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An ecological study of the bacterial flora of Westhampton Lake

Thomas Constantine Mandes

AN ECOLOGICAL STUDY OF THE BACTERIAL FLORA OF WESTHAMPTON LAKE

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An Ecological Study of the Bacterial Flora of Westhampton Lake

by

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August, 1952

PREFACE

The writer wishes to express his sincere gratitude to Dr. Robert F. Smart for his choice of the problem and for his assistance and direction throughout the problem. He wishes to thank Dr. William K. Clark, Dr. Nolan E. Rice, and Dr. John C. Strickland, of the Biology Department for their advice as to form and context. The writer also wishes to thank Dr. William E. Trout for his aid in the chemical aspects of the problem, and Professor Sherman E. Grable, Professor Austin Grigg, and Dr. Stanley Skiff for their assistance in the statistical methods employed in this thesis.

Thomas C. Mandes

The University of Richmond

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INTRODUCTION

Most natural bodies of water which have been investigated have been shown to contain bacteria. Exceptions apparently occur where we find those waters which, due to their chemical constitution and physical makeup, are inimical even to the growth of bacteria.

Since bacteria are virtually ubiquitous in waters, it would necessarily follow that biologists interested in this aspect of biology would have intensively studied bacteria and the part they play in the biology of water. Quite the contrary; instead of the study continuing along lines laid down by early scholars it has shifted to a study of bacteria which are not indigenous to water. Emphasis has been placed on the public health aspects of water and studies of pathogenic organisms, and manurial pollution have supplanted investigations largely taxonomic.

Ecological investigations on innate bacterial flora have to date been fragmentary. Kleiber (1894) investigated the fate of bacteria washed into lakes from inflowing streams. Ward (1897) made the first intensive study of microorganisms in water. Ward isolated eighty forms from the water of the River Thames. Classification of Ward's isolates was based entirely on pigmentation, gelatin liquefaction, and capsulation. Also along taxonomic lines there appeared the work of Fuller and Johnson (1899) in which they divided water bacteria found in the Ohio River at Cinncinati into, fluorescent and non-fluorescent types. The non-fluorescent types were subdivided into four groups, the bacteria of these groups being apportioned according to chromogenesis. Further subdivision of the

non-fluorescent forms depended upon the fermentation properties of the organisms in question, or whether they produced proteus-like or subtilis-like colonies on gelatin. The authors claimed that the characteristics upon which the classification depended were quite fixed, a view not in agreement with that of Ward. Boyce and Hill (1900) after a year's examination of Liverpool waters arrived at conclusions that largely supported those of Ward. They closely followed Ward's classification and supplemented it with certain types, "possibly variants," that he had not recorded. The most detailed study was that of Jordan (1903) in which he isolated 543 cultures from the waters of the Illinois, Mississippi, and Missouri Rivers and classified them on the basis of their cultural, morphological, and biochemical characteristics. Topley and Wilson (1946) found it convenient to divide bacteria found in water into three categories. The first included bacilli divided into fluorescent, chromogenic, or non-chromogenic types. The second consisted of chromogenic and non-chromogenic cocci, and the third contained the sarcinae.

There appear to be very few studies on the bacterial flora of lakes. Lavanchy (1914), Grovitch (1918), and Marca (1927) published theses on the bacterial flora of Lake Geneva, but the number of organisms studied was small. Fred and his colleagues (1924) isolated organisms from Lake Mendota. The majority of the bacteria were biochemically inactive, gelatin liquefaction being the only physiological test observed in the majority of cases. Chromogenic forms represented a large percentage of the isolations. Further studies by Fred and his colleagues were concerned with the significance of decreases in the

numbers of bacteria entering a lake from one of its tributaries. The most recent work on microorganisms found in water was that of Taylor (1940) who examined several lakes in England. Taylor's most significant finding was the predominance of gram negative rods, with sporulating rods appearing in only six percent of the cases. Biochemically the cultures showed little activity, gelatin being liquefied in approximately one-fourth of the cases. Chromogenic types in this study also represented a large proportion of the total number of organisms. Taylor's findings generally verified the findings of Fred and his co-workers. Further studies by Taylor dealt with ecological subjects such as the reduction in the numbers of bacteria that enter a lake and the several factors responsible for this: (1) sedimentation, (2) competition of organisms for food, (3) variations in temperature, (4) the presence of predatory protozoa.

Smither (1948) in a thesis concerned with the effects of chemical factors on plankton in Westhampton Lake, determined the amount of dissolved oxygen present from October to March. Pierce (1947) considered, among other things, the correlation between the dissolved oxygen content and temperature of an aquatic habitat. With few exceptions the dissolved oxygen content increased or decreased as the temperature fell or rose. Pierce also made hydrogen ion determinations throughout the year and found an increase of pH from September through the winter. A decrease occurred in the spring followed by a small rise as summer approached.

Previous research has been concerned in some instances with an enumeration and identification of bacterial forms found in particular

bodies of water. At other times work has dealt with environmental factors or chemical alterations affecting the numbers of bacteria. The purpose of the present investigation is a survey of environmental factors affecting the numbers of bacteria and a study of the species of bacteria found in a particular body of water.

WESTHAMPTON LAKE

Westhampton Lake, the body of water upon which this study was conducted, is an artificial lake. It is located on the University of Richmond campus where is came into existence approximately one hundred years ago by the construction of a dam across Westham Creek. At the northern head of the lake are two streams from which the lake derives part of its water. The stream flowing in from the northwest collects water from several drainage pipes at its beginning and picks up water from two small tributaries, one from the Westhampton College campus and the other from a tributary of Westham Creek, in its journey toward the lake. The stream entering from the northeast originates beyond Patterson Avenue near a housing development known as Sunset Hills. In addition to these two sources of water there are four springs within the lake. Three of these springs are located in the center of the lake bed, while the fourth is located on the west shore. Westhampton Lake is approximately fifteen feet deep at its deepest part, and covers an area of approximately twelve and one-half acres.

MATERIALS AND METHODS

Part 1 Bacteriological Analysis

Water samples were taken from Westhampton Lake from November 1951 to July 1952. Prior to collection of the samples, ground glass-stoppered bottles were cleansed by rinsing them, first, with strong sulfuric acid, then rinsing again with distilled water, and wrapping them in paper held secure by a string. The bottles were sterilized in the autoclave at a pressure of fifteen pounds per square inch for fifteen minutes, after which they were placed in cardboard containers. The sterilized and covered bottles after having received the above treatment were considered bacteriologically sterile.

In considering the selection of sampling points great care and discretion was necessary in order that the samples would be representative of all ecological situations and variations that might affect the numbers and kind of bacteria. To this end eight sampling stations were established and from each, samples were taken at regular intervals.

When collecting a sample care was exercised to eliminate the possibility of contamination from the air or the collector's hands.

In order to obviate the abnormal results occurring when collected samples are left alone for any great length of time, bacteriological examination was begun as soon as possible. In no case not more than six hours elapsed from the time of sampling until analyses were begun. The manual of Standard Methods for Examination of Water Supplies advises that examination be initiated before a six to twelve hour time lapse, because storage would reduce the bacterial numbers and suspended matter after this period.

Since Westhampton Lake cannot be considered as grossly polluted it was feasible to use water dilutions up to and including 1:1000 dilutions. The bottles containing the samples were shaken and then 1 cc. portions were removed and pipetted into each of two Petri plates. One of these plates was incubated at 37° C. and the other kept at room temperature (20° C. - 25° C.). From the same sample bottle 1 cc. of water was pipetted into a water dilution bottle and 0.1 cc. of sample pipetted into a second dilution bottle. The dilution bottles were shaken and from each, 1 cc. portions were pipetted into each of two Petri plates. One of these plates therefore, contained the 1:100 dilution of sample and the other, the 1:1000 dilution. After the lake water had been pipetted into the Petri dishes, melted but cool, (40° C.), agar was poured into the plates, and after gentle rotation to assure uniform distribution of the sample, the agar was allowed to harden. Three plates from each sample of water taken were incubated and the fourth kept at room temperature. After forty-eight hours the plates were examined for bacterial growth, and by using a Quebec Colony Counter the colonies were counted and recorded. The medium found most favorable for bacterial multiplication, and therefore one on which the greatest number of colonies would appear, was sodium caseinate agar, (Appendix B).

The plates were next examined for different types of colonies judged principally on morphology, consistency, color, and appearance through transmitted light. Different colonies were "fished" and restreaked on fresh Nutrient Agar, (Appendix B). All colonies that differed by the aforementioned characters were picked from the plates and streaked on agar slants. All agar slants were incubated at 37° C., and when a

period of twenty-four to forty-eight hours had elapsed a gram stain preparation, (Appendix C), and a hanging drop preparation were made of each slant. The staining schedule was as follows; the smears were stained for one minute with ammonium oxalate crystal violet and then washed in tap water. The smears were then immersed for one minute in iodine solution, washed in tap water and blotted dry. They were then decolorized for thirty seconds with gentle agitation in 95 per cent alcohol and blotted dry. The smears were counterstained ten seconds in safranin solution and washed in tap water. They were then dried and examined. Gram positive organisms stained blue and gram negative organisms stained red. Aside from obtaining the morphology of the bacterium by employing the gram's stain technique, the organism in question could be classified into a gram positive or gram negative category and detection of any contamination could be made.

In addition to the gram stain preparation a hanging drop preparation of each isolate was made. The hanging drop preparation consisted of a suspension of the organism on a cover slip which was inverted over a depression glass slide. The use of this method allowed the observer to verify his results obtained from the gram reaction as to the purity of the culture and the morphology of the bacterium, and also allowed for classification of the organism as a motile or non-motile form.

If contamination was observed by either of the above two methods, the contaminated culture was restreaked on an agar plate and an isolated colony picked, restreaked on an agar slant, and again subjected to the above procedures. In the event that various colonies were found to be pure cultures, they were stored in a refrigerator until the author was ready to perform diagnostic tests for their identification.

The tests employed for the identification of the isolated bacteria varied with the organism in question. The media used and the diagnostic tests performed most often in the identification of organisms were as follows:

Fermentations

Monosaccharides (hexoses)	Glucose
Disaccharides	Lactose
	Maltose
	Sucrose
Polysaccharides	Starch
Monohydric Alcohol	Glycerol
Polyhydric Alcohol	Mannitol

(Appendix B)

After inoculation of the unknown organism into the above media, the tubes were observed for a change of color of the indicator which was indicative of the fermentable properties of the bacterium in question. Gas production was detected by the displacement of the liquid medium in the fermentation tube inverted in the larger culture tube.

Physiological Reactions

Koser's Citrate Test: This test depended upon the ability of an organism to utilize the citrate medium, (Appendix B), as a sole source of carbon.

Litmus Milk: (Appendix B). This medium demonstrated the ability of an organism to produce a fermentation, or a peptonization. The indicator litmus detected acid production or an alkaline reaction.

Voges-Proskauer Test: This test detected the formation of acetyl-methyl-carbinol from glucose. Glucose-Peptone Water, (Appendix B),

was the medium employed with alpha-naphthol and potassium hydroxide, (Appendix B), as reagents.

Methyl Red Test: The same medium used to demonstrate the Voges-Proskauer reaction was employed here. The methyl red indicator was turned red by cultures having a pH of 5 or less, and yellow by organisms having a pH greater than 5.

Nitrate Reduction Test: Nitrate Broth, (Appendix B), was employed as a test of the ability of bacteria to reduce nitrates to nitrites. Sulphanilic acid and dimethyl-alpha-naphthylamine, (Appendix C), were the reagents used in this test.

Test for the production of Indole: Bacto-Tryptone in a 1 per cent concentration and Kovac's reagent, (Appendix C), were used to demonstrate the production of indole.

Test for the formation of Hydrogen Sulfide: Lead acetate agar, (Appendix B), detected hydrogen sulfide production by the unknown organisms.

Demonstration of Proteolysis by the use of Gelatin: Difco gelatin, (Appendix B), showed any proteolytic activity by its liquefaction after inoculation and incubation.

Cultural Characters

Potato Medium: The pigment production of organisms was enhanced by inoculation into this substratum.

Nutrient Broth: Difco nutrient broth, (Appendix B), was a medium in which most of the bacteria investigated produced characteristic growths, such as the formation of a pellicle, or a distinct sediment.

After these tests had been performed and interpreted, Bergey's Manual of Determinative Bacteriology was consulted as an aid in

identification. When certain fastidious organisms were encountered, special media supplemented the media previously mentioned.

Part 2 Chemical Analysis

Bacteria are, in the main, dependent upon oxygen for their continued well being. Nitrates and phosphates, their intermediate products and end products, are good indices of metabolic activity.

With this in mind chemical tests were performed on samples of water taken from the lake in order to determine the amount of oxygen, nitrates, and phosphates, present, and to correlate this data with the numbers of bacteria found at the same time. When a sample of water was taken from the lake and brought to the laboratory, part was retained for chemical analysis and the other portion was removed aseptically. This latter portion was pipetted into an undiluted petri plate, and into two other petri plates after having been first pipetted into dilution bottles in order to get 1:100 and 1:1000 dilutions. Cooled agar was poured into the plates and, after hardening, the plates were placed in the incubator and the colonies counted forty-eight hours later. The bacterial numbers were recorded with the chemical determinations obtained the same day the sample was taken.

In order to determine the amount of dissolved oxygen care was exercised so that atmospheric oxygen would not diffuse into the water and give erroneous results in the final titration. To eliminate this possibility an apparatus was used as described by Suckling (1944), and shown in Figure 1.

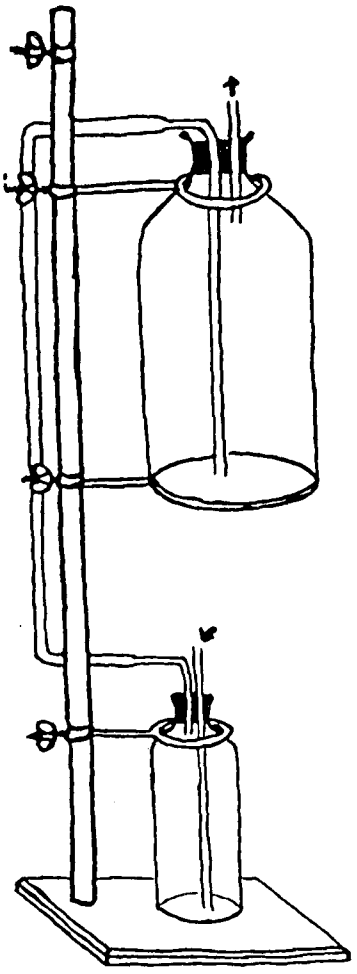


FIGURE 1

The arrows indicate the direction of flow. The apparatus shown above was employed in order that atmospheric oxygen would not interfere with the final results by diffusing into the water and being interpreted as dissolved oxygen. When the larger bottle had become filled, one could infer that a three-fold displacement of water had occurred in the smaller one, since the larger bottle was three times the volume of the smaller. A three-fold displacement was all that was necessary to eliminate the presence of any atmospheric oxygen.

The Winkler method was employed to determine the dissolved oxygen. The determination was divided into three stages as set forth by Suckling (1944).

1. Preliminary oxidation.

2. Fixation of the dissolved oxygen.

3. Measurement of the dissolved oxygen by liberation of iodine and titration with thiosulfate solution.

1. Preliminary Oxidation. After the sample of water had been obtained, 1 cc. of concentrated sulfuric acid was added by pipette, followed by sufficient $\frac{N}{8}$ potassium permanganate to leave a slight excess of the latter after the sample, (stoppered without an air bubble), had stood for fifteen minutes. One-half of a cubic centimeter was usually sufficient.

When a slight excess had been maintained for fifteen minutes, as shown by a faint pink color remaining, this excess was destroyed by the addition of 1 cc. of a 2 per cent potassium oxalate solution, the bottle being carefully re-stoppered.

2. Fixation of the Dissolved Oxygen. When the liquid had become colorless, 1 cc. of 30 per cent manganous sulfate was added by pipette, followed immediately by 3 cc. of a mixed solution containing 70 per cent potassium hydroxide and 10 per cent potassium iodide. The stopper was inserted and the sample taken to the laboratory where the final part, the measurement of oxygen, was begun.

3. Measurement of the Dissolved Oxygen. 5 cc. of concentrated sulfuric acid were run in from a pipette, the bottle re-stoppered and inverted until the precipitated hydroxides of manganese were dissolved.

The iodine set free in the acid mixture was then titrated by $\frac{N}{80}$ thiosulfate solution. The titration was carried out by measuring out 250 cc. of the liquid and transferring it to a separate flask for titration. Thiosulfate solution was run in until the yellow color was almost dispelled, and the titration completed after addition of a little freshly prepared starch solution, (Appendix C). Since 250 cc. of the liquid were used for the titration, the number of cc. of thiosulfate required, multiplied by $\frac{4}{100}$, gave mg. of oxygen per 100 cc. or parts per 100,000 by weight.

After the estimation of dissolved oxygen a nitrate nitrogen determination was carried out. A colorimetric determination was employed for this estimation. The color discs of the Hellige Aqua Tester were based upon the reaction between the nitrate content of a water sample and 1, 2, 4-phenol disulfonic acid, with the ultimate formation in alkaline solution of the yellow tripotassium salt of nitrophenoldisulfonic acid. A fresh sample of water was used, as nitrate can be converted uninterruptedly into ammonia by bacterial action.

Before making the nitrate determination, the nitrite content of the water sample was determined, since the presence of nitrites in excess of 1 part per million would lead to erroneous results. If the nitrite content had exceeded 1 part per million it would have been converted into nitrate by heating a few minutes with the addition of hydrogen peroxide. This situation never arose since in all the determinations the amount of nitrite present never exceeded an amount which would interfere with the nitrate nitrogen estimation.

Another preliminary test that had to be performed before the final

nitrate analysis could be made, was the determination of the hydrogen ion concentration. Aside from using this test as a preliminary to the Nitrate Test, pH determinations were made throughout the complete problem to ascertain the differences of acidity and/or alkalinity in the winter as compared to the summer. The Hellige pH meter was the apparatus employed for these determinations.

In using this apparatus when performing the preliminaries to the Nitrate Test, if the pH reading was definitely on the alkaline side, 0.02 N sulfuric acid was added until neutrality was reached.

After this preliminary treatment the nitrate determination was begun. A water sample of 100 ml. was evaporated to dryness. Two ml. of phenoldisulfonic acid solution, (Appendix C), was added.

