

## 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  $22 \times 10^6$  cells  $\text{ml}^{-1}$ ) 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 ecophysiological 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  $0.63 \text{ year}^{-1}$  (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  $128 \mu\text{gChl l}^{-1}$  in the mixed layer (Nõges & Kangro, 2005). Concentrations of total nitrogen and phosphorus were  $980 \text{ mg N m}^{-3}$  and  $55 \text{ mg P m}^{-3}$  in the surface layers (<2.5 m) and  $6322 \text{ mg N m}^{-3}$  and  $830 \text{ mg P m}^{-3}$  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  $10 \mu\text{g ml}^{-1}$ ) for 5 min in the dark. Samples were filtered onto black 0.22- $\mu\text{m}$ -pore-size polycarbonate filters (Poretics) and stored at  $-21^\circ\text{C}$  until counting with epifluorescence microscope (Leica DM RB) at  $1000\times$  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  $10 \text{ nM } ^3\text{H}$ -thymidine (Amersham; specific activity  $26 \text{ Ci mmol}^{-1}$ ). 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  $2 \times 10^{18}$  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  $^{14}\text{CO}_2$  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  $\sim 1$ – $1.5 \text{ m}$ , temperature in this layer was the highest. Thickness of the metalimnion was usually  $\sim 2 \text{ m}$ , and an extensive anoxic zone developed in hypolimnion with increased  $\text{H}_2\text{S}$  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 ( $9.5 \pm 0.5 \times 10^6 \text{ cells ml}^{-1}$ ) and lowest in the epilimnion ( $5.7 \pm 0.3 \times 10^6 \text{ cells ml}^{-1}$ ). 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  $5.8 \pm 3.6 \mu\text{gC l}^{-1}\text{h}^{-1}$ ). The lowest