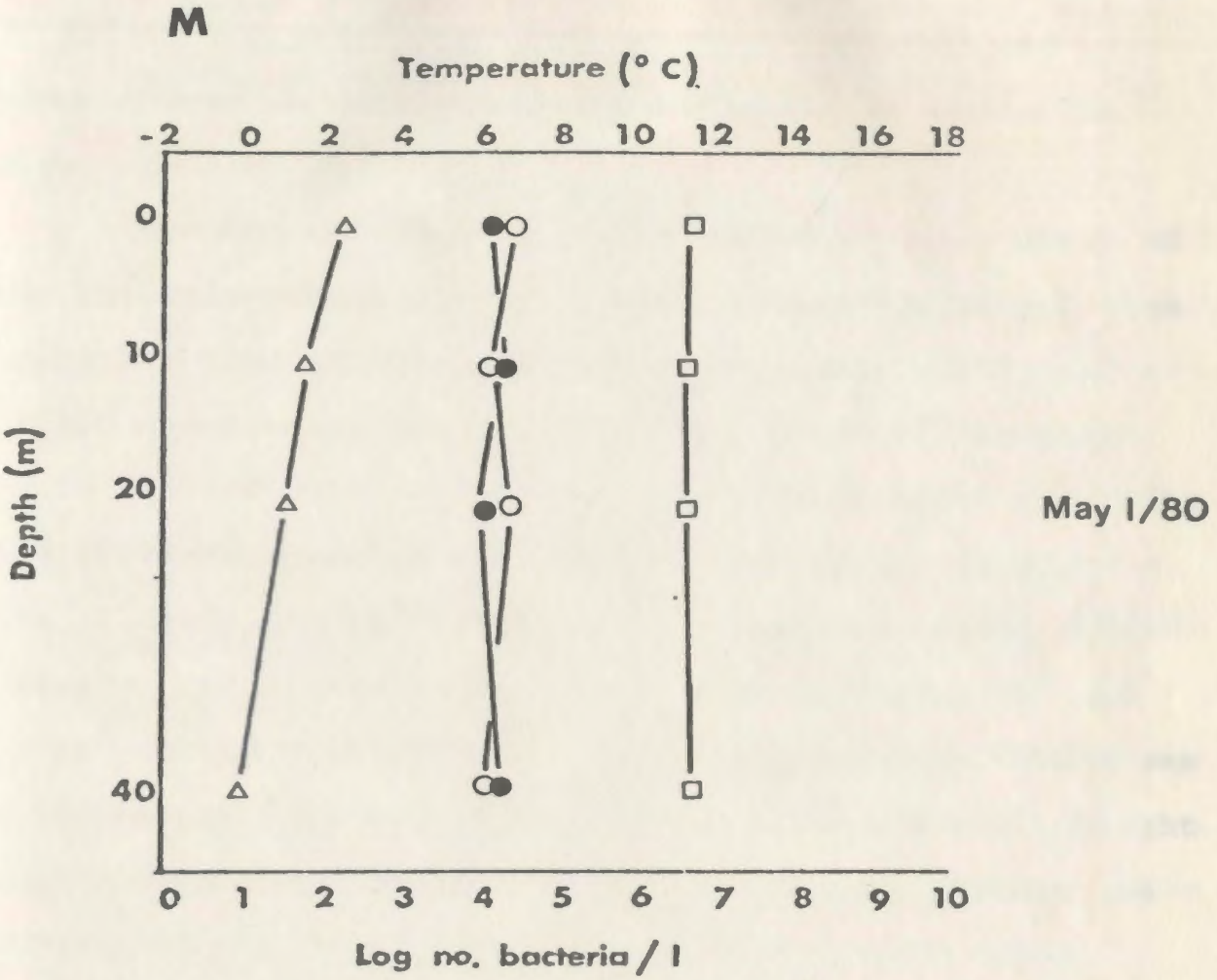
February 2 / 80



March 4 / 80



March 27 / 80


M
Temperature (° C)
-2 0 2 4 6 8 10 12 14 16 18
0
10
20
40
Depth (m)
0 1 2 3 4 5 6 7 8 9 10
Log no. bacteria / l
May 1/80

than at other times of the year. There was a 10 to 20 fold difference in total bacterial numbers between the extremes. At 0.5 m, AODC yielded a range of 2.8×10^7 to 3.8×10^8 bacteria per liter. The range was 3.8×10^7 to 3.5×10^8 per liter at 10 m, 2.8×10^7 to 4.6×10^8 per liter at 20 m, and 2.8×10^7 to 3.0×10^8 per liter at 40 m. The biggest seasonal change in total bacteria was at 0.5 m, while the smallest occurred at 10 m.

The MPN method generally yielded higher numbers of viable bacteria during the September to December period than at any other time although high values in June, 1979, and May, 1980 were exceptions. The largest seasonal variation (3.3×10^3 to 4.9×10^5 per liter) was a one hundred and fifty fold difference observed at 40 m. The variation at 20 m (1.1×10^4 to 1.1×10^6 per liter) gave a one-hundred fold difference. At 0.5 m and 10 m the range was 7.8×10^3 and 7.9×10^3 to 7.0×10^5 TVHB per litre respectively. This was an eighty-eight fold difference in bacterial numbers. Slight variation in numbers occurred with depth but no pattern was observed.

Viable bacteria determined by the standard plate count method were highest in June, 1979, for all depths tested. However, no consistent vertical trend was observed. The range of counts at each depth was 1.2×10^4 to 3.3×10^6 per liter at 0.5 m, 6.0×10^3 to 2.7×10^6 per liter at 10 m, 1.2×10^4 to 8.0×10^5 per liter at 20 m, and 2.6×10^4 to 5.9×10^5 per liter at 40 m. The widest range occurred at 10 m and the smallest at 40 m. Whereas, there was a four hundred

and fifty fold seasonal difference in bacterial numbers at 10 m there was only a twenty-two fold difference at 40 m.

The percentage of total bacteria which were viable by the standard plate count varied greatly over the year (Figure 3). Highest percentages for all depths occurred in June, 1979. The seasonal range at the four sampling depths was .004 - 3.370%, .003 - 2.950%, .006 to .860%, and .018 - .801%, respectively.

As a percentage of AODC, viable bacteria determined by the MPN method also exhibited variation over the course of the sampling period (Figure 4). However, the amount of variation was not as great as that of the standard plate count method. MPN percentages were highest for all depths in December, 1979. The seasonal range was .003 - 1.633% at 0.5 m, .003-1.556% at 10 m, .005 - .868% at 20 m, and .006 - 1.750% at 40 m.

The percentage of total bacteria which were determined viable by either method had a seasonal pattern. Figure 5 shows that at 10 m, plate counts gave higher percentages in the late spring and early summer than did the MPN method. This situation was reversed in the fall and early winter. Since this pattern was observed at the other sampling depths, as well, it appears that the bacteria were physiologically different during the spring than they were during the winter. Otherwise, the change in recovery pattern as a result of cultivating the bacteria by two different methods would not be expected. Further evidence to support the hypothesis of

Figure 3 Total viable heterotrophs by the plate count method as a percentage of the total direct count (AODC) at all four sampling depths.

Samples taken at 0.5 m. o—o

Samples taken at 10 m ●—●

Samples taken at 20 m △—△

Sample taken at 40 m ▲—▲

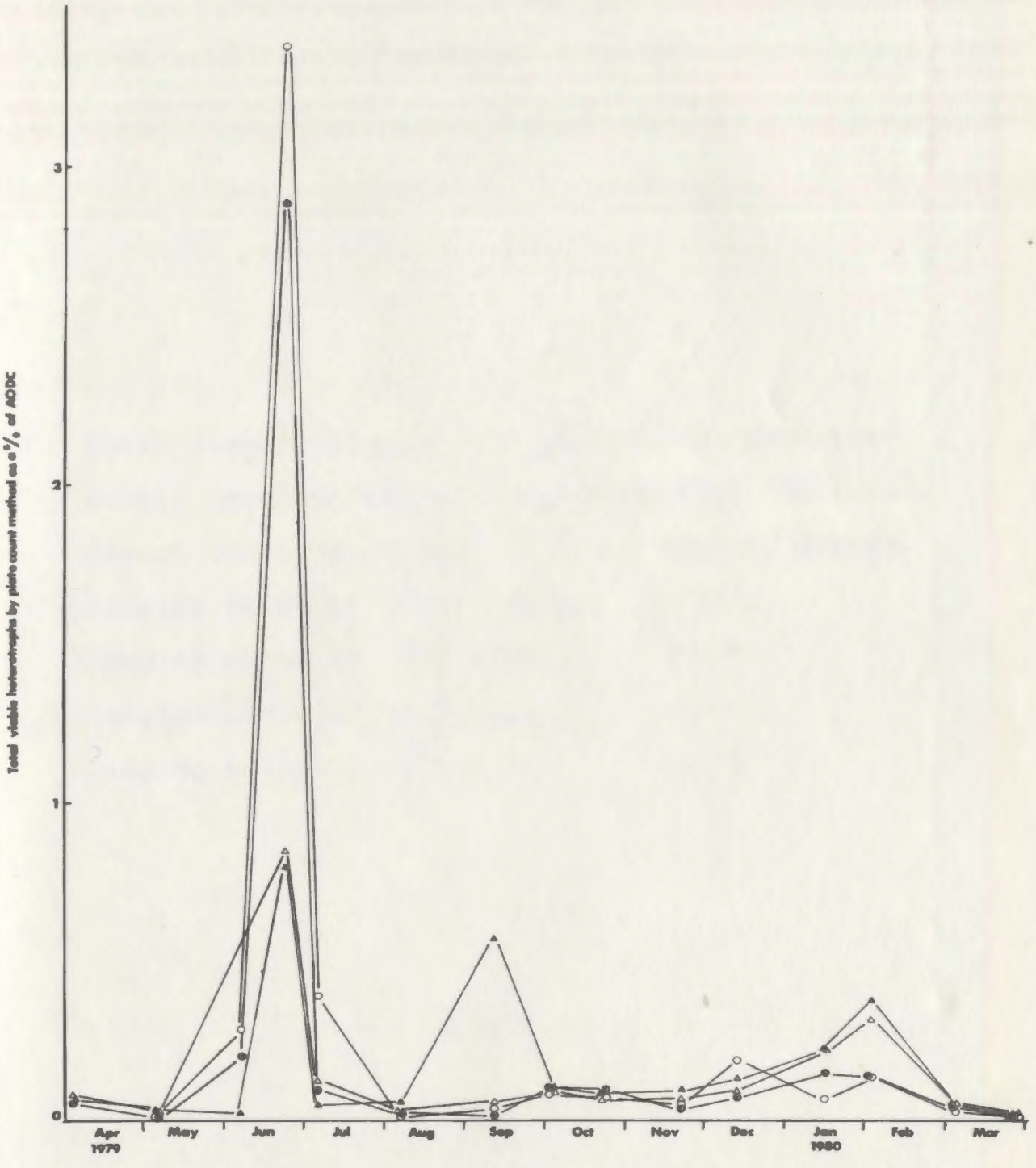

Total viable heterotrophs by plate count method as a % of AODC
3
2
1
0
Apr
1979
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
Jan
1980
Feb
Mar

Figure 4 Total viable heterotrophs by the most-probable-number (MPN) method as a percentage of the total direct count (AODC) at all four sampling depths.

Samples taken at 0.5 m depth o—o

Samples taken at 10 m depth ●—●

Samples taken at 20 m depth △—△

Samples taken at 40 m depth ▲—▲

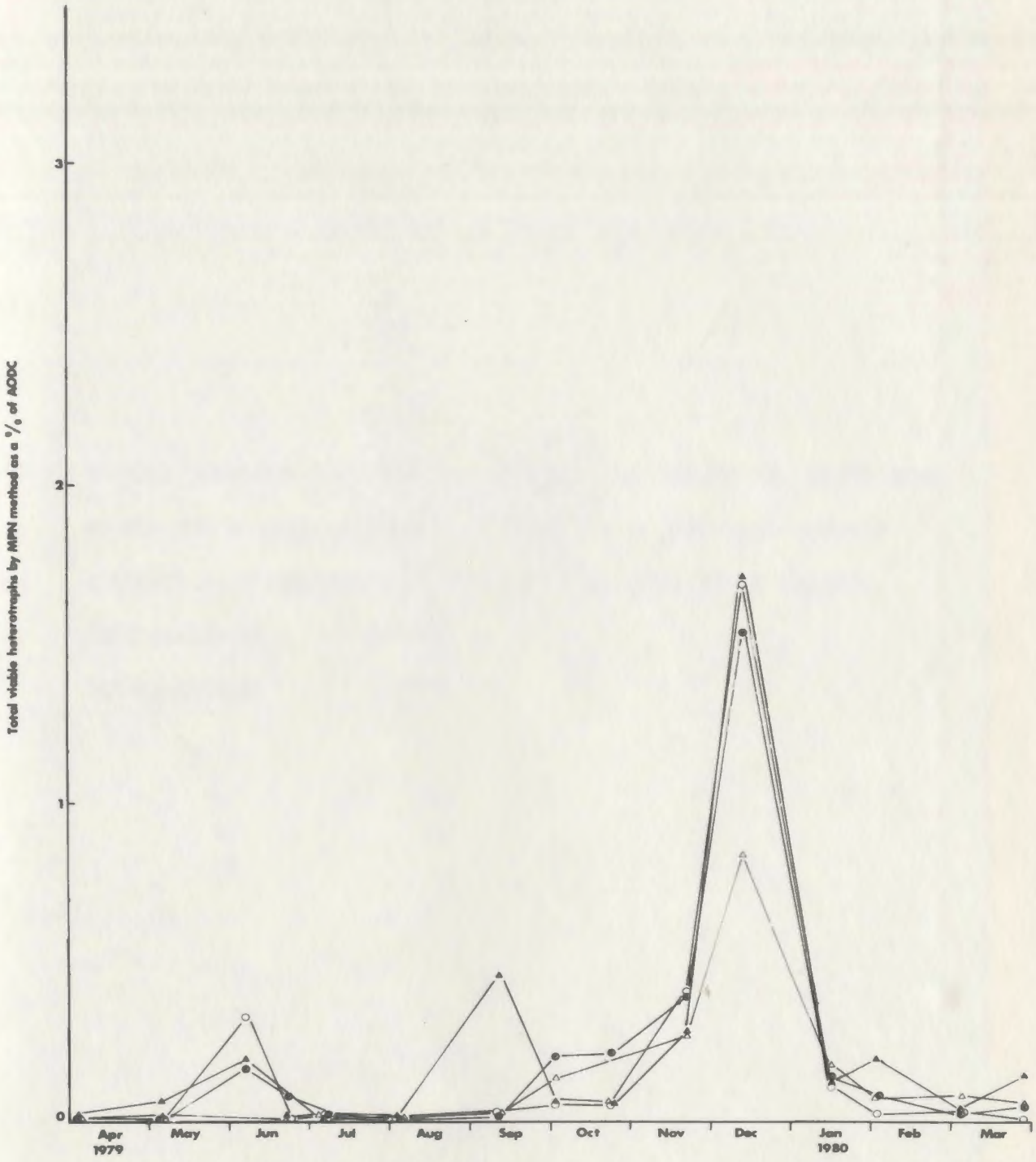
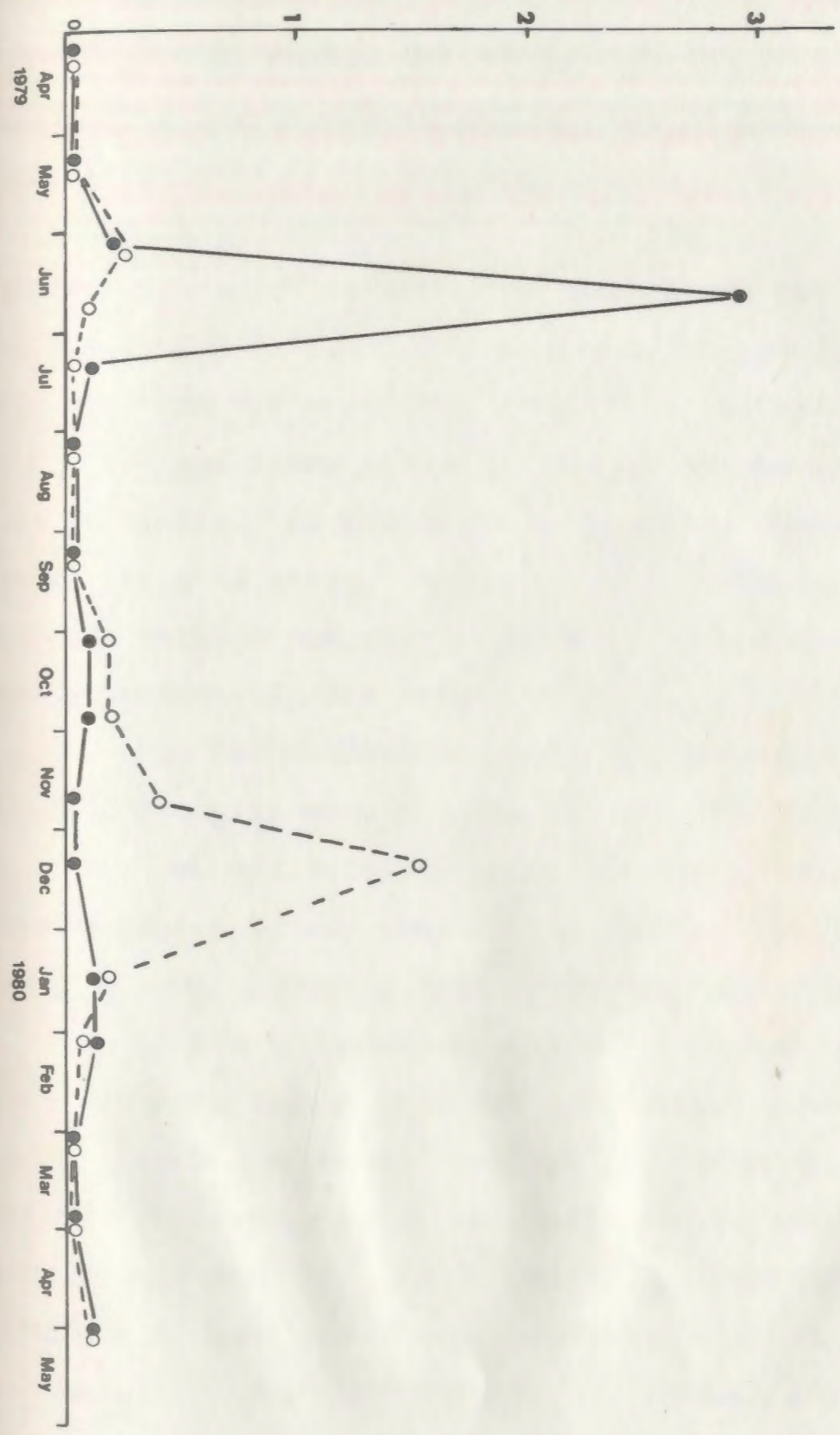

Total viable heterotrophs by MPN method as a % of AODC
3
2
1
0
Apr
1979
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
Jan
1980
Feb
Mar

Figure 5 Total viable heterotrophs by two methods (SPC and MPN) as a percentage of the total direct count (AODC). Results are shown for the 10 m depth.

