

DISSERTATIONES BIOLOGICAE UNIVERSITATIS TARTUENSIS

100

DISSERTATIONES BIOLOGICAE UNIVERSITATIS TARTUENSIS

100

**THE IMPACT OF NITROGEN AND
PHOSPHORUS CONCENTRATION AND
N/P RATIO ON CYANOBACTERIAL
DOMINANCE AND N₂ FIXATION
IN SOME ESTONIAN LAKES**

ILMAR TÖNNO



TARTU UNIVERSITY
PRESS

Institute of Zoology and Hydrobiology, University of Tartu, Estonia

The dissertation is accepted for the commencement of the degree of *Doctor philosophiae* in hydrobiology on August 25, 2004 by the Doctoral Committee of the Faculty of Biology and Geography of the University of Tartu

Opponent: PhD Lauri Arvola

Commencement: October 8, 2004, Room 301, Vanemuise 46

The publication of this dissertation is granted by the University of Tartu

© Ilmar Tõnno, 2004

Tartu Ülikooli Kirjastus

www.tyk.ut.ee

Tellimuse nr. 438

CONTENTS

LIST OF ORIGINAL PUBLICATIONS	6
1. INTRODUCTION	7
2. STUDY AREA	8
3. METHODS	9
Sampling	9
N ₂ -fixation, PO ₄ -P enrichment experiments, nitrification and water chemistry	10
Phytoplankton preservation and microscopy	11
4. SUMMARY AND DISCUSSION OF PAPERS	12
N ₂ -fixation and nitrification: occurrence and rates	12
Nutrients and nitrogen fixation in shallow lakes.....	15
Nitrogen dynamics in stratified Lake Verevi	20
5. CONCLUSIONS.....	21
REFERENCES	22
SUMMARY IN ESTONIA. Lämmastiku, fosfori ja N/P suhte mõju tsüanobakterite domineerimisele ning molekulaarse lämmastiku fikseerimisele mõnedes Eesti järvedes.....	25
ACNOWLEDGEMENTS	27
PUBLICATIONS	29

LIST OF ORIGINAL PUBLICATIONS

Present thesis is based on the following papers, which are referred to in the text with respective Roman numerals

- I Tõnno, I. & Nõges, T. 2003. Nitrogen fixation in a large shallow lake: rates and initiation conditions. *Hydrobiologia* 490: 23–30.
- II Nõges, T., Tõnno, I., Laugaste, R., Loigu, E. & Skakalski, B. 2004. The impact of changes in nutrient loading on cyanobacterial dominance in Lake Peipsi (Estonia/Russia). *Arch. Hydrobiol.* 160, 2: 261–279.
- III Tõnno, I., Ott, K. & Nõges, T. 2004. Nitrogen dynamics in steeply stratified temperate Lake Verevi, Estonia. (submitted to *Hydrobiologia*).
- IV Tõnno, I., Künnap, H. & Nõges, T. 2003. The role of zooplankton grazing in the formation of ‘clear water phase’ in a shallow charophyte-dominated lake. *Hydrobiologia* 506–509: 353–358.
- V Nõges, P., Tuvikene, L., Feldmann, T., Tõnno, I., Künnap, H., Luup, H., Salujõe, J. & Nõges, T. 2003. The role of charophytes in increasing water transparency: a case study of two shallow lakes in Estonia. *Hydrobiologia* 506–509: 567–573.
- VI Nõges, T., Tõnno, I., Blinova, I., Loigu, E., Jastremski, V., Laugaste, R. & Skakalski, B. 2003. Reduced nitrogen loading enhances cyanobacterial blooms in Lake Peipsi. In: Proceedings of the second international conference “Sustainable management of transboundary waters in Europe”. Poland, Miedzyzdroje: 501–504.

AUTHOR’S CONTRIBUTION

Publication (I): This study was initiated and designed by the author. He was responsible for the processing of the results and for the preparation of the manuscript.

Publication (II): Author participated in the processing of the results and was responsible for the preparation of the manuscript.

Publication (III): This study was a part of Lake Verevi investigation project. Nitrogen fixation and nitrification measurements were initiated and designed by the author. He was also responsible for the processing of the results and for the preparation of the manuscript.

Publication (IV): Author participated in the designing of this study. He was responsible for the processing of the results and for the preparation of the manuscript.

Publication (V): Author collected and analysed the phytoplankton data.

