THE GREEN BACTERIA

A PROBLEM IN HYDROBACTERIOLOGY

By

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A THESIS

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INTRODUCTION

In attempting to write a paper on aquatic bacteriology, the writer finds herself in an almost unexplored field. There are but few sign posts to guide the investigator and those which do exist are subject to debatable interpretations. It should be of value in explaining the "raison-d'etre" for this particular paper to offer some comments of other workers who have felt the need of exploratory and intensive work in the field of hydrobacteriology and for closer cooperation with limnologists and ecologists in general.

Thienemann (1927) comments as follows: "The greatest need of limnology, the satisfaction of which would be of benefit to many associated departments, is bacteriological information. It may be momentarily surprising if I insist that hydrobacteriology as such is virtually non-existent. It is true that there have been investigations regarding bacteria living in water, but for the most part they have had reference primarily to practical hygienic problems. It is well known that bacteria play an extremely important part in the cycle of life-materials; it may even be the most important part, if we are justified in assigning degrees of importance. No matter how detailed may be our methods in water chemistry, even the most intensive delving into purely chemical processes is not going to solve for us the mystery of the metamorphosis of matter, if we neglect bacterial action ..... What has been said here about the importance of bacteriological research to hydrobiology and limnology applies also to fisheries and biology in

general. Whenever we encounter the difficult problem of the cycle of substances on this earth, we also encounter the necessity of considering bacteria."

Welch (1935) says: "Pure limnology, as contrasted with applied limnology, is primarily concerned with the normal bacterial populations of uncontaminated waters, and particularly those which may be regarded as of more or less regular occurrence in the waters of different kinds of lakes. Unfortunately, this is an almost unknown field, since bacteriologists and others employing the methods of bacteriology have been mainly concerned in the past with pathogenic bacteria. Only a very few lakes have received any study of the bacteria native to them, and even in those few instances there are many gaps in the available information .... The student who desires a comprehensive list of specifically identified bacteria (to genera and to species) which compose the water bacteria is doomed to disappointment, since none is available for any unmodified lake in North America or, in so far as the writer knows, in other continents."

Huber-Pestalozzi (1938) states the problem in this way: "Je mehr der Einblock in das Leben eines Sees vertieft werden soll, desto mehr müssen auch die Bakterien in den Bereich der Untersuchung gezogen werden; ja man darf sogar behaupten, dass für das Verständnis der oft stark ineinandergreifenden Lebenszyklen in einem See, besonders im eutrophen See, überhaupt für den gesamten Stoffhaushalt in einem stehenden Gewässer, die Kenntnis der Tätigkeit sowohl der im freien Wasser als der im schlamme lebenden Bakterien unerlässlich ist."

However, the opposite side of the problem is presented when Jordan, in Ward and Whipple's Fresh WaterBiology states "There is no special and characteristic class of water bacteria but forms from the air, from the soil, from decomposing animal and plant substances and from the healthy and diseased tissues of animals and plants may at times find their way into water".

It is a sad commentary on the status of hydrobacteriology in the United States that the statement by Jordan is still perpetrated in one of the most important source books available to the aquatic biologist.

It is foolhardy to suggest that the investigation reported upon here will solve many of the problems suggested by Thienemann, Welch and Huber-Pestalozzi. However, after five years of study on the natural bacteriology of lakes and rivers, the author feels that there is some justification in attempting to present the results of an ecological study of one typically aquatic group of bacteria, the Chlorobacteriaceae. The present study represents, in so far as the author is aware, a first attempt to study a specific group of bacteria as it is organized in its natural environment.

In any aquatic environment wherein there exists a measurable amount of hydrogen sulphide gas, sufficient light for the process of photosynthesis to take place and a low oxygen threshold, there may develop organisms which have been designated as green bacteria. These bacteria, according to van Niel (1932), are capable of reducing carbon dioxide photochemically without the liberation of oxygen using hydrogen sulphide as the hydrogen donor. The green bacteria may be further

characterized as bacterio-viridin-containing organisms which develop in purely mineral media containing hydrogen sulphide and whose biochemical activities are activated by a supply of radiant energy.

Lauterborn (1915) established the family Chlorobacteriaceae for those organisms which should be included in the description given above. However, as nothing was known of the biochemical activities of the green bacteria until 1922, it is obvious that the Chlorobacteriaceae as represented by Lauterborn was a purely artificial group. In the family Chlorobacteriaceae Lauterborn created a new genus Chlorobacterium, which consisted of one species Chlorobacterium symbioticum; the genera Schmidlea, Pelogloea, Pelodictyon and Chlorochromatium were also included. The two latter genera were originated by Lauterborn in 1906. Geitler and Pascher (1925) followed the same main outlines of classification as did Lauterborn but included several other genera and species. In Bergey's Manual of Determinative Bacteriology, sixth edition (1948) is incorporated the following description of the Chlorobacteriaceae.

Order Eubacteriales
Suborder Rhodobacteriineae
Family III. Chlorobacteriaceae Geitler and Pascher.
(Cyanochloridinae-Chlorobacteriaceae Geitler and Pascher, Die Süsswasserflora Deutschlands, Osterreichs und der Schweiz, Jena, 12, 1925, 451; Chlorothiobacteria Bavendamm, Ergeb. Biol., 13, 1936, 49.

Green bacteria, usually of small size, occurring singly or in cell masses of various shapes and sizes, developing in environments containing rather high concentrations of hydrogen sulphide and exposed to light. As a rule not containing sulphur globules but frequently depositing elementary sulphur outside the cells. Contain green pigment of a chlorophyllous nature, though not identical with the common green plant chlorophylls nor with bacteriochlorophyll. Capable of photosynthesis in the presence of hydrogen sulphide; do not liberate oxygen.

Key

I. Free living bacteria not intimately associated with other microbes.

a. bacteria not united into well defined colonies
Genus I Chlorobium

aa. bacteria united into characteristic aggregates

b. bacteria without intracellular sulphur granules
Genus II Pelodictyon

bb. bacteria with intracellular sulphur granules
Genus III Clathrochloris

II. Green bacteria found as symbiotic aggregates with other organisms

a. aggregates composed of green bacteria and protozoa
Genus IV Chlorobacterium

aa. aggregates composed of two different types of bacteria

b. aggregates small, barrel shaped, actively motile, and consisting of a central, polarly flagellated, rod-shaped bacterium with a covering of green sulphur bacteria.
Genus V Chlorochromatium

bb. aggregates large, cylindrical, non motile and composed of a central filamentous bacterium with a more or less extensive covering of green sulphur bacteria.
Genus VI Cylindrogloea

Some comment is indicated as to the system of classification of the green bacteria as used by Skuja (1948). Skuja considers the Chlorobacteriales as one of six orders under the phylum Bacteriophyta. Skuja further incorporates three families, i.e., Pelosphaeraceae, Chlorobacteriaceae and Chlorochromatiaceae under the order Chlorobacteriales. It will be noted that Skuja's classification is somewhat different than that which is incorporated into the sixth edition of Bergey's Manual. In the Manual, the genera Pelosphaera, Tetrachloris and Pelochromatium are not recognized.

Of the six genera of green bacteria included in Bergey's Manual loc. cit., there is factual proof of the existence of but one legitimate genus i.e., Chlorobium. All other genera have been erected solely on the basis of preparations made from natural samples and have not taken into account any variation possibly caused by different environmental conditions. A member of the genus Chlorobium has been isolated and grown in pure culture by van Niel (1931).

The Problem

There exists then for the organisms designated as "green bacteria" but little information upon which to base an application of accepted taxonomical principles. It has been inferred that such data as are available have been obtained from preparations which, while designated as "pure" could not have been so in the true sense of the word. The term "pure culture" must be reserved for one which consists of the progeny of a single cell. The early investigators of the purple and green bacteria did not effect single cell isolations and cultures which appeared pure microscopically were accepted without exacting criteria. The expression "Damit war die Reinkultur erreicht" appears frequently in the early literature. However, it should be emphasized here that the pure cultures referred to in the literature were obtained by transferring from one liquid medium to another of the same consistency. It seems obvious that the use of liquid media would, in turn, necessitate the use of the single cell isolation technique.

It follows then that the various species of green bacteria have been established largely on the basis of size with no provision for variation within the species itself. Indeed, even genera have been erected on the same general basis of size or of cell conglomerates considered as static units. If size was not used as the distinguishing characteristic to separate genera, then an association with another bacterium or some lower organism was deemed sufficiently unique to merit a new described form.

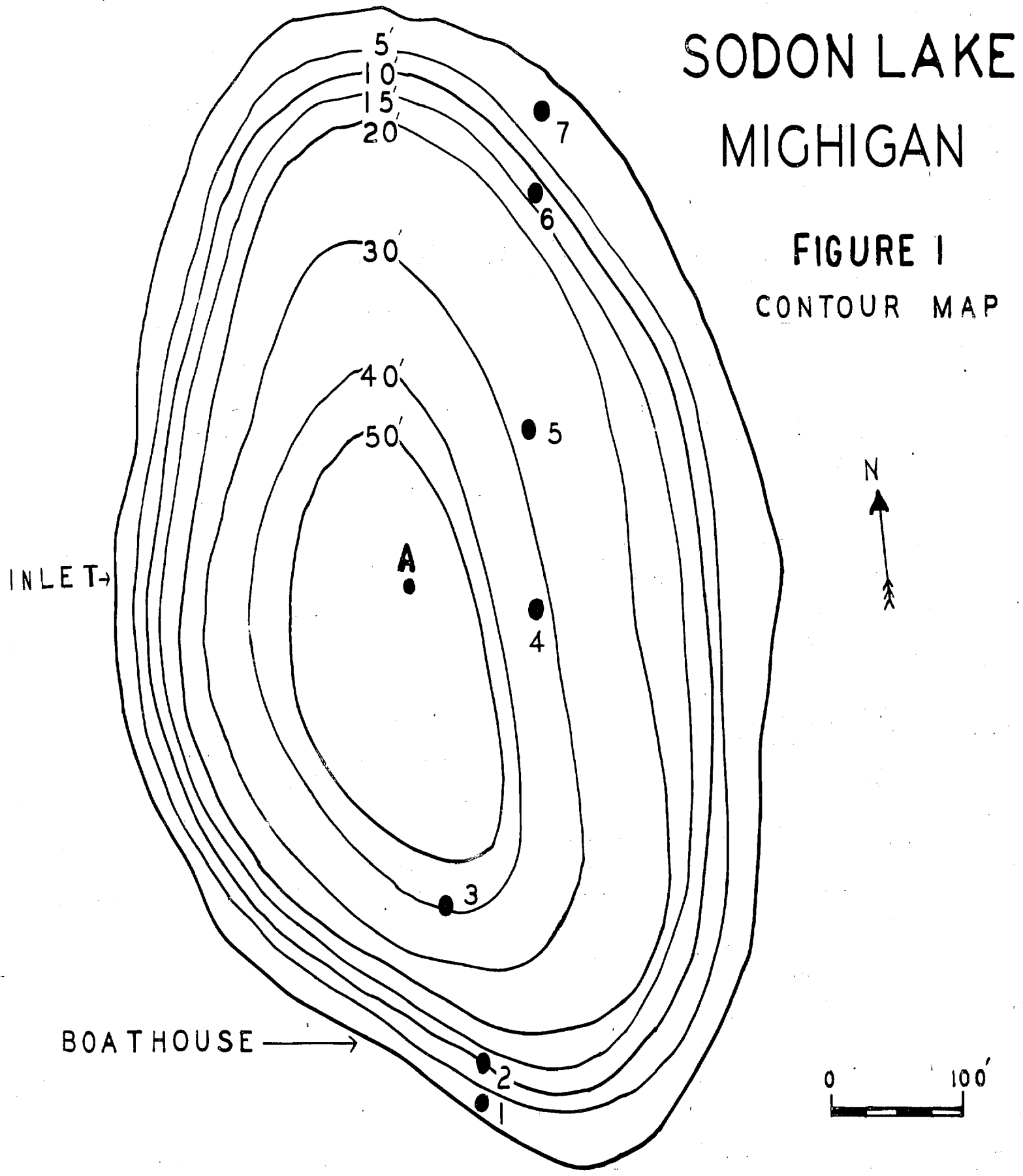
Scope of the Problem

It is the purpose then of this paper to present such information as has been obtained from an ecological investigation of the green bacteria and to supplement such information with data from pure culture studies. It seems not improbable that the information offered may be of value in interpreting the biological relationships in an ecosystem and that some light may be thrown upon the present taxonomical status of the group under investigation; the influence of the environment on morphological variation, while extremely difficult to evaluate, will be none the less evident.

REVIEW OF THE LITERATURE

Historically, the investigations on the green bacteria should parallel those of the purple sulphur bacteria; the literature on this latter group is voluminous and approximately 100 years old. However, while the early investigators of the purple bacteria noted the occurrence of the green forms no extensive studies of their morphology, physiology and ecological relationships were made. While Pringsheim (1949) has indicated that Szafer first described the green bacteria in 1911, actually the literature is much older. van Tieghem (1880) mentioned a Bacillus virens as being green in color. Engelman (1882) stated that Bacterium chlorinum contained a chlorophyll. Dangeard (1890) described forms which we now know must have been green bacteria. Nadson (1912) gave us the first, actual recognizable descriptions of morphological characteristics. Nadson's work was supplemented by the studies of Buder (1913), Perfiliev (1914), Pascher (1914), Lauterborn (1915) and Bavendamm (1924). It is extremely difficult to evaluate the work of these European investigators as it appears that only in rare instances did one biologist ever acknowledge the contributions of his colleagues. For instance, Perfiliev (1914) described much more extensively the forms characterized by Lauterborn early in 1914. Perfiliev loc. cit., also pointed out that the green bacteria described by Lauterborn had been previously depicted by Szafer in 1911. Perfiliev loc. cit., commented further that the Chlorochromatium aggregatum of Lauterborn was identical with the Chloronium mirabile of Buder. But Lauterborn in 1915 published an extensive paper "Die sapropelische

Lebewelt" in which he made no mention of the work of either Nadson or Perfiliev! Geitler and Pascher (1925) in an appendix to Pascher's Süsswasserflora, Heft 12, listed the known green bacteria and proposed a change from the older Chlorobacteriaceae to Cyanochloridinae, on the basis that definite proof was lacking as to the bacterial nature of the green organisms. However Metzner (1922) investigated the pigments of the green bacteria and offered proof that they did not contain either chlorophyll a or b or bacteriochlorophyll; Metzner loc. cit., called the pigment found in the green bacteria, bacterioviridin. Geitler and Pascher loc. cit., either were not aware of Metzner's work or else disregarded it. The most recent work on the green bacteria has been done by van Niel (1931). In addition to a revision of Geitler and Pascher's taxonomy of the green bacteria for the sixth edition of Bergey's Manual, van Niel has made the green bacteria the subject of extensive, theoretical research on the fundamental nature of photosynthesis.


SODON LAKE
MICHIGAN
FIGURE 1
CONTOUR MAP
5'
10'
15'
20'
30'
40'
50'
7
6
5
4
3
2
1
A
INLET→
BOATHOUSE →
N
0
100'

DESCRIPTION OF ENVIRONMENT SELECTED FOR ECOLOGICAL STUDY

The aquatic environment under investigation is located in Oakland County, Michigan (Bloomfield Township, Section 20, latitude 42° 19', longitude 83° 17'). The lake is a small glacial lake located in the Outer Defiance Moraine and has a maximum depth of 56 feet; it is approximately 5.7 acres in area at the surface, 3.2 acres within the 20 foot depth contour and 1.0 acre within the 40 foot isobath. The volume development of the lake is 1.21, indicating that morphologically the lake appears as a cone. The lake is protected from wind action by the surrounding hills and by a red maple-tamarack swamp which borders the shore. The littoral area, to a depth of 12 feet, is covered with the alga, Chara contraria. Beyond the 12 foot contour there is some Nitella and a moss of the genus Fontinalis.

METHODS OF PROCEDURE

1. General. During the period of field observations, January 22, 1949 to January 1, 1950, through the cooperation of the Cranbrook Institute of Science arrangements were made for the regular collection of water samples from Sodon Lake. Sampling was done bi-monthly, ordinarily at 9:30 in the morning. The deepest part of the lake (56 feet) was selected as a permanent station; this was designated as Station A, the location of which may be seen by reference to Figure 1, The Contour Map of Sodon Lake. Sampling was done from the surface of the lake to the mud-water interface, usually at intervals of five feet. However, other depth intervals were sampled for special studies and whenever any thermal disturbance was noted.

Samples for bacteriological analysis were collected in sterile bottles of three liter capacity. The samples were obtained from the desired depths by the use of a Kemmerer water sampler (see appendix for illustration); this same device served to secure material for chemical, physical and biological determinations. Any departure from routine procedures will be indicated under appropriate headings.

A word should be said here concerning the difficulties inherent in the obtaining of a water sample from a specific depth and, at the same time, insure that the sample is representative of that particular depth and not contaminated by an extraneous flora or fauna. The first objection, that the sample secured is not representative of a particular depth, is easily overcome. In the preliminary work concerned with this investigation, the desired samples were obtained in sterile glass

bottles fitted with an inlet tube which was broken at the desired depth by sending down a messenger. However, neither qualitatively nor quantitatively did the plankton differ from that obtained by using the Kemmerer sampler. The contamination from the sampler itself is more apparent than real. It seems to the author that there is considerable justification in using the Kemmerer bottle for securing samples for bacteriological analysis. It is recognized that metal bottles, in some instances, are bactericidal or bacteriostatic; however, this action has been observed only on stored water samples Zobell (1941). It should also be noted that the bactericidal action is more pronounced with brightly polished surfaces. Under the conditions reported upon here, samples were transferred from the sampler to sterile bottles in less than three minutes; and the sampler was, at all times, heavily coated with oxides. We should like to make one further point in connection with the use of the metal sampling device. If the metal is bactericidal, then the flora which remains attached to the bottle, while it is being stored, should be killed and the bottle would therefore be, in a sense, "sterile" when again placed in operation.

2. Field Investigations. The following procedures were carried out in the field at each bi-monthly sampling period:

1. Collection of samples for bacteriological analyses
2. Collection of samples for chemical analyses
3. Collection of samples for analyses of the hydrosol
4. Collection of euplankton (phytoplankton and zooplankton)

5. Vertical distribution of temperature
6. Measurement of light intensities at various depths
7. Suspension of standard 25 x 75 mm. glass slides for attachment studies

3. Laboratory Investigations. The following procedures were carried out in the laboratory at each bi-monthly sampling period:

1. Chemical determinations of dissolved oxygen, hydrogen sulphide, carbonate, bicarbonate, nitrate nitrogen, nitrite nitrogen, iron and phosphorous.
2. Determination of hydrogen-ion concentration
3. Enumeration and identification of plankton
4. Analysis of submerged slides as accessory information to population studies
5. Special bacteriological studies, which will be indicated under appropriate headings.

PRELIMINARY RECONNAISSANCE

In August of 1947 a green organism appeared in tremendous numbers in the phytoplankton of Sodon Lake; the organism was preserved but no study was made of it. Later, it was tentatively identified by the author as Chlorobium limicola Nadson a member of the green sulfur bacteria. From January 1949 to January 1950, in connection with a bacteriological survey on the ecology, distribution and nutrition of other aquatic bacteria, we have studied the seasonal distribution, the vertical and horizontal distribution, the culture, chemical, physical and biological environment and morphology of the green bacterium.

METHODS OF PLANKTON ENUMERATION

In order to determine the seasonal peaks and trends of the green bacteria and other members of the plankton, it was necessary that an enumeration standard be sought. While we are aware that many diverse quantitative plankton methods exist, (see particularly Lackey (1938) and Littleford et al. (1940), there is no general agreement as to what constitutes a counting unit. No attempt will be made here to review or to evaluate the various quantitative methods which have been proposed for treating planktonic forms; it seems obvious that the procedure adopted must be determined by the conditions of the field experiment, particularly the relative abundance and size of the organisms under consideration. Harvey (1934) has said "the only strictly quantitative method of sampling the population is to centrifuge a small sample of water and count the plants in it". We have, under the conditions of the present experiment, treated each organism as a unit and have not assigned colony factors to organisms which exist as aggregates of single cells. By using such a method, we have eliminated the personal factor as far as judgment of the number of cells in a colony is concerned.

