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Bacterioplankton abundance and activity in a small hypertrophic stratified lake

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Abstract

Bacterioplankton abundance and production were followed during one decade (1991–2001) in the hypertrophic and steeply stratified small Lake Verevi (Estonia). The lake is generally dimictic. However, a partly meromictic status could be formed in specific meteorological conditions as occurred in springs of 2000 and 2001. The abundance of bacteria in Lake Verevi is highly variable (0.70 to 22×10^6 cells ml^{-1}) and generally the highest in anoxic hypolimnetic water. In 2000–2001, the bacterial abundance in the hypolimnion increased probably due to meromixis. During a productive season, heterotrophic bacteria were able to consume about 10–40% of primary production in the epilimnion. Our study showed that bacterioplankton in the epilimnion was top-down controlled by predators, while in metalimnion bacteria were dependent on energy and carbon sources (bottom-up regulated). Below the thermocline hypolimnetic bacteria mineralized organic matter what led to the depletion of oxygen and created anoxic hypolimnion where rich mineral nutrient and sulphide concentrations coexisted with high bacterial numbers.

Introduction

Bacteria are the most numerous planktonic organisms in freshwater lakes, they can be responsible for transformation of all net primary production. In lakes bacteria contribute ~10–90% of the total respiration rate (Biddanda et al., 2001) and their importance seems to increase toward more oligotrophic systems (e.g., Baines & Pace, 1991). Large vertical heterogeneity and steep gradients are characteristic to thermally stratified lakes facilitating the sequence of specific ecophysiologically different microbial populations over small depth intervals. Changes in the concentrations of key environmental factors such as oxygen, sulphides, nitrogen and phosphorus compounds as well as in light intensity and quality lead to the differences in food web structure and in abundance of its major players.

In present study the inter-annual, seasonal and vertical distribution of the total abundance and activity of non-photosynthetic planktonic bacteria was followed in a small steeply stratified hypertrophic lake. This a temperate region lake (Lake Verevi, area 12.6 ha, mean depth 3.6 m, maximum depth 11 m) is a partly meromictic and strongly stratified hypertrophic freshwater lake in South Estonia protected from wind and has the average water exchange of 0.63 $year^{-1}$ (Loopmann, 1984). Summer stratification develops quickly after the ice-break in April leading to fast oxygen depletion in the hypolimnion. In 1991–2001 the average Secchi depth was ~2 m, chlorophyll *a* concentration varied from 3.5 to 128 μgChl l^{-1} in the mixed layer (Nõges & Kangro, 2005). Concentrations of total nitrogen and phosphorus were 980 mg N m^{-3} and 55 mg P m^{-3} in the surface layers (<2.5 m) and 6322 mg N m^{-3} and 830 mg P m^{-3} in the bottom layers (>5 m) in 1984–2001 (Ott et al., 2005). The aim of our study was to follow and understand the reasons of bacterial abundance and activity distribution on the background of

formation of the lake water stratification, on vertical gradients of environmental factors, and also the food web interactions.

Materials and methods

Sampling

Water samples were taken from 3 to 8 layers at the deepest point of the lake. In 1991, 1993, 1994 and 1998 sampling was carried out by Ruttner or van Dorn sampler. In 2000 and 2001 a water pump (Masterflex N 7533–60) with "easy-load" pump-head (model 7518–12) connected to a tube (diameter 8 mm), designed for study of thin (20–25 cm) water layers, was used for sampling. Temperature and oxygen concentration were measured before sampling. In a diurnal study the samples were taken at 1 m intervals from the layer of 0.5 to 7 m at 12:00 and 16:00 in August 2, and at 8:00 and 12:00 in August 3, 2001.

Analytical methods

Water temperature and the concentration of dissolved oxygen were measured by thermooxymeter Landorem 200 (Tartu University, Estonia). In 2000 and 2001, the parallel measurements were done with Aqua-Check Water Analyzer (O.I. Analytical Corporation). Chemical analyses were performed as described by Ott et al. (2005).

