A study on biological activity measurements and heterotrophic bacteria in a small freshwater lake

(9)

B. K. Tiwari & R. R. Mishra
Department of Botany, School of Life Sciences, North Eastern Hill University, Shillong 793014, India

Keywords: biological activity, heterotrophic bacteria, freshwater lake

Abstract

A 6-m-deep lake has been sampled to measure the temporal and depth-wise distribution of heterotrophic bacteria and biological activity in the water. Surface, mid-depth and bottom waters were analysed at monthly intervals for a period of one year. The coefficient of heterotrophic activity, alkaline phosphatase activity and biological oxygen demand are used as an index of biological activity. The bacterial community was at maximum during spring, coinciding with high values of biological activity. Highest biological activity was observed in the bottom waters. Dissolved organic carbon showed a significant positive correlation with most of the biological activity parameters. This suggests that biological activity, as measured by the coefficient of heterotrophic activity, was more closely related to the concentration of substrates than to population density of heterotrophic bacteria.

Introduction

Population estimates have been widely used as a measure of activity of microorganisms. However, many times they do not reflect the actual activity of the microbes (Strickland 1971). Populations of bacteria have been measured by direct microscopic counts and by plate counts. While the former does not reflect whether these cells are active or dead, the latter always underestimates the population, and the counts reflect certain ecophysiological groups which can grow on the medium used for culture. General awareness of this problem has increased and during the last two decades attempts have been made to measure the biological activity in various environments using enzymatic methods (Hoffman & Hoffman 1966; Lenhard 1967; Overbeck & Babenzien 1964; Reichardt *et al.* 1967; Reichardt 1971; Jones 1972a, b; Verstraete *et al.* 1976) and utilization of substrates (Azam & Holm Hansen 1973; Godlewska-Lipowa 1974a, 1976; Vaccaro & Jannash 1966; Wright & Hobbie 1965, 1966). Substrate utilization and enzymes activity measurements are the two methods most widely used to understand the involvement of microbial communities in the functioning of various ecosystems. Studies on heterotrophic activity of bacterial microflora (Wrgiht & Hobbie 1965; Vaccaro & Jannash 1966) have shown that bacteria assimilate organic substrates in relation to their concentration. Godlewska-Lipowa (1974a) measured the coefficient of heterotrophic activity by estimating the quantity of oxygen utilized by bacteria. She reported that the coefficient of heterotrophic activity is a direct measure of the degree of eutrophy and the process of destruction of organic matter in aquatic ecosystems.

Phosphatases are responsible for phosphate regeneration in aquatic environments. In such environments, phosphatases, first demonstrated by Steiner (1938), are produced by bacteria (Jones 1972a, b), algae (Brandes & Elton 1956) and zooplankton (Jansson 1976). Phosphatases may be associated with seston or exist as dissolved enzymes

Hydrobiologia 94, 257–267 (1982). 0018 8158/82/0943 0257/$02.20.

(Berman 1970; Jones 1972a; Overbeek & Babenzein 1964; Reichardt *et al.* 1967). The release of enzymes in soluble form has been often attributed to mineralization of planktonic organisms (Reichardt *et al.* 1967). Phosphatase activity has proved to be good indicator of degree of eutrophication (Jones 1972a).

The present paper takes into consideration various methods of biological activity measurements and relates them to physical and chemical characters of the water in time and depth. Three parameters, viz. alkaline phosphatase activity, coefficient of heterotrophic activity and biological oxygen demand (BOD), have been used to measure biological activity in the lake.

Description of the lake

The lake is man-made, built in 1893 and situated in Shillong (India) (altitude 1 460 m, latitude 25.34° N, longitude 91.25° E). The adjoining slopes of the valley are covered with dense managed grasses and scattered trees, predominantly pine. The lake receives water from an inflow throughout the year and has a controlled surface outlet which allows excess water to flow out during rainy months. Throughout the year the water level does not change appreciably except during January–February when it drops by approx. 30 cm. The bathymetric map and the general morphometry of the lake are given in Fig. 1. The sampling station is located 10 m from the shore at the deepest part of the lake where the bottom is silty with a cover of semi-decomposed plant litter, mainly pine needles. Secchi disk transparency during the study was 98 to 112 cm. *Chlorella* sp. and *Microcystis* spp. dominate the phytoplankton community. A phytoplankton maximum was observed during the month of March. The lake harbours a sizeable community of zooplankton and fish, and receives nutrients in the form of litter of adjoining vegetation during fall. The rainfall and temperature at the study area are shown in Fig. 2.

Materials and methods

Samples for bacteriological examination from 3 m and the bottom (6 m) were collected between 1 000 and 1 100 h using a JZ sampler (Zobell 1941).