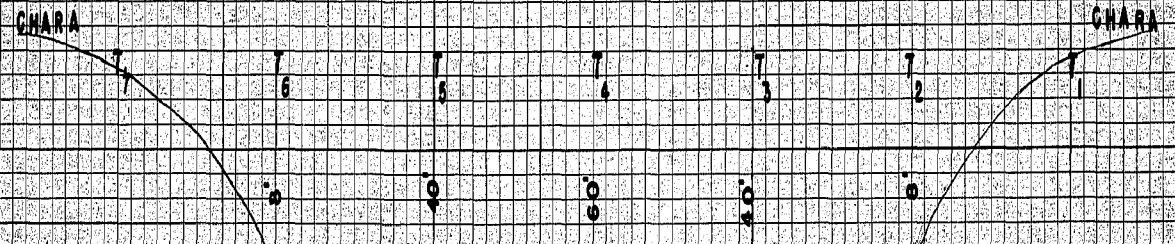
The specific technique which we have used for our enumerations is that of G. W. Martin (ms.) and is described in detail by Van Deusen (ms. 1947). Essentially, the method consists of enumeration by direct count and calculation of the number of organisms per liter of lake water.

A Spencer binocular microscope equipped with a combination of 25x oculars and a 97x objective was used for enumeration of the bacteria; for the phytoplankton, 15x oculars and a 43x objective were used; for enumeration of the zooplankton, a combination of 15x oculars and a 10x objective was used.

The three liter samples for bacteria counts and for the phytoplankton were passed through a Foerst Centrifuge (15,000 G). The concentrate was washed into a small vial and the original sample was recentrifuged. In all, this process was repeated three times. The concentrate was then composited and counted immediately or preserved for later study.

One liter samples for the zooplankton count were strained through a number 25 silk plankton net and a tally of each organism present in the concentrate was made. The total was expressed as the number of organisms per liter of lake water.

FIGURE 2
PROFILE OF SODON LAKE
LOCATION OF SAMPLING STATIONS



THE VERTICAL AND HORIZONTAL DISTRIBUTION

To determine the vertical and horizontal distribution profile of the green bacteria, seven stations were selected for sampling; The locations of these stations are shown in Figures 1 and 2. Samples were secured from the following depths, measured from the surface: four inches, four feet, eight feet, twelve feet, fourteen feet, sixteen feet, twenty feet, twenty-four feet and twenty-eight feet. Below the twenty-eight foot level, photolometer readings indicated zero transmission of light and hence an unfavorable environment for the growth of the green bacteria existed there. The seven stations located along the transect, hereafter designated as "T" are as follows:

T_1 and T_7 .....deep in Chara

T_2 and T_6 .....8 feet in depth

T_3 and T_5 .....30-40 feet in depth

T_4 .....50-56 feet in depth

Microscopic examination of the phytoplankton indicated what appeared to be a dense concentration of green bacteria existing at 25± 5 feet. This observation was further tested by determining the absorption spectra of the various samples in a Coleman Jr. Spectrophotometer. In every case, the zone of concentration was sharply delimited at 25± 2 feet. The horizontal distribution was determined over the entire horizontal transect of the lake and again showed that the zone of concentration appeared to be at 25± 2 feet and to extend shoreward to meet the lake bottom.

SEASONAL DISTRIBUTION

At the start of this investigation sterile 25 x 75 mm. glass slides were placed in special containers and suspended so that there existed a continuous attachment surface from just below the surface of the water to the mud-water interface. It was planned to treat the bacteria deposited upon the slides in a quantitative manner, and thus provide for seasonal data on the bacterial population of Sodon Lake. Such a method had been employed by Henrici (1933) in his pioneer work on the aquatic bacteria of Minnesota lakes. Furthermore, Zobell (1941) and others have emphasized that the bacterial population of the open water of lakes and oceans is largely periphytic; Zobell loc. cit., has postulated that the relatively small bacterial population reported for lakes and rivers is due to the fact that aquatic bacteria are removed from the phytoplankton by attaching themselves to solid surfaces, thus increasing their specific gravity and rate of sedimentation.

The assumption by Henrici, Zobell and others that the organic material deposited on slides represented growing bacterial cells has proved to be utterly fallacious. A chance observation, arising from inadvertently gram staining a sterile slide which had been suspended in sterile water, raised the question as to whether the material being deposited on the submerged slides represented bacteria or merely an accumulation of organic material. It was found that the amount of material which accumulated on a glass slide in a given period of time could be measured fairly accurately. A wet combustion method with potassium dichromate in 50% sulfuric acid was used as the oxidizing

agent. The dichromate solution was 0.015N. The amount of material accumulated was determined by the difference in blank titrations of equal volumes of the dichromate solution, after acting on the slide for 15 minutes at 100° C. It appeared definitely that an accumulation of organic material would take place in sterile water as well as in the natural lake water. Consequently, the data obtained from the submerged slides can not be used in a quantitative sense.

Our discussion of the seasonal distribution of the green bacteria in Sodon Lake is based on the plankton counts.

The seasonal appearance of the green bacteria has been erratic. Under the conditions of the present investigation, they were first noted in the plankton on January 22, 1949 and continued to be present, although in diminishing numbers, until April. The green bacteria did not reappear in the phytoplankton until July; intensive sampling, both daily and diurnal failed to reveal their presence. From July until November, they were again members of the plankton community; however, no trace of green bacteria has been found in the plankton from early November to the termination of this study. An examination of Figure 3 will show the seasonal distribution of the green bacteria in Sodon Lake. From the appearance of the graph, it seems possible that a numerical peak occurred in January of 1949; a second and obvious peak occurred in August. We cannot theorize concerning the fate of the green bacteria after the early part of November; it is tempting to postulate that, if the organisms continued to be members of the plankton

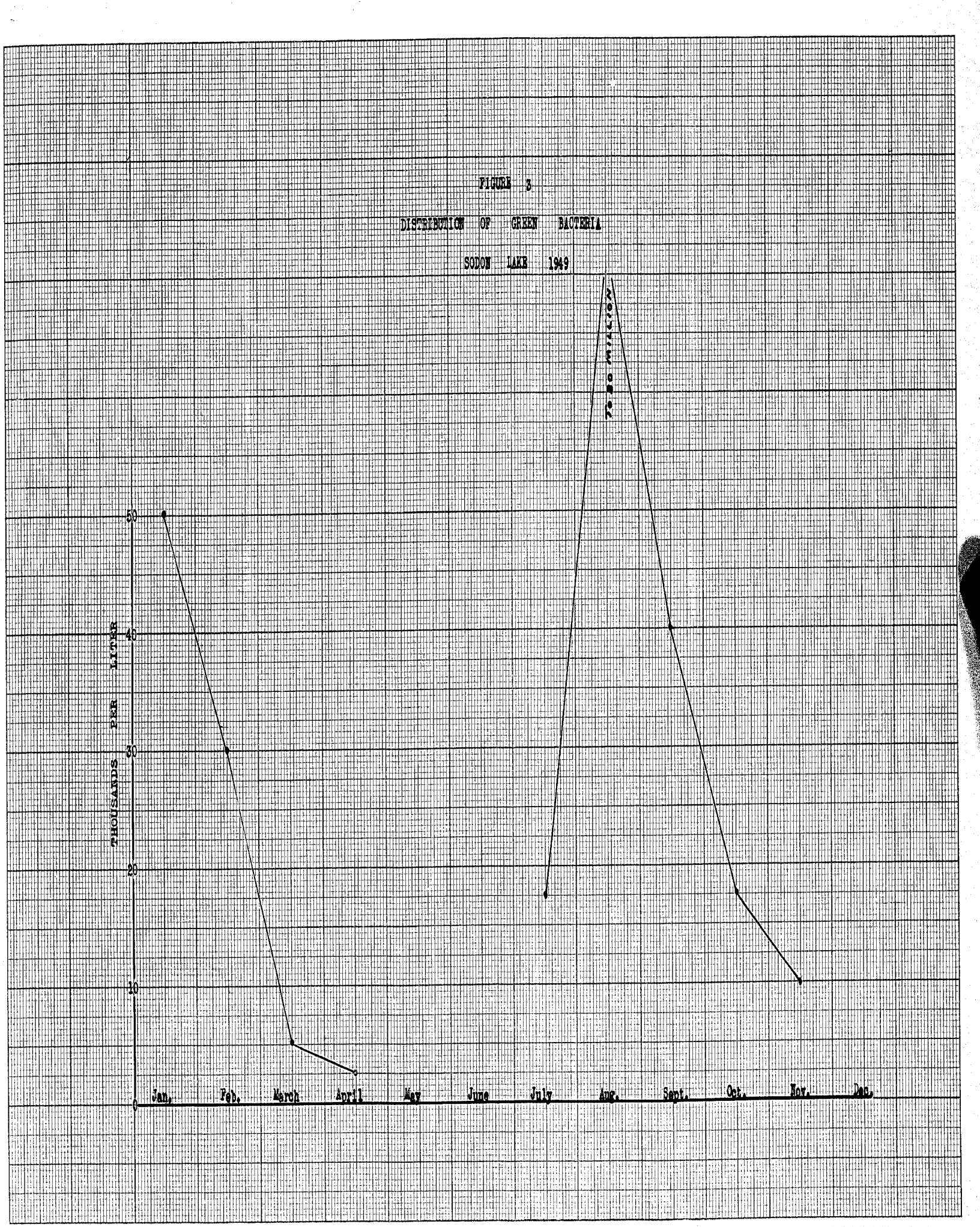
community, a numerical peak would have been reached in January or February of 1950.

We shall discuss somewhat later, possible reasons for the disappearance of the green bacteria from the phytoplankton of Sodon Lake. However, we should like to emphasize here that, coincident with the observation that the green bacteria were no longer residents of the plankton community, a complete reinvestigation of the lake was accomplished; several additional stations were established and all intervening depths were sampled. The results, in so far as determining the presence of the green bacteria was concerned, were completely negative.

FIGURE 3

DISTRIBUTION OF GREEN BACTERIA

SODOM LAKE 1949



ENVIRONMENTAL FACTORS POSSIBLY AFFECTING THE ZONATION OF THE GREEN BACTERIA

If each physical, chemical and biological factor under consideration is analyzed individually but with the realization that no one factor or set of conditions is ever responsible for a given situation, the environmental habitat of the green bacteria becomes clear. However, we do not, at this point, offer such information as possible clues to controlling mechanisms but rather as a guide to the overall ecological picture of Sodon Lake.

The following environmental factors have been investigated throughout the course of this investigation:

A. Physical

1. Temperature
2. Hydrogen-ion concentration
3. Light intensity, including relationship to turbidity

B. Chemical

1. Chemistry of the water
2. Spectrochemistry of the water

C. Biological

1. Phytoplankton
2. Zooplankton

A. Physical.

1. Temperature. The temperatures taken throughout the entire period of this investigation are shown in Table I (Appendix); temperature readings taken on a transect of the zone of green bacteria are indicated in Table II. These latter values are recorded as of August 4, 1949 and indicate a period when the green bacteria were at their

TABLE II

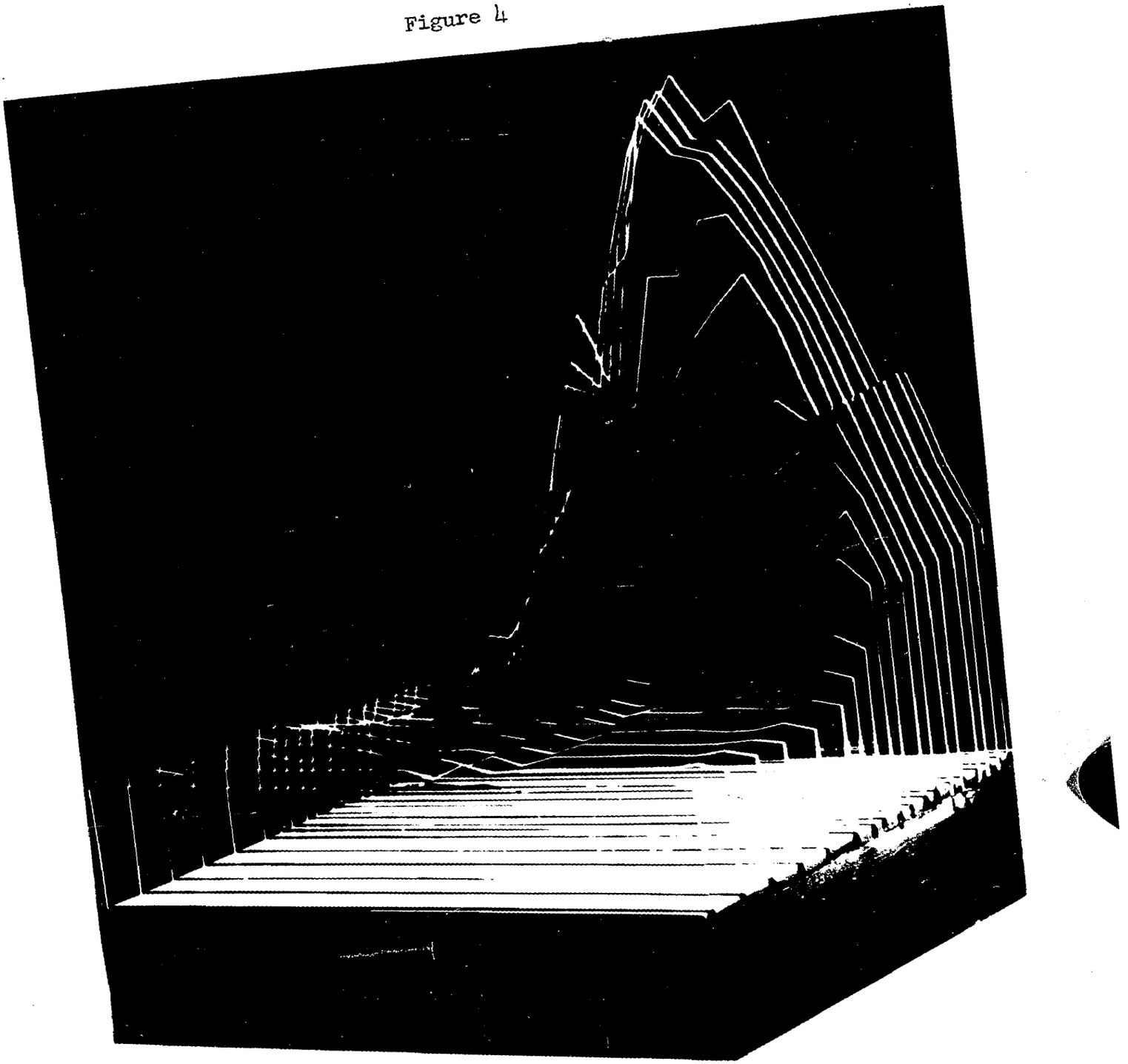
TRANSECT OF ZONE OF GREEN BACTERIA, SODON LAKE, 4 AUG. 49.

Temperature readings: Degrees Fahrenheit

Depth	T_3	T_4	T_5
0	80.6	79.0	82.3
1	80.5	79.0	81.1
2	80.2	78.9	80.5
3	78.7	78.7	79.8
4	78.1	78.3	78.8
5	77.6	77.4	77.7
6	76.8* 74.4	75.8	76.6
7	72.8	73.2	73.5
8	70.4-	69.4	70.7
9	66.9	66.3	67.1
10	63.3	63.4	63.2
11	59.1	60.3	60.5
12	56.7	56.5	55.9
13	54.2	53.2	53.7
14	51.7	51.2	52.1
15	49.1	49.1	49.2
16	47.3	47.3	47.2
17	45.8	45.6	45.3
18	44.6	44.4	44.3
19	43.6	43.8	43.5
20	43.0	43.0	42.8
21	42.2	42.4	42.1
22	41.7	41.7	41.5
23	41.1	41.2	41.2
24	40.9	41.1	41.0
25	40.5	40.8	40.9
26	40.2	40.7	40.7
27	40.2	40.7	40.7
28	40.1	40.6	40.5
29	40.0	40.5	40.2
30	40.1	40.4	40.2
31	40.1		40.2
32	40.1		40.2
33	40.0		40.2
34	40.1		40.3
35	40.2	40.5	40.3
36			40.4
37			40.5
38			40.5
39			
40		40.7	
45		40.9	
46			
47		40.9	
48		41.0	
49			
50		41.1	
55		41.2	

* Intermediate reading taken at 6.5 ft. depth.

Figure 4



A Three Dimensional Model Showing Temperature Trends in Sodon Lake

90° to Right — Zones (Surface to 56 ft.)
90° to Left — Time (Jan. to Dec. 1949)
Vertical — Temperature (32 - 75° F.)

numerical peak. A three dimensional graph showing seasonal temperature trends of Sodon Lake is presented in Figure 4.

It must be emphasized that the temperatures delimiting the zones of concentration vary but little throughout the year. It will be recalled that the zone of concentration for the green bacteria has been determined at 25± 2 feet.

While it is recognized that, in accordance with Van't Hoff's law, and considering the academic approach to the effect of temperature on populations, plankton populations are determined largely by the temperature characteristics of a body of water, it must also be noted that the temperature range from the upper limits of the green bacteria zone to the bottom of the lake is rarely more than 1° F.

Actually very little fundamental research has been done on the effects of temperature on the activities of individual planktonic organisms; for the period of observation of the present investigation, the factor of temperature does not seem to exert an appreciable effect upon the distribution of green bacteria.

In general, in the late spring and early summer, the temperature of surface waters varies owing to increased solar radiation; the resulting difference in density of the water gives rise to the formation of two distinct layers of water, the upper layer or epilimnion and the lower layer or hypolimnion. Under some conditions the temperature is approximately the same at all depths in the epilimnion. Below the epilimnion is the transitional zone, known as the thermocline. Here, the temperature falls rapidly with increasing depth.

In the hypolimnion, the temperature, which ordinarily is considerably lower than that of the epilimnion does not change much with increasing depth.

The factor of temperature here, seems, in this instance, to be more of an academic question than a practical biological one.

All temperature records throughout this investigation were taken with a Foxboro Electric Thermophone (See appendix for illustration). This is actually an electric thermometer which acts upon the principle that the resistance of a conductor to the passage of an electric current changes with the temperature, also that the rate of change in resistance due to temperature differs in different metals. The thermophone possesses advantages over most other kinds of sub-surface temperature measuring devices in that it is extremely sensitive and accurate. (Description largely taken from Welch 1948).

2. Hydrogen-ion concentration. An examination of Figure 5 will show the results of pH determinations, taken over a period of twelve months. In Table III are shown the pH values taken on a transect of the green bacteria zone at a period when the green bacteria reached a numerical peak. All pH values were measured electrometrically, using a Leed and Northrup pH meter.

Ordinarily, surface waters undergo relatively small changes in pH value from season to season. General changes in pH of a progressive, apparently permanent kind are absent or are so extremely slight as to be indistinguishable.

Each organism has its own toleration range of pH terminated by a minimum and a maximum and possesses an optimum at some intermediate position. These toleration ranges are extremely difficult, if not impossible, to determine under field conditions.

The literature contains numerous positive contentions that pH is an important limiting factor for many organisms; however, sometimes the evidence is conflicting for the same species. Many workers regard the pH as of at least secondary importance, and certain investigators have claimed that no correlation of any sort exists with the pH range as it occurs in natural waters. According to Welch[1], it has been shown that acclimitization effects may be manifested not only in individuals from widely separated parts of the geographical range but also in the instance of individuals of the same species which occupy waters closely adjacent but differing widely in the pH value.

It seems probable that pH, per se, can be of little significance; but rather is an indication of the chemical complex esiting in an aquatic environment.

3. Light Intensity. Measurements of the penetration of light through the water and ice were made with a submersible photometer designed by Dr. George Clark of Harvard University and executed by F. Schueler of Waltham, Mass., (see appendix for illustration). Essentially, the instrument consists of two Weston photronic cells, connected by a 60 foot waterproof cable to a galvanometer. Between the cells there is a

[1] Welch, P. S. Limnology. McGraw Hill, New York, 1935, 471 pp.