Publication (VI): Author participated in the processing of the results.

1. INTRODUCTION

Nitrogen (N) is one of the main building blocks for the production of organic matter and it is required in great quantities (Stolp, 1996; Williams et al., 2002). Most of the nitrogen on Earth is present in molecular form (N_2) being biologically unavailable except for organisms containing the enzyme nitrogenase. N_2 -fixation (N_2 fix) by micro-organisms is the only process in nature that counteracts the losses of nitrogen from the environment by denitrification (Fig. 1). Cyanobacteria appear responsible for most of planktonic N_2 fix in aquatic ecosystems, this ability gives a significant competitive advantage to these organisms during the periods of nitrogen limitation (Tilman et al., 1982; Howarth et al., 1988a; Leppänen et al., 1988). Many hypotheses have been presented to explain cyanobacterial dominance and blooms in lakes. One of the most common is resource ratio competition theory, predicting that cyanobacteria tend to dominate in lakes where the ratio of nitrogen and phosphorus (P) is low, mainly because of the ability of some of these species to use molecular nitrogen (Elser 1999). This theory has been proved both empirically and experimentally. Cyanobacteria, both fixing and not fixing N_2 , tend to dominate if the ratio of total nitrogen (TN) and total phosphorus (TP) in the water column is below ca. 5–10 by mass (Schindler 1977, Seip 1994, Michard et al., 1996, Bulgakov & Levich 1999), although some authors regard the critical TN/TP ratio to be much higher, even as much as 29 by Smith (1983). According to many authors (Smith et al., 1987, Willén 1992, Lathrop et al., 1998, Downing et al., 2001) cyanobacterial dominance and blooms couple more strongly to the variations in P and N concentrations, rather than changes in N/P ratio. Other factors such as water temperature, pH, light intensity and total carbon dioxide concentration are also important. Generally N_2 -fixing cyanobacteria are better nitrogen competitors, but poorer phosphorus competitors, than other groups of algae. In shallow lakes, however, cyanobacteria appear to be more efficient than other phytoplankton species in converting P into their biomass (Smith, 1983; Nixdorf & Deneke, 1997). Water in shallow lakes is permanently mixed up and therefore enabling more or less homogenous nutrient spreading in the water column. Unlike from shallow lakes, in stratified water-bodies phytoplankton (including cyanobacteria) takes up epilimnetic nutrients and transports them to the hypolimnion via sedimentation. Nutrients may accumulate in the hypolimnion during stratification period while nutrients deficiency may occur in the epilimnion if resupply from the inflows is limited (Scheffer, 1998). In earlier papers, however, it has not been studied whether cyanobacterial N_2 -fixation starts in lakes instantly after the set-up of favourable conditions or after some lag period.

