QUANTITATIVE AND QUALITATIVE ASPECTS OF BACTERIAL DISTRIBUTION, WITH SPECIAL REFERENCE TO THE GENUS PSEUDOMONAS MIGULA, IN A COLD SEAS ENVIRONMENT (AVALON PENINSULA NEWFOUNDLAND)

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by

© Joyce Elizabeth Noseworthy, B.Sc. (Hons.)

A Thesis submitted in partial fulfilment
of the requirements of the degree of
Master of Science

Department of Biology
Memorial University of Newfoundland

August 1975

St. John's Newfoundland

ABSTRACT

Both sea water and plankton tow samples were taken off Small Point, on the east coast of the Avalon Peninsula, Newfoundland, at intervals from September 12, 1971 to August 10, 1972. Samples were collected from the surface, bottom and middepth or thermocline. To determine total bacterial populations, the samples were plated onto YBP-SWA and incubated at 20C and 5C; there were no significant differences between the counts made at these two temperatures. Total pseudomonads were determined both by spreading plates of 0.03% cetrimide agar and by replication of colonies from YBP-SWA onto the cetrimide plates.

The pseudomonads were identified using a scheme devised for this study, since it was found that the schemes already in existence placed undue emphasis on motility and pigmentation. The fluorescent antibody technique was used in the differentiation of pseudomonads from non-pseudomonads and was found to be of value.

Seventy-eight percent of the plates spread with sea water produced no colonies. Total numbers of bacteria ranged from 10 to 4,329 per liter of plankton

tow; total pseudomonads ranged from 1 to 865 per liter. Numbers varied apparently randomly from trip to trip. No regular pattern of vertical bacterial distribution was detected and the thermocline apparently had no effect on vertical distribution. Bacterial and pseudomonad numbers seemed to be unrelated to either water temperatures or salinities.

Phytoplankton samples were counted and numbers of diatoms, dinoflagellates, and silicoflagellates were recorded. This data indicated that a diatom bloom, caused mainly by Chaetoceros species, occurred from March to May or June. For all three sampling depths, bacterial and pseudomonad numbers were lowest when the diatoms were at their peak, but started to increase as the bloom declined.

ACKNOWLEDGEMENTS

I acknowledge, with gratitude, the financial support I received from both the Provincial Government of Newfoundland and the National Research Council of Canada. I wish to thank my supervisor, Dr. G. Moskovits, for his advice on and interest in this project, as well as for the additional financial aid which enabled me to attend the Fluorescent Antibody Technique course at the Center for Disease Control in Atlanta, Georgia. I am deeply appreciative of the help and advice I received from the instructors, staff and fellow students at the C.D.C.

The sampling necessary for the project could not have been accomplished without the assistance of Mr. Wayne Burke, Ms. Anne Moore, and the late Mr. Martin Hickey. I am grateful to the M.S.R.L. for the use of the "Teal". The College of Fisheries, Navigation, Marine Engineering and Electronics most kindly placed the "Beiner" at our disposal when the "Teal" could not be used, and the captain and crew of the "Beiner" were most cooperative.

Mr. Burke analyzed the plankton samples. Mr. Roy Ficken, Biology Department photographer, photographed and printed the figures. Mr. Paul Murphy helped in the

drawing of the graphs and assisted in the proofreading of the thesis. Ms. Judy Conway typed the thesis. To these and to all others who may have helped in any way to bring this project to completion, I give my sincerest thanks.

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INTRODUCTION

Casual sampling (G. Moskovits, unpublished data) of the coastal waters of the Avalon Peninsula, Newfoundland, indicated that the bacterial populations of this region were extremely low. This is a type of environment, unprotected and characterized by high salinities and low water temperatures, on which few studies have been carried out. The very few microbiological investigations of the open North Atlantic Ocean (Fischer, 1894; Minervini, 1900; Kriss, 1963; 1970) all gave higher plate counts than were found along the Avalon Peninsula.

A complete examination of the distribution of marine bacteria in any region should involve qualitative as well as quantitative work. However, because of the time and effort inherent in such a project, only a single bacterial group could be studied in detail. The genus Pseudomonas Migula was chosen for a number of reasons. 1) Pseudomonads are widely distributed in the marine environment. 2) Pseudomonads, because of their metabolic versatility, are important in many processes occurring in the seas. 3) The biochemistry and metabolism of pseudomonads are better understood than

are those of any other group of marine bacteria. 4) The taxonomy of the pseudomonads, although far from satisfactory, is better organized and more detailed than is that of any of the other genera found in the marine environment. 5) A medium selective for pseudomonads was available.

The present study was undertaken: 1) to verify the existence of unusually low bacterial populations in Avalon Peninsula waters, 2) to study the fluctuations of these populations in time and space during the sampling period, 3) to characterize the kinds of bacteria recovered and to examine in detail the seasonal and depth distribution of bacteria of the genus Pseudomonas, and 4) to attempt to examine the possible influence of such environmental factors as water temperature and phytoplankton distribution on bacterial distribution.

MATERIALS AND METHODS

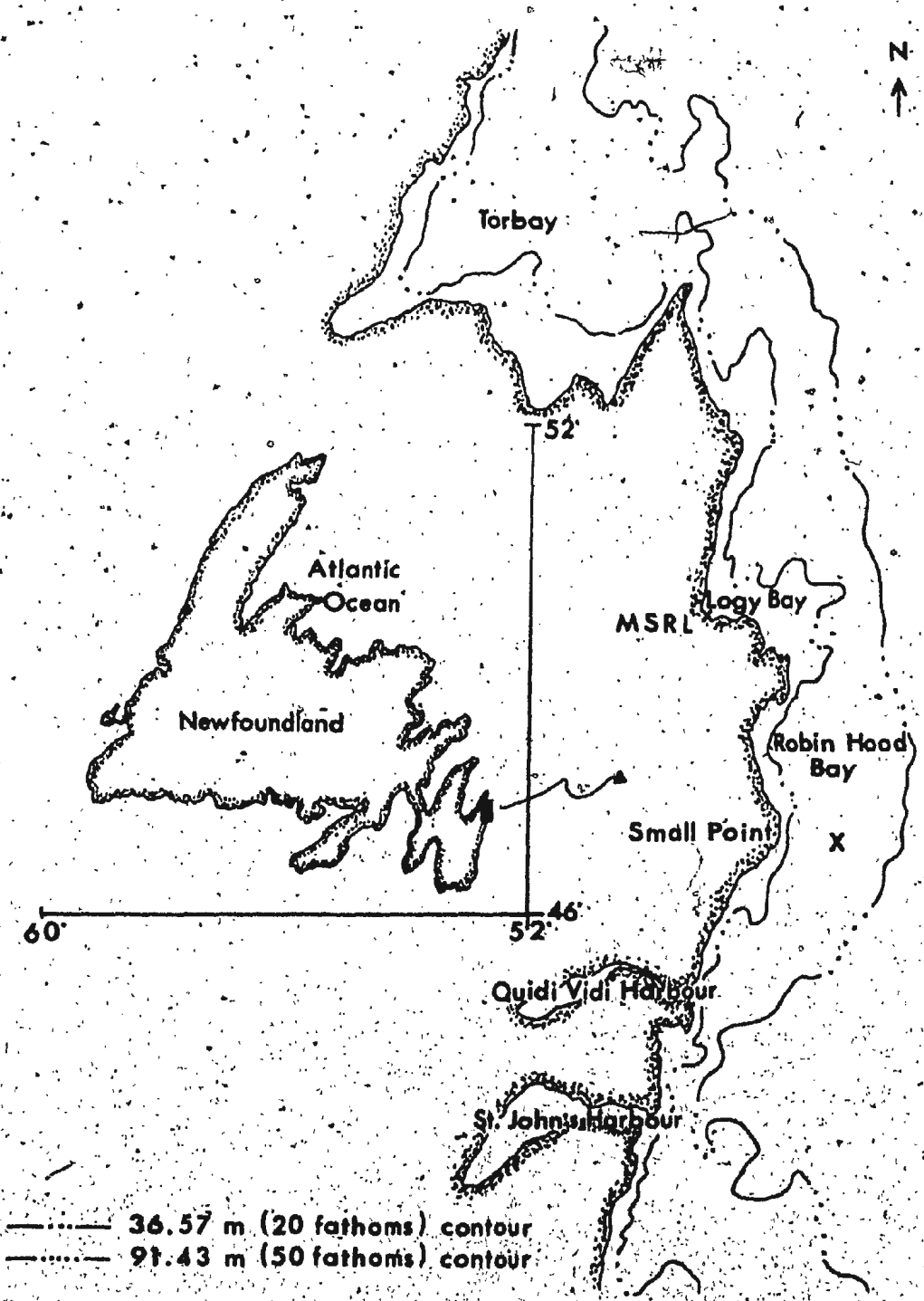
FIELD WORK

1. Sampling Site

The field work was carried out off Small Point (47° 36' N; 52° 39' W), adjacent to Robin Hood Bay on the east coast of the Avalon Peninsula, Newfoundland, and approximately 1.6 km southeast of the Marine Sciences Research Laboratory (M.S.R.L.) of the Memorial University of Newfoundland (Figure 1). Plankton tows were made north from this site, sometimes as far as the southern limit of Logy Bay. Work was done in approximately 65 m of water.

The sampling site was chosen for two reasons. First, it could be reached from Quidi Vidi Harbour where the sampling boat was moored, in 15 to 20 minutes, so that samples could be returned to the laboratory and processed the same day they were collected. Second, this area had no fresh water run-off, and, because of the southward trend of the coastal current, was free of the gross pollution associated with the harbours of St. John's and Quidi Vidi.

Figure 1. Section of the east coast of the Avalon Peninsula showing the sampling station (X).


N
Torbay
52°
Atlantic Ocean
Newfoundland
MSRL
Logy Bay
Robin Hood Bay
Small Point
X
60°
52°46'
Quidi Vidi Harbour
St. Johns Harbour
36.57 m (20 fathoms) contour
91.43 m (50 fathoms) contour

2. Sampling Period

The sampling was done in fifteen trips, from September, 1972 to August, 1973. Trips at two-week intervals had been planned, but were not possible because of bad weather or mechanical difficulties with the sampling vessel. Field work was done from the M.S.R.L.'s launch "Teal", except for the period from March 21 to May 29, 1973, when the research vessel "Beiner" of the College of Fisheries, Navigation, Marine Engineering and Electronics was used.

3. Measurements

On each trip, the following operations were carried out:

(a) Water depth determinations

The depth of water was measured with a sounding lead run over a meter wheel. A depth record was obtained using a bathythermograph (BT). The water depth value was used to determine the levels at which water samples were taken and plankton tows made.

(b) Water temperature determinations

Temperatures from surface to bottom were measured by means of a BT. A surface "bucket" temperature determination was also made and used to correct the BT

trace, where necessary.

(c) Other measurements

Air temperature was measured and observations were made on weather, wind force and direction, sea state, and swell.

These data are given in Appendix I.

4. Sampling Procedures

Samples were collected from three depths: approximately 1 m below the surface, approximately 1 m above the bottom, and middepth. When the presence of a thermocline was indicated upon examination of the BT trace, samples were taken from this level rather than from middepth.

The following samples were taken on each trip:

(a) Salinity samples

Water samples for salinity determinations were collected from bottom and middepth (or thermocline) using a 1200-ml capacity Nansen bottle, and from the surface with a bucket. In the laboratory, specific gravities of the samples were determined with a hydrometer. The hydrometer readings were then converted to salinities using Seawater Temperature and Density

Reduction Tables (Zerbe and Taylor, 1953). Salinity data are given in Table 6, Appendix I.

(b) Water samples for bacteriology

Water samples were collected in sterile bottles held in a ZoBell sampling frame (ZoBell, 1941a). Immediately the sample bottle was brought up and removed from the frame, its stopper with attached tubing was removed and replaced with a sterile Escher stopper. The bottle was then put into a clean, dry plastic bag and iced until it reached the laboratory.

(c) Plankton tow samples

Quantitative plankton tows were made using a Clarke-Bumpus sampler of 13-cm aperture, to which was attached a No. 20 nylon net (nominal mesh aperture of 0.076 mm). The operating depth of the sampler was estimated from the wire angle (that angle made by the cable with the water surface), and the meter wheel reading. A separate 15-min tow was made at each depth.

Plankton material retained by the net was recovered by rinsing the outer surface of the net, with the collecting cup attached, in a bucket of clean sea water. The cup was then carefully removed and its contents poured into a sterile jar. This rinsing process was carried out at least twice, after which the jar was capped tightly and iced.

LABORATORY WORK

1. Initial Work

(a) Culture media

Bacteriological data given in this study are based on the culture plate technique. Two media were used for the culture work; yeast-beef-peptone-seawater agar (YBP-SWA) and 0.03% cetrimide agar, the compositions of which are given in the Formulary. The YBP-SWA, a modification of ZoBell's Medium 2216 (ZoBell, 1941b), was used to give an indication of total numbers of bacteria. The cetrimide agar was used for total counts of pseudomonads, being selective for these organisms (Noseworthy & Moskovits, 1974).

All culture plates were poured 4 to 5 days prior to use and incubated at room temperature. They were then critically examined for contamination using a stereoscopic binocular microscope. The sterile plates were then stored in a cold room to prevent excessive dehydration.

(b) Treatment of samples

Upon returning to the laboratory, the water and plankton tow samples were immediately transferred to a cold room (1.5 - 4.5C). All processing of the samples up to and including preparation of dilutions was done in the cold room. Sterile 9-ml dilution blanks, prepared

with paper-filtered (Whatman No. 5) sea water, were used.

Each water sample bottle was shaken vigorously by hand for one minute. Ten ml of the sample were removed and shaken for one minute before preparation of a 1:10 dilution.

The plankton tow samples were treated in a different manner. After shaking each sample jar thoroughly for one minute, one-half the contents was poured into a sterile Waring Blendor cup and ground up for one minute. Ten ml were then removed and used to make dilutions of 1:10, 1:100, and 1:1000. The remaining half of each sample was preserved with sufficient neutralized formalin to give a final concentration of 4%, and set aside for subsequent examination.

The samples were ground up for two reasons. First, more accurate dilutions could be made with finely ground up plankton organisms than with large, intact ones. Second, more accurate plate counts could be obtained once the bacterial clumps were broken up and the bacteria dispersed.

Increases in the temperature of the plankton tow samples, due to friction between the grinding blades and the samples, were minimized by chilling the blender cups

in a freezer for at least 24 hr before use. This prevented temperature increases great enough to kill the bacteria (ZoBell & Conn, 1940).

(c) Plating technique

Plating was carried out using the spread plate technique of Buck and Cleverdon (1960), who found that it gave higher bacteria counts than did the pour plate method. Portions of the raw samples, dilutions and controls (sterile dilution blanks) were plated in triplicate, on YBP-SWA in the cold room, and on both culture media at room temperature. The dilution blanks were shaken for at least 30 sec before the 0.1-ml aliquots were removed from them, deposited on the surfaces of the plates and spread with individual, sterile glass spreaders.

(d) Bacteria counts

The plates spread at room temperature and those spread in the cold room were incubated at 20C and 1.5 - 4.5C, respectively, for 3 weeks. At one-week intervals, the total numbers of colonies on each plate were counted. A stereoscopic binocular microscope, which greatly facilitated the counting of the smaller colonies, was employed. Due to the very low numbers of bacteria found in the seawater samples, counts were made and retained even on those plates which had fewer than thirty colonies. With these exceptions, the counting procedure conformed to

that given in Standard Methods for the Examination of Water and Wastewater (American Public Health Association, 1971).

2. Isolation of Possible Pseudomonads

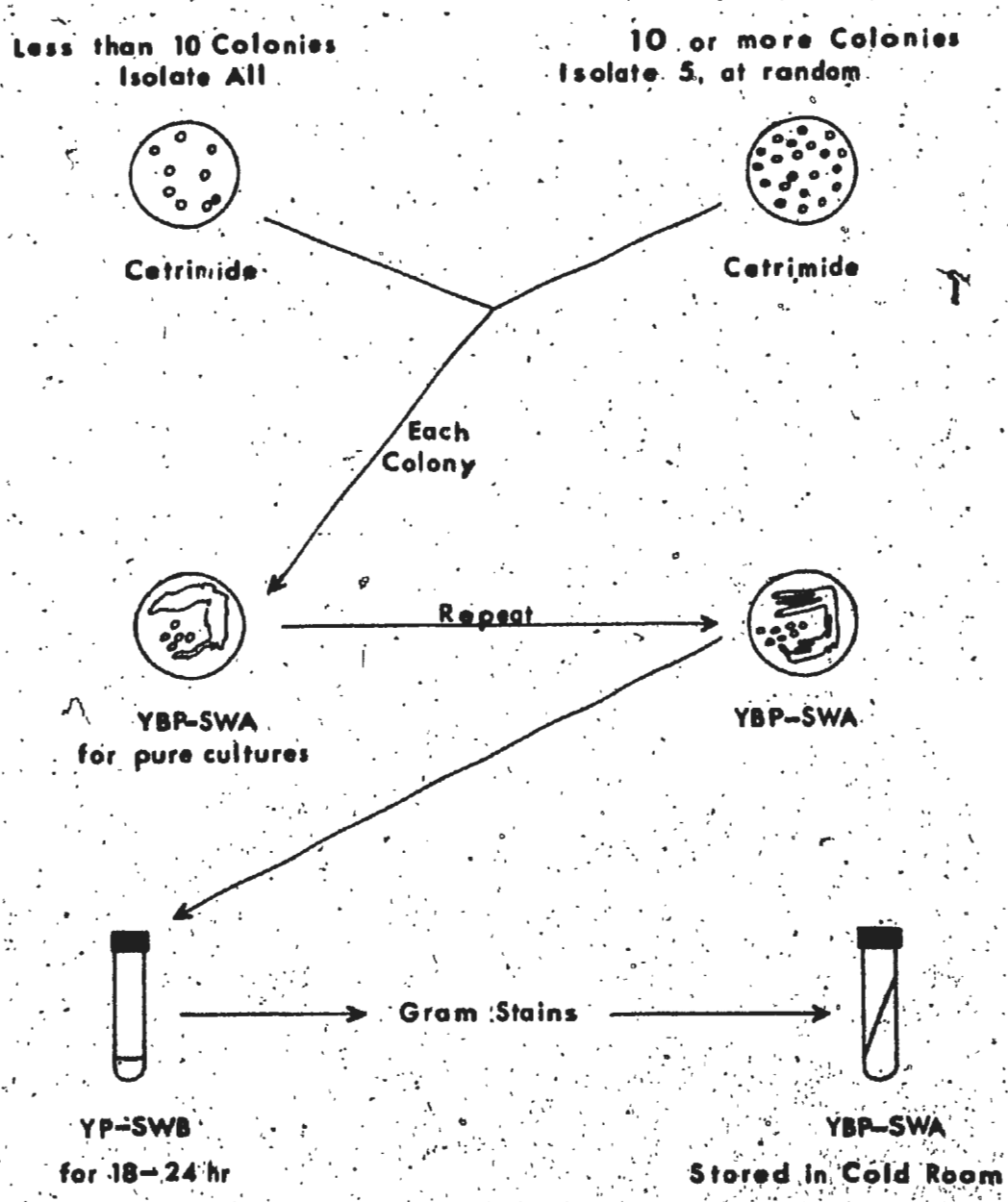
After the final count, each plate was examined for the presence of non-spreading colonies, either non-chromogenic or having a diffusible yellow-green pigment. These were regarded as possible pseudomonads. Plates with no colonies of this type or with colonies crowded so closely together as to make isolation of individual colonies difficult were discarded. The procedures used to isolate colonies from both media are shown schematically in Figures 2 and 3.

(a) Isolation from cetrimide agar

The number of possible pseudomonad colonies was noted. All the colonies were not isolated as this would have yielded too many isolates to work with. Generally, if there were less than ten such colonies on a plate, all were isolated; if ten or more were present, only five colonies, chosen at random, were examined.

To determine whether or not the chosen colonies were pure cultures, each was streaked out to give well-isolated colonies on YBP-SWA. After good growth was obtained, one colony was picked off and the process repeated; this was continued until all the colonies on the plate appeared

Figure 2. Flow diagram for the isolation of possible pseudomonads from cetrimide agar.


Less than 10 Colonies
Isolate All
10 or more Colonies
Isolate 5, at random
Cetrimide
Cetrimide
Each
Colony
Repeat
YBP-SWA
for pure cultures
YBP-SWA
Gram Stains
YP-SWB
for 18-24 hr
YBP-SWA
Stored in Cold Room

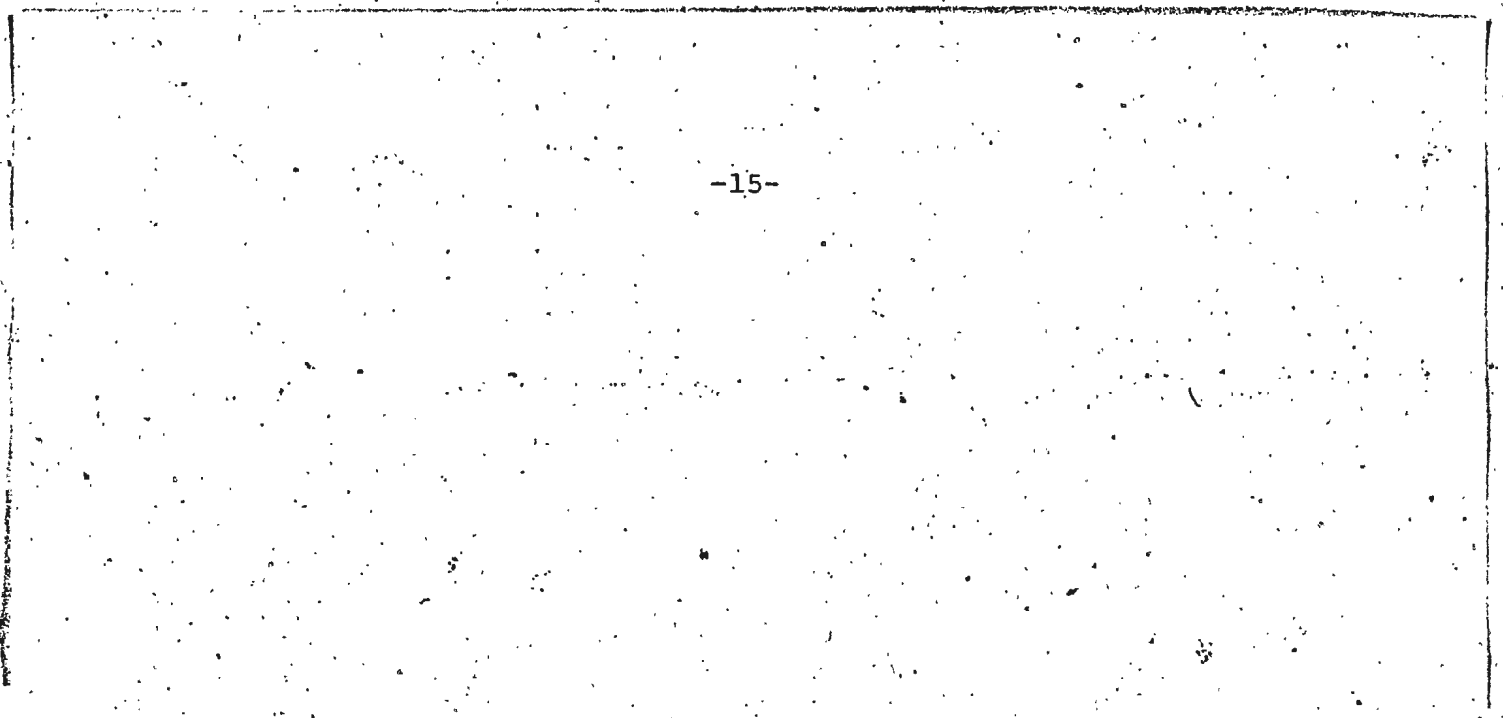
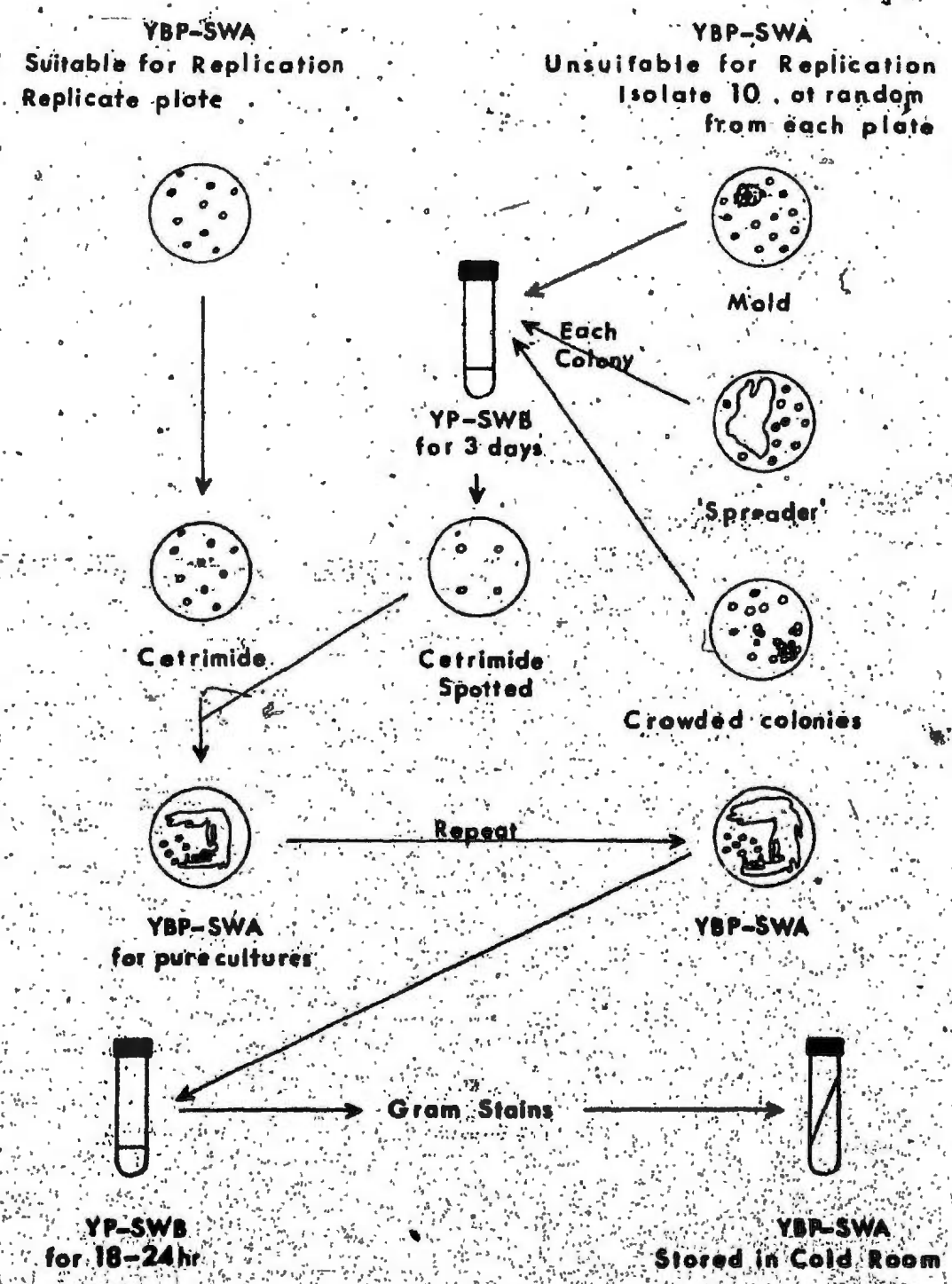


Figure 3. Flow diagram for the isolation of possible pseudomonads from YBP-SWA.


YBP-SWA
Suitable for Replication
Replicate plate
YBP-SWA
Unsuitable for Replication
Isolate 10 , at random
from each plate
Mold
Each
Colony
YP-SWB
for 3 days
'Spreader'
Cetrimide
Cetrimide
Spotted
Crowded colonies
Repeat
YBP-SWA
for pure cultures
YBP-SWA
Gram Stains
YP-SWB
for 18-24 hr
YBP-SWA
Stored in Cold Room

identical. A well-isolated colony was then transferred to yeast-peptone-seawater broth (YP-SWB) (See Formulary) and, after 18-24 hr incubation, a Gram stain was done. Gram-negative rods were transferred to YBP-SWA slants and, after sufficient incubation to give heavy growth, stored in the cold room until further tests could be done. Gram-positive cultures were discarded. Throughout the isolation procedure, all incubation was at 20C.

(b) Isolation from YBP-SWA

One manifestation of the communal growth response phenomenon, described by Roth, Stiles and Clegg (1972), is that the numbers of colonies recovered on selective media may be greater when the inocula consist of masses of bacteria than when they are single cells, as is the case in diluted material. This means that it is possible that more pseudomonads, capable of growth on cetrimide, were present in the samples than the numbers growing on the cetrimide agar plates indicated. Therefore, it was decided to isolate and identify probable pseudomonad colonies from the YBP-SWA plates as well as from the cetrimide agar plates so that, for each sample, a comparison of the numbers of pseudomonads isolated on both media could be made.

Those YBP-SWA plates with discrete and well-isolated

colonies were replicated to cetrimide agar using the Lederberg and Lederberg (1952) replica plating technique. Plates having "spreader" colonies, molds, or colonies crowded closely together on any part of the plate were not replicated. Instead, ten randomly-chosen suitable colonies from each plate were transferred to tubes of YP-SWB, and, after 72-hr incubation, spotted on cetrimide agar with a 2-mm loop. Both replicated and spotted plates were incubated and examined at one-week intervals. When growth occurred, the colonies were streaked out on plates of YBP-SWA and the isolation procedure continued, as described above. If no growth occurred after 3 weeks, the plates were discarded and no further tests were done on the original colonies. All incubation was at 20C.

