STUDIES OF FRESHWATER BACTERIA

IV. Seasonal Fluctuations of Lake Bacteria in Relation to Plankton Production[1]

ARTHUR T. HENRICI

Laboratories of Bacteriology at the University of Minnesota, Minneapolis, and at Station-on-Alexander, Cushing, Minnesota

Received for publication August 1, 1937

Inland lakes in the temperate zone undergo, through the year, a series of seasonal changes in physical and chemical characteristics dependent on changing temperature and light relationships. These changes in turn influence the quantity and kind of living organisms in the lake. Such changes in the life of inland lakes have been well studied so far as the larger organisms are concerned. But, although a number of studies of lake bacteria have been published, few of these have been carried on systematically over a period of time so that seasonal variations could be determined; and those papers which have reported on seasonal variations have been quite inconclusive.

Early studies were made at Lake Zürich by Kleiber (1894), Pfenniger (1902), and Minder (1920). Kleiber studied especially the fate of the large numbers of bacteria brought into the lake by inflowing streams. He noted that these had largely disappeared at a distance of 20 meters from the mouth. Pfenniger found that stream contamination affected the surface more than the deeper water. He reported the numbers of bacteria to be minimum during the summer stratification. The highest counts were obtained during the autumn following the death of plankton organisms. Minder confirmed the observation of Pfenniger, finding the highest bacterial counts in March and November, the lowest in midsummer. He believed that the summer mini-

[1] Aided by grants from the Graduate School of the University of Minnesota, and from the National Research Council.

mum was due to the lethal action of sunlight, and that food supply had little influence.

A summer minimum was also noted by Ruttner (1932) at Lake Lunz, and by Graham (1934) at Flathead Lake, with spring and autumn maxima. It is to be noted that all of these lakes are mountain lakes of the oligotrophic type. Ruttner believed that the turnover of the lake was responsible for the spring and fall maxima. Graham considered that autumn rains, and the melting of mountain snow in the spring, increased the bacteria by washing in material from the surrounding land.

The most extensive studies of seasonal fluctuations of lake bacteria have been made by Fred, Wilson and Davenport (1924) who reported observations on Lake Mendota, a stratified eutrophic lake of glacial origin in Wisconsin. Plate counts were made at regular intervals over a period of three years, and the results were found to vary considerably from year to year; there was a summer maximum in 1920, a fall maximum in 1921, and a spring maximum in 1922. They discuss the complexity of the factors affecting the numbers of bacteria in lake water, and emphasize the importance of rainfall. Lake Mendota is a drainage lake, and the bacterial counts are apparently considerably affected by the washing in of bacteria from the soil.

From this brief review of the scant literature it will be seen that such physical factors as sunlight, surface drainage, the stirring up of the water at the time of spring and fall turnover, and the summer stratification, have been considered more important in determining the seasonal fluctuations of lake bacteria than the production of organic matter by the plankton. It is true that the influence of the death of plankton in the water has been emphasized by Pfenniger (1902), Biega (1906) and Kolkwitz (1911), but their opinions are not well supported by the available data.

The observations here reported were made on a lake which does not stratify, and which was singularly free (during the period of observation) from surface drainage.

Lake Alexander is a hard-water, shallow, highly eutrophic lake, located in Morrison County near the center of Minnesota. It is

approximately 4½ miles long and 2 miles across in the widest portion, with a maximum depth of 15 meters and an estimated mean depth of 9 meters. It is usually frozen from the middle of November to the middle of April, and does not stratify in the summer because it is so shallow and subject to strong wind action. It is a lake of the so-called "spring-fed" or seepage type, receiving drainage from a few small brooks only, none of which is more than 2 miles long. The observations here recorded were made during a year (1933) of very low rainfall. All of the inflowing brooks were completely dry throughout the year, and the lake level dropped about one meter. Consequently the washing of bacteria from the surrounding land into the lake was a negligible factor.