The mixture was then diluted with distilled water, and approximately 12 N potassium hydroxide was slowly added until definitely alkaline. Eight ml. of the 12 N potassium hydroxide would neutralize about 2 ml. of the phenoldisulfonic acid. The yellow alkaline solution was diluted to 100 ml., allowed to stand overnight then filtered and poured into the Nessler Tube for comparison with the glass color standards.

The determination of phosphates is a color reaction based upon the reduction of the phosphomolybdate complex in acid solution by means of 1-amino, 2-naphthol, 4-sulfonic acid. One ml. of the test sample was measured into a 100 ml. graduated cylinder. Distilled water was added to bring the volume to 86 ml. Under constant stirring 10 ml. of molybdate reagent, (Appendix C), were added and mixed; then 4 ml. of aminonaphthol sulfonic acid solution, (Appendix C),

added and mixed. The time was noted. The developed color was compared against the glass color standards during the time interval of 10 to 15 minutes after the addition of reagents.

After these chemical tests had been performed, their results were recorded with the corresponding bacterial counts in order that any correlation between the data could be observed.

EXPERIMENTAL RESULTS AND DISCUSSION

I. Bacterial Population Studies in Relationship to Ecological Factors

Plate Counts

Bacterial plate counts were made from January through March and from May through July. Inspection of the data revealed an increase in the number of bacteria during the summer months as compared to their decrease during the winter.

Temperature, above anything else, was the factor responsible for this increase. During the winter months the temperature was low causing a decrease in the metabolic activities of the organisms, and thereby limiting their multiplication. On the other hand, during the summer months the high temperature allowed for an increase of metabolism resulting in an increase in bacterial numbers. The results bear this point out and one may conclude that similiar findings would be made by other investigators examining other bodies of water whose degree of pollution was the same as that of Westhampton Lake.

Taylor (1940) in his examination of several bodies of water in England arrived at conclusions that differed from the author's. Taylor's bacterial counts were the reverse of the counts of this work. This discrepancy may be attributed to the fact that the areas he considered were ones of gross pollution which resulted in a competition among microscopic life for sustenance. This, in turn, resulted in an eventual decrease in their number. During the winter months where the

temperature maintained bacterial metabolism at a minimum, the organisms present persisted and multiplied, because their food supply was always abundant since their population was not so great as to bring about a competition among the bacteria for the available nutriment. The results of the writer's studies of bacterial populations are recorded in table I and Graph I.

Table I. Plate Counts of bacteria per cubic centimeter of sample made from January to July.

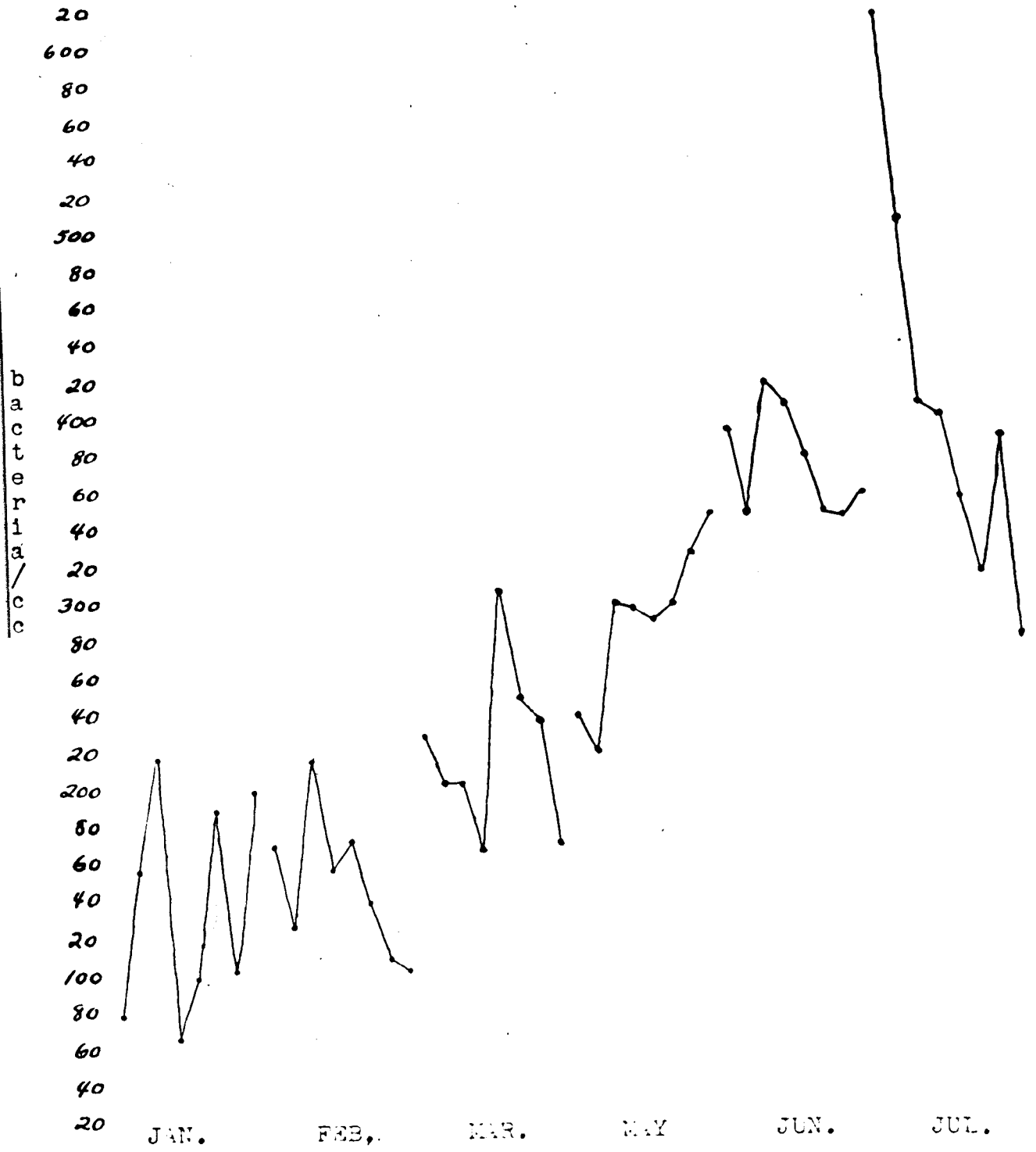
		Samples								
		#1	#2	#3	#4	#5	#6	#7	#8	mean temperature
	January	79	154	215	65	97	87	100	197	8.5°C.
Months	February	169	124	212	154	170	137	108	100	11.0°C.
	March	226	200	200	163	301	246	232	169	13.0°C.

		Samples								
		#1	#2	#3	#4	#5	#6	#7	#8	mean temperature
	May	237	217	298	292	287	296	322	344	28.8°C.
Months	June	390	344	417	402	376	344	341	353	30.6°C.
	July	614	501	403	398	351	311	388	279	32.9°C.

Reference to the above Table and Graph I reveals the fact that as the summer approached the bacterial numbers increased, and when midsummer was reached, the highest counts of bacteria were made.

Under normal conditions the bacterial population of Westhampton Lake passed through seasonal fluctuations involving an increased number

GRAPH I


20
600
80
60
40
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500
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400
80
60
40
20
300
80
60
40
20
200
80
60
40
20
100
80
60
40
20
bacteria/cc
JAN.
FEB.
MAR.
MAY
JUN.
JUL.

of microorganisms with increases in temperature, and a decrease of bacterial numbers with decreases in the temperature. There did occur however, abnormal increases in the bacterial population when rain fell. This increase of bacterial numbers persisted for forty-eight hours after the rain fell, after which, the count of microorganisms began to decrease. The return to normalcy came about, usually, after seventy-two hours had elapsed as evidenced by plate counts made at that time which revealed a count close to the counts made before precipitation had occurred.

Rain falling after a relatively dry spell would wash a large number of soil organisms into the water. Bacterial percolation through the soil, by introducing fresh wash from the surface ground, increased contamination of the lake. If more prolonged moderate rain fell an opposite effect would have been exerted. This opposite effect would be a dilution of the body of water causing in turn, a decrease in the number of bacteria found.

Table II shows the increase in the number of microorganisms after a rainfall of 0.47 inches.

Table II. Effects of Precipitation on Bacterial Counts

Precipitation = 0.47 inches

	Before precipitation	After precipitation	% increase
Collection # 1	2145 bacteria/cc.	3735 bacteria/cc.	57%
Collection # 2	1885 bacteria/cc.	3735 bacteria/cc.	54%
Collection # 3	1755 bacteria/cc.	2275 bacteria/cc.	77%

Table II - continued

	Before precipitation	After precipitation	% increase
Collection # 4	975 bacteria/cc.	2340 bacteria/cc.	42%
Collection # 5	1040 bacteria/cc.	1820 bacteria/cc.	57%
Collection # 6	715 bacteria/cc.	2275 bacteria/cc.	31%
Collection # 7	845 bacteria/cc.	1300 bacteria/cc.	65%
Collection # 8	652 bacteria/cc.	1214 bacteria/cc.	53%

That an increase in the number of organisms lasted for forty-eight hours with their return to normalcy occurring at the seventy-hour period is evident from Table III. The counts made in this table contain data collected after a 0.35 inch rainfall, showing that even when a rainfall as slight as this occurred the phenomenon of bacterial increase was still evident.

Table III. Time Intervals for the Return of Bacterial Populations to their normal Counts after Precipitation.

Precipitation = 0.35 inches

	Collection # 1	Collection # 2	Collection # 3
24 hrs. before pption.	575 bacteria/cc.	572 bacteria/cc.	463 bacteria/cc.
24 hrs. after pption	1750 bacteria/cc.	1700 bacteria/cc.	998 bacteria/cc.
48 hrs. after pption	1365 bacteria/cc.	1407 bacteria/cc.	992 bacteria/cc.
72 hrs. after pption	474 bacteria/cc.	411 bacteria/cc.	403 bacteria/cc.

Table IV illustrates the dilution effects occurring after a moderate rainfall. After 0.85 inches of rain had fallen samples

were collected in order to determine if this amount of precipitation was sufficient to produce dilution of the lake causing a decrease in the number of bacteria.

Table IV. Dilution and Flushing Effects of a Prolonged Rainfall

Precipitation = 0.85 inches

	Before precipitation	After precipitation
Collection # 1	393 bacteria/cc.	76 bacteria/cc.
Collection # 2	409 bacteria/cc.	81 bacteria/cc.
Collection # 3	473 bacteria/cc.	83 bacteria/cc.
Collection # 4	400 bacteria/cc.	76 bacteria/cc.
Collection # 5	362 bacteria/cc.	69 bacteria/cc.

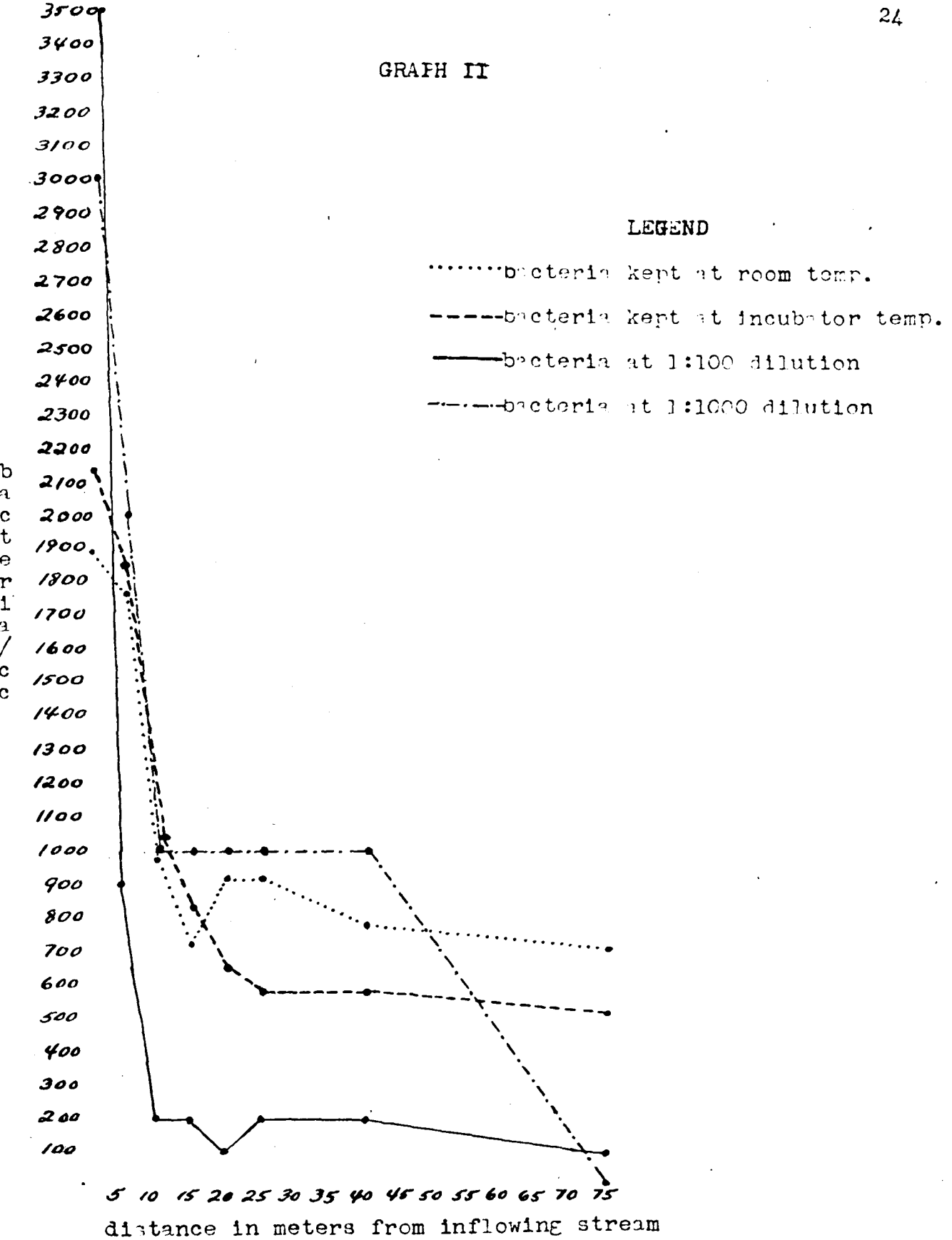
From Tables II, III and IV it is readily evident that the dilution and flushing effect of rain occurred between a rainfall range of 0.35 inches where increases of bacteria due to washed in soil were still evident, and 0.85 inches where decreases of bacteria due to dilution and flushing appeared. One can conclude that between these two amounts may be found that amount of precipitation where bacterial increase stops and bacterial decrease begins.

Another interesting phenomenon regarding bacterial numbers and the ecological factors influencing them, was the decrease in bacterial counts obtained when microorganisms entered a lake from an inflowing stream. Kleiber (1894) studied this ecological problem and arrived at the conclusion that the zone rich in bacteria due to an entering stream did not extend out in the lake beyond twenty meters. In the

author's treatment of this situation the data showed that in Westhampton Lake a few meters beyond twenty was where the bacterial counts tended to become constant. Taylor (1940) in his work on the number of bacteria at various distances from the mouth of the River Brathay in England, obtained data showing a steady decrease in bacterial numbers, although his initial decrease just beyond the river mouth was gradual and not in keeping with the author's figures where an abrupt drop in bacterial numbers was noted the first few meters beyond the inflowing stream.

The reasons for the decrease of the numbers of bacteria at various distances from the mouth of a tributary were: PROBABLY (1) The factor of sedimentation which was the falling of organisms caused by their introduction from a relatively fast moving body of water to a slow moving one. (2) There occurred a deprivation of food substances in the lake since there were more organisms competing for the available food in the lake. (3) Predatory protozoa were present consuming bacteria as part of their food intake. With the data at hand, the first factor, a physical one, is the most significant. Table V shows the bacterial numbers at different distances from the mouth of the stream flowing in from the northeast at the head of the lake. Graph II shows these results in graphic form. Table VI shows the results of collections made at a different time and after slight precipitation. Rainfall does not alter the results in any way other than to increase the bacterial numbers. In Table VI as in Table V the independent variable was the distances from the mouth of the inflowing stream, while the dependent variable was the bacterial numbers at various distances away from the mouth of the stream. Graph III shows the tabulations of Table VI graphically.