Total number of bacteria (TNB) was determined by DAPI staining (Porter & Feig, 1980). Formaldehyde or glutaraldehyde preserved samples (final concentration 2%) were incubated with DAPI (final concentration 10 μg ml^{-1}) for 5 min in the dark. Samples were filtered onto black 0.22-μm-pore-size polycarbonate filters (Poretics) and stored at −21 °C until counting with epifluorescence microscope (Leica DM RB) at 1000× magnification.

Bacterial activity and production was estimated by the tritiated thymidine incorporation method (Bell et al., 1983). Triplicate 10 ml subsamples of each sample (+3 formaldehyde killed blanks) were treated with 10 nM ^{3}H-thymidine (Amersham; specific activity 26 Ci mmol^{-1}). The subsamples were incubated 30 min at room temperature. Cold base–acid–ethanol extraction was used for purification of DNA as described by Wicks & Robarts (1987). The uptake of thymidine was converted to the number of produced cells by using conversion factor of 2×10^{18} cells per mole of incorporated thymidine.

For the estimations of chlorophyll *a* concentration (Chl *a*) plankton was filtered on Whatman GF/F filters. In 1991 and 1993 the pigments were extracted by 90% acetone (Edler, 1979), in 1998–2001 in parallel by 90% acetone and 96% ethanol (Jespersen & Christoffersen, 1987). The absorption of the extract between 430 and 750 nm was determined with a scanning UV-VIS spectrophotometer (Cecil-3000). When applying extraction both with acetone and with ethanol the maximal concentration of Chl *a* was used in further analysis as recommended by Nõges & Solovjova (2000). Primary production (PP) of phytoplankton was estimated *in situ* using $^{14}CO_2$ assimilation technique (Steeman-Nielsen, 1952). Detailed description of PP method is given by Nõges & Kangro (2005).

Results

Stratification

Morphometrical characteristics of the lake result in strong gradients of temperature and oxygen (Figs. 1, 2, and 4). In mid-summer aerobic epilimnion expanded only to the upper ~1–1.5 m, temperature in this layer was the highest. Thickness of the metalimnion was usually ~2 m, and an extensive anoxic zone developed in hypolimnion with increased H_2S concentration during the productive season (May–October). Total number of bacteria (TNB) was statistically significantly different between different layers (Tukey's *post-hoc* test ANOVA, $p < 0.001$). In average highest TNB was recorded in the hypolimnion ($9.5 \pm 0.5 \times 10^6$ cells ml^{-1}) and lowest in the epilimnion ($5.7 \pm 0.3 \times 10^6$ cells ml^{-1}). At the end of July, 1998, bacterial activity increased with depth in the epilimnion and peaked at the aerobic/anaerobic interface (Fig. 1). The average values of bacterioplankton production (BP) were not statistically different between epi-, meta- and hypolimnion, however the variation of BP was the highest in epilimnion (mean 5.8 ± 3.6 μgC l^{-1}h^{-1}). The lowest