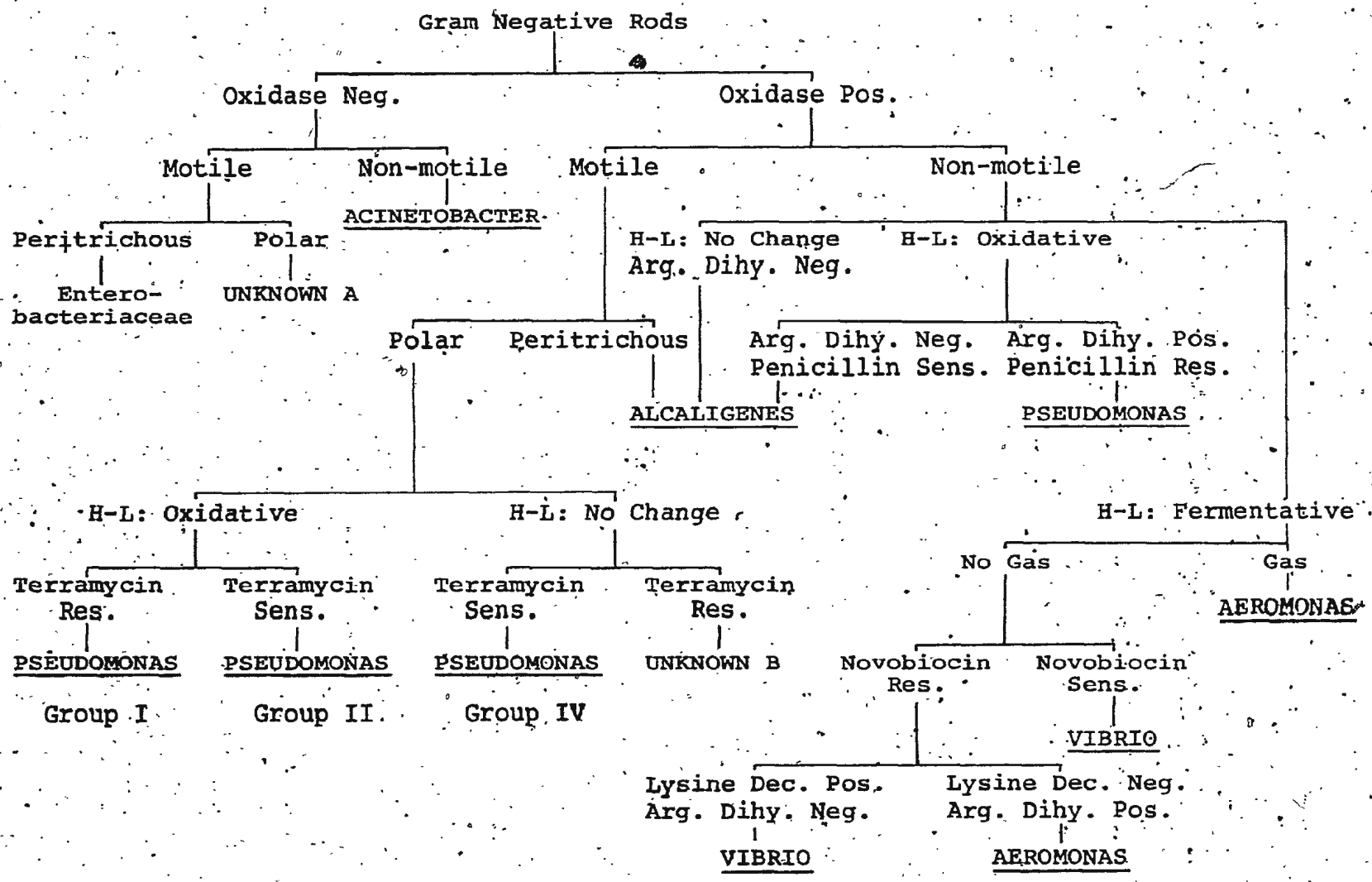
3. Identification of the Isolates

Sufficient characterization of the isolates to identify them as either pseudomonads or non-pseudomonads was carried out. In most cases, identification to the generic level was possible. The isolates were identified by following the scheme shown in Figure 4, based on that given by Scholes and Shewan (1964) and modified to conform to the revised taxonomy of Gram-negative rods given in Bergey's Manual of Determinative Bacteriology (1974). The fluorescent antibody (FA) technique was used as an

Figure 4. Scheme used for the identification of isolates.

Abbreviations used:

Arg. Dihy.	Arginine Dihydrolase
Lysine Dec.	Lysine Decarboxylase
H-L	Hugh-Leifson reaction
Neg.	Negative
Pos.	Positive
Res.	Resistant
Sens.	Sensitive


Gram Negative Rods
Oxidase Neg.
Oxidase Pos.
Motile
Non-motile
Motile
Non-motile
ACINETOBACTER
Peritrichous
Polar
H-L: No Change
Arg. Dihy. Neg.
H-L: Oxidative
Entero-
bacteriaceae
UNKNOWN A
Polar
Peritrichous
Arg. Dihy. Neg.
Penicillin Sens.
Arg. Dihy. Pos.
Penicillin Res.
ALCALIGENES
PSEUDOMONAS
H-L: Oxidative
H-L: No Change
H-L: Fermentative
No Gas
Gas
Terramycin
Res.
Terramycin
Sens.
Terramycin
Sens.
Terramycin
Res.
AEROMONAS
PSEUDOMONAS
PSEUDOMONAS
PSEUDOMONAS
UNKNOWN B
Novobiocin
Res.
Novobiocin
Sens.
Group I
Group II
Group IV
VIBRIO
Lysine Dec. Pos.
Arg. Dihy. Neg.
Lysine Dec. Neg.
Arg. Dihy. Pos.
VIBRIO
AEROMONAS

adjunct to the conventional identification methods.

(a) Biochemical tests

Formulae and methods of preparation for all media and test reagents used are given in the Formulary. The incubation temperature for all the tests was 20C, unless otherwise specified. The following tests were performed:

(i) Gram reaction and morphology:

Smears of 18-24 hr cultures in YP-SWB were made and Gram-stained, then were examined for gram-reaction and cell morphology. Cell dimensions, based on measurement of ~~at least 5~~ 5 to 15 cells per culture, were recorded.

(ii) Oxidase test:

A modification of the method described by Steele (1961) was used. A 7-day culture on YBP-SWA was tested by touching a capillary tube containing the oxidase reagent to the center of a colony and noting the time required for the colony to turn purple. At least five colonies were tested per plate, a fresh capillary tube being used for each colony. The average reaction time was calculated and the oxidase reaction of the culture determined according to the following scale:

Reaction in 0-10 sec	Positive
Reaction in 10-60 sec	Delayed positive
No reaction in 60 sec	Negative.

(iii) Motility:

This was determined by phase contrast examination of a 24-hr culture in YP-SWB. The incubation temperature was lowered to 15C for those cultures found to be non-motile at 20C. Before an isolate was regarded as non-motile, wet mounts were made and examined following each of four successive transfers of the culture at 24-hr intervals through YP-SWB.

(iv) Flagella:

Eighteen to twenty-four-hr YBP-SWA slant cultures of those isolates found to be motile were stained with BBL Flagella Stain, using the technique outlined by Leifson (1951).

(v) Dissimilation of glucose

Bacto-MOF medium (Difco) was used with the technique of Hugh and Leifson (1953).

(vi) Production of gas from glucose:

Durham tubes were used in tubes of basal medium, the composition of which was based on that of Hugh and Leifson (1953). Glucose to give a final concentration of 1% was added.

(viii) Sensitivity to antibiotics:

The following antibiotics were used: penicillin, 2.5 I U (Bacto-concentration discs) and 10 I U (Bacto-

sensitivity discs); novobiocin, 5 mcg (Bacto-sensitivity discs); and 2,4 - diamino - 6,7 - diisopropyl pteridine (0/129) prepared in the laboratory.

The surfaces of plates of yeast-beef-peptone-synthetic seawater agar (YBP-SYN-SWA) were swabbed with turbid broths (3-4-days growth) before the antibiotic discs were pressed firmly onto the plates. The plates were read at 48-hr intervals.

(viii) Arginine dihydrolase:

The method of Thornley (1960) was used.

(ix) Decarboxylase tests:

The method of Møller (1955) utilizing arginine, lysine and ornithine was followed. Bacto-Decarboxylase Base Moeller (Difco) was used.

(x) Production of fluorescein:

Bacto-Pseudomonas agar F was used. The technique of King, Ward, and Raney (1954) was followed, and the plates were observed in the dark under short-wave ultraviolet light.

(xi) Production of pyocyanin:

Bacto-Pseudomonas agar P was used.

(xii) Growth on fresh water medium:

Three-mm loopfuls of turbid (3-4 day) broth cultures

were inoculated onto slants of yeast-beef-peptone-fresh water agar (YBP-FWA). These were incubated for periods up to four weeks and examined at intervals for growth.

(b) Fluorescent antibody technique

The tentative identifications arrived at by means of the biochemical tests were verified by the use of the indirect fluorescent antibody (FA) staining method.

The procedure of Moskovits and Foelsche (1970) was used for the production of antibody to Pseudomonas fluorescens (ATCC 13525, neotype strain). The concentration of cells obtained for immunization was 4.37 - 4.5 x 10^9 per ml, by direct count.

Five New Zealand White rabbits were immunized subcutaneously for the production of immune serum, and two were used to produce normal sera. The animals received 4 injections per week with a 2-day rest period between weeks. The dosages given were: 0.2 ml, 0.4 ml, 0.8 ml, 1.0 ml, 1.0 ml, 1.5 ml, 1.5 ml, 2.0 ml, 2.0 ml, 3.0 ml, 3.0 ml, 5.0 ml. Prior to immunization, each rabbit was tested for the presence of antibodies to P. fluorescens antigen. Using the tube agglutination method (Evans, 1957), zero titers were obtained in all cases.

Eight days after the final immunization, trial bleedings

were made. Antibody titers of 1:640 to 1:1280 were found. Ear vein bleedings were made 2, 6, 9 and 11 days later, without reduction in titer. Sera from the four bleedings were pooled and filter-sterilized.

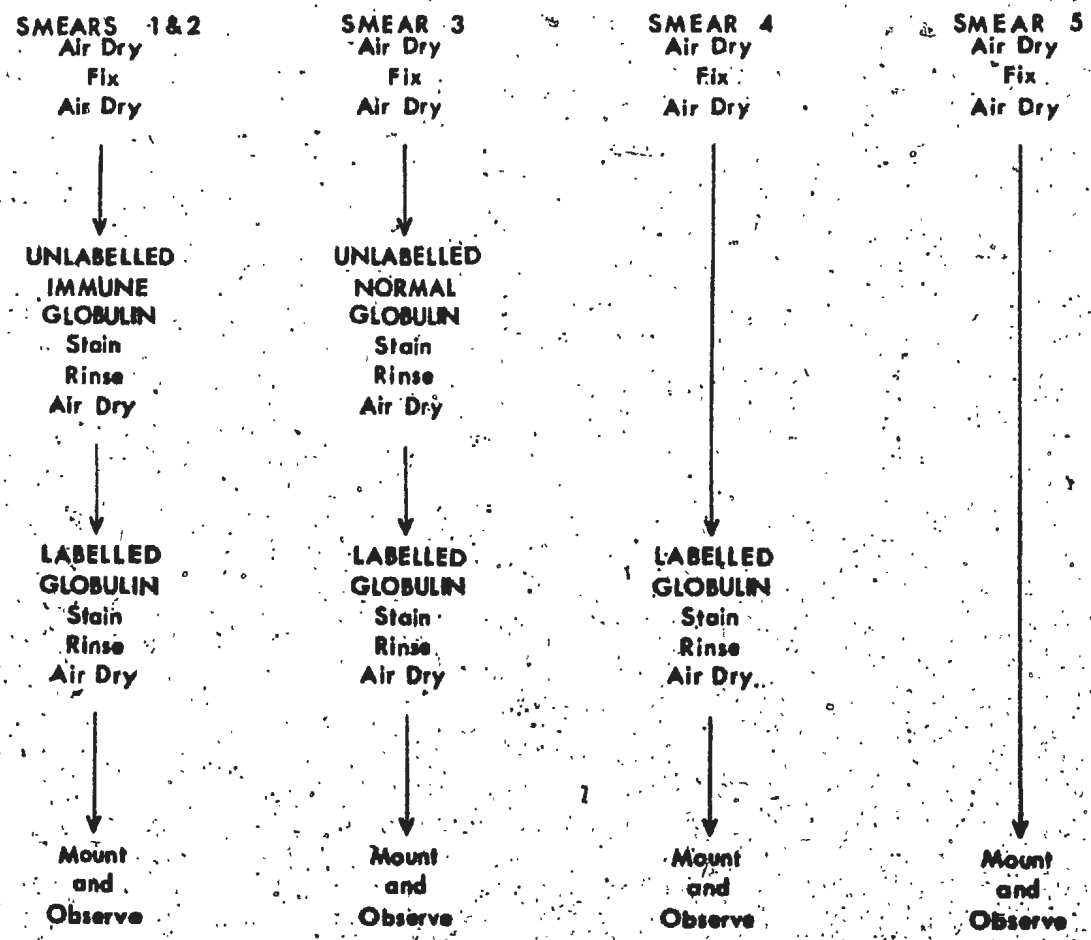
The procedure of Cherry, Goldman, Carski, and Moody (1960) was used for the fractionation of globulins from both the immune and the normal sera and for the performance of the indirect and control tests. The protein contents of the immune and normal sera, determined with a Bausch and Lomb serum protein meter, were 3.8 and 3.5% respectively. The globulins were not diluted and were frozen until used.

All staining was done in moist chambers at room temperature (20-25C) for 30 minutes. Bacto-FA buffer and Bacto-FA mounting fluid (both pH 7.2) were used for rinsing and mounting the slides. The labelled globulin used was Bacto-FA rabbit globulin antiglobulin (goat).

From 48-hr slants of each isolate, five smears were made, air-dried and fixed in 95% ethanol. Two smears were stained with both unlabelled immune globulin and labelled antiglobulin. Two were used as controls; one with unlabelled normal globulin in place of unlabelled immune globulin and the other without unlabelled immune globulin, both followed by labelled antiglobulin. The



Figure 5. Flow diagram for fluorescent antibody staining.


SMEARS 1&2
Air Dry
Fix
Air Dry
UNLABELLED IMMUNE GLOBULIN
Stain
Rinse
Air Dry
LABELLED GLOBULIN
Stain
Rinse
Air Dry
Mount and Observe
SMEAR 3
Air Dry
Fix
Air Dry
UNLABELLED NORMAL GLOBULIN
Stain
Rinse
Air Dry
LABELLED GLOBULIN
Stain
Rinse
Air Dry
Mount and Observe
SMEAR 4
Air Dry
Fix
Air Dry
LABELLED GLOBULIN
Stain
Rinse
Air Dry
Mount and Observe
SMEAR 5
Air Dry
Fix
Air Dry
Mount and Observe

last smear, used to detect autofluorescence, was processed without the use of globulins. A flow diagram for the staining procedures is given in Figure 5.

As soon as they were prepared, the slides were examined with a binocular Zeiss Universal microscope equipped with 12.5X wide field eyepieces and a 100X oil immersion objective. A brightfield condenser (N.A. = 1.4) was used. The illuminant was an Osram HBO-200W mercury vapor lamp. A UG-1 (3mm) exciter filter, a BG-38 red-absorbing filter, and a No. 41 barrier filter were used.

Fluorescence of the organisms was rated as follows:

4+	Brillant yellow-green color, very sharp
3+	Bright yellow-green color, sharp and clear
2+	Definite fluorescence, color definitely yellow-green, but of low intensity
1+	Definite fluorescence, color non-descript, not definite green
$\pm$	Borderline reaction between 1+ and 0 reaction
0	No fluorescence.

This scale was used by Moskovits and Foelsche (1970).

RESULTS

TEMPERATURE

The water temperatures at the depths sampled on each trip are shown in Table 1, Appendix I. Figures 6, 7 and 8 show the temperature changes which occurred for the surface, middepth and bottom, respectively.

From September 12 to January 25, the surface water temperature fell from 13.0C to -1.0C. The temperature remained at -1.0C until March 21, rose to 0.5C on May 15 and May 29, and then, increased steadily to 14.0C on August 10. The variation from minimum (-1.0C) to maximum (14.0C) temperatures was 15.0C. These data show the existence of an annual cycle of surface temperatures with maxima in late summer (August - September) and minima in winter (January - March).

The middepth (thermocline) water temperature fell continuously from a maximum of 11.8C on September 12 to -1.0C on January 25, where it remained until June 13. The temperature increased to 5.0C on July 10, fell to 2.8C on July 24, and rose again to 5.5C on August 10. The difference between minimum (-1.0C) and maximum (11.8C)

temperatures was 12.8C. The cyclic nature of the middepth temperature regime is still evident, but, due to the low temperatures recorded in the late 1973 trips, is not as pronounced as that of the surface.

From September 12 to October 24, the bottom water temperature decreased from 1.7C to -0.6C; then, on November 23, it rose to an anomalous 3.0C, the highest bottom water temperature recorded. On January 25, the temperature fell to -1.0C, where it remained until May 29, before dropping to the minimum of -2.0C on June 27. The temperature rose to -1.2C on July 10, decreased slightly to -1.3C on July 24 and increased to -0.8C on August 10. The difference between minimum (-2.0C) and maximum (3.0C) temperatures was 5.0C. There was little evidence of seasonal variation in bottom water temperature.

Data in Table 1 also show that, with respect to temperature, the water column was homogeneous from top to bottom from November 23 to March 21, and nearly homogeneous on May 15 and May 29, (top to bottom temperature differences were 1.5C).

The trips made in September, on October 24, and from June to August show that thermal stratification existed. Data on the extent of this stratification is given in Table 2, Appendix I. This began at depths below the

surface ranging from 5 to 22 m and showed thicknesses of 5 to 15 m. Stratification was most pronounced on September 12, July 24 and August 10, which showed a temperature change of 0.8C per meter of thermocline depth.

SALINITY

Table 6, Appendix I, shows the salinity changes which occurred during the sampling period at each of the depths sampled. Salinity values ranged from a minimum of 31.4°/oo on August 10 to a maximum of 36.3°/oo on June 27 for surface waters; from 3.14°/oo on August 10 to 35.7°/oo on January 25 for middepth (thermocline) waters; and from 34.5°/oo on August 10 to 38.5°/oo on June 13 for bottom waters. Generally, salinities increased with increasing depth. The values obtained are closely related to open ocean salinities, which range from 33.0°/oo to 37.0°/oo (Fairbridge, 1966), and reflect the absence of fresh water run-off into the sampling area.

TOTAL BACTERIA COUNTS

Table 1, Appendix II shows the numbers of bacterial colonies growing on the YBP-SWA plates spread with undiluted sea water for each trip. All counts were

extremely low and in 211 or 78% of the 270 plates, no colonies were produced. Because of this, all quantitative bacterial data were determined from the counts made on the plankton tow samples, after 3 weeks incubation.

Tables 3 and 4, Appendix II, give the total numbers of bacteria per liter of plankton tow based on growth on YBP-SWA following incubation at 20C and 5C, respectively. The calculations on which these numbers are based are provided in Table 2, Appendix II. Figures 6, 7 and 8 compare the counts per liter of plankton tow based on 20C incubation with those based on 5C incubation for surface, middepth (thermocline), and bottom, respectively.

The 20C and 5C counts per liter were compared statistically using the T test for paired observations. The calculations involved are provided in Table 5, Appendix II. The null hypothesis, that the two incubation temperatures produced no difference in bacterial counts, was accepted, even using a level of significance (α) of 0.10. This means that the results provide no evidence that either incubation temperature is more favourable than the other in allowing the growth of maximal numbers of bacterial colonies.

Figure 6 shows the numbers of bacteria per liter of surface plankton tow samples and the surface water

Figure 6. Top: Total bacteria per liter of surface plankton tow for the sampling period, September 12, 1971 to August 10, 1972, on YBP-SWA at 20C and 5C incubation.

Bottom: Surface water temperatures during the sampling period.

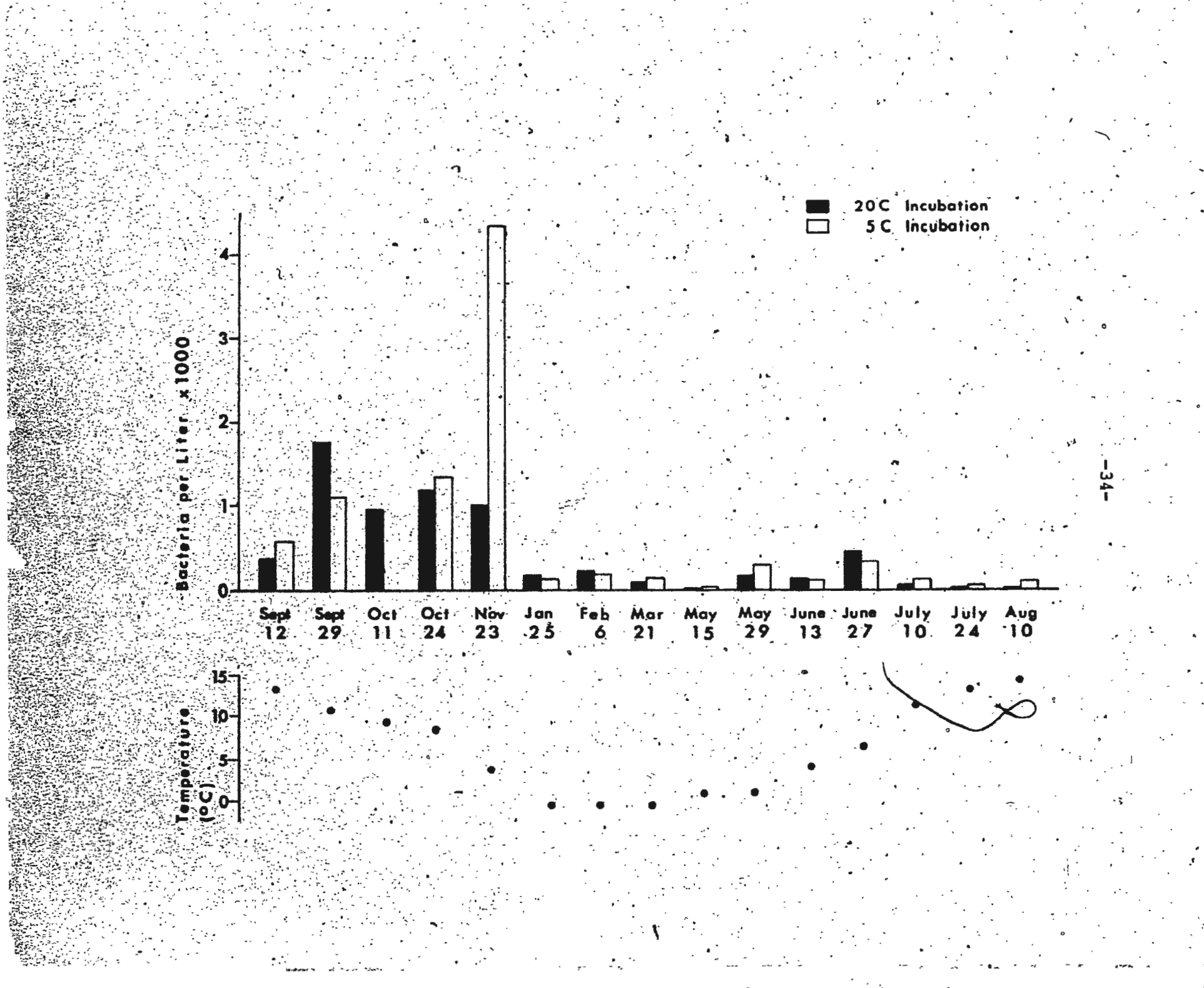

20C Incubation
5 C Incubation
Bacteria per Liter, x 1000
0
1
2
3
4
Sept 12
Sept 29
Oct 11
Oct 24
Nov 23
Jan 25
Feb 6
Mar 21
May 15
May 29
June 13
June 27
July 10
July 24
Aug 10
Temperature (°C)
0
5
10
15

Figure 7. Top: Total bacteria per liter of middepth (thermocline) plankton tow for the sampling period, September 12, 1971 to August 10, 1972, on YBP-SWA at 20C and 5C incubation.

Bottom: Middepth (thermocline) water temperatures during the sampling period.

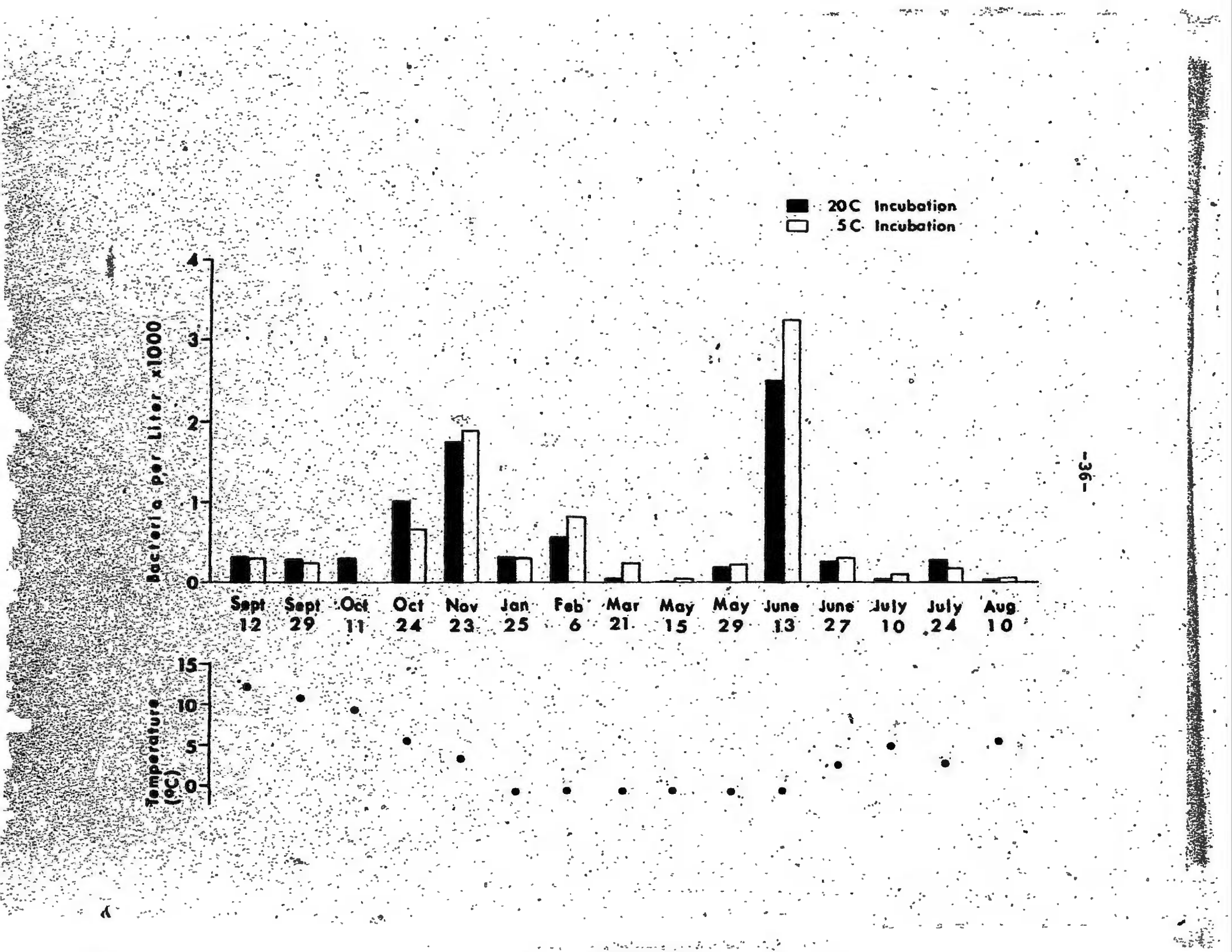
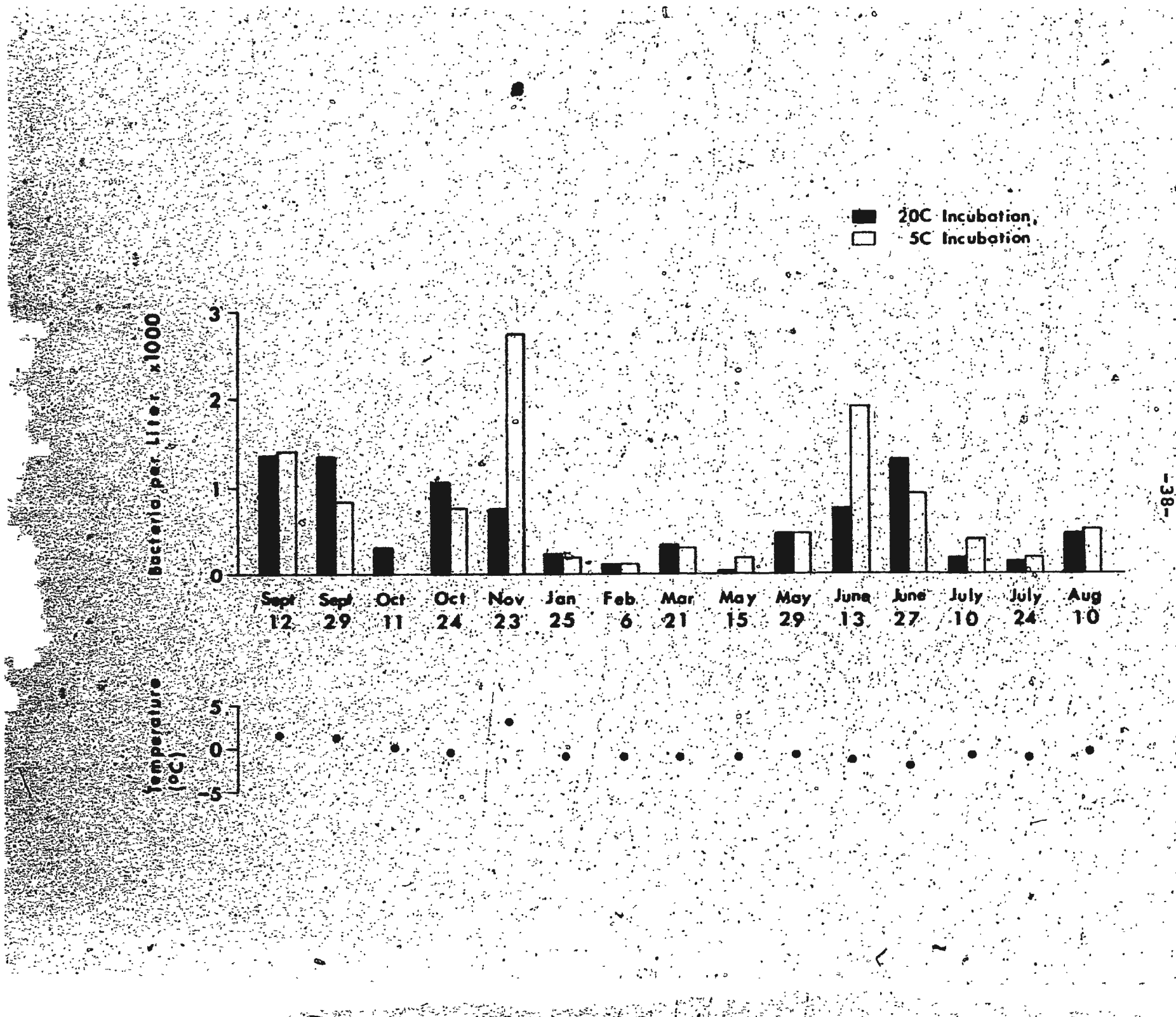

20C Incubation
5C Incubation
Bacteria per Liter x1000
4
3
2
1
0
Sept 12
Sept 29
Oct 11
Oct 24
Nov 23
Jan 25
Feb 6
Mar 21
May 15
May 29
June 13
June 27
July 10
July 24
Aug 10
Temperature (°C)
15
10
5
0

Figure 8. Top: Total bacteria per liter of bottom plankton tow for the sampling period, September 12, 1971 to August 10, 1972, on YBP-SWA at 20C and 5C incubation.

Bottom: Bottom water temperatures during the sampling period.


20C Incubation
5C Incubation
Bacteria per Liter x1000
3
2
1
0
Sept 12
Sept 29
Oct 11
Oct 24
Nov 23
Jan 25
Feb 6
Mar 21
May 15
May 29
June 13
June 27
July 10
July 24
Aug 10
Temperature (°C)
5
0
-5

temperatures recorded for each trip. The 20C counts ranged from a high of 1,732 per liter on September 29 to a low of 10 per liter on May 15. The 5C counts ranged from a maximum of 4,329 per liter on November 23 to a minimum of 23 per liter on May 15. The highest counts at both incubation temperatures were found from September to November when water temperatures were falling (13.0C to 3C). Except for the small increases in bacterial numbers on May 29 and June 27, counts at both incubation temperatures for the remainder of the sampling period were low, not only when the water temperatures were lowest (-1.0C to 0.5C) from January 25 to May 15, but also from July 10 to August 10 when water temperatures were increasing to the maximum (11.0C to 14.0C). The May 29 increase was associated with a low water temperature (0.5C), while the June 27 increase occurred at 6C.