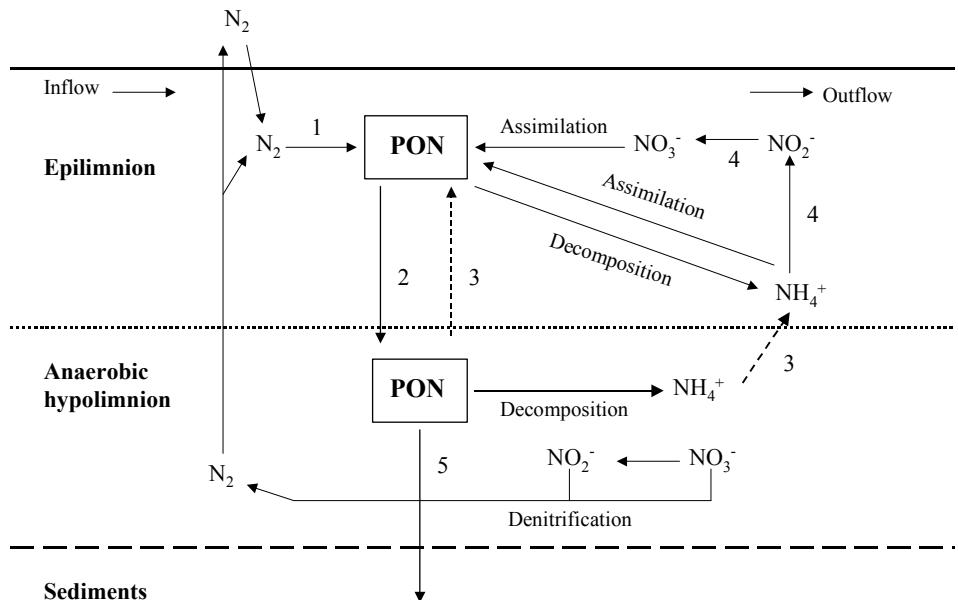


Figure 1. Conceptual scheme of the main processes of the nitrogen cycle in a stratified lake according to Lampert and Sommer (1997): 1 – planktic molecular nitrogen fixation (N_2 fix); 2 – a part of the PON (particular organic nitrogen) sinks to the hypolimnion; 3 – due to water mixing some PON is carried from hypolimnion to epilimnion; 4 – in epilimnion ammonium (NH_4^+) is subject to nitrification; 5 – a part of PON is switched off from nitrogen cycle due to sedimentation.

The majority of our studies were performed in very large shallow eutrophic lakes. One aim was to investigate the set-up of the initiation conditions and the induction of cyanobacterial N_2 -fixation in shallow eutrophic lakes. We also specified, how different factors (concentration of nitrogen and phosphorus, N/P ratio) influence the seasonal development and species succession of N_2 -fixing cyanobacteria. Besides this, the role of N_2 -fixation and nitrification in the nitrogen cycle of a strongly stratified lake was studied.

2. STUDY AREA

The study **I** was carried out in Lake Võrtsjärv, Estonia. Lake Võrtsjärv is a large (270 km^2) and shallow (mean depth 2.8 m, maximum depth 6 m) eutrophic (mean total nitrogen concentration is 2 mg l^{-1} and total phosphorus concentration 0.05 mg l^{-1}) lake in Central Estonia. The renewal of water takes 240–384 days. Lake is covered with ice for on an average 135 days (from mid-November till mid-April). The water is alkaline (pH 7.5–8.5) with a great buffering capacity and a high seston content (Haberman et al., 1998). Studies **II** and **VI** were carried out in Lake Peipsi, Estonia/Russia. Lake Peipsi ($3,555 \text{ km}^2$, mean depth 7.1 m, maximum depth 15 m) is the fourth largest lake of Europe. Located on the border of Estonia and Russia, it is an important transboundary waterbody and Europe's largest international lake. The catchment area of L. Peipsi ($47,800 \text{ km}^2$) includes parts of the Estonian, Russian and Latvian territories, and involves also the drainage basin of L. Võrtsjärv. The volume of water in L. Peipsi is 25 km^3 and the residence time of the water is about 2 years. The outflow, the River Narva, flows into the Gulf of Finland. Riverine transport is the most important pathway for the input of nutrients into L. Peipsi (Nõges et al., 1996; Jaani 2001).

Paper **III** focuses on small (0.126 km^2 , mean depth 3.6 m, maximum depth 11 m) stratified hypertrophic (mean total nitrogen and total phosphorus concentrations in the epilimnion are 0.95 mgN l^{-1} and 0.05 mgP l^{-1} , and in the hypolimnion 6.65 mgN l^{-1} and 0.8 mgP l^{-1} , respectively) Lake Verevi in South-Estonia. L. Verevi is strongly stratified from April to September and has permanently anoxic hypolimnion. The average water exchange rate in L. Verevi is 0.63 times per year. However, mostly the epilimnetic water is exchanged while the water from the deeper layers flows out only during a short vernal and a longer fall turnover. The temperature gradient in the metalimnion may exceed 10°C m^{-1} (Nõges & Nõges 1998) and is accompanied by steep gradients in dissolved oxygen content, nutrients, and biota.

Papers **IV** and **V** focuses on small (0.399 km^2) and shallow (mean depth 2.2 m, maximum depth 4.2 m) eutrophic (on average 1.0 mg l^{-1} of total nitrogen and 0.02 mg l^{-1} of total phosphorus) lake Prossa in East-Estonia. The bottom of this lake is covered with nearly 1.5 m thick mud layer (Mäemets, 1977). The whole bottom area of L. Prossa is covered by macrovegetation, dominated overwhelmingly by charophytes.

3. METHODS

Sampling

The water samples were collected by using Ruttner sampler (Papers **I**, **II**, **IV**, **V**, **VI**) or Masterflex pump (Paper **III**, for details see Zingel & Ott, 2000). Water samples were taken from one sampling station, near to the deepest part of the lake (Papers **I**, **III**) or from different sampling stations in case of L. Peipsi and L. Prossa (Papers **II**, **IV**, **V**, **VI**).