FIGURE 5

pH DETERMINATIONS

SODON LAKE

1949

SURFACE ----

25 FEET ___

TABLE III

pH DETERMINATIONS OF GREEN BACTERIA ZONE
TAKEN ON TRANSECT

Depth	Station		
	T_4	T_2	T_5
Surface	7.3	7.3	7.3
Two feet above upper limit of zone as judged by color	6.6	6.35	6.15
Upper limit of zone	6.6	6.25	6.4
Middle of zone	6.7	6.3	6.45
Lower limit of visible color	6.6	6.2	6.4

flashed opal diffusing screen; there is space for interchangeable glass filters and also for light-reducing filters. The instrument was calibrated[2] in terms of light intensity in foot-candles incident upon the face of the diffusing screen. Measurements of light intensity were made at 12 o'clock noon of each regular collecting day.

The transparency of the water at various depths was measured with a spectrophotometer; a graph was constructed so that the spectrophotometer readings could be translated into parts per million turbidity.

Almost nothing is known regarding the light requirements of the plankton of the inland lakes. It is entirely probable that each individual species has its own toleration point. Comments are included here only to emphasize the point that below the 25 foot level, 0.0 % transmission was always obtained. This value is the same regardless of ice covered with snow, ice alone or clear water. Since no member of the phytoplankton was secured much below the 25 foot level, this depth probably denotes the limit of the photosynthetic zone and, with the exception of the bacterial flora, probably coincides with the lower limit of the zone of biological activity.

Transparency determines the depth of water which can support photosynthesis and therefore this quality must show some relation to the penetration of light. Our turbidity values at the 25 foot level show a range of 7 to 20 p.p.m. Below the 25 foot level, turbidity values rapidly become higher but no detectable penetration of light occurs.

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[2] We are indebted to J. Harley and I. O'litzky for this work.

TABLE IV

DISSOLVED OXYGEN TRENDS - SODON LAKE JAN. 1949 - DEC. 1949

	Dissolved Oxygen p.p.m.						
	Depth						
Date	Surface	8'	15'	16'	20'	24'	25'
Jan.	No positive tests obtained						
Feb.	"	"	"	"			
March	11.4		8.2		5.0	0.0	
April	24.5				15.5	7.8	0.0
May	31.0				3.0	1.8	0.0
June	6.31	8.64	2.80	1.08		0.34	0.0
July	4.90	5.20		3.9	2.1	1.1	0.0
Aug.	7.7	8.3				1.7	0.0
Sept.	13.4	6.04		1.8	0.46	0.1	0.0
Oct.	5.5	2.5			0.0		
Nov.	5.5	5.5	5.5			1.1	0.0
Dec.	6.2	6.1	6.1		5.6	0.03	0.0

TABLE V

VERTICAL DISTRIBUTION OF DISSOLVED OXYGEN T TRANSECT

SODON LAKE -- AUGUST 1949

Location	Depth	D.O.(ppm)	D.O. (cc/l)	% Sat.
T_1	4"	11.04	7.85	142.0
T_2	4"	9.70	6.70	85.0
T_2	4'	7.50	5.18	92.3
T_2	8'	6.25	4.32	74.7
T_3	4"	6.88	4.81	86.8
T_3	4'	6.67	4.66	81.6
T_3	8'	5.99	4.19	70.0
T_3	12'	5.80	4.05	59.2
T_3	14'	1.80	1.26	16.9
T_3	16'	0.37	0.26	3.3
T_3	20'	0.19	0.13	1.6
T_3	24'	0.14	0.10	1.1
T_3	28'	0.00	0.00	0.0
T_4	4"	13.44	9.40	166.3
T_4	8'	6.65	4.66	81.3
T_4	12'	2.16	4.23	71.1
T_4	14'	2.69	1.42	22.2
T_4	16'	1.89	1.83	16.7
T_4	20'	0.46	1.32	3.8
T_4	24'	0.10	0.32	0.8
T_4	28'	0.00	0.00	0.00
T_5	4"	7.10	4.97	92.2
T_5	4'	6.76	4.73	83.4
T_5	8'	7.32	5.12	85.5
T_5	16'	1.57	1.10	14.2
T_5	24'	0.00	0.00	0.00
T_6	4"	12.83	8.99	8.99
T_6	4'	7.42	5.19	5.19
T_6	8'	6.79	4.75	4.75
T_7	4"	13.04	9.04	9.04

TABLE VI

DISSOLVED OXYGEN IN RELATION TO ZONATION OF GREEN BACTERIA

SODON LAKE AUGUST 1949

Depth (Ft.)	Dissolved Oxygen ppm		
	10:30 A.M.	2:00 P.M.	4:00 P.M.
21	0.24	0.24	0.24
22	0.24	0.24	0.24
23	0.11	0.13	0.16
24	0.11	0.13	0.16
25	0.08	0.12	0.12

B. Chemical Factors

1. Dissolved Oxygen. The rapid Winkler method of oxygen determination was used throughout this investigation. The procedure up to and including acidification was carried out in the field and titrations were effected immediately upon return to the laboratory. Concentrations of solutions were such that the final titration in c.c. was equal to parts per million of dissolved oxygen, Theriault (1925); the per cent saturation and cubic centimeters per liter were sometimes figured.

It is virtually impossible to present all the data which have been obtained. Many of the data are repetitious and can offer but little aid to an understanding of the problems involved in this study. We are therefore presenting in Table IV what we consider to be dissolved oxygen trends for the course of this experiment. In Table V are shown dissolved oxygen values for the entire T transect. In Table VI, dissolved oxygen values which are in direct relation to the zone of green bacteria are tabulated.

2. Hydrogen Sulphide. The vertical distribution of hydrogen sulphide was determined. The method used was that of Theroux, Eldridge and Mallmann (1936).

It is consistently stated in the literature that the green bacteria exist in an environment rich in hydrogen sulphide. Whether previous investigators have actually determined the presence of this gas by actual chemical tests or have been influenced in their deductions by the relatively strong and obvious odor, is hard to say. By actual chemical analysis, the amount of hydrogen sulphide in Sodon

Lake was, at all times, very small and frequently positive tests for this gas could not be obtained. Our values for hydrogen sulphide range from 1.065 ppm to 0.6 ppm. It is recognized that the values quoted are extremely low but it must also be obvious that where there exists a tremendous concentration of green bacteria, there must necessarily be but little more than trace amounts of hydrogen sulphide as the green bacteria are constantly converting this substance to sulphur.

3. Other Chemical Factors. Chemical analyses have been made routinely of the following chemical components of Sodon Lake: bicarbonate, carbon dioxide, carbonate, sulphate, iron, nitrate nitrogen and nitrite nitrogen. Determinations were also made of total, suspended and dissolved solids. Unless otherwise stated, all procedures are in accordance with Standard Methods, ninth edition, (1946). In general, determinations were made during the winter, spring, summer and fall when temperature records indicated thermal disturbances. However, numerous other analyses were made in an effort to determine intermediary products in sulphate metabolism; also, several determinations of total phosphorous were made. The results of the investigations are largely repetitious and are not presented in toto. Data representative of the various analyses are shown in Table VII.

C. Analysis of the hydrosol

Samples of the bottom soil were secured by means of an Ekman dredge (size 22.8 by 22.8 cm.); this dredge is a standard piece of limnological apparatus and can be used successfully for sampling a

TABLE VII

CHEMICAL ANALYSES SODON LAKE - 1949 (PARTS PER MILLION)

Depth	HCO_3	CO_2	CO_3	SO_4	Fe_2O_3	NO_2	NO_3	T.Solids	S.Solids	D.Solids
January										
25 ft.	185.0		0.0	39.6	0.1	0.029	0.0	292.0	0.0	292.0
bottom	295.0			20.4	0.15	0.016	0.0	346.0	0.0	346.0
February										
25 ft.	190.0		0.0	43.6	0.0	0.013	0.0	286.0	0.0	286.0
bottom	250.0		0.0	39.5	0.0	0.03	0.03	284.0	0.0	284.0
March										
25 ft.	192.0		0.0	42.7	0.0	0.02	0.0			
bottom	302.0		0.0	10.8	0.0	0.03	0.0			
April										
25 ft.	176.0		0.0	42.0	0.0	0.016	0.0	278.0	0.0	278.0
bottom	190.0		0.0	40.8	0.0	0.012	0.0	284.0	0.0	284.0
May										
25 ft.	180.0		0.0	38.4	0.0	0.024	0.0	282.0	8.0	274.0
bottom	287.0		0.0	15.8	0.0	0.008	0.0	350.0	12.0	338.0
June										
25 ft.	195.0		0.0	Trace	0.0	0.02	0.0			
bottom	304.0		0.0	0.0	0.0	0.01	0.0			
July										
25 ft.			0.0	Trace	0.0	0.02	0.0			
bottom			0.0	0.0	0.0	0.02	0.0			
August										
25 ft.			0.0	Trace	0.0	0.01	0.0			
bottom			0.0	0.0	0.0	0.01	0.0			
September										
25 ft.			0.0		0.0	0.02	0.0			
bottom			0.0		0.0	0.03	0.0			
October										
25 ft.	226.5		0.0		0.0	0.028	0.0			
bottom	340.2		0.0		0.0	0.02	0.0			
November										
25 ft.	199.2		0.0		0.0	0.01	0.0			
bottom	283.8		0.0		0.0	0.01	0.0			
December										
25 ft.	85.6		0.0		0.0	0.01	0.0			
bottom	179.9		0.0		0.0	0.01	0.0			

soft bottom. The samples were thoroughly mixed and aliquot parts selected for analysis by the Spurway soil tests. An examination of Table VIII will show the chemical composition of the hydrosol.

TABLE VIII

CHEMICAL COMPOSITION OF THE HYDROSOL - SODON LAKE - 1949

Parts per Million			
Sulphates	0.0	Magnesium	6.0 ppm
Nitrates	0.0	Potassium	10.0
Manganese	0.0	Phosphorous	trace
Calcium	200.0	Aluminum	trace
Ferric iron	trace	Ammonia nitrogen	trace

D. Biological Factors

1. The Phytoplankton. Our primary interest in determining the composition of the phytoplankton of Sodon Lake has been to see if there existed a more or less constant association of some member or members of the euplankton and the green bacteria. There is some evidence that such an association does exist. However, comments offered here must be interpreted as applying to the particular conditions present in Sodon Lake. Positive conclusions must necessarily be based upon the results of investigations of other lakes of the same general type as Sodon.

In January, 1949, at the start of the investigation reported upon here, Schroederia setigera coexisted with the green bacteria at depths

ranging from 22 to 27 feet. Quantitatively, there were approximately 59,941 Schroederia per liter of lake water. The number of green bacteria was about the same. A numerical peak of 1,712,683 was reached by Schroederia on February 19th. At this time, the number of green bacteria was constantly declining. Schroederia was absent from the plankton during the interval April to July; it will be recalled that the green bacteria were no longer members of the plankton at this time. From July to November, Schroderia again coexisted in the plankton with the green bacteria.

Perhaps no importance should be attached to the association described; however, such an association does seem significant enough to be commented upon here. Possibly some attention should be given by the phytologist to the physiology of Schroederia.

To complete the overall picture of the phytoplankton of Sodon Lake a preliminary list of the dominant forms encountered from January, 1949 to December, 1949 is presented here. While our data provide for seasonal quantitative and qualitative evaluations, the information provided could be of no value in determining the relationships of the green bacteria. The following list is by no means complete, but it should serve as a clue to the predominating algal flora of Sodon Lake.

The following members of the euplankton have been observed: Oscillatoria tenuis, Chroococcus minutus, Aphanothece nidulans, Aphanizomenon flos-aquae, Dactylococcopsis Smithii, Volvox sp., Eudorina elegans, Oöcystis lacustris, Schroederia setigera, Selenastrum sp., Microspora sp., Crucigenia alternaria, Crucigenia rectangularis,

Dinobryon sertularia, Closterium sp., Staurastrum sp., Cyclotella sp., Stephanodiscus sp., Tabellaria sp., Fragilaria sp., Synedra sp., Asterionella sp., Navicula sp., and Pinnularia sp.

2. The Zooplankton. It is the purpose of this phase of our study to present quantitative data showing the seasonal, vertical distribution of certain members of the zooplankton and to discuss briefly the possible relationship of selected chemical, physical and biological factors to this plankton. We are interested in determining here whether there is a typical zooplankton fauna accompanying the green bacteria.

In general it is true that the zooplankton of Sodon Lake is dominated by three entomostracans, Daphnia pulex, Diaptomus oregonensis and Cyclops prasinus; other plankters, Daphnia longispina and Bosmina coregoni, have appeared, but in such extremely small numbers that they may be considered as transient members of the plankton community. Daphnia longispina, for instance, was first seen in the plankton May 1st; its reappearance has not been observed. Whenever the transient forms have been noted in the plankton, their presence has been confined to the 15 $\pm$ 5 foot depth. Members of the zooplankton, other than those indicated above and nauplii of the copepods, have been virtually non-existent. The three dominant members of the zooplankton indicated above have been arranged according to depth and season in Tables IX (a), IX (b) and IX (c).

In tracing the seasonal periodicity of the individual zooplankters, under the conditions of this experiment, we are actually tracing the

TABLE IX (a)

NUMBERS PER LITER OF *DAPHNIA PULEX*, BY SEASON AND DEPTH, SODON LAKE, 1949

Samples taken about 11:00 A.M.

Depth in ft.	Date 2/19	3/5	3/19	4/2	4/16	4/30	5/14	5/28	6/11	6/25	7/9	7/23	8/4*	8/20	9/3	9/17	10/1	10/15	10/29	Totals
1	n.s.	n.s.	n.s.	4	21	3	n.s.	25	-	-	3	n.s.	4	12	n.s.	6	1	5	1	89
3	37	21	14	10	1	37	21	16	1	3	3	1	14	1	4	12	11	12	1	220
5	37	13	53	9	51	35	16	24	11	-	3	3	14	1	9	24	13	10	2	328
10	8	22	59	27	64	48	3	19	12	5	19	26	11	1	6	56	27	26	4	392
15	12	6	18	3	22	54	20	9	13	31	22	18	19	n.s.	8	68	28	44	36	431
20	15	4	19	-	5	5	7	3	33	-	13	27	12	n.s.	10	19	22	13	24	245
25	-	1	1	-	6	37	-	2	-	5	-	9	5	n.s.	7	5	8	3	3	82
30	-	2	1	-	2	-	2	5	2	1	2	2	1	n.s.	8	2	4	3	4	41
35	-	-	-	2	38	3	1	-	-	1	3	2	-	n.s.	1	-	1	4	1	57
40	-	-	1	2	5	1	-	-	1	3	-	-	2	n.s.	1	1	-	2	-	19
	109	69	166	57	215	223	70	103	73	49	68	88	82		54	193	115	122	76	1899

* Samples taken at 2:00 P.M.

TABLE IX (b)

NUMBERS PER LITER OF DIAPTOMUS OREGONENSIS, BY SEASON AND DEPTH, SODON LAKE, 1949

Samples taken about 11:00 A.M.

Depth in ft.	Date 2/19	3/5	3/19	4/2	4/16	4/30	5/14	5/28	6/11	6/25	7/9	7/23	8/4*	8/20	9/3	9/17	10/1	10/15	10/29	Totals
1	n.s.	n.s.	n.s.	1	3	3	n.s.	11	1	-	5	n.s.	11	1	n.s.	13	2	8	-	59
3	2	3	1	3	1	4	2	16	-	2	3	1	12	-	2	18	7	12	5	94
5	2	1	2	2	3	8	2	13	2	6	4	-	9	3	7	15	4	17	13	113
10	3	15	18	4	4	11	3	26	-	5	8	8	2	6	3	5	6	8	8	143
15	3	8	2	1	1	16	18	14	12	13	12	7	2	n.s.	2	4	2	7	2	126
20	2	4	-	1	-	3	9	3	43	15	9	6	2	n.s.	1	4	1	1	1	105
25	-	4	1	1	2	8	1	2	-	1	2	-	-	n.s.	-	1	-	1	-	24
30	1	-	-	-	-	-	-	2	2	-	-	1	1	n.s.	1	-	-	2	-	10
35	-	-	-	1	3	-	1	1	3	-	1	-	1	n.s.	-	2	-	1	-	14
40	-	-	-	-	-	-	-	1	2	-	-	1	-	n.s.	-	1	-	-	-	5
	13	35	24	14	17	53	36	89	65	42	44	24	40		16	63	22	57	29	693

* Samples taken at 2:00 P.M.

TABLE IX (c)

NUMBERS PER LITER OF CYCLOPS PRASINUS, BY SEASON AND DEPTH, SODON LAKE, 1949

Samples taken about 11:00 A.M.

Depth in ft.	Date 2/19	3/5	3/19	4/2	4/16	4/30	5/14	5/28	6/11	6/25	7/9	7/23	8/4*	8/20	9/3	9/17	10/1	10/15	10/29	Totals
1	n.s.	n.s.	n.s.	-	-	2	n.s.	7	1	-	1	n.s.	1	1	n.s.	9	14	5	4	45
2	-	-	1	-	-	7	10	12	4	1	-	-	2	1	5	11	14	8	16	92
5	-	-	2	-	2	9	13	16	8	3	1	-	1	-	5	18	4	16	12	110
10	-	1	10	4	2	4	10	15	20	5	4	2	4	-	1	12	8	5	14	121
15	-	1	28	2	3	11	15	15	19	11	6	-	10	n.s.	2	11	6	14	9	163
20	-	11	57	7	2	2	8	4	49	21	9	19	7	n.s.	-	2	5	10	6	219
25	-	14	11	2	-	12	1	2	3	2	3	9	1	n.s.	2	-	-	1	1	64
30	-	1	1	2	1	1	-	4	3	1	3	-	1	n.s.	-	-	3	-	2	23
35	-	-	1	2	-	-	2	1	2	2	1	-	1	n.s.	1	-	-	-	-	13
40	-	-	-	-	-	-	-	2	1	1	-	1	-	n.s.	-	-	-	-	1	6
	0	28	111	19	10	48	59	78	110	47	28	31	28		16	63	54	59	65	856

* Samples taken at 2:00 P.M.

seasonal history of the total net plankton as the net plankton is limited to the entomostracans listed above.

The universal concept that the annual total plankton curve is one showing but two sharp maxima and two well defined minima should not be accepted without reservation; under the conditions reported upon here, the zooplankton shows pronounced increases in numbers during March, May, June and September; several minima can also be observed, Figure 6. The classic curve also indicates that the curve of the zooplankton closely approximates that of the phytoplankton; our data also show a departure from this concept in that the phytoplankton, exclusive of bacteria, was almost non-existent until late March. This fact is of particular interest when an explanation of the nutrition of the entomostracans is sought.

It is usually stated or at least inferred, that the various chemical and physical factors operating on a body of water control the quality and quantity of the plankton; among others, the factors of hydrogen-ion concentration, dissolved oxygen, temperature and inorganic nutrients are included. However, Hutchinson (1944) states: "Clear cut correlations between chemical conditions and the qualitative composition of the phytoplankton (and zooplankton) are not to be expected." This concept may well be extended, in the present investigation, to include the quantitative composition of the plankton as well. We have previously indicated that the transient members of the zooplankton appear at the 25 ± 5 foot depth. The maximum numbers of the resident population also appear at this depth, Figure 7. From

information available in the literature on plankton distribution, an explanation of a preferential depth is not too clear.

The pH at any particular level of Sodon Lake tends to change but little throughout the season; also the values indicate slight change in vertical distribution. The figures are largely repetitious, the hydrogen-ion concentration always being in the alkaline range (pH 7.35 - 7.0) in the epilimnion and thermocline regions. The maximum vertical range is approximately 1.1 pH units. Also appreciable amounts of free CO_2 were rarely found, the range being from 3.0 to 0.5 p.p.m. Inasmuch as pH and free CO_2 are merely factors in a total chemical complex, it is improbable that they are, in themselves, indicators of environmental preference.

The vertical distribution of inorganic nutrients in Sodon Lake is approximately the same at all levels; no appreciable difference exists at the 5, 10, 15 and 20 feet levels nor is the seasonal variation significant. There are no extreme departures from the typical analysis as shown in Table X.