A float was anchored at the point marked *a* on the accompanying map (fig. 1). This point (Station 1) was chosen as being sufficiently far from shore to be truly representative of the open lake, yet convenient to the field laboratory. A second float, *b*, was anchored at a distance 50 meters from *a*, and a third one, *c*, at 300 meters. These were maintained in position throughout the period of observations, which were made at approximately weekly intervals from the time the ice went out in the spring until shortly before the freeze-up in the fall.

Six kinds of data were recorded, viz. the temperature of the water, the quantity of net plankton, the number of bacteria per cubic centimeter of water cultivable in an artificial medium (plate counts), the number of bacteria per square millimeter per day deposited on immersed glass slides (periphytic bacteria), the number of bacteria per cubic centimeter of the net plankton as determined by plate counts, and the number of bacteria per cubic centimeter of net plankton as determined by direct microscopic counts.

The temperature was determined by holding a laboratory thermometer about 0.5 meter below the surface until it came to equilibrium, and reading immediately.

The quantity of plankton was determined by towing a "Turtox" plankton net, conical in form, 24 cm. in diameter and 88 cm. long, of 20-mesh silk bolting cloth. This was towed for 300 meters (from *a* to *c*) when the plankton was scant, 50 meters

(from *a* to *b*) when it was abundant. The quantity of plankton has been recorded on the 50 meter basis, being divided by 6

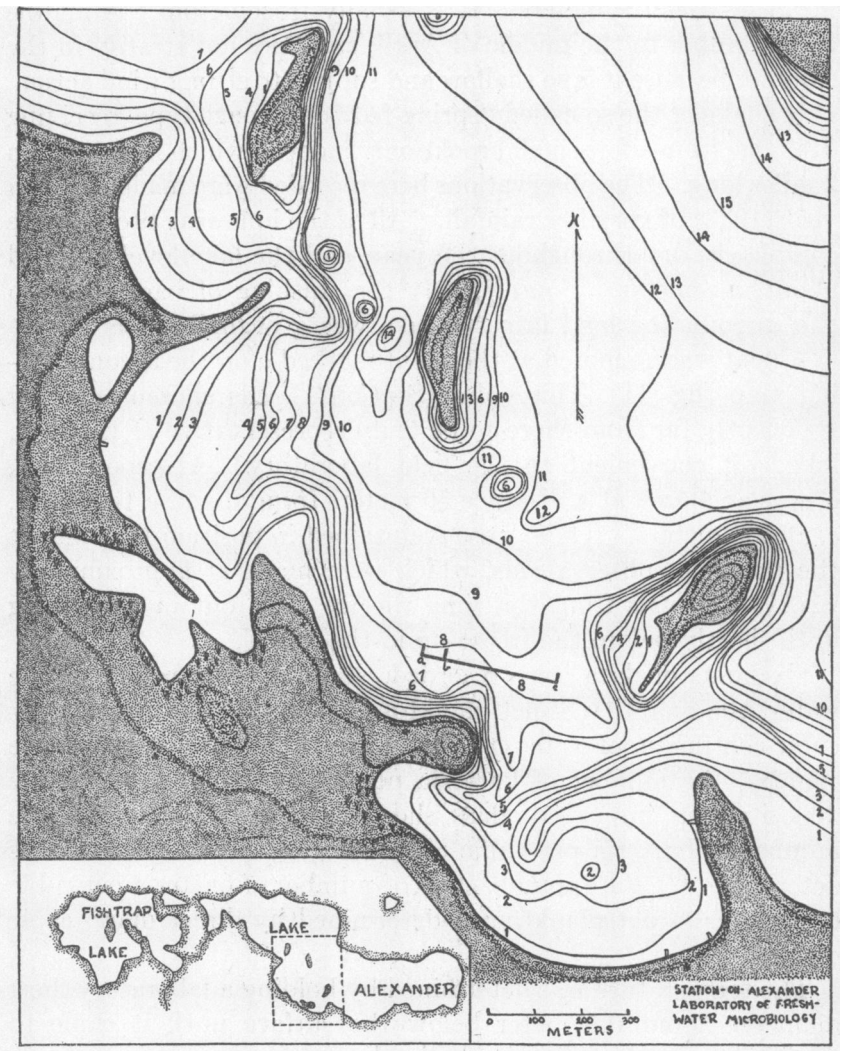

FIG. 1. MAP OF LAKE ALEXANDER, SHOWING LOCATION OF STATION WHERE DATA WERE COLLECTED

when the longer tows were made. Every effort was made to row the boat at a constant rate, so that the net, about 10 meters