SPC method ●—●

MPN method o—o


Total Viable Bacteria as a % of total bacteria by AODC
0
1
2
3
Apr
1979
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
Jan
1980
Feb
Mar
Apr
May

a change in the physiological state of the organisms will be presented later.

Hydrocarbon-degrading bacteria were more abundant during the May-September period than at other times of the year (Figure 6). The greatest number of oleoclasts was observed in September, 1979. Over the course of the sampling period, 1.1×10^3 to 2.2×10^5 oleoclasts per liter were recorded at 0.5 m, 7.7×10^2 to 9.2×10^5 per liter at 10 m, 8.0×10^2 to 9.2×10^5 per liter at 20 m, and 1.3×10^3 to 1.6×10^6 per liter at 40 m. The lowest number of oleoclasts was in winter. No depthwise pattern was observed during the course of this study. The average difference, for the 4 depths, between the yearly extremes in the number of oleoclasts was approximately one thousand fold. This is about ten fold higher than the difference observed when total viable heterotrophic bacteria were measured by the MPN method.

No coliforms or fecal coliforms were detected in the water samples at any time of the year. This indicated that Station C was probably free from terrestrial pollution.

Data on heterotrophic activity was obtained for all four sampling depths whenever possible. However, in April, June, August, September, October and November, 1979, data from one or more depths were not obtained. In April, only three depths were sampled. In the other five months, data were not obtained because the substrate uptake did not fit uptake kinetics. This occurred mainly in surface samples and at

Figure 6 Relationship between seasonal temperature and number of oleoclastic bacteria (MPN method). The number of oleoclastic bacteria at 4 depths is shown. The seasonal temperature profile is shown for the 10 m depth only.

Log no. oleoclasts at 0.5 m

Log no. of oleoclasts at 10 m

Log no. of oleoclasts at 20 m

Log no. of oleoclasts at 40 m

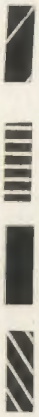

Log No. of Oleoclasts / l
1
2
3
4
5
6
Temperature (°C)
0
4
8
12
16
Apr 1979
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
Jan 1980
Feb
Mar 4
Mar 27

times when the water temperatures were relatively high. This problem did not present itself when water temperatures were low. Values for V_{max}, turnover time, $K_T + S_N$, and V_{max} specific activity index can be found in Appendix 4.

V_{max} followed a seasonal pattern (Figure 7). Values increased from spring to summer, then decreased throughout the fall, and remained low in winter. This pattern coincided with the rise and fall in temperature over the sampling period. V_{max} varied from 1.7 to 104.0 ng glutamate $l^{-1}h^{-1}$ at 0.5 m. At 10 m, 20 m, and 40 m, the ranges were 1.3 to 121.0, 1.6 to 59.0, and 2.1 to 40.6 ng glutamate $l^{-1}h^{-1}$, respectively. Except for a decrease in V_{max} as depth increased during summer and early fall, no consistent relationship between V_{max} and depth could be established.

V_{max} specific activity index, or V_{max}/cell, was generally higher during the summer months than at other times of the year (Figure 8). On the average, the highest index occurred in July. This preceded the maximum temperature which occurred in August. In fact, V_{max}/cell was considerably lower in August as compared to July. This supports the previous observation that the bacteria may have been in their most active state physiologically before the maximum temperature of the ocean was reached. With the exception of December, the winter, spring, early summer and fall, samples exhibited low V_{max} specific activity indices. No vertical pattern was observed. The seasonal range of V_{max}/cell was .024 to .378 fg glutamate h^{-1} $cell^{-1}$ at 0.5 m, .007 to 1.235 fg glutamate

Figure 7 Relationship between seasonal temperature and V_{max}. The V_{max} of the microbial population is shown for 4 depths. The seasonal temperature profile is shown for the 10 m depth only.



V_{max} at 0.5 m.

V_{max} at 10 m

V_{max} at 20 m

V_{max} at 40 m

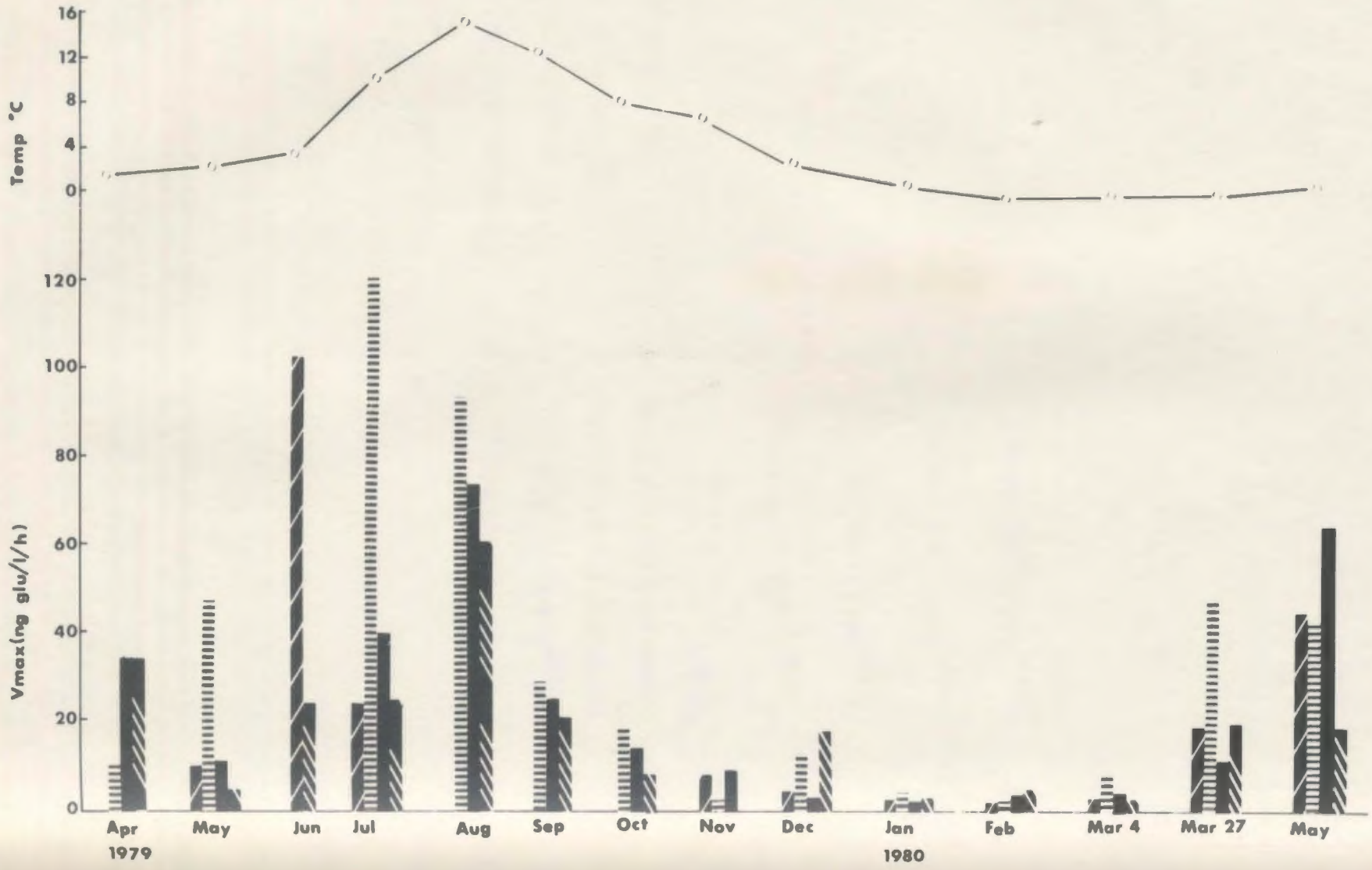
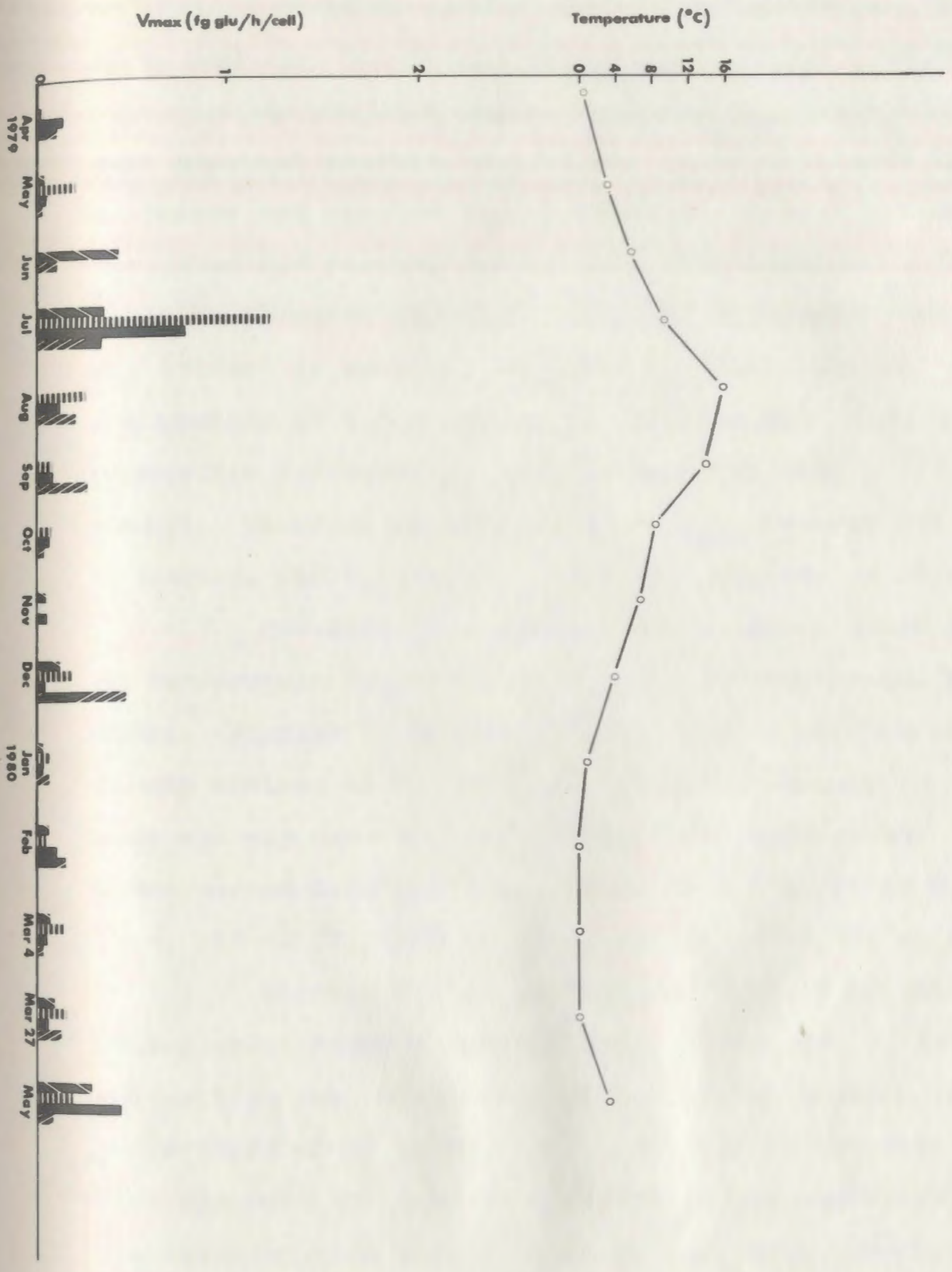

Temp °C
16
12
8
4
0
Vmax(ng glu/l/h)
120
100
80
60
40
20
0
Apr
1979
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
Jan
1980
Feb
Mar 4
Mar 27
May

Figure 8 Relationship between seasonal temperature and V_{max}/cell. The V_{max}/cell of the microbial population is shown for 4 depths. The seasonal temperature profile is shown for the 10 m depth only.


Vmax (fg glu/h/cell)
Temperature (°C)
0
1
2
0
4
8
12
16
Apr 1979
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
Jan 1980
Feb
Mar 4
Mar 27
May

h^{-1} $cell^{-1}$ at 10 m, .042 to .606 fg glutamate h^{-1} $cell^{-1}$ at 20 m, and .017 to .668 fg glutamate h^{-1} $cell^{-1}$ at 40 m.

At the 10 m depth, V_{max} and V_{max}/cell followed the same seasonal pattern (Figure 9). Both increased from spring to summer and reached their maximum in July. The values then decreased and reached their minimum in November. They rose again in December and then decreased once more. An increase was evident in spring. V_{max} and V_{max}/cell closely paralleled one another at 0.5 m, as well. At 20 m and 40 m, the two parameters followed the same pattern for most of the sampling period. However, at these depths, V_{max} reached its maximum in August, and V_{max}/cell reached its maximum in July.

Turnover time exhibited a seasonal trend (Figure 10). As temperature increased, turnover time decreased, and vice versa. Samples from April, 1979 did not fit this pattern. Stormy weather at the time of sampling had created very rough seas and may have altered hydrographic conditions. Turnover times varied from 285 to 13,074 h at 0.5 m, 66 to 8,068 h at 10 m, 185 to 12,704 h at 20 m, and 273 to 3,976 at 40 m.

Figures 11 and 12 show the effects of thermocline on bacterial numbers and activity. On August 7, 1979, the thermocline was established at a depth of 15-18 m (Figure 11). While total (AODC method) and total viable heterotrophic bacteria were not greatly affected by the thermocline, V_{max} and turnover time were. At 10 m, the water temperature was 15.5°C. V_{max} and turnover time were 93.6 ng glutamate $l^{-1}h^{-1}$ and 66 h, respectively. At 20 m (below the thermocline),

Figure 9 Relationship between seasonal temperature, V_{max} and V_{max}/cell at the 10 m sampling depth.

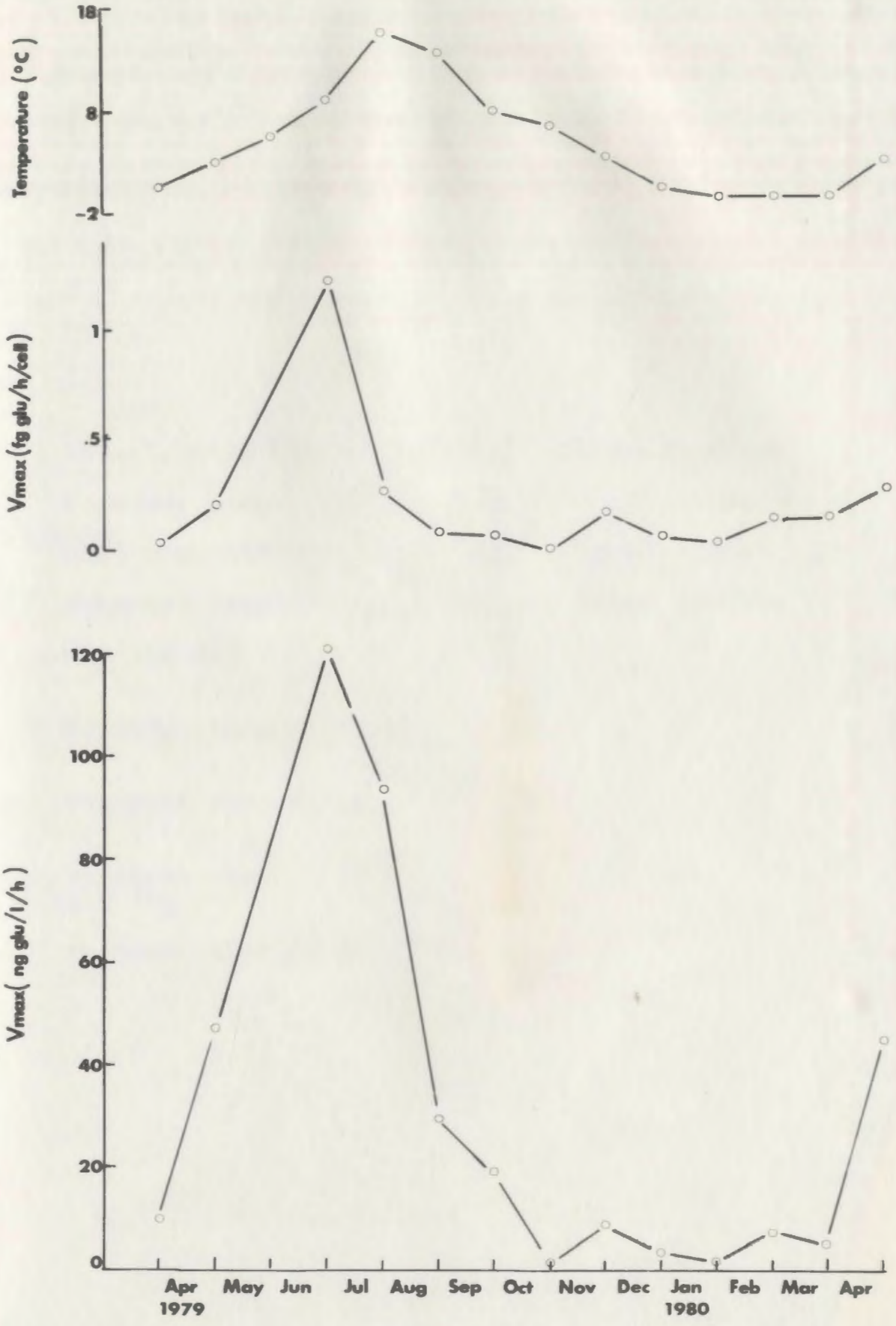
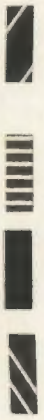

Temperature (°C)
18
8
-2
Vmax (fg glu/h/cell)
1
.5
0
Vmax(ng glu/l/h)
120
100
80
60
40
20
0
Apr
1979
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
Jan
1980
Feb
Mar
Apr

Figure 10 Relationship between seasonal temperature and turnover time. The turnover time of the microbial population is shown for 4 depths. The seasonal temperature profile is shown for the 10 m depth only.