Numbers of bacteria per liter of middepth (thermocline) plankton and middepth (thermocline) water temperatures are shown in Figure 7. At 20C incubation, the counts per liter ranged from a high of 2,573 on June 13 to a low of 18 on May 15. At 5C incubation, the counts ranged from 3,257 per liter on June 13 to 65 per liter on August 10. For both incubation temperatures, two maxima - a larger one on June 13 and a smaller one on November 23 -

were recorded. The June 13 maximum occurred at the lowest water temperature (-1.0C); the November 23 maximum occurred when the water temperature was 3.0C.

Bacteria counts per liter of bottom plankton and bottom water temperatures are shown in Figure 8. The 20C counts ranged from a high of 1,414 per liter on September 12 to a low of 41 per liter on May 15, and the 5C counts from 2,751 on November 23 to 140 on February 6. Peaks of approximately the same height for the 20C counts occurred on September 12 and 29 and on June 27. For the 5C counts, a large peak occurred on November 23 and a smaller peak on June 13. Although the September and November highs correspond to the highest recorded bottom water temperatures (1.7C, 1.0C, and 3.0C, respectively) the June peaks occurred when water temperatures were lowest (-1.5 and -2.0C).

In examining the bacterial numbers obtained at both 20C and 5C and the water temperatures observed, no clear relationship can be observed. ~~Low~~ Low, as well as high, bacterial numbers were found at the lowest water temperatures, ~~but much higher bacterial numbers were also found at the lowest water temperatures~~. On the other hand, in early September and from July to August, when surface water temperatures were highest, bacterial numbers were low or very low.

Temperature-wise, in going from surface to middepth (thermocline) to bottom waters, one passes from a pronounced cyclic pattern, to a less pronounced cyclic pattern, to a situation, in the bottom waters, where water temperatures tend to be uniformly quite low throughout the year.

The surface, middepth (thermocline) and bottom counts of total bacteria per liter were compared statistically using the T test for paired observations. The calculations involved are given in Table 6, Appendix II. The null hypothesis, that different sampling depths produced no difference in bacterial counts were accepted, using a level of significance (α) of 0.10.

IDENTIFICATION OF ISOLATES

Tables 1-6, Appendix III, show the characteristics determined for those genera to which the isolates examined were assigned. Identification to the species level was not attempted. More than one "type" of each genus was identified; these have been given the arbitrary designations of Types A, B, and so on. Only 20 isolates falling into two distinct groups, could not be identified to genus according to the scheme used and so were designated Unknown A and B.

None of the isolates studied were able to grow well on freshwater medium.

The range of cell dimensions and morphology for the genera isolated are given in Table 7, Appendix III.

Table 8, Appendix III, gives the results of the fluorescent antibody staining. Those isolates identified as pseudomonads generally gave brighter fluorescence than those regarded as non-pseudomonads. Group IV pseudomonads generally produced less intense fluorescence than did those of Groups I and II. Three of the seven isolates designated Unknown B showed a maximum fluorescence equivalent to that shown by Group IV pseudomonads. Autofluorescence was not observed with any of the isolates.

Altogether 2580 cultures (1321 isolated from samples plated onto YBP-SWA and 1259 isolated from samples plated onto cetrimide agar) were identified. Only 391 of these were non-pseudomonads. Tables 9 and 10, Appendix III, give the breakdown, by sample, of the distribution of the pseudomonad and non-pseudomonad isolates, respectively. It is possible to make comparisons between the numbers of Group I and II pseudomonads isolated on any trip. However, similar comparisons involving Group IV pseudomonads and non-pseudomonads should not be made as cetrimide, which is

known to inhibit the growth of these organisms, was used in the isolation procedure and, therefore, isolates so identified may be regarded as exceptions and not representative of all the Group IV pseudomonads and non-pseudomonads present. There are no data on the identity of the organisms present in the samples which were unable to grow on cetrimide.

PSEUDOMONAD COUNTS

The numbers of pseudomonads per liter based on counts of possible pseudomonads made on YBP-SWA and cetrimide agar are given in Tables 2 and 3, respectively, Appendix IV. The calculations involved are given in Table 1, Appendix IV. No values are given for the trips of July 24 and August 10. After 1 to 2 weeks incubation, large numbers of molds appeared on the original plates of all samples taken on these trips so that, although total counts could be made on these plates, no isolates could be taken from them.

Using the T test for paired observations, counts of pseudomonads per liter of plankton derived from YBP-SWA and cetrimide agar plates were compared. The calculations are presented in Table 4, Appendix IV. Even with a level of significance (α) of 0.10, the null hypothesis, that there were no differences in these

two sets of counts, was not rejected. There is, therefore, no evidence that more pseudomonads can be recovered by isolation onto YBP-SWA before transfer to cetrimide than by direct isolation onto cetrimide.

Figure 9 shows the numbers of pseudomonads per liter of surface plankton and the surface water temperature. On YBP-SWA, the counts per liter ranged from a high of 865 on September 29 to a low of 1 on May 15. The cetrimide counts went from the maximum of 650 per liter on October 24 to the minimum of 1 per liter on May 15. Highest numbers on both media occurred in the autumn when water temperatures were falling. A small peak occurred on June 27, according to the YBP-SWA counts, but the cetrimide counts showed no sign of a spring-summer maximum. For both media, lowest numbers were recorded during the period of lowest water temperature, but the low counts for July 10 corresponded to a high water temperature.

Pseudomonads per liter of middepth plankton and middepth water temperature are shown in Figure 10. The highest counts per liter, 1,013 on YBP-SWA and 801 on cetrimide, were recorded on November 23, while the lowest counts, 9 on YBP-SWA and 7 on cetrimide occurred on May 15. For both media, prominent peaks occurred on November 23 and smaller ones on June 13. The YBP-SWA counts showed

Figure 9. Top: Pseudomonads per liter of surface plankton tow for the sampling period, September 12, 1971 to July 10, 1972, on YBP-SWA and cetrimide agar at 20C incubation.

Bottom: Surface water temperatures during the sampling period.

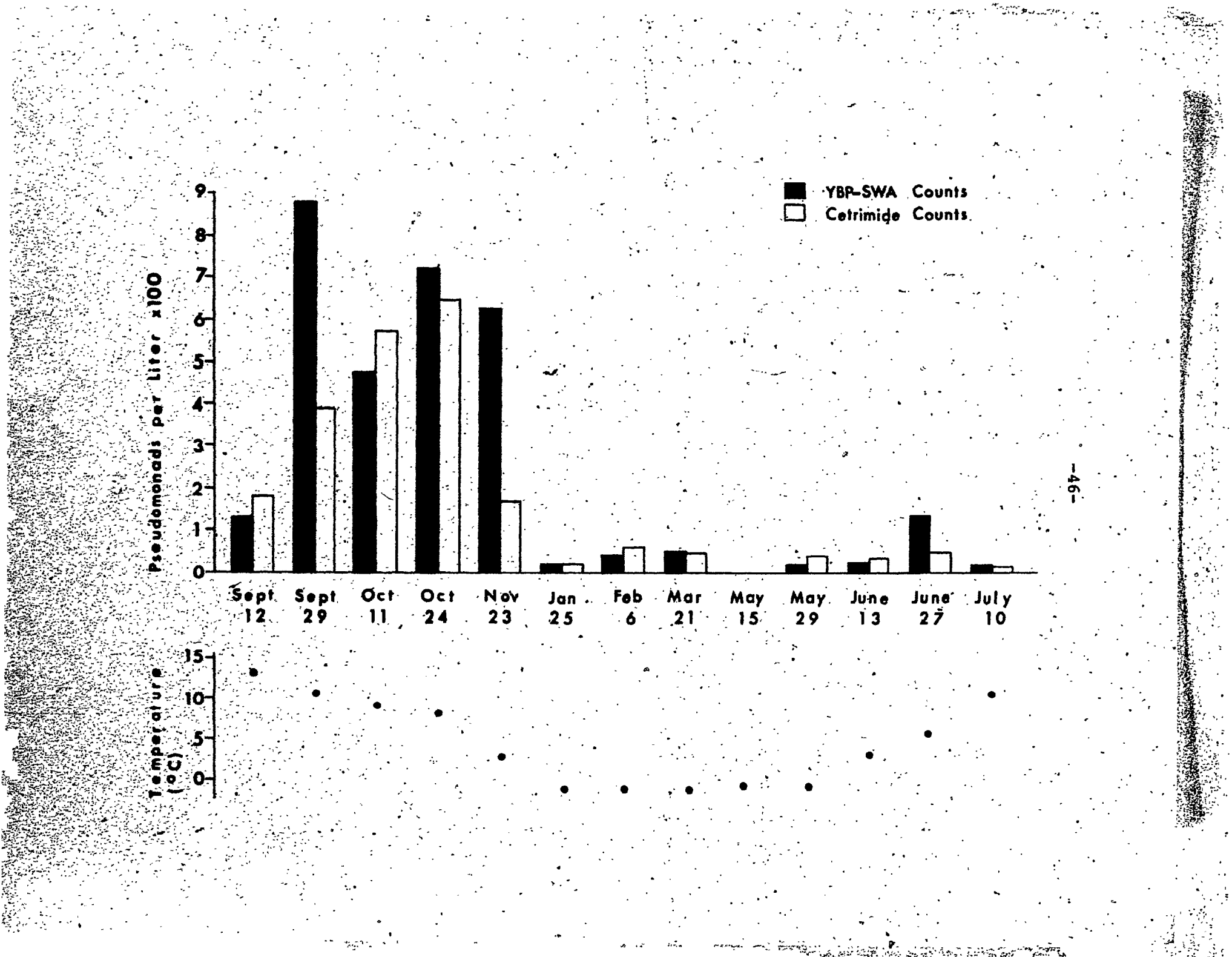
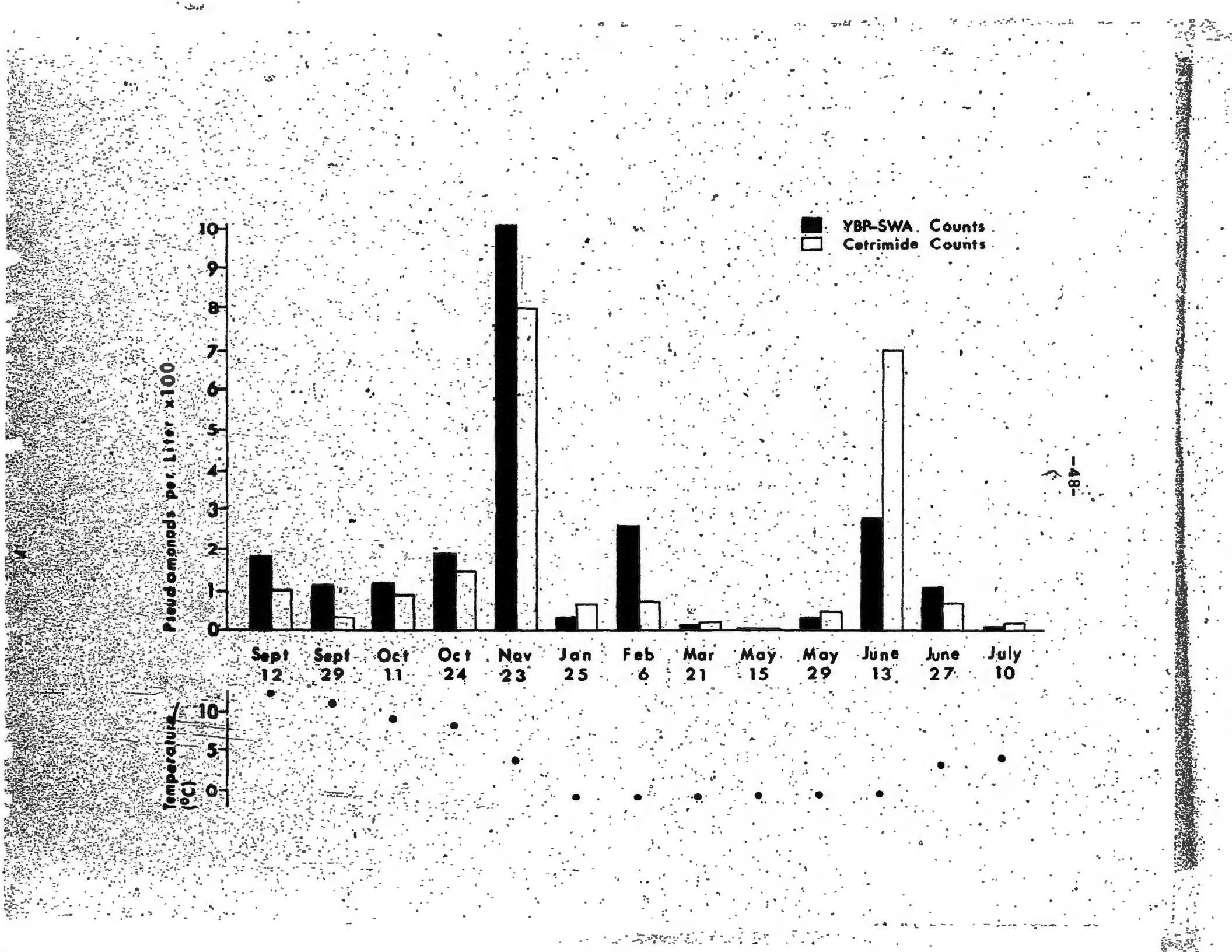

YBP-SWA Counts
Cetrimide Counts
Pseudomonads per Liter x100
0
1
2
3
4
5
6
7
8
9
Sept 12
Sept 29
Oct 11
Oct 24
Nov 23
Jan 25
Feb 6
Mar 21
May 15
May 29
June 13
June 27
July 10
Temperature (°C)
0
5
10
15

Figure 10. Top: Pseudomonads per liter of middepth (thermocline) plankton tow for the sampling period, September 12, 1971 to July 10, 1972, on YBP-SWA and cetrimide agar at 20C Incubation.

Bottom: Middepth (thermocline) water temperatures during the sampling period.


YBP-SWA Counts
Cetrimide Counts
Pseudomonads per Liter x 100
10
9
8
7
6
5
4
3
2
1
0
Sept 12
Sept 29
Oct 11
Oct 24
Nov 23
Jan 25
Feb 6
Mar 21
May 15
May 29
June 13
June 27
July 10
Temperature (°C)
10
5
0

a third peak, just slightly smaller than that of June 13, on February 6. The 1973 peaks occurred during the period of lowest water temperature but the November 23 peaks occurred when the water temperature was neither highest nor lowest.

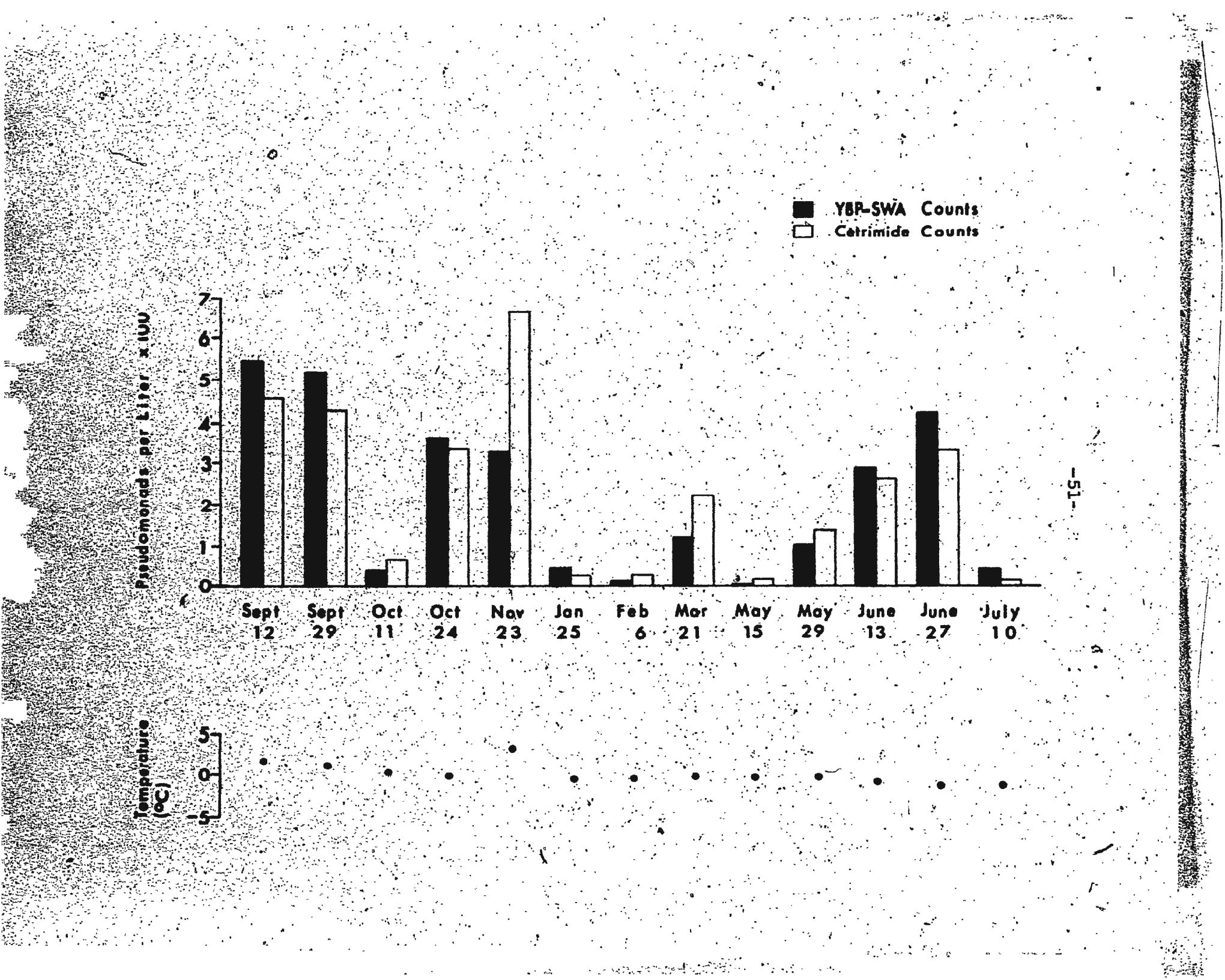
Figure 11 shows the pseudomonads per liter of bottom plankton and the bottom water temperature. The YBP-SWA counts ranged from a maximum of 553 per liter on September 12 to a minimum of 6 per liter on May 15, and cetrimide counts from 686 per liter on November 23 to 21 per liter on both May 15 and July 10. The YBP-SWA counts peaked in September of 1972 and June of 1973 while the cetrimide counts showed peaks in November and June. For both media, the September and November maxima occurred when water temperatures were highest and the June maxima when temperatures were lowest.

The numbers of pseudomonads per liter, regardless of medium used, at any level do not appear related to temperature, throughout the sampling period.

The surface, middepth (thermocline) and bottom counts of pseudomonads per liter were compared statistically using the T test. The calculations are shown in Table 6, Appendix IV. The null hypothesis, that different counts were not produced from samples from the different

Figure 11. Top: Pseudomonads per liter of bottom plankton tow for the sampling period, September 12, 1971 to July 10, 1972, on YBP-SWA and cetrimide agar at 20C incubation.

Bottom: Bottom water temperatures during the sampling period.


YBP-SWA Counts
Cetrimide Counts
Pseudomonads per Liter x 100
0
1
2
3
4
5
6
7
Sept 12
Sept 29
Oct 11
Oct 24
Nov 23
Jan 25
Feb 6
Mar 21
May 15
May 29
June 13
June 27
July 10
Temperature (°C)
5
0
-5

depths was accepted, using a level of significance (α) of 0.10. This indicated the lack of any regular pattern of vertical distribution of pseudomonads.

Table 5, Appendix IV compares the numbers of pseudomonads with total bacteria per liter for each sample and gives the percentages of pseudomonads. This is shown graphically, in Figures 12-14, for surface, middepth and bottom, respectively.

The percentage of bacteria which were pseudomonads in the surface samples ranged from a minimum of 10% on May 15 to a maximum of 76% on March 21. Except for the high percentage in March, pseudomonads were more dominant in the samples from September to November than in the January to July samples.

The percentage of pseudomonads in the middepth samples ranged from 11% on June 13 to 57% on November 23. It varied from trip to trip and no trend can be seen.

For the bottom samples, pseudomonads comprised from 13% on February 6 to 46% on November 23, of the total bacteria. No seasonal trend is apparent.

Table 6, Appendix IV, presents the results of T tests performed on these data and shows that the surface

Figure 12. Total bacteria and pseudomonads per liter of surface plankton tow for the sampling period, September 12, 1971 to July 10, 1972, on YBP-SWA at 20C incubation. The numbers represent percentages of the total bacteria which are pseudomonads.

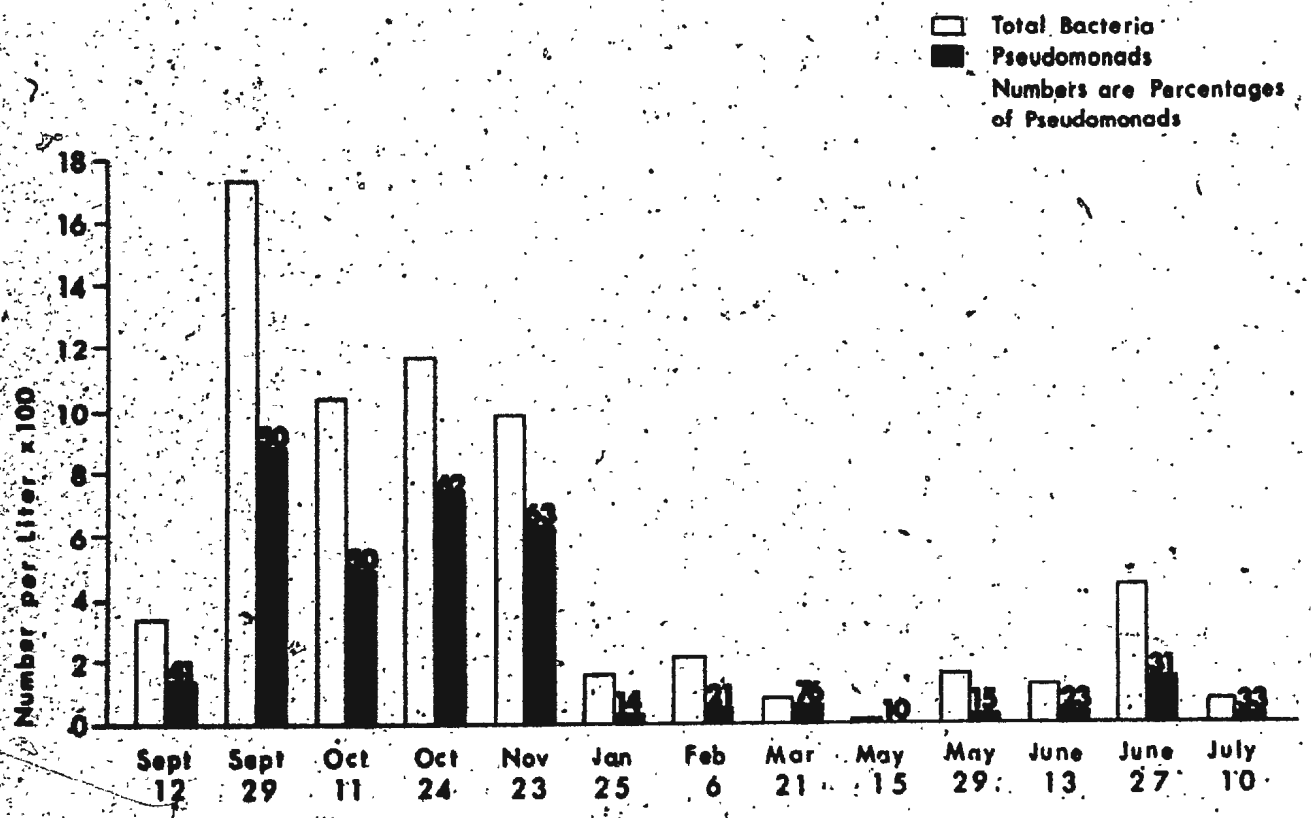

Total Bacteria
Pseudomonads
Numbers are Percentages of Pseudomonads
Number per Liter x 100
0
2
4
6
8
10
12
14
16
18
Sept 12
Sept 29
Oct 11
Oct 24
Nov 23
Jan 25
Feb 6
Mar 21
May 15
May 29
June 13
June 27
July 10

Figure 13. Total bacteria and pseudomonads per liter of middepth (thermocline) plankton tow for the sampling period, September 12, 1971 to July 10, 1972, on YBP-SWA at 20C incubation. The numbers represent percentages of total bacteria which are pseudomonads.

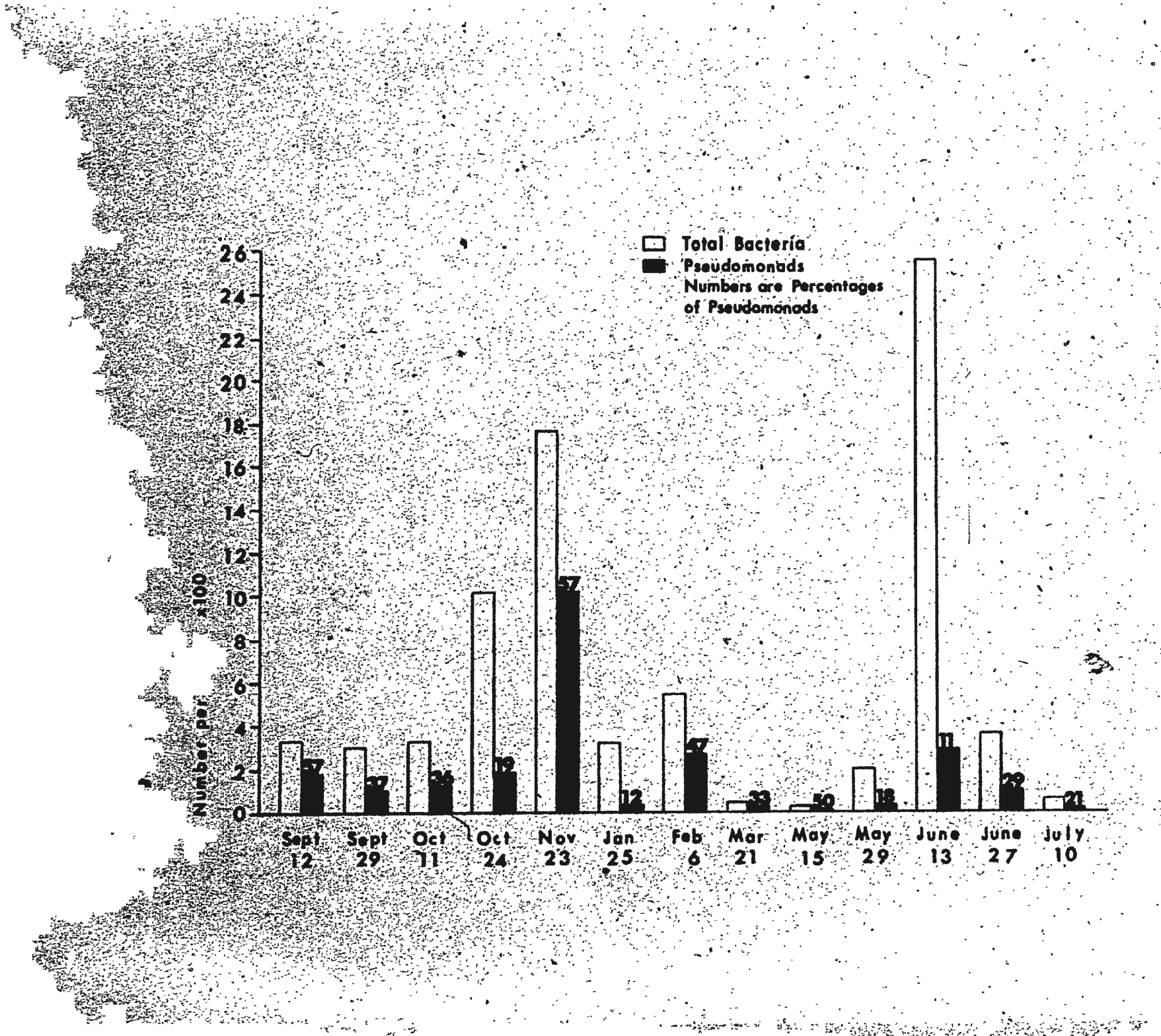
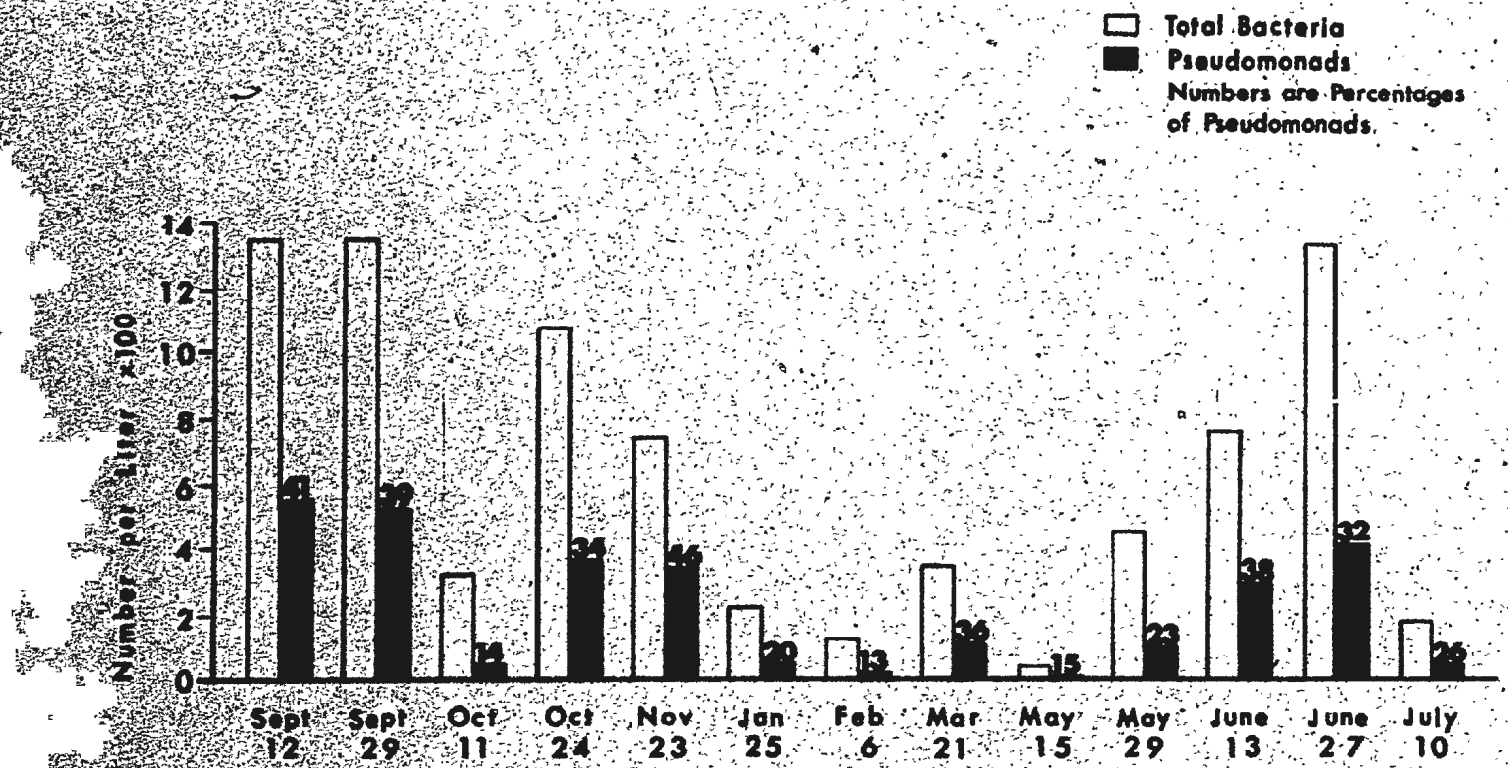

Total Bacteria
Pseudomonads
Numbers are Percentages of Pseudomonads
Number per x100
26
24
22
20
18
16
14
12
10
8
6
4
2
0
57
27
26
19
57
12
27
33
50
18
11
29
21
Sept 12
Sept 29
Oct 11
Oct 24
Nov 23
Jan 25
Feb 6
Mar 21
May 15
May 29
June 13
June 27
July 10

Figure 14. Total bacteria and pseudomonads per liter of bottom plankton tow for the sampling period, September 12, 1971 to July 10, 1972, on YBP-SWA at 20C incubation. The numbers represent percentages of total bacteria which are pseudomonads.