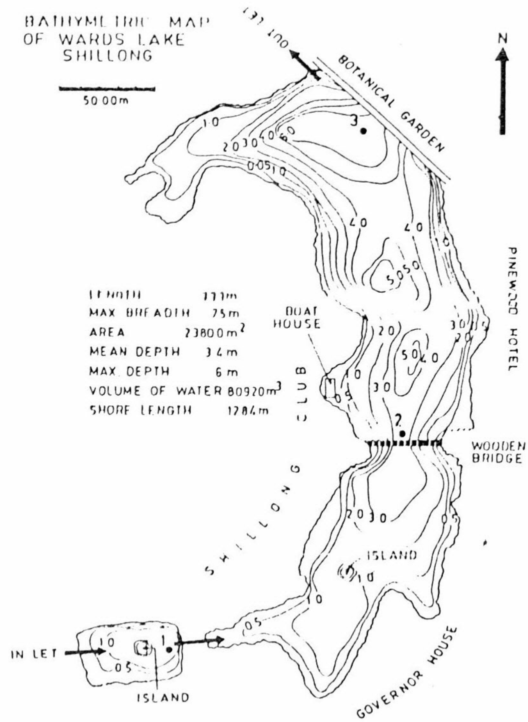

Fig. 1. Bathymetric map of the lake showing sampling station by closed circle marked by number 4.

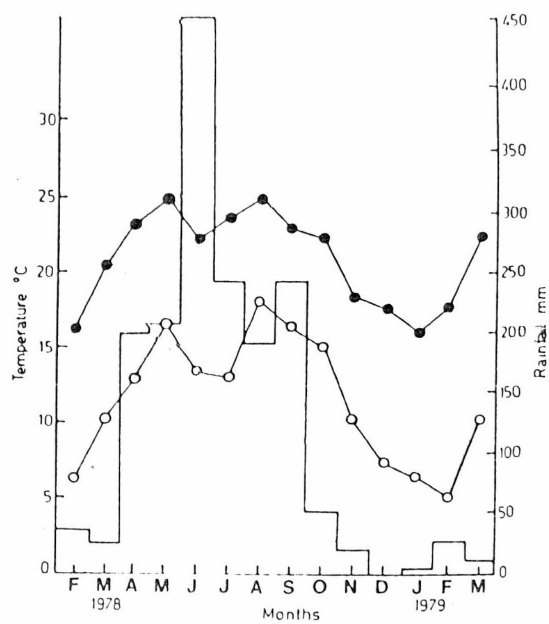

Fig. 2. Ombrothermic diagram of the study area. Rainfall: histograms, mean maximum temperature: closed circles and mean minimum temperature: open circles.

Surface samples were collected in sterile bottles, avoiding the surface film. Samples for all other analyses were collected using a 5-litre polythene Van Dorn bottle. Bottles were maintained in a dark chamber and transported to the laboratory within half an hour of sampling. Within 3 hours of collection the samples were inoculated for the estimation of viable bacteria by spread plate method. Casein peptone starch agar (CPS) (Collins & Willoughby 1962) was used for plate counts of bacteria. Aliquots of 0.5 ml in serial dilutions were inoculated to three replicates of partially dried medium. The plates were kept at 10 °C for 12 hours for absorption of the aliquot and incubated at 25 °C for 7 days. The plates having 30–300 visible colonies were counted. Direct microscopic counts were done following the method described by Sorokin & Kadota (1972) using 0.22 μm pore size millipore filters (Millipore Corporation, Massachussetts, U.S.A.).

The estimation of alkaline phosphatase followed the method of Verstraete *et al.* (1976), but reagents and distilled water were filter sterilized and aseptic precautions were observed. Soluble alkaline phosphatase was measured in the lake water filtered through 0.22 μm pore size millipore filter. Coefficient of heterotrophic activity was determined following Godlewska-Lipowa (1974). Lake water was filtered through a 40 μm pore-size sieve to remove phyto- and zooplankton. After measuring the initial dissolved oxygen concentration, the water was transferred to six BOD bottles. Bacteriological peptone (BDH) was added to three bottles to the concentration of 10 mg/l. All bottles were incubated at 20 ± 1 °C in a BOD incubator for 24 hours and dissolved oxygen concentration was measured. The coefficient of heterotrophic activity was calculated by dividing the oxygen consumption in lake water with peptone by the oxygen consumption in the lake water without peptone. The permanganate oxidizable dissolved organic carbon was measured as described by Gocke & Hoppe (1977). Estimation of soluble phosphate, nitrate, dissolved oxygen and BOD was done as outlined in Standard Methods (APHA 1971). Temperature was measured using a mercury thermometer of 0.1 °C accuracy. pH was measured with an electrical pH meter and conductivity with an Elico conductivity bridge.

Most sampling was done at weekly intervals; the data in the paper, however, are averaged per month. Simple correlation coefficients were used to test the relationship among the various factors.

Results

Population of bacteria

The population estimates of total and viable bacteria in the lake water are shown in Fig. 3. The total population of bacteria is 10 to 50 times the viable population, suggesting that only a fraction of total bacteria was estimated by the culture method. The trend of variation in total population and viable population was almost similar, which is evident from a highly significant correlation between the two parameters ($r = 0.99$; $p = 0.01$). In general, the bacterial population was high during the spring months (March–May) and declined to minimum during winter (December–January). During the other part of the year, the population remained almost stable with a slight increment in the late autumn (October–November).

Coefficient of heterotrophic activity

The coefficient of heterotrophic activity was highest during late spring (April–June) and lowest during winter (November–January). During the remaining part of the year the coefficient of heterotrophic activity was consistently higher in the bottom waters than at the surface and middle waters, except in August and September when it touched the lowest value. Besides distinct seasonality, marked variation was also observed at different depths. During winter the mid-depth water consistently harboured less heterotrophic ativity than the surface and bottom water (Fig. 4a).