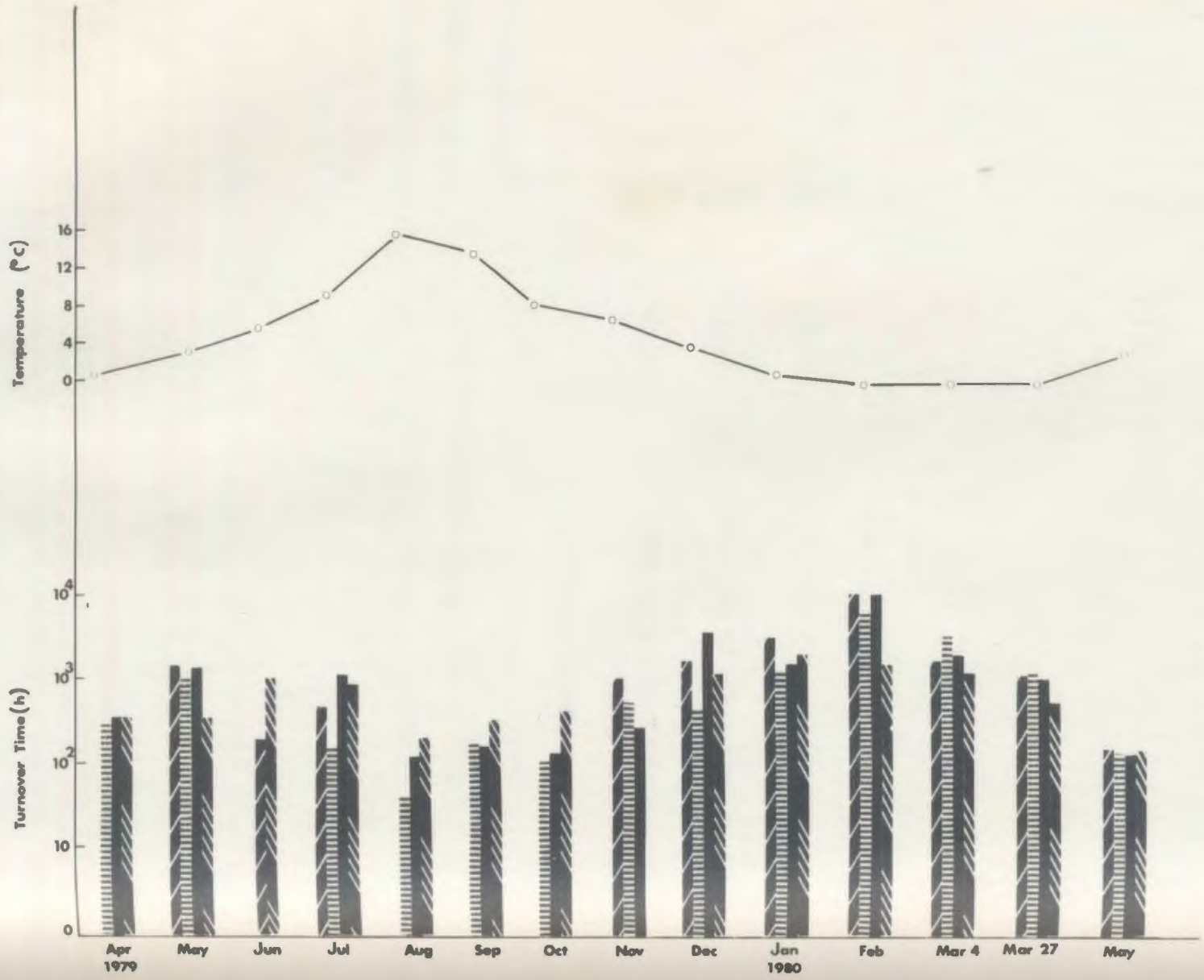

Temperature (°c)
16
12
8
4
0
Turnover Time (h)
10^4
10^3
10^2
10
0
Apr 1979
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
Jan 1980
Feb
Mar 4
Mar 27
May

Figure 11 Effect of the thermocline on bacterial numbers, V_{max} and turnover time (August 7, 1979). The thermocline (- - - -) was between 10 and 20 m on that date.

Bacterial numbers AODC method

Bacterial numbers SPC method

Bacterial numbers MPN method

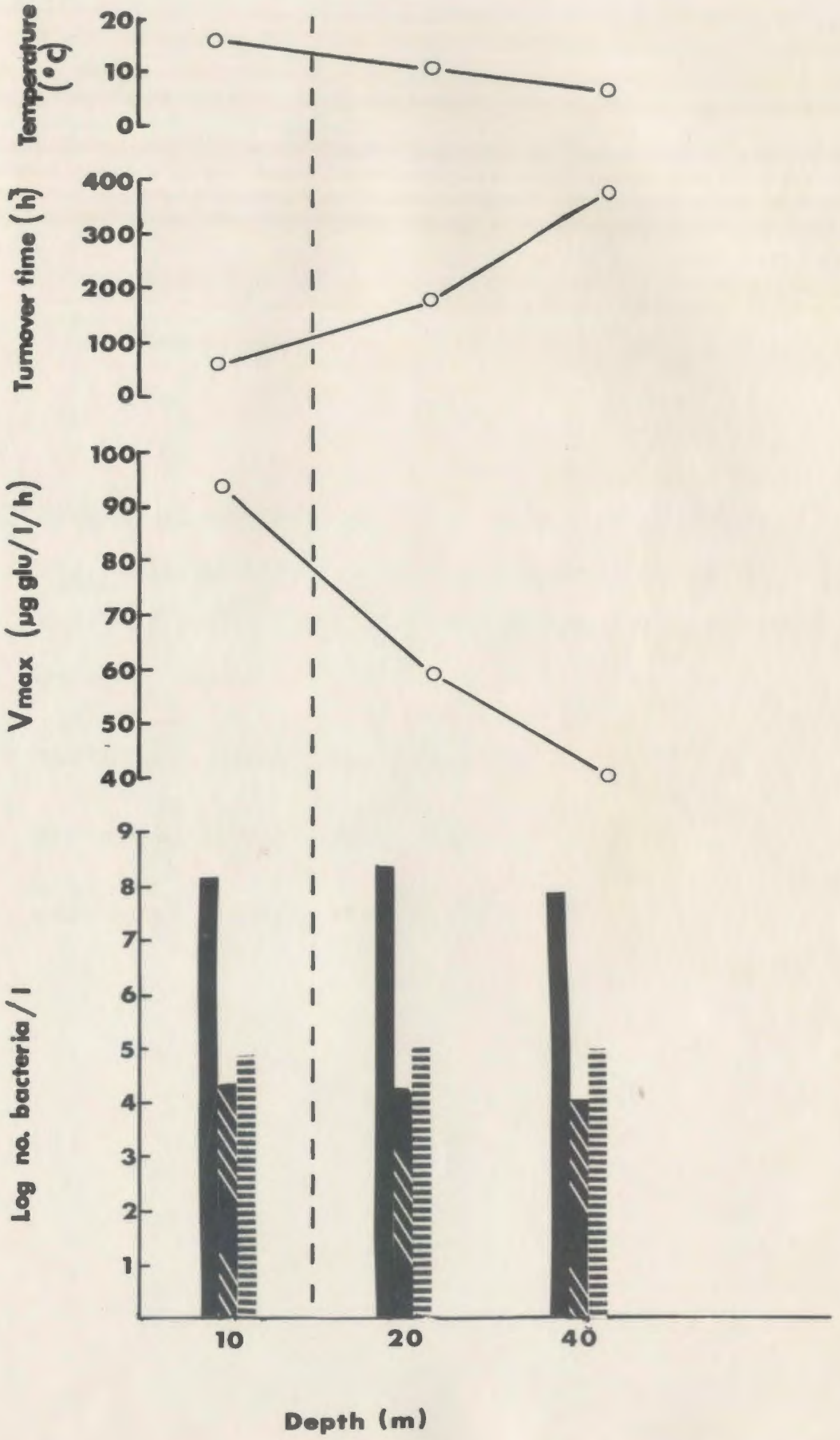
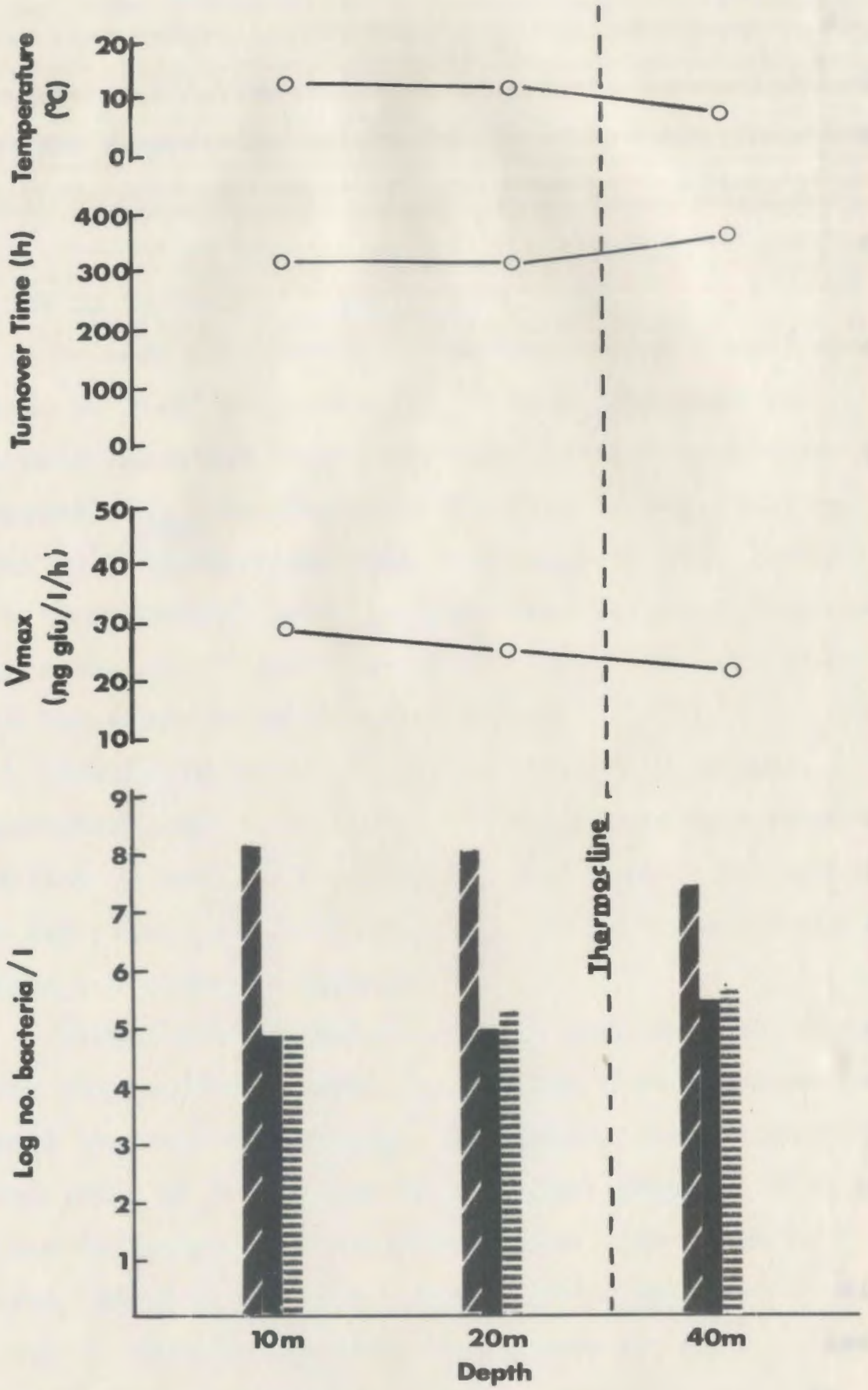

Temperature (°C)
20
10
0
Turnover time (h)
400
300
200
100
0
Vmax (µg glu/l/h)
100
90
80
70
60
50
40
Log no. bacteria/l
9
8
7
6
5
4
3
2
1
10
20
40
Depth (m)

Figure 12 Effect of the thermocline on bacterial numbers, V_{max} and turnover time (September 11, 1979). The thermocline (- - - -) was between 20 and 40 m on that date.

Bacterial numbers AODC method 

Bacterial numbers SPC method 

Bacterial numbers MPN method 


Temperature (°C)
20
10
0
Turnover Time (h)
400
300
200
100
0
Vmax (ng glu/l/h)
50
40
30
20
10
Log no. bacteria / l
9
8
7
6
5
4
3
2
1
Thermocline
10m
20m
40m
Depth

the water temperature was 10.0°C, V_{max} had decreased to 59.0 ng glutamate $l^{-1}h^{-1}$, and turnover time had increased to 185h. At 40 m, the temperature was 6.0°C, V_{max} was even lower and turnover time was twice that at 20 m. Thus, heterotrophic potential was not as high in the colder water below the thermocline as in the warmer water above.

On Sept. 11, 1979, the thermocline was established at a depth of 27-30 m (Figure 12). The differences in temperatures above and below the thermocline were not as great as in August. V_{max} decreased by 30% from 29.9 to 21.0 ng glutamate $l^{-1}h^{-1}$. Turnover time increased by 80%. Total bacteria (AODC method) were somewhat less below the thermocline as compared to above but total viable heterotrophic bacteria increased below the thermocline.

Figure 13 shows the concentrations of carbon, ortho-phosphate, and silicate in the 10 m samples throughout the sampling period. Nitrate levels are shown from September, 1979 to May 1980. The concentrations of these parameters at all depths are given in Appendix 5.

Carbon concentrations at 10 m rose and fell markedly during the April-September period, and then remained low throughout the fall and winter. Generally, carbon concentrations were lower at 0.5 m than at the other depths. They remained low throughout the sampling period with no peaks occurring. At 20 m and 40 m, carbon levels were relatively low except in August when high levels were present. These high concentrations coincided with the period of highest

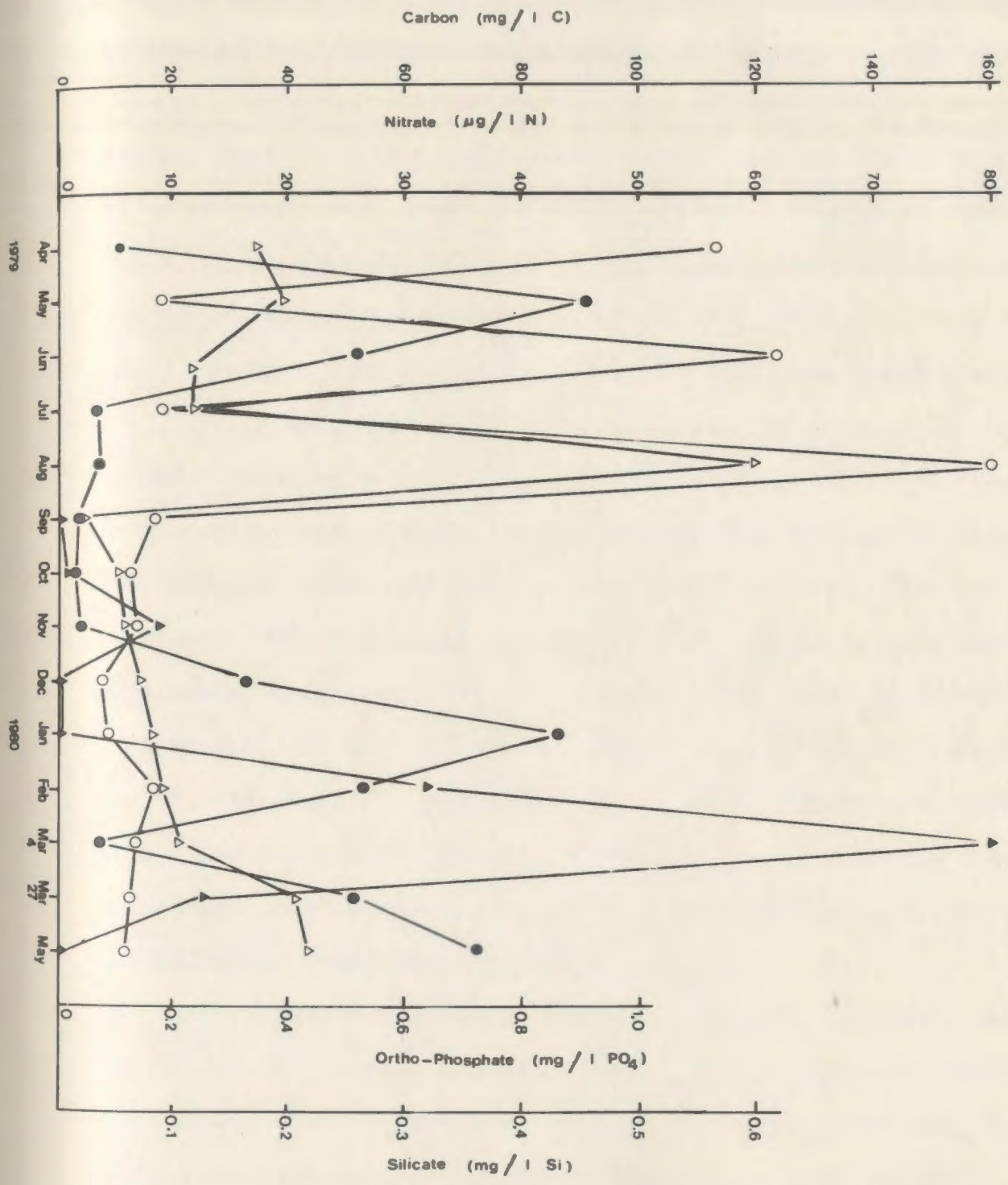
Figure 13 Relationship between amounts of carbon, nitrate, ortho-phosphate and silicate during the sampling period. Results shown are for the 10 m depth.

carbon o—c

nitrate ▲—▲

ortho-phosphate ●—●

silicate △—△


Carbon (mg / l C)
0
20
40
60
80
100
120
140
160
Nitrate (µg / l N)
0
10
20
30
40
50
60
70
80
1979
Apr
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
1980
Jan
Feb
Mar 4
Mar 27
May
0
0.2
0.4
0.6
0.8
1.0
Ortho-Phosphate (mg / l PO_4)
0.1
0.2
0.3
0.4
0.5
0.6
Silicate (mg / l Si)

heterotrophic potential. There was a noticeable bloom of phytoplankton at this time and these organisms may have been responsible for the high carbon levels.