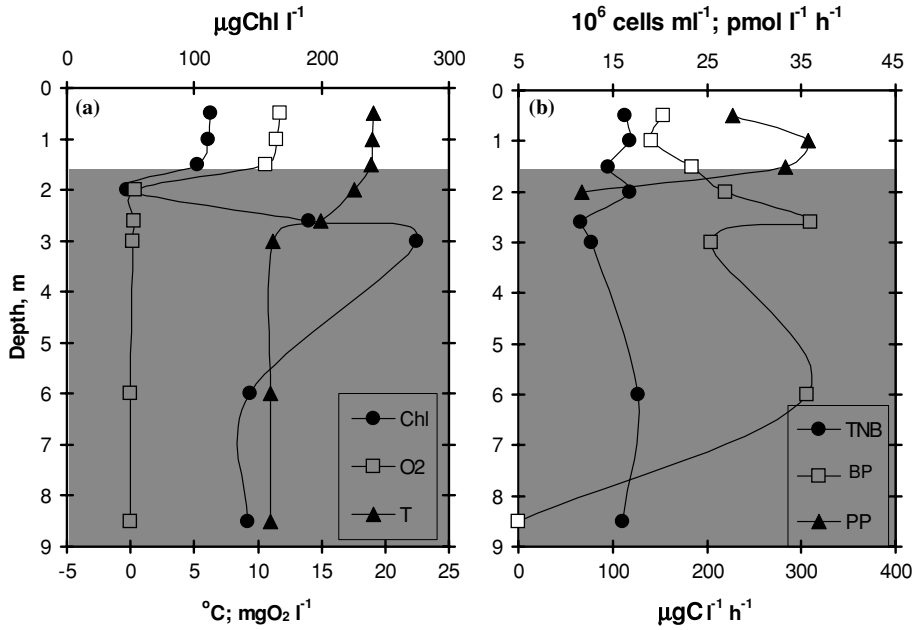

Figure 1. Vertical distribution of (a) temperature (T), oxygen (O_2), chlorophyll *a* concentration (Chl), and (b) primary production (PP), abundance (TNB), production of bacteria (BP) in Lake Verevi during strong summer stratification (in July 31, 1998). Shaded area on the plot shows the part of water-column below the light attenuation of 1% ($z_{1\%}$) (Reinart et al., 2005).

values and variability of activity were found in the hypolimnion (0.8 ± 0.3 μgC $l^{-1}h^{-1}$).

Seasonal dynamics and long term trends

TNB increased slightly during the productive season in all water layers, this increase was the most pronounced in metalimnion (Fig. 2). Hypolimnetic bacteria achieved the highest numbers when anoxic and sulphide rich waters spread further into water column. The increase of TNB in the hypolimnion was more pronounced compared between epi- or metalimnion. Typically in spring and late summer/autumn the additional peaks of TNB developed in the upper or lower metalimnion. Thymidine incorporation (TTI) was variable between the years, and no clear seasonal dynamics could be observed.

In 1990s, TNB did not change in the epi- and metalimnion but slightly increased in hypolimnion (Fig. 3, MANOVA of differences between co-effect of years and layers $p = 0.069$). Bacterial production data were scattered, however, a decrease of activity could be noticed in 1998, and 2001 as compared to the beginning of 1990s (Fig. 3, ANOVA $p = 0.001$, $n = 28$).

Diel dynamics of bacterioplankton

In August 2–3, 2001 when diurnal dynamics of bacteria (24 h cycle) was followed, the temperature and oxygen profiles were stable (Fig. 4b). TNB in epi- and metalimnion ($\sim$4–5 $\times$ 10^6 cells ml^{-1}) had low variability and did not change over time. In deeper layers of the hypolimnion TNB was significantly higher ($\sim$10–14 $\times$ 10^6 cells ml^{-1}) than in upper water layers. BP fluctuated highly in depth and during the diurnal cycle (Fig. 4a). The highest bacterial activity was estimated at the epilimnion/hypolimnion interface (average 0.218 μgC l^{-1} h^{-1}), in addition the quite high BP values (average 0.214 μgC l^{-1} h^{-1}) occurred also at the surface. Depth profiles of bacterial activity did not change during the diurnal cycle, though highest values were recorded in the afternoon (average 0.208 μgC l^{-1} h^{-1}), and lowest at noon (average 0.130 μgC l^{-1} h^{-1}).

Carbon flux

The average integrated bacterioplankton production in epilimnion was 354 mgC m^{-2} day^{-1} (assuming equal productivity over 24 h) while integral primary