GRAPH II


LEGEND
bacteria kept at room temp.
bacteria kept at incubator temp.
bacteria at 1:100 dilution
bacteria at 1:1000 dilution
bacteria/cc
3500
3400
3300
3200
3100
3000
2900
2800
2700
2600
2500
2400
2300
2200
2100
2000
1900
1800
1700
1600
1500
1400
1300
1200
1100
1000
900
800
700
600
500
400
300
200
100
5 10 15 20 25 30 35 40 45 50 55 60 65 70 75
distance in meters from inflowing stream

GRAPH III

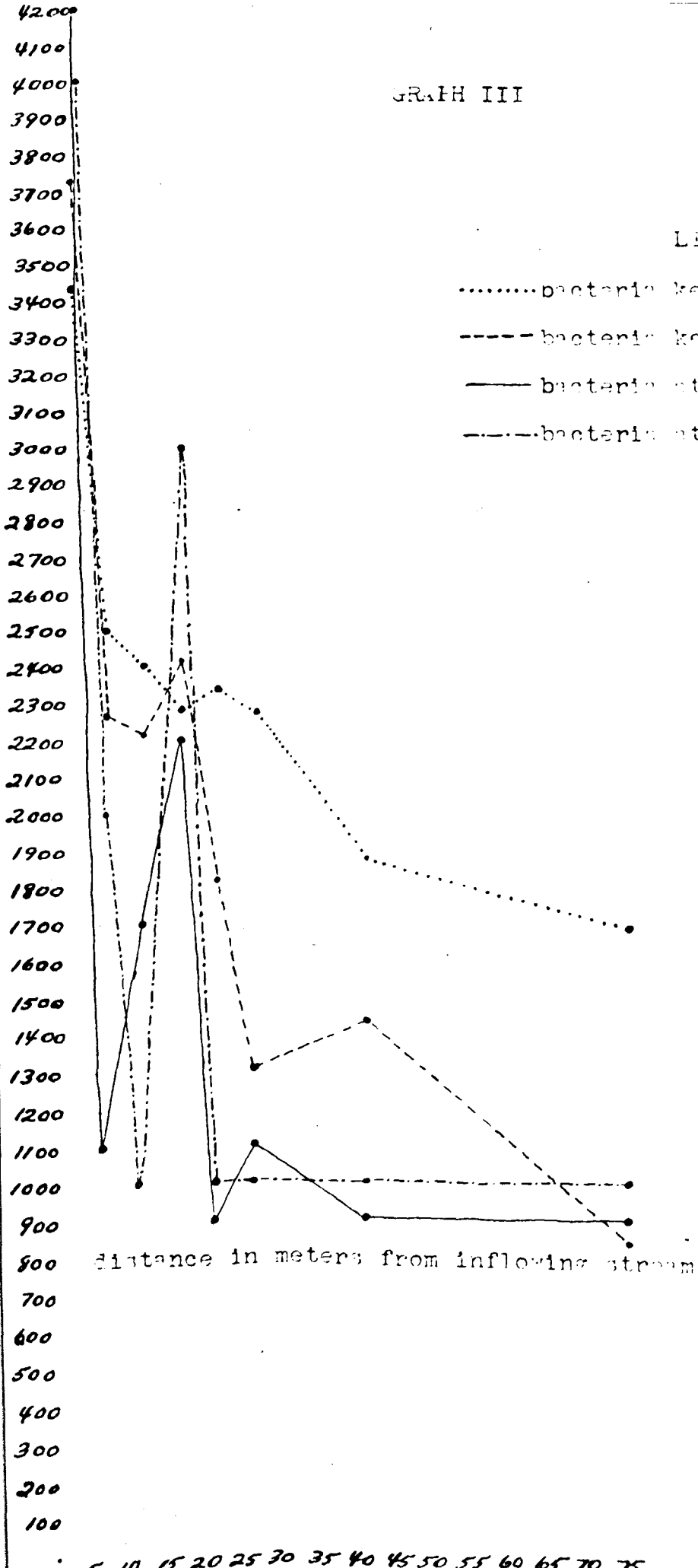

LEGEND
.....bacteria kept at room temp.
-----bacteria kept at incubator temp.
——— bacteria at 1:100 dilution
—·—·—bacteria at 1:1000 dilution
4200
4100
4000
3900
3800
3700
3600
3500
3400
3300
3200
3100
3000
2900
2800
2700
2600
2500
2400
2300
2200
2100
2000
1900
1800
1700
1600
1500
1400
1300
1200
1100
1000
900
800
700
600
500
400
300
200
100
5 10 15 20 25 30 35 40 45 50 55 60 65 70 75
distance in meters from inflowing stream

Table V. The Numbers of Bacteria as compared to their Distances from a Tributary

Distances in meters from inflowing stream	Plate Counts 48 hrs. room temp.	incubator temp.	1:100 dilution	1:1000 dilution
0 meters	1885 bact./cc.	2145 bact./cc.	3500 bact./cc.	3000 bact./cc.
5 meters	1755 bact./cc.	1950 bact./cc.	900 bact./cc.	2000 bact./cc.
10 meters	975 bact./cc.	1040 bact./cc.	200 bact./cc.	1000 bact./cc.
15 meters	715 bact./cc.	845 bact./cc.	200 bact./cc.	1000 bact./cc.
20 meters	910 bact./cc.	650 bact./cc.	100 bact./cc.	1000 bact./cc.
25 meters	910 bact./cc.	585 bact./cc.	200 bact./cc.	1000 bact./cc.
40 meters	780 bact./cc.	585 bact./cc.	200 bact./cc.	1000 bact./cc.
75 meters	715 bact./cc.	520 bact./cc.	100 bact./cc.	0 bact./cc.

Table VI. Bacterial Counts made after a slight precipitation as compared to Distances from a Tributary

distance in meters from inflowing stream	Plate Counts 48 hrs. room temp.	incubator temp.	1:100 dilution	1:1000 dilution
0 meters	3445 bact./cc.	3735 bact./cc.	4200 bact./cc.	4000 bact./cc.
5 meters	2500 bact./cc.	2260 bact./cc.	1100 bact./cc.	2000 bact./cc.
10 meters	2400 bact./cc.	2210 bact./cc.	1700 bact./cc.	1000 bact./cc.
15 meters	2275 bact./cc.	2405 bact./cc.	2200 bact./cc.	3000 bact./cc.
20 meters	2340 bact./cc.	1820 bact./cc.	900 bact./cc.	1000 bact./cc.

Table VI - continued

distance in meters from inflowing stream	room temp.	incubator temp.	1:100 dilution	1:1000 dilution
25 meters	2275 bact./cc.	1300 bact./cc.	1100 bact./cc.	1000 bact./cc.
40 meters	1875 bact./cc.	1430 bact./cc.	900 bact./cc.	1000 bact./cc.
75 meters	1690 bact./cc.	845 bact./cc.	900 bact./cc.	1000 bact./cc.

One may observe from this table and the preceding graph that after an initial decrease in numbers there occurred a very noticeable increase followed by a gradual decrease the farther one left the mouth of the inflowing stream. This the author attributed to the fact that these data were collected immediately following a slight rainfall. The rainfall was enough to cause a decided increase in the bacterial counts at all distances from the mouth of the inflowing stream, but even with the rainfall factor entering the picture the general trend of bacterial numbers was a decrease.

To further justify this part of the study dealing with ecological situations affecting the bacteria in Westhampton Lake, the author treated the data statistically in order to determine the degree of correlation between bacterial numbers and distances from inflowing streams. The "rank-difference" correlation coefficient was computed rather than the Pearson product-moment correlation coefficient because of the small numbers of pairs of observations. This coefficient measured the degree to which the two variables were associated and was symbolized by rho ( ).

could vary from +1.00 through zero to -1.00. A correlation coefficient of +1.00 would indicate a perfect positive relationship between

two variables; a zero coefficient would indicate no relationship; and -1.00 would indicate a perfect negative relationship. In terms of a formula the rank-difference correlation coefficient may be given thusly:

$$(\text{rho})\ \rho = 1 - \frac{6\Sigma D^2}{N(N^2-1)}$$

where

ρ = the rank-difference correlation coefficient

D^2 = the difference squared between each pair of ranks

N = the number of pairs of ranks

Table VII. Computation of Correlation of Room Temperature Counts of Table V

Distance	Bact. Counts	Rank Difference	Bact. Counts	D	D^2
0	1885	8	1	7	49
5	1755	7	2	5	25
10	975	6	3	3	9
15	715	5	7.5	2.5	6.3
20	910	4	4.5	.5	.3
25	910	3	4.5	.5	.3
40	780	2	6	4	16
75	715	1	7.5	6.5	42.3
					148.2

$$\rho = 1 - \frac{6(148.2)}{8(63)}$$

$$\rho = 1 - \frac{889.2}{504.0}$$

$$\rho = 1 - 1.76$$

$$\rho = -.76$$

-.76 indicates a strong negative correlation between the distances from the mouth of the tributary and the counts of bacteria kept at room temperature. Using this method the correlation coefficients of the bacterial counts at incubator temperature, 1:100 and 1:1000 dilutions of Tables V and VI were computed. The results were:

Correlation Coefficients of Data from Table V

Incubator temperature - .98
1:100 dilution - .50
1:1000 dilution - .37

Correlation Coefficients of Data from Table VI

Room temperature - .95
Incubator temperature - .73
1:100 dilution - .62
1:1000 dilution - .50

In order to observe the significance, if any, of these computed correlation coefficients, the writer referred to tables for converting the Rho Coefficient into its equivalent Pearson Product-Moment Correlation Coefficient, since no tables containing significant values of the Rho Coefficient could be found. With the exception of one instance the Pearson Product-Moment Coefficients had to be derived from the Rho Coefficients by interpolation.

Table VIII. Conversion Values of the Rho Coefficient (ρ) in terms of the Pearson Product-Moment Coefficient (r)

From Table V	ρ	r	from Table VI	ρ	r
room temperature	-.76	-.775	room temperature	-.95	-.954
incubator temperature	-.98	-.984	incubator temperature	-.73	-.748

Table VIII - continued

from Table V	ρ	r	from Table VI	ρ	r
1:100 dilution	-.50	-.518	1:100 dilution	-.62	-.639
1:1000 dilution	-.37	-.385	1:1000 dilution	-.50	-.518

The derived Pearson Product-Moment values were compared with (P) values at different levels of significance in tables giving this information, Fisher (1950).

Table IX. Comparison of Correlation Coefficients at different Levels of Significance

From table V		From table VI	
r = .775	P = .02	r = .954	P = .01
r = .984	P = .01	r = .748	P = .05
r = .518	P = (0.1)	r = .639	P = 0.1
r = .385	P = (0.1)	r = .518	P = (0.1)

From the above tabulation we see that the correlation coefficients were significant in five of the cases and not in three. The non-significant ones are indicated by parentheses around their P values. The two values having correlation coefficients of .518 were not too far removed from being significant. In all cases of bacterial counts made of plates that were not diluted, significant values were derived. The three values that were not significant were of diluted samples, two of these being the 1:1000 dilutions. This in itself seems significant, since the more dilution employed, the more difficult it became to arrive at a good correlation, the least correlated of all the values being the 1:1000 dilution. Since dilutions are usually employed in those examinations

of grossly polluted bodies of water, high dilutions in this examination were not necessary and were, in effect, responsible for the discrepancies shown among the correlation coefficients by not giving a close bacterial count by going from one large unit number of bacteria to another.

It was previously mentioned that microorganisms, for their continued well-being, are in part dependent upon the amount of dissolved oxygen present in a given body of water. As far as the metabolic activities of microorganisms are concerned, Nitrates and Phosphates play a more predominant role than any other of the chemical substances. With this in mind the author performed chemical tests on samples of water taken from the lake in order to determine if the aforementioned chemicals were of ecological import.

The principal sources of dissolved oxygen in water are, directly from the atmosphere through the exposed surface, and from the photosynthesis of chlorophyllaceous plants. The atmosphere being in contact with the surface of water becomes a consistent source of oxygen. The absorption of oxygen from the air is accomplished in two ways: (a) by direct diffusion at the surface and (b) through surface agitation such as wave motion.

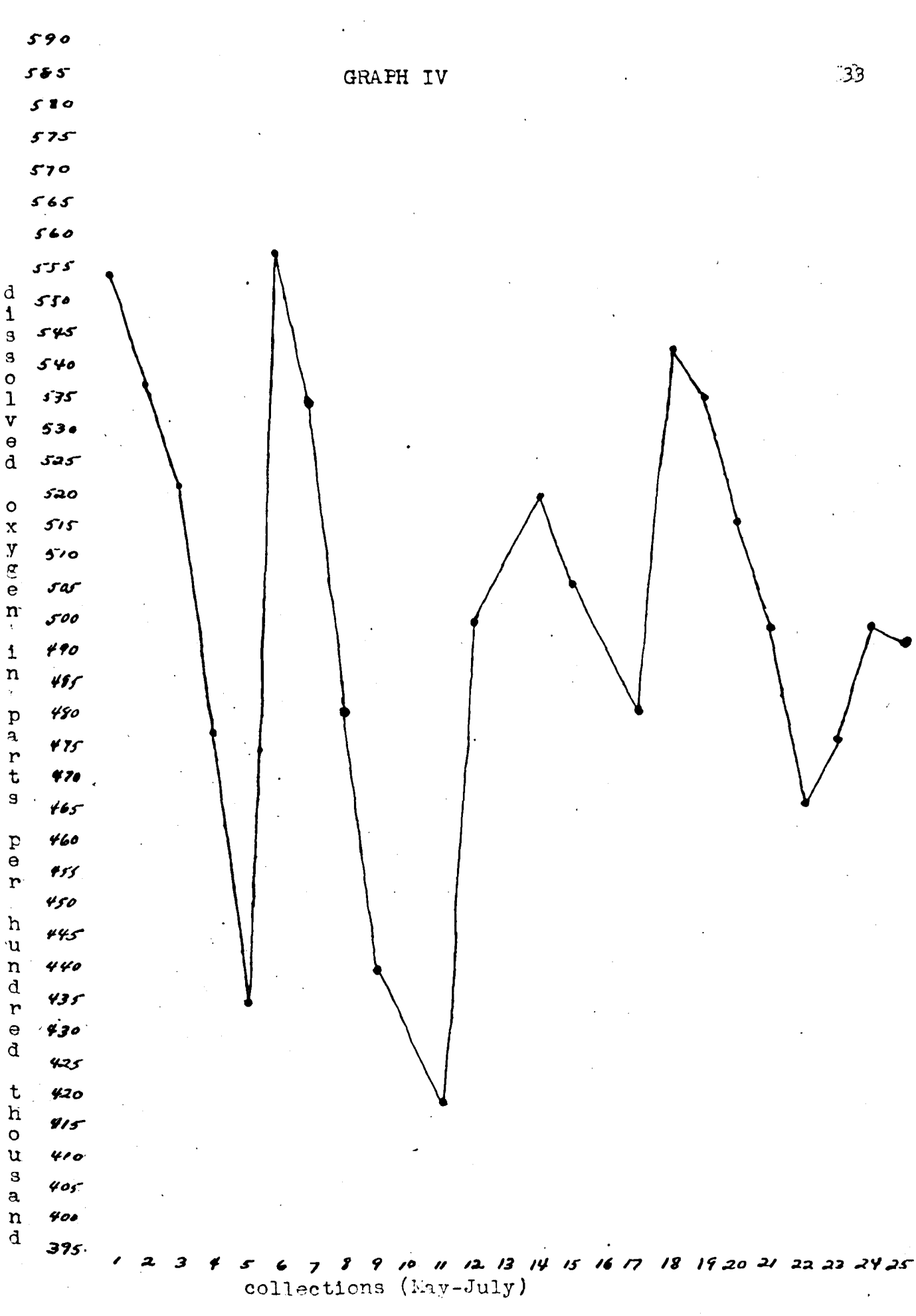
Unfortunately the amount of dissolved oxygen in Westhampton Lake during the winter months was not determined, but from past works, and one in particular, a general picture of the situation as it exists in the winter can be drawn. Smither (1948) found the amount of dissolved oxygen to range from 58 parts per million, at its maximum, to 10 parts per million, at its minimum. His study was begun in late October and terminated in late March. Pierce (1947) in a chemical study of four

aquatic habitats in Florida found the dissolved oxygen content of a body of water to fluctuate with the temperature. The amount of dissolved oxygen decreased as the temperature rose and increased as the temperature fell. During the winter months the amount of dissolved oxygen ranged from 6 parts per million to 11 parts per million. The average range of dissolved oxygen the year round was 5 parts per million. The dissolved oxygen determinations of Westhampton Lake made during the summer months by the author were very similiar to Pierce's findings.

Greater amounts of dissolved oxygen are found in bodies of water in the winter than in the summer. Pierce's year-round study showed this to be true in one aquatic locale and Smither's work combined with the author's show this to be true as far as Westhampton Lake is concerned. This reduction of dissolved oxygen is due to the respiration of plants and animals, and decomposition of organic matter. During the summer months both of these factors are operating at an increased rate, hence there comes a decrease in the amount of oxygen in water. Reduction may also occur by displacement of the dissolved oxygen with another gas, and also by the inflow of subterranean waters. The biggest single factor is due to the automatic release of dissolved oxygen from the water due to the oncoming of summer temperatures. Temperature affects the quantity of gas that can be absorbed, the solubility becoming less with an increase in temperature.

Graph IV shows the dissolved oxygen determinations for the months of May, June, and July. There are no major fluctuations, and the data are in keeping with the findings of Pierce regarding the dissolved

GRAPH IV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480
475
470
465
460
455
450
445
440
435
430
425
420
415
410
405
400
395
dissolved oxygen in parts per hundred thousand
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25
collections (May-July)

oxygen of a body of water during the summer months. Although temperature recordings were made every time a sample of water was obtained there exists no apparent correlation between the two in these data since the temperature did not decrease enough to show any increase in the amount of dissolved oxygen.

Table I showed how bacterial numbers decreased in the winter, and increased in the summer. From Graph IV one observes the amount of dissolved oxygen present in Westhampton Lake at various times during the summer. That this amount is lower than the amount that would be found in the same body of water in the winter, is true primarily because of physico-chemical laws in operation that are directly dependent on the temperature. If dissolved oxygen exerted any great influence on the number of bacteria, its presence in the summer would have to be greater than in the winter to correspond with the increase of bacteria in the summer. The amount of dissolved oxygen is less in the summer than in the winter, therefore it cannot be validly correlated with bacterial numbers, since bacterial numbers increased during the summer months and decreased during the winter months. This increase as the weather became warmer and decrease as the weather got colder shows that temperature affects bacteria more than any other factor, and that dissolved oxygen, although necessary, can proceed through reasonable fluctuations without adversely affecting the number of bacteria. The fluctuations of dissolved oxygen like bacterial numbers is dependent upon the temperature.