At 10 m, phosphate levels were relatively low during summer and fall but were much higher in winter and spring. This pattern was also observed at the other sampling depths. Thus, high concentrations of phosphate were present during periods of low heterotrophic activity, and low concentrations were present during periods of high heterotrophic activity.

Silicate levels were generally higher in spring and summer than at other times of the year. At 10 m, the highest silicate concentration occurred in August and the lowest in September. Concentrations increased through the fall and winter. This pattern was also observed at 0.5 m and 20 m. The same took place at 40 m except that the April silicate concentration was slightly higher than that of August. Thus, at all depths, a high silicate concentration was associated with the period of highest heterotrophic activity (August) although no relationship between the two parameters was established over the sampling period.

Nitrate concentrations were low in fall and increased in winter. The highest levels were recorded March 4, 1980 for 10 m, 20 m, and 40 m, and in January, 1980 for 0.5 m. The overall trend appeared to be that lower nitrate concentrations were associated with higher heterotrophic potential, and vice versa. This was not the case for samples taken from 0.5 m. However, with no nitrate data available for the period

from April to August, 1979, it would be impossible to draw any firm conclusions.

Figure 14 represents a summary of much of the data given previously for the 10 m depth. Total bacteria (AODC) followed no consistent seasonal pattern but were low in numbers during the winter. Plate counts and MPN's also failed to show a consistent pattern over the course of sampling. However, plate counts gave higher estimates of viable bacteria during the summer, and the MPN method gave higher estimates in the fall.

V_{max} and turnover time followed seasonal patterns. The former increased and decreased with temperature, and the latter did the opposite.

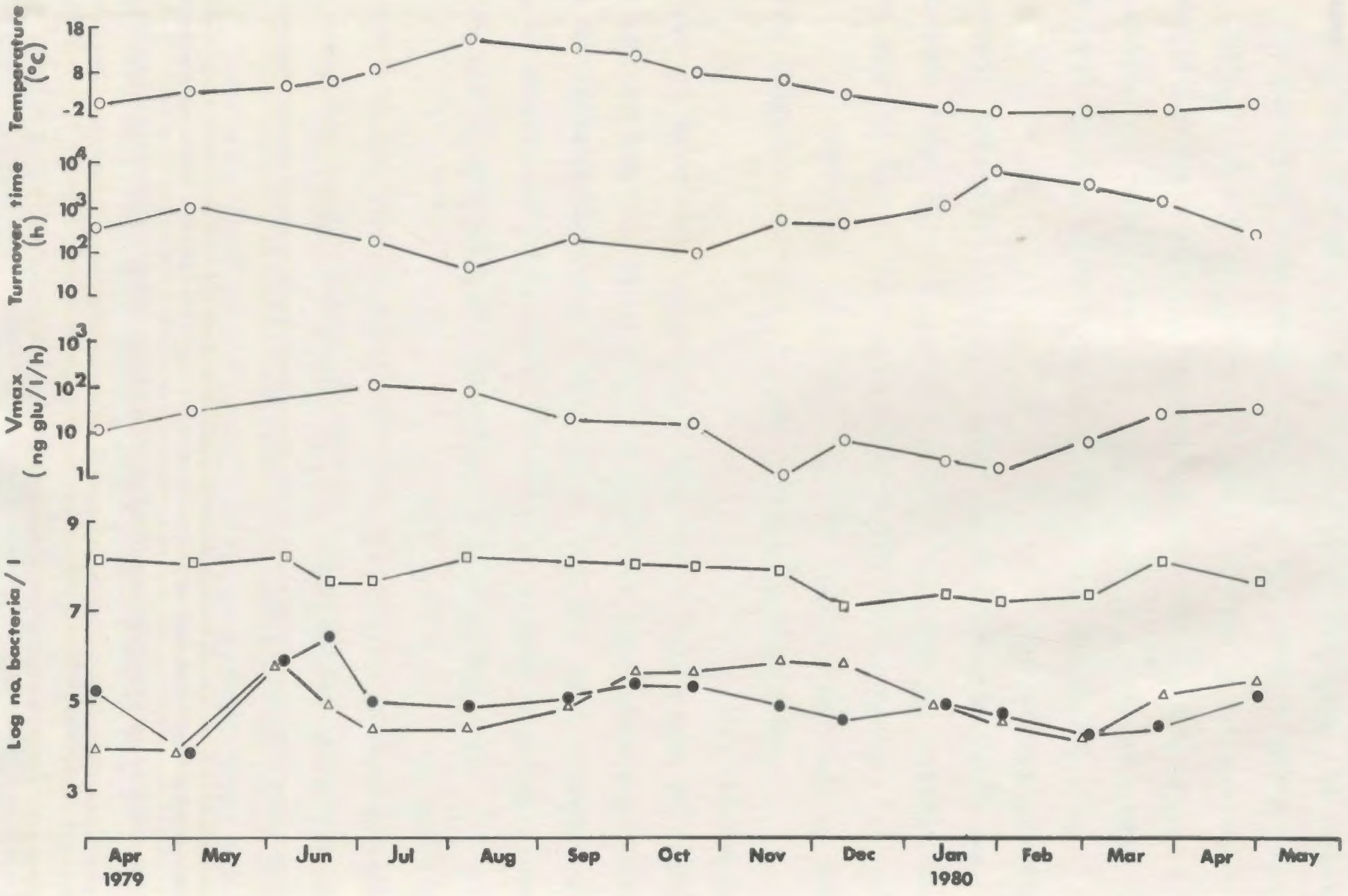
For the factors shown in figure 14, similar patterns to those at 10 m were observed at the 0.5, 20 and 40 m.

Figure 14 Relationship, by season, between temperature, turnover time, V_{max} and bacterial numbers at the 10 m depth.

Bacterial numbers AODC method □—□

Bacterial numbers SPC method ●—●

Bacterial numbers MPN method △—△


Temperature (°C)
18
8
-2
Turnover time (h)
10^4
10^3
10^2
10
Vmax (ng glu/l/h)
10^3
10^2
10
1
Log no. bacteria / l
9
7
5
3
Apr 1979
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
Jan 1980
Feb
Mar
Apr
May

Part B: Experiments That Were Not Related To Seasonal Changes.

On October 11, 1979, a water sample from the 10 m depth was collected at Station C. The following determinations were replicated twelve times; MPN, SPC, and AODC. Five replicates of the heterotrophic potential were done. The standard deviations of V_{max}, turnover time, and the three enumeration methods were calculated and are presented in Table 1.

The standard deviation on the AODC was 9.66% of the mean. The MPN method gave higher estimates of viable bacterial numbers than did SPC. This was seen consistently in fall data. However, the standard deviation of the MPN method was 43.67% of the mean, while that of the SPC was only 12.04% of the mean.

The standard deviations of V_{max} and turnover time were 54.10% and 19.59%, respectively, of the mean.

On July 18, 1979, four stations, B, C, X, and Y, were sampled at the 10 m depth. These samples were analyzed in the usual manner and the four stations compared. Data are given in Figure 15.

The water was cooler at Station C (8.0^{o}C) than at the other stations (9.0-9.5^{o}C). The low number of bacteria at Station C may have been a result of this. Total bacteria at stations B, X, and Y, were 1.5×10^{8} to 1.6×10^{8} per liter, whereas only 7.0×10^{7} per liter were observed at Station C. This represents only 45% of the average number

Table 1. Standard deviations of SPC, MPN, AODC, V_{max}, and turnover time.

Replicate	Bacteria per liter			V_{max} (ng glu $l^{-1}h^{-1}$)	T_t (h)
	SPC	MPN	AODC x 10^8		
1	197,000	79,000	1.51	18	400.7
2	144,000	230,000	1.51	24	378.5
3	142,000	460,000	1.51	7	487.1
4	168,000	330,000	1.49	35	635.6
5	153,000	330,000	1.27	10	436.4
6	177,000	230,000	1.21		
7	147,000	330,000	1.63		
8	173,000	130,000	1.47		
9	152,000	490,000	1.62		
10	203,000	170,000	1.61		
11	158,000	330,000	1.25		
12	157,000	490,000			
Total	1,971,000	3,599,000	16.08	94	2338.30
Mean	164,250	299,920	1.46	18.80	467.66
Variance	391,230	17,153,410	1.98×10^{14}	101.36	8398.4
Standard de-					

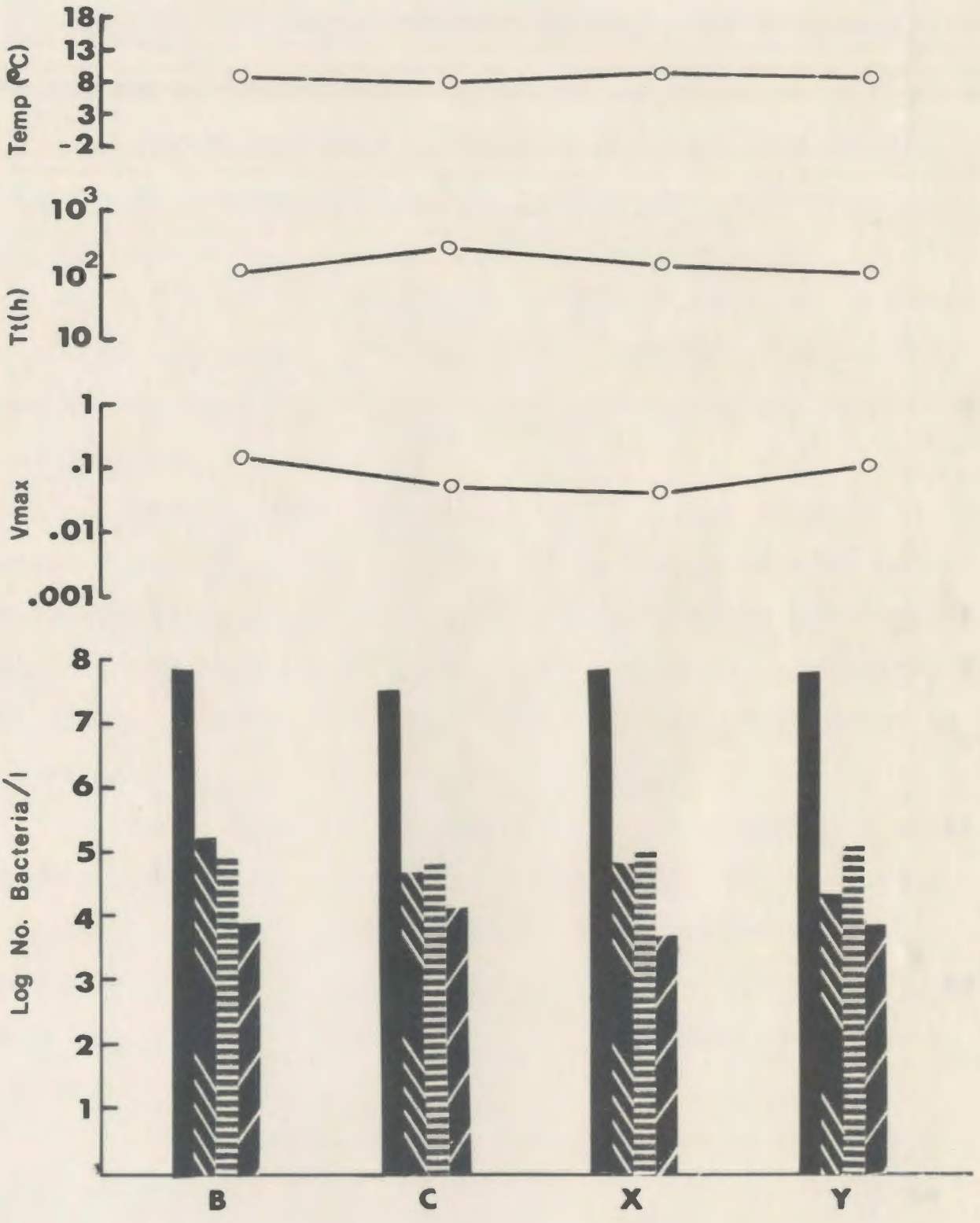
Figure 15 A comparison of bacterial numbers, V_{max} and turnover time (T_t) at 4 different stations (B, C, X and Y). All samples were collected at a depth of 10 m on July 18, 1980.

Bacterial numbers AODC method

Bacterial numbers SPC method

Bacterial numbers MPN method

No. of oleoclastic bacteria


Temp (°C)
18
13
8
3
-2
Tt(h)
10³
10²
10
Vmax
1
.1
.01
.001
Log No. Bacteria/l
8
7
6
5
4
3
2
1
B
C
X
Y

of total bacteria at the other stations. Total bacteria at stations B, X, and Y fall within one standard deviation (as shown in Table 1) of each other, while Station C lies outside the range.

Viable bacteria as determined by the MPN method, exhibit a wide range of numbers. The lowest estimate, 2.3×10^4 per liter, was observed at Station Y, and the highest, 1.7×10^5 per liter was seen at Station B. A comparison of the values at all stations revealed that only those at stations C and X were within one standard deviation of each other.

Plate counts gave lower results than MPN's at Station B but the opposite occurred at the other stations. The lowest plate count (6.5×10^4 per liter) was recorded at Station C and the highest (1.2×10^5 per liter) at Station Y. None of the plate counts were within one standard deviation of another.

In contrast to the relatively low numbers of total and viable bacteria at Station C, the number of oleoclasts per liter was higher at this station than at the others. While counts at B, X, and Y ranged from 4.9 to 7.9×10^3 per liter, 1.3×10^4 oleoclasts per liter were observed at Station C.

A wide range of V_{max} values was observed. V_{max} values at stations C and X were 83.2 and 76.0 ng glutamate $l^{-1}h^{-1}$, respectively, and were within one standard deviation of

each other. At stations B and Y, 265.1 and 226.3 ng glutamate $l^{-1}h^{-1}$, respectively, were recorded. These also were within one standard deviation of each other. V_{max} specific activity index was lowest at Station X (1.1 fg glutamate h^{-1} $cell^{-1}$) and ranged from 2.5 to 3.4 fg glutamate h^{-1} $cell^{-1}$ at the other stations. The longest turnover time, 495 h, was observed at Station C. At B, X, and Y, respective turnover times were 191 h, 256 h, and 137 h. None of the turnover times were within one standard deviation of another.

On November 6, 1979, water samples were collected from depths of 10 m and 100 m at Station Z. Table 2 contains data obtained from this sampling trip.

The water temperature dropped from 7.5°C at 10 m to 1.0°C at 100 m. Total and viable bacteria were lower at 100 m, as well. In the deeper sample, there were 25.6% fewer total bacteria. The MPN and SPC methods respectively yielded 53.5% and 39.3% fewer viable bacteria at the 100 m depth.

V_{max} and V_{max} specific activity index were higher at 10 m than at 100 m, However, turnover time was also higher at the 10 m depth.

MPN, SPC, and V_{max} values at the 100 m depth were typical of the values obtained at Station C in January 1980, when the water temperature was 0.5°C throughout the water column. However, V_{max}/cell and turnover time were lower in the deep sample than in the January samples, and total bacteria were higher. Total bacteria were also higher at

Table 2. Comparison of temperatures, bacterial numbers, V_{max}, V_{max}/cell, and turnover times at 10 m* and 100 m* depths.

Depth (m)	Temp (^{o}C)	Bacteria per liter AODC	MPN	SPC	Oleoclasts	V_{max} (ng glu $l^{-1}h^{-1}$)	V_{max}/cell (fg glu $h^{-1}cell^{-1}$)	T_t (h)
10	7.5	1.8×10^8	1.7×10^5	1.8×10^5	7.9×10^4	5.2	.029	1942
100	1.0	1.3×10^8	7.9×10^4	1.1×10^5	4.6×10^3	2.5	.019	1783

* Samples obtained at Station Z.

100 m than at 40 m in June, 1979, when the water temperature was 0.5°C.

On December 4, 1979, a 40 m water sample and a sediment sample were taken at Station S. Heterotrophic potential was obtained for the sediment by using a dilution of 1 g (wet) of sediment into 2000 ml sterile filtered seawater. Table 3 contains the data from this experiment. The concentrations of total bacteria and of oleoclasts were greater in the sediment sample than in the water sample. However, viable bacteria by the SPC method were less abundant in the sediment. This is reflected by the V_{max} specific activity indices which show that this parameter was lower in the sediment than in the 40 m sample. Turnover time and the lower plate count in the sediment sample may have resulted from a higher incidence of bacterial clumping in the sediment. V_{max} was greater in the sediment sample.

Table 3. Comparison of bacterial numbers, V_{max}, V_{max}/cell, and turnover times in a 40 m water sample and a sediment sample.

Sample	Number of Bacteria				T_t	V_{max} *	V_{max}/cell**
	AODC($\times 10^8$)	MPN($\times 10^5$)	SPC($\times 10^5$)	Oleoclasts($\times 10^5$)	(h)		
40 m	.59/l	1.80/l	.92/l	.07/l	381	27.8	.47
Sediment	21.26/g dry wt	2.61/g dry wt	.05/g dry wt	1.62/g dry wt	467	84.5	.040

* units for V_{max}: ng glu $l^{-1}h^{-1}$ for 40 m; ng glu/g dry wt/h for sediment

** units for V_{max}/cell: fg glu h^{-1} $cell^{-1}$.

Discussion

For this study, the standard plate count method and the most probable number method were used to enumerate viable bacteria. Total bacteria were enumerated by the acridine orange direct count method. All three enumeration techniques have some disadvantages which may distort the true picture of natural microbial populations.

Plate counts are influenced by many factors such that the end result is usually an underestimate of the natural population. The composition of the medium is one such factor. Buck (1974) compared plate counts of seawater samples using nine different media and reported a wide range of results. Highest counts were obtained on two media, one containing Gelysate and the other containing Trypticase. Buck (1974) compared these media by changing the concentrations of the two peptones. He found that high concentrations (5.0 and 10 g/l) resulted in low counts and low concentrations (0.1 and 1.0 g/l) gave high counts. Sieburth (1967) also found that the peptone content of a medium could be inhibitory. Vaatanen (1977) showed that a medium, which he designated medium V, gave higher counts for seawater samples than did ZoBell's medium 2216E (Oppenheimer and ZoBell, 1952). Simidu (1974) also found that medium composition affected counts.