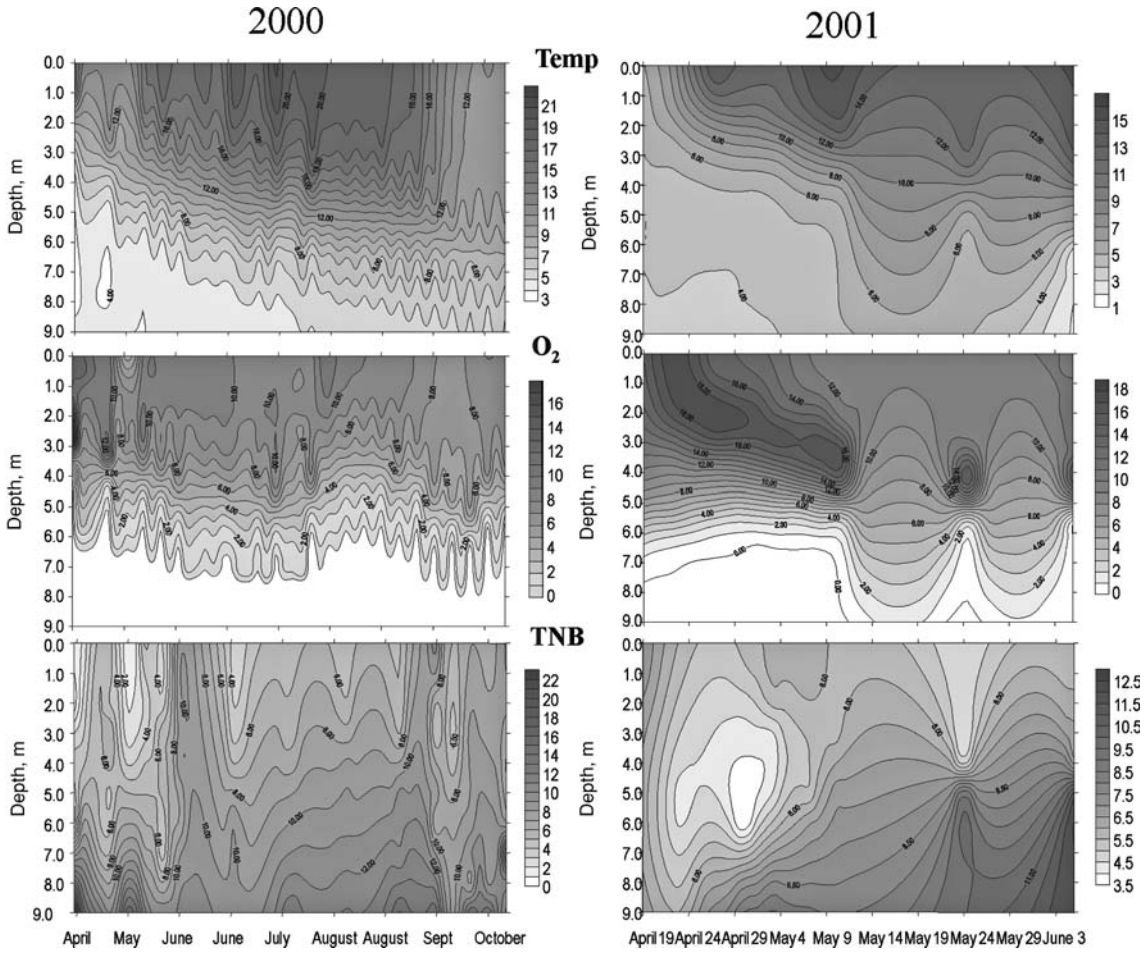

Figure 2. Time-depth distribution of temperature (Temp, °C), oxygen (O_2, mg O_2 l^{-1}), and total numbers of bacteria (TNB, 10^6 cells ml^{-1}) in 2000–2001.

production averaged 1195 mgC m^{-2} day^{-1}. Thus, integral BP consisted ~40% of primary production during the productive season. At the same time BP and PP did not couple with each other and the ratio between BP and PP was less than 10% in summer (June–August) and in most cases reached highest values in late autumn (up to 80% of integral PP).

Relationships between bacterioplankton with other biota and physico-chemical environment

Partial correlation analysis (interrelation between variables is adjusted) was used to estimate relationships between TNB and other available variables. Data of different layers (i.e. epi-, meta- and hypolimnion) were analyzed separately. The abundance of epilimnetic bacteria did not have significant partial correlation to physico-chemical variables ($p > 0.05$). The only pronounced negative correlation was found between TNB and the abundance of zooplankton groups Rotatoria and Cladocera (partial $r = -0.63$ and -0.56, $p < 0.001$, respectively). In the environment of steep gradients of temperature and oxygen (metalimnion), TNB showed significant positive correlation (partial $r > 0.40$, $p < 0.05$) with total and inorganic phosphorus, temperature and Chl *a*. In the mainly anaerobic hypolimnetic layer the bacterial number was strongly correlated (partial $r > 0.80$, $p < 0.01$) to the concentration of total nutrients (N, P) and phosphate, but not with the inorganic nitrogen. A strong correlation was also found