behind, would sink to a depth of approximately 1 meter. The plankton was collected in vials attached to the apex of the net, and immediately transferred to a 4 per cent formaldehyde solution. On reaching the laboratory this was made up to a volume of 100 cc. and stored in a tightly stoppered bottle. At the end of the season all of the samples were transferred to 100 cc. graduated cylinders, also stoppered, and allowed to sediment for two weeks, at the end of which time the volume of plankton was read.

The method used for collecting and measuring the plankton is admittedly crude. The largest error is due to the fact that the different types of plankton organisms vary in the degree to which they form a compact sediment. The filamentous blue-green algae which formed the bulk of the plankton during the summer make a much less compact sediment, and therefore the relative quantities are somewhat exaggerated during this period. But the plankton measurements were made only for comparison with the bacteriological observations, and are, in the author's opinion, sufficiently accurate to show the trends.

Immediately after collecting the sample for measuring the plankton, the apex of the net was tied shut, and the net was towed over the same course. The net was held up to drain, and allowed to continue draining until the laboratory was reached, when the apical opening was untied, and samples of the compact net plankton were removed for bacteriological analysis.

A 5 cc. sample was removed by means of a 10 cc. graduated pipette with an opening approximately 4 mm. in diameter. This was transferred to a large bottle containing 95 cc. of sterile tap water and glass beads. After thorough shaking of this 1:20 dilution, further dilutions were made with 99 cc. water blanks, and quantitative plate cultures made by the same procedure used for plating the water samples.

With a 1 cc. tuberculin syringe, the tip of which had been cut off, another portion of the compact net plankton was removed, and 0.2 cc. was discharged upon the surface of a 50 x 75 mm. microscope slide. This slide had a line ruled across it with a wax pencil 25 mm. from one end, marking off an area 50 x 50 mm. A similarly marked slide was inverted over the first one, and the

compact plankton was "worked" over the measured areas, by rubbing one slide over the other, much as one prepares slides of sputum, continuing the "working" until the plankton was nearly dry. In this way the larger plankton organisms were crushed, and the entire mass spread in a fairly uniform film over the two slides. These were fixed by heat, stained with crystal violet, and the number of bacteria determined by counting 50 fields on each of the two slides. From these direct microscopic counts the number per cubic centimeter of the concentrated plankton was computed.

An examination of fresh plankton, either unstained or (better) mounted in Amann's fluid with cotton blue, reveals the presence of large numbers of bacteria. These are either embedded in the slime surrounding plankton algae, or on the chitinous surface of plankton animals, or in independent zoogloeal masses embedded in their own gum. In the smeared slides as described, the plankton organisms are largely disintegrated, or crushed so flat that the individual bacteria may be readily seen.

Samples of water for plating were collected in test-tubes which had been drawn out to a capillary tip, exhausted, sealed and sterilized. They were held under the water at a depth of about 0.5 meter, the tip broken by hand, and taken immediately to the field laboratory where they were plated. The plating medium used contains 0.05 per cent each of peptone, sodium caseinate, glycerol, starch and dibasic potassium phosphate, with 1.5 per cent agar, in tap water. This was put up in screw-cap lotion bottles of 120 cc. capacity, 30 cc. of agar to a bottle. These bottles were used in place of Petri dishes, the agar being melted and inoculated in the bottle, which was then placed on its side so that the agar hardened in a thin layer. The bottles were incubated with the caps tightly screwed, at room temperature, in the field laboratory. They were held for 1 to 3 weeks, depending on the temperature, before counting. The colonies may be readily counted through the glass wall of the bottle, using a hand lens. All samples were plated in 1 cc. quantities, undiluted and diluted 1:10, making 5 replicate plates from each dilution.