TABLE X

AVAILABLE NUTRIENTS IN SODON LAKE, 1949

Parts per Million										
NO_2-N	NO_3-N	Fe_2O_3-FE	SO_4	HCO_3	CO_3	Ca	K	P	Mn	Mg
0.029	0.0	0.1	43.6	192.0	0.0	200.0	10.0	Trace	0.0	6.0

While it is recognized that the inorganic content of Sodon Lake is surprisingly small, the two ions frequently offered as limiting factors for the phytoplankton (nitrate-nitrogen and phosphate-phosphorous) are present in amounts equal to those reported for highly productive lakes.

We attach slight, if any, importance to temperature as a governing factor for the distributions under investigation. Let us consider again the fact that the majority of the entomostracans are found at the 15 + or - 5 foot depth. At these depths, we have to cite just two examples, a range of 10.1° in May and a range of 27.5° in August. Temperature obviously cannot, in itself, be a controlling factor and the thermocline, when present is no barrier to the migrations.

While the dissolved oxygen content is never considered to be a limiting factor in an unpolluted body of water, Sodon Lake does exhibit some interesting oxygen relationships. From January 22nd to March 5th, a positive test for dissolved oxygen could not be obtained at any depth for the station selected as the permanent sampling place. As this seemed to be a rather unusual situation, the tests were repeated by co-workers using several different methods; the results were negative. From March 19th to November 15th oxygen is present in the epilimnion and thermocline while the hypolimnion is completely anaerobic. We have been unable throughout the period of this investigation to obtain a positive dissolved oxygen test below the 25 foot level. However, it will be recalled that the bulk of the plankton population is found at about the 20 foot level where the oxygen values are adequate for

the plankton Entomostraca. These oxygen values are, however, always less than 0.2 cc. per liter which, according to Pennak (1946) is the critical point for most plankton Entomostraca. We are completely at a loss to explain the distributions during January, February and March when Sodon Lake was anaerobic. It is probable that a more delicate test for oxygen, i.e., the luminous bacteria method of Beijerinck should have been used to detect minute traces of oxygen at the times indicated. It is also possible that the reported absence of oxygen is more apparent than real and due to some interfering substance. However, tests for thiosulfate, an expected product of the bacterial oxidations of hydrogen sulphide, were negative. At any rate, it must be concluded that the entomostracans under investigation show an extremely high tolerance to marginal amounts of oxygen and that the figure cited by Pennak must be modified at least for this experiment. In this connection, it should be emphasized that the entomostracans also show considerable tolerance to hydrogen sulfide as measurable amounts of this gas are found just below the 25 foot level. Almost nothing is known regarding the light requirements of the plankton of inland lakes. It is entirely possible that each individual species has its own toleration point. A comment is included here only to emphasize the point that below the 25 foot level 0.0% transmission was obtained. Inasmuch as but few members of the plankton were obtained much below the 25 foot depth, This figure limits the extent of the photosynthetic zone and, with the exception of the bacterial flora, probably coincides with the lower limit of the zone of biological activity.

It is to the biological factors that we turn for a possible explanation of the distribution of the entomostracans. It has been emphasized that the zooplankton approached a peak before the phytoplankton appeared in measurable numbers. It would then appear that the entomostracans were independent of the algal flora for food and that they must turn either to colloidal material present in the water or to the bacteria. While an investigation of the digestive tract of the copepods shows, in some instances, the presence of minute green organisms (probably green bacteria) these did not appear to be in an active state of digestion. From our bacteriological studies, we have considerable evidence that the greatest concentration of bacteria exists just below the 20 foot level and that their numbers diminish as the vertical distance becomes less. It would appear then that there is circumstantial evidence, at least, that there is a definite nutritional response by the copepods to the bacterial flora in their immediate environment.

FIGURE 6

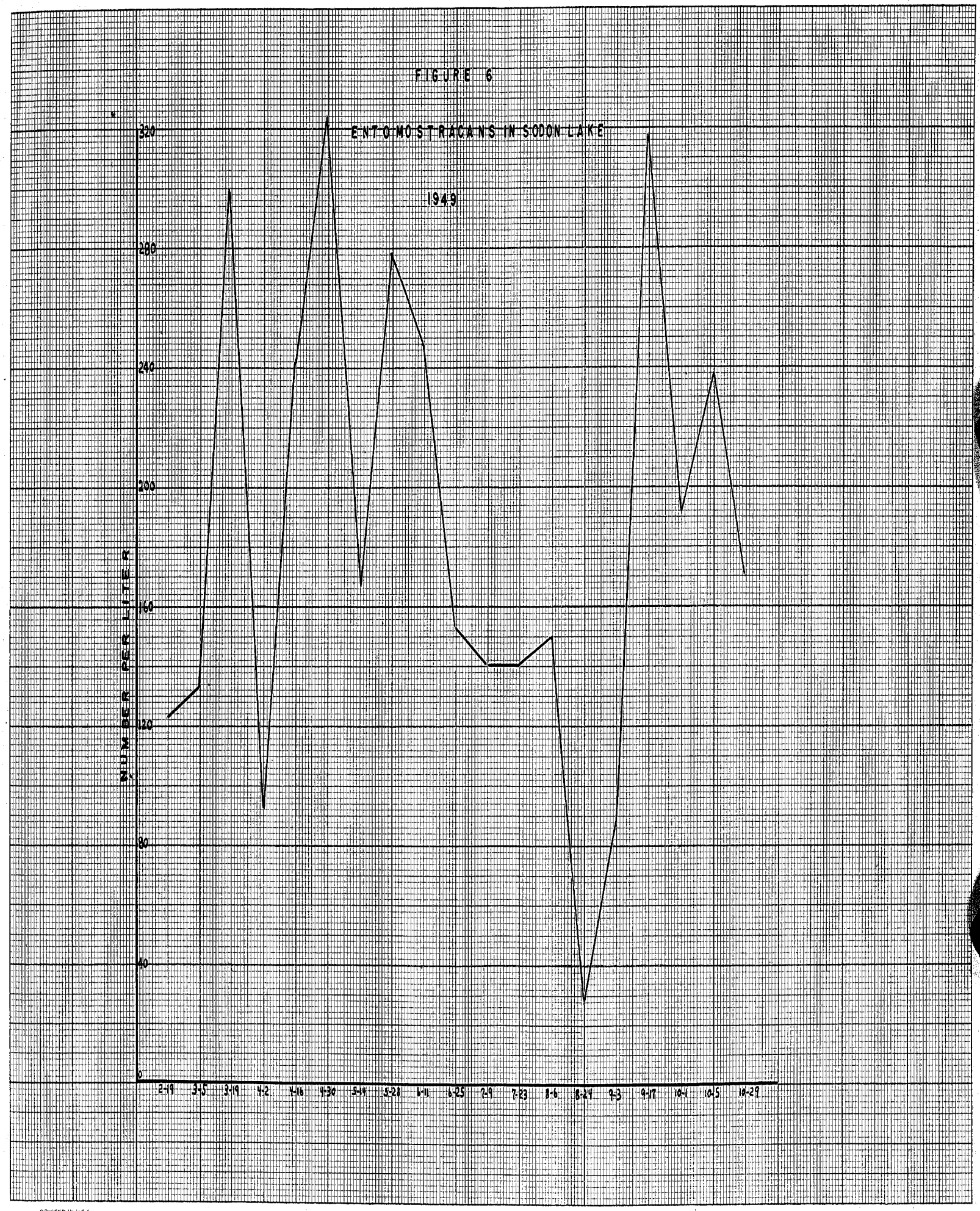
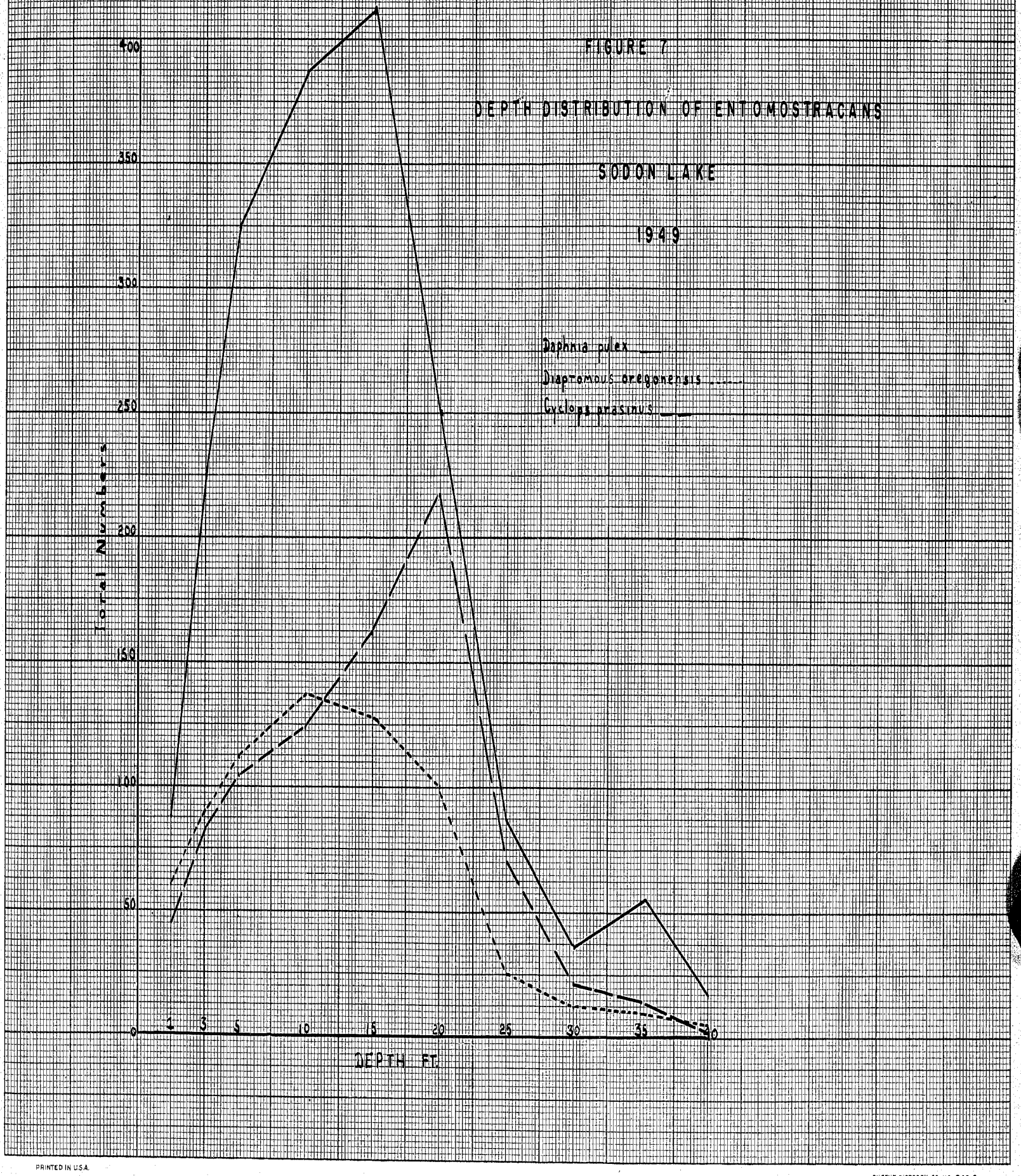

ENTOMOSTRACANS IN SODON LAKE
1949
NUMBER PER LITER
320
280
240
200
160
120
80
40
0
2-19
3-5
3-19
4-2
4-16
4-30
5-14
5-28
6-11
6-25
7-9
7-23
8-6
8-24
9-3
9-17
10-1
10-15
10-29

FIGURE 7

DEPTH DISTRIBUTION OF ENTOMOSTRACANS

SODON LAKE

1949



THE CULTURE OF THE GREEN BACTERIA

A. Crude Cultures

As has been stated in the introduction to this paper, published descriptions of genera and species of the green bacteria have been based largely on preparations made from crude cultures or from the study of the organisms as they existed in their natural environment. It seems obvious that the diversified processes of microbial decomposition take place in a manner that cannot be controlled and that the resulting crude cultures must necessarily contain a large variety of metabolic products. These, in turn, may influence, as an environmental response, the bacterial morphology; and may account for, in some measure, the number of green bacteria reported in the early literature as distinct genera and species.

Our interest in crude cultures has been of an exploratory nature only. To this end, glass cylinders were about one quarter filled with the black mud of the bottom deposits and upon the mud was deposited enough deoxygenated water to fill the cylinders. These were then covered with glass plates to make hermetically sealed units and stored in the light under the usual laboratory conditions. No attempt was made to control the experiments.

Examination of our crude cultures has shown a number of seemingly different types of green bacteria which include the spherical, rod, slightly curved and spiral shaped organisms reported upon by other investigators. In our opinion, conclusions drawn from the examination of crude cultures are worthless; such conclusions are only definitive in

that the presence of the organism is confirmed. For a conclusive and valid investigation of the green bacteria, crude culture methods must be supplemented by those in which the environmental conditions can be more accurately controlled and which can be reproduced by other investigators.

It is hard to conceive that the German School of Bacteriology, which owes much of its prestige to the work of Robert Koch, in part Koch's postulates and his pure culture studies, has published, as late as 1913, descriptions of bacteria derived wholly from observations on crude cultures.

B. Enrichment Cultures

It might be expected that the devising of a culture medium to enhance the growth of the green bacteria would be a comparatively simple task. However, such was not the case. The usual procedures, preparatory to the formulation of an experimental medium, would be the chemical analysis of the environment in which the organism in question is growing and an analysis of the bacterial ash; these two determinations should give values which are, at least, indicative of the gross inorganic salt requirements of the organism under investigation.

Most investigators of aquatic habitats have followed the standard procedures for chemical analyses as indicated by the American Public Health Association; but, it is felt, that such procedures are not at all suited to waters of extremely low mineral content. In many cases, tremendous quantities of water must be evaporated before the usual analytical methods can be applied. The time element is of great

importance here if it is necessary that many determinations be carried on simultaneously. A search[3] of the literature indicates that other methods of analysis are available for waters of low mineral content in which the total solids are in the range of 250 parts per million. Iron, for example, can be determined by the mercaptoacetate method; sodium, by the "triple acetate methods"; magnesium, by the method of Kolthoff and Gill and calcium, by the oxalate method of Lingane. Some further comments on these methods will be expressed elsewhere in this paper.

The results of our analyses by refined methods indicate nothing more than the mere presence of many inorganic substances in concentrations to which the term "trace" may conveniently be applied. Consequently, our enrichment medium has been formulated on the basis that carbon, hydrogen, oxygen, nitrogen, phosphorus and sulfur are indispensable components of all living cells. To these elements deemed necessary to the metabolism of the cell, magnesium has been added because of the fact that it is thought to be important in metabolic processes and also that it is a component of bacteriochlorophyll. It has been assumed that other salt requirements would be met by the presence of trace elements in otherwise chemically pure substances.

While Beijerinck early in his investigations of the purple bacteria used the principle of enrichment cultures, the technique, as such, has been employed but little by subsequent investigators.

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[3] With the assistance of Dr. Stone, Chemistry Department, Michigan State College.

Beijerinck probably added a source of hydrogen sulphide to ditch or canal water, thus making his cultures, to some extent at least, partially controllable. Instead of ditch or canal water, we have used a medium of the following composition as an enrichment for our crude cultures:

sterile, aged lake water.....	1000 ml.
NH_4Cl.....	0.1%
K_2HPO_4.....	0.1%
$MgCl_2$.....	0.1%
$NaHCO_3$.....	0.1%
$Na_2S.9H_2O$.....	0.1%

The pH was adjusted to 7.5 by the addition of Na_2CO_3 and H_3PO_4. The aqueous solutions of the inorganic salts, with the exception of the bicarbonate and the sulphide, were autoclaved for 15 minutes at 121° C.; the sulphide solution was autoclaved separately and added in the desired concentration to the other inorganic salts; the bicarbonate solution was sterilized by filtration.

The medium was dispensed into screw capped bottles, seeded with varying amounts of the crude culture, sealed to provide anaerobic conditions and incubated at room temperature, using a seven watt bulb as a source of illumination. Cultures were incubated for seven days. Following the incubation period, serial transfers were made and treated as indicated above; the transfer process was continued until the cultures appeared microscopically to consist of but one type of cell.

From the enriched and purified cultures, transfers were made to a medium of the same inorganic constitutents as indicated but with the addition of 0.3% agar to make a semi-solid medium; this medium was dispensed into screw cap tubes, inoculated, using the shake culture

technique, and sealed with sterile vaspar. The cultures were incubated as before and kept for comparative purposes.

C. Pure Cultures

To insure cultures which, in the true sense of the word, could be considered as "pure", the single cell isolation technique was practised. Using a deFonbrune micromanipulator, single cells from the natural environment were isolated, inoculated into the defined culture medium and treated in the same manner as the enriched cultures.

GENERAL MORPHOLOGY

A. From Enrichment Cultures

The striking variation in morphology of the green bacteria as reported from the literature must be reemphasized here. With the exception of one species, Chlorobium limicola, all descriptions of the green bacteria recorded in the literature have been derived from observations on crude cultures. As soon as a cell form was found which differed in but one minor respect from other described forms, a new species was created. Variability of the organism under the influence of different environmental conditions was not considered; and therefore all existing classifications are based upon morphology and size measurements as determined in a natural state; cell conglomerates were considered as static units. The phenomenon of environmental variation is so common that it merits no discussion other than to point out that by varying the environment of an organism morphological variants can be produced almost at will.

In preparations made from our enrichment cultures, the green bacteria appeared as rods, approximately 2.5 u by 1.0 u, as cocci, as spiral forms and as curved vibrio like organisms. No one morphological variety could be said to be dominant.

B. From Pure Cultures

In preparations made from cultures started from single cells, some general variation in morphology is again evident. Perhaps a non-motile rod about 1.0 u by 0.5 u appears more often than other morphological variants. However, neither shape nor size is a constant

morphological feature. Cell conglomerates are present, these being surrounded by capsular material which can be dissolved off by suitable bacteriological techniques.

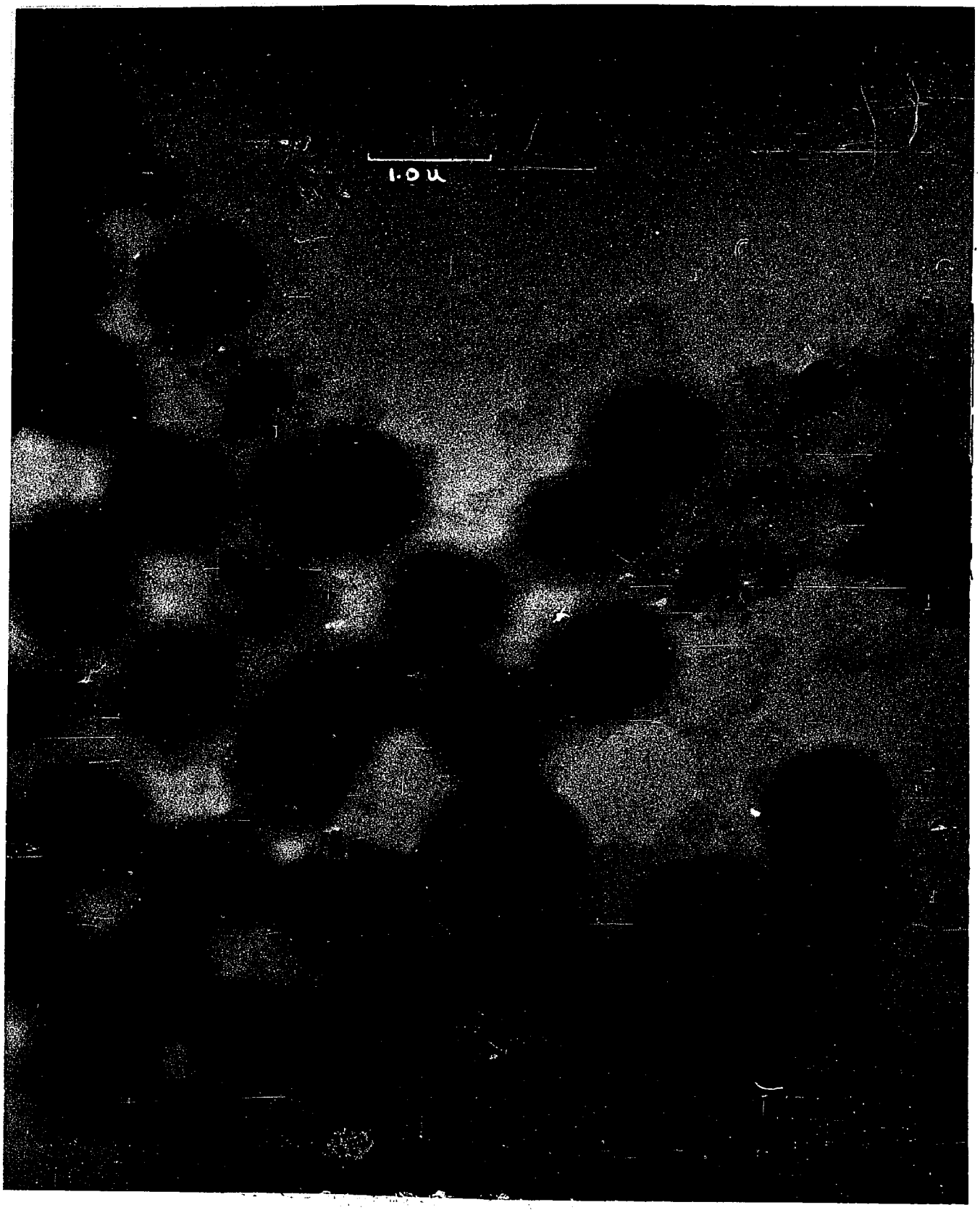
Electron microscope pictures show two to three areas of heavy condensation, approximately 0.25 u in diameter as determined by reference to particles of polystyrene latex. The condensed areas can be dispersed by extraction with ethyl alcohol; from a study of the absorption spectra of the green bacteria, there is some evidence that the zones of concentration may represent a nucleic acid component. In Figures 8, 9, 10 and 11, the morphology of the green bacteria as determined by the electron microscope is shown.