In papers **I**, **II** and **VI** depth-integrated water samples were used (water taken from the whole water column with an interval of 0.5 m was mixed in one bigger tank). In L. Verevi (Paper **III**) eight (in year 2000) or three to eight (in year 2001) vertical samples were taken at different depths of the epi-, meta- and hypolimnion. In case of papers **IV** and **V** depth-integrated water was taken from five sampling points on the transect from the lake centre to the shore, and mixed together.

N₂-fixation, PO₄-P enrichment experiments, nitrification and water chemistry

N₂-fixation in L. Võrtsjärv was measured during summer period (from June to September) in years 1998–2000 (Paper **I**). In study **III** N₂fix was measured in L. Verevi on 07.08.2000, 28.08.2000 and 02.08.2001 from different depth horizons in the epilimnion. Acetylene reduction method was applied (Stewart et al., 1967; Presing et al., 1996). Measurements were done in the same days when water chemistry and phytoplankton in the lakes were monitored. N₂fix rate was expressed as microgram of nitrogen per litre per day. In case of phosphorus enrichment experiments (Paper **I**) 20 l of the water from L. Võrtsjärv was collected into four plastic vessels and KH₂PO₄ was added to achieve final concentrations of 0.025; 0.05; 0.1 or 0.2 mgP l⁻¹. Vessels were exposed *in situ* for 48 (in 1998) and 72 h (in 2000). Nitrogen fixation was measured after every 24 h.

Nitrification was measured in year 2000 and 2001 from different depth horizons according to the supposed NH₄⁺ and oxygen content (Paper **III**). Modified ¹⁴C technique (dark carbon assimilation) was used for nitrification measurement (Steeman-Nielsen, 1952; Joye et al., 1999). Nitrification rate was expressed as milligram of nitrogen per cubic meter per day. Water chemical samples (Papers **I**, **II**, **III**, **IV**, **V**, **VI**) were analysed according to the methods described by Grasshoff et al. (1983).

Phytoplankton preservation and microscopy

Phytoplankton samples were fixed with acidified Lugol's solution (Papers **I**, **III**, **IV**, **V**, Papers **II**, **VI**: since 1996) or with formaldehyde (Papers **II**, **VI**: 1962–1995). Phytoplankton species were identified and the biomass was determined microscopically at x400 magnification in a settling chamber by using inverted microscope (Utermöhl, 1958) in studies **I**, **III**, **IV**, **V** and in studies **II**, **VI** since 1996. In studies **II**, **VI** in 1962–1996 a Fux-Rosenthal counting chamber was used. In case of papers **II** and **VI** the intercalibration of both methods proved that there was good comparability of the results for phytoplankton. Biomass was expressed as gram of wet weight per cubic meter. In paper **I** sixty filaments were measured and the number of heterocysts was counted for each N₂-fixing cyanobacterial species in each sample.

4. SUMMARY AND DISCUSSION OF PAPERS

Paper **I** focuses on N₂-fixation and on phosphorus enrichment experiments in Lake Võrtsjärv. The aim of the paper was to study the set-up of the initiation conditions and the induction of cyanobacterial N₂fix in a large shallow eutrophic lake. The aim of papers **II** and **VI** was to specify the conditions and factors controlling the development of N₂-fixing cyanobacteria in a large shallow polymictic eutrophic Lake Peipsi. Paper **III** focuses on nitrogen dynamics in stratified Lake Verevi. The aim of this study was to investigate the dynamics of different nitrogen compounds as well as the rates of N₂-fixation and nitrification in diverse habitats of a steeply stratified lake. The aim of paper **IV** was to study the role of zooplankton grazing in the formation of ‘clear water phase’ in shallow charophyte-dominated Lake Prossa. Paper **V** focuses to the hydrochemical regime and the biota of Lake Prossa. This study was performed in 2000–2001 within the frames of the EC project ECOFRAME, aimed to work out water quality criteria for shallow lakes in Europe.