The temperature at which plates are incubated will select for bacteria which can produce colonies at that temperature and will inhibit those bacteria which cannot. Sie-

burth (1967) counted a series of plates that were incubated at different temperatures and found that the incubation temperature influenced both the type and number of colonies formed.

The technique by which plates are inoculated can affect results as well. Traditional inoculating methods are the spread-plate and the pour-plate methods. The former method has been shown to produce higher counts than the latter (Buck and Cleverdon, 1960; Vaatanen, 1977). The northern marine environment has a low temperature. Pouring warm agar (45-50^{o}C) onto seawater inocula would probably kill any psychrophilic bacteria present. For spread-plating, Buck and Cleverdon (1960) found that spreading rods covered with a silicone solution (Desicote) yielded higher counts than did plain spreading rods. The technique used during this study (i.e., pipetting inoculum onto a spinning plate) provided well distributed colonies and was very rapid.

It is common for bacteria in seawater to occur in aggregates or clumps (ZoBell, 1946). However, unless a clump of bacteria is dispersed in some way, the clump, which is comprised of several bacteria, will give rise to only a single colony on an agar plate. Thus, when clumped bacteria are involved, the plate count will not give an accurate figure for the bacterial population in a sample. Jones and Jannasch (1959) found that treating sea water samples with surface active agents increased plate counts. These authors observed cells in large clumps by direct microscopy. Such clumps

were not observed in this study. Stevenson et al. (1974) attempted to disperse bacterial aggregates by shaking, blending, and adding Tween 80. For water samples, blending and blending with Tween 80 caused a decrease in plate counts and shaking had no effect. However, blending increased counts for sediment samples.

Some of the problems associated with the SPC method (i.e., medium composition and incubation temperature) are also drawbacks of the MPN method. In addition, the MPN method gives only a statistical estimate of the number of viable bacteria in a unit volume of sample. The results obtained in this study showed that the MPN method gave a wide range of values (7.9×10^4 to 4.9×10^5 per liter) for twelve subsamples of a single sample.

The MPN method of Mills et al, (1978) was used for the enumeration of oleoclasts. The main disadvantage of this method was that only some growth-positive tubes exhibited turbidity. Non-turbid tubes had to be assayed for protein before they could be designated negative or positive for growth.

Epifluorescence microscopy is a convenient method of determining total bacterial numbers because bacterial cells are easily distinguished from detrital particles. However, dead and dormant cells are not readily distinguished from actively metabolizing cells. According to Hobbie et al., (1977), cells of the latter type should fluoresce red-orange when stained with arcidine orange due to the interaction of

the dye with RNA, the predominant nucleic acid in active cells. DNA is more abundant in inactive cells and these cells fluoresce green. Delattre et al. (1979) considered green-fluorescing cells to be active as opposed to red-fluorescing cells. In this study, only green-fluorescing cells were observed. Zimmerman et al. (1978) developed a technique whereby total and respiring aquatic bacteria can be determined simultaneously. The dye 2-(p-iodophenyl)-3-(p-nitrophenyl)- 5-phenyl tetrazolium chloride (INT) is added to samples. Bacteria can reduce the dye to INT-formazan which accumulates as dark red spots in respiring cells. Total and viable bacteria can be determined by alternating epifluorescence and transmitted bright-field microscopy. Kogure et al. (1979) added 0.025% yeast extract and 0.002% nalidixic acid to seawater samples and incubated them for 6 h. They found that viable cells grew but did not divide under such conditions. Thus, viable cells appeared larger than non-viable cells when viewed by epifluorescence microscopy and a direct count of viable cells was possible.

Bacterial numbers in the waters of the northwestern Atlantic have not been studied widely. Even less work has been carried out in Newfoundland waters. A large proportion of the quantitative data available for the North Atlantic has been supplied by the Russian microbiologist Kriss. From samples taken along 30^{o}W and 40-70^{o}N, most yielded no bacteria or one bacterium from filtering 40 ml of sample (Kriss, 1963). Numbers increased in the subtropical zone and were highest in

the equatorial-tropical area. These findings, plus observations elsewhere in the world, led Kriss (1963) to suggest that heterotrophic bacteria decreased in number as latitude increased. However, Kriss incubated his filters at 18-35°C. These temperatures would not be suitable for growth of psychrophiles which may be the predominant type of bacteria in high latitude waters. Also, the incubation time was only 4-7 days which, likely, is insufficient for a high yield of colonies. Filters used by Kriss had a pore size of 0.5 µm. Many marine bacteria are small enough to pass through pores of this size (Anderson and Heffernan, 1965; Ferguson and Rublee, 1976; Hobbie et al., 1977; Zimmerman et al., 1978) and this factor may have been responsible for the low counts obtained by Kriss.

Later work by Kriss and colleagues (Kriss, 1970; Kriss et al., 1967) also indicated low numbers of viable heterotrophic bacteria in the Northern Atlantic. Sieburth (1971) recorded higher numbers for samples from the Caribbean and Atlantic. Seventy-three percent of his samples had less than five bacteria per ml and 27% had more than five. Sieburth's findings did not support Kriss's idea that bacterial numbers decreased as latitude increased (Kriss, 1963). Wright (1978) obtained an average plate count of 1.45×10^5 bacteria per liter for samples from the Gulf of Maine. Samples from the South Davis Strait yielded 1.66×10^3 to 4.57×10^6 bacteria per liter in spring and 2.51×10^4 to 4.37×10^6 per liter in summer. Samples from Woody Point and Outer Cove (both

in Newfoundland) contained 2.1×10^7 and 3.4×10^8 bacteria per liter, respectively, by an MPN method (Mulkins-Phillips and Stewart, 1974). In the latter study, MPN values were high because the authors recorded the highest possible MPN values. The results of Mulkins-Phillips and Stewart (1974), Wright (1978), and Bunch (1979) are considerably higher than those of Kriss (1963; 1970) and Kriss et al., (1967), and are in the same range as the results obtained in this study. A study by Noseworthy (1976) of the waters off Newfoundland's east coast showed that viable heterotrophic bacteria were not abundant in these waters. It was found that 78% of plates inoculated with seawater failed to produce even a single colony. Resorting to plankton tows, Noseworthy observed plate counts of 10 to 4.3×10^3 bacteria per liter of plankton tow. Such figures are considerably lower than those obtained in this study. The medium used by Noseworthy, a modification of ZoBell's Medium 2216 (ZoBell, 1941) called yeast-beef-peptone sea water agar (YBP-SWA), contained six times the concentration of peptone as the medium used in this study. Noseworthy's plate counts could have been reduced due to the high peptone content of the medium which is known to inhibit bacterial growth on agar plates (Sieburth, 1967; Buck, 1974). Also, the sampling site (Logy Bay, Newfoundland) was different and until more extensive studies are completed it will not be known if the bacterial numbers are the same in all regions of the Newfoundland coast.

ZoBell (1946) found that bacterial numbers increas-

ed over the first 40 or 50 m of depth and then decreased. An opposite pattern was observed by Jannasch and Jones (1959). Others (Sieburth, 1971; Hobbie et al., 1972; Noseworthy, 1976) have detected no regular pattern of vertical distribution of marine bacteria. In this study, no distinct trend up to 40 m was observed.

ZoBell (1946) stated that although temperature can affect bacterial activity, it has little influence on the numbers of bacteria in the sea. Except for short-lived fluctuations in bacterial numbers associated with temperature, he reported little seasonal change in numbers in California surface waters where an annual temperature change of 8^{o}C was recorded. During a study of Akkeshi Bay, Japan, Ezura et al. (1974) observed maximum numbers of bacteria in summer and minimum numbers in winter. However, in two other sampling years, the maximum numbers occurred in winter. The data supplied by Zimmerman (1977) indicated that total bacteria in the Kiel Fjord area followed a seasonal distribution pattern with highest numbers occurring in spring and early summer. Hoppe (1978) observed that total bacterial numbers in the Kiel Bight stayed relatively constant over the year whereas active bacteria exhibited a distinct minimum in winter. In a study of the coastal waters of northern France, Delattre et al., (1979) found that total bacteria correlated significantly with temperature. Plate counts followed no seasonal pattern, however. Noseworthy (1976) observed that temperature and bacterial numbers were unrelated in waters

off eastern Newfoundland. The annual temperature changes recorded by Noseworthy (1976) were 5.0°C in bottom water, 12.8°C at mid-depth, and 15.0°C in surface waters. The current study showed that no apparent relationship existed between bacterial numbers and temperature in Newfoundland waters. Temperature changes were 17.0°C at 0.5 m, 16.0°C at 10 m, 14.0°C at 20 m, and 9.0°C at 40 m, but numbers of bacteria exhibited no pronounced seasonal trend at either depth except for a sharp increase in late spring and early summer.

Values for total bacteria in seawater are generally much higher than those of viable bacteria. From samples taken off the coast of North Carolina, Ferguson and Rublee (1976) observed 6.6×10^8 bacteria per liter. Watson et al. (1977) reported 3.6×10^9 bacteria per liter in seawater near Woods Hole, Massachusetts. Beaufort Sea samples yielded 1.5×10^8 bacteria per liter in winter and 4.5×10^8 and 3.7×10^8 per liter in two successive summers (Griffiths et al., 1978). Wright (1978) observed total bacteria ranging from 6.1×10^8 to 1.9×10^9 per liter in samples from the Gulf of Maine. Bunch (1979) reported a wider range of bacterial numbers than did most authors. Samples taken from the Davis Strait, contained 7.9×10^6 to 3.2×10^8 bacteria per liter in spring, and 1.8×10^7 to 1.2×10^9 per liter in summer. Delattre et al., (1979) also reported a wide range (1.5×10^8 to 5.2×10^{10} bacteria per liter) for coastal waters off France. In this study of Conception Bay total

bacteria were in the range of 10^7 to 10^8 per liter. No vertical pattern was observed but, seasonally, numbers were lower in winter.

Using a modified MPN procedure, Mulkins-Phillips and Stewart (1974) determined bacterial numbers in Atlantic sediments. From the top layer of sediment cores, MPN values of 1.0 - 7.7 x 10^4, 2.4 x 10^5 to 1.1 x 10^8, and 2.2 x 10^5 to 3.5 x 10^6 bacteria per g wet weight were recorded for South Shore (Nova Scotia), Halifax, and Frobisher Bay, respectively. In sediments from the Beaufort Sea, 6.6 x 10^8 to 1.1 x 10^{10} total bacteria per g dry weight were observed in summer and 1.0 x 10^9 per g dry weight was observed in winter (Griffiths et al., 1978). In this study, the single sediment sample analyzed yielded 2.13 x 10^9 bacteria per g dry weight by AODC, 1.80 x 10^5 per g dry weight by MPN, and 9.2 x 10^4 per g dry weight by SPC. The high MPN values reported by Mulkins-Phillips and Stewart (1974) arise from the fact that these authors reported the maximum possible values.

The study of oleoclastic marine bacteria has become increasingly important with the recent increase in development of offshore oil resources. Colwell et al., (1977) enumerated oleoclasts in seawater along the eastern coast of the United States. From Miami, Florida to Cape Hatteras, North Carolina, oleoclasts ranged from 10 per liter to 9.0 x 10^4 per liter. In the Northwest Providence Channel, the minimum number recorded was 1 per liter and the maximum

was 2.0×10^4 per liter. Chesapeake Bay yielded 100 to 6.8×10^5 oleoclasts per liter. In Newfoundland waters, 9.52×10^6 and 8.19×10^5 oleoclasts per liter were observed at Outer Cove and Woody Point, respectively (Mulkins-Phillips and Stewart, 1974). In sediments, Mulkins-Phillips and Stewart (1974) reported 8.0×10^2 to 8.7×10^5 oleoclasts per g wet weight from stations near Halifax, 1.1×10^3 to 8.0×10^3 per g in Chedabucto Bay, and 1.4×10^2 to 1.2×10^3 per g in Frobisher Bay. In the current study of Conception Bay it has been shown that oleoclastic bacteria are present in numbers ranging from 8.0×10^2 to 1.6×10^6 per liter of seawater. Oleoclastic bacteria were present at a level 7.0×10^3 per g dry weight in the sediment.

Plate counts and MPN results indicate that most of the bacteria seen by direct microscopy are not viable. Jannasch and Jones (1959) reported that plate counts represent only 1/3 to 1/10,000 of the total bacteria present in a sample. Wright (1978) obtained plate counts that were less than 1% of the total bacteria. Delattre et al (1979) found that on the average, 2.3% of total bacteria were colony-forming units. Bunch (1979) also found that plate counts were considerably lower than total counts. In this study I have reported that 0.003-3.370% of the total bacteria formed colonies on agar plates. MPN values showed that 0.003-1.750% of the total bacteria were able to grow in broth culture.

For reasons discussed earlier, the SPC and MPN methods cannot give an accurate estimate of the actual

population of viable heterotrophs. Other techniques have shown that higher percentages of the total population are viable. The method of Zimmerman et al. (1978), wherein INT is added to samples, showed that 6 to 12% of bacteria in the Baltic Sea were respiring cells. The method of Kogure et al. (1979) revealed that viable bacteria were one order of magnitude lower than total bacteria but three orders of magnitude higher than colony-forming bacteria. Using autoradiography, Hoppe (1978) found that, in samples taken from the western Baltic in summer, up to 56 % of the total bacteria were metabolically active, but only 0.01% of active cells produced colonies. In winter, an average of 20% exhibited activity. Of these, up to 12.5% produced colonies.

The use of ^{14}C-labeled substrates in the measurement of heterotrophic activity involves several basic assumptions. These have been reviewed by Wright (1973). The kinetic model assumes that substrate uptake by bacteria exhibits saturation kinetics. When bacteria are exposed to increasing substrate concentrations, an uptake rate is revealed which will not increase with subsequent increases in substrate concentrations. A second assumption is that all users of the substrate respond identically to variations in the substrate concentration. Thirdly, one must assume that the number of bacteria does not change during the course of the experiment. The kinetic model also assumes an experimental medium in which the concentrations of all solutes is known. This is not the case for natural waters and the amount of

interference of substrate uptake by similar molecules is hard to determine. A fifth assumption is that during an experiment, the substrate concentration does not change significantly. Most researchers discard data when a concentration change of greater than 5% is encountered (Thompson and Hamilton, 1974) although Delattre et al. (1979)accepted a change of up to 10%. The final assumption associated with the kinetic model is that substrate transport occurs in one direction only (i.e., into the cell). Transport out of the cell is related to pooling of substrate in bacterial cells. In this situation, substrate is taken up but is not used or incorporated by the cells.

The problems associated with the heterotrophic potential method of Wright and Hobbie (1966) have been reviewed by Wright (1973) and Thompson and Hamilton (1974). The concentrations of individual organic compounds in seawater are very low, generally less than 10 µg per liter (Wright, 1973). Glutamic acid in an estuary varied from 0.71 to 3.60 µg glutamate l^{-1} over a one-year period and concentrations of other amino acids were similar to this (Crawford et al., 1974). The amount of labeled substrate added to a sample should not be sufficient to radiobiologically damage cells, and it should not create a situation that differs greatly from the natural environment.

The fixation technique which ends the incubation period is known to affect the counts per minute (cpm) obtained by liquid scintillation counting. Wright and Hobbie (1965)

found that Lugol's acetic acid was a better fixative than formalin which was used by Parsons and Strickland (1962) in that higher counts per minute were obtained with the former. Wright (1970) found that rapid filtration at the end of incubation gave better results than a fixative. This method was applied in the current study. Samples in respiration bottles were fixed with 50% phosphoric acid which also promoted recovery of $^{14}CO_2$. Acidifying a sample results in conversion of inorganic carbon to gaseous form.

Incubation time and incubation temperature are important factors to be considered. The latter has been shown to affect substrate uptake (Wright, 1970). Incubation time should not be so long as to allow bacteria to take up enough substrate to significantly change the substrate concentration. Conversely, it should not be so short that an insufficient amount of substrate is taken up. Ideally, the incubation time should be just long enough to get measurable radioactivity at the lowest substrate concentration used (Wright, 1973). In this study, some samples yielded no heterotrophic potential data because samples were incubated for too long and consequently, greater than 5% of the added glutamic acid was taken up. Such a situation occurred on June 22, 1978.

Several authors (Vaccaro and Jannasch, 1967; Vaccaro, 1969; Hamilton and Preslan, 1970) have reported a high incidence of failure when using the heterotrophic uptake method. Problems arose from either uptake levels being too low or from a high degree of variability in uptake from

one substrate concentration to the next. In this study, the latter situation made it impossible to obtain 0.5 m results in August, September, and October, 1979, and 40 m results in November, 1979.

Heterotrophic potential in the marine environment varies from one region of the world to the next. A portion of the variation may be due to modifications of the technique made by different researchers, but environmental factors also contribute to observed differences. With ^{14}C-glucose, V_{max} of 8.3 - 50 ng $l^{-1}h^{-1}$ and turnover times of 420 to 4300 h were recorded in the Pacific (Takahashi and Ichimura, 1971). V_{max} and turnover time in Antarctic waters were 9.0 ng glucose $l^{-1}h^{-1}$ and 1,090 h, respectively (Gillespie et al, 1976). Gocke (1977) observed V_{max} of 10 to 137.5 ng glucose $l^{-1}h^{-1}$ and turnover times of 10 to 395 h in the Baltic Sea. Values from stations in the Newport River estuary were 1.4 to 19.3 ng glucose $l^{-1}h^{-1}$ and 25 to 94 h respectively (Palumbo and Ferguson, 1978). V_{max} in coastal waters off northern France varied from 1.5 to 1,040 ng glucose $l^{-1}h^{-1}$ (Delattre et al., 1979).