In Figure 8 is shown an aggregation of cells surrounded by mucous material; the rod and coccus forms predominate here. In Figure 9, the same type of grouping is indicated but an attempt has been made to shadow cast the objects. The result is not entirely successful but the surface topography is evident; particles of polystyrene latex have been incorporated into this preparation in order to offer a comparative basis of size. In Figure 10 is shown a single cell of the green bacterium. Incorporated into Figure 11 is a rod shaped cell and a coccus shaped cell; these are the two morphological variants of C. limicola which appear in our cultures.

We have identified the organism studied in pure culture as Chlorobium limicola Nadson. It seems possible that the Chlorochromatium aggregatum of Lauterborn, his Pelodictyon clathratiforme and his genus Pelogloea probably represent growth forms or environmental variants of

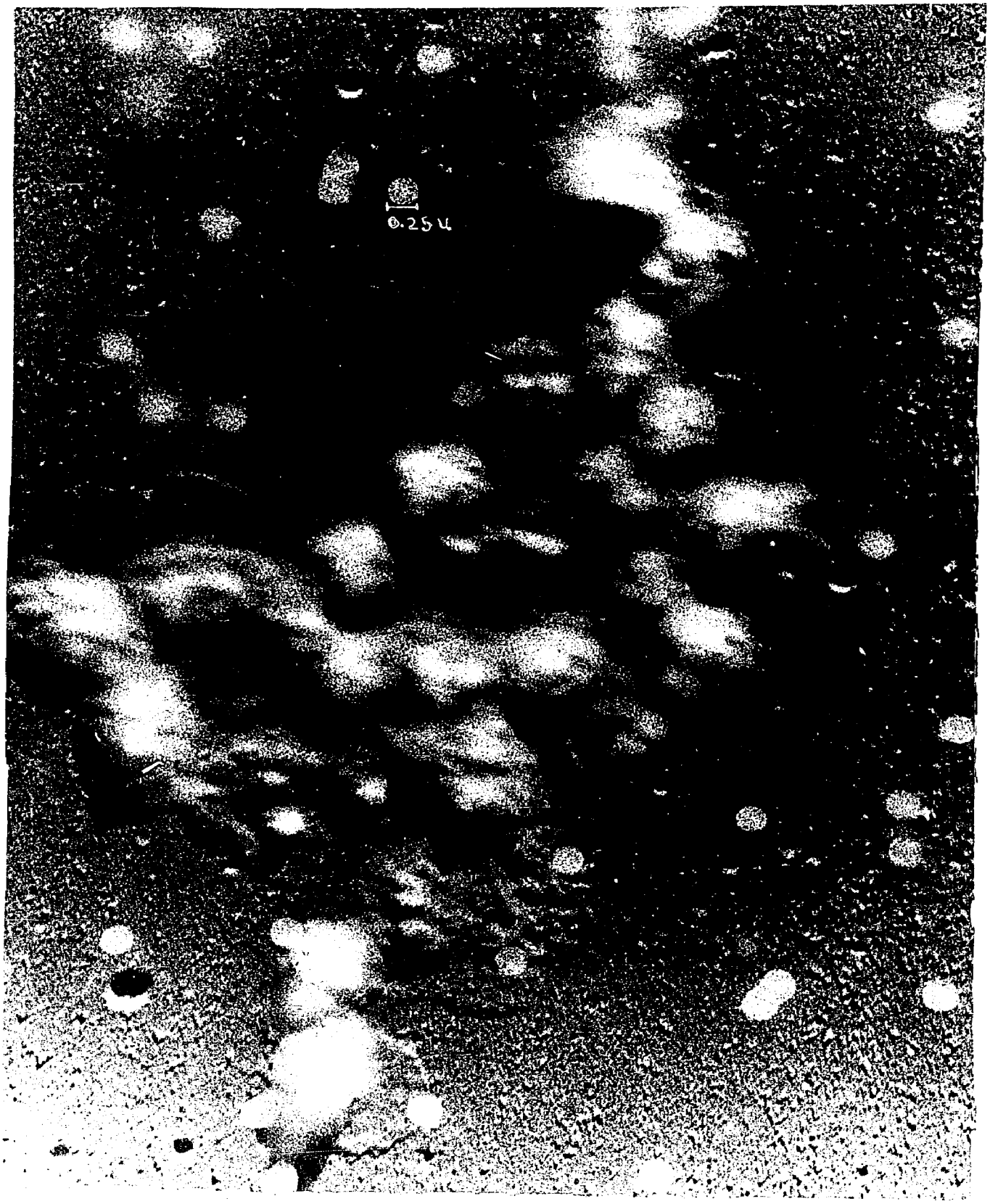
Chlorobium limicola. Some conclusions in regard to the morphology of these latter organisms can be drawn by reference to Figure 12. It appears rather obvious that variation is to be expected regardless of whether pure cultures or crude cultures are analyzed. However, we do not have sufficient data to justify more conclusive statements. The several cultures of Chlorobium limicola which we have studied do show different morphology and cell aggregations, indicating close circumstantial evidence, at least, that such variations resemble those described for other distinct species of green bacteria.

Figure 8.



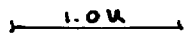
Cell aggregation of Chlorobium limicola.

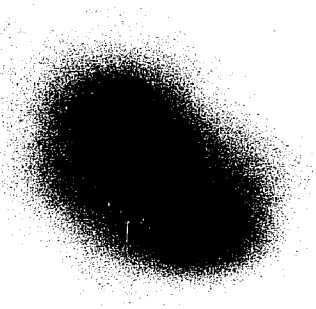
Figure 9.



C. limicola, shadow casted.

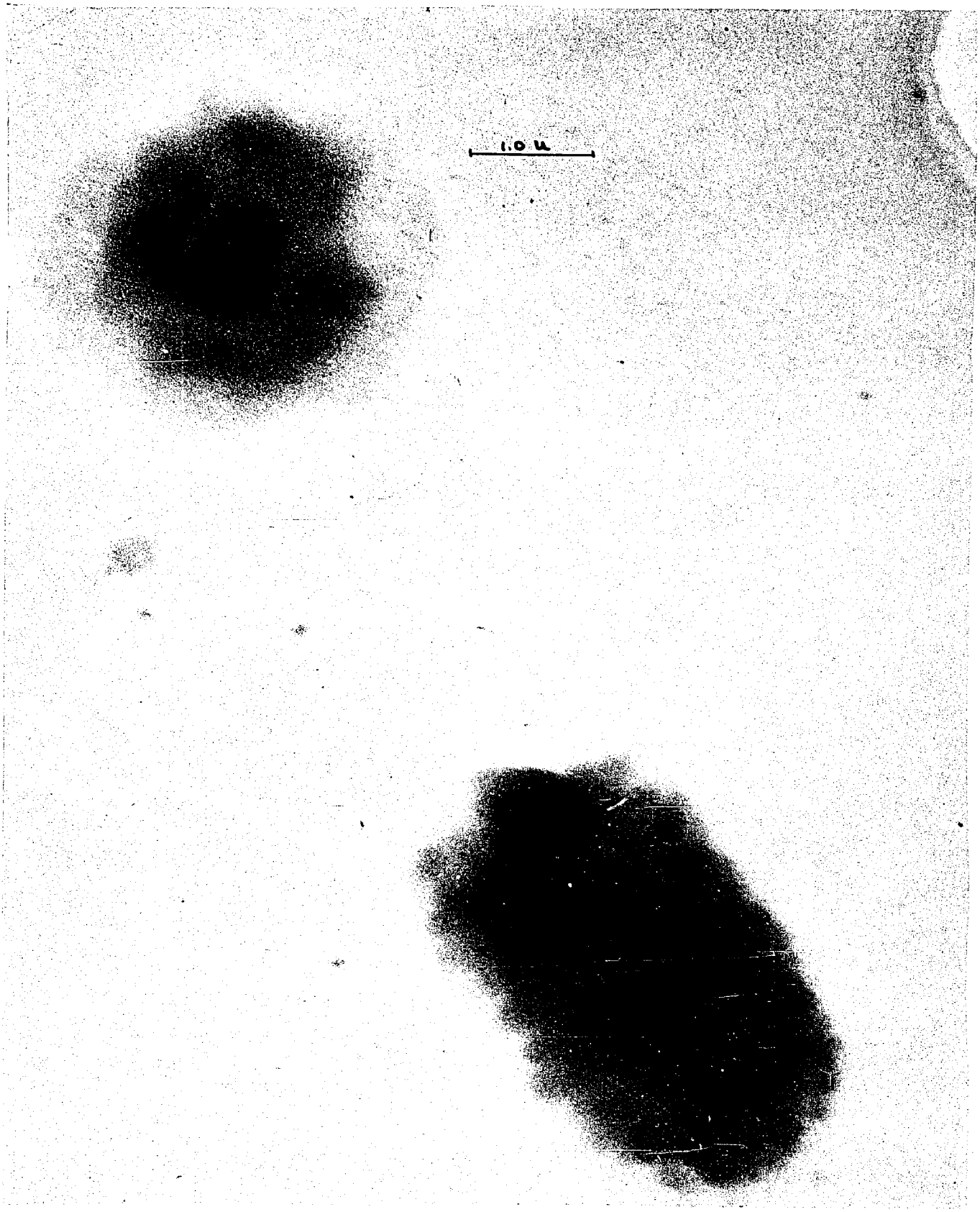
Figure 10.





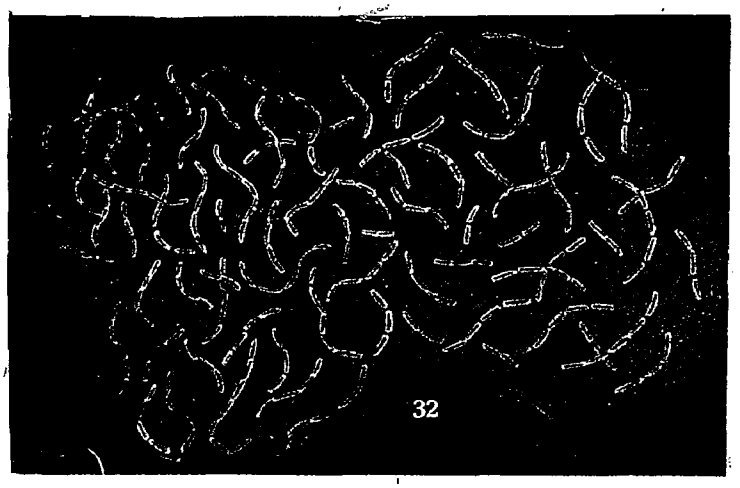
Single cell of C. limicola.

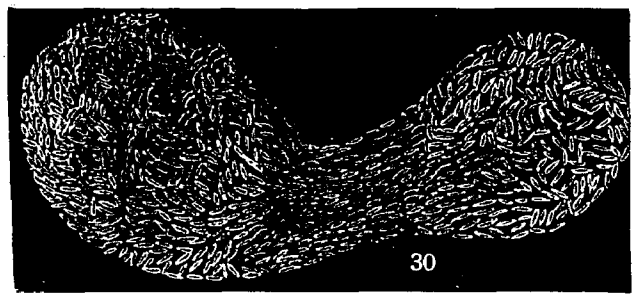
Figure 11.

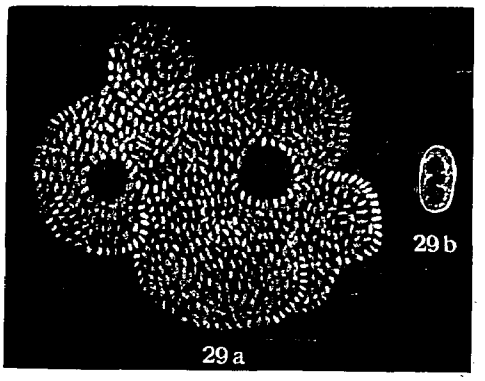


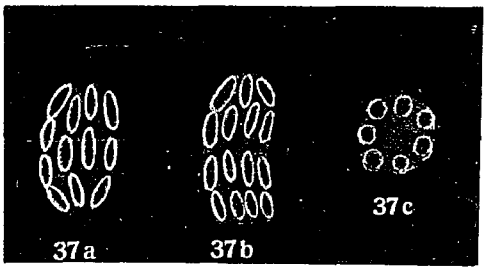
Morphological variants of C. limicola.

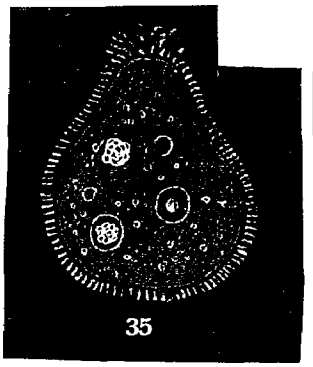
Figure 12.

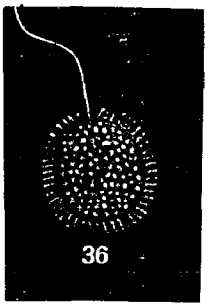


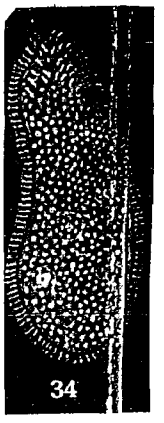


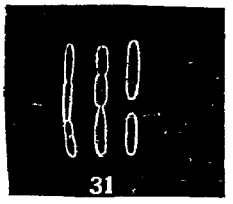












Key to Figure 12

Taken from Die sapropelisch Lebewelt by Lauterborn

34-36	Chlorobacterium symbioticum nov. spec.
29	Schmidlea luteola Schmidle
32	Pelogloea chlorina Lauterborn
30-31	Pelogloea bacillifera nov. spec.
32	Pelodictyon clathratiforme Szafer
37	Chlorochromatium aggregatum Lauterborn

THE PIGMENTS OF THE GREEN BACTERIA

Very few data exist on the spectral properties of the green bacteria. Metzner (1922) has indicated that the green bacteria do not contain phycocyanin, the presence of which would place these organisms close to the blue-green algae, the Cyanophyceae. Metzner, loc. cit., has also intimated that the chlorophyll of the green bacteria, bacterioviridin, differs from the chlorophyll of the higher plants and from that of the green algae. vanNiel (1931) has accepted the ideas expressed above in regard to the pigments of the green bacteria but his conclusions are based upon the work of Metzner and do not represent either a reinvestigation of Metzner's work or exploratory work of his own. Therefore a reexamination of the pigments of the green bacteria is indicated here.

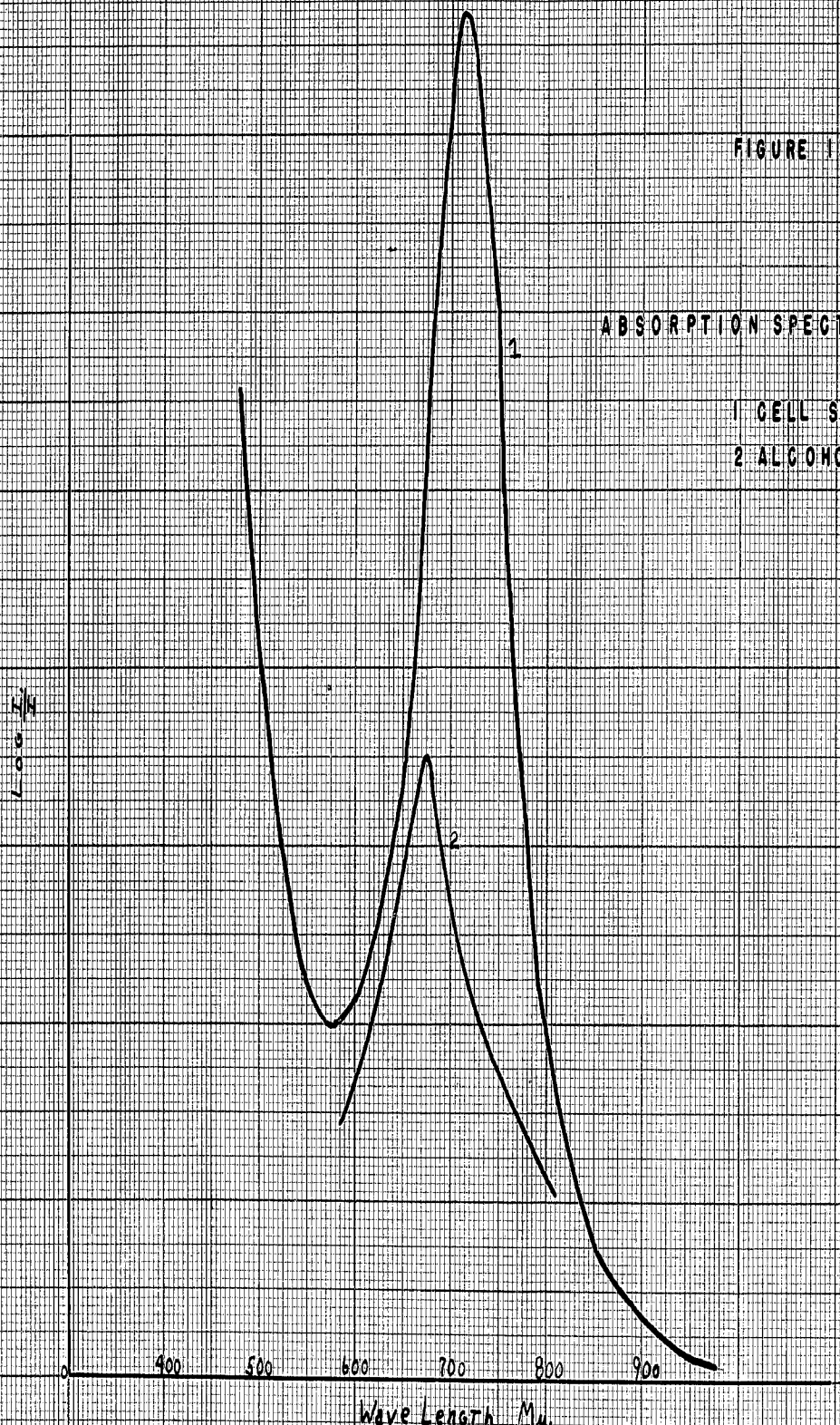
Absorption spectra were determined for cell suspensions and for cell extracts of the green bacteria, using pure cultures as the source of material. A Beckman Spectrophotometer was used for this work. For the cell extracts, the cellular material was extracted in the dark with ethyl alcohol; the residue from this alcoholic extract was treated with various solvents i.e., carbon disulphide, benzene, carbon tetrachloride, chloroform, dioxane and petroleum ether. However, absorption spectra of the extractions with the various solvents mentioned proved to be dependent on the refractive index of the solvent. For a standard, petroleum ether was adopted as the secondary solvent. An examination of Figure 13 will show the absorption spectrum from (1) cell suspensions of green bacteria and (2) the alcoholic extract.