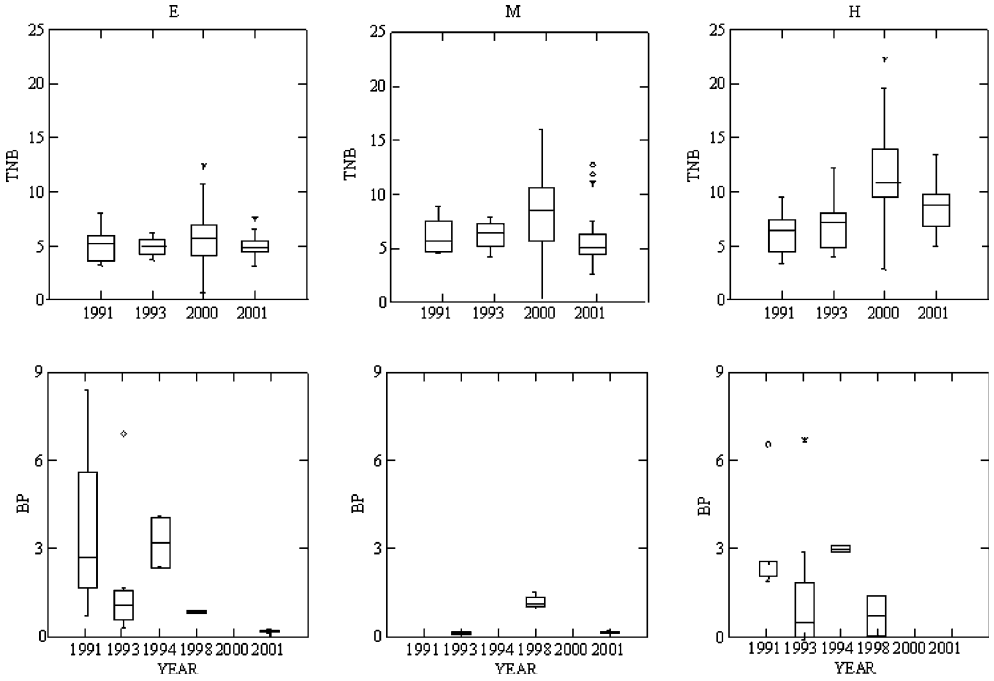

Figure 3. Summarized box-plot (with mean, standard deviation and absolute range) of abundance and activity of bacterioplankton in different layers in Lake Verevi through 1991–2001: (a) total number of bacteria (TNB, 10^6 cells ml^{-1}); (b) bacterioplankton production (BP, μg C l^{-1} h^{-1}); E: epilimnion M: metalimnion, H: hypolimnion.