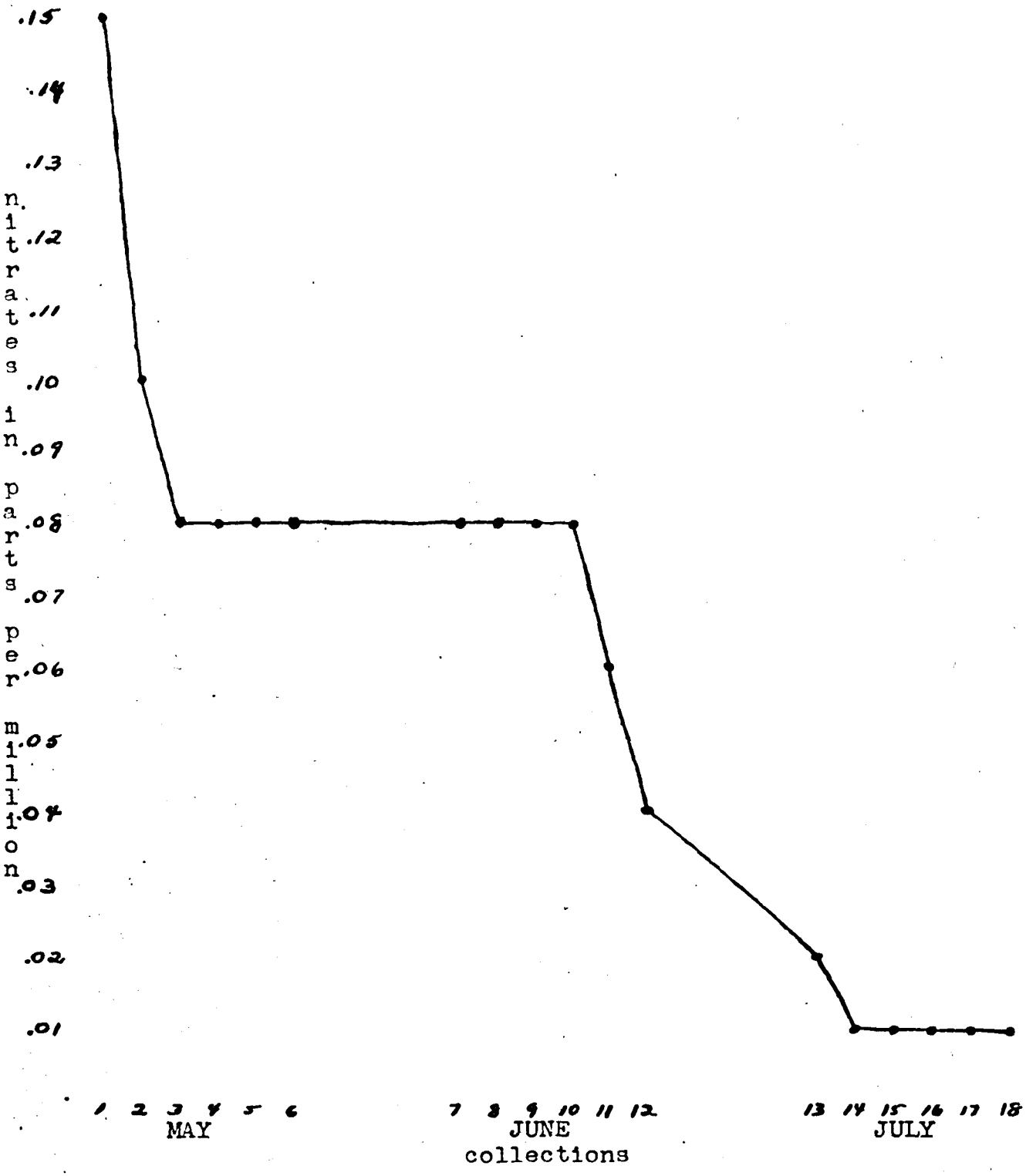
The amount of nitrates present in a body of water is significant because of the role that nitrates play in the biochemical

activities of microorganisms. Some bacteria are capable of reducing nitrates to nitrites, others can oxidize nitrites to nitrates. Nitrates, because of these biochemical reactions, have been recognized as indicators of the amount of oxidation and decomposition of organic matter in water.

Examination of Westhampton Lake for the amount of nitrate present showed that there occurred a gradual decrease in the nitrates present from spring to mid-summer. Unfortunately a year-round survey was not made, but from the data available the nitrate content diminished steadily from early spring to late summer. Because of this apparent uninterrupted decrease, and the fact that Pierce (1947), and Whipple (1933) have indicated that nitrate nitrogen increased in the winter and gradually decreased through the spring and summer, the author concluded that the same general situation held true for Westhampton Lake. Graph V shows the nitrate diminution occurring from early spring to late summer.

The phosphorous of all living tissue, vegetable and animal, in the process of disruption is finally oxidized to phosphates. Phosphates are, therefore found in manure, sewage, and soil, and consequently in water. The element phosphorous is never found in the free state but in the oxidized form of phosphates. These phosphates have been thought of by some workers as being the limiting factor affecting plankton numbers. In the field of bacterial ecology there have been no data regarding the significance of phosphates. The data obtained by the author concerning phosphates yielded no definite conclusions. The study was not conducted throughout the year and the data collected through the spring and summer months were few.

GRAPH V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.14
.13
.12
.11
.10
.09
.08
.07
.06
.05
.04
.03
.02
.01
nitrates in parts per million
1 2 3 4 5 6
MAY
7 8 9 10 11 12
JUNE
13 14 15 16 17 18
JULY
collections

Table X. Phosphates present in parts per million as compared to the Bacterial Counts per cubic centimeter of sample

Phosphates (parts per million)	Bacteria/cc.
90	575
90	622
70	431
90	542
70	418
70	474
70	391
90	500
90	544
70	411
70	419

As seen from Table X all the phosphate analyses of Westhampton Lake water showed 70 parts per million as the minimum amount present and 90 parts per million as the maximum amount. There were no intervening values. That there must be some correlation between the phosphates present and number of microorganisms can be observed in Table X. In all cases where the minimum value for phosphates was found the lowest number of bacteria appeared and vice versa.

Determinations of the pH of the Westhampton Lake water were made in the winter and summer. Table XI compares the pH determinations of the summer and winter.

Table XI. pH determinations of the Winter as compared to those of the Summer

	Winter pH determinations	Summer pH determinations
determination # 1	7.3	8.3
2	7.2	8.7
3	7.2	8.5
4	7.4	8.5
5	7.2	8.3
6	7.3	8.5
7	7.2	8.7
8	7.4	8.5
9	7.6	8.3
10	7.3	8.5
11	7.3	8.5
12	7.4	8.7
mean pH	7.32	8.5

That an increase toward alkalinity occurred in the summer is evident from the above table. The water was at all times definitely alkaline.

A correlation was found to exist between rainfall and pH

Table XII. Effects of rainfall on the pH of Westhampton Lake

	pH before pption.	pH after pption.
Observation # 1	8.7	8.5
Observation # 2	8.7	8.5

Table XII continued

	pH before pption.	pH after pption.
Observation # 3	8.7	8.5
Observation # 4	8.5	8.3
Observation # 5	8.7	8.3
Observation # 6	8.3	8.1
Observation # 7	8.5	8.3
Observation # 8	8.5	8.3

The pH decreased 0.2 units every time calculations were made. This observed decrease of pH was attributed to the washed-in soil which was acidic in nature.

An interesting observation made in passing, in connection with the highly alkaline nature of the water, was that chromogenic forms both in numbers and variety occurred in greater abundance during the summer months when the lake water was so definitely alkaline. This fact together with the fact that almost all chromogenic forms reacted in an alkaline manner when inoculated into litmus milk seems to indicate that an alkaline situation enhances color production. In vitro, pigment production is better manifested at room temperature, therefore at a lower temperature than that of the incubator. In vivo, pigment production is better manifested at the high temperatures of the summer months rather than the low winter temperatures. This paradoxical situation seems to rule out the temperature factor and its influence on chromogenesis, placing greater emphasis on the factor of alkalinity which showed positive correlation in every instance.

II. Nature of the Bacterial Flora of Westhampton Lake

The introduction to this work spoke of the treatment the question of indigenous flora had received from past workers. There have been contributions but not nearly as numerous as the contributions that have stemmed from studies concerned with the presence of excretal bacteria in water. Past workers have identified aquatic forms by biochemical means or staining properties. In the first case they would enumerate the percentage of proteolytic forms as compared to the non-proteolytic forms, or the percentage of biochemically active forms as distinguished from the biochemically inactive ones. Others mentioned the percentage of gram negative rods as compared to the percentage of gram positive sporulating rods. In this work the author, by employing various biochemical criteria and cultural characters, has obtained results of the kinds of natural water bacteria found in Westhampton Lake.

In a situation dealing with the enumeration of bacteria found in fresh water the point, that aquatic forms alone do not comprise the indigenous flora of water, should be emphasized. Practically all of the organisms of the Family Bacillaceae are inhabitants of the soil, and by various means, principally by percolation through the soil, find their way into a body of water. That this bacterial egress is brought about by precipitation is evident from Table II. The occurrence of these forms in water is considered natural and one may conclude that many of the bacteria found in water are thus derived from soil and vegetation. Falling rain introduces the bacteria of air into a body of water. Therefore it is correct to speak of natural water bacteria if one understands by this that besides aquatic forms, soil, vegetation, and air

make noticeable contributions to the natural situation. On the other hand we find forms introduced into Westhampton Lake that do not comprise the natural flora. These forms, which the author has chosen to refer to as "Xenotypes", belong, in the majority of cases, to the Family Enterobacteriaceae. Members of this family have as their source the intestinal tract. Xenotypes may then be regarded as "foreigners" or "strangers," with the implication of not belonging in a natural situation of this sort. Some members of the Family Enterobacteriaceae, which are characterized by their production of acid and gas when inoculated into lactose broth, are found occurring in nature, but differentiation of natural forms from intestinal forms cannot be made. Aside from these intestinal forms members of the Genus Alcaligenes of the Family Achromobacteriaceae were encountered. These too are intestinal forms and therefore are foreign to a natural water arrangement. Several species of micrococci, namely, Micrococcus ureae, Micrococcus candidus, and Micrococcus epidermidis, which are derived from human sources, were isolated. These were found to be present in Westhampton Lake and could also be considered as xenotypes. Pseudomonas aeruginosa, another xenotypic form, which according to Hitchens (1948) may be found in polluted water or sewage, also occurred in Westhampton Lake. Disregarding the small percentage of xenotypic forms the rest of the bacteria identified could be considered as the true water bacteria, the indigenous flora of Westhampton Lake. The Family Achromobacteriaceae was well represented, as was the Family Bacillaceae. Many members of the former family, with the exception of the Genus Alcaligenes, are inhabitants of water. Members of the Family Bacillaceae were found in greater numbers

during the winter than any other represented family. From this the author concluded that the indigenous flora of Westhampton Lake derived most of its forms from the soil because of their greatet number at this time of year.

In a work concerned with the indigenous flora of a body of water coliform organisms, ordinarily, would not be dealt with. This is true in most cases but in respect to Westhampton Lake the ecological situation has been so modified that a brief discussion is necessary.

Twenty years ago a bacteriological study of the lake would have been wholly concerned with the natural flora. At this time, however, with the growth of communities in areas surrounding the lake the natural flora, although still very much evident, have been joined by bacterial forms that are inhabitants of the intestinal tract. To the pre-existing natural arrangement there has been added the coliform organisms. These xenotypes have found their way into the lake via streams that drain into this body of water originating near areas of human habitation. Differentiation of these forms was not difficult since all were lactose-fermenting organisms, while the natural water bacteria were not. The only difficulty that arose in regard to means of differentiation was that all identified members of the Family Enterobacteriaceae are widely distributed in nature and a distinction between the forms found in nature from the forms found inhabiting the intestinal canal could not be made. Although this is true, in America any presence of these lactose-fermenting organisms is considered an index of pollution and the body of water under consideration would be regarded as being polluted.

In the bacterial examination of Westhampton Lake samples were collected from every body of water that entered the lake. Coliform

organisms were found in every stream that entered the lake, the stream entering from the northwest contributing more of these intestinal forms than any other tributary. Samples were collected along the entire lengths of every tributary in hopes that the source of contamination could be traced. In every instance lactose-fermenting organisms were present well into the upper-most reaches of these streams.

Even though the presence of these inhabitants of the intestinal tract was readily evident, their number did in no way alter the natural arrangement. Bacteria, both soil and water forms, that may be considered as being indigenous were found in greater abundance than this intestinal group. The gram-negative rods belonging to the Family Achromobacteriaceae, with the exception of members of the genus alcaligenes, are considered as being widely distributed in nature. The members of this family were well represented. Hence one can conclude that Westhampton Lake although being polluted, is not grossly polluted, or polluted to the extent where there would be an exclusion of naturally occurring bacteria.

The bacteria identified and described on the following pages represent the flora collected by the author in his examination of Westhampton Lake. The enumeration is divided into two sections, the first containing the organisms making up the winter flora, and the second, representing the bacteria of the summer flora. The biochemical tests employed in identification of the organisms and the results of these tests may be found in the tables in Appendix A. The following section merely lists the organisms and discusses certain ones which are of interest, morphologically, taxonomically or otherwise. If a medium or test was required

in the identification of the organism that was not ordinarily used, mention is made of it in this section also.

Winter Flora

Family Achromobacteriaceae

Isolate # 2 . . . . . Flavobacterium marinum

Isolate # 8 . . . . . Alcaligenes metalcaligenes

Isolate # 9 . . . . . Alcaligenes viscosus var. dissimilis

Isolate # 10 . . . . . Alcaligenes metalcaligenes

Isolate # 12 . . . . . Alcaligenes faecalis

Isolate # 15 . . . . . Achromobacter superficiale

Isolate # 22 . . . . . Flavobacterium aquatile

Isolate # 31 . . . . . Flavobacterium aquatile

Isolate # 33 . . . . . Achromobacter delicatulum

Isolate # 39 . . . . . Flavobacterium aquatile

Isolate # 40 . . . . . Alcaligenes viscosus

Isolate # 41 . . . . . Alcaligenes recti

Isolate # 42 . . . . . Alcaligenes faecalis

Isolate # 44 . . . . . Alcaligenes recti

Isolate # 48 . . . . . Alcaligenes bookeri

Isolate # 49 . . . . . Alcaligenes faecalis

Isolate # 51 . . . . . Flavobacterium devorans

Isolate # 57 . . . . . Achromobacter iophagum

Isolate # 59 . . . . . Flavobacterium marinum

Isolate # 61 . . . . . Alcaligenes bookeri

Isolate # 66 . . . . . Alcaligenes faecalis

Isolate # 77 . . . . . Flavobacterium aquatile

Isolate # 82 . . . . . Flavobacterium devorans

Isolate # 83 . . . . . Alcaligenes bookeri

Isolate # 89 . . . . . Alcaligenes faecalis

Family Bacillaceae

Isolate # 3 . . . . . Bacillus megatherium

Isolate # 6 . . . . . Bacillus cereus var. mycoides

Isolate # 13 . . . . . Bacillus cereus

Isolate # 14 . . . . . Bacillus megatherium

Isolate # 17 . . . . . Bacillus cereus var. mycoides

Isolate # 20 . . . . . Bacillus cereus var. mycoides

Isolate # 21 . . . . . Bacillus cereus var. mycoides

Isolate # 23 . . . . . Bacillus lentus

Isolate # 24* . . . Bacillus megatherium - Bacillus mycoides intermediate

Isolate # 26 . . . . . Bacillus megatherium

Isolate # 27* . . . Bacillus megatherium - Bacillus cereus intermediate

Isolate # 28* . . . Bacillus megatherium - Bacillus cereus intermediate

Isolate # 29* . . . Bacillus megatherium - Bacillus cereus intermediate

Isolate # 32* . . . Bacillus megatherium - Bacillus mycoides intermediate

Isolate # 36 . . . . . Bacillus cereus var. mycoides

Isolate # 37* . . . Bacillus megatherium - Bacillus mycoides intermediate

Isolate # 38* . . . Bacillus megatherium - Bacillus mycoides intermediate

Isolate # 43 . . . . . Bacillus megatherium

Isolate # 45 . . . . . Bacillus cereus

Isolate # 50 . . . . . Bacillus pumilis

* Vid. discussion at end of this section concerning intermediates.

Isolate # 52 . . . . . Bacillus megatherium

Isolate # 53 . . . . . Bacillus cereus

Isolate # 56* . . . Bacillus megatherium - Bacillus mycoides intermediate

Isolate # 60 . . . . . Bacillus pumilis

Isolate # 62* . . . Bacillus megatherium - Bacillus mycoides intermediate

Isolate # 64 . . . . . Bacillus subtilis

Isolate # 68 . . . . . Bacillus pumilis

Isolate # 69 . . . . . Bacillus cereus var. mycoides

Isolate # 71* . . . Bacillus megatherium - Bacillus mycoides intermediate

Isolate # 73 . . . . . Bacillus pumilis

Isolate # 74 . . . . . Bacillus pumilis

Isolate # 75 . . . . . Bacillus pumilis

Isolate # 76 . . . . . Bacillus pumilis

Isolate # 78 . . . . . Bacillus subtilis

Isolate # 79* . . . Bacillus megatherium - Bacillus mycoides intermediate

Isolate # 80 . . . . . Bacillus sphaericus

Isolate # 81* . . . Bacillus megatherium - Bacillus mycoides intermediate

Isolate # 85* . . . Bacillus megatherium - Bacillus cereus intermediate

Isolate # 86* . . . Bacillus megatherium - Bacillus cereus intermediate

Isolate # 88* . . . Bacillus megatherium - Bacillus mycoides intermediate

Isolate # 91 . . . . . Bacillus pumilis

Isolate # 92* . . . Bacillus megatherium - Bacillus cereus intermediate

Isolate # 93* . . . Bacillus megatherium - Bacillus cereus intermediate

Isolate # 94 . . . . . Bacillus circulans

Isolate # 95 . . . . . Bacillus pumilis

Isolate # 96 . . . . . Bacillus pumilis

* Vid. discussion at end of this section concerning intermediates.

Isolate # 97 . . . . . Bacillus pumilis

Isolate # 98 . . . . . Bacillus subtilis

Family Enterobacteriaceae

Isolate # 1 . . . . . Escherichia freundii

Isolate # 7 . . . . . Escherichia intermedium

Isolate # 4 . . . . . Escherichia freundii

Isolate # 11 . . . . . Escherichia intermedium

Isolate # 18 . . . . . Aerobacter aerogenes

Isolate # 19 . . . . . Escherichia intermedium

Isolate # 30 . . . . . Escherichia freundii

Isolate # 34 . . . . . Aerobacter aerogenes

Isolate # 35 . . . . . Paracolobactrum intermedium

Isolate # 46 . . . . . Escherichia intermedium

Isolate # 54 . . . . . Escherichia freundii

Isolate # 55 . . . . . Escherichia freundii (variant)

Isolate # 58 . . . . . Escherichia intermedium

Isolate # 65 . . . . . Aerobacter aerogenes

Isolate # 67 . . . . . Aerobacter aerogenes

Isolate # 87 . . . . . Aerobacter aerogenes

Family Micrococcaceae

Isolate # 5 . . . . . Micrococcus Candidus

Isolate # 25 . . . . . Micrococcus epidermidis

Isolate # 70 . . . . . Micrococcus ureae

Isolate # 72 . . . . . Micrococcus rhodochrous

Isolate # 90 . . . . . Micrococcus ureae

Isolate # 84 . . . . . Sarcina flava

Family Pseudomonadaceae

Isolate # 47 . . . . . Pseudomonas aeruginosa

Isolate # 63 . . . . . Pseudomonas aeruginosa

Isolate # 16 . . . . . Spirillum virginianum

Isolates # 2 and # 59 were identified as Flavobacterium marinum which, according to Hitchens et al., (1948) was originally isolated from fish in the Pacific Ocean. Because of this the organism under discussion was given the specific name marinum. The author has unquestionably found this organism present in the fresh water of Westhampton Lake in two instances and has concluded that this form is present in both salt water and fresh water.