FIGURE 13

ABSORPTION SPECTRA OF GREEN BACTERIA

1 CELL SUSPENSION

2 ALCOHOLIC EXTRACT



In order to interpret the absorption spectrum of the green bacteria, it seems necessary that an analytical comparison be made of the absorption spectra of other unicellular chlorophyll-containing organisms. Special attention should be paid to the absorption spectrum of the blue-green algae because it is to this group that Geitler would assign the green bacteria. It will be remembered that Geitler proposed a change in status of the green bacteria from the Chlorobacteriaceae of Lauterborn to the Cyanochlorodineae (Geitler and Pascher). This change was recommended largely on the basis that definite proof was lacking as to the existence, in the green bacteria, of a chlorophyllous pigment different from that which existed in the blue-green algae.

Many data are at hand for the comparison of the absorption spectra of chlorophyll-containing organisms. An extensive literature is available from investigations in the field of photosynthesis. However, it must be emphasized that the specific techniques used to study photosynthesis in-vitro are not generally available to the bacteriologist. We refer here, primarily, to the disruption of the cell by ultra sonic vibrations; this technique permits the use of an extract which gives the same absorption bands as that of the pigment when present in the plastids.

As we have not been able to prepare "cell juice" extracts of green bacteria, our data and the interpretation thereof must be based on cell suspensions and extractions made with organic solvents. However, such comparisons as will be made between the absorption spectra of the green

bacteria and those of other one celled chlorophyll-containing organisms will be based on similar extractions of these plants with the same organic solvent.

In Figure 13 is shown the absorption spectrum of (1) a cell suspension of green bacteria and (2) the alcoholic extract. A strong maximum at about 740 mu. is indicated for the cell suspension; however, it is obvious that there is a shift to the shorter wave lengths for the alcoholic extract, which places the absorption maximum at about 670 mu.

In a series of beautiful experiments Hubert (1935) was able to show that the spectra of chlorophyll (that is, the position of the maxima) in organic solvents depended on the refractive index of the solvent used. Accordingly, the comparisons now to be noted are based on cell suspensions and extracts with ethyl alcohol; thus duplicating the conditions under which the absorption curves of the green bacteria were drawn.

The data to be summarized regarding the absorption spectra of the blue-green algae and the green algae are so well known that it is not thought necessary to document them. The data are reproducible even with relatively crude instruments. For the blue-green algae, using Oscillatoria sp. as an example, and using a cell suspension, two maxima exist; one of these maxima is at 680 mu., the other, at 620 mu. 680 is the position on the absorption band of chlorophyll and 620 represents the phycocyanin. Now in an alcoholic extract of Oscillatoria, the chlorophyll maximum is shifted to about 670.5 mu. Using Chlorella sp. as an example of the green algae, the absorption maximum is at 680 mu;

in an alcoholic extract, this maximum is shifted to 664 mu. It is apparent that a shift to the shorter wave lengths takes place in the alcoholic extracts of the green bacteria, the blue-green algae and the green algae. To clarify these data for further discussion, we may look at them in tabular form.

	Absorption Maxima (mu.) of		
	Green Bacteria	Green Algae	Blue-green Algae
Cell Suspension	740	680	680 and 620
Alcoholic Extract	670	664	670.5

What are we to conclude from a comparison of the absorption spectra of the green algae, the blue-green algae and the green bacteria? vanNiel (1944) has said "The green sulphur bacteria contain a chlorophyll which is most certainly not identical with either bacteriochlorophyll or chlorophyll a or b." But this very strong statement is based upon one determination made by Metzner in 1922. Obviously, when using cell suspensions, there is a small difference (60 mu.) between the absorption spectra of the green algae and the green bacteria; however, for the alcoholic extracts of these organisms the difference in absorption maxima is reduced to 6 mu. Such a difference is hardly a valid reason for the statement quoted from vanNiel. Contrary to the opinion expressed by Pringshein (1949) that the pigments of the green bacteria will probably be the most suitable features upon which to base a classification, it appears that a more valid rationale must be found if a clear demarkation of the lower algae and the green bacteria is sought.

METABOLISM OF THE GREEN BACTERIA

The term "autotrophic-photosynthetic" has been coined to describe the metabolism of the green bacteria. The expression is singularly descriptive inasmuch as the green bacteria develop in entirely inorganic media, containing hydrogen sulphide. Light of sufficient intensity and quality to activate a photochemical reaction must also be provided.

The classic equation for photosynthesis of the green plants is represented as: $CO_2 + H_2O \xrightarrow{\text{Light}} CH_2O + O_2$. Formaldehyde, in this equation represents a compound formed by the reduction of carbon dioxide. For photosynthesis as carried on by the green bacteria, a generalized equation would be represented as: $CO_2 + 2H_2S \xrightarrow{\text{Light}} CH_2O + H_2O + 2\ S$. In the photosynthesis of the green plant, the reaction of water with carbon dioxide gives rise to the production of oxygen. This phenomenon is so well known that it merits no discussion. However, photosynthesis as effected by the green bacteria differs in at least two major aspects from that carried on by the higher plants. With the green bacteria, the conversion of carbon dioxide into a reduction product is dependent entirely upon the presence of sulphide in the environment. A second major difference in the metabolism of the two groups of plants is the observable fact that no oxygen is produced as the result of the photosynthetic activities of the green bacteria. The most sensitive test known to demonstrate the presence of oxygen is the luminous bacteria method developed by Beijerinck. This method will detect the presence of oxygen in quantities of the order of magnitude of a millionth of a

microgram. Even the use of Beijerinck's method fails to show the presence of oxygen as a by-product of bacterial photosynthesis.

Hypothetically, the process of bacterial photosynthesis can be reconstructed as indicated in the following equations:

$$CO_2 + 2H_2S \xrightarrow{\text{Light}} CH_2O + H_2O + 2S$$

or

$$2CO_2 + H_2S + 2H_2O \xrightarrow{\text{Light}} 2(CH_2O) + H_2SO_4$$

The two equations given above can be generalized as

$$CO_2 + 2H_2A \xrightarrow{\text{Light}} CH_2O + 2A + H_2O$$

Here, H_2A is used to designate any reducing agent or hydrogen donor while A is the oxidation or dehydrogenation product.

DISCUSSION

Preliminary Discussion

We have, thus far, in this paper presented data on certain ecological factors which operate within a body of water and which have been interpreted by students of aquatic biology as exerting positive or negative effects upon aquatic populations. It must be emphasized here that ecological elements can never operate independently of each other but that they must be considered as members of an infinitely complex ecosystem, each component of which is necessary and vital to the dynamism of the environment.

In attempting to analyze or to evaluate the data obtained during the course of this investigation, one is confronted by a multitude of factors all of which would appear to influence a distribution. It is generally conceded that for most plants and animals such a distribution does exist and that a suitable criterion such as Chauvenets may be applied. It remains to be seen whether the bacteria under investigation can be treated as an ecological unit in a manner comparable to other members of the flora and fauna.

We shall, in the following pages, attempt to analyze individually each possible controlling factor under consideration but always with the realization that no one factor or set of conditions is ever responsible for a given distribution.

Factors Possibly Influencing the Distribution of Green Bacteria

It seems justifiable to use as a major premise the statement that the number of green bacteria found in a particular environment at a

stated time is a function of two complex variables; these variables undoubtedly are (1) the resultant of forces favoring reproduction of the species and (2) the resultant of forces favoring death or extinction of the species. Even though optimal environmental conditions for reproduction may exist, survival conditions may be unfavorable and the resulting bacterial population will be limited; if survival conditions are favorable, a large population may result in spite of the fact that the elements for growth and reproduction are not optimal. The remarks expressed above may be summarized in the form of a differential equation wherein the time is expressed as the constant K; F and F' represent the resultant of favorable and unfavorable forces respectively.

$$\frac{dN}{dF} \sim KdF \quad \text{or} \quad \frac{dN}{dF} \sim KdF'$$

Temperature as a Controlling Factor

We shall first discuss temperature as a possible controlling factor affecting or regulating the distribution of Chlorobium limicola. We have previously indicated, in a preliminary discussion of the factor of temperature, that most aquatic environments show at least two major thermal characteristics which can be interpreted as having some ecological significance. These two main thermal characteristics are (1) some form of seasonal change and (2) the existence of an area called the thermocline. This latter term was coined by Birge in 1897 to account for a thermal layer wherein the fall in temperature was at least 0.548° F. per foot of vertical distance. The areas above and below the thermocline are known respectively as the epilimnion and the hypolimnion;

here, the temperature fall is less than 0.548° F. per foot of vertical distance.

The waters of Sodon Lake depart from the classic interpretation of temperature expressed above in at least two major aspects. These are (1) an increase in temperature from the top to the bottom of the hypolimnion, and (2) a permanent stagnation of the hypolimnion throughout the year.

While the first of these observable phenomena has been reported by Newcombe (1948) and substantiated by the author, the range of temperature involved is rarely more than 0.5° F. (39.2°-39.7° F.) at the thirty foot level, representing the middle portion of the hypolimnion; and a poorly defined range of 0.1° F. (41.0°-40.9° F.) at the mud-water interface. While it can be shown that some metabolic properties of a lake bear a direct relationship to its thermal conditions, it can, in no way, be demonstrated that dicothermy or temperature inversion of the hypolimnion is a controlling factor for aquatic populations.

The second thermal characteristic of Sodon Lake to be discussed i.e., the permanent stagnation of the hypolimnion throughout the year, has, it seems to us, an important bearing on the bacterial populations and all other populations investigated during the course of this study. It has repeatedly been emphasized that the depth interval of 25 $\pm$ 5 feet delimits the zone of biological productivity of Sodon Lake. This statement must not be construed as including the activities of the anaerobic bacteria, for these organisms reach their maximum numbers at the mud-water interface. However, in respect to the possible influence

of the hypolimnion, if we consider that the 30 foot depth interval represents the middle portion of the hypolimnion, then there is conclusive evidence that the green bacteria, other members of the phytoplankton and the zooplankton reach their maxima near this hypolimnetic zone of demarkation. Reference to Table I will show that an almost constant temperature existed throughout the year at the 30 foot depth. However, considerable caution must be used in assigning a degree of importance to temperature as a regulatory factor for any distribution. Certainly, temperature may be expected to influence the metabolic activities of bacteria and may cause restriction as to the kinds present in a given environment; but temperature, per se, can influence a bacterial distribution only in that it may regulate the availability of materials for synthesis. It is disconcerting to read, in much of the available limnological literature, of the positive effects of temperature upon populations. All such statements should be made definitive by stating the definite role that temperature is thought to play. If food material is present for the growth of bacteria, a rise in temperature may accelerate metabolism but only while sufficient material is present for synthesis. Once this point is reached, the temperature may actually cause a diminishing of the bacterial population. With the exceptions indicated, it is entirely probable that the bacterial population is independent of the factor of temperature.

However, we look upon the demarkation zone of the hypolimnion as a convenient depth which represents an ecotene level; it is here,

probably, that the thermal influences of the shallow water and deep water merge and possibly offer a barrier to biological migrations.

Dissolved Oxygen

We have indicated, in the preceding paragraph that temperature, per se, probably has little, if any, effect upon the distribution of the green bacteria. However we must now consider the factor of oxygen depletion as a function of temperature and biological activity. It is extremely difficult, in light of present day knowledge, to do more than theorize concerning the absolute hypolimnial oxygen deficit. We are aware of the work of Alsterberg (1930), Hutchinson (1938) and of Kusnetzow and Karsinkin (1931); these investigators have attempted to explain the depletion of oxygen in the hypolimnion by various means. Alsterberg has proposed the microstratification theory which infers that the chief consumption of oxygen is in the bottom deposits; when these deposits are exhausted of oxygen, the depletion continues into the hypolimnion. Hutchinson computes the "real" oxygen deficit by adding to the apparent deficit the amount of oxygen necessary to oxidize such substances as methane and hydrogen sulphide which emanate from the bottom deposits and bubble through the hypolimnion. Kusnetzow and Karsinkin support the theory that the oxygen depletion in the hypolimnion is due to the oxygen consumption by bacteria. It is probable that no one theory can account for the anaerobic condition found in the hypolimnion; our data would offer some support to the theory of Kusnetzow and Karsinkin in that enormous numbers of bacteria are present

in the hypolimnion of Sodon Lake. However, to the effect of bacterial action must be added that of the hydrogen sulphide gas bubbling through the hypolimnion and thus removing quantities of oxygen.

It is not difficult to correlate our data on the distribution of the green bacteria with the observed hypolimnial deficit. The green bacteria are entirely anaerobic and we have not been able to stimulate the growth of these organisms with even minute traces of oxygen. Many of the other groups of bacteria which we have found in the hypolimnion while classified as "aerobic" may properly be designated as microaerophilic or facultative anaerobic and would therefore be able to survive for a time under strictly anaerobic conditions. These groups exhibit toleration ranges extending at least to the forty foot depth interval.

While we have demonstrated that, throughout the period of this investigation, an anaerobic condition has prevailed below the 25 ± 2 feet depth interval, it was thought that additional data in regard to anaerobiosis could be obtained if the oxidation-reduction potentials at various depths were known. It is generally conceded that the potentials existing in lake water and muds are oxidation-reduction potentials.

To this end, an apparatus for measuring Eh and pH, at any desired depth, has been designed by R. J. Harley, of the Bacteriology Department of Michigan State College. Essentially, the measuring apparatus consists of a Leeds and Northrup vacuum tube potentiometer; the required electrodes are embedded in Wards Bio-Plastic. The glass electrode for

pH readings, the platinum electrode for Eh determinations and the calomel half-cell are arranged as indicated in Figure 15. Each electrode wire is attached to one wire of a three conductor shielded waterproof cable 60 feet in length; the wires from the cable are connected to a Leeds and Northrup Assembly Unit, number 7663-Al. This potentiometer is equipped with a scale, calibrated in pH units; temperature corrections for the pH readings are made by a compensating dial on the panel board of the instrument. By means of a toggle switch, Eh measurements are made on the same scale as for pH measurements. All measurements of potentials are referred to the standard hydrogen electrode.

Determinations of Eh and pH were made in situ on March 11, 1950; at this time, an eight inch ice cover existed together with approximately seven inches of snow. At the surface 2.0 p.p.m. of dissolved oxygen were detected while the corresponding value at 20 feet was 1.0 p.p.m. A positive test for dissolved oxygen could not be obtained below the 25 foot depth interval. Hydrogen sulphide could not be detected in the upper layers of water but was present in values ranging from 2.0 p.p.m. at the 40 foot level to 7.5 p.p.m. at the 50 foot level. Ferrous iron was present in trace amounts. The pH range was from 8.5 at the surface to 6.5 at the mud-water interface.

The presence of the green bacteria could not be demonstrated. Therefore, in approaching a discussion of the results of our Eh measurements, we are considering a situation wherein the green bacteria either do not exist or their numbers are so small as to render them undectectable by conventional collecting methods.

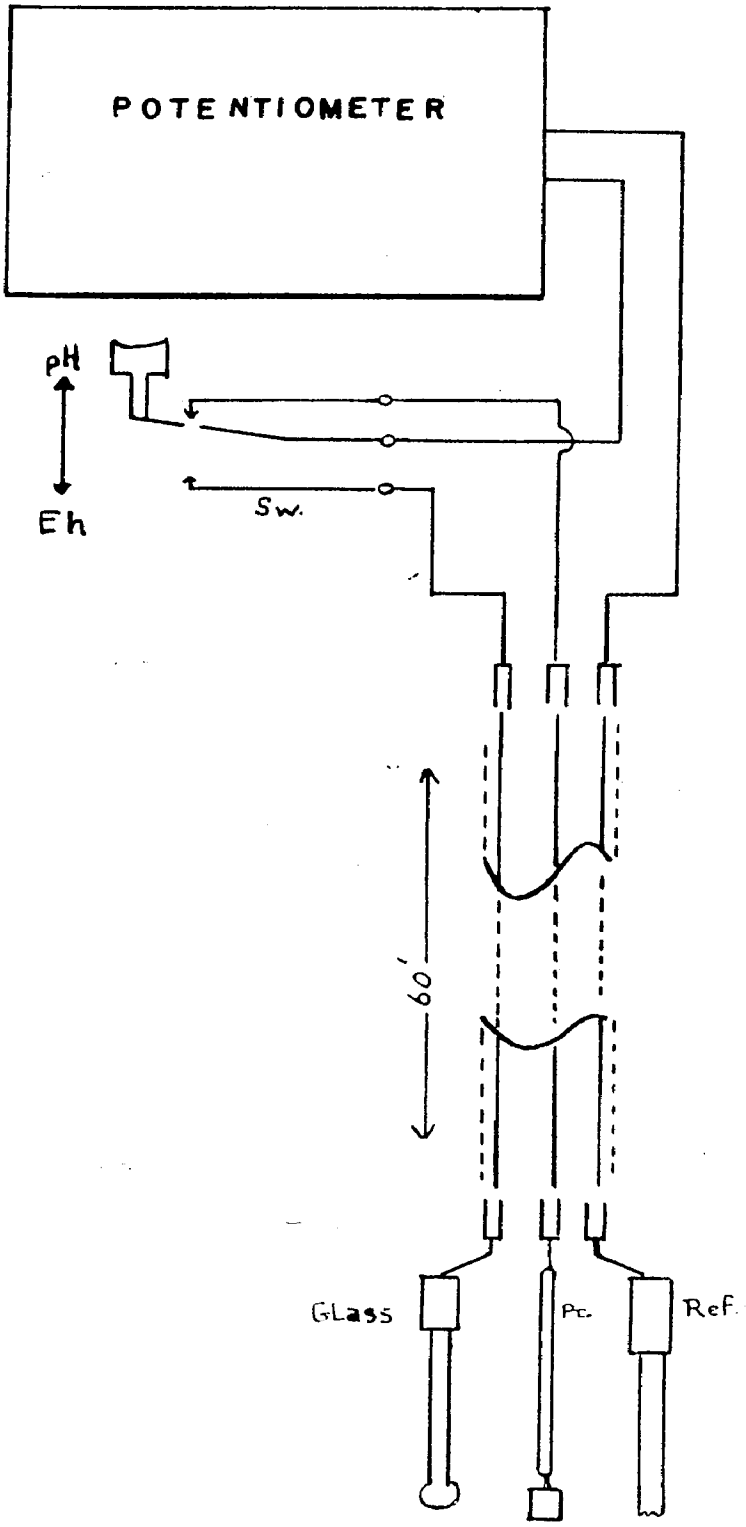
FIGURE 15

DIAGRAM OF VACUUM TUBE POTENTIOMETER

AND ASSOCIATED SUBMERSION ELECTRODE ASSEMBLY

FOR

MEASUREMENT OF EH AND pH



At the beginning of the anaerobic zone, we obtained an Eh of +0.2748 volts; this figure was relatively constant until a depth of 50 feet was reached. At the 50 foot depth interval, an Eh of +0.2558 volts was obtained.

The important question to be discussed is: "How is an electrode potential of a particular magnitude to be interpreted?" It is true that there have been several investigations involving the determination of potentials as they exist in natural waters. But the only valid conclusion to be drawn from the studies is that the potentials vary in different lakes. While quantitative methods should give results which may be interpreted directly, it seems that a static or controlled system must be present in order for the results to be meaningful. Much of the data which are available have come from studies on the laboratory cultivation of anaerobes; here, conditions are controlled insofar as is possible under laboratory cultivation. However, we must realize that even in a so-called "controlled state" not only is the oxidation-reduction potential changing but also the hydrogen-ion concentration and the chemical composition of the medium or, more properly speaking, the milieu is in a state of flux. If attempts are made to standardize conditions further as, for instance, by controlling pH, we will no longer be dealing with a natural system. Now if our attention is directed to a natural body of water as a culture medium for untold types and numbers of organisms, the problem and the interpretation of experimental data are raised to exponential heights.