Values for glutamic acid also show a wide range of regional variation. In the eastern tropical Pacific, Hamilton and Preslan (1970) observed a V_{max} of 15.2 ng glutamate $l^{-1}h^{-1}$. High V_{max} values (6.10×10^2 to 1.90×10^4 ng glutamate $l^{-1}h^{-1}$) were found in estuarine waters (Crawford et al., 1974). Turnover times were correspondingly low (0.64 to 43.40 h). Williams et al. (1976) recorded a V_{max} of 0.08 to

20.5 ng glutamate $l^{-1}h^{-1}$ in waters off California. In the Antarctic, V_{max} of 11.2 ng glutamate $l^{-1}h^{-1}$ was observed (Gillespie et al., 1976). Winter water samples from the Beaufort Sea exhibited an average V_{max} of 3.1 ng glutamate $l^{-1}h^{-1}$ and sediment samples showed a V_{max} of 0.06 ng glutamate per g dry weight h^{-1} (Griffiths et al., 1978). In two successive summers, V_{max} values of 44 and 21 ng glutamate $l^{-1}h^{-1}$ were observed for water samples, and 0.61 and 0.83 ng glutamate $l^{-1}h^{-1}$ for sediments from the Beaufort Sea (Griffiths et al., 1978). Bunch (1979), from studies conducted in the Davis Strait, reported average V_{max} values of 300 and 95.8 ng glutamate $l^{-1}h^{-1}$ in April and August, respectively. During the present study, V_{max} values which ranged from 1.3 to 121 ng glutamate $l^{-1}h^{-1}$ at Station C, and turnover times from 66 h to 13,074 h were recorded. V_{max} in the sediment sample was 84.5 ng glutamate per g dry weight h^{-1}.

The results of Takahashi and Ichimura (1971) indicated that V_{max} for glucose decreased with depth. For glutamic acid, Williams et al. (1976) obtained higher V_{max} values in 25 m samples than in 100 m samples. On two sampling days, these authors found that V_{max} was 45 and 23 times higher in the 25 m samples. The data of Gocke (1977) did not correspond with these findings. For glucose, he found little vertical difference in V_{max} except at a highly eutrophic station. However, the depths sampled by Gocke (1977) were not as deep as those sampled by Takahashi and

Ichimura (1971) and Williams et al. (1976), nor was there as great a difference between the depths. Bunch (1979) obtained V_{max} and turnover times for glutamic acid at depths ranging from 1 m to 200 m at various stations in the South Davis Strait. He found that heterotrophic activity was low at depths greater than 50 m. However, his figures do not indicate a regular pattern of V_{max} with depth. Data from the four depths sampled at Station C (0.5 m to 40 m) showed a relationship between depth and heterotrophic potential only when a strong thermocline had been established. At Station Z, V_{max} at 10 m was more than twice that at 100 m.

Heterotrophic activity in marine waters often exhibits a seasonal pattern. In an estuarine environment, a one-year study revealed that uptake of glutamic acid did not follow a regular seasonal pattern (Crawford et al., 1974). In the Baltic Sea, Gocke (1977) observed that the maximum V_{max} for glucose coincided with the highest water temperature (August) and that V_{max} and temperature decreased together throughout the fall and winter. However, a spring peak for V_{max} when water temperatures were low indicated that temperature was not the only factor affecting V_{max}. On the other hand, Hoppe (1978), also working in the Baltic, concluded that water temperature was the main factor affecting bacterial activity. Griffiths et al. (1978) found that microbial activity in both waters and sediments of the Beaufort Sea was lower in winter than in summer. In the Davis Strait, Bunch (1979) observed a higher V_{max} for glutamic acid in

April than in August. Bacterial numbers were higher in August but a phytoplankton bloom in April may have contributed to the high V_{max} at that time. A seasonal study by Delattre et al. (1979) indicated that V_{max} for glucose correlated closely with temperature. At all four depths examined in the current study, V_{max} and turnover time followed a seasonal pattern. The former increased and decreased with temperature but was slightly out of phase, while the latter was inversely related to temperature. The highest V_{max} observed from 0.5 m and 10 m samples occurred in June and July, respectively. This preceded the maximum temperature attained at these depths. Similarly, V_{max} values at 20 m and 40 m were highest in August although the maximum temperatures at these depths did not occur until September. Thus, at each depth, the period of highest microbial activity occurred prior to the maximum temperature at that depth. V_{max} values at 0.5 m and 10 m were highest when the water temperatures at those depths was 9.0°C. At 20 m, the maximum V_{max} occurred when the temperature was 10.0°C. V_{max} at 40 m was highest when the temperature was 6.0°C. However, the temperature at this depth never rose beyond 8.0°C. It may be that the bacterial population has an optimum temperature for microbial activity at 9 to 10°C. Either that or the cells may go through a short period during June and July in which the cells are rejuvenated and that this period does not last throughout the entire period that the body of water is warming up.

The specific activity indices of Wright (1978) have not been widely adopted yet. However, many authors have included total bacterial counts and V_{max} values in their tables and thus it is possible to convert the data to V_{max}/cell. In the Gulf of Maine, V_{max}/cell for glucose ranged from 0.003 to 0.028 fg h^{-1} $cell^{-1}$ (Wright, 1978). V_{max}/cell values of 4.6×10^{-4} and 3.6×10^{-3} fg glucose $h^{-1}cell^{-1}$ were reported at two stations in the Newport River estuary (Palumbo and Ferguson, 1978). These low values probably would have been even lower had the authors used 0.2 µm filters rather than 0.45 µm filters for direct counting. Conversion of the average V_{max} values and average total bacterial counts of Griffiths et al. (1978) into V_{max}/cell values, revealed ranges in the vicinities of 0.021 - 0.098 fg glutamate h^{-1} $cell^{-1}$ for water samples, and 0.060 - 0.924 fg glutamate h^{-1} $cell^{-1}$ for sediments. Conversion of data from the 20 m depth from the South Davis Strait (Bunch, 1979) yielded values of 0.205 - 1.27 fg glutamate h^{-1} $cell^{-1}$ in spring, and 0.129 - 0.708 fg glutamate h^{-1} $cell^{-1}$ in summer. V_{max}/cell in this study ranged from 0.017 to 1.24 fg glutamate h^{-1} $cell^{-1}$. The maximum V_{max}/cell for 40 m was observed in December whereas the maximum at the other depths occurred in July. No vertical pattern was established during the course of the study. Seasonally, V_{max}/cell was low in the fall and winter and highest in the summer. As was the case with V_{max}, V_{max}/cell values were highest before the maximum water temperature occurred. V_{max}/cell at 40 m was also higher in July than it

was in September when the maximum water temperature at that depth occurred.

Stevenson (1978) hypothesized that the majority of bacteria in aquatic environments exist in a dormant state. A wide range of factors affect bacterial populations in the sea. These include such things as fluctuations in temperature, salinity and nutrients. In response to these fluctuations, bacteria can either die, adjust to the change, or become dormant (Stevenson, 1978). If some factor changes drastically enought to inhibit bacterial growth, dormancy provides a means of survival for bacteria until favorable conditions reoccur (Lamanna and Malette, 1965).

The literature contains evidence that supports Stevenson's hypothesis. Vaccaro and Jannasch (1966) failed to obtain a good kinetic response in seawater samples but the bacterial population responded quickly to enrichment with glucose and a good kinetic response was obtained within twelve hours. Wright (1973) stated that the response was too rapid to be attributed to bacterial multiplication. He interpreted the results as indicating that a portion of the population which had been dormant had become active. Jannasch (1967) showed that cultures of marine bacteria under conditions of limiting substrate concentration would not divide but would survive in an inactive state. Sieburth (1967) found that on the basis of seasonal temperature, different physiological types dominated at different times of the year. Williams and Gray (1970) examined amino acid uptake by marine

bacteria. An increase in uptake occurred immediately after substrate addition. Presumably, this was due to the active portion of the population. A second increase in uptake occurred 20 to 35 h after substrate was added. This may have been the result of microbial growth or possibly due to the activation of a previously dormant portion of the bacterial population. Novitsky and Morita (1976) starved a culture of a psychrophilic marine vibrio and observed that cells decreased in size and changed in shape from rods to cocci. Electron microscopy of thin sections of these small cells revealed that normal cell structure was maintained. When inoculated onto fresh medium, the cells grew without lag, reverted back to their rod shape, and regained their normal size. Wright (1978) observed that natural marine bacteria exhibited a marked increase in V_{max} specific activity related to nutrient enrichment. Hoppe (1978) reported that although the numbers of total bacteria remained fairly constant throughout the year, up to 56% of these were active in summer, but as low as 10% were active in winter.

The data from this study supports the concept that a portion of the marine bacterial population exists in a physiologically inactive state. In June, a sharp increase in the number of viable bacteria was detected by the SPC method. Total bacteria did not increase in number at this time thus indicating that previously dormant cells had become active. Increases in V_{max}/cell during June and July also indicated that there was increasing activity in the

bacterial population. Oleoclasts were more abundant in September than at any other time of the year and since total numbers (AODC) had not increased at this time, it would appear that the portion of the total population that was capable of degrading hydrocarbons was larger at this time than during other times of the year.

Usually the MPN method of counting viable bacteria gives higher numbers than the SPC method does (A.P.H.A., 1976). During this study values by both methods were approximately equal on 9 out of the 14 times that samples were taken. There was a seasonal pattern to the differences that were observed. During June and July, 1979 the counts were greater by the SPC method and during October, November and December, 1979 the counts were greater by the MPN method. From the V_{max}/cell data it can be assumed that the bacteria were undergoing rejuvination during June and July and conversely that they were entering a period of dormancy during late fall and early winter. The plating (SPC) procedure apparently causes more stress to the cells than the MPN procedure does because counts by the latter method are generally higher as reported by the A.P.H.A. (1976). The cells may have been able to withstand the stress of plating more readily during the period of rejuvenation and this could account for the observed increase in total viable cells by the SPC method during June and July. This, in turn, further supports the concept of a period of dormancy and a period of rejuvenation. However, this conclusion should be substantiated by future

studies in which there are more frequent samplings during the periods in which the phenomena of rejuvenation and dormancy were observed in this study.

Summary of Results

1. Total bacterial numbers (AODC) were generally lower in winter than at other times of the year.
2. Total viable heterotrophic bacteria were higher by the standard plate count (SPC) method in June and July but were higher by the most probable number (MPN) method in October, November, and December. It is postulated that the bacterial population differed physiologically at the different times of year.
3. The percentage of total bacteria which were viable was 0.003-3.370% by the SPC method, and 0.003-1.750% by the MPN method.
4. Oleoclasts were more abundant during the May-September period than at other times of the year.
5. No consistency in the vertical pattern of distribution was observed for either total, viable, or oleoclastic bacteria.
6. Heterotrophic activity exhibited a seasonal pattern. V_{max} increased from spring to summer, decreased throughout the fall, and remained low in winter. Turnover time followed an opposite pattern.
7. Heterotrophic activity exhibited no vertical pattern over the first 40 m depth except on sampling days when a thermocline existed. On these days, activity was lower below the thermocline than above.
8. Evidence was obtained that the optimum temperature for

bacterial activity in Conception Bay was 9-10°C. At 0.5, 10 and 20 m, the highest V_{max} values were obtained when the water temperature was in this range.

9. Results obtained indicated that only a small proportion of the total bacterial population was active during most of the year. This supports Stevenson's hypothesis that the majority of aquatic bacteria exist in a dormant state.

REFERENCES

Alexander, M. 1965. Most-probable-number method for microbial populations. In Methods of soil analysis. Vol. II. Edited by C.A. Black, D.D. Evans, J.L. White, L.E. Ensminger, and F.E. Clark. Amer. Soc. of Agron., Madison. pp. 1467-1472.

American Public Health Association. 1976. Standard methods for the examination of water and wastewater, 14th edition. American Public Health Association, Inc., New York.

Anderson, J.I.W., and W.P. Hefferman. 1965. Isolation and characterization of filterable marine bacteria. J. Bacteriol. 90: 1713-1718.

Andrews, P. and P.J. LeB. Williams. 1971. Heterotrophic utilization of dissolved organic compounds in the sea. III. Measurement of the oxidation rates and concentrations of glucose and amino acids in seawater. J. Mar. Biol. Ass. U.K. 51: 111-125.

Armstrong, F.A.J., C.R. Sterns, and J.D.H. Strickland. 1967. The measurement of upwelling and subsequent biological processes by means of the Technicon Auto Analyzer and associated equipment. Deep-Sea Res. 14: 381-389.

Berman, T., O. Hadas, and U. Marchaim. 1979. Heterotrophic glucose-uptake and respiration in Lake Kinneret. Hydrobiol. 62: 275-282.

Brown, K.A. 1979. Modified procedure for determining $^{14}CO_2$ produced by respiration in water or sediment samples. Limnol. Oceanogr. 24: 1141-1145.

Buck, J.D. 1974. Effects of medium composition on the recovery of bacteria from seawater. J. Exper. Mar. Biol. Ecol. 15: 25-34.

Buck, J.D. and R.C. Cleverdon. 1960. The spread plate as a method for the enumeration of marine bacteria. Limnol. Oceanogr. 5: 78-80.

Bunch, J.N. 1979. Microbiological observations in the south Davis Strait. Fish. Mar. Serv. MS Rep. 1515: X + 92 p.

Burnison, B.K. and R.Y. Morita. 1974. Heterotrophic potential for amino acid uptake in a naturally eutrophic lake. Appl. Microbiol. 27: 488-495.

Cavari, B.Z. and O. Hadas. 1979. Heterotrophic activity, glucose-uptake, and primary productivity in Lake Kinneret. Freshwater Biology. 9: 329-338.

Colwell, R.R., J.D. Walker, B.F. Conrad, and I.A. Seesman. 1977. Microbiological studies of Atlantic Ocean water and sediment from potential off-shore drilling sites. In L.A. Underkoffer (ed.). Developments in Industrial Microbiology, Vol. 17, Proceedings of the 22nd General Meeting, Univ. of Rhode Island, R.I. Aug. 17-22, 1975. Society for Industrial Microbiology, Arlington.

Colwell, R.R. and W.G. Wiebe. 1970. "Core" characteristics for use in classifying aerobic heterotrophic bacteria by numerical taxonomy. Bull. Georgia Acad. Sci. 28: 165-185.

Crawford, C.C., J. E. Hobbie, and K.L. Webb. 1974. The utilization of dissolved free amino acids by estuarine microorganisms. Ecology 55: 551-563.

Delattre, J.M., R. Delasmont, M. Clabaux, C. Oger, and H. Leclerc. 1979. Bacterial biomass, production, and heterotrophic activity of the coastal seawater at Gravelines (France). Oceanologica Acta 2: 317-324.

DiGeronimo, M.J., M. Nikaido, and M. Alexander. 1978. Most-probable-number technique for the enumeration of aromatic degraders in natural environments. Microb. Ecol. 4: 263-266.

Ezura, Y., K. Daiku, K. Tajima, T.Kimura and M. Sakai. 1974. Seasonal differences in bacterial counts and heterotrophic bacterial flora in Akkeshi Bay. In Effects of the ocean environment on microbial activities. pp. 249-257. Edited by R.R. Colwell and R.Y. Morita. University Park Press, London.

Ferguson, R.L. and P. Rublee. 1976. Contribution of bacteria to the standing crop of coastal plankton. Limnol. Oceanogr. 21: 141-145.

Gillespie, P.A., R.Y. Morita, and L.P. Jones. 1976. The heterotrophic activity for amino acids, glucose and

acetate in Antarctic waters. J. Oceanogr. Soc. Japan. 32: 74-82.

Gocke, K. 1977. Heterotrophic activity. In Microbial ecology of a brackish water environment. Vol 25. Edited by G. Rheinheimer, Springer-Verlag pp. 198-222.

Griffiths, R.P., S.S. Hayasaka, T.M. McNamara, and R.Y. Morita. 1978. Relative microbial activity and bacterial concentrations in water and sediment samples taken in the Beaufort Sea. Can. J. Microbiol. 24: 1217-1226.

Hamilton, R.D. and J.E. Preslan. 1970. Observations on heterotrophic activity in the eastern tropical Pacific. Limnol. Oceanogr. 15: 395-401.

Hanson, R.B. and J. Snyder. 1979. Microheterotrophic activity in a salt-marsh estuary, Sappelo Island, Georgia. Ecology. 60: 99-107.

Harrison, M.J., R.I. Wright, and R.Y. Morita. 1971. Method for measuring mineralization in lake sediments. Appl. Microbiol. 21: 698-707.

Herbert, D., P. Phipps, and R.E. Strange. 1971. Chemical analysis of microbial cells, pp. 209-344. In Methods in microbiology 5B. Edited by J.R. Norris and D.W. Ribbons. Academic Press, London and New York.

Hobbie, J.E. and C.C. Crawford. 1969. Respiration corrections for bacterial uptake of dissolved

organic compounds in natural waters. Limnol. Oceanogr. 14: 528-532.

Hobbie, J.E., C.C. Crawford, and K.L. Webb. 1968. Amino acid flux in an estuary. Science 159: 1463-1464.

Hobbie, J.E., R.J. Daley, and S. Jasper. 1977. Use of nuclepore filters for counting bacteria by fluorescence microscopy. Appl. Environ. Microbiol. 33: 1225-1228.

Hobbie, J.E., O. Holm-Hansen, T.T. Packard, L.R. Pomeroy, R.W. Sheldon, J.P. Thomas, and W.J. Wiebe. 1972. A study of the distribution and activity of micro-organisms in ocean water. Limnol. Oceanogr. 17: 544-555.

Hoppe, H.G. 1978. Relations between active bacteria and heterotrophic potential in the sea. Netherlands J. Sea Res. 12: 78-98.

Jannasch, H.W. 1967. Growth of marine bacteria at limiting concentrations of organic carbon in seawater. Limnol Oceanogr. 12: 264-271.

Jannasch, H.W., and G.E. Jones. 1959. Bacterial populations in seawater as determined by different methods of enumeration. Limnol. Oceanogr., 4: 128-139.

Jones, G.E. and H.W. Jannasch. 1959. Aggregates of bacteria in seawater as determined by treatment with surface active agents. Limnol. Oceanogr. 4: 269-276.

Kogure, K., U. Simidu, and N. Taga. 1979. A tentative direct microscopic method for counting living marine bacteria. Can. J. Microbiol. 25: 415-420.

Kriss, A.E. 1963. Marine microbiology. John Wiley and Sons, Inc. New York.

Kriss, A.E. 1970. Ecological-geographical patterns in the distribution of heterotrophic bacteria in the Atlantic Ocean. Microbiology 39: 313-320.

Kriss, A.E., I.E. Mishustina, N. Mitskevich, and E.V. Zemtsova. 1967. Microbial populations of oceans and seas. Edward Arnold Ltd. London.

Lamanna, C. and M.F. Mallett. 1965. Basic bacteriology: Its biological and chemical background. 3rd ed. Williams and Wilkins Co., Baltimore.