The periphytic bacteria were determined by suspending slides

from the under surface of the paraffined wooden float at the point *a* shown on the map. In all cases, two 50 x 75 mm. slides were used for each observation, and counts of 50 fields were made on each of the two slides. The technique of fastening, staining, and counting the slides has been described in previous publications, (Henrici, 1933, 1936). The middle portion of these slides was about 10 cm. below the water surface. Slides were immersed for varying periods, depending on the amount of growth, but in most cases for three weeks.

These data are presented in graphic form in figures 2 and 3. The total plankton, plate counts of water bacteria, and slide counts of periphytic bacteria are grouped together in figure 2; they seem to vary together in a significant manner. The counts of bacteria in the concentrated net plankton are presented in figure 3, together with the temperatures.

It will be seen from figure 2 that the plankton was produced in three distinct pulses, the first reaching its peak about June 1. During this period the dominant plankton organisms were diatoms (*Melosira, Fragilaria, Tabellaria,* and *Asterionella*). These decreased rapidly during June, reaching a minimum at July 1. During this period the plankton became quite heterogeneous, various green algae, and Protozoa (*Volvox, Ceratium,* and *Dinobryon*) becoming prominent. During July, when the water was constantly warm, the plankton again increased markedly, this increase being due almost entirely to blue-green algae (*Microcystis, Anabaena, Lyngbya*) which reached a maximum early in August when the lake was "blooming," i.e., on still days the entire surface of the water was covered with a greenish scum.

This pulse of blue-green algae decreased rapidly during August, the period of decrease being marked by a great increase of microscopic crustacea, which also disappeared rapidly. As the temperature of the water began to fall during the latter part of August, there was initiated a third plankton pulse that continued through September. The dominant organisms were again diatoms.

The plankton pulses of Lake Alexander differ somewhat from those which have been reported from other lakes. In most

cases, plankton studies have been made on deep, stratified lakes. These usually show two maxima, obviously associated with the spring and fall turnover of the stratified water which leads to general aeration and a distribution of food elements from the bottom deposit into the upper layers of water. Such deep lakes do not usually bloom during the summer. The very pronounced