Total Bacteria
Pseudomonads
Numbers are Percentages of Pseudomonads.
Number per Liter x 100
0
2
4
6
8
10
12
14
41
39
14
34
46
20
13
26
15
23
38
32
26
Sept 12
Sept 29
Oct 11
Oct 24
Nov 23
Jan 25
Feb 6
Mar 21
May 15
May 29
June 13
June 27
July 10

percentages were significantly higher than the bottom percentages when the level of significance (α) equals 0.10. There is no evidence to show that significant differences exist between surface and middepth or between middepth and bottom.

PHYTOPLANKTON COUNTS

Phytoplankton count data are presented in Table 1, Appendix V, and shown graphically in Figures 15 to 17, for surface, middepth and bottom, respectively.

In the surface samples, numbers of diatoms increased slowly from 1 per liter on September 12 to 203 per liter on February 6, and then more quickly to reach a peak of 23,740 per liter on May 15. From May 15 to July 10, their numbers decreased and they were not found in the remaining samples. Dinoflagellate numbers peaked at 168 per liter on September 12, decreased gradually to 11 cells per liter on January 25, then increased gradually to 53 cells per liter on June 27, before reaching a maximum population of 566 cells per liter on July 24. Dinoflagellate numbers were lowest during the winter months. Silicoflagellates were present only from November to May with a peak population of 341 cells per liter on March 21.

Figure 15. Top: Total numbers of diatoms, dinoflagellates, and silicoflagellates per liter of surface plankton tow for the sampling period, September 12, 1971 to August 10, 1972.

Bottom: Surface water temperatures during the sampling period.

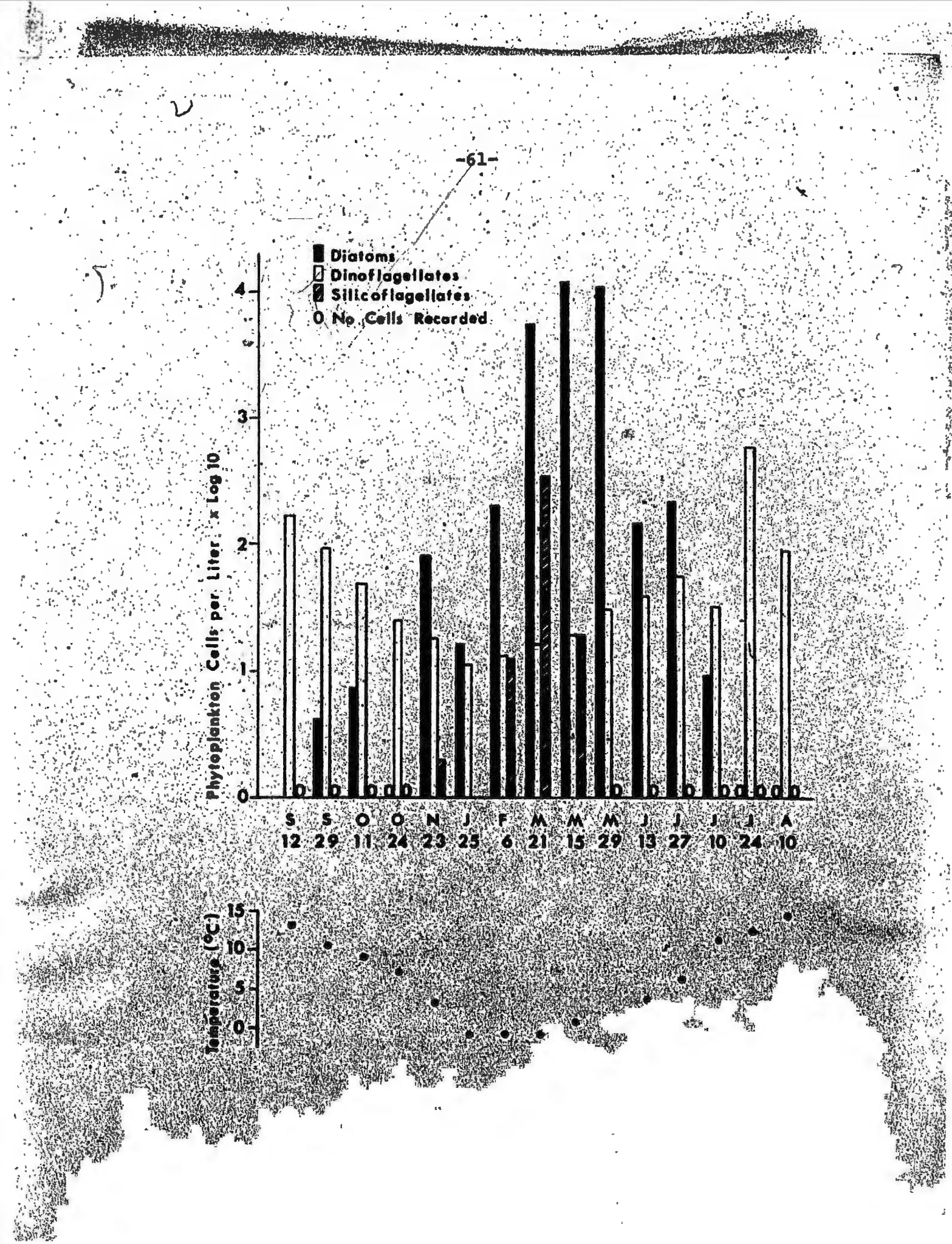
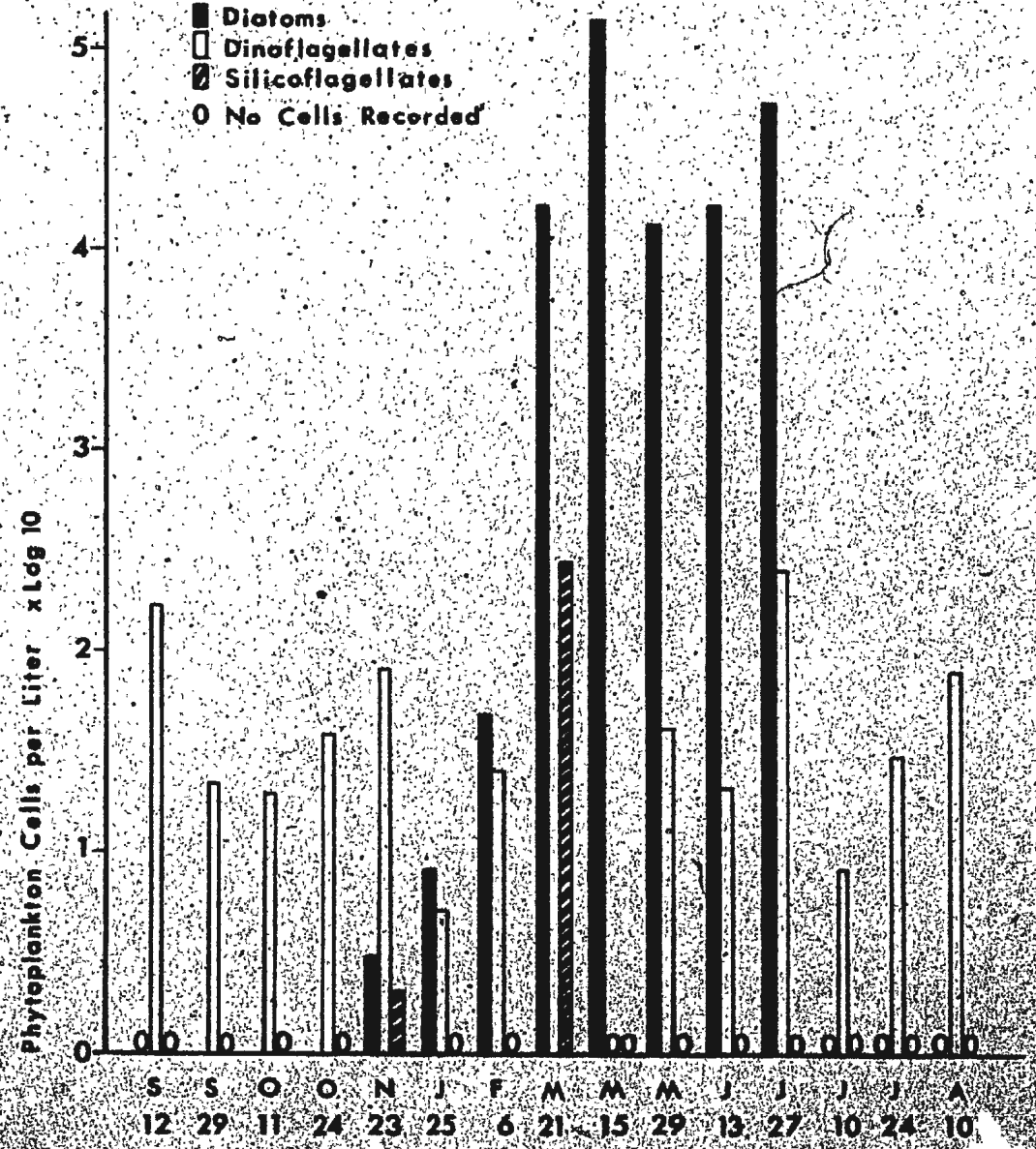

Diatoms
Dinoflagellates
Silicoflagellates
0 No Cells Recorded
Phytoplankton Cells per Liter, x Log 10
0
1
2
3
4
S 12
S 29
O 11
O 24
N 23
J 25
F 6
M 21
M 15
M 29
J 13
J 27
J 10
J 24
A 10
Temperature (°C)
0
5
10
15

Figure 16. Top: Total numbers of diatoms, dinoflagellates, and silicoflagellates per liter of middepth (thermocline) plankton tow for the sampling period, September 12, 1971 to August 10, 1972.

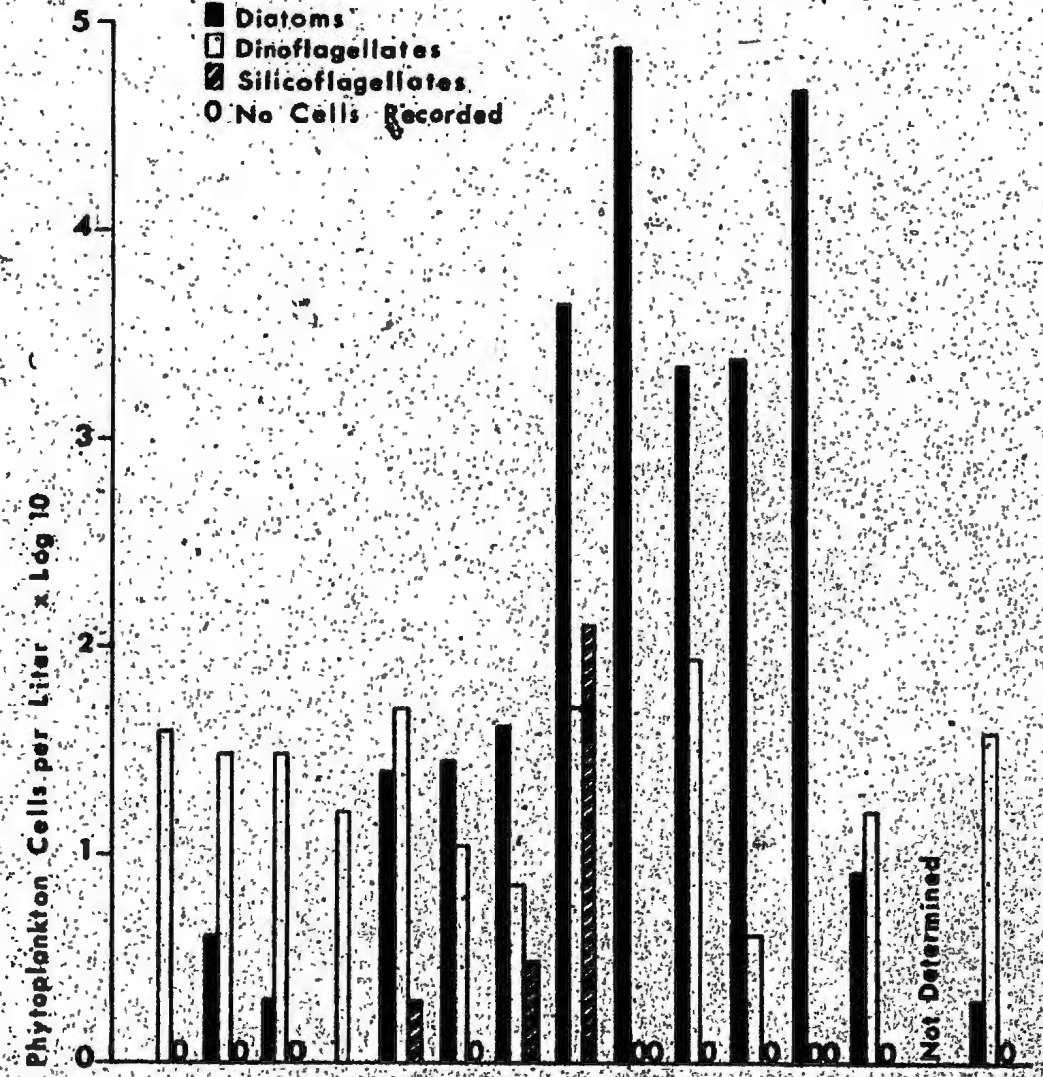
Bottom: Middepth (thermocline) water temperatures during the sampling period.

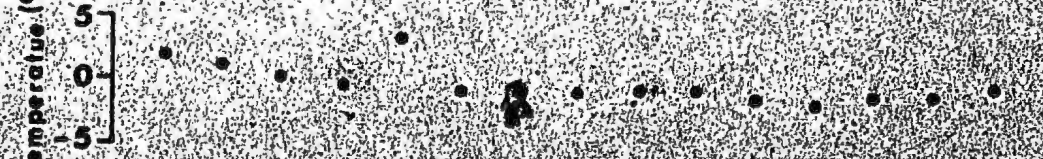

Diatoms
Dinoflagellates
Silicoflagellates
0 No Cells Recorded
Phytoplankton Cells per Liter x Log 10
5
4
3
2
1
0
S 12
S 29
O 11
O 24
N 23
J 25
F 6
M 21
M 15
M 29
J 13
J 27
J 10
J 24
A 10


Temperature (°C.)
10
5
0

Figure 17. Top: Total numbers of diatoms, dinoflagellates, and silicoflagellates per liter of bottom plankton tow for the sampling period, September 12, 1971 to August 10, 1972.

Bottom: Bottom water temperatures during the sampling period.


Diatoms
Dinoflagellates
Silicoflagellates
0 No Cells Recorded
Phytoplankton Cells per Liter x Log 10
5
4
3
2
1
0
Not Determined
S 12
S 29
O 11
O 24
N 23
J 25
F 6
M 21
M 15
M 29
J 13
J 27
J 10
J 24
A 10


Temperature (°C)
5
0
-5

In the middepth samples, diatoms numbered only 1 per liter from September 29 to October 24, then increased steadily to a high of 129,870 per liter on May 15. After dropping to 11,247 per liter on May 29, they reached a secondary peak of 51,119 per liter on June 27, before disappearing completely. Dinoflagellates dominated the phytoplankton in the samples taken from September to November and from July to August. Maximum counts of 164 and 241 cells per liter were obtained on September 12 and June 27, respectively, although at the later date the diatoms were the dominant forms. Following a drop to 8 cells per liter on July 10, there was an increase to 74 cells per liter on August 10. Silicoflagellates were found in the samples of November 23 and March 21 only, and peaked at 260 cells per liter on March 21.

Diatoms were present in all the bottom samples. Extremely low numbers (1 to 8 cells per liter) were encountered from September 12 to October 24 and on July 10 and August 10. The greatest numbers were recorded from March 21 to June 27 with peaks of 73,570 cells per liter and 46,002 cells per liter, respectively. Dinoflagellates were present in all the samples, except those of May 15 and June 27. From September 12 to October 24, their numbers decreased from 37 to 16 cells per liter, then the numbers increased slightly to 51 on November 23 before

decreasing further to 7 per liter on February 6. They rose to 51 cells per liter on March 21, fell to 0 on May 15 and reached their maximum of 84 per liter on May 29. After falling to 0 cells per liter on June 27, dinoflagellates increased slowly to 37 per liter on August 10. Silicoflagellates were present only in samples taken on October 24, November 23, February 6 and March 21, with a peak of 121 cells per liter on March 21.

Samples from all three depths show a similar overall picture. Diatoms were absent or present in low numbers only from September to October and from July to August, but increased explosively to form a "bloom" in March to May or June. Dinoflagellates generally peaked in September and July or August when diatoms were absent or in very low numbers, and almost disappeared during the diatom "bloom". Silicoflagellates never dominated the entire population and appeared only from November to March or May. As this sequence characterized all three depths, it is not directly related to water temperature.

The phytoplankton species composition of the plankton tow samples is given in Table 2, Appendix V. The diatom blooms, at all three sampling depths, were dominated by species of Chaetoceros. Species of Fragilaria also contributed, but to a lesser extent, to the May 15 peaks, while Leptocylindrus danicus was quantitatively important

in the June 27 peaks which occurred at the middepth (thermocline) and bottom.

Table 3, Appendix V, compares total phytoplankton with total bacteria and total pseudomonads. This information is presented graphically in Figures 18-20 for surface, middepth, and bottom, respectively.

From September 12 to October 24, the surface phytoplankton numbers decreased steadily, while both total bacteria and pseudomonads increased then showed only slight fluctuations. There was a slight rise in phytoplankton numbers in November, but total bacteria and pseudomonad numbers dropped slightly. On January 25, numbers of bacteria, pseudomonads, and phytoplankton dropped. The phytoplankton then increased constantly to reach their maximum on May 15. Total bacteria and pseudomonads, on the other hand, increased slightly at first but dropped to their minima on May 15. From May 15 to August 10, phytoplankton numbers dropped, then showed small increases and decreases. Numbers of bacteria and pseudomonads increased from May 15 to June 27, and then fell off.

Middepth (thermocline) phytoplankton counts fell from September 12 to October 11, increased until November 23, and then dropped on January 25. Numbers of total bacteria

Figure 18. Total phytoplankton, total bacteria, and numbers of pseudomonads per liter of surface plankton tow for the sampling period, September 12, 1971 to August 10, 1972.

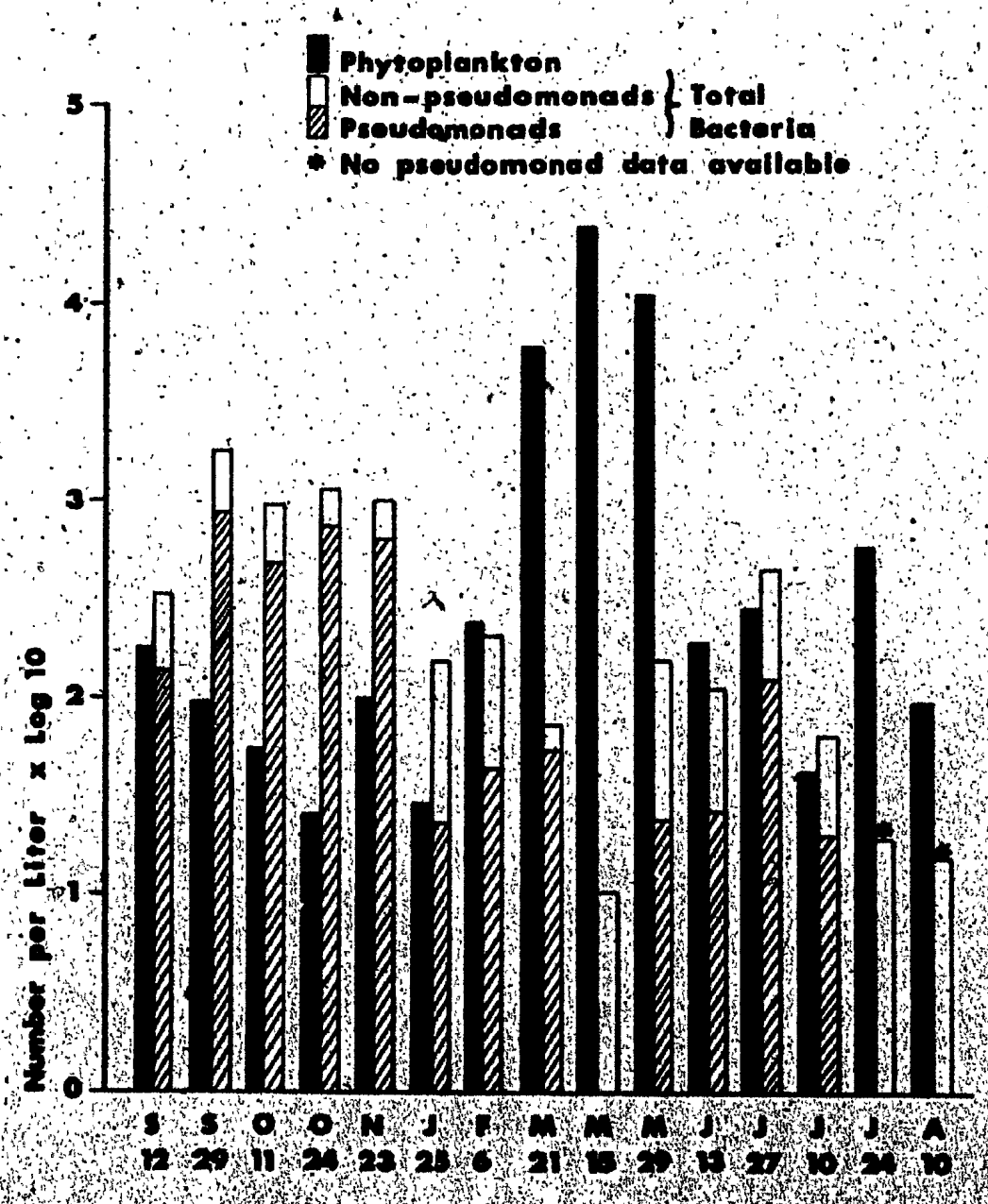

Phytoplankton
Non-pseudomonads } Total
Pseudomonads } Bacteria
* No pseudomonad data available
Number per Liter x Log 10
0
1
2
3
4
5
S 12
S 29
O 11
O 24
N 23
J 25
F 6
M 21
M 15
M 29
J 15
J 27
J 10
J 24
A 10

Figure 19. Total phytoplankton, total bacteria, and numbers of pseudomonads per liter of middepth (thermocline) plankton tow for the sampling period, September 12, 1971 to August 10, 1972.

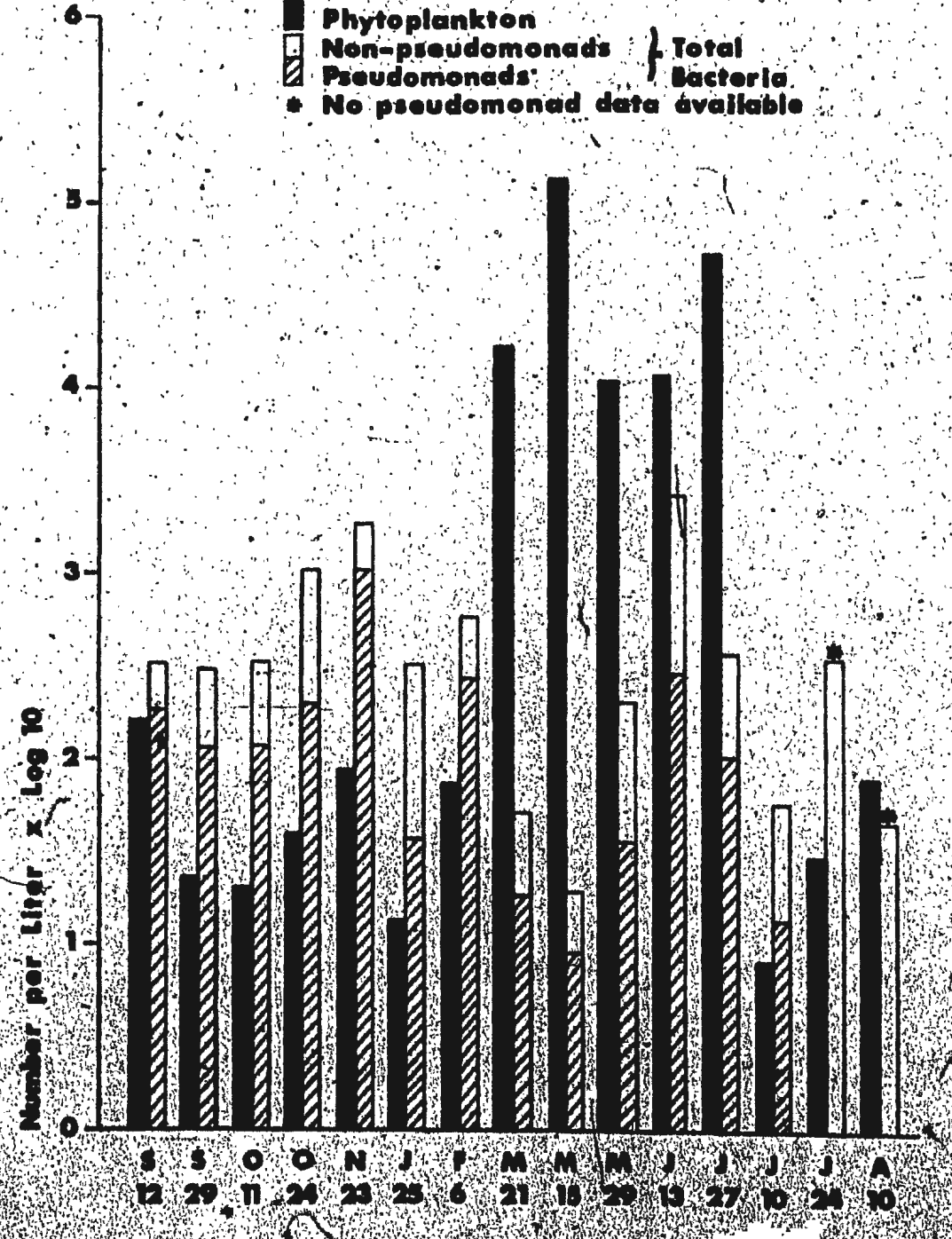
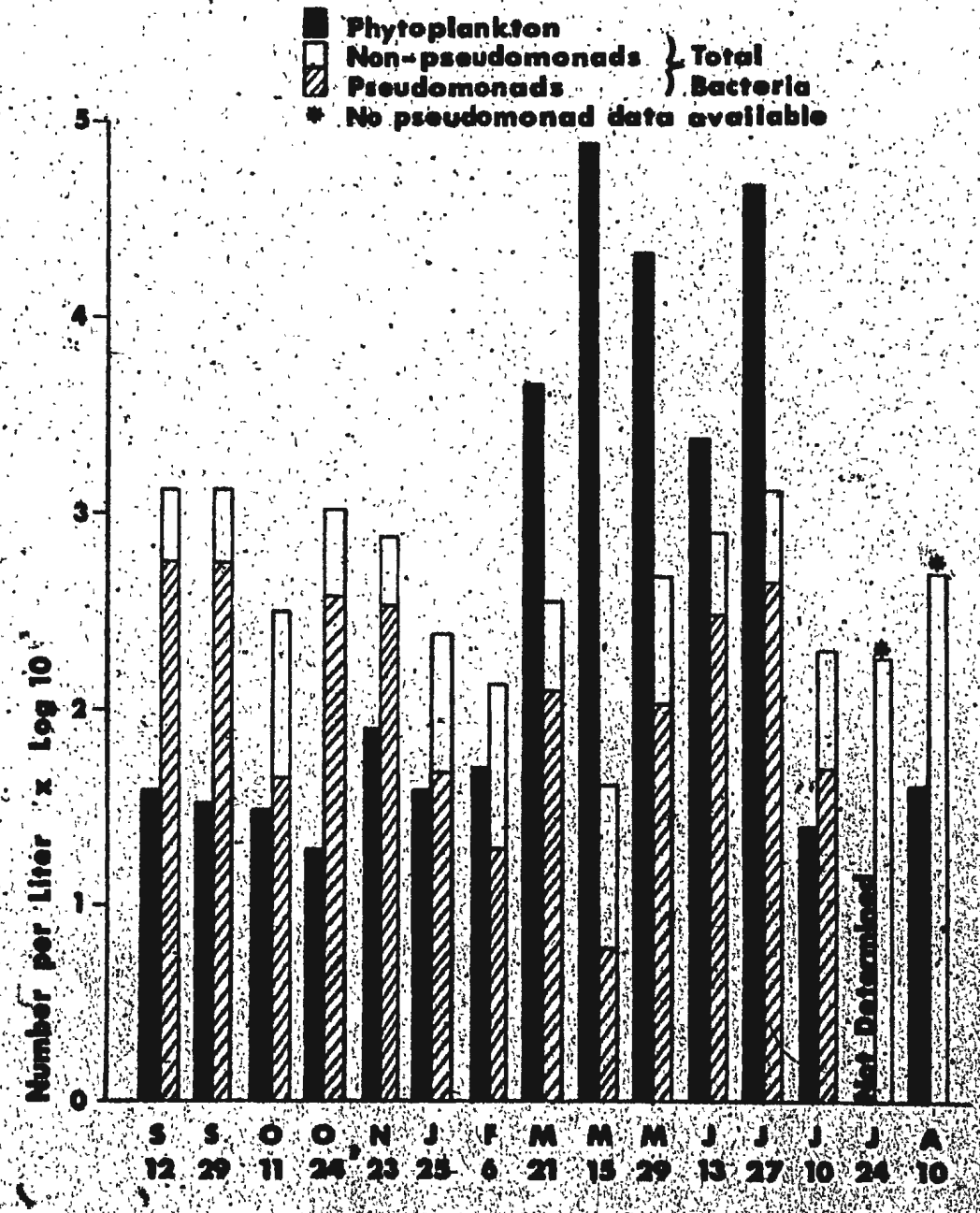

Phytoplankton
Non-pseudomonads
Pseudomonads*
Total Bacteria
* No pseudomonad data available
Number per Liter x Log 10
0
1
2
3
4
5
6
S 12
S 29
O 11
O 24
N 23
J 25
F 6
M 21
M 18
M 29
J 13
J 27
J 10
J 24
A 10

Figure 20. Total phytoplankton, total bacteria, and numbers of pseudomonads per liter of bottom plankton tow for the sampling period, September 12, 1971, to August 10, 1972.