In the last analysis, we can only raise questions concerning the Eh of natural waters. Perhaps further investigations along this line will be profitable. Some of the questions to be answered before positive interpretations of electrode potentials in a natural system can be made are: does the electrode potential represent the state of some particular reversible system or systems; does the potential represent a positive correlation with oxygen tension; and, does the potential represent an average of several systems, to some extent, in equilibrium with each other?

If it can be shown that there is a significant difference in electrode potentials when the green bacteria are present in detectable quantities, some light will be shed upon the conditions of existence of not only the green bacteria but also of the fauna and flora which co-exist with the green bacteria in the anaerobic zone.

The Presence of Hydrogen Sulphide

At no time during the course of this investigation has the hydrogen sulphide content of Sodon Lake been large and frequently a positive test for hydrogen sulphide could not be obtained. The annual range has been from 0 to 1.065 p.p.m. Some attention should be given to these rather small quantities. Comparatively large values for hydrogen sulphide in the immediate environment of the green bacteria, 0.1 grams per liter at a depth of 18.7 meters, 0.282 grams per liter, and 0.3 grams per liter at a depth of 43 meters have been reported by Szafer (1910), Nadson (1912) and Bavendamm (1924). It is rather hard to accept the

values cited above when compared with a demonstrable fact; namely, that in the zone of concentration of the green bacteria, the hydrogen sulphide is being converted into elemental sulphur. Thus there must necessarily be minute amounts of the gas in the immediate environment of the green bacteria. While our values for hydrogen sulphide increase with the vertical distance (an expected result) the concentration of the green bacteria does not increase with the vertical distance; in fact, the zone of concentration of the green bacteria is sharply delimited at the twenty-five foot depth. We are proposing here that the small amount of hydrogen sulphide found in the zone of concentration is the conditio sine qua non for the existence of the green bacteria. The dehydrogenation of hydrogen sulphide and the reduction of carbon dioxide with the resulting loss of oxygen creates an anaerobic condition without which the green bacteria cannot exist.

Light

There must be, under a given set of conditions, a critical value for light intensity which marks the point at which light becomes the limiting factor for growth of the green bacteria. A discussion of the intricacies of light evaluation is beyond the scope of this paper; it is certain that light measurements and interpretation of the resulting data belong in the hands of a competent physicist. We can only indicate here that a sufficient amount of light to activate a photochemical process does penetrate through the water of Sodon Lake to the upper limits of the anaerobic zone. Below the 25 foot level 0.0% transmission was always obtained; this value is the same for ice with snow, ice alone or water.

It may be of interest to cite some examples of light values obtained by various workers in aquatic investigations. Clark (1939) indicates that the compensation intensity i.e., that intensity of light which will activate a photosynthetic process and below which photosynthesis is overbalanced by the process of respiration, is approximately 30 to 40 foot candles. Wilson (1935) cites figures of 400 to 600 foot candles for the same set of conditions; and Greenbank (1945) states that the amount of light which will penetrate one and one-half feet of clear ice is sufficient for photosynthesis to take place. It is obvious that serious disagreements exist as to the quantity of light necessary to activate a photochemical process. Our own data show extremely low values for the photosynthetic zone, ranging from 1.5 to 1.0 foot candles.

The Chemical Constituents of the Water, Other than Dissolved Oxygen and Hydrogen Sulphide

Literature dealing with the biology of lakes abounds with tables of chemical determinations purporting to be accurate analyses of the chemical nature of aquatic environments. If this chemical literature is examined carefully, one undebatable conclusion must be drawn. This conclusion, which we shall later qualify by citing pertinent examples, is that many chemical data reported in the literature are untenable with what is known about chemical behavior.

A basic assumption in water analysis, considering now phenolphthalein-methyl orange alkalinity, is that the bicarbonate and hydroxide

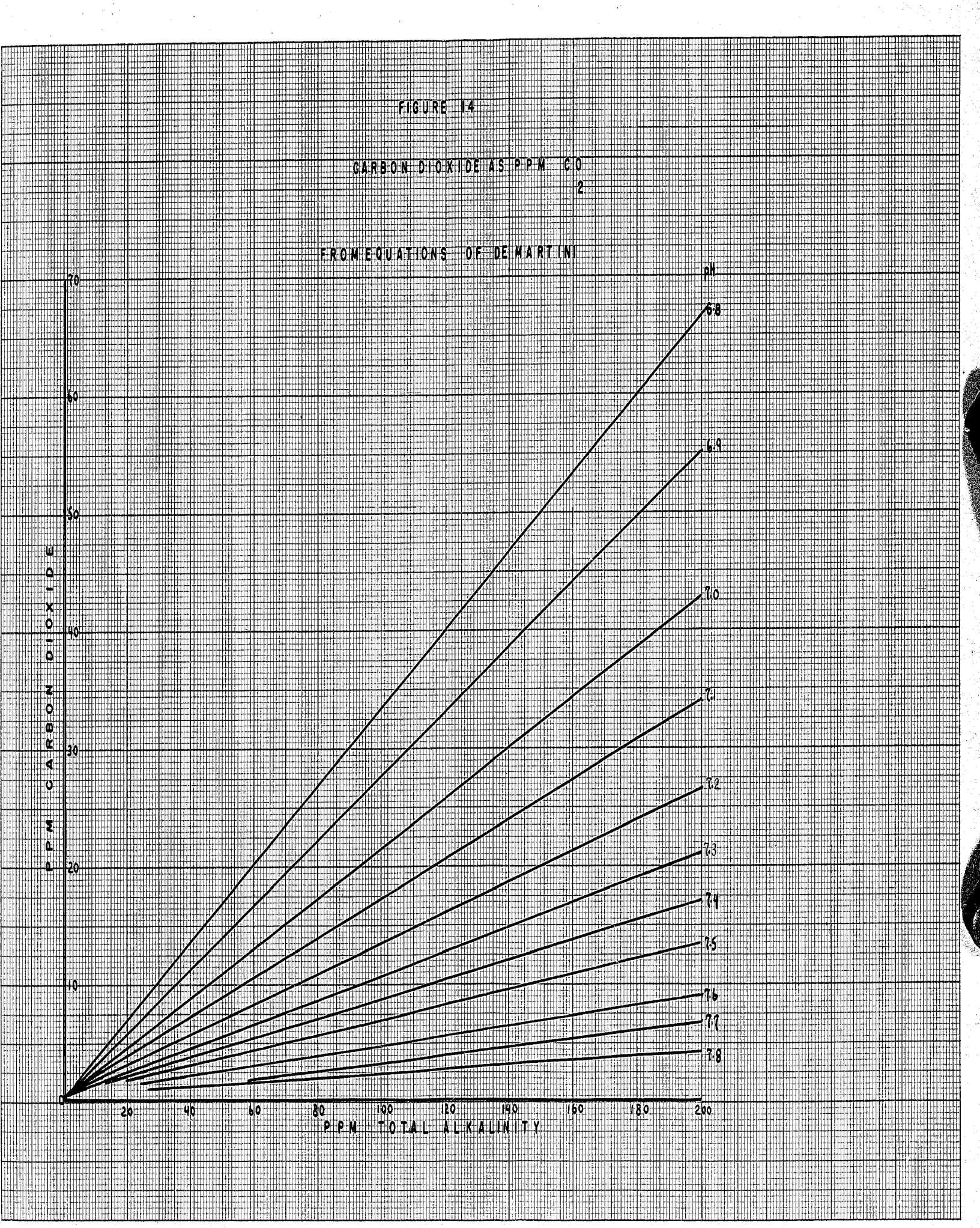
ions may not exist in the same water; this assumption is certainly not correct. DeMartini (1938) has called attention to this very obvious fact and also to the inadequacies of free carbon dioxide determinations. Again, the values obtained for hydroxide alkalinity computed by titration are not in agreement with the hydroxyl ion content as determined from the hydrogen-ion concentration of water. The suggestion is therefore offered that the carbon dioxide, bicarbonate, normal carbonate and hydroxide content of natural waters should be calculated from the pH and total alkalinity of these waters by means of equations based on physical chemistry. By this method the differences in individual techniques will be minimized and a more suitable rationale will be available for comparing the chemistry of different types of water. An examination of Figure 14 will show a physical chemistry approach to the determination of the relationship between pH, total alkalanity and carbon dioxide; this graph was constructed by using the equations of DeMartini, loc. cit. Similar figures can be constructed to show the relationship for bicarbonate alkalanity and normal alkalanity; and, knowing the pH and the total alkalanity, the desired component can be read directly from the chart.

In view of the attention currently being directed to a consideration of trace elements as limiting factors in aquatic environments, it should be emphasized that the standard methods of analyses as suggested by the American Public Health Association are not suitable for detecting small amounts of iron, sodium, magnesium, potassium and other elements; unfortunately, many of the reports in the literature are based

FIGURE 14

CARBON DIOXIDE AS PPM CO_2

FROM EQUATIONS OF DE MARTINI



upon Standard Methods. Some means which are suitable for the determination of the four elements listed above will be offered; these methods are adequate for waters which contain 150-250 parts per million of total solids.

Iron: The thiocyanate method as indicated in Standard Methods is not satisfactory here because such a procedure is not particularly sensitive. Among the more sensitive methods available from the literature are the mercaptoacetate, the orthophenanthroline and the salicyladoxime methods. Of these, only the mercaptoacetate method gives a color which is suitable for use in Nessler tubes. In addition, this method does not require concentration of the sample even at a concentration of 0.1 p.p.m. of iron. Anions do not affect the color and low concentrations of the heavy metals do not interfere.

Sodium: The chloroplatinate method for potassium and the calculation of sodium from the weight of the mixed chlorides is at least only a crude approximation when the sample contains only a few p.p.m. of the alkalies. The "triple acetate" methods are practical for such a condition; the technique of Barber and Kolthoff (1928) is recommended.

Magnesium: Kolthoff and Gillam (1941) use a colorimetric determination of magnesium by adsorbing titan yellow on precipitated magnesium hydroxide.

Potassium: When the picric acid test for potassium is negative, the method of Caley (1931) may be used.

It was decided to subject a vertical series of water samples from Sodon Lake to spectrographic analysis. These analyses were thought to

be necessary inasmuch as Newcomb (1950) has reported for Sodon Lake a biochemical stratification of soluble phosphorus, silica, calcium and iron. Neither our chemical analyses by routine procedures nor the results of spectrographic analyses support Newcomb's contention of chemical stratification in Sodon Lake.

The procedure involved in a spectrographic analysis can be summarized as follows: the sample is burned in an arc and spark stand by a high voltage spark. The light emitted by the sample is dispersed into a spectrum and photographed by a spectrograph. The lines on the plate are identified by a comparator and their blackness measured by a microphotometer. Jarrell-Ash equipment was used for the spectrochemical work, and the assistance of Dr. J. Bedell, President of the National Spectrographic Laboratories, is gratefully acknowledged.

Much of the data obtained are largely repetitious; this is an expected result inasmuch as Sodon Lake is known to exhibit an incomplete overturn. The results of the spectrographic analyses are indicated in Table XI.

The specific chemical conditions found in Sodon Lake are not particularly unique. Alkalinity is due entirely to bicarbonates which are present in large amounts. The quantities of free carbon dioxide are small as would be expected in a hard water lake. Nitrate nitrogen and phosphate phosphorous, the two ions given considerable attention as limiting factors for plankton production, are present in amounts equal to those reported for productive lakes. The elements reported as being present in trace amounts in Sodon Lake may be of great

TABLE XI

SODON LAKE - SPECTROGRAPHIC ANALYSIS*

Major	Minor	Low	Very Low	0.01%	0.001%
0.0141 g/l over 10%	1-10%	0.0041 g/l 0.1-1.0%	0.0004 g/l 0.1%	0.000041 g/l	0.0000041 g/l
Mg		B	Fe	P	Ni
Ca			Si	Al	Bi
				Cu	Cr
				Ti	Mo
				Mn	Co
				V	Pb
					Sn
					Na(trace)
					Ag Trace

* Based upon three liter samples taken at various depths.

importance to the organisms present or they may be of no importance. It is the contention of this author that, until information is available showing the amount of each available nutrient or element present in a lake and actually used by individual organisms, routine chemical determinations are of little value in explaining the compositional differences and distributions. The idea suggested may be impossible of execution and purely in the realm of theory; but it is hoped that scientific theorizing regarding some of the problems of lake biology may lead to new methods of approach.

It is not the purpose of this paper to discuss the chemistry of Sodon Lake and its possible effects on productivity. Rather, we are interested in knowing if the chemical composition of the water has some observable consequence upon the distribution of the green bacteria.

Mortimer (1939) has indicated that the chemical composition of water is approximately the same at all depths and varies but slightly with the seasons. Our investigations, involving the chemistry of the water of Sodon Lake over a period of one year seem to support Mortimer's contention. The conclusion is therefore reached that the fluctuations in the amounts of dissolved substances in the water give no clue as to the distribution of aquatic bacteria.

Biological Factors

It would be interesting if we could conclude from an analysis of our data that a significantly unique phytoplankton and zooplankton accompanied the distribution of the green bacteria. Such, apparently, is not the case. Daphnia pulex, Diaptomus oregonensis and Cyclops

prasinus (the three most common members of the zooplankton of Sodon Lake) are cosmopolitan in their distribution; these organisms do, however, under the environmental conditions present in Sodon Lake, exhibit an adaptive tolerance to minute amounts of oxygen and to measurable quantities of hydrogen sulphide. A total of 117 Daphnia pulex, 29 Diaptomus oregonensis and 45 Cyclops prasinus were collected at depths where a positive test for dissolved oxygen could not be obtained. It is planned, in the near future, to attempt duplication of the data on the copepod distribution under laboratory conditions.

The phytoplankton, while qualitatively restricted, does not seem to be peculiarly different from that of other hard water lakes. While a more detailed study of the phytoplankton will form the basis of another study, it is sufficient to state here that there are no indications of compositional differences between Sodon Lake and other small glacial lakes. The Bacillariae were perhaps the most numerous of the planktonic forms, both as to the number of species and the number of individuals. The term "selection biota", an expression denoting a group of organisms ordinarily widely distributed and able to withstand a variety of environmental conditions, is perhaps descriptive of both the phytoplankton and the zooplankton of Sodon Lake.

Taxonomical Considerations of the Green Bacteria

While it is possible that future research may disclose many distinct species of green bacteria, it is certain that, at present, the Chlorobacteriaceae represent a very artificial grouping. Only as

bacteriological information from a large number of aquatic habitats is obtained and analyzed, can meaningful advances be made. It is certain that pure culture studies must be carried on in chemically defined and reproducible media. While it is recognized that the laboratory cultivation of bacteria creates an unnatural environment (unnatural in the sense that such a method cannot recreate an ecological system) it is the only means by which control of the environment can be assured.

The type species of the green bacteria, Chlorobium limicola Nadson, is the only member of the Chlorobacteriaceae which we have isolated and grown in pure culture. A description of C. limicola follows: Cells: various shapes and sizes, markedly dependent upon environmental conditions; spherical to ovoid, about 0.5 u to 1.0 u in diameter; rods, generally 0.7 u by 1.0 to 2.5 u; regularly produce mucus, causing the formation of cell conglomerates of different size and shape, but not, as a rule, of characteristic appearance; color, yellowish-green; nonmotile; involution forms common; strictly anaerobic; apparently dependent upon hydrogen sulphide and light. No development in organic media.

As we have observed no green bacteria in our collections which cannot be placed in the genus Chlorobium, a discussion of other reported genera must be based upon descriptions existing in the literature.

The genus Pelodictyon Lauterborn and the genus Clathrochloris Geitler are supposedly distinguishable by the fact that the former includes green bacteria without intracellular sulphur globules; the genus Clathrochloris includes those green bacteria which possess intracellular sulfur globules. Both genera are described as occurring as

cell aggregates. Representatives of neither genus have been obtained in pure culture. The reported presence of intracellular sulfur globules in Clathrochloris sulfurica is inconsistent with all other published descriptions of the Chlorobacteriaceae; to date, C. sulfurica is the only genus described as containing intracellular sulfur granules.

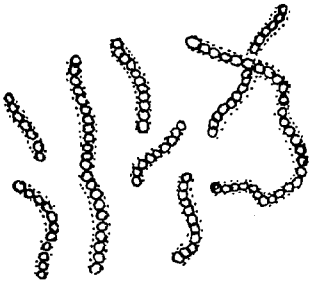
The genera Chlorobacterium Lauterborn, Chlorochromatium Lauterborn and Cylindrogloea Perfiliev seem to have no taxonomical legitimacy; these genera comprise those green bacteria found as symbionts with other organisms. The genera have been erected provisionally on the assumption that generic rank may be used to designate a stable complex. However, there is some evidence that the complexes mentioned above are not stable and hence the green constitutents should be assigned to either new or existing genera.

It is once more tempting to suggest that Chlorobium represents, insofar as present knowledge is concerned, the only legitimate genus of green bacteria. Pelodictyon may properly be an environmental variant of Chlorobium limicola.

Pringsheim (1949), commenting on organisms whose relationship with the bacteria and the blue-green algae is disputed, states: "The variability of van Niel's Chlorobium is remarkable..... This polymorphism surpasses that of every other microorganism and should be confirmed by thorough scrutiny". In Figure 16 the morphology of C. limicola as determined by van Niel, Skuja and the author is presented.

Figure 16.

Chlorobium limicola (various authors)



Chlorobium limicola after Skuja x 2000

Chlorobium limicola after van Niel
(from pure culture)

Chlorobium limicola camera lucida x 1900
(from pure culture)
Bicknell

It must be forcibly emphasized that the pleomorphism as shown by van Niel cannot be demonstrated from our pure culture studies; it is also obvious that C. limicola, as figured by Skuja shows none of the pleomorphism stressed by van Niel.

SUMMARY

1. The bacteriological study reported upon here represents one phase of an intensive limnological investigation of a small, glacial lake.
2. Various ecological factors were studied in an effort to determine the effect of an ecological complex upon the distribution of a specific member of the green bacteria i.e., Chlorobium limicola.
3. The following possible controlling factors thought to influence the distribution of green bacteria were investigated: temperature, pH, light and chemical components of the water.
4. The character of the euplankton was determined in an attempt to ascertain if a peculiar phyto and zooplankton accompanied the distribution of the green bacteria.
5. The absorption spectra of C. limicola were determined and compared with those of a green alga and a blue-green alga.
6. Electron microscope studies of the morphology of C. limicola were presented.
7. Pure culture studies of C. limicola were described.

CONCLUSIONS

The following tentative conclusions are offered with the realization that future research may cause some reversal of opinion:

1. Changes in temperature cannot be shown to correlate with the observed distribution of green bacteria.
2. The hypolimnetic oxygen deficit is thought not to be the primary reason for restricting the green bacteria to an oxygenless zone.
3. Changes in concentrations of dissolved and total solids probably have no effect upon the distribution under consideration; this statement also appears to be true in regard to the possible effects of slight changes in the chemical composition of the water on the distribution.
4. Light intensity, in that it restricts the depth of the photosynthetic zone, is a positive factor in the distribution of the green bacteria.
5. The presence of hydrogen sulphide, the subsequent dehydrogenation of this substance and the reduction of carbon dioxide with the creation of an anaerobic environment are probably the most important factors in determining the environment of the green bacteria.