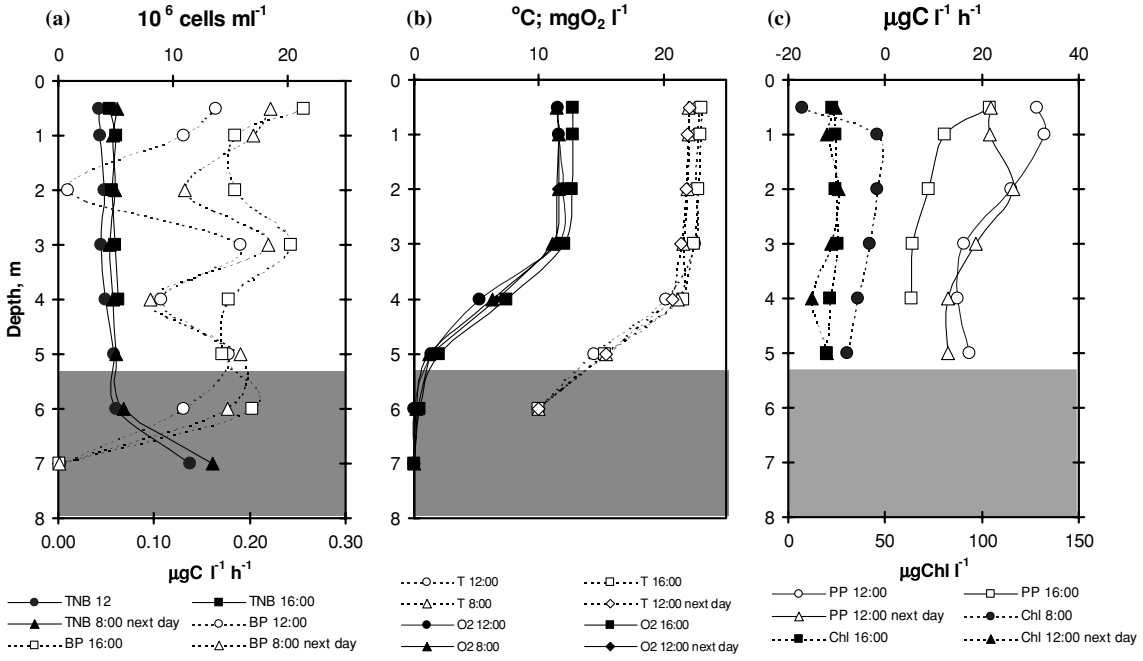

Figure 4. Diel dynamics of (a) the bacterial abundance (TNB) and activity (TTI-tritiated thymidine incorporation), (b) temperature and oxygen concentration and (c) chlorophyll *a* concenctration (Chl*a*) and primary production (PP). Shaded area on the plot shows the part of water-column below the light attenuation of 1% ($z_{1\%}$) (Reinart et al., 2005) at mid-day (12:00).

between H_2S concentration and TNB (partial $r = 0.61$, $p = 0.035$) whereas correlations to temperature, Chl *a* and to any other analyzed variable were insignificant.

In order to evaluate the prevalence of the 'bottom-up' or 'top-down' regulation of bacterial growth the relationship between TNB and BP was analyzed using linear regression analysis. In the upper layer of the lake TNB and BP were not related (strong top-down control by predation), in the metalimnion TNB and BP showed strong and significant relationship ($R^2 = 0.90$, $p < 0.000$) demonstrating the bottom-up regulation of bacterial growth. An insignificant negative regression ($p > 0.05$) was found in hypolimnion indicating weak N and P limitation in the deepest layers of the lake.

Discussion

Strongly stratified dimictic and monomictic, and in particular meromictic lakes produce extreme types of aquatic environments at comparatively small scales. Thermal stratification influences a wide variety of biological, chemical and physical processes in such lakes. This includes depth distribution of microorganisms as well as general energy and nutrient fluxes. Therefore these lakes constitute a good opportunity to study the relationships between several distinct habitats at small scales.

The absence of significant turbulence at the thermo- and oxycline prevented dispersion of plankton populations and ensured stability of the chemical gradients in Lake Verevi. Pronounced thermal and chemical stratification occurred from the end of April until September. In years 2000 and 2001, partial meromixis appeared because of the water was not completely mixed after the ice-break in April (Nõges & Kangro, 2005). This caused rapid nutrient depletion in the epilimnion and continuous anoxic situation in the bottom layers during productive season providing to the bacteria at least three distinct habitats with clearly different environmental conditions: euphotic and aerobic surface layer, euphotic but microaerobic/anoxic metalimnion, and aphotic and anoxic (with H_2S) hypolimnion. Generally, the seasonal thermocline depth is influenced by lake size, nutrients load and water transparency, as lake area increases, wind fetch increases and seasonal thermocline deepens. Wind fetch is small in Lake Verevi and nutrients load high, leading to a thermocline at only 1–2 m depth, however some fluctuations due to weather conditions are possible (Fig. 2). Resource availability and grazing by protozoans which are the major known mechanisms for controlling bacterial production (e.g., Gasol, 1994) are also important in Lake Verevi. The number of epilimnetic bacterioplankton in Lake Verevi had the typical range of hyper- and eutrophic lakes ($\sim 5 \times 10^6$ cells ml^{-1}). Productivity of bacteria was the highest and most variable in this compartment (0.001–64 $\mu gC\ l^{-1}\ h^{-1}$, mean 3.5). The epilimnion usually is more subjected to disturbances and bacteria have to grow irregular or erratic bursts, thus, epilimnetic bacteria were highly possibly prevailed by opportunistic populations growing on labile substrates.