Biological oxygen demand (BOD)

Biological oxygen demand showed distinct seasonal variation. It was maximum during spring and decreased with the onset of summer. Lowest values were recorded in November–January, followed by a slight increase in February. In general, the highest BOD values were associated with bottom waters. In most of the months, the middle waters had higher values than the surface but not during March, April, June and February (Fig. 4b).

Alkaline phosphatase activity

The alkaline phosphatase activity showed a distinct seasonality. The trend of variation at the three

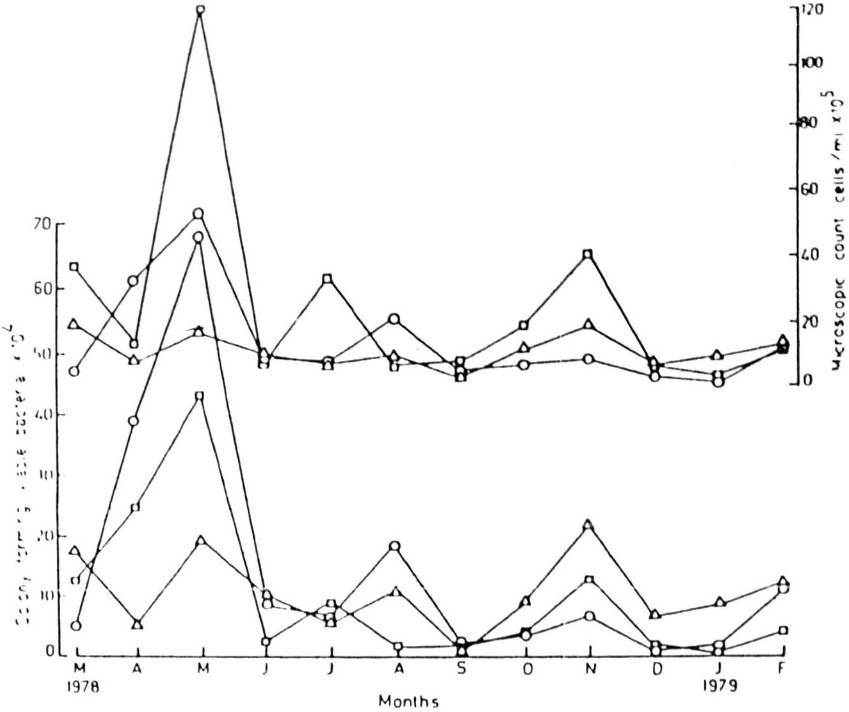

Fig. 3. Monthly variation in numbers of bacteria. Surface water: circles; mid-depth water: triangle; bottom-water: squares.

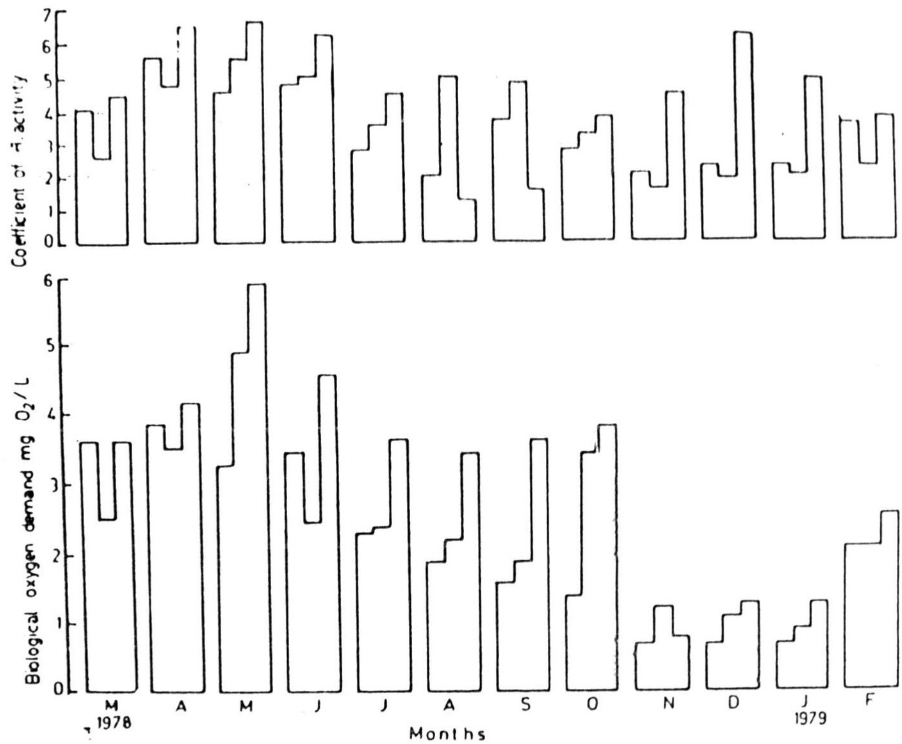

Fig. 4. (a) Monthly variation in the coefficient of heterotrophic activity. (b) Biological oxygen demand values for waters of three depths first bar for surface water, second bar for mid depth water and third for the bottom waters.

depths, however, did not follow the same pattern. While surface waters showed maximum activity during March–June, bottom water exhibited a maximum in November. Depth-wise, the activity decreased during March–June, after which the maxima tend to shift to bottom waters and from August to January, maximum activity was recorded at the bottom. Minimum activity was recorded in January and February. The soluble enzyme remained at fairly constant levels throughout most of the year except during winter when the values were comparatively low (Fig. 5).