Phytoplankton
Non-pseudomonads
Pseudomonads
Total Bacteria
* No pseudomonad data available
Number per Liter x Log 10
0
1
2
3
4
5
S 12
S 29
O 11
O 24
N 23
J 25
F 6
M 21
M 15
M 29
J 13
J 27
J 10
J 24
A 10
No Data Taken

and pseudomonads remained at a nearly constant level from September 12 to October 11, increased on November 23, and then dropped on January 25. Numbers of phytoplankton, total bacteria and pseudomonads increased on February 6, then phytoplankton numbers increased to a maximum on May 15, while total bacteria and pseudomonads decreased to a minimum on the same date. Phytoplankton numbers dropped on May 29, rose on June 27, fell again on July 10, and then rose to the end of the sampling period. Total bacteria and pseudomonads increased from May 15 to June 13, and then decreased until July 10. Numbers of total bacteria and pseudomonads rose and fell slightly on July 24 and August 10, respectively.

Bottom phytoplankton numbers decreased slowly from September to October 24, then after some small fluctuations, increased until May 15, when the maximum was reached. At the same time bacteria and pseudomonad numbers appeared to be decreasing, although there was considerable fluctuation. Minima of both total bacteria and phytoplankton occurred on May 15. Phytoplankton numbers dropped off to June 13, then rose almost to the May 15 maximum on June 27, before dropping off drastically at the end of the sampling period. Bacteria and pseudomonads increased in numbers from May 15 to June 27, then decreased and increased again.

While the relationship between phytoplankton numbers, total bacterial numbers and pseudomonad numbers at each of the depths sampled is not completely clear, it is evident that the phytoplankton maximum observed at each depth on May 15 is associated with the lowest numbers of both total bacteria and pseudomonads found during the sampling period.

DISCUSSION

CULTURE METHODS

Throughout this study, the culture plate (spread plate) method was used for quantitative bacterial determinations. The chief criticism of this method is that it results in an underestimation of the numbers of bacteria present in the samples studied. In spite of this, however, the culture plate method was chosen as the most appropriate enumeration technique for the purposes of this study.

It had originally been planned to compare numbers of bacteria per unit volume of sea water with those per unit volume of plankton tow and it was decided that both types of samples should be processed in the same way. The membrane filter technique, which might have provided higher bacterial counts in the case of the sea water samples, had to be rejected because of the difficulties involved in attempting to filter the plankton samples. Fragments of plankton organisms would occlude the filters. Because bacteria are thought to occur attached to the plankton, the resulting bacterial colonies would be clumped together on or near the plankton particles rather

than spread over the entire filter surface. Replication onto cetrimide agar plates would have been more difficult from a filter than from a plate because colonies are very easily detached from the filter surface. Also, because of the smaller surface area of the 47-mm diameter filter compared to that of a standard 100-mm diameter culture plate, colonies would have been smaller and closer together, making both replication and isolation more difficult.

The results obtained in this study have been expressed in terms of bacteria or pseudomonads per liter, but, of course, the plate count method determines, not total numbers of organisms per unit volume, but numbers of colony-generating or colony-forming units. These are referred to as C.G.U.'s or C.F.U.'s by Lewin (1974) and Wiebe and Hendricks (1974), respectively. The choice of terminology reminds one of two things. First, not all bacteria present in the sample are able to produce colonies under the particular cultural conditions used. Second, not every colony produced is the result of the multiplication of just one bacterial cell. A C.G.U. or C.F.U. may, therefore, be either a single cell or an aggregate of two to many cells. One of the reasons for grinding up the plankton samples was to break up such aggregates. Thus, Moskovits (1951) found in ten out of

eleven samples studied, grinding increased, by factors of 1.3 to 13.0, the numbers of bacterial colonies produced from plankton samples.

The accuracy of viable bacteria or C.G.U. counts in the estimation of actual bacterial populations from aquatic environments is questionable. Wood (1953) suggested that the discrepancy between direct microscopic (total bacteria) counts and plate (C.G.U.) counts is decreased as the number of bacteria present in the sample is decreased. Thus, the difference is likely to be much less in open ocean-type environments, where the numbers of bacteria per unit volume are low, than in estuarine or shallow coastal environments, where the numbers are higher. In fact, Wood (1953) stated that in the former situation, plate counts might be assumed to be of the same order as total bacteria. He was doubtful, however, whether this applied to samples from plankton "swarms" because the adsorption and subsequent clumping of bacteria on the plankton served to underestimate plate counts. However, as the bacterial clumps are broken up by grinding, C.G.U. counts of the plankton samples should, according to his statements, provide a rough estimate of total bacteria counts in those situations where numbers of bacteria are low.

Other workers, such as Cholodny (1929), Wilson and Kullman (1931), Bere (1933), ZoBell (1946), Oppenheimer (1952), Carlucci and Pramer (1957), Collins and Kipling (1957), Jannasch (1958) and Seki (1971), have been more critical of the plate count method than Wood (1953) even when only relatively small bacterial populations are involved. Kriss, Mishustina, Mitskevich, and Zemtsova (1967) have indicated that, aside from the limitations of this method already mentioned, plate counts can be further greatly distorted, especially on small areas of solid medium, because of antagonism. All these factors taken together ensure that the number of colonies recovered on the plates will be lower than the number of bacteria present in the sample. Jannasch and Jones (1959) stated that as few as 1/3 to 1/10,000 of the cells present, as seen by direct microscopic count, show up on the culture plates and other estimates of the fraction appearing have been as few as 1/1,000 (Waksman, 1934), and from 1/200 to 1/500 (Butkevitch, 1938). Despite the fact that some of the cells recorded by direct count are dead, it is apparent, according to these estimates, that the number of colonies recovered may bear no relationship to the number of living bacteria in the sample.

Noseworthy and Moskovits (1974) tested the ability of four agents to select for marine pseudomonads, while

eliminating non-pseudomonads, and found 0.03% cetrimide to be the most effective. However, it was also found that, in order to eliminate all non-pseudomonads, those Group III and IV pseudomonads tested were also completely inhibited. Therefore, cetrimide was primarily a selective agent for pseudomonads of Groups I and II.

The results of the present study indicate that there are non-pseudomonads and pseudomonads of Group IV which are capable of growth on 0.03% cetrimide. The difference in the results is probably due to the fact that, whereas 2580 isolates were examined in the present study, a considerably smaller number of cultures, which did not include cetrimide-resistant forms of these organisms, was used in the previous one.

MOTILITY

Forty-five percent, or 1153 of the 2580 isolates studied were determined to be non-motile. Such a high proportion of non-motile organisms appears unusual, since it is agreed that polar-flagellated rods comprise the bulk of the bacteria in sea water (Waksman, 1934; ZoBell, 1946; Leifson, Cosenza, and Murchelano , 1964; Wood, 1967; Johnson, Schwent and Press, 1968). Motile bacteria were found to comprise 75% and 79% of the total bacteria in Narragansett Bay, Rhode Island and in the nearby Atlantic

Ocean, respectively. In Noank Harbour, Rhode Island, where there was more terrigenous contamination, only 39% of the bacteria were motile (Leifson, et al., 1964). Pfister and Burkholder (1965) studying 114 Antarctic and 37 Puerto Rican isolates, found that 66.2% were gram-negative, motile bacteria with a single flagellum. Kriss, et al. (1967) reported that the bulk of the thousands of micro-organisms they obtained from various geographical zones of the Atlantic, Indian, and Pacific Oceans, over a 7-year period, consisted of non-sporing, gram-negative, motile rods.

Unless the high proportion of non-motile organisms among the current isolates is to be regarded as unique, there are two possible explanations for the situation. First, it may be that many of these isolates were motile in the natural environment but, because of the nature of the testing procedure, appeared non-motile. Second, it may be possible that a high percentage of non-motile organisms is not as rare as the first brief look at the literature makes it appear.

The high proportion of non-motile isolates, coupled with the fact that most of them were, according to their biochemical characteristics, members of the "motile" genera, Pseudomonas, Vibrio, and Aeromonas, leads one to

question the reliability of the motility testing procedure. The method and medium used were standard ones which, with over one-half the isolates, gave reliable results in that the cultures determined to be motile showed flagella upon subsequent staining. However, the cultures had not been tested for motility immediately after isolation but, instead, had first been stored for periods up to one year with one sub-culture after six months. It is possible that the flagella of originally motile bacteria were lost or became non-functional during the long storage period. The literature of the motility test gives no indication of how soon after initial isolation a culture should be tested for motility, and those studies which found that motile bacteria were the dominant forms (Leifson, et al., 1964; Pfister and Burkholder, 1965; Kriss, et al., 1967) failed to state the time interval which had elasped between isolation and motility testing.

Cultures motile in the marine environment may have become non-motile by spontaneous genetic mutation, but aside from this, loss of flagella can also arise in a purely phenotypic manner (Rhodes, 1965). Studies on the genetics of flagellation have not been carried out on the bacterial groups concerned but have been performed on other genera, so that it is probable that the same general rules apply. Quadling and Stocker (1962) working with Salmonella, found

that under conditions which prevented the synthesis of new flagella, the flagella on a dividing cell were shared equally during cell division so that, with each new generation, the number of flagella per cell decreased while the proportion of non-flagellated cells increased, until eventually the entire culture became non-motile. Their work also suggested that each active normal flagellum was associated with a "motility-conferring particle" which was not replicated but, instead, retained by one of the daughter cells and passed down from generation to generation in a unilinear manner (Quadling, 1958). Thus, if growth conditions precluded the synthesis of these particles, non-motile cultures would result, regardless of whether or not flagella production were possible.

Flagella can be lost under a variety of cultural conditions. Too acid or too alkaline media can cause flagellin disintegration (Rhodes, 1965). Trace amounts of iron and aluminum can supress flagellation in Bacillus and Proteus (Weinberg and Brooks, 1963; Rhodes, 1965), and possibly in other genera as well. According to Lacey (1961), variations in such factors as temperature, light, pH, salt balance, degree of aeration and surface-active compounds can cause loss of flagellation in a number of micro-organisms.

In addition to this, lack of motility does not necessarily mean the absence of flagella. Leifson (1960) stated that flagellated cells may be non-motile if changes in the medium (e.g. low pH) damage the flagella, if the flagella are in a "paralyzed state", or if the flagella are rendered non-functional "from various causes". He did not say what these causes were, nor did he differentiate between non-functional and paralyzed flagella. Nevertheless, it appears that non-motile flagellated cells, whatever the reason for this state, are not unusual.

It is often impossible to determine either the number or percentage of motile cultures isolated in a particular study because of the manner in which the data are presented. When isolation and characterization of bacteria from marine sources are carried out, the results are usually presented in terms of numbers of organisms assigned to genera and although the particular determinative scheme used to characterize the isolates is provided, there is no indication of any variation with regard to motility (or for that matter, to any other characteristic).

Sieburth (1967), for example, isolated a total of 1119 cultures from a series of semi-monthly samples from Narragansett Bay during 1962-1963. Using a scheme based on that of Shewan, Hobbs, and Hodgkiss (1960) he determined

that the dominant taxonomic groups were: flavobacteria - cytophagas, pseudomonads, vibrios, achromobacters, and arthrobacters. For each sample, he determined what percentage of the total isolates was assigned to each of these groups; but he provided in his data no further characterization, so that the percentages of motile bacteria present in the samples were unknown. According to the scheme used, pseudomonads and vibrios are motile. Considering the large numbers of isolates involved, it does not appear unreasonable to assume that some non-motile pseudomonads and vibrios were encountered. However, there is no mention of this, nor was there any provision in the identification scheme for this contingency.

When one considers that relatively few qualitative studies of marine bacteria have been made, and that the presentation of Sieburth's results is typical of that used in most other studies of this type (Anderson, 1962; Simidu and Aiso, 1962; Altschuler and Riley, 1967; Johnson, Katarski, and Weisrock, 1968; Johnson, Schwent, and Press, 1968; Sieburth, 1971; Simidu, Ashino, and Kameko, 1971; Ezura, Daiku, Tijame, Kimura, and Sakai, 1974), it is easy to understand why more data on the kinds of marine bacteria from different marine environments cannot be given. Therefore, it is possible that non-motile bacteria are more common in the marine environment than

heretofore suspected.

Forty-three percent, or 941 of the 2189 isolates assigned to the genus Pseudomonas were non-motile, as were 32% or 15 of the 47 Vibrio isolates, 38% or 14 of the 37 Aeromonas isolates, 43% or 75 of the 174 Alcaligenes isolates and 100% of the 113 Acinetobacter isolates. Of these genera, only Acinetobacter is, by definition, strictly non-motile, while members of Alcaligenes are either motile or non-motile. This means that the vast majority (81% or 934) of the 1153 non-motile isolates studied here were assigned to the "motile" genera Pseudomonas, Vibrio and Aeromonas. Determinative schemes, such as those given by Scholes and Shewan (1964), Bain and Shewan (1968), and Hendrie and Shewan (1966), which are used to identify marine bacteria make no provision for non-motile forms of these genera. It is in this respect that the main difference between the scheme used in this study (Figure 4) and those used by other workers in this field lies.

Despite the ease with which it can be lost, motility and type of flagellation are given prime importance in the identification schemes designed for marine bacteria. It would be wise to question whether flagellation is as fundamental a characteristic in bacteria as in the higher protists, or whether by utilizing this feature we

are being misled by the opinions inherited from our pioneer, botanically-trained systemists, Migula, Beijerinck, Winogradsky, and others (Rhodes, 1965). The value of such a highly variable characteristic in taxonomy has been questioned by a number of workers, such as Bartholomew (1949), Gaby and Free (1953), Sneath (1956), Rhodes (1959), and Lysenko (1961). Surely, it would be wiser to define the taxonomic groups concerned on the basis of more stable and fundamental traits, such as DNA base composition (Colwell and Mandel, 1964; Colwell, Citarella, and Ryman, 1965; Ley, 1968, 1969; Baumann, et al., 1972).

FLUORESCEIN PRODUCTION

According to the scheme used in this study, 2166 or 84% of the 2580 isolates examined were identified as pseudomonads of Groups I and II, the organisms for which cetrimide was found to be selective (Noseworthy and Moskovits, 1974). Shewan, Hobbs, and Hodgkiss (1960) who devised the groupings, stated that these pseudomonads were characterized by the production of fluorescein, a yellow-green, diffusible, fluorescent pigment. Although King's Medium B (King, et al., 1954) was used in the present study, none of the isolates examined produced this pigment.

The ability to produce fluorescein is variable and

related to the composition of the medium (Rhodes, 1959). It has been found that Mg^{2+}, SO_4^{2-} and PO_4^{3-} are necessary for fluorescein production in Pseudomonas fluorescens, and that the type and concentration of peptone used is also of great importance (Georgia and Poe, 1931, 1932). Totter and Moseley (1953) studied fluorescein production by Pseudomonas aeruginosa and found that the amount of fluorescein produced varied inversely with the concentration of iron in the medium, but they did not determine the concentration necessary to completely inhibit fluorescein production. Their observations were extended also to P. fluorescens "which behaved in a manner very similar to P. aeruginosa". According to Garabaldi (1967), a low concentration of iron is generally regarded as the most important cultural requirement for the production of this pigment. In the present study, King's Medium B, which is recommended largely because of its very low iron concentration was used, but it was prepared with synthetic seawater instead of distilled water. It is possible that the trace amounts of iron in the seawater constituents may have exceeded the limit permitting fluorescence. However, pseudomonads of Groups I (NCMB[1] 404, IN_3FL) and II (NCMB 104 and 403), when grown on King's Medium B

[1] NCMB = National Collection of Marine Bacteria Torrey Research Station, Aberdeen, Scotland.

with synthetic seawater in a previous study (Noseworthy and Moskovits, 1974) did produce fluorescein, although when used in this study as controls, they did not.

Even when cultural conditions are standardized, fluorescein production is not constant (Rhodes, 1959), as non-pigmented strains have been shown to arise spontaneously from pigmented cultures (Seleen and Stark, 1943). Many pseudomonads lose the property of forming pigment because of age and continued sub-culture (ZoBell, 1946; Shewan, Hodgkiss and Liston, 1954; Lysenko, 1961). It has also been shown that lyophilization caused 20% to 22% of Pseudomonas cultures tested by Lysenko (1961) to lose the ability to produce fluorescein.

Despite its variability, fluorescein production has long been used as an important taxonomic characteristic. Turfitt (1936) concluded that pigment production on an "appropriate" medium was constant and recommended that chromogenic function be used as a basis for the division of the genus Pseudomonas into two species - one of which produced only fluorescein (P. fluorescens) and the other of which produced both fluorescein and pyocyanin (P. aeruginosa). In spite of later work which showed that pigment production was far from constant, fluorescein and pyocyanin production have continued to be important criteria in the identification of Pseudomonas species.

The reliance on pigmentation in taxonomy has been questioned by a number of workers (Tobie, 1938; Haynes, 1951; Rhodes, 1959; Bühlman, Vischer, and Bruhin, 1961; Lysenko, 1961; Stanier, Palleroni, and Doudoroff, 1966; Johnson, Katarski, and Weisrock, 1968). Although the production of fluorescein by a gram-negative, polar-flagellated rod is a useful indicator of a pseudomonad, pigment production should not be relied upon for the identification of all pseudomonads (Sherris, et al., 1959; Park, 1962). Indeed, Stanier, et al., (1966) even questioned the recognition of the fluorescent pseudomonads as a special subgroup of the genus, until their analyses had indicated that these organisms shared additional properties which permitted their identification even when no pigment was produced. Because of the existence of such properties, which do not depend upon cultural conditions for their manifestation, the role of pigmentation in the taxonomy of this genus appears unnecessary.

SENSITIVITY TO 0/129

Collier, Campbell, and Fitzgerald (1950) found that the growth of Vibrio cholerae was inhibited by 0/129. Shewan, Hodgkiss and Liston (1954) tested the effect of this compound against a number of cultures isolated from both terrestrial and marine sources. They found that it

inhibited the growth of vibrios, but not that of other bacteria, and recommended that this test be used routinely as an aid to the identification of Vibrio isolates. As a result, the identification schemes of Bergey's Manual of Determinative Bacteriology (1974), Shewan (1963), Scholes and Shewan (1964), and Bain and Shewan (1968) used the vibriostatic agent 0/129 in the differentiation of the genera Vibrio and Aeromonas.

None of the isolates examined in the present study were sensitive to this agent. Apparently, vibrios resistant to 0/129 have been encountered before, since Bain and Shewan (1968) stated that "several workers have claimed that the use of the vibriostatic agent is of doubtful value", but unfortunately, they did not offer any suggestions as to why 0/129 should be effective in the hands of some workers but not others. Merkel (1972) may have provided a possible explanation on finding that the vibriostatic activity of 0/129 was inhibited when used on media prepared with synthetic or natural seawater, or containing concentrations of NaCl or $MgCl_2$ equivalent to those found in seawater. The inhibition is apparently due to an ionic effect, rather than to the presence of any specific metal ions. Although the medium used by Shewan, et al. (1954) was not specified, that used by Bain and Shewan (1968) and, presumably, that used in previous,

related studies which showed vibriostatic activity, was blood agar, essentially a freshwater medium. It is possible that "those workers" referred to by Bain and Shewan (1968) as having had difficulty with 0/129 may have used media with higher concentrations of NaCl, $MgCl_2$, or both. Synthetic sea water was used to prepare the sensitivity test medium employed in the present study, so that the resistance to 0/129 of those isolates identified at Vibrio spp. may, therefore, be explained. It was not possible to test the effect of 0/129 on the vibrios using a medium prepared with distilled water, because none of the isolates produced good growth on freshwater medium.

UNKNOWN ISOLATES

Twenty-three of the cultures isolated, all identical in the biochemical and morphological characteristics studied, could not be identified according to the scheme used and were designated Unknown A. Their fermentative reactions in Hugh-Leifson medium indicated that they belonged to the Family Vibrionaceae (Bergey's Manual, 1974), but their pattern of reactions did not conform to that of any of the genera in this family. Photobacterium is the only genus of this family which can be oxidase-negative, but this genus is also characterized by the

ability to luminesce and by the production of gas from glucose. Unknown A showed no indication of luminescence, but because of the transitory nature of this property and the long interval between isolation and testing, special media designed to enhance this ability were not used. Similarly, there was no evidence of gas production by any of the isolates.

Seven of the isolates conformed to none of the genera in the identification scheme and were called Unknown B. They appeared to be more closely related to the pseudomonads than were any of the other non-pseudomonads studied. Except for their reaction to terramycin, they had the same biochemical and morphological characteristics as the Group IV pseudomonads and gave a fluorescent antibody reaction almost as intense as that group. These organisms might have been considered as Group III pseudomonads (none of which were recovered in this study), but they gave no reaction in Hugh-Leifson medium, whereas Group III pseudomonads give an alkaline reaction.

FLUORESCENT ANTIBODY TECHNIQUE

In the determination of the identity of the isolates examined in this study, good agreement was obtained between the results of the standard identification procedures and

those of the fluorescent antibody technique. All the isolates identified as pseudomonads produced a definite to bright (1+ to 3+) fluorescence. Pseudomonads of Groups I and II, which are more closely related to P. fluorescens, generally gave brighter and more intense reactions (2+ to 3+) than those of Group IV (1+ to 2+), although there was some overlap. However, with the exception of those isolates designated Unknown B, the non-pseudomonads gave fluorescent reactions ranging from none to non-descript (0 to 1+). Moskovits and Foelsche (1970) suggested that the fluorescent antibody technique might be an effective alternative to laborious identification procedures in the differentiation of marine pseudomonads from non-pseudomonads. The results obtained in the present study appear to support this suggestion.

NUMERICAL TAXONOMY

Few studies have been performed on the groups of marine bacteria, so that the taxonomy of these micro-organisms is still in a state of confusion. One of the difficulties is that bacterial taxonomists apparently have not decided whether morphological, biochemical or physiological characters should be of prime importance, so that the criteria for identification are unclear (Wood, 1967).

According to Ingram and Shewan (1960), overshadowing this are the "difficulties of defining even genera, which spring from misgivings about the validity of characters like flagellation and pigmentation, long thought to be stable and fundamental". They suggested that, in regards to Pseudomonas and other marine genera, "it may be necessary to relinquish the old conception of the genus as a distinct systematic unit characterized by basic homogeneity in morphology and metabolism", and opt instead for the "agnostic application of the Adansonian techniques" of Sneath's (1957) numerical taxonomy.

Sneath and Sokal (1973) defined numerical taxonomy as "the grouping by numerical methods, of taxonomic units into taxa on the basis of their character states". Numerical taxonomy differs from traditional taxonomy mainly on the basis of the fact that no character is "non-mutable" (Colwell, 1964). Therefore no single character is indispensable in defining a taxonomic unit, nor is it a sufficient single criterion. What is important, then, in defining any taxon is not the possession of certain "key" characters by all its members, but the overall similarity of the characters the members possess. Obviously, it follows that the greater the content of information in the taxa of any classificiation and the more characters on which it is based, the better

that classification will be (Colwell, 1964; Sneath and Sokal, 1973).

Several studies involving numerical taxonomy have been performed on marine bacteria or on strains of those genera to which the isolates examined in the present study have been assigned. Liston (1960), in a study of Pseudomonas and related genera, found that within the pseudomonads, there was a large group (related to P. aeruginosa) which produced a green fluorescent pigment, were biochemically active and had no requirement for sea water. His results also indicated the possible existence of two other groups of pseudomonads, one of which was unpigmented, biochemically inactive and able to grow without sea water while the other was psychrophilic, biochemically inactive and unable to grow without sea water. He suggested a possible relationship between his groups and those of Shewan, Hobbs and Hodgkiss (1960) but, although a later study (Colwell and Liston, 1961) showed 4 distinct clusters within the genus, these clusters, according to biochemical characterization, did not correspond with Shewan's groupings. Unfortunately, further investigations along these lines have not been pursued. Liston's (1960) results, along with those of Goodfellow (1967) who found that pseudomonads were a distinct group with high phenotypic similarity, supported

the suggestion of Rhodes (1959, 1961) that the genus Pseudomonas be reduced to 3 closely related species. However, Lysenko (1961), while agreeing that many similarities existed among the strains of Pseudomonas he studied, felt that Rhodes' definition of genus was too narrow and that her definition of species was too broad in scope. He found two distinct groups - one containing both P.aeruginosa and P. fluorescens along with some closely related species, and the other containing 11 different species.

The results of Liston (1960) and Goodfellow (1967) indicated that the genus Aeromonas was closely related to the Enterobacteriaceae. They may have been partially responsible for the movement of this genus from the Family Pseudomonadaceae (Bergey's Manual of Determinative Bacteriology, 1957) to the Family Vibrionaceae (Bergey's Manual Determinative Bacteriology, 1974). Similarly, Baumann, Doudoroff and Stanier (1968), Thornley (1967, 1968) and Pinter and Bende (1967) may have been responsible for the abolition of the genus Achromobacter and the clarification and redefinition of the genera Acinetobacter and Alcaligenes.

Numerical taxonomy studies have indicated that Vibrio appears to be the major difficulty in the classification

of gram-negative marine rods, as strains assigned to this genus have been found which are closely related to almost all the other marine genera: Pseudomonas, Spirillum, Photobacterium, Flavobacterium, Alcaligenes, Aeromonas (Hugh and Leifson 1953; Liston, 1960; Colwell and Liston, 1961; Colwell and Mandel, 1964; Johnson, Katarski and Weisrock, 1968). This is not at all surprising since this genus has traditionally been defined on the basis of cell morphology and flagellation, both of which are very mutable characteristics.

The results of a study of 200 marine strains by Johnson, Katarski, and Weisrock (1968), using numerical taxonomy, suggested that a continuum of marine bacteria with all possible inter-generic strains existed. This was attributed, not only to the fact that the organisms tested were members of the largest group of bacteria (gram-negative rods) indigenous to the acknowledged site of origin of all life (the seas), but also to the fact that the generic criteria used were designed to identify evolved and established land and pathogenic organisms. The unsuitability of the tests used to identify marine isolates had previously been mentioned by Liston (1960), who pointed out the ambiguous position of those marine strains (such as Acinetobacter) notable for their lack of reactivity in the usual biochemical and physiological tests.

These failed to cluster with any other groups in his analyses and those of other workers (Beers, Fisher, Megraw and Lockhart, 1962; Focht and Lockhart, 1965; Pintér and Bende, 1967). Liston (1960) recommended that, in order to create an orderly system of classification, new tests to which marine genera could give positive results, probably based on nutritional requirements, be developed. DNA base analysis might be of considerable importance here, for high degrees of similarity between this characteristic and the results of numerical analysis have been found (Colwell and Mandel, 1964; Colwell, et al., 1965; Ley, 1968, 1969; Baumann, et al., 1972).

BACTERIAL NUMBERS

The quantitative bacterial data obtained in this study from plankton tow samples show that, over the one-year period studied and for all depths sampled, the number of bacteria per liter of plankton tow ranged from 10 to 4,329 (0.01 to 4.33 per ml).

A number of workers have attempted to relate the numbers of bacteria present in a plankton tow to the numbers of free bacteria found in the sea water in which the tow was made. Waksman, Reuszer, Carey, Hotchkiss, and Renn (1933) found that from 500 to 2,270 times more

bacteria were associated with phytoplankton than were found free in the sea water of the Gulf of Maine. Velankar (1950, 1955) reported that the ratios of free bacteria in sea water to bacteria attached to phytoplankton ranged from 1:5 to 1:1,900 for the Bay of Bengal, from 1:70 to 1:1,300 for the Gulf of Mannar, and from 1:30 to 1:2,400 for Palk Bay, between India and Ceylon. However, Moskovits (1951), working in Vinyard Sound, Massachusetts, found that there were only 0.001 to 0.09 times as many bacteria associated with the plankton as were free in the sea water. Even when the plankton were ground up to disperse the bacterial clumps, there were still only 0.008 to 0.2 times as many bacteria in the plankton as there were in the sea water. He suggested (Moskovits, 1961) that the difference between his results and those of Waksman, et al. (1933) and Velankar (1950, 1955) might have been due to the fact that he used a quantitative plankton sampling procedure, whereas the other workers did not.

The relationship found between free and attached bacteria is further complicated by the method used to enumerate the bacteria. Taga and Matsuda (1974) found that when the plate count method was used, the average counts were 10^5 attached, and 10^3 free, bacteria per liter from North and Equatorial Pacific Ocean water.

However, when the numbers were determined by the direct microscopic method, the free bacterial population was found to be larger than the population of attached bacteria by a factor of 6.5.

It is, therefore, impossible to accurately determine the bacterial population of sea water from plate counts of plankton tow material. However, regardless of the exact relationship involved in the present study, the plate counts of both plankton tow material and seawater indicate that the bacterial populations of the sampling area were very low.

Few quantitative bacteriological studies of the North Atlantic Ocean have been made. Fischer (1894) sampled from southern Greenland to the Carribean Sea and, using the plate count method, found that the highest count, 10,000 colonies per ml, occurred just south of the Grand Banks. Minervini (1900) sampled the North Atlantic approximately mid-way between Europe and North America and found that his plate counts ranged from 50 to 108 colonies per ml. Because of the nature of their sampling and culturing techniques, the results of both of these workers are questionable. For almost 60 years after these studies, no further quantitative work was done on cold water environments of the North Atlantic Ocean.

Kriss and his colleagues in 1959 sampled in a transect along 30°W from the Arctic to the Tropic of Capricorn, and found that 90% of the samples taken between 40°N and 48°N had from 0 to 9 colonies per ml, while the remaining 10% has from 10 to 99 colonies per ml (Kriss, 1963). Samples in which only single heterotrophic organisms or none at all were found made up 99% of all the samples taken from the North Atlantic (Kriss, et al., 1967). Sampling was repeated from the Arctic to the Antarctic in 1969 and the results were similar, for in approximately the same area, 94.6% of the samples had from 0 to 9 colonies and 5.4% had from 10 to 99 (Kriss, 1970). In both cases, the figures were based on the number of colonies produced after 40 to 50 ml of sea water had been membrane-filtered.

Kriss' extensive sampling in the world oceans have led him to suggest (Kriss, 1963) that the number of bacteria per unit volume of sea water decreases with increasing distance from the Equator to the Poles. If this be the case, bacterial populations in Newfoundland waters should be very small, because of the island's geographical position. However, Sieburth (1971), sampling from the Panama Canal to Rhode Island, did not find a regular decrease in bacterial concentrations

with increasing latitude. The majority of his samples all had less than 5 colonies per ml, although there were sporadic high counts. This situation was attributed to "spotty" productivity rather than to geography. It is unfortunate that the sampling was not continued farther northward as even "low" counts of 5 colonies per ml are higher than the counts obtained in the present study. The results of Wiebe and Hendricks (1974), who sampled along a transect in the Antarctic Ocean, are also at variance with the global geographical pattern of bacterial numbers suggested by Kriss, for they found that C.F.U.'s per ml ranged from less than 1 to a few 100, which are much higher than both the values obtained by Kriss (1963, 1970) and those obtained in the present study.

Regardless of whether or not they are part of an overall geographic pattern, very low bacterial populations, such as those encountered in the samples of the present study may be caused by a number of factors (Renn, 1937; Waksman and Hotchkiss, 1937). Chief among these are: low concentrations of suitable nutrient material, near-freezing temperatures, and unfavourable relationships with other organisms.

ORGANIC MATTER

The vast majority of bacteria in the sea, including all those involved in the present study, are heterotrophic and, therefore, require, for their growth and metabolism, pre-formed organic material, of which two types, dissolved and particulate, are present in the marine environment. Although some of the dissolved organic portion enters the sea as land run-off, most is autochthonous (Provasoli, 1963), and consists of the decay products of dead organisms, the extracellular substances produced by algae, and the excretion of marine animals (Riley and Chester, 1971). Particulate organic matter is of three types: living phytoplankton, detritus, and organic aggregates. Parsons (1963) estimated that living phytoplankton comprised only 20% of the total particulate organic matter, which, in turn, is exceeded by the dissolved fraction by a factor of at least 10 to 20.