Isolate # 57, identified as Achromobacter iophagum, was found to be fermentatively more active than Hitchens et al., (1948) would lead one to believe. According to Hitchens acid is produced from glucose and sucrose and occasionally from maltose and glycerol. The author in his investigation of this organism found it capable of fermenting Glucose, Lactose, Maltose, Sucrose, Mannitol, and Glycerol. No gas was produced.

Isolate # 15, identified as Achromobacter superficiale would show no growth on potato (Jordan), limited growth (Bergey), or abundant growth (Steinhaus), according to Hitchens et al., (1948). The author found that this organism would produce a scanty flesh colored growth when cultivated on potato.

Isolate # 33, Achromobacter delicatulum, gave an acid reaction in litmus milk, Hitchens et al., (1948), or underwent slow reduction

and peptonization according to Steinhaus' report found in Bergey's Manual of Determinative Bacteriology. This worker reports that Achromobacter superficiale gives an acid reaction in litmus milk which no subsequent indications of being reduced and peptonized.

Among the organisms identified in the Family Bacillaceae, Isolate # 23, Bacillus lentus was of significance because according to Hitchens et al., (1948) this organism produces no growth on potato. The author found Bacillus lentus capable of producing a moderate, butyrous, creamy growth on potato medium after twenty-four hours of incubation.

Bacillus pumilis, one of the most numerous of the organisms comprising the winter flora, was of interest to the author because of its action on litmus milk. Although no time for the organism to react in this medium was given in Bergey's Manual, one would consider a period of twenty-four to seventy-two hours sufficient for this particular organism to react. On the contrary it consistently took all isolates a minimum of seventy-hours to begin to give their characteristic reaction in litmus milk, peptonization.

Bergey's Manual of Determinative Bacteriology in treating of the members of the Family Bacillaceae reserves a section in which it discusses those forms which, according to certain authorities, are intermediate between Bacillus megatherium and Bacillus cereus in regard to certain physiological reactions. In the author's investigation these intermediate forms appeared and the basis upon which their intermediate position rested depended upon their action in Lactose, Mannitol, Nitrate, and Voges-Proskauer media. Seventeen forms were isolated which were found to be intermediate between the typical Bacillus megatherium and

the typical Bacillus cereus and its variant, Bacillus cereus var. mycoides. Of the seventeen forms Isolates 27, and 28, gave identical reactions, isolates 85, 86, and 93 gave similiar reactions, 88, and 71, did likewise, as did 32, and 24, 79, and 81, and 37, 56, 62. Therefore only three isolates exhibited any individuality with regard to biochemical reactions. The following table illustrates this and another significant point, that is, that when Isolate # 88 is encountered we find rhizoid growth present in all cases for the remainder of the table. Rhizoidal growth in agar is a characteristic typical of Bacillus cereus and coincident with the appearance of rhizoidal growth in the following table, the biochemical reactions lean more toward the typical Bacillus cereus reactions rather than the reactions of Bacillus megatherium.

	Lactose	Mannitol	Nitrate Test	Voges-Proskauer	Growth
Typical Bacillus megatherium	acid	acid	negative	negative	
Isolate # 27	acid	negative	negative	negative	
Isolate # 28	acid	negative	negative	negative	
Isolate # 85	acid	acid	negative	positive	
Isolate # 86	acid	acid	negative	positive	
Isolate # 93	acid	acid	negative	positive	
Isolate # 29	negative	negative	negative	negative	
Isolate # 92	negative	acid	negative	positive	
Isolate # 88	acid	acid	positive	positive	rhizoid
Isolate # 71	acid	acid	positive	positive	rhizoid
Isolate # 38	acid	negative	negative	positive	rhizoid
Isolate # 32	negative	negative	positive	negative	rhizoid
Isolate # 24	negative	negative	positive	negative	rhizoid
Isolate # 79	negative	acid	positive	positive	rhizoid
Isolate # 81	negative	acid	positive	positive	rhizoid
Isolate # 62	negative	negative	negative	positive	rhizoid
Isolate # 56	negative	negative	negative	positive	rhizoid
Isolate # 37	negative	negative	negative	positive	rhizoid
Typical Bacillus cereus	negative	negative	positive	positive	growth can be rhizoidal or not rhizoidal.

Table XIII. Biochemical Reactions of Bacillus megatherium - Bacillus mycoides intermediates

	Lactose	Mannitol	Nitrate Test	Voges-Proskauer	Growth
Typical Bacillus megatherium	acid	acid	negative	negative	
Isolate # 27	acid	negative	negative	negative	
Isolate # 28	acid	negative	negative	negative	
Isolate # 85	acid	acid	negative	positive	
Isolate # 86	acid	acid	negative	positive	
Isolate # 93	acid	acid	negative	positive	
Isolate # 29	negative	negative	negative	negative	
Isolate # 92	negative	acid	negative	positive	
Isolate # 88	acid	acid	positive	positive	rhizoid
Isolate # 71	acid	acid	positive	positive	rhizoid
Isolate # 38	acid	negative	negative	positive	rhizoid
Isolate # 32	negative	negative	positive	negative	rhizoid
Isolate # 24	negative	negative	positive	negative	rhizoid
Isolate # 79	negative	acid	positive	positive	rhizoid
Isolate # 81	negative	acid	positive	positive	rhizoid
Isolate # 62	negative	negative	negative	positive	rhizoid
Isolate # 56	negative	negative	negative	positive	rhizoid
Isolate # 37	negative	negative	negative	positive	rhizoid
Typical Bacillus cereus	negative	negative	positive	positive	growth can be rhizoidal or not rhizoidal.

This condition is not unlike the taxonomic situation of Escherichia coli and its many intermediate forms, although there is no paucity of typical forms of Bacillus megatherium and Bacillus cereus, as there is of Escherichia coli. Five typical forms of Bacillus megatherium and nine typical forms of Bacillus cereus and its variant, Bacillus cereus var. mycoides, were isolated. It is evident from the accumulated data that Bacillus mycoides, the "variant," of Bacillus cereus, is no longer in the dubious position of whether it should be given the dignity of a variety or merely the designation of a stage of growth (morphotype). The data show that not only does Bacillus mycoides vary from Bacillus cereus in its rhizoid growth on agar, but that variation occurs in the biochemical reactions in six different situations encompassing fourteen intermediate forms.

In the Family Enterobacteriaceae Isolate # 55, Escherichia freundii gave a negative nitrate reduction test. The test was repeated and the same results were obtained. The writer concluded that this organism was a variant of the true Escherichia freundii where nitrites are always produced from nitrates.

In the identification of organisms belonging to this group Brilliant Green Bile Broth, (Appendix B), was used as a presumptive test for gas production. Eosin-methylene-blue Agar, (Appendix B), was employed as a means of distinction between colonies of the genus Escherichia and the genus Aerobacter. The colonies of the former were small, isolated, and possessed a metallic sheen when cultivated on this differential medium. The colonies of the latter were not small and isolated but ran together, becoming confluent, when cultivated on E.M.B. Agar.

In the identification of organisms belonging to the Family Micrococcaceae, Ammonium hypophosphate, (Appendix B), and Urea were employed aside from the usual tests. Some members possessed the ability to utilize $NH_4H_2PO_4$ as a sole source of nitrogen. The test was interpreted as positive if a visible turbidity appeared in an inoculated culture and negative if no such turbidity was evident.

Micrococcus ureae was identified twice in the investigation of the winter flora. This micrococcus, unlike other members of its group, possessed the property of decomposing Urea, therefore Urea broth, (Appendix B), was employed in the identification of these organisms. The orange colored solution would become red in positive cases. No color change occurred if the reaction was negative.

Spirillum virginianum, a member of the Family Pseudomonadaceae, was the only member of this family that warranted mention here. This organism was biochemically inactive and identification depended upon its size, and the use of Uschinsky's Medium, (Appendix B). Spirillum virginianum produced a marked turbidity when cultivated in this medium.

Ninety-eight organisms were identified as members of the winter flora. Twenty-five forms belonged to the Family Achromobacteriaceae, forty-eight forms to the Family Bacillaceae, sixteen forms to the Family Enterobacteriaceae, six forms to the Family Micrococcaceae, and three forms to the Family Pseudomonadaceae. Thirty different species were isolated. 48% of the total number of isolates were sporulating rods, 44% were gram negative rods, and 6% were cocci. 12% possessed chromogenic properties. 16% were lactose fermenters.

Summer Flora

Family Achromobacteriaceae

Isolate # 3	Flavobacterium marinum
Isolate # 6	Achromobacter eurydice
Isolate # 7	Achromobacter stationis
Isolate # 10	Flavobacterium harrisonii
Isolate # 13	Flavobacterium sewanense
Isolate # 25	Alcaligenes metalcaligenes
Isolate # 26	Alcaligenes faecalis
Isolate # 28	Alcaligenes recti
Isolate # 29	Alcaligenes metalcaligenes
Isolate # 31	Achromobacter liquefaciens
Isolate # 32	Alcaligenes recti
Isolate # 41	Achromobacter eurydice
Isolate # 43	Alcaligenes bookeri
Isolate # 47	Alcaligenes marshallii
Isolate # 48	Flavobacterium aquatile
Isolate # 49	Alcaligenes bookeri
Isolate # 51	Flavobacterium marinum
Isolate # 56	Flavobacterium devorans
Isolate # 57	Alcaligenes faecalis
Isolate # 59	Alcaligenes recti
Isolate # 64	Alcaligenes faecalis
Isolate # 66	Alcaligenes recti
Isolate # 73	Flavobacterium devorans

Isolate # 75 . . . . . Achromobacter delicatulum

Isolate # 76 . . . . . Alcaligenes metalcaligenes

Isolate # 77 . . . . . Achromobacter liquefaciens

Isolate # 80 . . . . . Flavobacterium marinum

Isolate # 82 . . . . . Achromobacter liquefaciens

Isolate # 90 . . . . . Flavobacterium aquatile

Family Bacillaceae

Isolate # 18 . . . . . Bacillus cereus var. mycoides

Isolate # 19 . . . . . Bacillus megatherium

Isolate # 21 . . . . . Bacillus cereus var. mycoides

Isolate # 22 . . . . . Bacillus sphaericus

Isolate # 23 . . . . . Bacillus megatherium

Isolate # 33 . . . . . Bacillus pumilus

Isolate # 36 . . . . . Bacillus circulans

Isolate # 40 . . . . . Bacillus pumilus

Isolate # 52 . . . . . Bacillus cereus var. mycoides

Isolate # 54 . . . . . Bacillus subtilis

Isolate # 60 . . . . . Bacillus pumilus

Isolate # 65 . . . . . Bacillus megatherium

Isolate # 69 . . . . . Bacillus pumilus

Isolate # 72 . . . . . Bacillus cereus var. mycoides

Isolate # 81 . . . . . Bacillus subtilis

Isolate # 86 . . . . . Bacillus cereus

Isolate # 87 . . . . . Bacillus pumilus

Isolate # 91 . . . . . Bacillus megatherium

Isolate # 92 . . . . . Bacillus megatherium

Isolate # 93 . . . . . *Bacillus cereus* var. *mycoides*

Isolate # 95 . . . . . *Bacillus cereus* var. *mycoides*

Isolate # 97 . . . . . *Bacillus megatherium*

Isolate # 99 . . . . . *Bacillus pumilus*

Family Enterobacteriaceae

Isolate # 1 . . . . . *Aerobacter aerogenes*

Isolate # 2 . . . . . *Aerobacter cloacae*

Isolate # 5 . . . . . *Aerobacter aerogenes*

Isolate # 15 . . . . . *Serratia plymuthicum*

Isolate # 16 . . . . . *Serratia marcescens*

Isolate # 24 . . . . . *Escherichia freundii*

Isolate # 30 . . . . . *Serratia marcescens*

Isolate # 34 . . . . . *Paracolobactrum aerogenoides*

Isolate # 35 . . . . . *Paracolobactrum aerogenoides*

Isolate # 37 . . . . . *Serratia marcescens*

Isolate # 38 . . . . . *Escherichia intermedium*

Isolate # 42 . . . . . *Paracolobactrum aerogenoides*

Isolate # 45 . . . . . *Aerobacter aerogenes*

Isolate # 50 . . . . . *Escherichia intermedium*

Isolate # 53 . . . . . *Escherichia freundii*

Isolate # 55 . . . . . *Escherichia coli*

Isolate # 62 . . . . . *Escherichia freundii*

Isolate # 63 . . . . . *Paracolobactrum intermedium*

Isolate # 67 . . . . . *Aerobacter aerogenes*

Isolate # 68 . . . . . *Escherichia freundii*

Isolate # 70 . . . . . Escherichia intermedium

Isolate # 71 . . . . . Aerobacter cloacae

Isolate # 74 . . . . . Escherichia intermedium

Isolate # 78 . . . . . Escherichia intermedium

Isolate # 79 . . . . . Escherichia intermedium

Isolate # 83 . . . . . Escherichia coli

Isolate # 84 . . . . . Aerobacter aerogenes

Isolate # 85 . . . . . Paracolobactrum intermedium

Isolate # 88 . . . . . Paracolobactrum intermedium

Isolate # 89 . . . . . Escherichia freundii

Isolate # 96 . . . . . Escherichia freundii

Isolate # 98 . . . . . Escherichia freundii

Family Micrococcaceae

Isolate # 14 . . . . . Micrococcus varians

Isolate # 17 . . . . . Micrococcus roseus

Isolate # 58 . . . . . Micrococcus ureae

Isolate # 20 . . . . . Sarcina flava

Isolate # 46 . . . . . Sarcina flava

Family Pseudomonadaceae

Isolate # 4 . . . . . Pseudomonas geniculata

Isolate # 11 . . . . . Pseudomonas geniculata

Isolate # 12 . . . . . Pseudomonas jaegeri

Isolate # 27 . . . . . Pseudomonas geniculata

Isolate # 44 . . . . . Pseudomonas aeruginosa

Isolate # 61 . . . . . Pseudomonas aeruginosa

Isolate # 94 . . . . . Pseudomonas aeruginosa

Isolate # 8 . . . . . Spirillum tenue

Family Rhizobiaceae

Isolate # 9 . . . . . Chromobacterium violaceum

Isolate # 39 . . . . . Chromobacterium violaceum

Among the organisms comprising the summer flora, Isolates # 2 and # 71, Aerobacter cloacae appear for the first time. Aerobacter cloacae was distinguished from Aerobacter aerogenes by not producing gas in glycerol broth.

Isolates # 3, # 51, and # 80, Flavobacterium marinum appeared in the summer flora, justifying the author's contention that this organism occurs in fresh water as well as salt water.

Isolates # 6 and # 41, Achromobacter eurydice, occur as secondary invaders of European foulbrood of bees according to Hitchens (1948). The habitat of this bacterium is unknown and from the findings of this study it appears that this organism is widely distributed in nature.

Isolate # 7, Achromobacter stationis is found in sea water according to Hitchens (1948). This organism was found in Westhampton Lake, leading the author to believe that this form may also be found in fresh water. The biochemical and cultural reactions of Isolate # 7 were not exactly as those given by Hitchens (1948) and the possibility exists that this may be a new form.

Isolate # 13, Flavobacterium sewanense, was first isolated from sea water. This form, as were a good number of the forms inhabiting sea water, was found in Westhampton Lake.

Isolates # 9, and # 39, Chromobacterium violaceum, appeared for the first time in the summer collections. This organism produces a deep violet pigment.

Bacillus pumilus was well represented among the members of the Family Bacillaceae that comprised the summer flora. In every case this organism did not peptonize litmus milk until after seventy-two hours had elapsed. This situation was also found to occur in the isolations of Bacillus pumilus in the winter collections.

Isolates # 55, and # 83 were identified as Escherichia coli. This is the first appearance of this intestinal form. The biochemical reactions given by Isolates # 55 and # 83 were identical to those given by Hitchens (1948). The author did not review the literature to see if this form was a typical one or not.

Many organisms belonging to the Genus Paracolobactrum appeared in the summer collections as compared to the winter collections. This was not only true for members of this genus, since all intestinal forms made up a larger percentage of the summer flora than the winter flora. The warm weather seemed to favor their multiplication.

Ninety-nine organisms were identified as members of the winter flora. Twenty-nine forms belonged to the Family Achromobacteriaceae, twenty-three forms to the Family Bacillaceae, thirty-two forms to the Family Enterobacteriaceae, five forms to the Family Micrococcaceae, eight forms to the Family Pseudomonadaceae, and two forms to the Family Rhizobiaceae. Thirty-eight different species were isolated. 23% of the total number of isolates were sporulating rods, 77% were gram negative rods, and 5% were cocci. 18% possessed chromogenic properties. 32% were lactose fermenters.

SUMMARY

The writer has carried out a study of the ecological factors influencing the bacterial population of a fresh water lake, and has enumerated the species of organisms encountered in this study.

The fresh water lake investigated was Westhampton Lake located on the University of Richmond campus. This lake was under investigation from January to July.

Plate counts of the bacterial population of Westhampton Lake were made. Sodium caseinate agar was employed in making these plate counts. The lowest bacterial populations were encountered during the month of January. A progressive rise in bacterial population occurred from this month to July where the highest plate counts were made.

Precipitation of the magnitude of 0.47 inches caused a mean percentage increase of 54.5% in the number of bacteria, whereas precipitation of the magnitude of 0.85 inches caused a decrease in bacterial population. The increase was attributed to the introduction of soil forms into the lake by rain draining through the soil. The decrease in the numbers of bacteria was due to the dilution and flushing effects of the rain.

It was shown that after 0.35 inches of rainfall a time interval of seventy-two hours elapsed before bacterial populations returned to their normal number.

Statistical methods showed a strong negative correlation existed between the number of bacteria in a lake and the distances they occurred

from the mouth of a tributary. The numbers of bacteria decreased the farther away they were from the mouth of an inflowing stream.

Dissolved oxygen determinations showed that bacterial numbers are not as dependent upon the amount of dissolved oxygen in the lake as they are upon the temperature.

Nitrate determinations showed an uninterrupted decrease in the amount of nitrates present indicating that they are dependent upon temperature also.