Permanganate oxidizable dissolved organic carbon (PODOC)

In general, PODOC values were higher during spring and early summer (March–May), and declined subsequently. The minimum values were recorded during colder months (November–January), while during the remainder of the year a fairly constant level was maintained except for a peak in October, at mid-depth. Organic carbon was always highest in the bottom waters. The PODOC values increased with depth except in March, April and June (Fig. 6).

Soluble reactive phosphate

Soluble phosphate values also showed distinct seasonality. In general, higher values were recorded during spring and summer and showed a definite increase along depth. During winter, however, the pattern was either opposite (November, December, February) or it did not vary significantly (October, January). Throughout the year soluble phosphate remained at fairly high levels (Fig. 7).

Estimates of nitrate showed an interesting pattern of variation throughout the study period. A minimum was recorded in May and remained quite low during April–July. The higher values for nitrate were recorded during August–October (Fig. 7).

Dissolved oxygen (DO)

Throughout the study, dissolved oxygen remained fairly constant at the surface. Distinct vertical gradients are depicted in Fig. 8. The oxygen

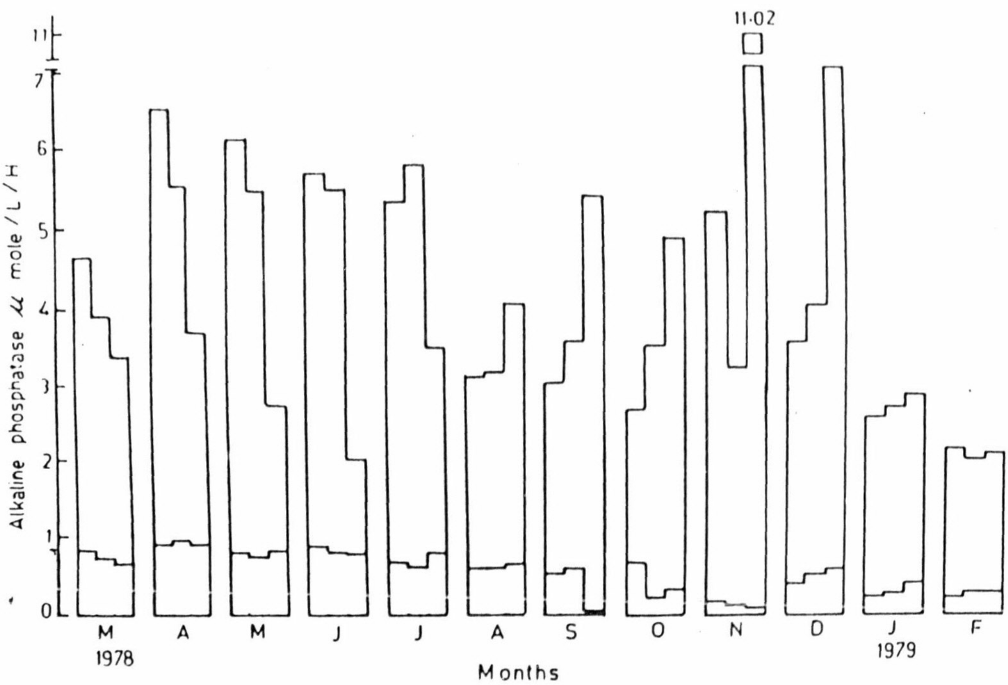

Fig. 5. Monthly variation in alkaline phosphatase activity for waters at different depths. Partition at base indicates the values for soluble alkaline phosphatase. Depths shown as in Fig. 4.

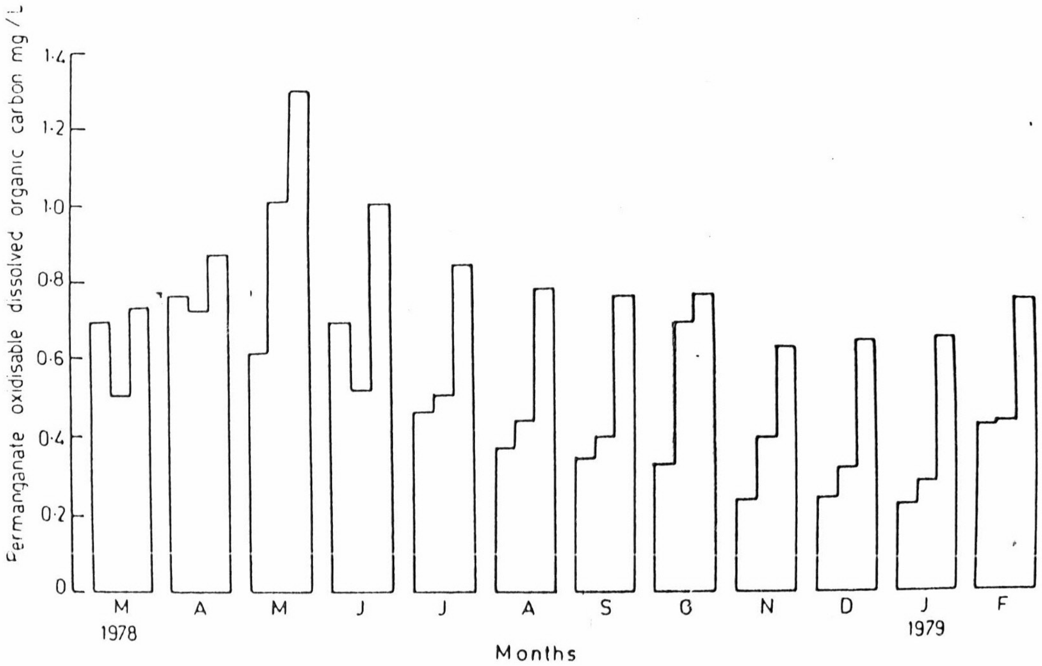

Fig. 6. Monthly variation in permanganate oxidizable dissolved organic carbon values. Depths shown as in Fig. 4.