N₂-fixation and nitrification: occurrence and rates

In L. Võrtsjärv N₂-fixation usually started in July or August and continued up to mid-September, which is a relatively short-term if to compare with the whole vegetation period. In 1998 N₂fix occurred from July 21st to September 15th, being the highest in 21st of July ($2.18 \mu\text{gN l}^{-1} \text{ day}^{-1}$) and decreasing after that. In 2000 N₂fix started much later (August 15th) and lasted until September 12th. The highest N₂fix rate ($2.61 \mu\text{gN l}^{-1} \text{ day}^{-1}$) was measured in August 15th (Fig. 2). N₂-fixation was not detected in 1999, probably due to extremely low biomass of N₂-fixing cyanobacteria ($0.006\text{--}1.6 \text{ gWW m}^{-3}$). In steeply stratified Lake Verevi N₂fix can occur in the epilimnion where temporal nitrogen limitation could appear and N₂-fixing cyanobacteria exist. In L. Verevi such conditions are usually met in August. In 2000 N₂fix occurred on August 7th and 28th at depths of 2m ($2.80 \mu\text{gN l}^{-1} \text{ day}^{-1}$) and 0.5m ($0.30 \mu\text{gN l}^{-1} \text{ day}^{-1}$), respectively. In 2001 N₂fix ($0.38 \mu\text{gN l}^{-1} \text{ day}^{-1}$) was detected only on August 2nd at a depth of 3m

In PO₄-P enrichment experiments (years 1998 and 2000) N₂-fixation started earlier than N₂fix was detected in L. Võrtsjärv. In enriched mesocosms N₂fix was also more intensive than in lake water. The most intensive N₂-fixation occurred in the vessel where phosphorus concentration was 0.1 mg l^{-1} . N₂-fixation was most intensive in the second day (48h) of the experiment. On the third day (72h) of experiment N₂fix descended in all experimental vessels (Fig. 3A;B).

In L. Verevi nitrification occurred in the epilimnion and in the upper part of the hypolimnion (5–5.5m), where some oxygen was present, as it is an O₂ demanding process (Fig. 4). According to Knowles *et al.* (1981), nitrification has been reported to occur more rapidly at low O₂ concentrations. Thereby in L. Verevi also more intensive nitrification occurred in the upper hypolimnion (except August 2, 2001; Fig. 4).

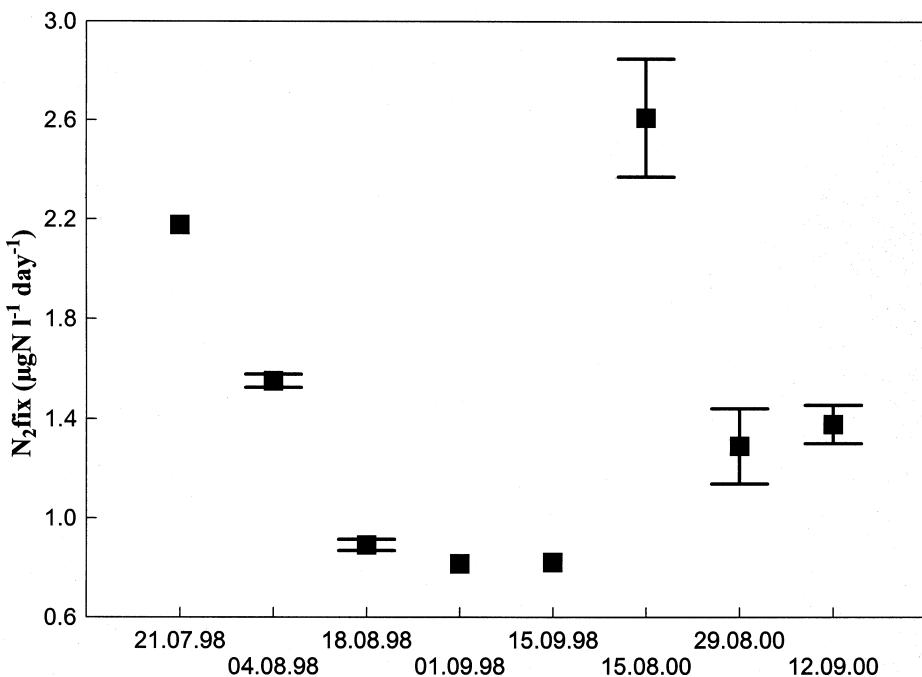


Figure 2. Molecular nitrogen fixation in L. Võrtsjärv in 1998 and 2000. Minimum, maximum and median of replicates are represented.