Lehmicke, L.G., R. T. Williams, and R.L. Crawford. 1979. ^{14}C-most-probable number method for enumeration of active heterotrophic microorganisms in natural waters. Appl. Environ. Microbiol. 38: 644-649.

Mills, A.L., C.Breuil, and R.R. Colwell. 1978. Enumeration of petroleum-degrading marine and estuarine microorganisms by the most probable number method. Can. J. Microbiol. 24: 552-557.

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

Neary, M.P. 1971. Nuclear Laboratory Equipment Manual. Beckman Instruments, Inc. Fullerton, Calif.

Noseworthy, J.E. 1976. Quantitative and qualitative aspects of bacterial distribution, with special reference to the genus Pseudomonas Migula, in a cold seas environment (Avalon Peninsula, Newfoundland). M.Sc. Thesis, Memorial University of Newfoundland, St. John's, Nfld.

Novitsky, J.A. and R.Y. Morita. 1976. Morphological characterization of small cells resulting from nutrient starvation of a psychrophilic marine vibrio. Appl. Environ. Microbiol. 32: 617-622.

Oppenheimer, C.H. and C.E. Zobell. 1952. The growth and viability of sixty-three species of marine bacteria as influenced by hydrostatic pressure. J. Mar. Res. 11: 10-18.

Palumbo, A.V. and R.L. Ferguson. 1978. Distribution of suspended bacteria in the Newport River Estuary, N. Carolina. Est. Coastal Mar. Sci. 7 : 521-530.

Parsons, T.R. and J.D.H. Strickland. 1962. On the production of particulate organic carbon by heterotrophic processes in the sea. Deep Sea Res. 8: 211-222.

Roubal, G. and R. Atlas. 1978. Distribution of hydrocarbon-utilizing organisms and hydrocarbon biodegradation potentials in Alaskan continental shelf areas.

Appl. Environ. Microbiol. 35: 897-905.

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

Sieburth, J. McN. 1971. Distribution and activity of oceanic bacteria. Deep-Sea Res. 18: 1111-1121.

Simidu, U. 1974. Improvement of media for enumeration and isolation of heterotrophic bacteria in seawater. In Effects of the ocean environment on microbial activities. pp. 249-257. Edited by R.R. Colwell and R.Y. Morita. University Park Press, London.

Stevenson, L.H. 1978. A case for bacterial dormancy in aquatic systems. Microbial Ecology 4: 127-133.

Stevenson, L.H., E.C. Millwood, and B.H. Hebeler. 1974. Aerobic, heterotrophic bacterial populations in estuarine water and sediments. In Effects of the ocean environment on microbial activities. pp. 566-575. Edited by R.R. Colwell and R.Y. Morita. University Park Press, Baltimore.

Strickland, J.D.H. and T.R. Parsons. 1972. A practical handbook of seawater analysis. 2nd ed. Fish. Res. Board Can., Ottawa.

Takahashi, M. and S. Ichimura. 1971. Glucose uptake in ocean profiles with special reference to temperature. Mar. Biol. 11: 206-213.

Thompson, B. and R.D. Hamilton. 1974. Some problems in heterotrophic uptake methodology. In Effects of

the ocean environment on microbial activities. pp. 566-575. Edited by R.R. Colwell and R.Y. Morita. University Park Press, Baltimore.

Vaatanen, P. 1977. Effects of composition of substrate and inoculation technique on plate counts of bacteria in the northern Baltic Sea. J. Appl. Bacteriol. 42: 437-443.

Vaccaro, R.F. 1969. The response of natural microbial populations in seawater to organic enrichment. Limnol. Oceanogr. 14: 726-735.

Vaccaro, R.F. and H.W. Jannasch. 1966. Studies on heterotrophic activity in seawater based on glucose assimilation. Limnol. Oceanogr. 11: 596-607.

Vaccaro, R.F. and H.W. Jannasch. 1967. Variations in uptake kinetics for glucose by natural populations in seawater. Limnol. Oceanogr. 12: 540-542.

Walker, J.D., and R.R. Colwell. 1976. Measuring potential activity of hydrocarbon degrading bacteria. Appl. Environ. Microbiol. 31: 189-197.

Watson, S.W., T.J. Novitsky, H.L. Quinby, and F.A. Valois. 1977. Determination of bacterial number and biomass in the marine environment. Appl. Environ. Microbiol. 33: 940-946.

Williams, P.J. LeB. 1970. Heterotrophic utilization of dissolved organic compounds in the sea. I. Size distribution of population and relationship between respiration and incorporation of growth

substrates. J. Mar. Biol. Ass. U.K. 50: 859-870.

Williams, P.J. LeB. and C. Askew. 1968. A method of measuring the mineralization by micro-organisms of organic compounds in sea-water. Deep Sea Res. 15: 365-375.

Williams, P.J. LeB., T. Berman, and A. Holm-Hansen. 1976. Amino acid uptake and respiration by marine heterotrophs. Mar. Biol. 35: 41-47.

Williams, P.J. Le.B. and R.W. Gray. 1970. Heterotrophic utilization of dissolved organic compounds in the sea. II. Observations on the response of heterotrophic marine populations to abrupt increases in amino acid concentrations. J. Mar. Biol. Assoc. U.K. 50: 871-881.

Wright, R.T. 1970. Glycollic acid uptake by planktonic bacteria. In Organic matter in natural waters, pp. 521-536. Edited by D.H. Wood. Institute of Marine Science, Occasional Publication No. 1. College, Alaska: Univ. of Alaska.

Wright, R.T. 1973. Some difficulties in using ^{14}C-organic solutes to measure heterotrophic bacterial activity. In Estuarine microbial ecology, Vol. 1, pp. 199-217. Edited by H.L. Stevenson and R.R. Colwell. Univ. South Carolina Press, Columbia, S.Car.

Wright, R.T. 1978. Measurement and significance of specific activity in the heterotrophic bacteria in natural waters. Appl. Environ. Microbiol. 36: 297-305.

Wright, R.T. and J.E. Hobbie. 1965. The uptake of organic solutes in seawater. Limnol. Oceanogr. 10: 22-28.

Wright, R.T. and J.E. Hobbie. 1966. Use of glucose and acetate by bacteria and algae in aquatic ecosystems. Ecology 47: 447-464.

Zimmerman, R. 1977. Estimation of bacterial number and biomass by epifluorescence microscopy and scanning electron microscopy. In Microbial ecology of a brackish water environment, Vol. 25, pp. 103-120. Edited by G. Rheinheimer. Springer-Verlag.

Zimmerman, R., R. Itturiaga, and J. Becker-Buck. 1978. Simultaneous determination of the total number of aquatic bacteria and the number thereof involved in respiration. Appl. Environ. Microbiol. 36: 926-935.

ZoBell, C.E. 1941. Studies on marine bacteria. I. The Cultural requirements of heterotrophic aerobes. J. Mar. Res. 4: 42.75.

ZoBell, C.E. 1946. Marine Microbiology. Chronica Botanica, Waltham, Mass.

Appendix 1

Calculation of Kinetic Parameters

The Beckman LS3150T produces a printout showing three separate columns of numbers (Appendix Table 1). The column on the left of the printout sheet represents the numbers of the individual vials which are counted. The second column consists of external standard channels ratio (ESCR) values. The right-hand column represents the radioactive counts per minute (cpm) obtained from each vial. Each figure in parentheses denotes the accuracy to which the contents of a particular vial was counted.

One problem associated with liquid scintillation counting is that of quenching. Quenching is the attenuation of photon output due to compounds in the liquid scintillator. It results in reduced counts per minute. To correct for quenching, the method of Neary (1971) was followed. A series of vials containing Nuclepore Filters and appropriate amounts of toluene based scintillation fluid and Protosol were prepared. A known amount of ^{14}C-toluene was added to each vial and different amounts of CH_3NO_2, a quenching agent, were added. After counting the vials, each cpm value was expressed as a percentage of the added disintegrations per minute (dpm) by the following formula:

$$\% \text{ counting efficiency} = \frac{\text{cpm (observed) x 100}}{\text{dpm (theoretical)}}$$

Each counting efficiency was plotted against the corresponding ESCR value to produce a quench correction curve (Appendix Fig. 1).

Using the quench correction curve, individual cpm values were corrected for quenching and converted to dpm by

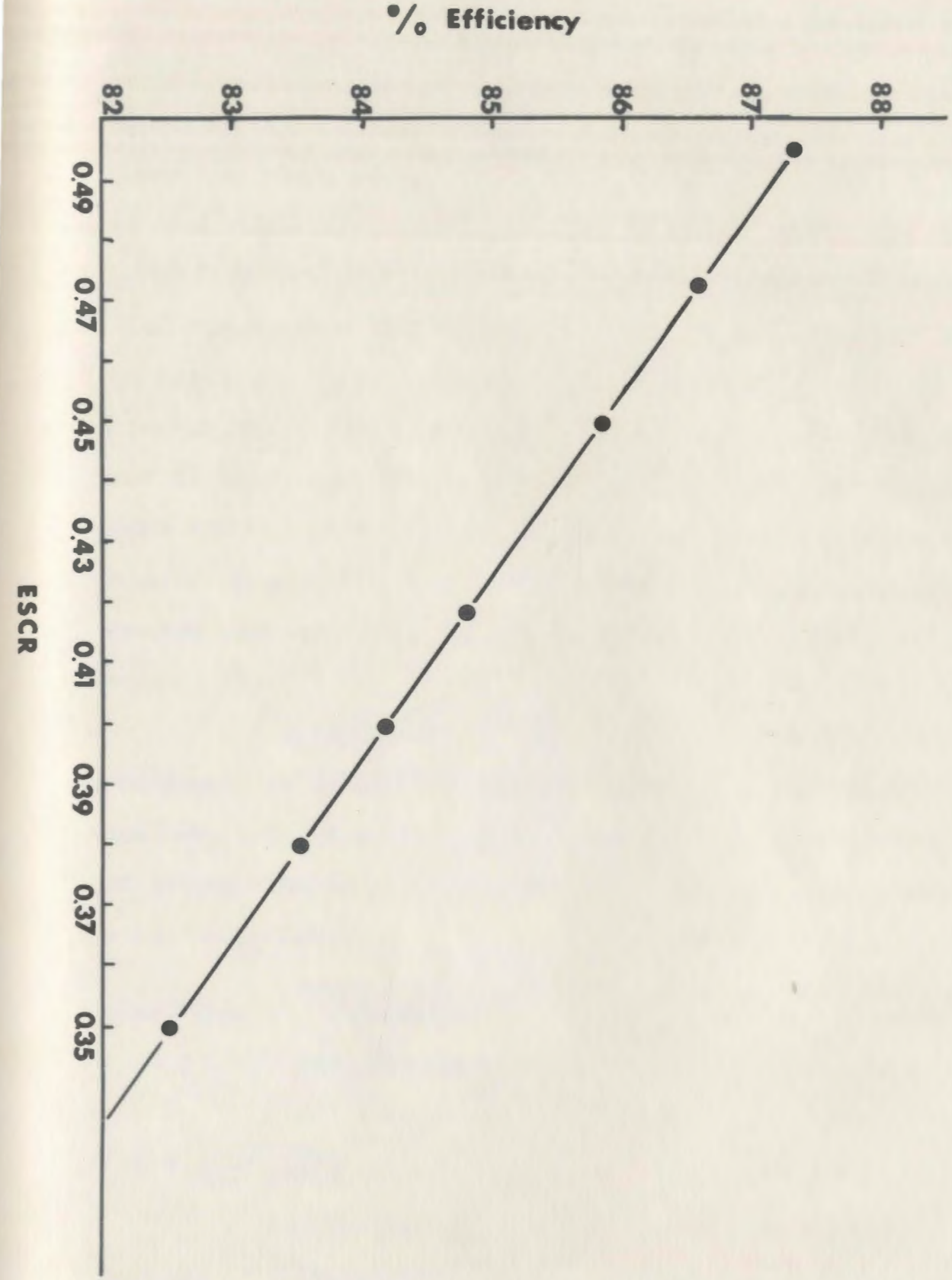
Appendix 1, Table 1. Sample of type of printout obtained from the Beckman Model LS3150T liquid scintillation counter.

Vial no.	ESCR*	CPM**
1	0.412	3250.7(1.0%)
2	0.420	3202.6(1.0%)
3	0.470	917.8(2.0%)
4	0.409	5808.4(1.0%)
5	0.410	5502.2(1.0%)
6	0.450	1584.4(1.5%)
7	0.407	7109.5(1.0%)
8	0.407	8178.9(1.0%)
9	0.453	2418.8(1.0%)
10	0.400	9374.0(1.0%)
11	0.406	9253.0(1.0%)
12	0.447	2501.3(1.0%)
13	0.403	11452.8(1.0%)
14	0.403	9528.3(1.0%)
15	0.456	2398.9(1.0%)
16	0.398	12479.4(1.0%)
17	0.400	14977.2(1.0%)
18	0.466	779.9(2.0%)
19	0.400	154.6(5.0%)
20	0.403	274.9(3.0%)
21	0.403	103.6(5.0%)

* ESCR = external standard channels ratio

** cpm = counts per minute

Appendix 1 Figure 1: Quench correction curve showing the relationship between the external standard channels ratio (ESCR) and the counting efficiency (%) for ^{14}C of the Beckman LS3150T liquid scintillation counter.


% Efficiency
82
83
84
85
86
87
88
ESCR
0.49
0.47
0.45
0.43
0.41
0.39
0.37
0.35

the following formula:

$$dpm = \frac{cpm\ (observed)\ x\ 100}{\%\ counting\ efficiency}$$

Appendix Table 1 represents the printout obtained for the 0.5 m sample taken March 27, 1980. Vial numbers 1 and 2 correspond to the vials that contained the filters used with the lowest concentration of ^{14}C-glutamic acid and number 3 represents the vial containing the respired $^{14}CO_2$ associated with the concentration. Vial numbers 4, 5, and 6 correspond to the next concentration of substrate, and so on. Vial numbers 19, 20 and 21 represent the vials associated with the subsamples that were killed with 50% phosphoric acid prior to incubation. The counts obtained in these vials were due to non-biological processes and were subtracted from the counts obtained from the other vials.

In determining the kinetic parameters, it was necessary to plot the concentration of added substrate (A) against t/f (the incubation time (t) divided by the fraction of added substrate utilized (f)). The following calculations were involved:

$$\text{final dpm} = \begin{matrix}\text{dpm of filtered} \\ \text{subsample} \\ + \\ \text{dpm of respir-} \\ \text{ation}\end{matrix} - \begin{matrix}\text{dpm of filtered} \\ \text{killed subsample} \\ + \\ \text{dpm of respiration} \\ \text{(killed)}\end{matrix}$$

$$f = \frac{\text{final dpm}}{\text{dpm added}}$$

An example can be illustrated using values from Appendix 1, Table 1.

Vial 1 cpm = 3250.7

ESCR = 0.412

% counting efficiency = 84.60

$$\text{dpm of filtered subsample} = \frac{3250.7 \times 100}{84.60}$$

$$= 3842.4$$

Vial 3 cpm = 917.8

ESCR = 0.470

% counting efficiency = 86.50

$$\text{dpm of respiration} = \frac{917.8 \times 100}{85.50}$$

$$= 1061.0$$

Vial 19 cpm = 154.6

ESCR = 0.400

% counting efficiency = 84.20

$$\text{dpm of filtered killed subsample} = \frac{154.6 \times 100}{84.20}$$

$$= 183.6$$

Vial 21 cpm = 103.6

ESCR = 0.440

% counting efficiency = 85.50

$$\text{dpm of respiration (killed)} = \frac{103.6 \times 100}{85.50}$$

$$= 121.2$$

Final dpm = (3842.4 + 10610.0) - (183.6 + 121.2)

= 4903.4 - 304.8

= 4598.6

Fraction of added substrate utilized (f):

$$f = \frac{\text{final dpm}}{\text{dpm added}} = \frac{4598.6}{444,000}$$

$$= .0104$$

The incubation time for this experiment was 20.25 h. Thus,

$$\frac{t}{f} = \frac{20.25h}{.0104}$$

$$= 1947.1 \text{ h}$$

All t/f values were determined in this manner. Eventually a table (Appendix Table 2) was made. A linear regression of the data in the table was carried out using a STATl1 computer package and a plot of A versus t/f could be made (Appendix Fig. 2).

The turnover time (T_t) is represented by the Y-intercept and equals 1645.2 h. V_{max} is the reciprocal of the slope. Thus,

$$V_{max} = \frac{1}{51.1}$$

$$= 0.0196$$

$K_T + S_N$ is represented by the X-intercept, or mathematically by

$$K_T + S_N = \frac{T_t}{\text{Slope}}$$

$$= \frac{1645.2}{51.1}$$

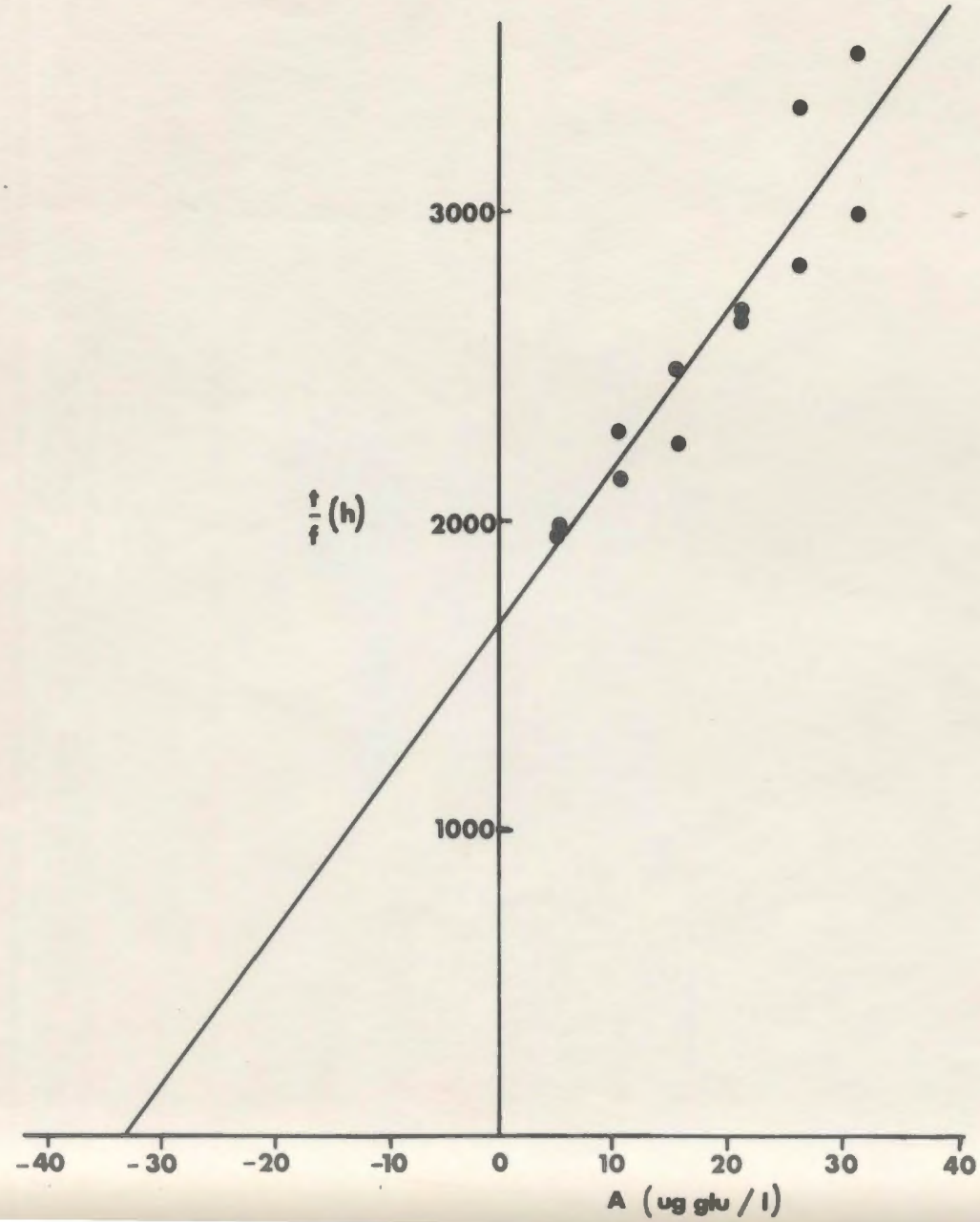
$$= 32.1$$

Appendix Table 2

Values of t/f obtained from a typical experiment.