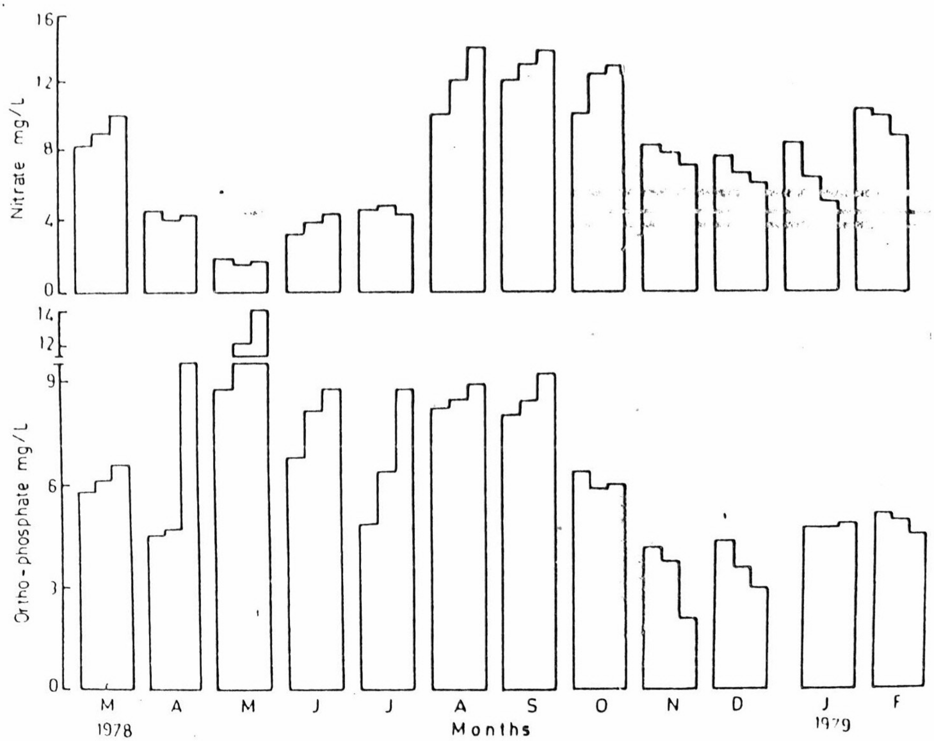

Fig. 7. Monthly variation in nitrate and orthophosphate values. Depths shown as in Fig. 4.

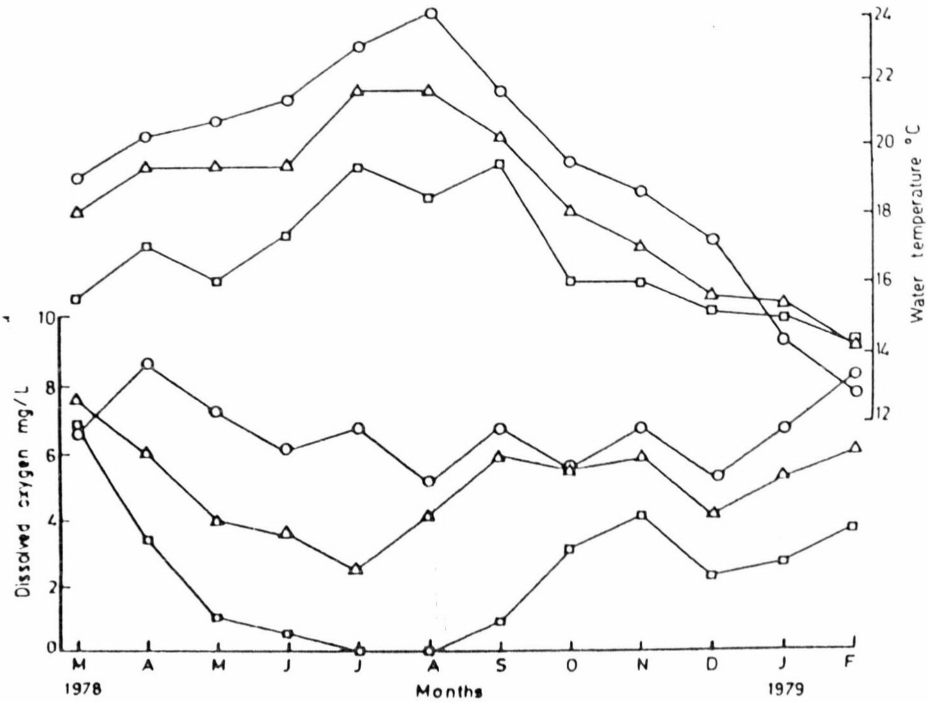

Fig. 8. Monthly variation in water temperature and dissolved oxygen values. Symbols as in Fig. 3.