Phosphates, as indices of metabolic activity, were investigated to see if any correlation existed between the amount of phosphates present and the numbers of bacteria. The phosphates fluctuated somewhat with the numbers of bacteria, but the data were too few to draw any conclusions.

Hydrogen ion determinations showed a mean pH of 7.32 in the winter, and 8.5 in the summer. Rainfall decreased the pH of the lake water.

Although evidences of manurial pollution and human contamination were detected in Westhampton Lake, bacteria regarded as being indigenous to a body of water were found in greater abundance than intestinal forms.

Ninety-eight organisms were identified in the winter flora. Five families, eleven genera, and thirty-one different species made up the summer flora group. Seventeen intermediate forms of Bacillus megatherium and Bacillus cereus var. mycoides were isolated. Many of these seventeen forms showed the same biochemical reactions.

Ninety-nine organisms comprised the summer flora. Six families, thirteen genera, and thirty-eight different species represented the summer flora.

In the winter collections 48% were sporulating rods, 44% were gram negative rods, and 6% were cocci. 12% possessed chromogenic properties. 16% were lactose fermenters. In the summer collections 23% were sporulating rods, 77% were gram negative rods, and 5% were cocci. 18% possessed chromogenic properties. 32% were lactose fermenters.

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VITA

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This thesis was typed by Frances B. Forsyth

APPENDIX A

The following tables list the results of the morphological, gram's stain and biochemical tests performed on the organisms of the winter and summer flora.

Legend

Media

Dex. = Dextrose

Lac. = Lactose

Mal. = Maltose

Suc. = Sucrose

Gly. = Glycerol

Man. = Mannitol

Cit. = Citrate

L.M. = Litmus milk

V.P. = Voges-Proskauer

M.R. = Methyl Red

Nit. = Nitrate

Ind. = Indole

PbAc. = Lead Acetate

Gel. = Gelatin

Pot. = Potato

Nut. Br. = Nutrient Broth

E.M.B. = Eosin-methylene-blue Agar

B.G.B. = Brilliant Green Bile Broth

$NH_4H_2PO_4$ = Ammonium Hypophosphate

St. = Starch

Tests

Fermentations

a & g = acid and gas

\- = negative

Litmus milk

a = acid

alk. = alkaline

pep. = peptonized

coag. = coagulated

red. = reduced

dig. = digested

n.c. = no change

Voges-Proskauer

Methyl Red

Nitrate

Indole

Lead Acetate

\+ = positive

\- = negative

Gelatin

\+ = liquefied

\- = not liquefied

Legend - continued

Tests

Starch

+ = hydrolized

- = not hydrolized

Nutrient Broth

cl. = clear

t. = turbid

v.s. = viscid sediment

g.s. = granular sediment

pell. = pellicle

Characteristics

motility: + = motile

- = non-motile

gram's reaction: + = gram positive

- = gram negative

Endospores: + = endospores present

- = endospores absent

Growth on Potato: yel. = yellow

brn. = brown

cr. = cream

w. = white

sc. = scanty

ab. = abundant

shin. = shiny

wr. = wrinkled

WINTER FLORA

TABLE I - Results of Morphological, Gram's Stain, and Biochemical Tests performed on identified Isolates found in Westhampton Lake

FAMILY ACHROMOBACTERIACEAE

Isolations with their Identifications	Gm's react	Morph	Size*	Endo	Mot	Dex	Lac	Mal	Suc	St	Gly	Man	Cit	L.M	V.P	M.R	Nit	Ind	PbAc	Gel	Nut.Br.	Pot
Isolate # 2 Flavobacterium marinum	-	rod	0.8 by 1.5	-	-	a								alk			-	-	-	+	t.	yel & shin
Isolate # 8 Alcaligenes metalcaligenes	-	rod	0.6 by 1.5	-	-				-					alk			-	-			pel.	sc., w. shin
Isolate # 9 Alcaligenes viscosus var. dissimilis	-	rod	0.7 by 1.5	-	-									alk	-	-	-	-	-		pel. & t.	ab., w., & shin.
Isolate # 10 Alcaligenes metalcaligenes	-	rod	0.6 by 1.5	-	-				-					alk			+	-		-	pel.	sc., w., shin.,
Isolate # 12 Alcaligenes faecalis	-	rod	0.6 by 1.5	-	+									alk			-	-		-	t., pel. v.s.	sc., & yell.
Isolate # 15 Achromobacter superficiale	-	rod	1.0 by 2.0	-	+									n.c.			-	-		+	t.	sc., flesh-colored

* all measurements are in microns

Table II

Family Achromobacteriaceae - continued

Isolations with their Identifications	Gm's react	Morph	Size*	Endo	Mot	Dex	Lac	Mal	Suc	St	Gly	Man	Cit	L.M	V.P	M.R	Nit	Ind	PbAc	Gel	Nut.Br.	Pot
Isolate # 22 Flavobacterium aquatile	-	rod	0.7 by 2.5	-	-									n.c			-	-		+	t.	sc., yel., butyrous
Isolation # 31 Flavobacterium aquatile	-	rod	0.5 by 2.2	-	-									n.c.			-	-		+	t.	sc., yel., butyrous
Isolate # 33 Achromobacter delicatulum	-	rod	1.0 by 2.0	-	+	a	a	a	a	-				a			+	-	+	-	t.	cr.- yel.
Isolate # 39 Flavobacterium aquatile	-	rod	0.5 by 2.2	-	-									n.c.			-	-		-	t.	sc., yel., butyrous
Isolate # 40 Alcaligenes viscosus	-	rod	0.7 by 1.5	-	+									alk & ropy	-	-	-	-	-	-	t., & pel.	w., shin., ab.
Isolate # 41 Alcaligenes recti	-	rod	0.5 by 1.7	-	+									alk.			+	-		+	t.	shin., tan, ab.
Isolate # 42 Alcaligenes faecalis	-	rod	0.5 by 1.0	-	+									alk.			-	-		-	t., v.s.	shin., tan
Isolate # 44 Alcaligenes recti	-	rod	0.5 by 1.7	-	+									alk.			+	-		+	t.	shin., tan, ab.

Table III

Family Achromobacteriaceae - continued

Isolations with their Identifications	Gm's react	Morph	Size*	Endo	Mot	Dex	Lac	Mal	Suc	St	Gly	Man	Cit	L.M	V.P	M.R	Nit	Ind	PbAc	Gel	Nut.Br.	Pot.
Isolate # 48 Alcaligenes bookeri	-	rod	0.5 by 1.5	-	+									pep.			-	-		+	t., v.s.	shin., yel.-w, ab.
Isolate # 49 Alcaligenes faecalis	-	rod	0.5 by 1.0	-	+									alk.			-	-		-	t., v.s.	shin., brn.-red
Isolate # 51 Flavobacterium devorans	-	rod	0.8 by 1.2	-	+									n.c.			-	-		+	t.	dirty yel.
Isolate # 57 Achromobacter iophagum	-	rod	1.0 by 2.5	-	+	a	a	a	a	+	a	a		n.c.			+				t.	brn.-w.
Isolate # 59 Flavobacterium marinum	-	rod	0.8 by 1.5	-	+	a								alk.			-	-	-	+	t.	yel., shin.
Isolate # 61 Alcaligenes bookeri	-	rod	0.5 by 1.5	-	+									pep.			-	-		+	t., v.s.	shin., yel.-w., ab.
Isolate # 66 Alcaligenes faecalis	-	rod	0.5 by 1.0	-	+									alk.			-	-		-	t., v.s.	shin., brn.-red
Isolate # 77 Flavobacterium aquatile	-	rod	0.5 by 2.5	-	-									n.c.			-	-		+	t.	dull yel.

Table IV

Family Achromobacteriaceae - continued

Isolations with their Identifications	Gm's react	Morph	Size	Endo	Mot	Dex	Lac	Mal	Suc	St	Gly	Man	Cit	L.M	V.P	M.R	Nit	Ind	PbAc	Gel	Nut.	Br.	Pot
Isolate # 82 Flavobacterium devorans	-	rod	0.8 by 1.2	-	+								n.c.				-	-		+	t.		dirty yel.
Isolate # 83 Alcaligenes bookeri	-	rod	0.5 by 1.5	-	-								pep.				-	-		-	t., v.s		shin., yel.-w., ab.
Isolate # 89 Alcaligenes faecalis	-	rod	0.5 by 1.0	-	+								alk.				-	-		-	t., v.s.		shin., tan

Table V

FAMILY BACILLACEAE

Isolations with their identifications	Gm's react	Morph	Size	Endo	Mot	Dex	Lac	Mal	Suc	St	Gly	Man	Cit	L.M	V.P	M.R	Nit	Ind	PbAc	Gel	Nut.Br.	Pot
Isolate # 3 Bacillus megatherium	+	rod	1.5 by 3.0	+	-	a	a	a	a	+	a	-	+	pep	-		-			+	t.	w., ab.
Isolate # 6 Bacillus cereus var. mycoides	+	rod	1.0 by 3.0	+	-	a	a	a	a	+	a	-	-	pep	+		+			+	t.	cr.-w., ab.
Isolate # 13 Bacillus cereus	+	rod	1.2 by 4.0	+	-	a	-	a	-	+	a	-	-	pep	+		+			+	t.	thick cr.-w.
Isolate # 14 Bacillus megatherium	+	rod	1.0 by 2.5	+	+	a	-	a	a	+	a	-	+	pep	-		+			+	t.	cr.-yel.
Isolate # 17 Bacillus cereus var. mycoides	+	rod	1.0 by 3.5	+	+	a	+	a	a	+	a	-	-	pep	+		+			+	t.	shin, cr.
Isolate # 20 Bacillus cereus var. mycoides	+	rod	1.0 by 3.0	+	-	a	-	a	a	+	a	-	-	pep	+		+			+	t.	w., rough.
Isolate # 21 Bacillus cereus var. mycoides	+	rod	1.0 by 3.0	+	-	a	-	a	-		a	-	-	pep	+		+			+	t.	shin., w.
Isolate # 23 Bacillus lentus	+	rod	0.6 by 2.0	+	+	a	a		a	+			+	n.c.	-		-			-	t.	slight cr. growth

Table VI

Family Bacillaceae - continued

Isolations with their identifications	Gm's react	Morph	Size	Endo	Mot	Dex	Lac	Mal	Suc	St	Gly	Man	Cit	L.M	V.P	M.R	Nit	Ind	PhAc	Gel	Nut.Br.	Pot.
Isolate # 24 B. meg. - B. cereus intermediate	+	rod	1.3 by 2.5	+	-	a	-	a	a	+	a	-	-	pep	-		+		+	t.	w., cr.
Isolate # 26 Bacillus megatherium	+	rod	1.5 by 4.0	+	+	a	-	a	a	+	a	a	+	pep	-		-		+	t.	cr. ab.
Isolate # 27 B. meg.-B. cereus intermediate	+	rod	1.2 by 3.0	+	+	a	a	a	a	+	a	-	+	pep	-		-		+	t.	shin., cr.
Isolate # 28 B. meg.-B.cereus intermediate	+	rod	1.0 by 3.0	+	+	a	a	a	a	+	a	-	+	pep	-		-		+	t.	gray-w., ab.
Isolate # 29 B. meg. -B.cereus intermediate	+	rod	1.0 by 3.5	+	-	a	-	a	a	+	a	-	+	pep	-		-		+	pell.	dull, w.
Isolate # 32 B. meg. - B.cereus intermediate	+	rod	1.0 by 3.2	+	-	a	-	a	a	+	a	-	-	pep	-		+		+	pell. & t.	shin., w.
Isolate # 36 Bacillus cereus var. mycoides	+	rod	1.1 by 3.2	+	-	a	-	a	a	+	a	-	+	pep	+		+		+	t.	shin., w.

Table VII

Family Bacillaceae - continued

Isolations with their Identifications	Gm's react	Morph	Size	Endo	Mot	Dex	Lac	Mal	Suc	St	Gly	Man	Cit	L.M	V.P	M.R	Nit	Ind	PhAc	Gel	Nut.Br.	Pot.
Isolate # 37 B. meg. -B.cereus intermediate	+	rod	1.2 by 3.5	+	-	a	-	a	a	+	a	-	+	pep	+		-			+	t.	rough yel.-w.
Isolate # 38 B. meg.-B.cereus intermediate	+	rod	1.0 by 3.5	+	-	a	a	a	a	+	a	-	+	pep	+		-			+	t.	w.
Isolate # 43 Bacillus megatherium	+	rod	2.0 by 5.0	+	+	a	-	a	a	+	a	a	+	pep	-		-			+	t.	w.
Isolate # 45 Bacillus cereus	+	rod	1.5 by 4.0	+	-	a	-	a	-	+	a	-	-	pep	+		+			+	t.	shin., w., ab.
Isolate # 50 Bacillus pumilis	+	rod	0.6 by 2.0	+	+	a	-	a	a	-	a	a	+	pep	+		-			+	pell. & t.	shin., wr., yel.
Isolate # 52 Bacillus megatherium	+	rod	1.5 by 4.0	+	+	a	-	a	a	+	a	a	+	pep	-		-			+	t.	w.
Isolate # 53 Bacillus cereus	+	rod	1.0 by 4.0	+	-	a	-	a	-	+	a	-	+	pep	+		+			+	t.	shin., cr.-w., ab.
Isolate # 56 B. meg.-B.cereus intermediate	+	rod	1.2 by 4.0	+	-	a	-	a	a	+	a	-	+	pep	+		-			+	t.	w., ab.

Table IX

Family Bacillaceae - continued

Isolations with their Identifications	Gm's react	Morph	Size	Endo	Mot	Dex	Lac	Mal	Suc	St	Gly	Man	Cit	L.M	V.P	M.R	Nit	Ind	PbAc	Gel	Nut.Br.	Pot.
Isolate # 75 Bacillus pumilis	+	rod	0.6 by 2.0	+	+	a	a	a	a	-	a	a	+	pep	+	-		+		pell., & t.	wr., & yell.
Isolate # 76 Bacillus pumilis	+	rod	0.7 by 1.9	+	+	a	-	a	a	-	a	a	+	pep	+	-		+		pell., & t.	wr., & yell.
Isolate # 78 Bacillus subtilis	+	rod	0.8 by 2.0	+	+	a	a	a	a	+	a	a	+	pep	+	+		+		pell., & t.	wr., & red
Isolate # 79 B.meg - B.cereus intermediate	+	rod	1.2 by 3.5	+	-	a	-	a	a	+	a	a	+	pep	+	+		+		t.	shin., w ab.
Isolate # 80 Bacillus sphaericus	+	rod	0.7 by 1.5	+	+	-	-	-	-	-	-	-	+	n.c.	-	-		-		t.	dirty tan
Isolate # 81 B. meg.-B.cereus intermediate	+	rod	1.2 by 3.2	+	-	a	-	a	a	+	a	a	+	pep	+	+		+		t.	shin., w., ab.
Isolate # 85 B.meg.-B.cereus intermediate	+	rod	1.0 by 3.2	+	-	a	a	a	a	+	a	a	-	pep	+	-		+		t.	w., ab.
Isolate # 86 B.meg.-B.cereus intermediate	+	rod	1.0 by 3.5	+	-	a	a	a	a	+	a	a	-	pep	+	-		+		t.	w., ab.

Table X

Family Bacillaceae - continued

Isolations with their Identifications	Gm's react	Morph	Size	Endo	Mot	Dex	Lac	Mal	Suc	St	Gly	Man	Cit	L.M	V.P	M.R	Nit	Ind	PbAc	Gel	Nut.	Br.	Pot.
Isolate # 88 Bacillus cereus var. mycoides	+	rod	1.5 by 3.5	+	-	a	a	a	a	+	a	a	+	pep	+		+		+	pell., & t.	shin., w., ab.
Isolate # 91 Bacillus pumilis	+	rod	0.6 by 1.8	+	+	a	-	-	a	-	a	a	+	pep	+		-		+	pell., & t.	wr., yel.
Isolate # 92 Bacillus cereus	+	rod	1.0 by 3.5	+	-	a	-	a	a	+	a	a	+	pep	+		-		+	t.	gray-brn.
Isolate # 93 B.meg.-B.cereus intermediate	+	rod	1.1 by 3.0	+	-	a	a	a	a	+	a	a	-	pep	+		-		+	t.	shin., cr.
Isolate # 94 Bacillus circulans	+	rod	0.8 by 1.5	+	+	a	a	a	a	+	a	a	-	a	-		-		+	t.	yel.-w.
Isolate # 95 Bacillus pumilis	+	rod	0.6 by 2.0	+	+	a	a	a	a	-	a	a	+	pep	+		-		+	pell., & t.	shin., yel.
Isolate # 96 Bacillus pumilis	+	rod	0.6 by 1.8	+	+	a	a	a	a	-	a	a	+	pep	+		-		+	pell., & t.	shin., yel.