The concentration of total organic matter in the sea has been estimated to be 4-5 mg per l by Krogh (1931), 2 mg per l by Skopintsev (1948), and 0.2-2.7 mg per l by Provasoli (1963). Riley and Chester (1974) stated that the portion of dissolved organic material in the oceans ranged from 0.3 to 3 mg per l, but added that in coastal areas, the concentration could be as high as 20 mg per l, because of increased pollution or higher phyto-

plankton populations. Skopintsev (1959) determined that the concentration of dissolved organics in the North Atlantic Ocean ranged from 1.04 mg per l to 1.94 mg per l, and referred to other Russian workers who found values of 2.40 to 2.48 mg per l for the same area. Duursma (1961) also investigated dissolved organic matter in the North Atlantic, but obtained much lower values, i.e., 0.2-1.3 mg per l.

Not all of the organic material present in sea water can be utilized by bacteria, as most of the dissolved portion and a considerable part of the detritus is resistant to bacterial decay (Krogh, 1934; Menzel and Goering, 1966; Riley and Chester, 1971). According to ZoBell (1946), the concentration of organic nutrients available to bacteria in the marine environment is at or below the minimum required by many bacteria and considerably less than that which is claimed to be essential for heterotroph multiplication, i.e., 10-100 mg per l. Therefore, organic carbon has been regarded as the principal factor limiting bacterial populations in the seas (Waksman and Carey, 1935; Waksman and Renn, 1936; ZoBell, 1946; Kriss, 1963).

Because the vast majority of bacteria living in the sea have been found attached to particulate matter such as detritus or living plankton (Lloyd, 1937; Prescott

and Winslow, 1931; Waksman, et al., 1933; Stark, Stadler, and McCoy, 1938; ZoBell, 1934, 1946; Bader, Hood, and Smith, 1960; Seki, 1971), it has been suggested that bacteria are able to utilize the dilute nutrient material in the sea, only if it be accumulated or concentrated onto solid surfaces. According to Kriss (1963), solid surfaces not only adsorb organic material, but also, at the solid-liquid interfaces, cause a change in the properties of some types of organic matter so that a stable form becomes more susceptible to bacterial enzyme action. In addition, solid surfaces retard the diffusion of exoenzymes and partially digested food away from the bacteria (ZoBell, 1946), so that decomposition processes are more effective in their vicinity (Duursma, 1961). Bader, et al. (1960) noted that inorganic particles in the sea water are also of importance in concentrating nutrient material and, thus, affect the number of bacteria present.

The amount of organic material present in the oceans varies horizontally (geographically), vertically, and seasonally (Duursma, 1961, 1965; Horne, 1969).

Kriss, et al. (1967) suggested that labile organic matter decreased in concentration with distance from the Equator to the Poles and that this situation explained the

corresponding decrease in bacterial numbers which they found. Tropical seas, which were rich in microbial life, received considerable enrichments from terrestrial sources, as many large rivers, draining through areas of rich plant and animal life, empty into them. Such material was little modified, "unhumidified", and of allochthonous origin (Kriss, 1963), and, therefore, readily available to bacteria. However, in higher latitudes, despite the rich productivity of plankton, less organic material was available to bacteria because this material was mainly autochthonous in origin and was, therefore, more resistant to bacterial attack (Kriss, 1963). Duursma (1961) found wide horizontal variations in the concentration of organic matter in the North Atlantic, but he did not report any relationship to latitude.

No information is available on the concentrations of organic material in the water at the sampling site of the present study, nor are there data on the types and source of this material. Considering the absence of freshwater run-off into the sampling area, it does not appear likely that much of the organic matter here is allochthonous. But is is possible that the concentrations of organic material here are quite low or that the material is resistant to bacterial attack, and that this, in part,

may explain the low bacterial numbers encountered.

The concentrations of organic matter in the seas varies with depth (Duursma, 1961, 1965; Parsons and Strickland, 1952; Horne, 1969), and these variations are reflected in the differences in bacterial numbers with depth.

According to ZoBell (1946), there is generally a regular increase in the number of colonies from unit volumes of sea water downwards to depths of 40 to 50 m and then a decrease until the sea bottom is reached. This statement was based both on his results and on those presented in earlier studies (Schmidt - Nielsen, 1901; Otto and Neumann, 1904; Bertel, 1912; Föyn and Gran, 1928). However, the results of some other workers were not consistent with ZoBell's (1946) statement. Drew (1912) found a regular decrease in bacterial numbers with increasing depth in the West Indies, and Lloyd (1930) found similar results in the North Sea. Jannasch and Jones (1959) noted a situation apparently opposite to that described by ZoBell (1946), in that they found that counts off the California coast were highest at the surface, decreased steadily to a depth of about 75 m, and then increased. Kriss (1963) found differences in counts from samples taken at different depths of the same station, from samples taken at the same depth of different

stations, and even from multiple samples taken at the same depth of the same station. Kriss (1963) attributed these differences to variations in the concentrations of organic material present. Other examples of apparently random and irregular vertical distribution were reported by Brisou and Rautlin de la Roy (1966), Castellví (1967), and Cvilic (1967). Ezura, et al. (1974) found no significant differences between samples taken from Akkeshi Bay, Japan, at different depths, for most of the year. In the present study, no relationship was observed between the size of bacterial populations and sampling depth.

It has been suggested (Schmidt - Nielsen, 1901; ZoBell, 1946) that regular differences in bacterial numbers with depth occur, not in well-mixed, but in stratified waters, such as those associated with the presence of a thermocline. The rapid density change at the thermocline should be expected to slow down the sinking of the particles to which the bacteria are attached, leading to their accumulation at that level (Harder, 1968; Tanaka, Nakanishi, and Kadota, 1974). Assuming that the bacteria are able to utilize the particulate matter as nutrients and multiply, an increase in bacterial numbers at the thermocline should be expected. However several workers (Kriss and Rukina, 1952; Jannasch

and Jones, 1959; Ezura, et al., 1974) have obtained the lowest bacterial counts at the thermocline. In the present study, the existence of a thermocline had no apparent effect on bacterial numbers.

In the surface waters (upper 100 m) of the oceans, the concentrations of all types of organic matter vary seasonally. Populations of living phytoplankton undergo a yearly cycle of maxima (spring and autumn blooms, depending on latitude) and minima and, because almost all the organic material in the sea is ultimately derived from the photosynthetic activity of phytoplankton, the amounts of such organics also have a seasonal variation. Duursma (1961) found that the highest concentrations of dissolved organic material occurred in spring and early summer, a little later than the plankton bloom, with a minor maximum following the autumn bloom. This was attributed to the release of large amounts of organic matter into the oceans due to the breakdown of the phytoplankton cells. The same cycle was found to be characteristic of both coastal and open-seas environments, although the seasonal changes were less pronounced in the latter (Duursma, 1960). Because they are formed from dissolved organic material (Riley, 1963; Riley, Wangersky, and Van Hemert, 1964; Sheldon, Evelyn, and Parsons, 1967) the number of organic aggregates present in the oceans

is also dependent upon phytoplankton populations. The amount of detritus is also directly related to phytoplankton and was found to be highest shortly after the bloom (Horne, 1969; Menzel and Ryther, 1970) when the number of dead or dying cells was greatest.

Phytoplankton are the prime source of nutrients for bacteria and they also provide solid surfaces for bacterial attachment and the concentration of dissolved nutrients, so it is not surprising that numbers of bacteria are closely related to numbers of phytoplankton (ZoBell, 1946). A number of workers (Gran, 1933; Waksman, et al., 1933; Von Brand, Rakestraw, and Renn, 1937; Voroschilova and Dianova, 1937; ZoBell, 1946; Moskovits, 1951; Williams, 1951; Deveze, 1955, Seki, 1971) have investigated this relationship and all have found similar results. They found that, especially during the bloom, bacterial populations rose and declined as the phytoplankton populations rose and fell. It was also demonstrated that as plankton numbers were declining at the end of the bloom, bacterial populations rose, often reaching higher numbers than when the numbers of phytoplankton were greatest. This was attributed to the utilization of the increased amounts of labile organic material in the waters supplied by the dead and dying plankton organisms (Waksman, et al., 1933; Moskovits,

1951; Williams, 1951).

In the present study, the relationship between the bacterial and phytoplankton populations is not clear, except for the bloom period. During that time, at each depth sampled, minimum numbers of bacteria occurred when phytoplankton populations were highest. This might be due to the secretion of large amounts of antibacterial substances by the plankton (Seki, 1971). Numbers of bacteria started to increase once plankton numbers had reached their peak and started to decline. This is in accordance with the results of studies already mentioned and was probably due to the utilization of the organic material liberated from the decaying phytoplankton cells.

TEMPERATURE

Temperature profoundly affects the growth and metabolism, cell size and morphology, cell yield, nutritional requirements, enzyme constitution, and chemical composition of micro-organisms (Ingraham, 1962; Farrell and Rose, 1967), yet the effects of low temperatures (5C or below), characteristic of 95% of the ocean, on marine bacteria are not well understood (Morita, 1975). However, it is doubtful that temperature has much effect on the numbers of bacteria found in the seas, because while low temperatures slow down the rates of

metabolism and growth, they also increase generation time and decrease the death rate (ZoBell, 1946; Ingraham, 1962).

In the present study, bacterial numbers and water temperatures were apparently unrelated. Several other studies conducted over periods of months or years (Moskovits, 1951; Velankar, 1955, Seki, 1966; Castellví 1967) indicated that only vague relationships or none at all existed between the sizes of bacterial populations and water temperatures. Wood (1953) reported that bacterial numbers off the coast of Australia were greatest when the water temperature was highest, while ZoBell and Feltham (1934) found that highest bacterial numbers were obtained when the water was coldest off La Jolla, California. However, in both these studies, a relationship between bacterial populations and water temperatures was not apparent over the entire period studied.

Altschuler and Riley (1967) and Sieburth (1967) studied the bacterial populations of Long Island Sound and Narragansett Bay, respectively, over extended periods of time. At regular intervals, they collected samples from the sea water and isolated bacteria from them, using a number of different incubation temperatures. It was found that, in both studies, the largest numbers of bacteria grew on those plates incubated at the temperatures

which were closest to the water temperatures from which the particular samples were taken. In the present study, two incubation temperatures, 20C and 5C, were used, but the situation as described by Altschuler and Riley (1967) and Sieburth (1967) was not encountered. No significant differences between the 20C counts and the 5C counts were found, despite the fact that the water temperature was 5C or lower throughout the entire year at the bottom sample level and over a large portion of the year at the surface and middepth (thermocline).

Both Altschuler and Riley (1967) and Sieburth (1967) indicated that, although water temperature selected "thermal types" (bacteria best adapted to the particular water temperatures prevailing), it did not determine the generic composition (based on the identification scheme of Shewan, et al., 1960) of the bacterial population. Although generic composition of the samples taken in the present study was not determined, the numbers of pseudomonads and their percentages of the total bacterial populations were. Except for the surface samples, where they appeared to be more prevalent in the autumn than in the winter to summer period, pseudomonads did not appear to undergo a seasonal selection. According to Altschuler and Riley (1967), Pseudomonas, along with Spirillum and Vibrio, "contributed significantly" to the summer samples but

were of little importance at any other time of the year in Long Island Sound. Sieburth (1967) found that, in Narragansett Bay, the quantitative importance of Pseudomonas varied randomly throughout the year.

The term "psychrophile", coined by Schmidt - Nielsen (1902), is used to describe those bacteria able to grow and multiply at low temperatures. Considerable confusion exists about the maximal, minimal, and optimal temperatures for the growth of psychrophiles, since the term has been defined in a variety of ways (Morita, 1966). However, the most recent and probably the most useful definition states that psychrophiles are those organisms "having an optimal temperature for growth at about 15C or lower, a maximal temperature for growth at about 20C and a minimal temperature for growth at 0C or below" (Morita, 1975).

Because of the low temperatures characteristic of most of the marine environment, it is generally agreed (Ingraham, 1962; Morita, 1966, 1975; Farrell and Rose, 1967) that the large majority of marine bacteria, especially members of the genus Pseudomonas, are psychrophiles. The results of the present study, however, do not indicate how many psychrophiles, if any, were present, because both temperatures used (20C and 5C) lie within the temperature range of psychrophilic bacteria.

EFFECTS OF MICRO-ORGANISMS

Bacterial populations in the oceans are affected by the activities of a variety of micro-organisms, including phytoplankton, bacteriophage and other bacteria.

Phytoplankton and their activities closely affect the bacterial populations of the seas. As has already been discussed, phytoplankton provide nearly all the nutrients needed by the bacteria and also supply solid surfaces for attachment.

Seki (1971) found that although many bacteria attached themselves to phytoplankton following the bloom, few, if any, attached before or during the bloom period and were found, instead, on detritus. Moskovits (1951) noted that while the number of bacteria attached to phytoplankton was greater than the number free in the water when the bloom was declining, it was less than the number of free bacteria before and during the bloom. Plankton cells are alive and healthy before and during the bloom, but are dead and decaying after this period has ended. A number of workers (Steeman - Nielsen, 1955; Oppenheimer and Vance, 1960; Moskovits, 1961; Droop and Elson, 1966) have found that bacteria readily attach themselves to dead phytoplankton but are rarely, if ever, attached to living ones.

Droop and Elson (1966) concluded that healty diatoms have some "defense mechanism" which prevents bacterial attachment and which is lost when the diatoms die. Seki (1971) suggested that the mechanism involved is the production of some antibacterial agent released into the environment, similar in effect to acrylic acid which was found to produce bacterial inhibition in certain plankton bloom areas of the Antarctic (Sieburth, 1959, 1960, 1961). A wide variety of phytoplankton organisms manufacture and secrete antibacterial agents (Steeman - Nielson, 1955; Sieburth, 1964, 1968). However, Sieburth (1964) stated that the production of algal antibiotics in detectable amounts appeared to occur only during periods of active growth. Such products, if produced, would, therefore, tend to accumulate in large quantities during phytoplankton blooms.

In the present study, lowest numbers of bacteria were encountered when phytoplankton populations were at their peak.

Little is known of either the occurrence or activity of marine bacteriophage. Although some phages active against marine bacteria have been isolated from the sea (ZoBell, 1946; Spencer, 1955, 1960; Wiebe and Liston, 1968) so far none have been found in the open North Atlantic Ocean or from the Atlantic coast of North America.

Both ZoBell (1946) and Spencer (1955) were able to isolate phages only after many unsuccessful attempts and, so, concluded that they occurred only sparsely and sporadically in sea water far from land.

According to ZoBell (1946), "bacteriophage is generally found associated with large numbers of rapidly multiplying bacteria, so it is doubtful if the sparse bacterial populations characteristic of open ocean environments are conducive to the development or activity of phage". He added that there were "no experimental data or theoretical considerations to suggest that bacteriophage are factors which limit the bacterial populations of the open ocean". The area sampled in the present study is a coastal one, but it has sparse bacterial populations characteristic of oceanic environments, and, therefore, it is doubtful that bacteriophage activity is important here. Unfortunately, no data on the presence of phage in the sampling area is available.

Some types of bacteria are able to adversely affect the activities and numbers of other bacteria in marine environments. Such an effect is accomplished either by direct action on the part of the bacteria (as in the case of predatory bacteria) or by the release of certain inhibitory substances into the environment.

The predatory bacterium, Bdellovibrio, preys on other bacteria present in many natural environments, including the sea (Shilo, 1966). Unfortunately, most of the marine work done with members of this genus has involved predation on coliforms (Mitchell, Yankofsky, and Jannasch, 1967) in coastal waters, rather that predation on bacteria indigenous to the marine environment. Because of this, little is known of either the distribution or the ecological importance of Bdellovibrio in the sea.

Some marine bacteria are able to inhibit the growth of other bacterial types by the production of inhibitory substances. Lewis (1929) found that, in nutrient medium, a culture of P. fluorescens he had isolated from the sea excreted a toxin which inhibited the growth of both bacteria and fungi, and Burkholder, et al. (1966) isolated a pseudomonad which was active against a variety of other gram-negative bacteria. Bacterial antagonism is, according to Kriss, et al. (1967), partially responsible for the underestimation of bacterial numbers by the culture plate method. Waksman and Carey (1935) stated that the antagonistic effects of microorganisms, including bacteria, might help to explain the low numbers of bacteria usually found in sea water.

No information is available on the presence of either Bdellovibrio or antagonistic bacteria in the sampling area.

FURTHER STUDY

While the present study has been only a brief first look at the bacteriology of the coastal waters of the Avalon Peninsula, it has already indicated the complexities of the relationship between the bacteria and their environment. However, any attempts to clarify the problem must involve laboratory studies, where some of the many variables present in the natural environment can be controlled.

A study of the growth rates of selected bacteria, isolated from the sampling site, by the continuous culture method (Herbert, Elsworth, and Telling, 1956; Jannasch,1969) in sea water collected from the same area at different times of the year could possibly explain the low levels of bacteria encountered. Low growth rates might indicate a shortage of nutrients, a lack of surfaces for attachment, or both. These possibilities might then be further investigated by the addition to the culture set-up of metabolizable organic compounds, inert solid surfaces, or both. Simultaneous culture of bacteria and

phytoplankton, especially those present at times of highest or lowest bacterial counts, could yield important information on the possible effect on the bacteria of metabolites produced by the algae.

SUMMARY OF RESULTS

1. Seventy-eight percent of the plate counts of sea water samples produced no colonies.

2. The plate counts of the plankton tow material indicated that bacterial numbers were very low (10 to 4,329 per liter) throughout the year for all three sampling depths.

3. No regular vertical pattern of bacterial distribution was found. The presence of a thermocline apparently had no effect on the vertical distribution of the bacteria.

4. Bacterial populations did not appear to be related to water temperatures or salinities.

5. No significant differences between the 20C plate counts and the 5C plate counts were found.

6. The number of pseudomonads per liter of plankton tow varied from season to season. Pseudomonads dominated the autumn surface samples, but no trend could be detected for the other depths.

7. The percentages of pseudomonads in the total bacterial populations were higher in the surface samples than in the bottom ones.

8. Cetrimide (0.03%) medium was not as effective a selective agent for pseudomonads as had been previously determined. No significant differences could be detected between numbers of pseudomonads which developed on cetrimide after direct isolation onto this medium and numbers which developed after replication from YBP-SWA.

9. The use of motility and pigmentation in the identification of pseudomonads is of doubtful value. An identification scheme stressing the importance of characteristics other than motility and pigmentation was devised.

10. The fluorescent antibody technique was found to be of value in the differentation of pseudomonads from other organisms.

11. For all three sampling depths, diatoms bloomed (caused mainly by Chaetoceros species) from March, to May or June, but were quantitatively unimportant throughout the rest of the year. Dinoflagellates peaked when diatoms were absent, and almost disappeared during the diatom bloom. Silicoflagellates

were present, in low numbers, only during the autumn and winter samples.

12. There was no apparent relationship between phytoplankton populations and numbers of total bacteria or pseudomonads, except for the bloom period. Lowest numbers of both total bacteria and pseudomonads occurred when the phytoplankton bloom was at its peak, then, as phytoplankton numbers declined, the numbers of bacteria and pseudomonads started to rise.

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FORMULARY

Cetrimide Agar (Noseworthy and Moskovits, 1974)

2% solution of cetrimide (hexadecyltrimethylammonium bromide) in distilled water.

Add membrane-filtered cetrimide solution to cooled (45C) Bacto-Pseudomonas agar F (prepared with synthetic sea water) to give a final concentration of 0.03%. Pour plates immediately.

Decarboxylase Media

Make up Bacto-Decarboxylase Base Moeller (Difco) in synthetic sea water. Check pH (reads approximately 5.95). Divide the medium into 4 equal amounts and add L-amino acid hydrochlorides as follows:

1.	1% arginine	(pH approximately 6.00)
2.	1% lysine	(pH approximately 6.00)
3.	1% ornithine	(pH approximately 5.95)
4.	0 amino acid	(Control)

Dispense 3-ml amounts of these portions in 13 x 100-mm tubes and plug with foam-plastic stoppers. Autoclave at 15 lb for 15 min. When cool enough to handle, layer with sterile mineral oil.

Fluorescein Medium

Make up Bacto-Pseudomonas agar F (Difco) as directed in synthetic sea water. Check pH (runs 6.85-6.90). Autoclave at 15 lb for 15 min.

Gas Production Medium (modified from Hugh and Leifson, 1953)

Peptone	2.0 g
NaCl	5.0 g
K_2HPO_4	0.3 g
Synthetic sea water	1000.0 ml

Dissolve all components. Adjust pH to 7.1. Dispense 5-ml portions in 13 x 100-mm foam-plastic plugged tubes. Add an inverted Durham tube to each. Autoclave at 15 lb for 20 min. Cool and hold at 50C in water bath. To each tube of medium, add, aseptically, 0.5-ml sterile (membrane-filtered) glucose solution (10%). Mix thoroughly.

Gram Stain

1. Ammonium Oxalate Crystal Violet

Solution A:	
Crystal violet (90% dye content)	2.0 g
Ethyl alcohol (95%)	20.0 ml
Solution B:	
Ammonium oxalate	0.8 g
Distilled water	80.0 ml

Mix solutions A and B.

2. Iodine Solution

Iodine	1.0 g
KI	2.0 g
$NaHCO_3$	1.0 g
Distilled water	300.0 ml

3. Safranin Counterstain

Safranin O (2.5% in 95% ethanol)	10.0 ml
Distilled water	100.0 ml

Staining Schedule:

Stain 1 min with ammonium oxalate crystal violet. Pour off excess stain.

Immerse 1 min in Iodine solution. Rinse in tap water and shake off excess.

Decolorize 10 sec in 95% ethanol. Rinse. Shake off excess water.

Counterstain for 30 sec with safranin. Wash. Blot dry.

Flagella Stain

Make up BBL-Flagella stain as directed. Pour into dark glass bottle and keep tightly stoppered. Discard stain more than 1 week old.

Medium for Antigen Production (Moskovits and Foelsche, 1970)

NH_4Cl	3.0 g
KH_2PO_4	1.0 g
K_2HPO_4	2.0 g
$MgSO_4 \cdot 7H_2O$	0.5 g
NaCl	3.0 g
D-galactose	6.0 g
Distilled water	1000.0 ml

Adjust pH to 7.8. Autoclave at 7 lb for 15 min. Cool in ice bath immediately afterward.

Mineral Oil Overlay

Dispense 2-ml portions of Nujol mineral oil in small vials (approximately 13 x 45 mm). Cap each vial with double-thickness aluminum foil. Hot-air sterilize for 2 h at 170-180C.

MOF Medium (Difco)

Make up as directed. Steam to melt agar. Dispense in 100-ml amounts. Autoclave at 15 lb for 15 min. Cool and hold at 50C in water bath. To each 100-ml medium, add, aseptically, 10-ml sterile (membrane-filtered) glucose solution (10%). Mix thoroughly. Dispense, aseptically, 3-ml portions in 13 x 100-mm sterile, foam-plastic plugged tubes.

O/129 Sensitivity Discs

Prepare 0.1% solution of O/129 (2,4-diamino-6,7-diisopropyl pteridine) in acetone. Soak Bacto-Concentration Discs (sterile blanks, 1/4") in O/129 solution for 24 h. Drain and place in sterile glass Petri dish. Dry overnight at 35C.

Oxidase Reagent

Prepare 1% solution of N,N,N',N',-tetramethyl-p-phenylene-diamine-dihydrochloride in distilled water. Use immediately.

Pyocyanin Medium

Make up Bacto-Pseudomonas agar P (Difco) as directed in synthetic sea water. Check pH (runs 6.65-6.80). Autoclave at 15 lb for 15 min.

Synthetic Sea Water (Lyman and Fleming, 1940; modified by Moskovits and Flanagan, 1967)

NaCl	23.476 g
$MgCl_2.6H_2O$	10.629 g
Na_2SO_4 (anhydrous)	3.917 g
$CaCl_2.2H_2O$	1.459 g
KCl	0.664 g
$NaHCO_3$	0.192 g
KBr	0.096 g
Glass-distilled water	1000.000 ml

Add the salts directly to the distilled water. Dissolve each reagent completely before adding the next.

Thornley's Arginine Medium (Thornley, 1960)

Peptone	1.00 g
K_2HPO_4	0.30 g
L-arginine monohydrochloride	10.00 g
Phenol red	0.01 g
Oxoid agar No. 3	12.00 g
Synthetic sea water	1000.00 ml

Dissolve all components. Adjust pH to 7.2. Add agar and steam to melt. Dispense 3-ml portions in 13 x 100-mm tubes and plug with foam-plastic stoppers. Autoclave at 15 lb for 10 min.

Yeast-Beef-Peptone Sea Water Agar (YBP-SWA)

Beef extract	1.5 g
Yeast extract	3.0 g
Peptone	6.0 g
Oxoid Agar No. 3	12.0 g
Natural sea water	1000.0 ml

Dissolve all components. Adjust pH to 7.8. Add agar and steam to melt. Filter hot through double-layer of cheese-cloth and Pyrex glass wool. Autoclave at 15 lb for 20 min.

Yeast-Beef-Peptone Fresh Water Agar (YBP-FWA)

Make up as directed for Yeast-beef-peptone sea water agar, but substitute distilled water for sea water.

Yeast-Beef-Peptone Synthetic Sea Water Agar (YBP-SWN-SWA)

Make up as directed for Yeast-beef-peptone sea water agar, but substitute synthetic sea water for natural sea water.

Yeast-Peptone Sea Water Broth (YP-SWB)

Make up as directed for yeast-beef-peptone sea water agar, but omit the beef extract and agar. Check, but do not adjust, pH. Filter cold through double thickness Whatman No. 5 filter paper.

APPENDIX I: OCEANOGRAPHIC AND METEOROLOGICAL DATA

Table 1. Water temperatures during the sampling period.

Date	Temperatures (C) Surface	Middepth	Bottom
1972			
Sept. 12	13.0	11.8	1.7
Sept. 29	10.5	10.5	1.0
Oct. 11	9.0	8.8	0
Oct. 24	8.0	7.8	-0.6
Nov. 23	3.0	3.0	3.0
1973			
Jan. 25	-1.0	-1.0	-1.0
Feb. 6	-1.0	-1.0	-1.0
Mar. 21	-1.0	-1.0	-1.0
May 15	0.5	-1.0	-1.0
May 29	0.5	-1.0	-1.0
June 13	3.5	-1.0	-1.5
June 27	6.0	2.5	-2.0
July 10	11.0	5.0	±1.2
July 24	12.0	2.8	-1.3
Aug. 10	14.0	5.5	-0.8

Table 2. Thermal stratification of water masses sampled.

Date	Depth of Thermocline (Meters)	Change in Depth (Meters)	Temperature Gradient (C)	Temperature Change (C)	Temperature Change with Depth (C/M)
1972					
Sept. 12	15-20	5	7-11	4	0.80
Sept. 29	22-30	8	4-10	6	0.75
Octo. 24	15-24	9	2- 8	6	0.67
1973					
June 13	15-20	5	0- 3	3	0.60
June 27	10-25	15	0- 6	6	0.40
July 10	5-15	10	3-10	7	0.70
July 24	5-10	5	3- 7	4	0.80
Aug. 10	5-10	5	5- 9	4	0.80

Table 3. Sampling depths during the sampling period.

Date	Water Depth (Meters)
1972	
Sept. 12	64.0
Sept. 29	62.5
Oct. 11	70.1
Oct. 24	73.2
Nov. 23	73.2
1973	
Jan. 25	64.0
Feb. 6	64.0
Mar. 21	69.5
May 15	71.3
May 29	73.2
June 13	69.5
June 27	61.0
July 10	61.0
July 24	64.0
Aug. 10	61.0

Table 4. Meteorological data during the sampling period.

Date	Weather*	Air Temperature (C)	Wind* Force	Direction
1972				
Sept. 12	Mainly cloudy	16.0	F B	W
Sept. 29	Fair	7.0	L B	W
Oct. 11	Fair	7.0	M B	NW
Oct. 24	Overcast sky	6.5	L A	W
Nov. 23	Broken clouds	3.0	L B	W
1973				
Jan. 25	Overcast sky	3.0	L B	W
Feb. 6	Fair	-3.0	M B	NW
Mar. 21	Overcast sky	1.5	F B	N
May 15	Overcast sky	8.0	M B	W
May 29	Overcast sky Rain	3.0	F B	SW
June 13	Overcast sky	6.5	L B	NNW
June 26	Overcast sky	18.0	G B	SW
July 10	Fair	20.0	F B	WSW
July 24	Overcast sky Drizzle, Fog	15.0	L B	SW
Aug. 10	Blue sky	15.0	M B	W

*Descriptions based on Manual for Coding and Punching Oceanographic Data on Cards (1960).

Weather:

Blue sky	clear or hazy atmosphere
Fair	few clouds; scattered sky
Mainly cloudy	1/2 sky overcast
Broken clouds	3/4 sky overcast
Overcast sky	whole sky overcast

Wind Force:

		Knots	approx. m/sec
C	calm	0-1	0.00- 0.52
LA	light air	1-3	0.52- 1.55
LB	light breeze	4-6	2.06- 3.09
GB	gentle breeze	7-10	3.61- 5.15
MB	moderate breeze	11-16	5.67- 8.24
FB	fresh breeze	17-21	8.76-10.82
SB	strong breeze	22-27	11.33-13.91
MG	moderate gale	28-33	14.42-17.00

Table 5. Sea conditions during the sampling period.

Date	Sea State*	Sea Swell
1972		
Sept. 12	Slight	Moderate
Sept. 29	Slight	Moderate
Oct. 11	Moderate	Moderate
Oct. 24	Calm - rippled	Moderate
Nov. 23	Smooth wavelet	Slight
1973		
Jan. 23	Smooth wavelet	Moderate
Feb. 6	Slight	Moderate
Mar. 21	Moderate	Moderate - heavy
May 15	Smooth wavelet	Moderate
May 29	Slight - moderate	Moderate - heavy
June 13	Smooth wavelet	Heavy
June 27	Smooth wavelet	None
July 10	Moderate	Moderate - heavy
July 24	Smooth wavelet - slight	Moderate
Aug. 10	Slight	Moderate

*Descriptions based on Manual for Coding and Punching Oceanographic Data on Cards (1960).

Sea State:	Height in m
Calm - glassy	0
Calm - rippled	0 -0.1
Smooth wavelet	0.1-0.5
Slight	0.5-1.2
Moderate	1.2-2.4
Rough	2.4-4.0
Very rough	4.0-6.1

Table 6. Salinities during the sampling period.

Date	Salinities (°/oo) Surface	Middepth	Bottom
1972			
Sept. 12	33.5	33.5	35.4
Sept. 29	32.5	34.0	34.5
Oct. 11	31.4	32.7	34.6
Oct. 24	32.9	32.9	34.2
Nov. 23	32.8	33.5	33.5
1973			
Jan. 25	30.3	35.7	36.3
Feb. 6	33.3	33.3	32.0
Mar. 21	34.0	34.0	34.0
May 15	31.2	33.8	35.4
May 29	31.5	34.1	36.7
June 13	34.6	34.6	38.5
June 27	36.3	33.6	37.6
July 10	35.1	35.1	36.4
July 24	33.2	34.5	35.8
Aug. 10	31.4	31.4	34.5

APPENDIX II: TOTAL BACTERIA COUNT DATA

Table 1. Numbers of bacterial colonies produced from 0.1 ml undiluted sea water after incubation at 20C and 5C*.