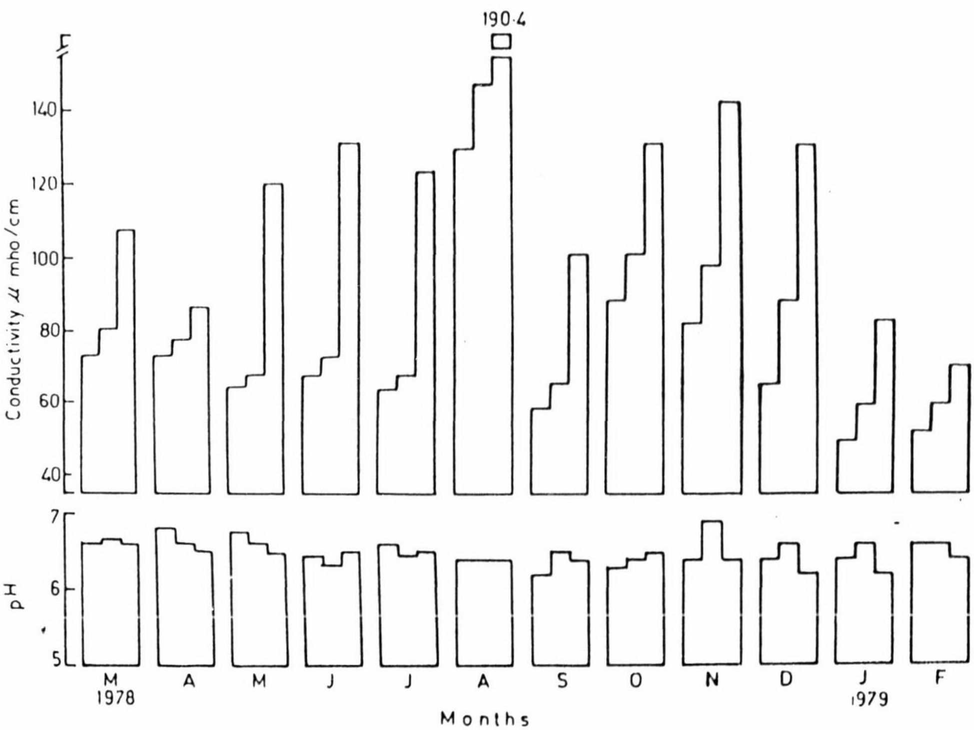

Fig. 9. Monthly variation in conductivity and pH values. Depths shown as in Fig. 4.

Table 1. Simple correlation coefficient values among various parameters.

	Microscopic count of bacteria	Viable count of bacteria	Biological oxygen demand	Coefficient of heterotrophic activity	Total alkaline phosphatase activity	Soluble alkaline phosphatase activity	Permanganate oxidizable dissolved organic carbon
Microscopic count of bacteria		0.9926**	0.5404	0.5201	0.5430	0.4500	0.5024
Viable count of bacteria			0.5505	0.5497	0.5754	0.4523	0.5178
Biological oxygen demand				0.8831**	0.6519*	0.8359**	0.69932**
Coefficient of heterotrophic activity					0.6015*	0.7072**	0.89501**
Total alkaline phosphatase activity						0.6157*	0.6903*
Soluble alkaline phosphatase activity							0.8344**

*, ** Significant at 0.05 and 0.01 p, respectively.

level at the bottom showed strong seasonal variation. It was fairly high in March and decreased steadily up to July. In July and August the bottom water was anoxic. Thereafter, re-oxygenation was noted and the value steadily increased until November, with a drop during December.

The water temperature varied between 12.8 and 24.0 °C. The bottom temperature remained quite low as compared to the surface and mid-depth waters. Single departure from this general pattern was observed in the month of February when the surface water was cooler than the bottom water (Fig. 8). Figure 9 shows that conductivity values increased with the depth. Conductivity was maximum in August and minimum in late winter (January, February). This also showed that the bottom waters were always rich in inorganic ions. pH did not vary appreciably and remained near neutrality throughout (Fig. 9).

These various variables, viz. dissolved oxygen temperature, conductivity, organic carbon and others, suggest that the lake remained stratified for most of the year and that mixing took place in January–February. Correlation coefficients are given in Table 1. A significant correlation was noted between permanganate oxidizable dissolved organic carbon and BOD, heterotrophic activity and alkaline phosphatase activity. A comparison of the depth-wise distribution pattern of ortho-phosphate and alkaline phosphatase activity shows a negative relationship between both (Figs. 5 and 7).

Discussion

On comparison of bacterioplankton population estimates by two methods, it appears that both methods depict similar trends of variation with depth and time. However, the depth-wise distribution of bacterial populations estimated by one method did not always correspond with the estimate obtained by the other method. This was particularly true with the low population estimates in the bottom water samples by plate count, such as in March, May, September, October and November, where the bottom samples contained maximum bacterial populations by the microscopic counting but not with the plate count method. This may reflect a difference in physiological state of bacteria in the bottom water. Indeed, this layer is anoxic for most of the year and rich in dissolved inorganic and organic nutrients. Therefore, the bacteria of the bottom waters might not grow well on a CPS medium when incubated aerobically. Thus, it may be concluded that the viable count gives an underestimation of the bacterial community, which is more pronounced in extreme environments.