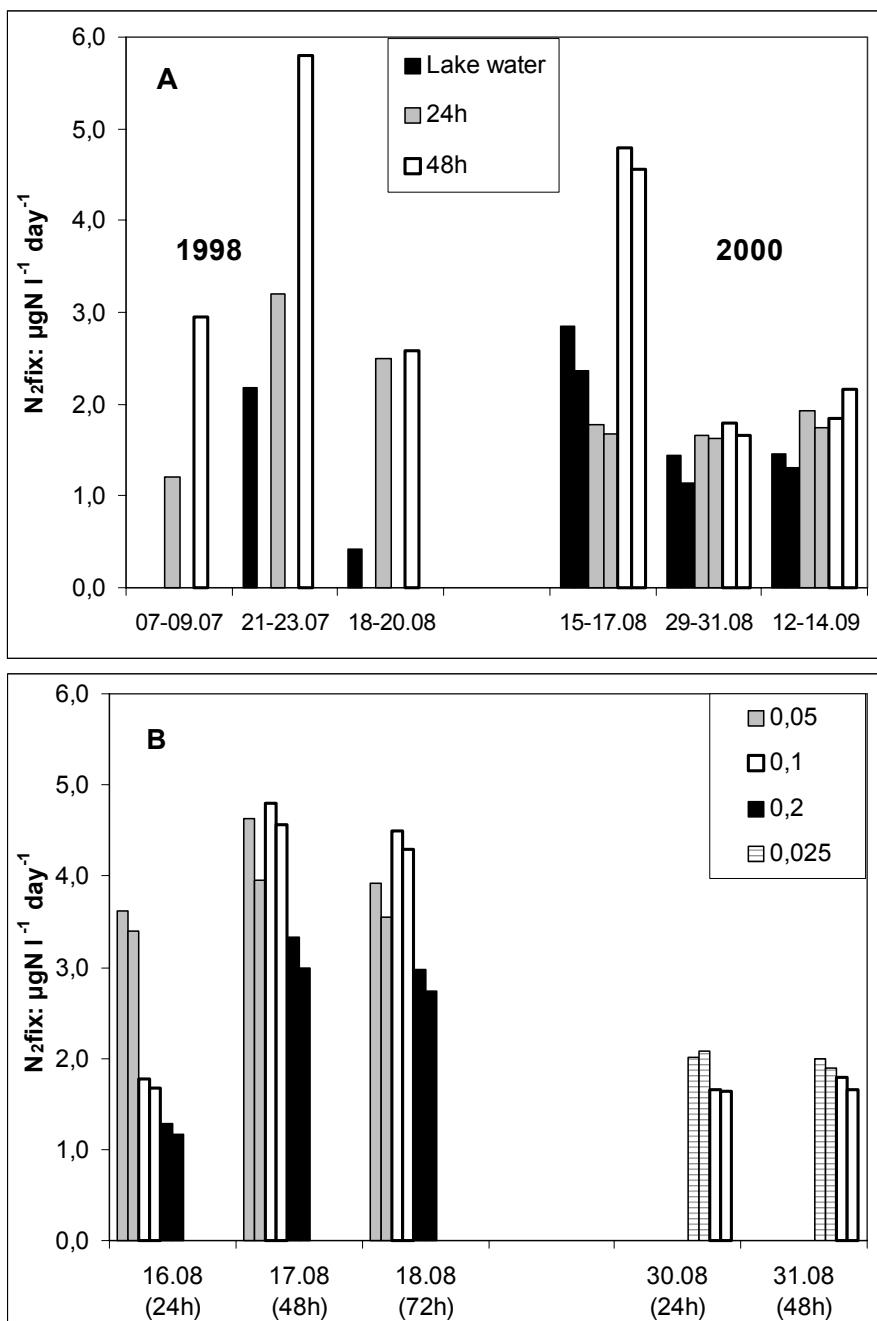


Figure 3. Molecular nitrogen fixation in PO₄-P enrichment experiments in L. Vörtsjärv in 1998 and 2000. N₂fix was measured after every 24 hour. A: Concentration of PO₄-P in vessels was 0.1 mgP l⁻¹. B: Influence of different PO₄-P concentrations (0.025 mgP l⁻¹; 0.05 mgP l⁻¹; 0.1 mgP l⁻¹; 0.2 mgP l⁻¹) on N₂ fixation in 2000.