Autochthonous primary production provided energy and carbon for heterotrophic bacteria, however, the growth of bacteria was instead controlled by predators and therefore no relationship between abundance and activity of bacteria was found. Also TNB and BP did not correlate neither with algal biomass nor primary production but instead TNB was negatively correlated with zooplankton biomass. Generally, algal and bacterial productions were unbalanced, therefore more organic carbon was produced during the productive season than utilized by heterotrophic bacteria. The results of present study also indicated that the excess of primary production was partly consumed during the clear water phase in June (after phytoplankton bloom in May) and in late autumn when phytoplankton activity had collapsed but bacteria still remained highly productive afterwards. Also the diurnal dynamics of PP and BP showed uncoupled variations of algal and bacterial activity: BP was the highest in the afternoon when PP decreased (Fig. 4).

In the metalimnion usually two peaks of abundance and activity of bacteria occurred at the interface between epi- and metalimnion (Fig. 4) or at the transition from metalimnion to hypolimnion (Fig. 1). Very similar depth profiles of bacterial abundance were found in L. Plußsee (Weinbauer & Höfle, 1998). As typical to eutrophic lakes the

productive epilmnion was dominated by production of particulate organic matter (Biddanda et al., 2001), and in the process of sedimentation these particles were trapped in the upper part of the thermocline (Ott et al., 2005) and were possibly utilized by aerobic heterotrophic bacteria. Another peak of BP associated with microaerobic/anoxic conditions and with bacteriochlorphyll maximum. This zone had better access to H_2S together with light favouring development of phototrophic sulfur bacteria (e.g. Camacho et al., 2001). Bacteria in the thermocline could depend more on abiotic environment at the same time remineralizing organic matter and releasing inorganic nutrients, as correlation of bacterial abundance and BP with inorganic nutrients and temperature were strong ($p > 0.40$, $p < 0.05$). Water temperature was below the range (10–14 °C) what is reported to limit growth rate of bacteria (Hoch & Kirchman, 1993; Carlsson & Caron, 2001). Thus, temperature could also be an important factor controlling the development of bacteria in thermocline. Also BP and TNB were positively related to each other indicating bottom-up regulation of bacterioplankton activity.

Similar to other lakes (Weinbauer & Höfle, 1998; Kasprzak et al., 2000), TNB reached the highest values ($15–20 \times 10^6$ cells ml^{-1}) in the physically most homogenous hypolimnion. Abundance of bacteria in deep layers increased from 1990s to 2000s because of more rapid oxygen depletion in deep layers, caused most probably because of spring meromixis in warmer springs (Nõges & Kangro, 2005). At the same time anoxic and rich in H_2S environment was created by bacteria itself. This was expressed by a good correlation between number of bacteria and H_2S concentration. However, main energy and carbon still originated mostly from the upper highly productive layers. Hypolimnetic bacteria were not highly active (or measurements of BP failed in anoxic waters) and their high numbers were supported rather by specific conditions (lack of most eukaryotic organisms, therefore no grazing, undisturbed environment, etc) than high productivity. Such growth is typical to equilibrium populations (K-strategists) growing in stable environments (Andrews & Harris, 1986). However, the bacterial activity in binding of nutrients in hypolimnion is important as the concentrations of total phosphorus and nitrogen were strongly correlated with TNB. Probably bacteria were one of the most important nutrient pools in hypolimnion. At the same bacteria also re-mineralized organic compounds and released excesses of nutrients as the concentrations of inorganic N and P were very high in hypolimnion.

In conclusion, small steeply stratified water bodies such as Lake Verevi provide a promising environment for studying bacterial physiological and species diversity. Very strong stratification is stabilized by the small size of the lake ensuring that certain populations of bacteria develop under the specific environmental conditions. Ecophysiological studies of these bacteria would provide a deeper insight into energy and matter fluxes of the ecosystems of such kind of lakes.

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