The coefficient of heterotrophic activity corresponds well with bacterial population estimates and dissolved organic carbon values. Similarly, BOD estimates were generally in correspondence with the dissolved organic carbon values. It appears, therefore, that BOD values give an etimate of oxidizable organic carbon. The estimation of organic carbon by permanganate oxidation method underestimates the total value but its correspondence with BOD and other parameters of heterotrophic activity confirms that it is a good measure of biologically oxidizable organic carbon, at least in our experimental conditions.

Earlier studies on alkaline phosphatase have well established their role in phosphate regeneration (Jansson 1977) and as an indicator of eutrophication and microbial biomass (Reichardt *et al.* 1977; Jones 1972a). These and most of the other attempts to relate phosphatase activity with microbial biomass and other limnological characters have been based on short-term studies, except for those of Stevens & Parr (1977) and Health & Cooke (1975). During this study higher values of alkaline phosphatase activity were coupled with higher bacterial populations and other biological activity measurements during April–July, which was consistent with the observations of Jones (1972a). The second maximum in phosphate activity was observed in the bottom waters during November–December and associated with the lowest values of soluble phosphate concentration. It may, therefore, be inferred that during the period of high microbial activity (March–June) the phosphatase activity was not repressed by higher ortho-phosphate values as observed by Heath & Cooke (1975). But low values of soluble phosphates could trigger the enzymatic activity (November–December) in order to release phosphate from organic compounds. During the period of high phosphatase activity the depth-wise distribution of soluble phosphates showed a trend opposite to phosphatase activity, clearly depicting a negative relation between the two. The relationship between phosphate and phosphatase activity observed during this study is in conformity with work by Stevens & Parr (1977). Therefore, in natural waters, phosphorus limitations may enhance phosphatase activity but the repression of phosphatase activity by soluble phosphates may not occur if it coincides with high microbial activity. The absolute values of phosphatase activity often cannot be compared with other reports because of differences in units as well as methods employed. However, data of the present study are within the range reported by Heath & Cooke (1975). The depth-wise distribution of phosphatase activity suggests that during spring and warm periods of the year, more phosphatase was produced in the surface waters than in the bottom waters, largely by phytoplankton. Jones (1972a) also observed some increase in phosphatase activity in deeper water in late summer. Berman (1970) did not find any induction repression and/or activation of phosphatase in Lake Kinneret with soluble phosphate. Similarly Jones (1972b) did not find such a relationship and suggested that without a detailed survey, the control mechanism of enzymes could not be determined satisfactorily. Results also clearly depict that major portion of the alkaline phosphatase activity remains associated with particulate matter and only a small fraction of it was in soluble form (Fig. 5). Coefficient of heterotrophic activity has often been used as a measure of biological activity measurement (Godlewska-Lipowa 1973, 1974b). The present study revealed that, although correlated with the microbial population estimates, it was rather directly related to dissolved organic carbon concentrations. BOD has been the most widely used single parameter to estimate biologically utilizable organic matter. BOD values in this study were significantly related with the dissolved organic carbon. Although maximum and minimum values also corresponded to maxima and minima in bacterial populations, a general relationship was not observed. The variation of nitrate was related to its utilization by phytoplankton as evident from its lowest values during and after the phytoplankton peak.

Maximum biological activity as measured by the coefficient of heterotrophic activity as well as maximum microbial population levels were noted during spring, which may be ascribed to the mixing of the water column in February (clearly evident from the bottom oxygen level in March) resulting into a March phytoplankton bloom followed by a bacterioplankton bloom. The fresh input of extraneous nutrients in the form of plant litter during late February and early March from the adjoining vegetation may also have contributed to this.

It may be concluded that in shallow stratified lakes, distinct seasonal variations in the biological activity and microbial populations take place. Mixing during the end of winter results in a bloom of phytoplankton in March–April, followed by a bacterioplankton bloom in May. The spring blooms also coincide with maxima in alkaline phosphatase activity, coefficient of heterotrophic activity and BOD. All these variables were positively correlated (Table 1).

Summary

The temporal and depth-wise variation in planktonic heterotrophic bacteria, coefficient of heterotrophic activity, BOD and alkaline phosphatase activity, permanganate oxidizable dissolved organic carbon, soluble reactive phosphate, nitrate, pH, conductivity, dissolved oxygen, and water temperature have been measured in a small freshwater lake for a period of one year at monthly intervals. Distinct spring (March May) maxima and winter (November January) minima were observed in the community of heterotrophic bacteria and the biological activity estimates. On statistical analysis of the data, clear positive relationship among heterotrophic bacteria, coefficient of heterotrophic activity, BOD, alkaline phosphatase and permanganate oxidizable dissolved organic carbon has been noted. However, phosphate, nitrate, pH, conductivity, dissolved oxygen and water temperatures did not show any significant correlation with any of the other parameters measured. The bottom waters harboured higher biological activity. The data also suggest that the biological activity as measured by the coefficient of heterotrophic activity was more closely related to substrate concentration (dissolved organic carbon) than to the population density of heterotrophic bacteria.