A (ug glutamic acid/l)	$t/_f$
5.2	1947.1
5.2	1958.3
10.4	2131.6
10.4	2275.3
15.6	2469.5
15.6	2225.3
20.8	2629.9
20.8	2664.5
26.0	2812.5
26.0	3319.7
31.2	3491.5
31.2	2977.9

Appendix 1 Figure 2: Sample plot of substrate concentration added (A) versus the reciprocal of incubation time (t) and fraction of substrate taken up (f). The value for V_{max} is the reciprocal of the slope. The y-intercept represents the turnover time.


3000
2000
1000
$\frac{f}{f}$ (h)
-40
-30
-20
-10
0
10
20
30
40
A (ug glu / l)

Appendix II

Calculation of Total Bacteria

The estimation of total bacterial numbers by the acridine orange direct count (AODC) method was done using a Zeiss IV FL epifluorescence condenser microscope. A focusing eyepiece (12.5x) containing a 1 mm x 1 mm net micrometer was used. For each sample, two 10 ml subsamples were counted and the average of the two was used in calculating the number of bacteria per liter.

Generally the micrometer represented one counting field. However, when bacterial numbers were high, a section of the micrometer was taken as one field. For each 10 ml subsample, the bacteria on ten different fields were counted, summed, and divided by 10 to give the average number of bacteria per field. With the average of both subsamples, the following formula was used:

$$N = \frac{Sn}{sV}$$

where N = number of bacteria

S = filtering area of filter

n = average no. of bacteria per field

s = area of field

V = volume filtered

Values for S, s, and V were:

$S = 2.01 \times 10^8 \ \mu m^2$

$s = 1 \times 10^4 \ \mu m^2$

V = 10 ml

The following is a sample calculation:

Field	Number of Bacteria	
	Subsample 1	Subsample 2
1	25	24
2	29	25
3	30	36
4	28	26
5	25	22
6	31	28
7	29	25
8	24	23
9	27	24
10	28	25
Total	276	248
Average	27.6	24.8

Average of two subsamples = $\frac{27.6 + 24.8}{2} = 26.2$

Using $N = \frac{Sn}{sV}$,

$$N = \frac{2.01 \times 10^8 \ \mu m^2 \times 26.2}{10^4 \ \mu m^2 \times 10 \ ml}$$

$$= 5.27 \times 10^4/ml$$

$$= 5.27 \times 10^7/l$$

APPENDIX III: BACTERIAL NUMBERS

Table 1. Numbers of total, viable, and oleoclastic bacteria at 0.5 m depth.

DATE	Bacteria per liter			
	AODC x 10^8	MPN x 10^5	SPC x 10^5	Oleoclasts x 10^5
Apr. 3/79	——*	——	——	——
May 4/79	3.03	0.079	0.12	0.022
June 22/79	0.98	0.17	33.07	0.033
July 5/79	0.64	0.14	2.58	0.033
Aug. 7/79	3.79	0.33	0.57	0.049
Sept. 11/79	2.84	1.10	1.16	2.20
Oct. 23/79	1.93	1.10	1.40	0.023
Nov. 21/79	1.65	7.00	0.87	0.070
Dec. 12/79	0.30	4.90	0.56	0.049
Jan. 15/80	0.70	0.79	0.51	0.011
Feb. 2/80	0.28	0.078	0.41	0.033
Mar. 4/80	0.40	0.13	0.12	0.023
Mar. 27/80	2.10	0.33	0.18	0.033
May 1/80	1.51	2.20	4.70	0.033

* —— = datum was not obtained

Table 2. Numbers of total, viable, and oleoclastic bacteria at 10 m depth.

DATE	Bacteria per liter			
	AODC x 10^8	MPN x 10^5	SPC x 10^5	Oleoclasts x 10^5
Apr. 3/79	2.80	0.079	1.74	0.0078
May 4/79	2.29	0.079	0.060	0.35
June 22/79	0.93	0.79	27.46	0.049
July 5/79	0.98	0.22	0.95	0.17
Aug. 7/79	3.43	0.23	0.77	0.49
Sept. 11/79	3.52	0.79	0.80	9.20
Oct. 23/79	2.25	4.90	2.22	0.023
Nov. 21/79	1.95	7.90	0.85	0.046
Dec. 12/79	0.45	7.00	0.35	0.049
Jan. 15/80	0.53	0.79	0.81	0.013
Feb. 2/80	0.38	0.33	0.58	0.033
Mar. 4/80	0.49	0.13	0.19	0.017
Mar. 27/80	2.99	1.70	0.28	0.033
May 1/80	1.00	3.30	2.00	0.023

Table 3. Number of total, viable, and oleoclastic bacteria at 20 m depth.

DATE	Bacteria per liter			
	AODC x 10^8	MPN x 10^5	SPC x 10^5	Oleoclasts x 10^5
Apr. 3/79	2.31	0.11	1.84	0.013
May 4/79	2.08	0.12	0.12	0.17
June 22/79	0.93	0.28	8.00	0.17
July 5/79	0.81	0.17	1.08	0.049
Aug. 7/79	4.62	0.22	1.02	0.070
Sept. 11/79	2.90	1.10	1.88	9.20
Oct. 23/79	1.89	1.10	1.27	0.023
Nov. 21/79	1.76	4.90	1.21	0.033
Dec. 12/79	0.38	3.30	0.40	0.023
Jan. 15/80	0.45	0.79	0.98	0.033
Feb. 2/80	0.28	0.23	.87	0.023
Mar. 4/80	0.78	0.64	0.43	0.008
Mar. 27/80	2.08	1.30	0.23	0.013
May 1/80	1.36	11.0	3.00	0.023

Table 4. Number of total, viable, and oleoclastic bacteria at 40 m depth.

DATE	Bacteria per liter			
	AODC x 10^8	MPN x 10^5	SPC x 10^5	Oleoclasts x 10^5
Apr. 3/79	3.01	0.33	1.99	0.022
May 4/79	1.93	1.30	0.83	0.17
June 22/79	0.74	0.23	5.93	0.049
July 5/79	0.68	0.033	0.33	0.033
Aug. 7/79	1.80	0.11	1.10	0.095
Sept. 11/79	0.74	3.50	4.33	16.00
Oct. 23/79	1.97	1.10	1.53	0.013
Nov. 21/79	1.55	4.60	1.61	0.017
Dec. 12/79	0.28	4.90	0.39	0.023
Jan. 15/80	0.36	0.49	0.82	0.033
Feb. 2/80	0.25	0.49	0.95	0.049
Mar. 4/80	0.49	0.22	0.26	0.013
Mar. 27/80	1.63	2.40	0.29	0.017
May 1/80	1.97	2.30	1.60	0.033

APPENDIX IV: KINETIC PARAMETERS

Table 1. V_{max}, V_{max} specific activity index, T_t, and $K_T + S_N$ at 0.5 m depth.

DATE	V_{max} (ng glu $l^{-1}h^{-1}$)	V_{max} s.a.i. (fg glu $h^{-1}cell^{-1}$)	T_t (h)	$K_T + S_N$ (ug glu l^{-1})
May 4/79	9.2	0.030	2470	22.8
June 22/79	——*	——	—	—
July 5/79	24.2	0.378	712	17.2
Aug. 7/79	——	——	—	—
Sept. 11/79	——	——	—	—
Oct. 23/79	——	——	—	—
Nov. 21/79	7.6	0.046	1315	10.0
Dec. 12/79	3.6	0.120	3277	12.1
Jan. 15/80	1.7	0.024	5827	10.1
Feb. 2/80	1.8	0.064	13074	23.9
Mar. 4/80	2.9	0.073	3122	9.0
Mar. 27/80	19.6	0.093	1646	32.2
May 1/80	45.5	0.301	285	13.0

* —— = datum was not obtained

Table 2. V_{max}, V_{max} specific activity index, T_t and $K_T + S_N$ at 10 m depth.

DATE	V_{max} (ng glu $l^{-1}h^{-1}$)	V_{max} s.a.i. (fg glu $h^{-1}cell^{-1}$)	T_t (h)	$K_T + S_N$ (ug glu l^{-1})
Apr. 3/79	10.0	0.036	509	5.1
May 4/79	47.2	0.206	1046	49.3
June 22/79	——*	——	——	—
July 5/79	121.0	1.235	267	32.2
Aug. 7/79	93.6	0.273	66	6.2
Sept. 11/79	29.9	0.085	315	9.4
Oct. 23/79	19.1	0.085	109	2.0
Nov. 21/79	1.3	0.007	738	15.7
Dec. 12/79	8.4	0.186	669	5.6
Jan. 15/80	3.5	0.066	1969	7.0
Feb. 2/80	1.8	0.047	8068	15.0
Mar. 4/80	7.3	0.149	5699	24.6
Mar. 27/80	47.1	0.158	1796	84.5
May 1/80	19.5	0.195	243	13.0

* —— = datum was not obtained

Table 3. V_{max}, V_{max} specific activity index, T_t, and $K_T + S_N$ at 20 m depth.

DATE	V_{max} (ng glu $l^{-1}h^{-1}$)	V_{max} s.a.i. (fg glu $h^{-1}cell^{-1}$)	T_t (h)	$K_T + S_N$ (ug glu l^{-1})
Apr. 3/79	33.4	0.145	585	19.6
May 4/79	10.8	0.052	2368	25.6
June 22/79	——*	——	——	—
July 5/79	49.1	0.606	1397	68.5
Aug. 7/79	59.0	0.128	185	10.9
Sept. 11/79	25.3	0.087	311	7.9
Oct. 23/79	14.1	0.075	227	3.2
Nov. 21/79	9.5	0.054	496	4.7
Dec. 12/79	1.6	0.042	6136	10.2
Jan. 15/80	1.7	0.036	2363	4.0
Feb. 2/80	2.9	0.104	12704	33.7
Mar. 4/80	3.7	0.047	3764	13.7
Mar. 27/80	11.4	0.055	1219	13.9
May 1/80	63.0	0.463	212	13.4

* —— = datum was not obtained

Table 4. V_{max}, V_{max} specific activity index, T_t, and $K_T + S_N$ at 40 m depth.

DATE	V_{max} (ng glu $l^{-1}h^{-1}$)	V_{max} s.a.i. (fg glu $h^{-1}cell^{-1}$)	T_t (h)	$K_T + S_N$ (ug glu l^{-1})
Apr. 3/79	34.7	0.115	593	20.6
May 4/79	3.2	0.017	593	19.7
June 22/79	—— *	——	——	—
July 5/79	24.0	0.353	944	22.2
Aug. 7/79	40.6	0.226	377	15.3
Sept. 11/79	21.0	0.284	564	11.9
Oct. 23/79	8.0	0.041	650	5.2
Nov. 21/79	——	——	——	—
Dec. 12/79	18.7	0.668	1672	31.4
Jan. 15/80	2.5	0.069	3976	10.0
Feb. 2/80	4.2	0.168	2620	11.1
Mar. 4/80	2.1	0.043	1933	4.1
Mar. 27/80	20.5	0.126	791	16.2
May 1/80	17.6	0.089	273	4.8

* —— = datum was not obtained

APPENDIX V: TEMPERATURE, SALINITY, AND CHEMICAL DATA

Table 1. Temperature, salinity, and chemical data at 0.5m depth.

Date	Temp (°C)	Salinity (°/oo)	NO_3^- (mg/lN)	o-$PO_4^{=}$ (mg/l$PO_4^{=}$)	Carbon (mg/lC)	Silicate (mg/lSi)
Apr. 3/79	——*	——	——	——	——	——
May 4/79	3.5	30.88	——	0.098	27	0.076
June 22/79	13.0	30.83	——	0.336	20	0.113
July 5/79	12.5	30.71	——	0.066	21	0.169
Aug. 7/79	16.5	30.88	——	0.046	13	0.470
Sept. 11/79	13.8	30.99	<0.001	0.030	13	0.025
Oct. 23/79	8.8	30.47	0.007	<0.005	17	0.060
Nov. 21/79	6.5	30.43	0.007	0.038	7	0.054
Dec. 12/79	3.5	30.92	0.003	0.048	10	0.067
Jan. 15/80	0.5	32.56	0.020	0.703	4	0.086
Feb. 2/80	-0.5	32.34	0.001	0.553	10	0.107
Mar. 4/80	-0.5	32.08	<0.001	0.029	6	0.118
Mar. 27/80	-0.5	31.90	0.016	0.241	16	0.148
May 1/80	2.8	31.15	<0.005	0.068	14	0.043

* —— = datum was not obtained.

Table 2. Temperature, salinity, and chemical data at 10m depth.

Date	Temp (°C)	Salinity (°/oo)	NO_3^- (mg/lN)	o-$PO_4^{=}$ (mg/l$PO_4^{=}$)	Carbon (mg/lC)	Silicate (mg/lSi)
Apr. 3/79	0.5	31.36	——*	0.104	113	0.173
May 4/79	3.0	31.24	——	0.908	18	0.198
June 22/79	6.0	30.71	——	0.062	53	0.103
July 5/79	9.0	31.07	——	0.070	18	0.119
Aug. 7/79	15.5	31.32	——	0.077	160	0.599
Sept. 11/79	13.5	31.17	<0.001	0.040	17	0.025
Oct. 23/79	8.0	30.87	0.004	0.024	21	0.054
Nov. 21/79	6.5	24.85	0.007	0.044	14	0.060
Dec. 12/79	3.5	30.91	<0.001	0.326	8	0.074
Jan. 15/80	0.5	32.19	<0.001	0.864	6	0.084
Feb. 2/80	-0.5	32.49	0.032	0.530	11	0.091
Mar. 4/80	-0.5	32.41	0.080	0.076	9	0.108
Mar. 27/80	-0.5	31.61	0.013	0.517	13	0.207
May 1/80	2.8	31.65	<0.005	0.723	12	0.219

* —— = datum was not obtained.

Table 3. Temperature, salinity, and chemical data at 20 m depth.

Date	Temp (°C)	Salinity (°/oo)	NO_3^- (mg/lN)	$0\text{-}PO_4^=$ (mg/l $PO_4^=$)	Carbon (mg/lC)	Silicate (mg/lSi)
Apr. 3/79	-1.0	31.80	——*	0.090	24	0.286
May 4/79	-1.0	31.51	——	0.260	13	0.092
June 22/79	2.0	31.19	——	0.074	13	0.184
July 5/79	5.0	31.80	——	0.072	16	0.129
Aug. 7/79	10.0	31.08	——	0.048	139	0.455
Sept. 11/79	13.0	31.10	<0.001	0.046	28	0.034
Oct. 23/79	8.0	31.06	0.004	0.044	23	0.054
Nov. 21/79	6.5	30.29	0.012	0.052	8	0.060
Dec. 12/79	3.5	29.76	<0.001	0.066	3	0.067
Jan. 15/80	-0.5	32.52	<0.001	0.473	3	0.087
Feb. 2/80	-0.5	31.94	0.011	0.507	47	0.094
Mar. 4/80	-1.0	32.15	0.081	0.059	3	0.109
Mar. 27/80	-1.0	32.00	0.017	0.243	7	0.132
May 1/80	2.0	31.76	0.033	0.924	8	0.255

* —— = datum was not obtained.

Table 4. Temperature, salinity, and chemical data at 40 m depth.

Date	Temp (°C)	Salinity (°/oo)	NO_3^- (mg/lN)	o-$PO_4^{=}$ (mg/l$PO_4^{=}$)	Carbon (mg/lC)	Silicate (mg/lSi)
Apr. 3/79	-1.0	31.80	——*	0.090	24	0.393
May 4/79	-1.0	31.32	——	1.300	32	0.092
June 22/79	0.5	31.87	——	0.082	15	0.110
July 5/79	2.5	31.56	——	0.116	8	0.158
Aug. 7/79	6.0	31.56	——	0.074	153	0.390
Sept. 11/79	8.0	31.54	0.002	0.058	18	0.034
Oct. 23/79	6.5	30.03	0.010	0.050	22	0.080
Nov. 21/79	6.0	30.96	<0.001	0.163	7	0.054
Dec. 12/79	3.0	30.91	<0.001	0.067	11	0.060
Jan. 15/80	-0.5	32.08	0.006	0.438	4	0.082
Feb. 2/80	-0.5	32.85	——	0.968	22	0.122
Mar. 4/80	-1.0	32.81	0.063	0.078	21	0.117
Mar. 27/80	-1.0	31.90	0.025	1.206	13	0.372
May 1/80	2.0	31.86	0.005	0.066	9	0.044

* —— = datum was not obtained