Table XI

Family Bacillaceae - continued

Isolations with their Identifications	Gm's react	Morph	Size	Endo	Mot	Dex	Lac	Mal	Suc	St	Gly	Man	Cit	L.M	V.P	M.R	Nit	Ind	PhAc	Gel	Nut.	Br.	Pot.
Isolate # 97 Bacillus pumilis	+	rod	0.6 by 2.0	+	+	a	-	a	a	-	a	a	+	pep	+		-		+	pell., & t.		wr., yel.
Isolate # 98 Bacillus subtilis	+	rod	0.8 by 2.0	+	+	a	a	a	a	+	a	a	+	pep	+		+		+	pell., & t.		wr., red

Table XII

FAMILY ENTEROBACTERIACEAE

Isolations with their Identifications	Gm's react	Morph	Size	Endo	Mot	Dex	Lac	Mal	Suc	Gly	Man	Cit	L.M.	V.P	M.R	Nit	Ind	PbAc	Gel	Pot	B.G.B.	E.M.B.
Isolate # 1 Escherichia freundii	-	rod	0.5 by 1.0	-	-	a & g	a & g	a & g	a & g	a & g	a & g	+	a	-	+	+	-	+	-	ab. yel. w.	g	g
Isolate # 4 Escherichia freundii	-	rod	0.5 by 1.0	-	-	a & g	a & g	a & g	a & g	a & g	a & g	+	a	-	+	+	-	+	-	ab. yel. w.	g	g
Isolate # 7 Escherichia intermedium	-	rod	0.5 by 1.0	-	-	a & g	a & g	a & g	a & g	a & g	a & g	+	a	-	+	+	+	-	-	ab. w.	g	g
Isolate # 11 Escherichia intermedium	-	rod	0.5 by 1.0	-	-	a & g	a & g	a & g	a & g	a & g	a & g	+	a	-	+	+	+	-	-	ab. w.	g	g
Isolate # 18 Aerobacter aerogenes	-	rod	0.5 by 1.5	-	-	a & g	a & g	a & g	a & g	a & g	a & g	+	a & coag.	+	-	+	-	-	-	cr. w.	g	g
Isolate # 19 Escherichia intermedium	-	rod	0.5 by 1.2	-	-	a & g	a & g	a & g	a & g	a & g	a & g	+	a	-	+	+	+	-	-	ab. w.	g	g
Isolate # 30 Escherichia freundii	-	rod	0.5 by 1.2	-	-	a & g	a & g	a & g	a & g	a & g	a & g	+	a	-	+	+	-	+	-	ab. yel.- w.	g	g
Isolate # 34 Aerobacter aerogenes	-	rod	0.6 by 1.2	-	-	a & g	a & g	a & g	a & g	a & g	a & g	+	a & coag.	+	-	+	-	-	-	cr.	g	g

Table XIII

Family Enterobacteriaceae - continued

Isolations with their Identifications	Gm's react	Morph	Size	Endo	Mot	Dex	Lac	Mal	Suc	Gly	Man	Cit	L.M.	V.P	M.R	Nit	Ind	PbAc	Gel	Pot	B.G.B.	E.M.B.
Isolate # 35 Paracolobactrum intermedium	-	rod	0.5 by 1.0	-	-	a & g	a&g 96 hrs	a & g	a & g	a & g	a & g	+	a	-	+	+	-		-	yel.- w.	g	g
Isolate # 46 Escherichia intermedium	-	rod	0.5 by 1.4	-	-	a & g	a & g	a & g	a & g	a & g	a & g	+	a	-	+	+	+	-	-	ab., w.	g	g
Isolate # 54 Escherichia freundii	-	rod	0.5 by 1.0	-	-	a & g	a & g	a & g	a & g	a & g	a & g	+	a	-	+	+	-	+	-	ab., yel.- w	g	g
Isolate # 55 Escherichia freundii	-	rod	0.5 by 1.2	-	-	a & g	a & g	a & g	a & g	a & g	a & g	+	a	-	+	+	-	+	-	ab., yel.- w	g	g
Isolate # 58 Escherichia intermedium	-	rod	0.6 by 1.0	-	-	a & g	a & g	a & g	a & g	a & g	a & g	+	a	-	+	+	+	-	-	ab., w	g	g
Isolate # 65 Aerobacter aerogenes	-	rod	0.7 by 1.5	-	-	a & g	a & g	a & g	a & g	a & g	a & g	+	a & coag.	+	-	+	-	-	-	cr.	g	g
Isolate # 67 Aerobacter aerogenes	-	rod	0.6 by 1.2	-	-	a & g	a & g	a & g	a & g	a & g	a & g	+	a	+	-	+	-	-	-	shin., w.	g	g
Isolate # 87 Aerobacter aerogenes	-	rod	0.6 by 1.2	-	-	a & g	a & g	a & g	a & g	a & g	a & g	+	a	+	-	+	-	-	-	shin., w.	g	g

Table XIV

FAMILY MICROCOCCACEAE

Isolations with their Identifications	Gm's react	Morph	Size	Endo	Mot	Dex	Lac	Mal	Suc	St	Gly	Man	L.M.	Nit	Ind	Gel	Nut.Br.	Pot.	Urea	$NH_4H_2PO_4$
Isolate # 5 *Micrococcus candidus*	+	coccus	0.5	-	-	a	a	a	a	-	a	a	a	-	-	-	t.	w.	-	-
Isolate # 25 *Micrococcus epidermidis*	+	coccus	0.5	-	-	a	a	a	a	-	a	-	a	+	-	-	t.	w.	-	-
Isolate # 70 *Micrococcus ureae*	+	coccus	0.8	-	-	a	a	-	a	-	-	a	red.	-	-	+	t. & v.s.	gray	-	-
Isolate # 72 *Micrococcus rhodochrous*	+	coccus	0.7	-	-	a	-	-	-	-	-	-	alk.	+	-	-	pell., & t.	red	-	+
Isolate # 90 *Micrococcus ureae*	+	coccus	0.7	-	-	a	a	-	a	-	-	a	red.	-	-	+	t & v.s.	gray	+	-
Isolate # 84 *Sarcina flava*	+	cocci	1.0	-	-	-	-	-	-	-	-	-	alk.	-	-	+	t.	yel.	-	-

Table XV

FAMILY PSEUDOMONADACEAE

Isolations with their Identifications	Gm's react	Morph	Size	Endo	Mot	Dex	Lac	Mal	Suc	St	Gly	Man	Cit	L.M	V.P	M.R	Nit	Ind	PbAc	Gel	Nut.Br.	Pot.
Isolate # 57 *Pseudomonas aeruginosa*	-	rod	0.5 by 1.5	-	+	-	-	-	-		-	-		red			+	-		-	pell. & t.	blue green
Isolate # 63 *Pseudomonas aeruginosa*	-	rod	0.5 by 1.5	-	+	-	-	-	-		-	-		red			+	-		+	pell. & t.	blue green
Isolate # 16 *Spirillum virginianum*	-	spir-illum	0.7 by 6.0	+	-	-	-	-	-	-	-	-	-	n.c.	-	-	-	-	-	-	-	-

Uschinsky's Medium abundant growth

SUMMER FLORA

TABLE I - Results of Morphological, Gram's Stain, and Biochemical Tests performed on identified Isolates found in Westhampton Lake

FAMILY ACHROMOBACTERICEAE

Isolations with their Identifications	Gm's react	Morph	Size	Endo	Mot	Dex	Lac	Mal	Suc	Gly	Man	L.M.	Nit	Ind	Gel	Nut.	Br. Pot
Isolate #3 *Flavobacterium marinum*	-	rod	0.8 by 1.2	-	-	a						alk	-	-	/	t.	yel., shin
Isolate #6 *Achromobacter eurydice*	-	rod	0.7 by 1.2	-	-	a	-	-	-	-	-	n.c.	-		-	t., v.s.	gray, butyrous
Isolate #7 *Achromobacter stationis*	-	rod	0.5 by 1.0	-	-	a	-	-	-	-	-	alk	/		/		no growth
Isolate #10 *Flavobacterium harrisonii*	-	rod	0.7 by 2.0	-	/	-	-	-	-	-	-	alk	-		/	t., v.s.	yel. brn. growth
Isolate #13 *Flavobacterium sewanense*	-	rod	0.8 by 3.0	-	-	-	-	-	-	-	-	n.c.			/	t	yel., shin
Isolate #25 *Alcaligenes metalcaligenes*	-	rod	0.6 by 1.3	-	-	-	-	-	-	-	-	alk			-	pell.	tan, & sc.
Isolate #26 *Alcaligenes faecalis*	-	rod	0.5 by 1.7	-	/	-	-	-	-	-	-	alk			-	t., v.s.	yel.- brn. growth

TABLE II

Family Achromobacteriaceae - continued

Isolations with their Identifications	Gm's react	Morph	Size	Endo	Mot	Dex	Lac	Mal	Suc	Gly	Man	L.M.	Nit	Ind	Gel	Nut.	Br. Pot
Isolate #28 Alcaligenes recti	-	rod	0.5 by 1.5	-	+	-	-	-	-	-	-	alk	+		+	t.	brn.-red growth
Isolate #29 Alcaligenes metalcaligens	-	rod	0.6 by 1.3	-	-	-	-	-	-	-	-	alk			-	t.	tan, & sc.
Isolate #31 Achromobacter liquefaciens	-	rod	0.6 by 2.0	-	+	-	-	-	-	-	-	n.c.	-	-	+	t.	yel streak
Isolate #32 Alcaligenes recti	-	rod	0.5 by 2.5	-	+	-	-	-	-	-	-	alk	+		+	t.	brn.-red growth
Isolate #41 Achromobacter eurydice	-	rod	0.7 by 1.2	-	-	a	-	-	-	-	-	n.c.	-		-	t., v.s.	gray buryrous
Isolate #43 Alcaligenes bookeri	-	rod	0.5 by 1.8	-	+	-	-	-	-	-	-	alk & pep	-	-	+/	t., v.s.	yel.-brn growth
Isolate #47 Alcaligenes marshallii	-	rod	0.4 by 1.2	-	-	-	-	-	-	-	-	alk & pep	-	-	+	t., v.s.	yel.

TABLE III

Family Achromobacteriaceae - continued

Isolations with their Identifications	Gm's react	Morph	Size	Endo	Mot	Dex	Lac	Mal	Suc	Gly	Man	L.M.	Nit	Ind	Gel	Nut.	Br. Pot
Isolate #48 Flavobacterium aquatile	-	rod	0.7 by 2.5	-	-							n.c.	-	-	≠	t.	sc., yel.
Isolate #49 Alcaligenes bookeri	-	rod	0.5 by 2.0	-	≠	-	-	-	-	-	-	alk & pep	-	-	≠	t., v.s.	yel.- brn. growth
Isolate #51 Flavobacterium marinum	-	rod	0.8 by 1.3	-	-	a						alk	-	-	≠	t.	yel.
Isolate #56 Flavobacterium devorans	-	rod	0.8 by 1.2	-	≠							n.c.	-	-	≠	t.	dirty yel.
Isolate #57 Alcaligenes faecalis	-	rod	0.5 by 1.7	-	≠	-	-	-	-	-	-	alk			-	t., v.s.	yel. brn. growth
Isolate #59 Alcaligenes recti	-	rod	0.5 by 2.5	-	≠	-	-	-	-	-	-	alk	≠		≠	t.	brn. red growth
Isolate #64 Alcaligenes faecalis	-	rod	0.5 by 1.7	-	≠	-	-	-	-	-	-	alk			-	t., v.s.	yel. brn. growth
Isolate #66 Alcaligenes recti	-	rod	0.5 by 2.5	-	≠	-	-	-	-	-	-	alk	≠		≠	t.	brn. red growth

Table IV

Family Achromobacteriaceae - continued

Isolations with their Identifications	Gm's react	Morph	Size	Endo	Mot	Dex	Lac	Mal	Suc	Gly	Man	L.M.	Nit	Ind	Gel	Nut.	Br. Pot
Isolate #73 Flavobacterium devorons	-	rod	0.8 by 1.2	-	≠							n.c.	-	-	≠	t.	dirty yel.
Isolate #75 Achromobacter delicatulum	-	rod	1.0 by 2.0	-	≠	a	a	a	a			a	≠	-	-	t.	cr.-yel.
Isolate #76 Alcaligenes metalcaligenes	-	rod	0.6 by 1.3	-	-	-	-	-	-	-	-	alk			-	t.	tan, sc.
Isolate #77 Achromobacter liquefaciens	-	rod	0.6 by 2.0	-	≠	-	-	-	-	-	-	n.c.	-	-	≠	t.	yel. streak
Isolate #80 Flavobacterium marinum	-	rod	0.8 by 1.2	-	-	a						alk	-	-	≠	t.	yel
Isolate #82 Achromobacter liquefaciens	-	rod	0.6 by 2.0	-	≠	-	-	-	-	-	-	n.c.	-	-	≠	t.	yel. streak
Isolate #90 Flavobacterium aquatile	-	rod	0.5 by 2.3	-	-							n.c.	-	-	-	t.	sc., yel.

TABLE V - Family Bacillaceae

Isolations with their Identifications	Gm's react	morph	Size	Endo	Mot	Dex	Lac	Mal	Suc	St	Gly	Man	Cit	L.M	V.P	Nit	Gel	Nut.	Br Pot
Isolate #18 *Bacillus cereus* var. Mycoides	+	rod	1.5 by 3.5	+	-	a	a	a	a	+	a	a	+	pep	+	+	+	pell. & t.	shim., w., ab.
Isolate #19 *Bacillus megatherium*	+	rod	2.0 by 5.0	+	+	a	-	a	a	+	a	a	+	pep	-	-	+	t.	w.
Isolate #21 *Bacillus cereus* var. mycoides	+	rod	1.5 by 3.5	+	-	a	a	a	a	+	a	a	+	pep	+	+	+	pell. & t.	shim., w. ab.
Isolate #22 *Bacillus Sphaericus*	+	rod	0.7 by 1.5	+	+	-	-	-	-	-	-	-	+	n.c.	-	-	-	t.	dirty tan
Isolate #23 *Bacillus megatherium*	+	rod	2.0 by 5.0	+	+	a	-	a	a	+	a	a	+	pep	-	-	+	t.	w.
Isolate #33 *Bacillus pumilus*	+	rod	0.6 by 2.0	+	+	a	-	a	a	-	a	a	+	pep	+	-	+	pell. & t.	shim., ws., yel.
Isolate #36 *Bacillus circulans*	+	rod	0.6 by 2.0	+	+	a	a	a	a	+	a	a	-	a	-	+	+	t.	yel.-w.
Isolate #40 *Bacillus pumilus*	+	rod	0.6 by 2.0	+	+	a	-	a	a	-	a	a	+	pep	+	-	+	pell. & t.	shim., ws., yel.

TABLE VI

Family Bacillaceae - continued

Isolations with their Identifications	Gm's react	Morph	Size	Endo	Mot	Dex	Lac	Mal	Suc	St	Gly	Man	Cit	L.M	V.P	Nit	Gel	Nut.Br	Pot
Isolate #52 Bacillus cereus var.mycoides	+	rod	1.5 by 3.5	+	-	a	a	a	a	+	a	a	pop		+	+	+	pell., & t.	shin., w., ab.
Isolate #54 Bacillus subtilis	+	rod	0.8 by 2.0	+	+	a	a	a	a	+	a	a	pop		+	+	+	pell., & t.	wr. & red
Isolate #60 Bacillus pumilus	+	rod	0.6 by 2.0	+	+	a	-	a	a	-	a	a	pep		+	-	+	pell., & t.	shin., ws. yel.
Isolate #65 Bacillus megatherium	+	rod	2.0 by 5.0	+	+	a	-	a	a	+	a	a	pop		-	-	+	t.	shin., w.
Isolate #69 Bacillus pumilus	+	rod	0.6 by 2.0	+	+	a	-	a	a	-	a	a	pep		+	-	+	pell., & t.	ws., shin., yel.
Isolate #72 Bacillus cereus var. mycoides	+	rod	2.0 by 3.5	+	-	a	a	a	a	+	a	a	pep		+	+	+	pell., & t.	shin., w., ab.
Isolate #81 Bacillus subtilis	+	rod	0.8 by 2.0	+	+	a	a	a	a	+	a	a	pep		+	+	+	pell., & t.	ws. & red.
Isolate #86 Bacillus cereus	+	rod	1.5 by 3.5	+	-	a	-	a	a	+	a	a	pep		+	+	+	t.	shin., cr.-w.

TABLE VII

Family Bacillaceae - continued

Isolations with their Identifications	Gm's React	Morph	Size	Endo	Mot	Dex	Lac	Mal	Suc	St	Gly	Man	Cit	L.M	V.P	Nit	Gel	Nut	Bi	Pot
Isolate #87 Bacillus pumilus	+	rod	0.6 by 2.0	+	+	a	a	a	a	-	a	a	+	pep	+	-	+	pell., p&l., t.	ws., & yel.
Isolate #91 Bacillus megatherium	+	rod	2.0 by 4.0	+	+	a	-	a	a	+	a	a	+	pep	-	-	+	t.	w.
Isolate #92 Bacillus megatherium	+	rod	3.0 by 5.0	+	+	a	-	a	a	+	a	a	+	pep	-	-	+	t.	w.
Isolate #93 Bacillus cereus var.mycoides	+	rod	1.2 by 3.5	+	-	a	a	a	a	+	a	a	+	pep	+	+	+	pell., p&l t.	shin., w., ab.
Isolate #95 Bacillus cereus var.mycoides	+	rod	1.3 by 3.5	+	-	a	a	a	a	+	a	a	+	pep	+	+	+	pell., & t.	shin., w., ab.
Isolate #97 Bacillus megatherium	+	rod	3.0 by 5.0	+	+	a	-	a	a	+	a	a	+	pep	-	-	+	t.	w.
Isolate #99 Bacillus pumilus	+	rod	0.6 by 2.0	+	+	a	a	a	a	-	a	a	+	pep	+	-	+	pell., & t.	ws., & yel.