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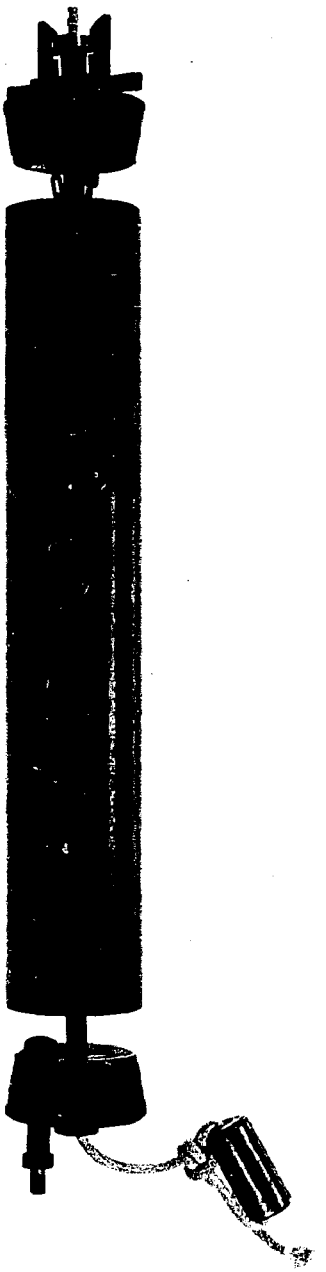
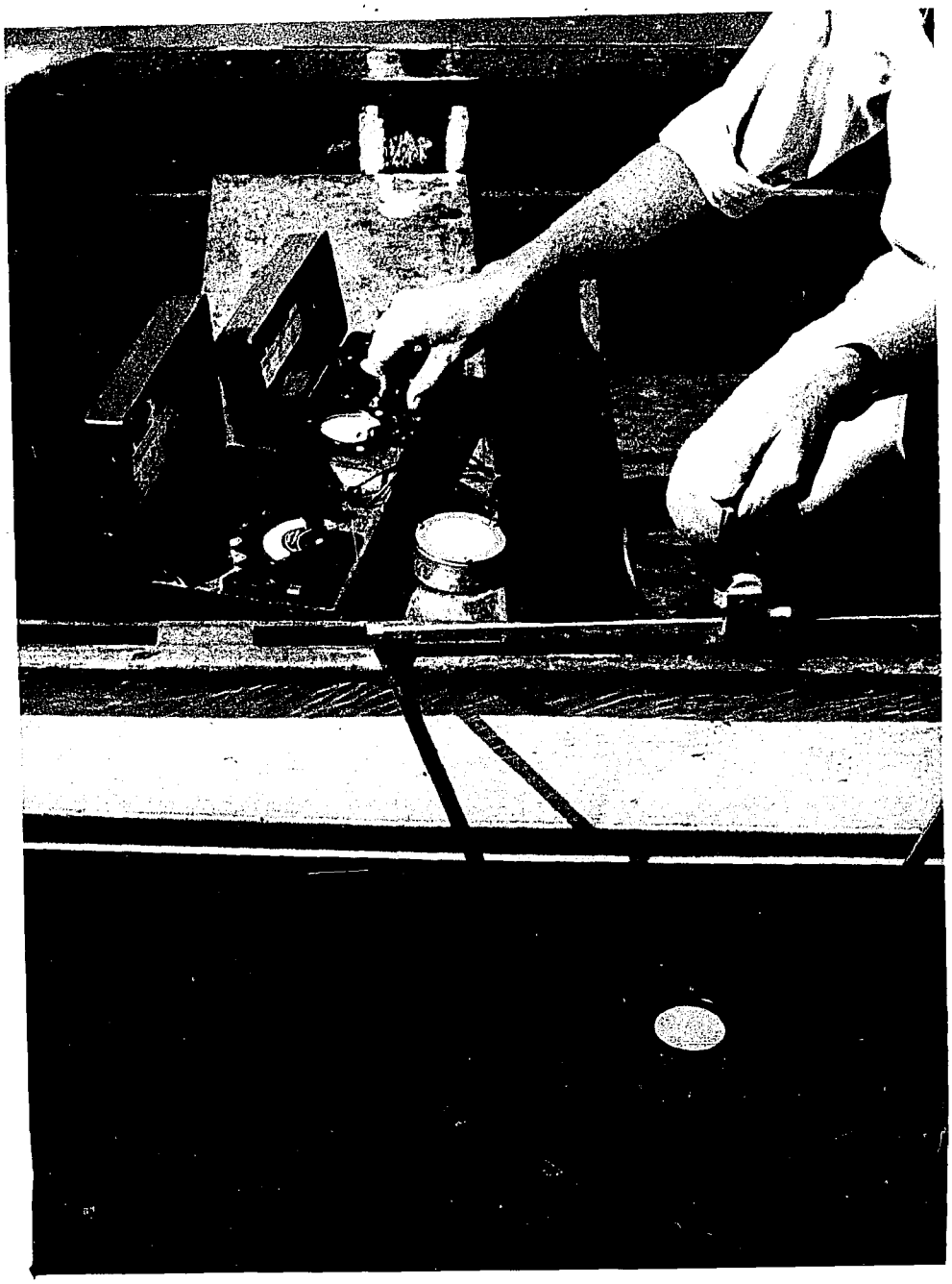
APPENDIX



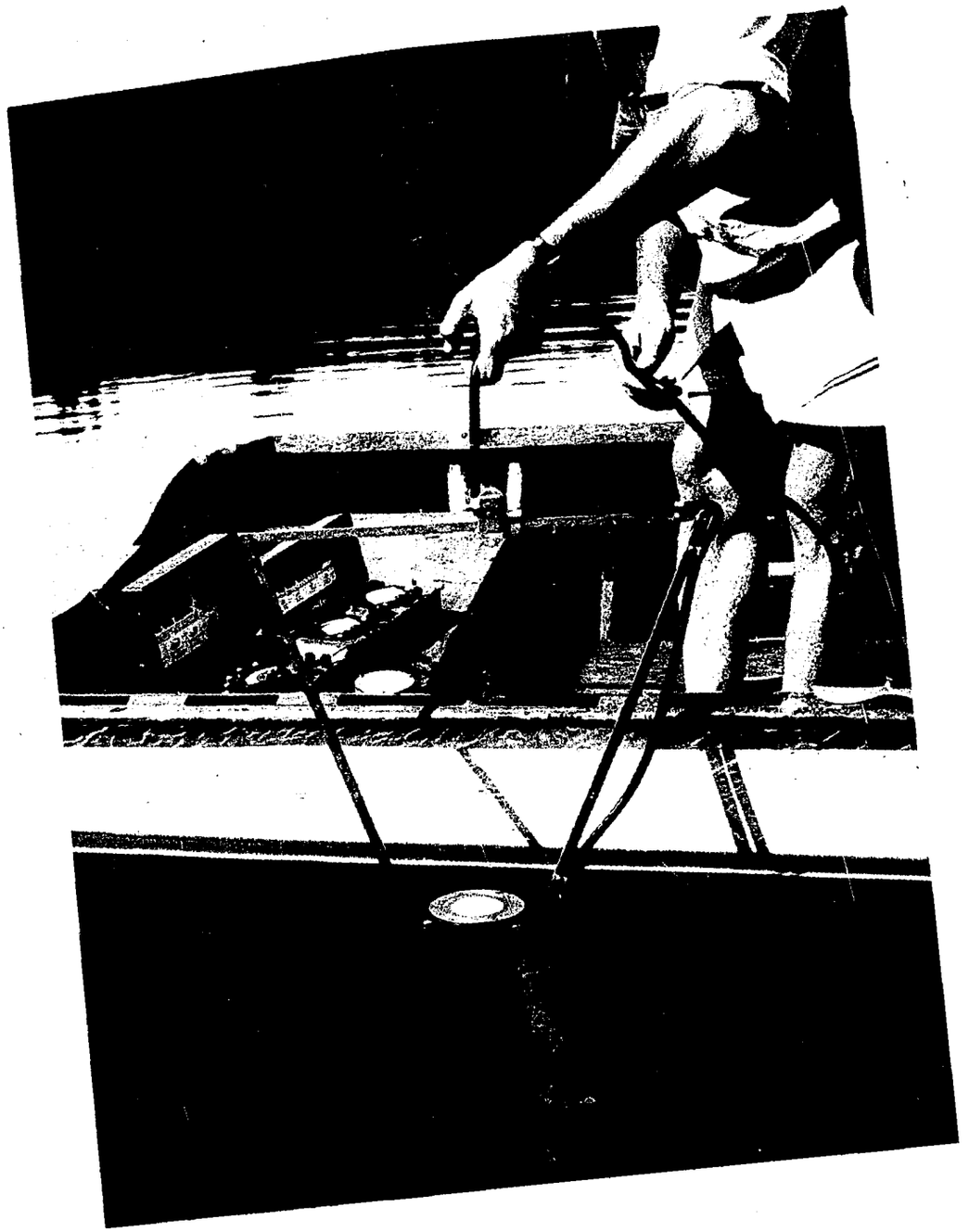
KEMMERER WATER SAMPLER

Sodon Lake with Ice Cover

Sodon Lake without Ice Cover



Submersible Photolometer



Submersible Photolometer

TABLE 1

SOLON LAKE WATER TEMPERATURES

STATION A DEGREES FAHRENHEIT 1949

Limits Of Thermocline Indicated By Black Line

Depth	1/22	2/5	2/19	3/5	3/19	4/3	4/16	4/30	5/14	5/28	6/11	6/25	7/9	7/23	8/4	8/20	9/3	9/17	10/1	10/15	10/29	11/5	11/12	11/26	12/10
0-1	36.4	32.1	35.6	39.6	39.6	46.2	47.7	58.2	67.0	61.2	73.4	79.5	83.0	79.4	80.9	75.0	70.5	65.5	59.7	60.4	52.9	48.7	48.9	40.0	34.7
1-2	38.5	36.5	39.0	39.6	39.2	46.2	47.8	58.0	66.6	61.1	72.5	79.2	82.5	79.2	79.0	74.5	70.5	65.3	59.5	60.4	53.8	48.5	48.9	40.0	38.2
2-3	38.5	38.5	39.5	39.6	40.1	46.2	47.8	57.6	66.4	61.1	71.7	79.0	82.2	78.4	78.0	74.5	70.4	65.3	59.2	60.4	53.6	48.6	48.9	"	38.4
3-4	38.6	38.6	39.5	40.1	"	"	47.7	57.0	63.9	"	71.6	78.8	82.0	"	"	"	70.3	"	"	"	"	48.5	"	"	"
4-5	38.8	38.9	39.0	40.3	40.5	45.8	47.7	56.3	63.2	61.0	71.2	77.6	81.9	78.2	77.5	"	"	"	59.1	"	"	48.4	48.1	"	"
5-6	"	"	39.0	40.3	"	45.8	47.6	55.5	62.1	60.9	70.3	74.9	81.6	78.2	77.2	74.5	70.3	"	5 9	60.2	53.7	"	48.1	"	"
6-7	"	"	38.8	40.0	"	45.6	47.5	54.1	60.7	60.8	68.8	71.2	80.9	77.6	77.2	74.0	70.2	"	59.0	"	"	"	48.1	"	"
7-8	"	"	38.8	39.6	"	45.2	47.4	52.9	58.3	60.9	66.3	67.5	79.1	76.3	76.9	74.0	70.2	"	59.0	"	"	"	47.7	"	"
8-9	"	"	38.6	39.4	"	44.8	47.1	50.7	55.6	59.7	64.7	62.4	74.0	73.0	76.4	74.0	70.2	"	59.0	"	"	"	47.7	"	"
9-10	"	"	"	39.2	40.5	44.6	46.3	49.6	53.0	56.6	59.3	60.0	69.4	69.3	72.2	72.5	70.2	"	"	60.2	"	48.4	"	"	"
10-11	"	"	"	39.2	39.9	44.4	45.9	48.1	50.8	53.3	57.4	57.5	64.7	65.4	69.0	72.0	70.2	"	"	60.1	"	48.4	"	"	"
11-12	"	"	"	39.1	39.7	43.7	45.3	47.2	48.7	51.1	54.4	54.1	59.6	61.7	65.0	67.5	68.5	"	"	60.1	"	48.3	"	"	"
12-13	"	"	"	39.0	39.4	42.7	44.2	45.9	47.2	49.6	52.9	51.3	56.8	58.6	60.3	64.5	66.5	65.0	"	60.0	"	48.3	"	"	"
13-14	"	"	"	38.9	"	41.7	42.5	44.7	45.9	47.7	49.2	48.7	53.6	54.0	57.2	60.5	62.1	62.5	"	59.4	53.6	48.2	"	"	"
14-15	39.0	"	"	38.9	"	40.7	41.4	43.6	44.7	46.2	47.1	46.8	50.9	51.2	55.0	57.5	56.0	60.5	58.9	58.7	53.6	48.2	"	"	"
15-16	"	"	"	38.9	"	40.2	40.7	42.9	43.9	44.2	45.6	45.0	48.6	48.9	52.5	54.0	55.7	57.9	58.5	57.8	53.6	48.2	"	"	"
16-17	"	39.0	"	38.9	"	40.1	40.1	41.7	43.1	43.2	44.2	44.0	46.7	46.5	49.5	52.0	53.4	55.4	57.1	56.6	53.6	48.2	"	"	"
17-18	"	"	"	38.9	"	40.0	39.8	40.9	42.3	42.4	43.5	43.2	45.4	45.0	47.0	50.0	51.2	53.5	54.7	55.0	53.6	48.2	"	"	"
18-19	"	"	"	38.9	"	39.9	39.7	40.5	41.5	41.9	42.9	42.6	44.0	44.1	45.1	47.5	48.4	50.2	53.1	53.0	53.6	48.2	"	"	"
19-20	"	"	38.6	38.9	39.4	"	39.6	40.3	41.1	41.5	42.2	41.7	44.0	43.0	44.0	46.5	46.1	47.7	49.1	51.0	53.7	48.2	47.1	"	38.4
20-21	"	"	39.0	39.1	39.4	"	39.6	40.0	40.7	41.1	41.4	41.2	42.4	42.1	43.0	44.5	44.7	46.0	47.0	48.7	51.7	48.2	47.1	"	"
21-22	"	"	39.0	39.1	39.6	"	"	40.0	40.5	40.7	41.1	40.9	42.0	41.6	42.4	43.0	42.6	45.0	45.4	46.4	50.2	48.2	47.1	"	"
22-23	"	"	39.0	39.1	39.6	"	"	39.9	40.4	40.5	41.0	40.6	41.5	41.2	41.9	42.5	42.9	47.9	44.1	44.9	47.7	47.5	46.7	"	"
23-24	"	39.2	39.0	39.4	39.6	"	"	39.9	40.3	40.4	40.8	40.5	41.1	40.6	41.4	42.0	42.3	42.0	43.5	44.0	46.2	44.1	45.8	"	"
24-25	"	39.4	39.2	39.4	40.0	"	"	39.9	40.2	40.3	40.7	40.5	41.1	40.6	40.7	41.5	41.8	42.0	42.9	47.2	45.2	43.2	44.1	38.9	"
25-26	39.7	39.8	39.5	39.7	40.0	"	39.7	39.9	40.2	40.2	40.5	40.5	40.6	40.2	40.7	41.0	41.3	41.5	42.2	42.4	44.3	42.6	43.1	38.9	"
26-27	40.1	40.0	39.6	39.8	40.4	40.2	"	39.9	40.2	40.2	40.4	40.5	40.7	40.2	40.7	41.0	41.1	41.4	41.6	41.5	43.1	42.1	42.6	38.9	"
27-28	40.2	40.2	40.0	40.0	40.5	40.3	"	39.9	40.2	40.2	40.3	40.5	40.7	40.3	40.7	40.5	40.9	41.1	41.4	41.1	42.6	41.7	42.0	38.9	"
28-29	40.2	40.2	40.0	40.1	40.5	40.2	"	39.9	40.2	40.2	40.3	40.5	40.7	40.2	40.7	40.5	40.7	41.0	41.0	41.1	42.1	41.3	41.6	38.9	38.4
29-30	40.2	40.2	40.0	40.1	40.5	40.2	"	39.9	40.2	40.2	40.3	40.1	40.5	40.2	40.7	40.5	40.7	41.0	40.9	40.9	41.5	41.1	41.0	40.3	38.4
30-31	40.2	40.2	40.0	40.2	40.5	40.1	39.8	39.9	40.1	40.1	40.3	"	"	40.0	"	"	"	41.0	40.8	40.7	41.3	41.1	41.0	4 1.9	39.6
31-32	40.2	40.4	40.0	40.2	40.5	40.1	39.8	39.9	40.1	40.1	40.3	"	"	"	"	"	"	40.7	40.6	40.6	41.3	41.0	41.0	41.7	40.6
32-33	40.3	40.5	40.2	40.2	40.5	40.4	39.8	39.9	40.1	40.1	40.3	"	"	"	"	"	"	40.7	40.5	40.5	41.3	40.8	41.0	41.6	40.7
33-34	40.3	40.5	40.2	40.2	40.6	40.6	39.8	39.9	40.1	40.1	40.3	"	"	"	"	"	"	40.7	40.5	40.5	41.3	40.8	40.5	41.4	40.7
34-35	40.4	40.5	40.2	40.2	40.7	40.6	39.8	39.9	40.1	40.1	40.4	"	"	"	"	"	"	40.5	40.4	40.5	41.0	40.7	40.5	41.3	40.8
35-36	40.4	40.5	40.4	40.3	40.7	40.5	40.0	40.0	40.2	40.2	40.4	"	"	"	40.5	"	40.4	40.4	40.4	40.5	41.0	40.7	40.5	41.3	40.8
36-37	40.5	40.6	40.4	40.4	41.1	40.5	40.0	40.0	40.2	40.2	40.4	"	"	"	"	"	"	40.4	40.4	40.5	41.0	40.7	40.5	41.3	40.8
37-38	40.5	40.6	40.4	40.6	41.0	40.7	40.0	40.1	40.2	40.2	40.4	"	"	"	"	"	"	40.4	40.4	40.5	41.0	40.7	40.5	41.3	40.8
38-39	40.5	40.6	40.5	40.6	41.0	40.7	40.0	40.1	40.2	40.2	40.4	"	"	"	"	"	"	40.4	40.4	40.5	41.0	40.7	40.5	41.3	40.8
39-40	40.7	40.6	40.6	40.6	41.0	40.8	40.0	40.1	40.2	40.2	40.4	"	"	"	"	"	"	40.4	40.4	40.5	40.8	40.7	40.5	41.3	41.0
40-41	"	40.6	"	"	"	40.5	"	"	40.3	40.3	"	"	"	"	"	"	40.5	"		"	"	"	"	"	"
41-42	"	40.6	"	"	"	40.4	"	"	40.4	"	"	"	"	"	"	"	"	"		"	"	"	"	"	"
42-43	"	40.6	"	"	"	40.5	"	"	40.3	"	"	"	"	"	"	"	"	"		"	"	"	"	"	"
43-44	"	40.8	"	"	"	40.6	"	"	40.3	"	"	"	"	"	"	"	"	"		"	"	"	"	"	"
44-45	"	"	"	"	"	40.6	"	"	40.3	"	"	40.1	40.7	"	"	"	"	40.6		"	"	"	"	"	"
45-46	"	"	"	40.7	"	40.7	"	"	40.4	"	"	"	40.7	"	"	"	40.6	"		"	"	"	"	"	"
46-47	"	"	"	"	"	40.7	"	"	"	"	"	"	40.7	"	"	"	"	"		"	"	"	"	"	"
47-48	"	"	"	"	"	40.7	"	"	"	"	"	"	40.7	"	"	"	"	"		"	"	"	"	"	"
48-49	"	"	"	"	"	40.7	"	"	"	"	"	"	40.7	"	"	"	"	"		"	40.9	"	"	"	"
49-50	"	40.9	"	"	"	40.8	"	"	"	"	40.7	"	41.0	40.4	"	"	"	"		40.7		40.8	40.5	41.4	41.1
50-51	"	40.9	40.6	40.8	41.0	40.8	40.3	40.1	40.4	40.4	40.7	40.1	41.0	40.4	"	"	"	"		"	"	"	"	"	"
51-52	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"		"	"	"	"	"	"
52-53	40.8	40.9	41.0	40.8	"	"	"	"	"	"	"	"	"	"	"	"	"	"		"	"	"	"	"	"
53-54	40.8	41.0	41.0	40.8	41.0	40.8	"	"	"	"	"	"	"	"	"	"	"	"		"	"	"	"	"	"
54-55	40.9	41.0	41.0	40.8	40.9	"	"	"	"	"	40.7	40.1	41.1	40.8	40.5	"	40.6	40.6		40.9	40.9	40.8	41.5	41.2	41.3
55-56	41.0	41.0	41.0	41.0	41.1	41.0	40.4	40.4	40.7	40.7	40.7	40.4	41.1	40.8	40.9										
56-57	41.0	41.0	41.0	41.0	41.1	41.0	40.4	40.4	40.7	40.7	40.7	40.4	41.1	40.8	40.9	40.5	40.8	40.5		40.9	40.9	40.8	41.5	41.2	41.3
57-58	41.0	41.0	41.0	41.0	41.2	41.0																			
58-59	41.0	41.0	41.0																						
59-60	41.0	41.0	41.0	41.0	41.5		40.4																		