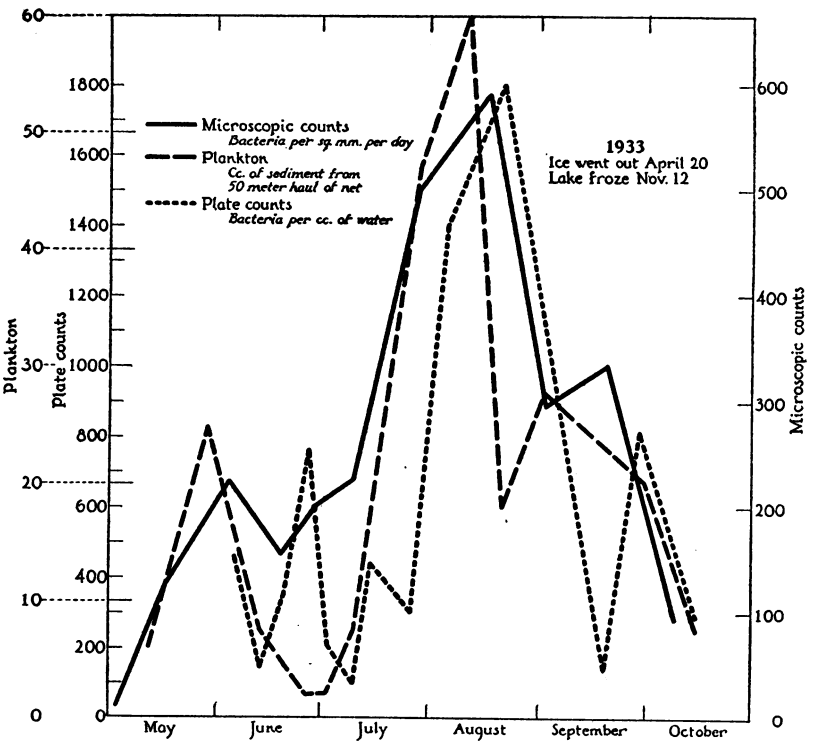

FIG. 2. SEASONAL FLUCTUATIONS OF TOTAL NET PLANKTON AND OF WATER BACTERIA IN LAKE ALEXANDER

midsummer plankton pulse of Lake Alexander may be due to the fact that the lake is not stratified, but is warmed and aerated to the bottom, and nutrient elements continuously circulated by wind action.

It will be seen from figure 2 that the curves for water bacteria also show three pulses during the year, which lag behind the

plankton pulse. The curve for the periphytic bacteria shows consistently a shorter lag than the curve for the plate counts of lake water. The amount of lag has also varied through the season. It must be remembered that the slides for the periphytic bacteria were immersed, on the average, for three weeks, the counts being plotted on the median date of the period of immersion. The curve does not, therefore, accurately indicate the time of greatest deposition of bacteria on the slides.

Since both curves for bacteria reproduce fairly well the curve for net plankton, these elements of life in the lake must be related, and the most obvious explanation of this relationship is that the plankton organisms provide organic matter which serves as food for the bacteria. Such a relationship has not been demonstrated in previous studies of lake bacteria because such studies have been made on lakes where the bacterial counts have been markedly affected by two other factors,—the semi-annual turnover which brings large numbers of bacteria from the bottom mud into the water, and the drainage of bacteria from the soil of the watershed into the lake.

The curves for bacteria in the concentrated net plankton are presented in figure 3. Since these represent bacteria per cubic centimeter of plankton, regardless of the volume of plankton in the lake water, it is not to be expected that they would follow the curves shown in figure 2. The bacteria trapped in the net plankton consist either of large zoogloeal colonies, or of smaller colonies attached to other plankton organisms. It was the author's impression, from microscopic studies of the wet plankton, that such bacteria were more abundant during midsummer when blue-green algae were dominant, but this impression is not supported by the actual counts. The very high peak in the curve for microscopic counts occurring at the beginning of June coincides with the dominance of the colonial flagellate, *Dinobryon*, in the plankton, the loricae and stalks of which were very heavily coated with bacteria. There is, almost throughout the series of observations, a curious negative correlation between the plate counts and the microscopic counts. This is probably accidental; at least the author can present no plausible explanation. Clumps

were very thoroughly broken up in preparing the smears, but probably not very well disintegrated by the shaking with beads preparatory to plating. While a study of the association of individual bacteria species with particular species of plankton