Date	Sample**	Incubation Temperature (°C) 20 Replicate 1	2	3	5 Replicate 1	2	3
1972							
Sept. 12	S	0	0	0	1	0	0
	T	1	0	0	0	0	0
	B	0	0	0	0	0	0
Sept. 29	S	0	0	0	1	2	0
	T	2	3	0	1	0	0
	B	3	0	0	0	0	0
Oct. 11	S	0	0	0	1	0	0
	M	0	0	0	2	1	0
	B	0	0	0	1	0	0
Oct. 24	S	0	0	0	0	0	0
	T	1	3	0	0	0	0
	B	3	1	0	0	0	0
Nov. 23	S	0	0	0	3	0	0
	M	2	1	0	0	0	0
	B	6	1	0	1	1	0
1973							
Jan. 25	S	5	0	0	0	0	0
	M	0	0	0	0	0	0
	B	1	0	0	0	0	0
Feb. 6	S	0	0	0	0	0	0
	M	0	0	0	0	0	0
	B	0	0	0	0	0	0

Table 1 Continued.

Date	Sample	Incubation Temperature (°C) 20 Replicate 1	2	3	5 Replicate 1	2	3
Mar. 21	S	0	0	0	0	0	0
	M	0	0	0	3	0	0
	B	0	0	0	0	0	0
May 15	S	0	0	0	0	0	0
	M	1	0	0	0	0	0
	B	0	0	0	0	0	0
May 29	S	3	4	7	3	1	0
	M	0	0	0	2	0	0
	B	1	0	0	0	0	0
June 13	S	3	0	0	3	1	0
	T	1	0	0	0	0	0
	B	2	0	0	2	0	0
June 27	S	1	1	0	0	0	0
	T	0	0	0	1	0	0
	B	0	0	0	1	0	0
July 10	S	0	0	0	0	0	0
	T	0	0	0	1	1	0
	B	0	0	0	1	0	0
July 24	S	2	3	9	1	0	0
	T	1	0	0	1	0	0
	B	0	0	0	0	0	0
Aug. 10	S	0	0	0	0	0	0
	T	2	2	0	1	3	0
	B	1	0	0	1	0	0

*Because of the low numbers involved, these counts were not converted to bacteria per unit volume for each sample.

**Sample designations: S Surface
M Middepth
T Thermocline
B Bottom

Table 2. Calculation of total numbers of bacteria per liter of plankton tow based on total colony counts on YBP-SWA after incubation at 20C and 5C.

Procedure for converting number of colonies per plate to number of bacteria per liter of plankton tow:

1. Determine average number of colonies per plate for the replicate plates of that dilution producing 30-300 colonies.

2. Multiply average number per plate by the appropriate dilution factor to get number of bacteria per ml of sample.

3. Multiply the number per ml of sample by the volume of the sample (expressed in ml) to get total bacteria in sample.

4. Divide bacteria in sample (which is a concentration of the total volume of water filtered through the plankton net) by total volume of water filtered (expressed in liters) to get number of bacteria per liter of plankton.

20C Counts

Date and Sample*	Total Bacteria per ml	Volume of Sample (ml)	Total Bacteria per Sample	Volume of Water Filtered (Liters)	Total Bacteria per Liter
1972					
Sept. 12					
S	6,000	500	3,000,000	9,268	324
T	2,140	605	1,294,704	3,996	315
B	12,000	590	7,079,832	5,256	1,347
Sept. 29					
S	19,333	570	11,019,810	6,364	1,732
T	3,233	570	1,842,810	6,092	302
B	21,333	520	11,093,160	8,260	1,343
Oct. 11					
S	11,533	510	5,881,830	6,268	938
M	4,300	410	1,763,000	5,456	323
B	3,800	550	2,090,000	6,580	318
Oct. 24					
S	16,767	520	8,718,840	7,400	1,172
T	8,167	490	4,001,832	3,960	1,011
B	15,467	560	8,661,520	8,044	1,077
Nov. 23					
S	16,677	540	9,000,180	9,064	993
M	15,333	570	8,739,810	4,928	1,774
B	7,900	550	4,345,000	5,864	741
1973					
Jan. 25					
S	2,500	570	1,425,000	9,364	152
M	4,300	570	2,451,000	7,888	311
B	2,967	520	1,542,840	6,564	235
Feb. 6					
S	1,763	590	1,040,172	5,124	203
M	3,533	520	1,837,160	3,356	547
B	1,433	580	831,140	6,200	134

Table 2 Continued

Date and Sample	Total Bacteria per ml	Volume of Sample (ml)	Total Bacteria per Sample	Volume of Water Filtered (Liters)	Total Bacteria per Liter
Mar. 21					
S	1,193	610	727,730	10,340	70
M	303	610	184,830	3,612	51
B	3,800	580	2,204,000	6,312	349
May 15					
S	170	550	93,500	9,084	10
M	110	590	64,900	3,600	18
B	440	600	264,000	6,400	41
May 24					
S	7,267	165	1,199,055	8,000	150
M	4,500	175	787,500	4,000	197
B	9,933	280	2,781,240	6,000	464
June 13					
S	1,087	450	489,150	4,320	113
T	6,200	420	2,604,000	1,012	2,573
B	7,167	400	2,866,800	3,712	772
June 24					
S	5,167	420	2,170,140	4,980	436
T	787	400	318,800	888	359
B	9,300	360	3,348,000	2,504	1,337
July 10					
S	1,017	420	427,140	7,116	60
T	673	380	255,740	4,508	57
B	2,900	590	1,711,000	9,120	188
July 24					
S	600	340	204,000	11,256	18
T	5,333	390	2,079,870	6,176	337
B	3,500	520	1,820,000	10,416	175
Aug. 10					
S	297	400	118,800	7,740	15
T	1,403	350	491,050	11,264	44
B	8,600	400	3,440,000	7,208	477

Table 2 Continued

5C Counts

Date and Sample	Total Bacteria per ml	Volume of Sample (ml)	Total Bacteria per Sample	Volume of Water Filtered (Liters)	Total Bacteria per Liter
1972					
Sept. 12					
S	11,325	500	5,662,748	9,268	611
T			1,190,808	3,996	298
B	12,329	580	7,274,304	5,256	1,384
Sept. 29					
S	12,002	570	6,841,300	6,364	1,075
T	2,672	570	1,523,000	6,092	250
B	13,295	520	6,913,620	8,260	837
Oct. 24					
S	19,226	620	9,997,400	7,400	1,351
T	5,471	490	2,680,920	3,960	677
B	11,003	560	6,161,704	8,044	766
Nov. 23					
S	12,663	540	39,238,056	9,064	4,329
M	16,332	570	9,308,992	4,928	1,889
B	29,331	550	16,131,864	5,864	2,751
1973					
Jan. 25					
S	1,906	570	1,086,224	9,364	116
M	4,165	570	2,374,288	7,888	301
B	2,196	520	1,142,136	6,564	174
Feb. 6					
S			845,460	5,124	165
M	5,312	520	2,761,988	3,356	823
B	1,497	580	868,000	6,200	140

Table 2 Continued.

Date and Sample	Total Bacteria per ml	Volume of Sample (ml)	Total Bacteria per Sample	Volume of Water Filtered (Liters)	Total Bacteria per Liter
Mar. 21					
S	1,729	610	1,054,680	10,340	102
M	1,599	610	975,240	3,612	270
B	3,613	580	2,095,584	6,312	332
May 15					
S	380	550	208,932	9,084	23
M	439	590	259,200	3,600	72
B	2,304	600	1,382,400	6,400	216
May 29					
S	13,236	165	2,184,000	8,000	273
M	5,371	175	940,000	4,000	235
B	9,943	280	2,784,000	6,000	464
June 13					
S	893	450	401,760	4,320	93
T	7,848	420	3,296,084	1,012	3,257
B	17,716	400	7,086,208	3,712	1,909
June 27					
S	3,652	420	1,533,840	4,980	308
T	699	400	279,720	888	315
B	6,803	360	2,448,912	2,504	978
July 10					
S	1,728	420	725,832	7,116	102
T	1,400	380	531,944	4,508	118
B	6,075	590	3,584,160	9,120	393
July 24					
S	1,291	340	438,984	11,256	39
T	2,930	390	1,142,560	6,176	185
B	4,327	520	2,249,856	10,416	216
Aug. 10					
S	1,606	400	642,420	7,740	83
T	2,092	350	732,160	11,264	65
B	8,974	400	3,589,584	7,208	498

Table 2 Continued

*Sample designations: S Sample
M Middepth
T Thermocline
B Bottom

Table 3. Total bacteria per liter of plankton tow based on 20C YBP-SWA counts.

Date	Surface	Middepth	Bottom
1972			
Sept. 12	324	315*	1,347
Sept. 29	1,732	302*	1,343
Oct. 11	938	323	318
Oct. 24	1,172	1,011*	1,077
Nov. 23	993	1,774	741
1973			
Jan. 25	152	311	235
Feb. 6	203	547	134
Mar. 21	70	51	349
May 15	10	18	41
May 29	150	197	464
June 13	113	2,573*	772
June 27	436	359*	1,337
July 10	60	57*	188
July 24	18	337*	175
Aug. 10	15	44*	477

*Thermocline samples.

Table 4. Total bacteria per liter of plankton tow based on 5C YBP-SWA counts.

Date	Surface	Middepth	Bottom
1972			
Sept. 12	611	298*	1,384
Sept. 29	1,075	250*	837
Oct. 11		Not determined	
Oct. 24	1,351	677*	766
Nov. 23	4,329	1,889	2,751
1973			
Jan. 25	116	301	174
Feb. 6	165	823	140
Mar. 21	102	270	332
May 15	23	72	216
May 29	273	235	464
June 13	93	3,257*	1,909
June 27	308	315*	978
July 10	102	118*	393
July 24	39	185*	216
Aug. 10	83	65*	498

*Thermocline samples.

Table 5. Comparison of numbers of total bacteria per liter of plankton tow based on 5C counts and 20C counts using the T test for paired observations.

5C Counts	20C Counts	Difference d_i	d_i^2
	Surface Samples		
611	324	287	82,369
1,075	1,732	- 657	431,649
1,351	1,172	179	32,041
4,329	993	3,336	11,128,896
116	152	- 36	1,296
165	203	- 38	1,444
102	70	32	1,024
23	10	13	169
273	150	123	15,129
93	113	- 20	400
308	436	- 128	16,384
102	60	42	1,764
39	18	21	441
83	15	68	4,624
	Middepth Samples		
298	315	- 17	289
250	302	- 52	2,704
677	1,011	- 334	111,556

Table 5 Continued.

5C Counts	20C Counts	Difference d_i	d_i^2
1,889	1,774	- 334	13,225
301	311	- 10	100
832	547	285	81,225
270	51	219	47,961
72	18	54	2,916
235	197	38	1,444
3,257	2,573	684	467,856
315	359	- 44	1,936
118	57	61	3,721
185	337	- 151	23,104
65	44	21	441
	Bottom Samples		
1,384	1,347	37	1,369
837	1,343	- 506	256,036
766	1,077	- 311	96,721
2,751	741	2,010	4,040,100
174	235	61	3,721
140	134	6	36
332	349	17	289
216	41	175	30,625
464	464	0	0
1,909	772	2,137	4,721,929

Table 5 Continued.

5C Counts	20C Counts	Difference d_i	d_i^2
978	1,337	- 359	128,881
393	188	205	42,025
216	175	41	1,681
498	477	21	441

Sample	Σd_i	Σd_i^2	$\bar{d}$	s_d*	t**
Surface	3,222	11,717,630	230.14	918.87	0.94
Middepth	869	758,478	62.07	232.80	1.00
Bottom	3,378	9,323,854	241.29	809.03	1.12
All	7,469	21,799,962	177.83	724.62	1.59

$$*s_d = \sqrt{\frac{n\Sigma(d_i^2) - (\Sigma d_i)^2}{n(n-1)}}$$

$$**t = \frac{\bar{d} - d_o}{s_d/\sqrt{n}}$$

Hypothesis tested:

There is a difference between 5C and 20C counts.

Table 5 Continued.

$H_o: \mu_5 - \mu_{20} = 0 = d_o$

$H_1: \mu_5 - \mu_{20} \neq 0$

$\alpha = 0.10$

Critical region: $T < -1.771$ and $T > 1.771$* for Surface, Middepth or Bottom Samples

$T < -1.645$ and $T > 1.645$ for all samples.

∴ Accept H_o in all cases.

*T values from Introduction to Statistics (Walpole, 1968).

Table 6. Comparison of numbers of total bacteria per liter of plankton tow from different depths based on 20C and 5C counts.

A. Surface and Middepth (20C)

Surface Counts	Middepth Counts	Difference d_i	d_i^2
324	315	9	81
1,732	302	1,430	2,044,900
938	323	615	378,225
1,172	1,011	- 161	25,921
993	1,774	- 781	609,961
152	311	- 160	25,600
203	547	- 344	118,336
70	51	19	361
10	18	- 8	64
150	197	- 47	2,209
113	2,573	-2,460	6,051,600
436	359	77	5,929
60	57	3	9
18	337	- 319	101,761
15	44	- 29	841

B. Middepth and Bottom (20C)

Middepth Counts	Bottom Counts	Difference d_i	d_i^2
315	1,347	-1,032	1,065,024

Table 6 Continued.

Middepth Counts	Bottom Counts	Difference d_i	d_i^2
302	1,343	-1,041	1,083,681
323	318	5	25
1,011	1,077	- 66	4,356
1,774	741	1,033	1,067,089
311	235	76	5,776
547	134	413	170,569
51	349	- 298	88,804
18	41	- 23	529
197	464	- 267	71,289
2,573	772	1,801	3,243,601
359	1,337	- 978	956,484
57	188	- 131	17,161
337	175	162	26,244
44	477	- 433	187,489

C. Surface and Bottom (20C)

Surface Counts	Bottom Counts	Difference d_i	d_i^2
324	1,347	-1,023	1,046,529
1,732	1,343	389	151,321
938	318	620	384,400
1,172	1,077	95	9,025
993	741	252	63,504

Table 6 Continued.

Surface Counts	Bottom Counts	Difference d_i	d_i^2
152	235	- 83	6,889
203	134	69	4,761
70	349	- 279	77,841
10	41	- 31	961
150	464	- 341	98,596
113	772	- 659	434,281
436	1,337	- 901	811,801
60	188	- 128	16,384
18	175	- 157	24,649
15	477	- 462	213,444

D. Surface and Middepth (5C)

Surface Counts	Middepth Counts	Difference d_i	d_i^2
611	298	313	97,969
1,075	250	825	680,625
1,351	677	674	454,276
4,329	1,889	2,440	5,953,600
116	301	- 185	34,225
165	823	- 658	432,964
102	270	- 168	28,224
23	72	- 49	2,401
273	235	38	1,444
93	3,257	-3,164	10,010,896

Table 6 Continued.

Surface Counts	Middepth Counts	Difference d_i	d_i^2
308	315	- 7	9
102	118	- 16	256
39	185	- 146	21,316
83	65	18	324

E. Middepth and Bottom (5C)

Middepth Counts	Bottom Counts	Difference d_i	d_i^2
298	1,384	-1,086	1,179,396
250	837	- 587	344,569
677	766	- 89	7,921
1,889	2,751	- 862	743,044
301	174	127	16,129
823	140	683	466,489
270	332	- 62	3,844
72	216	- 144	20,736
235	464	- 229	52,441
3,257	1,909	1,348	1,817,104
315	978	- 663	439,569
118	393	- 275	75,625
185	216	- 31	961
65	498	- 433	187,489

Table 6 Continued.

F. Surface and Bottom (5C)

Surface Counts	Middepth Counts	Difference d_i	d_i^2
611	1,384	- 773	597,529
1,075	837	238	56,644
1,351	766	585	342,225
4,329	2,751	1,578	2,490,084
116	174	- 58	3,364
165	140	25	625
102	332	- 230	52,900
23	216	- 193	37,249
273	464	- 191	36,481
93	1,909	-1,816	3,297,856
308	978	- 670	448,900
102	393	- 291	84,681
39	216	- 177	31,329
83	498	- 415	172,225

Comparison	d_i	d_i^2	$\bar{d}$	s_d*	t**
A	-1,834	9,365,798	122.27	816.86	0.58
B	- 779	606,841	51.90	753.45	0.27
C	-2,312	5,822,544	154.13	459.52	1.30
D	- 85	17,718,569	6.07	1,167.44	0.02
E	-2,302	5,355,317	164.41	618.71	0.99
F	-2,388	7,652,092	170.57	746.52	0.85

Table 6 Continued.

$$* \; S_d = \sqrt{\frac{n\,\Sigma(d_i^2) - (\Sigma d_i^2)}{n(n-1)}}$$

$$** \; t = \frac{\bar{d} - d_o}{S_d/\sqrt{n}}$$

Hypothesis tested:

There are no differences between the counts from different depths.

H_o: $u_S - u_M = 0$; $u_m - u_B = 0$; $u_S - u_B = 0$

H_i: $u_S - u_M \neq 0$; $u_m - u_B \neq 0$; $u_S - u_B \neq 0$

$\alpha = 0.10$

Critical region: $T < -1.761$ and $T > 1.761$* for A, B, and C

$T < -1.771$ and $T \geq 1.771$ for D, E, and F

∴ Accept H_o in all cases.

* T values from Introduction to Statistics (Walpole, 1968).

APPENDIX III: CHARACTERIZATION AND IDENTIFICATION OF ISOLATES

Table 1. Characteristics of isolates identified as *Pseudomonas* spp.

Characteristic	Group I* Type A	Group I* Type B	Group II Type A	Group II Type B	Group IV Type A	Group IV Type B
Oxidase	+	+	+	+	+	+
Motile; Polar flagella	+	-	+	-	+	-
Hugh-Leifson**	O	O	O	O	NC	NC
Sensitive to Penicillin	-	-	-	-	-	-
Sensitive to Terramycin	-	-	+	+	+	+
Arginine dihydrolase	+	+	+	+	+	+
Decarboxylases:						
Lysine	-	-	-	-	-	-
Ornithine	-	-	-	-	-	-
Arginine	-	-	-	-	-	-
Pigment:						
Fluorescein	-	-	-	-	-	-
Pyocyanin	-	-	-	-	-	-

*Grouping is that of Shewan, Hobbs, and Hodgkiss (1960).

**Hugh-Leifson reactions: O = Oxidative
NC = No change

Table 2. Characteristics of isolates identified as Acinetobacter spp.

Characteristic	Type A	Type B
Oxidase	-	-
Motile	-	-
Hugh-Leifson*	O	NC
Sensitive to Penicillin	-	-
Sensitive to Terramycin	-	-
Arginine dihydrolase	-	-
Decarboxylases:		
Lysine	-	-
Ornithine	-	-
Arginine	-	-

*Hugh-Leifson reations: O = Oxidative
NC = No change

Table 3. Characteristics of isolates identified as *Aeromonas* spp.

Characteristic	Type A	Type B	Type C	Type D
Oxidase	+	+	+	+
Motile; Polar flagella	+	+	-	-
Hugh-Leifson fermentative	+	+	+	+
Gas produced from Glucose	+	-	+	-
Sensitive to Novobiocin	-	-	-	-
Sensitive to 0/129	-	-	-	-
Arginine dihydrolase	+	+	+	+
Decarboxylases:				
Lysine	-	-	-	-
Ornithine	+	+	+	+
Arginine	+	+	+	+

Table 4. Characteristics of isolates identified as *Alcaligenes* spp.

Characteristic	Type A	Type B	Type C	Type D
Oxidase	+	+	+	+
Motile; Peritrichous flagella	+	+	-	-
Hugh-Leifson*	NC	O	NC	O
Sensitive to Penicillin	+	+	+	+
Sensitive to Terramycin	-	-	-	-
Arginine dihydrolase	-	-	-	-
Decarboxylases:				
Lysine	-	-	-	-
Ornithine	+	+	+	+
Arginine	+	+	+	+

*Hugh-Leifson: O = Oxidative
NC = No change

Table 5. Characteristics of isolates identified as *Vibrio* spp.

Characteristic	Type A	Type B	Type C	Type D	Type E	Type F
Oxidase	+	+	+	+	+	+
Motile; Polar flagella	+	-	+	-	+	-
Hugh-Leifson fermentative	+	+	+	+	+	+
Gas produced from Glucose	-	-	-	-	-	-
Sensitive to Novobiocin	+	+	-	-	+	+
Sensitive to 0/129	-	-	-	-	-	-
Arginine dihydrolase	-	-	-	-	-	-
Decarboxylases:						
Lysine	+	+	+	+	+	+
Ornithine	-	-	+	+	+	+
Arginine	-	-	-	-	-	-

Table 6. Characteristics of unidentified isolates.

Unknown A	Unknown B.
Oxidase negative	Oxidase positive
Motile; Polar flagella	Motile; Polar flagella
Hugh-Leifson fermentative	Hugh-Leifson no change
No gas from Glucose	Resistant to Penicillin
Sensitive to Novobiocin	Resistant to Terramycin
Resistant to 0/129	Arginine dihydrolase present
Arginine dihydrolase present	Decarboxylases:
Decarboxylases:	Lysine absent
Lysine present	Ornithine absent
Ornithine present	Arginine absent
Arginine present	

Table 7. Morphological descriptions and range of cell dimensions of the isolates studied.

Isolates	Morphology and Arrangement	Dimensions (μm)
Acinetobacter	Short, wide rods and coccobacilli. In pairs and chains of 4-8 cells.	0.8-1.2 x 1.2-1.8
Aeromonas	Rods and coccobacilli. Singly, in pairs and in chains of 3-12 cells.	1.0-1.5 x 1.2-1.8
Alcaligenes	Small rods and coccobacilli. Singly, with a few pairs.	0.5-1.0 x 0.5-1.5
Pseudomonas	Straight and curved rods of varying length. Singly.	0.5-1.0 x 1.2-2.2
Vibrio	Straight and curved slender rods. Singly, with a few pairs and short chains (3-5) cells.	0.5-0.8 x 1.5-2.0
Unknown A	Rods and coccobacilli. Singly.	0.8-1.0 x 1.0-1.5
Unknown B	Short rods. Singly	0.5-0.8 x 1.2-1.5

Table 8. Results of Fluorescent Antibody staining.

Identification of Isolates	Intensity of Fluorescence
Pseudomonas Group I	2+ - 3+
Pseudomonas Group II	2+ - 3+
Pseudomonas Group IV	1+ - 2+
Acinetobacter	0 - ±
Aeromonas	0 - 1+
Alcaligenes	0 - ±
Vibrio	± - 1+
Unknown A	0 - 1+
Unknown B	± - 2+

Table 9. Breakdown of Pseudomonad Isolates, by sample.

Date and Sample*	Pseudomonads Isolated: Group I A	Group I B	Group II A	Group II B	Group IV A	Group IV B	Total	Total Isolates
A. From samples plated onto YBP-SWA								
1972								
Sept. 12								
S	9	3	18	6	1		37	42
T	11		11	4			26	33
B	13	6	21		1		41	44
Sept. 29								
S	12	5	18	2			37	41
T	9	1	15	3			28	38
B	8	1	14	2			25	29
Oct. 11								
S	21	10	17	5			53	55
M	10		15	1			26	30
B	3	1	11				15	17
Oct. 24								
S	12	9	16	14	1		52	60
T	8	5	8				21	28
B	24	10	4	12			50	53
Nov. 23								
S	17	3	8				28	29
M	17	8	4	1			30	30
B	27		8			1	36	43
1973								
Jan. 25								
S	16						16	16
M	4	1	6	7	2		20	26
B	6	3	4	6			19	25

Table 9 Continued.

Pseudomonads Isolated

Date and Sample*	Group I A	Group I B	Group II A	Group II B	Group IV A	Group IV B	Total	Total Isolates
Feb. 6								
S		12	7				19	19
M	6	7	4	11			28	36
B			8	2			10	11
Mar. 21								
S	16	10	8	15	2		51	57
M	3	7	11	1			22	31
B	12			10	1		23	27
May 15								
S				5			5	5
M		9	5	3			17	21
B		6	1	14			21	21
May 29								
S	12	15	7	12			46	52
M	6	2	11	3			22	30
B	17	1	19	3	2	1	43	46
June 13								
S	9	4	6	17			36	41
T	5		10	3			18	37
B	14	6	2	15			37	45
June 27								
S	18	7					25	30
T	13	14	13		1		27	37
B	3		11	6	1		35	42
July 10								
S	21		10	2			33	39
T	9		5	10	1	2	19	33
B		9	10				19	32

B. From samples plated onto cetrimide agar

Table 9 Continued.

Date and Sample*	Pseudomonads Isolated							
	Group I		Group II		Group IV		Total	Total Isolates
	A	B	A	B	A	B		
1972								
Sept. 12								
S	12	1	16				29	32
T	8	2	6	10			27	37
B	4		22		1		27	30
Sept. 29								
S	18	1	5	8			32	38
T	1	10	5	4		1	21	30
B		6		22			28	28
Oct. 11								
S		15	14	6			35	38
M	7	3	10				20	24
B	3	12	7	4			26	33
Oct. 24								
S	9	19					28	30
T	2		3	12			17	28
B	6	7	3	6		2	24	38
Nov. 23								
S	5		17	3			25	34
M	4	8	9	7			28	31
B	13		8	6			27	32
1973								
Jan. 25								
S	3	5	8	8			24	31
M	14		10	11			35	36
B	4	11	3	10			28	33
Feb. 6								
S	10	5	8	9			32	41
M		7	6	9	1		23	33
B	3	14		12			29	30

Table 9 Continued

Date and Sample*	Pseudomonads Isolated: Group I A	Group I B	Group II A	Group II B	Group IV A	Group IV B	Total	Total Isolates
Mar. 21								
S		7		21			28	32
M	4	8	3	10			25	30
B	6	11	8	4			29	36
May 15								
S				3			3	3
M		10		7			17	23
B	3	4		27		1	35	40
May 29								
S	4	13	3	10			30	30
M		16	1	9			26	34
B	7	1	15	8			31	35
June 13								
S	3	12	7	9			31	36
T	14	4	12	4			34	36
B	5	11	15	3			34	34
June 27								
S	16		9	3			28	31
T	7		9	8			24	32
B	26	3	7				36	37
July 10								
S	14	8		13			35	38
T	3	11	9	9			32	33
B	8	5	6	10			30	32

*Sample designations: S Surface
M Middepth
T Thermocline
B Bottom.

Table 10. Breakdown of non-pseudomonad isolates, by sample.

Date and Sample	Acineto-bacter		Aeromonas				Alcaligenes				Vibrio						Unknown		Total
	A	B	A	B	C	D	A	B	C	D	A	B	C	D	E	F	A	B	
A. From samples plated onto YBP-SWA																			
1972																			
Sept. 12																			
S		1		1				2	1										5
T		2						2	1			1	1						7
B		2						1											3
Sept. 29																			
S		3							1										4
T		3		2				3	1								1		10
B	1	1						2											4
Oct. 11																			
S	1								1										2
M								1	2		1								4
B		1		1															2
Oct. 24																			
S	1	2						3	1								1		8
T	3							3									1		7
B								1	2										3

Table 10 Continued.

Date and Sample	Acineto-bacter A	Acineto-bacter B	Aeromonas A	Aeromonas B	Aeromonas C	Aeromonas D	Alcaligenes A	Alcaligenes B	Alcaligenes C	Alcaligenes D	Vibrio A	Vibrio B	Vibrio C	Vibrio D	Vibrio E	Vibrio F	Unknown A	Unknown B	Total
Nov. 23																			
S		1																	1
M																			0
B		3					2	2											7
1973																			
Jan. 25																			
S																			0
M		1					3				1			1					6
B		4					1		1										6
Feb. 6																			
S																			0
M		4					1	1			1						1		8
B												1							1
Mar. 21																			
S		1					2	1	1									1	6
M			1		1		4					1					2		9
B		3																1	4
May 15																			
S																			0
M		3																	4
B																			0

Table 10 Continued.

Date and Sample	Acineto-bacter		Aeromonas				Alcaligenes				Vibrio						Unknown		Total
	A	B	A	B	C	D	A	B	C	D	A	B	C	D	E	F	A	B	
May 29																			
S		1					1		4										6
M				2			3									1		2	8
B							2		1										3
June 13																			
S		3							2										5
T	2	3		1	3		6							3				1	19
B		1						3	3				1						8
June 27																			
S		4													1				5
T					1		4						2					3	10
B	2						1		4										7
July 10																			
S							2		4										6
T		2		2	1				3					4				2	14
B							3												3

B. From samples plated onto cetrimide agar

1972																			
Sept. 12																			
S							2		1										3
T		1		1	3				5										10
B							3												3

Table 10 Continued.

Date and Sample	Acineto-bacter		Aeromonas				Alcaligenes				Vibrio						Unknown		Total
	A	B	A	B	C	D	A	B	C	D	A	B	C	D	E	F	A	B	
Sept. 29																			
S		1					3							1				1	6
T		2		1			1		2				3						9
B																			0
Oct. 11																			
S							3												3
M		1					1						1		1				4
B	1	2		2		1		1											7
Oct. 24																			
S		2																	2
T		2					3				1		3					2	11
B	2	3		1	2				5				1						14
Nov. 23																			
S		5					1	2		1									9
M		2						1											3
B		2					1		2										5
1973																			
Jan. 25																			
S		3		1	1				2										7
M											1								1
B	1						4												5

Table 10 Continued.

Date and Sample	Acinetobacter A	Acinetobacter B	Aeromonas A	Aeromonas B	Aeromonas C	Aeromonas D	Alcaligenes A	Alcaligenes B	Alcaligenes C	Alcaligenes D	Vibrio A	Vibrio B	Vibrio C	Vibrio D	Vibrio E	Vibrio F	Unknown A	Unknown B	Total
Feb. 6																			
S		3							3	1				2					9
M		4	2								1	2					1		10
B														1					1
Mar. 21																			
S									4										4
M	1	1				1	1		1										5
B		2							4					1					7
May 15																			
S																			0
M					2				4										6
B	2	2								1									5
May 29																			
S																			0
M							1		4		1	2							8
B		2					2												4
June 13																			
S		2					1		2										5
T				1										1					2
B																			0

Table 10 Continued.

Date and Sample	Acinetobacter A	Acinetobacter B	Aeromonas A	Aeromonas B	Aeromonas C	Aeromonas D	Alcaligenes A	Alcaligenes B	Alcaligenes C	Alcaligenes D	Vibrio A	Vibrio B	Vibrio C	Vibrio D	Vibrio E	Vibrio F	Unknown A	Unknown B	Total
June 27																			
S							3												3
T		5	2								1								8
B														1					1
July 10																			
S							3												3
T													1						1
B									2										2

*Sample designations: S Surface
M Middepth
T Thermocline
B Bottom

APPENDIX IV: PSEUDOMONAD COUNT DATA

Table 1. Calculation of numbers of pseudomonads per liter of plankton tow based on possible pseudomonad counts on YBP-SWA and cetrimide agar.

Procedure for calculating number of Pseudomonads per liter:

1. Determine number of isolates from each sample which were identified as pseudomonads of Groups I, II and IV.

2. Divide total number of isolates into number found to be pseudomonads and multiply result by 100% to get the percentage of pseudomonads of the sample.

3. Determine the number of possible pseudomonads per liter from the plate count data.

4. Multiply the number of possible pseudomonads per liter by the percentage of pseudomonads to get the number of pseudomonads per liter of plankton tow.

Date and Sample*	Number of Isolates Tested	Number Identified as Pseudomonads	Percentage of Pseudomonads	Number of Possible Pseudomonads per Liter	Number of Pseudomonads per Liter
A. From samples plated onto YBP-SWA					
1972					
Sept. 12					
S	47	37	78.7	169	133
T	35	26	74.3	241	179
B	58	41	70.7	782	553
Sept. 29					
S	48	37	77.1	1,122	865
T	41	28	68.3	165	113
B	33	25	75.8	692	525
Oct. 11					
S	58	53	91.4	516	472
M	38	26	68.4	170	116
B	37	15	40.5	109	44
Oct. 24					
S	62	52	83.9	860	722
T	47	21	44.7	425	190
B	58	50	86.2	427	368
Nov. 23					
S	30	28	93.3	670	625
M	32	30	93.8	1,080	1,013
B	45	36	80.0	428	342

Table 1 Continued.