TABLE VIII-Family Enterobacteriaceae

Isolations with their Identifications	Gm's React	Morph	Size	Endo	Mot	Dex	Lac	Mal	Suc	Gly	Man	Cit	L.M	V.P	M.R	Nit	Ind	PbAc	Gel	Pot	BGB	EMB
Isolate #1 Aerobacter aerogenes	-	rod	0.5 by 1.5	-	-	a & g	a & g	a & g	a & g	a & g	a & g	≠	a & Coag	≠	-	≠	-	-	-	cr.- w	g	g
Isolate #2 Aerobacter cloacae	-	rod	0.5 by 1.5	-	-	a & g	a & g	a & g	a & g	a	a & g	≠	a	≠	-	≠	-		-	shin., w	g	g
Isolate #5 Aerobacter aerogenes	-	rod	0.5 by 1.5	-	-	a & g	a & g	a & g	a & g	a & g	a & g	≠	a & coag	≠	-	≠	-	-	-	cr.- w	g	g
Isolate #15 Serratia plymuthicum	-	rod	0.5 by 1.5	-	≠	a & g	a & g	a & g	a & g	a & g	a & g		a & coag	≠		≠	-		≠	pink		
Isolate #16 Serratia marcescens	-	rod	0.5 by 1.0	-	≠	a & g	a & g	a & g	a & g	a & g	a & g		a	≠		≠	-		≠	red		
Isolate #24 Escherichia freundii	-	rod	0.5 by 1.0	-	-	a & g	a & g	a & g	a & g	a & g	a & g	≠	a	-	≠	≠	-	≠	-	ab., yel.- w	g	g
Isolate #30 Serratia marcescens	-	rod	0.5 by 1.0	-	≠	a & g	a & g	a & g	a & g	a & g	a & g		a	≠		≠	-		≠	red		

TABLE IX

Family Enterobacteriaceae - continued

Isolations with their Identifications	Gm's React	Morph	Size	Endo	Mot	Dex	Lac	Mal	Suc	Gly	Man	Cit	L.M	V.P	M.R	Nit	Ind	PbAc	Gel	Pot	BGB	EMB
Isolate #34 Paracolobacterium bacterum Aerogenodies		rod	0.5 by 1.0	-	-	a & g	a & g	a & g	a & g	a & g	a & g	≠	a & coag 96 hrs.	≠	-	≠	-	-	-	cr.-w.	g	g
Isolate #35 Paracolo-bacterum aerogenoides	-	rod	0.5 by 1.0	-	-	a & g	a & g	a & g	a & g	a & g	a & g	≠	a & coag 96 hrs.	≠	-	≠	-	-	-	cr.-w.	g	g
Isolate #37 Serratia marcescens	-	rod	0.5 by 1.0	-	≠	a & g	a & g	a & g	a & g	a & g	a & g		a	≠		≠	-		≠	red		
Isolate #38 Escherichia intermedium	-	rod	0.5 by 1.0	-	-	a & g	a & g	a & g	a & g	a & g	a & g	≠	a	-	≠	≠	≠	-	-	ab. w.	g	g
Isolate #42 Paracolo-bactrum aerogenoides	-	rod	0.5 by 1.0	-	-	a & g	a & g	a & g	a & g	a & g	a & g	≠	a & coag 96 hrs.	≠	-	≠	-	-	-	cr.-w.	g	g
Isolate #45 Aerobacter aerogenes	-	rod	0.5 by 1.2	-	-	a & g	a & g	a & g	a & g	a & g	a & g	≠	a & coag	≠	-	≠	-	-	-	ab. w.	g	g
Isolate #50 Escherichia intermedium	-	rod	0.5 by 1.0	-	-	a & g	a & g	a & g	a & g	a & g	a & g	≠	a	-	≠	≠	≠	-	-	ab. w.	g	g

TABLE X

Family Enterobacteriaceae - continued

Isolations with their Identifications	Gm's React	Morph	Size	Endo	Mot	Dex	Lac	Mal	Suc	Gly	Man	Cit	L.	M.	V.	P	M.	R	Nit	Ind	PbAc	Gel	Pot	BGB	EMB
Isolate #53 Escherichia freundii	-	rod	0.5 by 1.0	-	-	a & g	a & g	a & g	a & g	a & g	a & g	≠	a	-	≠		≠		-	≠		-	ab., yel.-w.	g	g
Isolate #55 Escherichia coli	-	rod	0.5 by 1.5	-	-	a & g	a & g	a & g	a & g	a & g	a & g	-	a	-	≠		≠		≠	-		-	yel.-brn.	g	g
Isolate #62 Escherichia freundii	-	rod	0.5 by 1.0	-	-	a & g	a & g	a & g	a & g	a & g	a & g	≠	a	-	≠		≠		-	≠		-	ab., yel.-w.	g	g
Isolate #63 Paracolobactrum intermedium	-	rod	0.5 by 1.0	-	-	a & g	a & g 96 hrs.	a & g	a & g	a & g	a & g	≠	a	-	≠		≠		-			-	yel., w.	g	g
Isolate #67 Aerobacter aerogenes	-	rod	0.6 by 1.3	-	-	a & g	a & g	a & g	a & g	a & g	a & g	≠	a & coag	≠	-		≠		-	-		-	cr.	g	g
Isolate #68 Escherichia freundii	-	rod	0.5 by 1.0	-	-	a & g	a & g	a & g	a & g	a & g	a & g	≠	a	-	≠		≠		-	≠		-	ab., yel.-w.	g	g

TABLE XI

Family Enterobacteriaceae - continued

Isolations with their Identifications	Gm's React	Morph	Size	Endo	Mot	Dex	Lac	Mal	Suc	Gly	Man	Cit	L.M	V.P	M.R	Nit	Ind	PbAc	Gel	Pot	BGB	EMB
Isolate #70 Escherichia intermedium	-	rod	0.5 by 1.0	-	-	a & g	a & g	a & g	a & g	a & g	a & g	≠	a	-	≠	≠	≠	-	-	ab., w.	g	g
Isolate #71 Aerobacter aerogenes	-	rod	0.5 by 1.0	-	-	a & g	a & g	a & g	a & g	a & g	a & g	≠	a & coag	≠	-	≠	-	-	-	cr.	g	g
Isolate #74 Escherichia intermedium	-	rod	0.5 by 1.0	-	-	a & g	a & g	a & g	a & g	a & g	a & g	≠	a	-	≠	≠	≠	-	-	w.	g	g
Isolate #78 Escherichia intermedium	-	rod	0.5 by 1.0	-	-	a & g	a & g	a & g	a & g	a & g	a & g	≠	a	-	≠	≠	≠	-	-	ab., w.	g	g
Isolate #79 Escherichia intermedium	-	rod	0.5 by 1.0	-	-	a & g	a & g	a & g	a & g	a & g	a & g	≠	a	-	≠	≠	≠	-	-	ab., w.	g	g
Isolate #83 Escherichia coli	-	rod	0.5 by 1.0	-	-	a & g	a & g	a & g	a & g	a & g	a & g	-	a	-	≠	≠	≠	-	-	yel., brn.	g	g
Isolate #84 Aerobacter aerogenes	-	rod	0.5 by 1.0	-	-	a & g	a & g	a & g	a & g	a & g	a & g	≠	a & coag	≠	-	≠	-	-	-	cr.	g	g

TABLE XII

Family Enterobacteriaceae - continued

Isolations with their Identifications	Gm's React	Morph	Size	Endo	Mot	Dex	Lac	Mal	Suc	Gly	Man	Cit	L.M	V.P	M.R	Nit	Ind	PbAc	Gel	Pot	BGB	EMB
Isolate #85 Paracolobactrum intermedium	-	rod	0.5 by 1.0	-	-	a & g	a & g	a & g	a & g	a & g	a & g	≠	a	-	≠	≠	-		≠	yel.-g w.	g	
						96 hrs.																
Isolate #88 Paracolobactrum intermedium	-	rod	0.5 by 1.0	-	-	a & g	a & g	a & g	a & g	a & g	a & g	≠	a	-	≠	≠	-		≠	yel.-g w.	g	
						96 hrs.																
Isolate #89 Escherichia freundii	-	rod	0.5 by 1.0	-	-	a & g	a & g	a & g	a & g	a & g	a & g	≠	a	-	≠	≠	-	≠	-	ab., yel.-g w.	g	
Isolate #96 Escherichia freundii	-	rod	0.5 by 1.0	-	-	a & g	a & g	a & g	a & g	a & g	a & g	≠	a	-	≠	≠	-	≠	-	ab., yel.-g w.	g	
Isolate #98 Escherichia freundii	-	rod	0.5 by 1.0	-	-	a & g	a & g	a & g	a & g	a & g	a & g	≠	a	-	≠	≠	-	≠	-	ab., yel.-g w.	g	

TABLE XIII-Family Micrococcaceae

Isolations with their Identifications	Gm's React	Morph	Size	Endo	Mot	Dex	Lac	Mal	Suc	Gly	Man	L.M	Nit	Gel	Nut.	Bi.Pot	Urea	$NH_4H_2PO_4$
Isolate #14 Micrococcus varians	≠	coccus	0.9	-	-	a	a	-	a	a	a	a	≠	-	t.	shin., yel.	-	≠
Isolate #17 Micrococcus roseus	≠	coccus	1.0	-	-	-	-	-	-	a	a	alk		≠	t.	shin., red	-	≠
Isolate #58 Micrococcus ureae	≠	coccus	0.8	-	-	a	a	-	a	-	a	red	-	≠	t. & v.s.	gray	≠	-
Isolate #20 Sarcina flava	≠	cocci	1.0	-	-	-	-	-	-	-	-	alk	-	≠	t.	yel.	-	-
Isolate #16 Sarcina flava	≠	cocci	1.0	-	-	-	-	-	-	-	-	alk	-	≠	t.	yel.	-	-

TABLE XIV- Family Pseudomonadaceae

Isolations with their Identifications	Gm's React	Morph	Size	Endo	Mot	Dex	Lac	Mal	Suc	Gly	Man	L,M	Nit	Ind	Gel	Nut,Bi,	Pot
Isolate #4 Pseudomonas geniculata	-	rod	0.7 by 2.0	-	+	-	-	-	-	-	-	alk	-	-	+	t.	shin., brn.
Isolate #11 Pseudomonas geniculata	-	rod	0.7 by 2.0	-	+	-	-	-	-	-	-	alk	-	-	+	t.	shin., brn.
Isolate #12 Pseudomonas jaegeri	-	rod	0.5 by 1.2	-	+	-	-	-	-	-	-	not coag	-	-	+	pell., & t.	brn. medium becomes green
Isolate #27 Pseudomonas geniculata	-	rod	0.7 by 2.0	-	+	-	-	-	-	-	-	alk	-	-	+	t	shin., brn.
Isolate #44 Pseudomonas aeruginosa	-	rod	0.5 by 1.5	-	+	-	-	-	-	-	-	red	+	-	+	pell., & t.	blue-green
Isolate #61 Pseudomonas aeruginosa	-	rod	0.5 by 1.5	-	+	-	-	-	-	-	-	red	+	-	+	pell., & t.	blue-green
Isolate #94 Pseudomonas aeruginosa	-	rod	0.5 by 1.5	-	+	-	-	-	-	-	-	red	+	-	+	pell., & t.	blue-green

TABLE XV

Family Pseudomonadaceae - continued

Isolations with their Identifications	Gm's React	Morph	Size	Endo	Mot	Dex	Lac	Mal	Suc	Gly	Man	L.M.Nit	Ind	Gel	Nut.Bi	Pot
Isolate #8 *Spirillum tenue*	-	spir-illum	0.7 by 4.0	-	+	a	-	-	-	a	-	N.c.	-	-	-	light brn.

TABLE XVI- Family Rhizobiaceae

Isolations with their Identifications	Gm's react	Morph	Size	Endo	Mot	Dex	Lac	Mal	Suc	Gly	Man	L.M	Nit	Gel	Nut.Bi.	Pot
Isolate #9 Chromobacterium violaceum	-	rod	0.8 by 2.5	-	+	a	-	-	a	-	-	alk	+	+	t.	violet
Isolate #39 Chromobacterium violaceum	-	rod	0.8 by 2.5	-	+	a	-	-	a	-	-	alki	+	+	t.	violet

APPENDIX B

MEDIA

Ammonium Hypophosphate 1 per cent solution

Ammonium Hypophosphate	10.0 gms.
Distilled Water	2000.0 cc.

Brilliant Green Bile Broth 2 per cent solution

Bacto-Peptone	10.0 gms.
Bacto-Lactose	10.0 gms.
Bacto-Oxgall	20.0 gms.
Bacto-Brilliant Green	0.0133 gms.
Distilled Water	1000.0 cc.

E.M.B. Agar, Levine

Bacto-Peptone	10.0 gms.
Bacto-Lactose	10.0 gms.
Dipotassium Phosphate	2.0 gms.
Bacto-Agar	15.0 gms.
Bacto-Eosin Y	0.4 gms.
Bacto-Methylene Blue	0.065 gms.
Distilled Water	1000.0 cc.

Gelatin, Nutrient Bacto

Bacto-Beef Extract	3.0 gms.
Bacto-Peptone	5.0 gms.
Bacto-Gelatin	120.0 gms.
Distilled Water	1000.0 cc.

Glucose (Bacto-Dextrose) 1 per cent solution

Glucose	10.0 gms.
Phenol Red Broth Base	16.0 gms.
Distilled Water	1000.0 cc.

Glucose-Peptone Water

Proteose Peptone (Difco)	5.0 gms.
Glucose (c.p.)	5.0 gms.
Dipotassium Phosphate	5.0 gms.
Distilled Water	1000.0 cc.

Glycerol 1 per cent solution

Glycerol	10.0 cc.
Phenol Red Broth Base	16.0 gms.
Distilled Water	1000.0 cc.

Glycerol Ammonium Lactate Solution, Uschinsky's

1. Distilled Water	1000.0 cc.
2. Glycerol	45.0 gms.
3. Sodium Chloride	6.0 gms.
4. Calcium Chloride	0.1 gms.
5. Magnesium Sulfate	0.2 gms.
6. Dipotassium Acid Phosphate	1.0 gms.
7. Ammonium Lactate	10.0 gms.

Dissolve 2, 3, 4, 5, 6, and 7 in 1.

Koser Citrate Medium

Sodium Ammonium Phosphate	1.5 gms.
Monopotassium Phosphate	1.0 gms.
Magnesium Sulfate	0.2 gms.
Sodium Citrate	3.0 gms.

Lactose 1 per cent solution

Lactose	10.0 gms.
Phenol Red Broth Base	16.0 gms.
Distilled Water	1000.0 cc.

Lactose Broth, Bacto 1.5 per cent solution

Bacto-Beef Extract	3.0 gms.
Bacto-Peptone	5.0 gms.
Bacto-Lactose	5.0 gms.
Distilled Water	1000.0 cc.

Lead Acetate Agar

Lead Acetate Agar (Difco)	36.0 gms.
Distilled Water	1000.0 cc.

Litmus Milk, Bacto

Bacto-Skim Milk	100.0 gms.
Bacto-Litmus	5.0 gms.
Distilled Water	2000.0 cc.

Maltose 1 per cent solution

Maltose	10.0 gms.
Phenol Red Broth Base	16.0 gms.
Distilled Water	1000.0 cc.

Mannitol 1 per cent solution

Mannitol	10.0 gms.
Phenol Red Broth Base	16.0 gms.
Distilled Water	1000.0 cc.

Nitrate Broth, Bacto

Bacto-Beef Extract	3.0 gms.
Bacto-Peptone	5.0 gms.
Potassium Nitrate	1.0 gms.
Distilled Water	1000.0 cc.

Nutrient Broth

Bacto-Beef Extract	3.0 gms.
Bacto-Peptone	5.0 gms.
Distilled Water	1000.0 cc.

Nutrient Agar, Bacto

Bacto-Beef Extract	3.0 gms.
Bacto-Peptone	5.0 gms.
Bacto-Agar	15.0 gms.
Distilled Water	1000.0 cc.

Sodium Caseinate Agar

Peptone	0.5 gms.
Sodium Caseinate	0.5 gms.
Soluble Starch	0.5 gms.
Glycerol	1.0 cc.
Dipotassium Acid Phosphate	0.2 gms.
Magnesium Sulfate	0.05 gms.
Nutrient Agar	15.0 gms.
Distilled Water	1000.0 cc.

Starch Agar

Bacto-Beef Extract	3.0 gms.
Bacto-Peptone	5.0 gms.
Bacto-Agar	15.0 gms.
Soluble Starch	2.0 gms.
Distilled Water	1000.0 cc.

Sucrose 1 per cent solution

Sucrose	10.0 gms.
Phenol Red Broth Base	16.0 gms.
Distilled Water	1000.0 cc.

Urea Broth, Bacto

Bacto-Yeast Extract	0.1 gms.
Monopotassium Phosphate	9.1 gms.
Disodium Phosphate	9.5 gms.
Urea, Difco	20.0 gms.
Bacto-Phenol Red	0.01 gms.

APPENDIX C

REAGENTS

Alpha-naphthol solution (Voges-Proskauer Test)

a. Alpha naphthol (reagent quality) . . . . . 6.0 gms.

Alcohol (95%) . . . . . 100.0 cc.

b. Potassium Hydroxide . . . . . 16.0 gms.

Distilled Water to make . . . . . 100.0 cc.

Aminonaphthol Sulfonic Acid Solution (Phosphate Test)

1. Sodium Bisulfite Solution

Sodium Bisulfite (C.P.) . . . . . 150.0 gms.

Distilled Water . . . . . 1000.0 cc.

2. Sodium Sulfite Solution

Sodium Sulfite (C.P.) . . . . . 20.0 gms.

Distilled Water . . . . . 100.0 cc.

3. 1 - Amino, 2 - naphthol, 4 - sulfonic acid . . . . 0.5 gms.

Mix 195 cc. of sodium bisulfite solution with 5 cc. of sodium sulfite solution and add 0.5 gms. of ingredient number 3. Dissolve by stirring and gentle warming. Filter. Keep in a dark place.

Gram's Stain (Hucker's Modification)

Solution A

Crystal Violet (90% dye content) . . . . . 2.0 gms.

Ethyl Alcohol (95) . . . . . 20.0 cc.

Solution B

Ammonium Oxalate . . . . . 0.8 gms.

Distilled Water . . . . . 80.0 cc.

Mix Solutions A and B

Iodine . . . . . 1.0 gms.

Potassium Iodide . . . . . 2.0 gms.

Distilled Water . . . . . 300.0 cc.

Ninety-five per cent Alcohol

Safranin O (2.5% Solution in 95% alcohol) . . . . . 10.0 cc.

Distilled Water . . . . . 100.0 cc.

Ilosvay's Solution (Nitrate Test)

1. Sulphanilic Acid . . . . . 0.5 gms.

   Glacial Acetic Acid . . . . . 30.0 cc.

   Distilled Water . . . . . 120.0 cc.

   Dissolve.

2. Naphthylamine . . . . . 0.1 gms.

   Glacial Acetic Acid . . . . . 30.0 cc.

   Distilled Water . . . . . 120.0 cc.

Kovac's Reagent (Indol Test)

n-Amyl Alcohol . . . . . 75.0 cc.

Hydrochloric Acid (concentrated) . . . . . 25.0 cc.

Para-dimethylamino-benzaldehyde . . . . . 5.0 gms.

Molybdate Solution (Phosphate Test)

Ammonium Molybdate (C.P.) . . . . . 25.0 gms.

Distilled Water . . . . . 200.0 cc.

Dissolve.

Sulfuric Acid (10 N) . . . . . 600.0 cc.

Distilled Water to make . . . . . 1000.0 cc.

Phenoldisulfonic Acid

Phenol (pure white) . . . . . 25.0 gms.

Sulfuric Acid (concentrated) . . . . . 150.0 cc.

Dissolve.

Fuming Sulfuric Acid . . . . . 75.0 cc.

Stir and heat for two hours at 100°C.

Starch Indicator Solution

Soluble Starch . . . . . 4.0 gms.

Zinc Chloride . . . . . 20.0 gms.

Distilled Water . . . . . 1000.0 cc.

Dissolve the starch in 100.0 cc. of water, and filter. Mix the starch with a few cubic centimeters of cold water into a thin paste and pour into the boiling solution of zinc chloride with constant stirring. Dilute to one liter. Allow the flocculent material to settle and filter the supernatant fluid.