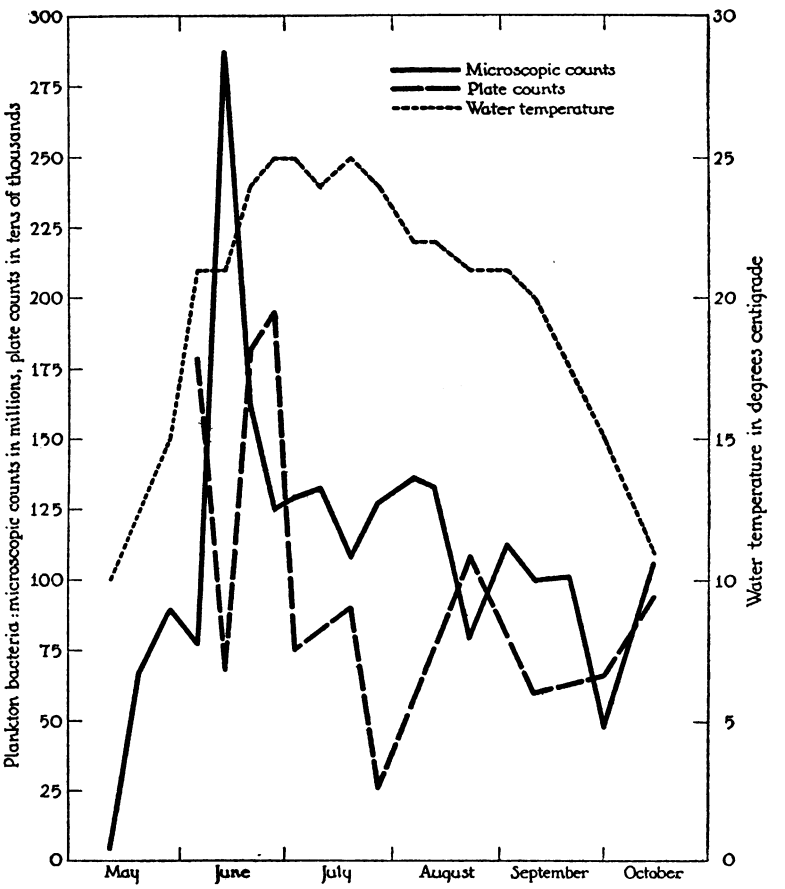

FIG. 3. SEASONAL FLUCTUATIONS OF WATER TEMPERATURE AND OF BACTERIA IN THE CONCENTRATED NET PLANKTON

organisms may prove fruitful, the general quantitative observations here reported do not seem to have yielded significant results.

SUMMARY

The quantity of total net plankton, plate counts of bacteria in the water, and microscopic counts of periphytic bacteria have

been determined in a shallow, unstratified lake of the seepage type, during a drought year when the lake received no surface drainage. Under these conditions it was found that the numbers of bacteria, as estimated by both methods, followed closely the curve for total plankton, with a lag which was greater in the case of the plate counts.

It is concluded that the production of organic matter by plankton organisms is an important factor in determining the number of bacteria in the water. Microscopic and plate counts of bacteria in the concentrated net plankton did not appear to fluctuate significantly through the seasons.

REFERENCES

BIEGA, M. 1906 Uber Wasserversorging aus dem Bodensee und die Beschaffenheit des Seewassers. J. f. Gasbel. u. Wasservers, **49**. (Quoted by Baier: Arch. f. Mikrobiol., 1935, **29**, 183–264).

FRED, E., WILSON, F., AND DAVENPORT, A. 1924 The distribution and significance of bacteria in Lake Mendota. Ecology, **5**, 322–339.

GRAHAM, V. 1934 A bacteriological study of Flathead Lake, Montana. Ecology, **15**, 101–109.

HENRICI, A. 1933 Studies of Freshwater Bacteria. I. A Direct Microscopic Method. Jour. Bact., **25**, 277–286.

HENRICI, A. 1936 III. Quantitative Aspects of the Direct Microscopic Method. Jour. Bact., **32**, 265–280.

KLEIBER, A. 1894 Qualitative und quantitative bakteriologische Untersuchungen des Zürichseewassers. Inaug. Diss. Zürich. (quoted by Fred, Wilson and Davenport).

KOLKWITZ, R. 1911 Zur Biologie der Talsperren, insbesondere der Eschbachtalsperre bei Remscheid. Mitt. Prüf.-Anst. Wasservers. (quoted by Baier).

MINDER, L. 1920 Zur Hydrophysik des Zürich- und Walensees, nebst Beitrag zur Hydrochemie und Hydrobakteriologie des Zürichsees. Arch. f. Hydrobiol., **12**, 122–194.

PFENNIGER, A. 1902 Beiträge zur Biologie des Zürichsees. Z. f. Gewässerkunde., **6**, 321. (Quoted by Fred, Wilson and Davenport.)

RUTTNER, F. 1932 Anhang zu "Beiträge zur Bakteriologie der Lunzer Seen," by A. Zih. Int. Rev. ges. Hydrobiol. und Hydrographie, **26**, 431–438.