Acknowledgement

We thank Dr. J. G. Jones, Freshwater Biological Association, Ambleside, England for critical review and helpful suggestions on the manuscript. This work was financially assisted by the University Grants Commission, New Delhi, India.

References

Azam, F. & Holm Hansen, O., 1973. Use of tritiated substances heterotrophy in sea water. Mar. Biol. 23: 191-196.

American Public Health Association, 1971. Standard Methods for the Examination of Water and Waste Water, 13th edn. APHA Association, Washington.

Berman, T., 1970. Alkaline phosphatase and phosphorus availability in Lake Kinneret. Limnol. Oceanogr. 15: 663 674.

Brandes, D. & Elton, R. N., 1956. An electron microscopical study of the histochemical localization of alkaline phosphatase in cell wall of Chlorella vulgaris. Nature 177: 274 275.

Collins, V. G. & Willoughby, L. G., 1962. The distribution of bacteria and fungal spores in Blelham Tarn with particular reference to an experimental overturn. Arch. Microbiol. 43: 294 307.

Godlewska-Lipowa, W. A., 1973. Heterotrophic activity of bacteria in the water of Mazurian Lakes of different trophic types. Pol. Arch. Hydrobiol. 20.

Godlewska-Lipowa, W. A., 1974a. Methods of microbiological investigations of water in the light of the hydrobiology. Pol. Arch. Hydrobiol. 21: 19 28.

Godlewska-Lipowa, W. A., 1974b. Heterotrophic activity of bacterial microflora in Mazurian lake of various trophy. Pol. Arch. Hydrobiol. 21: 51 58.

Godlewska-Lipowa, W. A., 1976. Effect of heated waters on biocenosis of the moderately polluted Narew river: microbiological characteristics. Pol. Arch. Hydrobiol. 23: 499 506.

Gocke, K. & Hoppe, H. G., 1977. Determination of organic substance and respiration potential. In: Rheinheimer, G. (Ed.) Microbial Ecology of a Brackish Water Environment, pp. 61-70. Springer-Verlag, Berlin.

Heath, R. T. & Cooke, G. D., 1975. The significance of alkaline phosphatase in a eutrophic lake. Verh. int. Verein. Limnol. 19: 959 965.

Hoffmann, E. D. & Hoffmann, G. G., 1966. Die Bestimmung der biologischen Tatigkeit in Boden mit Enzymenmethoden. Adv. Enzymol. 28: 365 390.

Jansson, M., 1976. Phosphatases in lake water: characterization of enzymes from phytoplankton and zooplankton by gel filtration. Science 194: 320-321.

Jansson, M., 1977. Enzymatic release of phosphate in water from subarctic lakes in northern Sweden. Hydrobiologia 56: 175 180.

Jones, J. G., 1971. Studies on freshwater bacteria: factors which influence the population and its activity. J. Ecol. 59: 593 613.

Jones, J. G., 1972a. Studies on freshwater bacteria: association with algae and alkaline phosphatase activity. J. Ecol. 60: 59-75.

Jones, J. G., 1972b. Studies on freshwater microorganisms: phosphatase activity in lakes of differing degree of eutrophication. J. Ecol. 60: 777 791.

Lenhard, G., 1967. Determination of protease activity in bottom deposite of sewage stabilization ponds. Hydrobiologia 29: 67-79.

Overbeck, J. & Babenzien, H. D., 1964. Über den Nachweis von freien Enzymen in Gewasser. Arch. Hydrobiol. 60: 107-114.

Reichardt, W., 1971. Catalytic mobilization of phosphate in lake water and Cyanophyta. Hydrobiologia 38: 377-394.

Reichardt, W., Overbeck, J. & Steubing, L., 1967. Free dissolved enzymes in lake waters. Nature 216: 1345-1347.

Sorokin, Ju. I. & Kadota, H. (Eds.), 1972. Techniques for the Assessment of Microbial Production and Decomposition in Freshwater. IBP Handbook No. 23. Blackwell Scientific, Oxford.

Steiner, M., 1938. Zur Kentnis des Phosphatkreislaufs in Seen. Naturwissenschaften 26: 723 724.

Stevens, R. J. & Parr, M. P., 1977. The significance of alkaline phosphatase activity in Lough Neagh. Freshwater Biological Investigation Unit Annual Reports, pp. 351 355. Department of Agriculture for Northern Ireland.

Strickland, J. D. H., 1971. Microbial activity in aquatic environments. In: Hughes, F. D. & Rose, A. H. (Eds.) Microbes and Biological Productivity, pp. 231–253. Cambridge University Press.

Vaccaro, R. F. & Jannash, H. W., 1966. Studies on heterotrophic activity in sea water based on glucose assimilation. Limnol. Oceanogr. 11: 596–607.

Van Dorn, W. G., 1956. Large volume water samplers. Trans. Am. Geophys. Union. 37: 682–684.

Verstraete, W. J. P., Voets, J. P. & Vanlancker, P., 1976. Evaluation of some enzymatic methods to measure the bioactivity of aquatic environments. Hydrobiologia 19: 257–266.

Wright, R. T. & Hobbie, J. E., 1965. The uptake of organic solutes in lake water. Limnol. Oceanogr. 10: 22–28.

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

Zobell, C. E., 1941. Apparatus for collecting water samples from different depths for bacteriological analysis. J. mar. Res. 4: 173–188.

Received 17 June 1981; in revised form 26 November 1981.