Date and Sample	Number of Isolates Tested	Number Identified as Pseudomonads	Percentage of Pseudomonads	Number of Possible Pseudomonads per Liter	Number of Pseudomonads per Liter
1973					
Jan. 25					
S	20	16	80.0	28	22
M	46	20	43.4	82	36
B	31	19	61.3	77	47
Feb. 6					
S	32	19	59.4	73	43
M	37	28	75.7	341	258
B	16	10	62.5	28	18
Mar. 21					
S	58	51	87.9	60	53
M	36	22	61.6	28	17
B	33	23	69.7	181	126
May 15					
S	7	5	71.4	1	1
M	22	17	77.3	11	9
B	34	21	61.8	9	6
May 29					
S	49	46	93.9	24	23
M	34	22	64.7	54	35
B	58	43	74.1	146	108

Table 1 Continued.

Date and Sample	Number of Isolates Tested	Number Identified as Pseudomonads	Percentage of Pseudomonads	Number of Possible Pseudomonads per Liter	Number of Pseudomonads per Liter
June 13					
S	56	36	64.3	43	26
T	46	18	39.1	719	281
B	48	37	77.1	383	295
June 27					
S	35	25	71.4	187	134
T	46	27	58.7	177	104
B	50	35	70.0	604	423
July 10					
S	46	33	71.7	28	20
T	40	19	47.5	25	12
B	26	19	73.1	67	49

B. From samples plated onto cetrimide agar

1972

Sept. 12					
S	32	29	90.6	201	182
T	37	27	72.9	143	104
B	30	27	90.0	505	455

Table 1 Continued.

Date and Sample	Number of Isolates Tested	Number Identified as Pseudomonads	Percentage of Pseudomonads	Number of Possible Pseudomonads per Liter	Number of Pseudomonads per Liter
Sept. 29					
S	37	32	86.5	451	390
T	32	21	65.6	53	35
B	28	28	100.0	434	434
Oct. 11					
S	38	35	91.2	624	575
M	24	20	83.3	115	96
B	33	26	78.9	85	67
Oct. 24					
S	30	28	93.3	697	650
T	26	17	63.4	218	138
B	36	24	66.7	506	338
Nov. 23					
S	34	25	73.5	238	175
M	31	28	90.3	887	801
B	32	27	84.4	813	686
1973					
Jan. 25					
S	31	24	77.4	30	23
M	36	35	97.2	71	69
B	33	28	84.8	36	31

Table 1 Continued.

Date and Sample	Number of Isolates Tested	Number Identified as Pseudomonads	Percentage of Pseudomonads	Number of Possible Pseudomonads per Liter	Number of Pseudomonads per Liter
Feb. 6					
S	41	32	78.0	79	62
M	33	23	69.7	103	72
B	30	29	96.7	44	43
Mar. 21					
S	32	28	87.5	55	48
M	31	25	80.6	30	24
B	36	29	80.6	285	230
May 15					
S	3	3	100.0	1	1
M	23	17	73.9	10	7
B	40	35	87.5	24	21
May 29					
S	30	30	100.0	42	42
M	34	26	76.5	63	48
B	35	31	88.6	160	142
June 13					
S	36	31	86.1	43	37
T	36	34	94.4	747	705
B	34	34	100.0	273	273
June 27					
S	31	28	90.3	68	61
T	32	24	75.0	94	71
B	37	36	97.3	345	336

Table 1 Continued.

Date and Sample	Number of Isolates Tested	Number Identified as Pseudomonads	Percentage of Pseudomonads	Number of Possible Pseudomonads per Liter	Number of Pseudomonads per Liter
July 10					
S	38	35	92.1	18	17
T	33	32	97.0	20	19
B	32	30	93.8	22	21

*Sample designations: S Surface
M Middepth
T Thermocline
B Bottom

Table 2. Number of Pseudomonads per liter of plankton tow based on YBP-SWA counts.

Date	Surface	Middepth	Bottom
1972			
Sept. 12	133	179*	553
Sept. 29	865	113*	525
Oct. 11	472	116	44
Oct. 24	722	190*	386
Nov. 23	625	1013	342
1973			
Jan. 25	22	36	47
Feb. 6	43	258	18
Mar. 21	53	17	126
May 15	1	9	6
May 29	23	35	108
June 13	26	281*	295
June 27	134	104*	423
July 10	20	12*	49

*Thermocline samples.

Table 3. Number of Pseudomonads per liter of plankton tow based on cetrimide agar counts.

Date	Surface	Middepth	Bottom
1972			
Sept. 12	182	104*	455
Sept. 29	390	35*	434
Oct. 11	575	96	67
Oct. 24	650	138*	338
Nov. 23	175	801	686
1973			
Jan. 25	23	69	31
Feb. 6	62	72	43
Mar. 21	48	24	230
May 15	1	7	21
May 29	42	48	142
June 13	37	705*	273
June 27	61	71*	336
July 10	17	19*	21

*Thermocline samples.

Table 4. Comparison of numbers of pseudomonads per liter of plankton tow based on counts made on YBP-SWA and cetrimide agar.

YBP-SWA Counts	Cetrimide Counts	Difference d_i	d_i^2
	Surface Samples		
133	182	- 49	2,401
865	390	475	225,625
472	575	-103	10,609
722	650	72	5,184
625	175	450	202,500
22	23	- 1	1
43	62	- 19	361
53	48	5	25
1	1	0	0
23	42	- 19	361
26	37	- 11	121
134	61	73	5,329
20	17	3	9
	Middepth Samples		
179	104	75	5,625
113	35	78	6,084
116	96	20	400
190	138	52	2,704

Table 4 Continued.

YBP-SWA Counts	Cetrimide Counts	Difference d_i	d_i^2
1,013	801	212	44,944
36	69	- 33	1,089
258	72	186	34,596
17	24	- 7	49
9	7	2	4
35	48	- 13	169
281	705	-424	179,776
104	71	33	1,089
12	19	- 7	49
	Bottom Samples		
553	455	93	9,604
525	434	91	8,281
44	67	- 23	529
368	338	- 30	900
342	686	-344	118,336
47	31	16	256
18	43	- 25	625
126	230	213	45,369
6	21	- 15	225
108	142	- 34	17,956
295	273	22	484

Table 4 Continued.

YBP-SWA Counts	Cetrimide Counts	Difference d_i	d_i^2
423	336	87	7,569
49	21	28	784

Sample	Σd_i	$\Sigma(d_i^2)$	$\bar{d}$	s_d*	t**
Surface	876	452,526	67.38	181.08	1.34
Middepth	174	276,578	13.38	151.18	0.32
Bottom	79	210,918	6.08	132.43	0.17
All	1,129	940,022	28.95	154.52	1.17

$$*s_d = \sqrt{\frac{n\Sigma(d_i^2) - (\Sigma d_i)^2}{n(n-1)}}$$

$$**t = \frac{\bar{d} - d_o}{s_d/\sqrt{n}}$$

Hypothesis tested:

There is a difference between isolation onto YBP-SWA and isolation onto cetrimide.

Table 4 Continued.

$H_o: u_y - u_c = 0 = d_o$

$H_1: u_y - u_c \neq 0$

$\alpha = 0.10$

Critical region: $T < -1.782$ and $T > 1.782$* for surface, Middepth or Bottom samples.

$T < -1.645$ and $T > 1.645$ for all samples.

$\therefore$ Accept H_o in all cases.

*T values from Introduction to Statistics (Walpole, 1968)

Table 5. Comparison of numbers of pseudomonads with total bacteria.

Date	Number of Bacteria*	Number of Pseudomonads	Percentage of Pseudomonads
	A. Surface samples		
1972			
Sept. 12	324	133	41
Sept. 29	1,732	865	50
Oct. 11	938	472	50
Oct. 24	1,172	722	62
Nov. 23	993	625	63
1973			
Jan. 25	152	22	14
Feb. 6	203	43	21
Mar. 21	70	53	76
May 15	10	1	10
May 29	150	23	15
June 13	113	26	23
June 27	436	134	31
July 10	60	20	33
	B. Middepth samples		
1972			
Sept. 12**	315	179	57

Table 5 Continued.

Date	Number of Bacteria*	Number of Pseudomonads	Percentage of Pseudomonads
Sept. 29**	302	113	37
Oct. 11	323	116	36
Oct. 24**	1,011	190	19
Nov. 23	1,774	1,013	57
1973			
Jan. 25	311	36	12
Feb. 6	547	258	47
Mar. 21	51	17	33
May 15	18	9	50
May 29	197	35	18
June 13**	2,573	281	11
June 27**	359	104	29
July 10**	57	12	21
C. Bottom samples			
1972			
Sept. 12	1,347	553	41
Sept. 29	1,343	525	39
Oct. 11	318	44	14
Oct. 24	1,077	368	34
Nov. 23	741	342	46

Table 5 Continued.

Date	Number of Bacteria*	Number of Pseudomonads	Percentage of Pseudomonads
1973			
Jan. 25	235	47	20
Feb. 6	134	18	13
Mar. 21	349	126	36
May 15	41	6	15
May 29	464	108	23
June 13	772	295	38
June 17	1,337	423	32
July 10	188	49	26

* Total bacterial numbers for July 24 and August 10 are given in Table 3, Appendix IV

** Thermocline Samples

Table 6. Comparisons of numbers of pseudomonads in plankton tow samples from different depths based on counts on both YBP-SWA and cetrimide agar.

A. Surface and Middepth (YBP-SWA counts)

Surface Counts	Middepth Counts	Difference d_i	d_i^2
133	179	- 46	2,116
865	113	752	565,504
472	116	356	126,736
722	190	532	283,024
625	1,013	-388	150,544
22	36	- 14	196
43	258	-215	46,225
53	17	36	1,296
1	9	- 8	64
23	35	- 12	144
26	281	-255	65,025
134	104	30	900
20	12	8	64

B. Middepth and Bottom (YBP-SWA counts)

Middepth Counts	Bottom Counts	Difference d_i	d_i^2
179	553	-374	139,876
113	525	-412	169,744
116	44	72	5,184
190	368	-178	31,684
1,013	342	671	450,241
36	47	- 11	121
258	18	240	57,600
17	126	-109	11,881
9	6	3	9
35	108	- 73	5,329

Table 6 Continued.

Middepth Counts	Bottom Counts	Difference d_i	d_i^2
281	295	- 14	196
104	423	-319	101,761
12	49	- 37	1,369

C. Surface and Bottom (YBP-SWA counts)

Surface Counts	Bottom Counts	Difference d_i	d_i^2
113	553	-440	193,600
865	525	340	115,600
472	44	428	183,184
722	368	354	125,316
625	342	283	80,089
22	47	- 25	625
43	18	25	625
53	126	- 73	5,329
1	6	- 5	25
23	108	- 85	7,225
26	295	-269	72,361
134	423	-289	83,521
20	49	- 29	841

D. Surface and Middepth (Cetrimide agar counts)

Surface Counts	Middepth Counts	Difference d_i	d_i^2
182	104	78	6,084
390	35	355	126,025
575	96	479	229,441
650	138	512	262,144
175	801	-625	390,625
23	69	- 46	2,116
62	72	- 10	100
48	24	24	576

Table 6 Continued.

Surface Counts	Middepth Counts	Difference d_i	d_i^2
1	7	- 6	36
42	48	- 6	36
37	705	-668	446,224
61	71	- 10	100
17	19	- 2	4

E. Middepth and Bottom (Cetrimide agar counts)

Middepth Counts	Bottom Counts	Difference d_i	d_i^2
104	455	-351	123,201
35	434	-399	159,201
96	67	29	841
138	338	-200	40,000
801	686	115	13,225
69	31	38	1,444
72	43	29	841
24	230	-206	42,436
7	21	- 14	196
48	142	- 94	8,836
705	273	432	186,624
71	336	-265	70,225
19	21	- 2	4

F. Surface and Bottom (Cetrimide agar counts)

Surface Counts	Bottom Counts	Difference d_i	d_i^2
182	455	-273	74,529
390	434	- 44	1,936
575	67	508	258,064
650	338	312	97,344
175	686	-511	261,121
23	31	- 8	64

Table 6 Continued.

Surface Counts	Bottom Counts	Difference d_i	d_i^2
62	43	19	361
48	230	-202	40,804
1	21	- 20	400
42	142	-100	10,000
37	273	-236	55,696
61	336	-275	75,625
17	21	- 4	16

Comparison	d_i	(d_i^2)	$\bar{d}$	S_d*	t**
A	776	1,241,838	59.69	315.64	0.68
B	-541	974,995	41.61	281.73	0.53
C	215	868,341	16.54	268.45	0.22
D	75	1,463,511	5.77	349.18	0.06
E	-888	647,074	68.31	221.06	1.11
F	774	875,960	59.54	262.98	0.82

* $$S_d = \sqrt{\frac{n\Sigma(d_i^2) - (\Sigma d_i)^2}{n(n-1)}}$$

** $$t = \frac{\bar{d} - d_o}{S_d/\sqrt{n}}$$

Hypothesis tested:

There are no differences in the numbers of pseudomonads present in the samples from different depths.

H_o: $u_S - u_M = 0$; $u_M - u_B = 0$; $u_S - u_B = 0$

Table 6 Continued.

H_1: $u_S - u_M \neq 0$; $u_M - u_B \neq 0$; $u_S - u_B \neq 0$

$\alpha = 0.10$

Critical region: $T \leq -1.782$ and $T \geq 1.782$*

$\therefore$ Accept H_o in all cases.

* T values from Introduction to Statistics (Walpole, 1968)

Table 7. Comparisons of percentages of pseudomonads in plankton tow samples from different depths based on counts made on YBP-SWA.

A. Surface and Middepth

Surface Counts	Middepth Counts	Difference d_i	d_i^2
41	57	-16	256
50	37	13	169
50	36	14	196
62	19	43	1,849
63	57	6	36
14	12	2	4
21	47	-26	676
76	33	43	1,849
10	50	-40	1,600
15	18	- 3	9
23	11	12	144
31	29	2	4
33	21	12	144

B. Middepth and Bottom

Middepth Counts	Bottom Counts	Difference d_i	d_i^2
57	41	16	256
37	39	- 2	4
36	14	22	484
19	34	-15	225
57	46	11	121
12	20	- 8	64
47	13	34	1,156
33	36	- 3	9

Table 7 Continued.

Surface Counts	Bottom Counts	Difference d_i	d_i^2
50	15	35	1,225
18	23	- 5	25
11	38	-27	729
29	32	- 3	9
21	26	- 5	25

C. Surface and Bottom

Surface Counts	Bottom Counts	Difference d_i	d_i^2
41	41	0	0
50	39	11	121
50	14	36	1,296
62	34	28	784
63	46	17	289
14	20	- 6	36
21	13	8	64
76	36	40	1,600
10	15	- 5	25
15	23	- 8	64
23	38	-15	225
31	32	- 1	1
33	26	7	49

Comparison	Σd_i	$\Sigma(d_i^2)$	$\bar{d}$	s_d*	t**
Surface-Middepth	62	6,936	4.77	23.52	0.73
Middepth-Bottom	50	4,332	3.85	18.57	0.75
Surface-Bottom	112	4,554	8.62	17.29	1.80

$$*s_d = \sqrt{\frac{n\Sigma(d_i^2) - (\Sigma d_i)^2}{n(n-1)}}$$

Table 7 Continued.

$$**t = \frac{\bar{d} - d_o}{s_d/\sqrt{n}}$$

Hypothesis tested:

There are no differences in the percentages of pseudomonads present in the samples.

H_o: $u_S - u_M = 0 = d_o$; $u_M - u_B = 0 = d_o$; $u_S - u_B = 0 = d_o$

H_1: $u_S - u_M \neq 0$; $u_M - u_B \neq 0$; $u_S - u_B \neq 0$

$\alpha = 0.10$

Critical region: $T < -1.782$ and $T > 1.782$*

∴ Accept H_o for Surface-Middepth and Middepth-Bottom Comparisons.

Reject H_o for Surface-Bottom Comparison.

* T values from Introduction to Statistics (Walpole, 1968)

APPENDIX V: PHYTOPLANKTON COUNT DATA

Table 1. Numbers of phytoplankton organisms per liter.*

Date and Sample**	Diatoms	Dino-flagellates	Silico-flagellates	Total
1972				
Sept. 12				
S	1	168	0	169
T	0	164	0	164
B	1	37	0	38
Sept. 29				
S	4	89	0	93
T	1	21	0	22
B	4	29	0	33
Oct. 11				
S	7	47	0	54
M	1	19	0	20
B	2	29	0	31
Oct. 24				
S	0	25	0	25
T	1	36	0	37
B	1	16	1	18
Nov. 23				
S	77	17	2	96
M	3	79	2	84
B	25	51	2	78
1973				
Jan. 25				
S	16	11	1	28
M	8	5	0	13
B	27	11	0	38
Feb. 6				
S	203	13	12	228
M	47	25	0	72
B	41	7	3	51

Table 1 Continued.

Date and Sample**	Diatoms	Dino-flagellates	Silico-flagellates	Total
Mar. 21				
S	5,516	16	341	5,873
M	16,284	0	260	16,544
B	4,318	51	121	4,490
May 15				
S	23,740	37	37	23,814
M	129,870	0	0	129,870
B	73,570	0	0	73,570
May 29				
S	10,997	30	0	11,027
M	11,247	38	0	11,285
B	21,188	84	0	21,272
June 13				
S	138	37	0	175
T	11,618	21	0	11,639
B	2,356	4	0	2,360
June 27				
S	219	53	0	272
T	51,119	241	0	51,360
B	46,002	0	0	46,002
July 10				
S	9	31	0	40
T	0	8	0	8
B	8	16	0	24
July 24				
S	0	566	0	566
T	0	28	0	28
B		Not determined		
Aug. 10				
S	0	89	0	89
T	0	74	0	74
B	2	37	0	39

Table 1 Continued.

* A Palmer Slide, holding a volume of 0.1 ml, was used to obtain the quantitative plankton data. Using a 16X objective and a wide angle 12.5X eyepiece, there were 838.3 microscope fields on the slide. At least 60 fields on 3 slides were examined for each sample, and the numbers of plankton organisms in each field noted. From these figures, the numbers of plankton organisms in the original samples were calculated.

The qualitative plankton data are based on the identification schemes given in Brunel (1962), Cupp (1943), Hendey (1964) and Lebour (1925).

**Sample designations:
S Surface
M Middepth
T Thermocline
B Bottom

Table 2. Phytoplankton species composition per liter of plankton tow samples.

Date and Type of Phytoplankton	Surface	Middepth	Bottom
Sept. 12			
Diatoms:			
Thalassiothrix sp.	1	0*	1
Dinoflagellates:			
Ceratium arcticum	2	0	2
C. fusus	2	0	0
C. longipes	157	162	16
Ceratium sp.	5	0	1
Peridinium depressum	2	2	18
Sept. 29			
Diatoms:			
Chaetoceros atlanticus	0	0*	2
Leptocylindrus sp.	4	0	0
Licmophora sp.	0	1	0
Nitzschia sp.	0	0	2
Dinoflagellates:			
Ceratium longipes	78	19	14
C. fusus	7	1	0
Ceratium sp.	0	0	2
Peridinium depressum	3	1	13
P. pellucidum	1	0	0
Oct. 11			
Diatoms:			
Licmophora sp.	0	1	0

Table 2 Continued.

Date and Type of Phytoplankton	Surface	Middepth	Bottom
Nitzschia sp.	0	0	1
Rhizosolenia styliformis	0	0	1
Thalassonema nitzschioides	7	0	0
Dinoflagellates:			
Ceratium arcticum			
C. fusus	3	0	1
C. longipes	42	15	15
Ceratium sp.	1	0	0
Peridinium conicum	0	2	0
P. depressum	1	1	12
Peridinium sp.	0	1	1
Oct. 24			
Diatoms:			
Licmophora sp.	0	0*	1
Rhizosolenia hebetata	0	1	0
Dinoflagellates:			
Ceratium arcticum	0	0	2
C. fusus	2	8	0
C. longipes	21	26	1
Ceratium sp.	2	0	0
Peridinium depressum	0	1	11
P. pellucidum	0	0	1
Peridinium sp.	0	1	0
Phalacroma rotundatum	0	0	1
Silicoflagellates:			
Distephanus speculum	0	0	1

Table 2 Continued.

Date and Type of Phytoplankton	Surface	Middepth	Bottom
Nov. 23			
Diatoms:			
Chaetoceros brevis	2	0	0
C. convolutum	2	0	0
C. debilis	7	0	0
C. decipiens	11	0	7
Chaetocerus sp.	0	0	5
Grammatophora sp.	1	0	0
Leptocylindrus danicus	43	0	0
Licmophora sp.	1	1	0
Nitzschia seriata	4	0	0
Pleurosigma sp.	1	0	0
Rhizosolenia styliformis	0	2	1
Rhizosolenia sp.	1	0	0
Synedra sp.	1	0	0
Thalassiothrix frauenfeldii	1	0	0
Thalassonema nitzschioides	2	0	0
Unknown pennates	0	0	12
Dinoflagellates:			
Ceratium arcticum	3	5	8
C. fusus	1	0	1
C. longipes	12	68	39
Ceratium sp.	1	0	1
Peridinium depressum	0	2	1
P. pellucidum	0	3	0
Peridinium sp.	0	1	1
Silicoflagellates:			
Distephanus speculum	2	2	2
Jan. 25			
Diatoms:			
Chaetoceros convolutum	1	0	1

Table 2 Continued.

Date and Type of Phytoplankton	Surface	Middepth	Bottom
C. debilis	6	3	6
C. decipiens	4	0	0
Chaetoceros sp.	2	1	10
Coscinodiscus centralis	1	1	2
Coscinodiscus sp.	1	2	2
Licmophora sp.	0	1	0
Nitzschia sp.	0	0	4
Rhizosolenia styliformis	0	0	1
Thalassonema nitzschioides	1	0	0
Unknown pennate	0	0	1
Dinoflagellates:			
Ceratium arcticum	5	0	0
C. longipes	3	3	2
Ceratium sp.	1	0	3
Peridinium conicum	1	1	2
P. depressum	1	0	3
P. pellucidum	0	0	1
Peridinium sp.	0	1	0
Silicoflagellates:			
Distephanus speculum	1	0	0
Feb. 6			
Diatoms:			
Chaetoceros atlanticus	5	2	0
C. convolutum	9	0	11
C. debilis	88	0	14
C. decipiens	33	0	3
Chaetoceros sp.	25	2	4
Cocconeis disculus	1	0	1
Coscinodiscus centralis	1	2	1
Coscinodiscus sp.	2	9	1

Table 2 Continued.

Date and Type of Phytoplankton	Surface	Middepth	Bottom
Fragilaria sp.	0	24	0
Leptocylindrus danicus	9	0	0
Licmophora sp.	2	5	1
Navicula sp.	0	0	1
Nitzschia longissima	1	0	0
N. seriata	8	0	0
Nitzschia sp.	2	2	3
Rhizosolenia hebetata	0	1	0
R. styliformis	1	0	0
Skeletonema costatum			
Stauroneis sp.	0	0	1
Thalassiothrix delicatula	1	0	0
Thalassonema nitzschioides	6	0	0
Dinoflagellates:			
Ceratium arcticum	2	2	1
C. longipes	5	12	2
Ceratium sp.	5	0	3
Peridinium conicum	0	5	1
P. depressum	1	2	0
P. pellucidum	0	2	0
Peridinium sp.	0	2	0
Silicoflagellates:			
Distephanus speculum	12	0	3
Mar. 21			
Diatoms:			
Biddulphia sp.	0	65	0
Chaetoceros affinis	146	0	52
C. atlanticus	0	0	104
C. compressus	0	260	0
C. convolutum	487	845	518
C. debilis	0	65	138

Table 2 Continued.

Date and Type of Phytoplankton	Surface	Middepth	Bottom
C. decipiens	276	0	242
C. sociale	0	4,550	0
Chaetoceros sp.	568	2,925	604
Coscinodiscus sp.	16	0	0
Fragilaria sp.	519	130	0
Licmophora sp.	0	33	0
Navicula sp.	16	0	0
Rhizosolenia hebetata	195	423	242
R. styliformis	81	0	0
Skeletonema costatum	406	293	242
Thalassiosira aestivalis	811	195	0
T. gravida	243	520	0
T. nordenskioldii	16	195	0
T. rotula	97	1,885	708
Thalassiosira sp.	1,509	3,900	1,468
Thalassonema nitzschioides	130	0	0
Dinoflagellates:			
Ceratium sp.	0	0	17
Peridinium conicum	16	0	0
P. depressum	0	0	17
Peridinium sp.	0	0	17
Silicoflagellates:			
Distephanus speculum	341	260	121
May 15			
Diatoms:			
Bacteriosira fragilis	0	519	157
Chaetoceros affinis	0	389	0
C. convolutum	148	0	314
C. debilis	369	0	236
C. decipiens	74	130	0
Chaetoceros sp.	19,124	90,299	61,859
Eucampia zodiacus	0	389	0

Table 2 Continued.

Date and Type of Phytoplankton	Surface	Middepth	Bottom
Fragilaria sp.	2,031	29,840	6,995
Navicula sp.	37	0	393
Nitzschia sp.	0	0	79
Pleurosigma sp.	37	0	0
Rhizosolenia hebetata	148	130	79
Thalassiosira aestivalis	1,145	4,411	0
T. gravida	258	0	0
T. nordenskioldii	369	3,114	2,515
Thalassiosira sp.	0	649	943
Dinoflagellates:			
Peridinium depressum	37	0	0
Silicoflagellates:			
Distephanus speculum	37	0	0
May 29			
Diatoms:			
Bacteriosira fragilis	30	0	0
Chaetoceros affinis	0	113	28
C. atlanticus	0	0	56
C. compressus	0	1,258	112
C. convolutum	0	0	84
C. debilis	80	88	0
Chaetoceros sp.	10,299	8,227	18,391
Eucampia zodiacus	0	13	0
Fragilaria sp.	349	1,359	1,565
Navicula sp.	0	0	168
Nitzschia seriata	30	0	0
Nitzschia sp.	20	113	84
Rhizosolenia hebetata	30	0	56
Rhizosolenia sp.	0	13	0
Thalassiosira aestivalis	159	0	252
T. nordenskioldii	0	25	196
Thalassiosira sp.	0	13	196
Thalassonema nitzschioides	0	25	0

Table 2 Continued.

Date and Type of Phytoplankton	Surface	Middepth	Bottom
Dinoflagellates:			
Ceratium sp.	0	0	0
Miniscula bipes	0	0	28
Peridinium depressum	0	38	0
Peridinium sp.	20	0	56
June 13			
Diatoms:			
Chaetoceros affinis	0	0*	9
C. compressus	0	104	26
C. convolutum	0	0	13
C. debilis	0	124	26
C. decipiens	0	41	0
Chaetoceros sp.	83	10,851	2,029
Cocconeis sp.	0	21	0
Eucampia zodiacus	0	0	17
Eucampia sp.	0	62	0
Fragilaria oceanica	0	0	43
Fragilaria sp.	0	0	124
Leptocylindrus danicus	12	62	13
Navicula sp.	4	0	0
Nitzschia sp.	4	21	0
Rhizosolenia hebetata	25	228	0
Rhizosolenia sp.	10	0	21
Stauroneis sp.	0	0	9
Thalassiosira sp.	0	21	17
Thalassonema nitzschioides	0	62	0
Unknown pennates	0	21	9
Dinoflagellates:			
Ceratium sp.	2	0	0
Peridinium ovatum	2	0	0
P. pellucidum	4	0	0
Peridinium sp.	29	21	4

Table 2 Continued.

Date and Type of Phytoplankton	Surface	Middepth	Bottom
June 27			
Diatoms:			
Chaetoceros affinis	0	562*	402
C. compressus	0	21,587	8,637
C. debilis	0	0	1,272
C. decipiens	0	0	268
Chaetoceros sp.	0	21,025	30,265
Eucampia zodiacus	0	80	402
Fragilaria sp.	0	0	1,206
Leptocylindrus danicus	182	7,544	2,143
Nitzschia sp.	17	0	536
Rhizosolenia hebetata	20	0	134
R. styliformis	0	241	134
Thalassiosira gravida	0	0	603
Thalassiosira sp.	0	80	0
Dinoflagellates:			
Ceratium macrocerus	2	0	0
Ceratium sp.	15	0	0
Dinophysis sp.	3	0	0
Peridinium depressum	13	80	0
Peridinium sp.	20	161	0
July 10			
Diatoms:			
Chaetoceros sp.	0	0*	6
Cosenodiscus centralis	0	0	2
Thalassonema nitzschioides	9	0	0
Dinoflagellates:			
Ceratium macroceros	5	0	0
C. longipes	4	0	0
Ceratium sp.	21	0	6

Table 2 Continued.

Date and Type of Phytoplankton	Surface	Middepth	Bottom
Dinophysis sp.	0	4	0
Peridinium depressum	1	0	6
P. ovatum	0	0	2
Peridinium sp.	0	4	0
Pyrocystis sp.	0	0	2
July 24			
Dinoflagellates:			
Ceratium arcticum	15	11*	**
C. longipes	60	0	
C. macroceros	75	0	
Ceratium sp.	416	14	
Peridinium depressum	0	3	
August 10			
Diatoms:			
Coscenodiscus centralis	0	0*	2
Dinoflagellates:			
Ceratium arcticum	4	16	7
C. longipes	0	4	0
Ceratium sp.	85	46	7
Peridinium depressum	0	8	19
Peridinium sp.	0	0	4

* Thermocline samples

** No qualitative data available for this sample.

Table 3. Numbers of phytoplankton cells, total bacteria, and total pseudomonads per liter based on counts made on YBP-SWA after 20C incubation.

Date	Sample*	Total Phytoplankton Cells per Liter	Total Bacteria per Liter	Total Pseudomonads per Liter
1972				
Sept. 12	S	169	324	133
	T	164	315	179
	B	38	1,347	553
Sept. 29	S	93	1,732	865
	T	22	302	113
	B	33	1,343	525
Oct. 11	S	54	938	472
	M	20	323	116
	B	31	318	44
Oct. 24	S	25	1,172	722
	T	37	1,011	190
	B	18	1,077	386
Nov. 23	S	96	993	625
	M	84	1,774	1,013
	B	78	741	342

Table 3 Continued.

Date	Sample*	Total Phytoplankton Cells per Liter	Total Bacteria per Liter	Total Pseudomonads per Liter
1973				
Jan. 25	S	28	152	22
	M	13	311	36
	B	38	235	47
Feb. 6	S	228	203	43
	M	72	547	258
	B	51	134	18
Mar. 21	S	5,873	70	53
	M	16,544	51	17
	B	4,490	349	126
May 15	S	23,814	10	1
	M	129,870	18	9
	B	73,570	41	6
May 29	S	11,027	150	23
	M	11,285	197	35
	B	21,272	464	108
June 13	S	175	113	26
	T	11,639	2,573	281
	B	2,360	772	295
June 27	S	272	436	134
	T	51,360	359	104
	B	46,002	1,337	423

Table 3 Continued.

Date	Sample*	Total Phytoplankton Cells per Liter	Total Bacteria per Liter	Total Pseudomonads per Liter
July 10	S	40	60	20
	T	8	57	12
	B	24	188	49
July 24	S	566	18	**
	T	28	337	**
	B	Not Determined	175	**
Aug. 10	S	89	15	**
	T	74	44	**
	B	39	477	**

*Sample designations: S Surface
M Middepth
T Thermocline
B Bottom

** Not Determined.