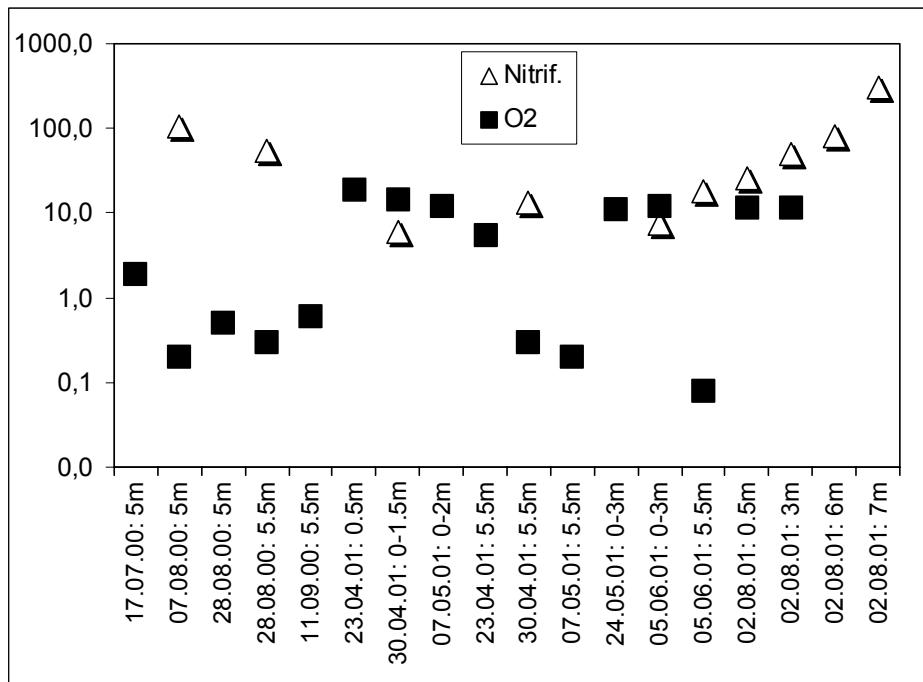


Figure 4. Dynamics of nitrification (Nitrif: $\text{mgN m}^{-3} \text{ day}^{-1}$) and dissolved oxygen (O_2 : mg l^{-1}) in L. Verevi in 2000–2001.

Nutrients and nitrogen fixation in shallow lakes

Biomass of N_2 -fixing cyanobacteria in L. Võrtsjärv (*Aphanizomenon skujae* Kom.-Legn. & Cronb., *Anabaena* sp.) and in L. Peipsi (*Gloeotrichia echinulata* J. E. Smith, *Aphanizomenon flos-aquae* L. Ralfs, *Anabaena* sp.) increased substantially in July and August. By this time biologically consumable nitrogen had been decreased and reached the lowest level during the vegetation period. In L. Peipsi N_2 -fixing cyanobacteria occupy a dominant position among blue-greens in July-August (up to 56%). Dominance of cyanobacteria in the summer phytoplankton of L. Võrtsjärv and L. Peipsi is quite ordinary as cyanobacteria are in general successful in shallow lakes (Nixdorf & Deneke, 1997). Basing on theoretical considerations, we expected that N_2fix starts when mineral nitrogen is on low level and N_2 -fixing cyanobacteria are represented in phytoplankton. Our measurements in L. Võrtsjärv did not assert this assumption as N_2fix started in both years, 1998 and 2000, a couple of weeks later than favourable conditions appeared. It seems not possible to predict the start and the duration of N_2fix from the hydrochemical conditions, phytoplankton species composition

and the amount of heterocysts. Many other factors as temperature, light, iron and molybdenum availability etc. have to be considered as well. In their studies Ferber et al. (2004) also find that the ability to fix N₂ is not the only factor for heterocystous cyanobacteria to outcompete other algae.

In L. Võrtsjärv phosphorus affected significantly nitrogen fixation. As shown above, N₂fix started in PO₄-P enrichment experiments a couple of weeks earlier than that occurred in lake water and achieved its highest value 48h after enrichment (Fig. 3A;B). Obviously, it takes some time for cyanobacteria to form new heterocysts and develop N₂fix after the assimilation of PO₄-P. In year 1998 during the period from July 21st to September 15th average N₂fix in L. Võrtsjärv was 1.079 µgN l⁻¹ day⁻¹, forming about 15% of the external nitrogen loading in this period. However, on yearly basis N₂fix formed only 2.34% of the annual external nitrogen loading to the lake. This corresponds to the values identified for the oligotrophic lakes by Kostjaev (1986). In L. Peipsi the biomass of N₂-fixing cyanobacteria increased considerably in the course of the increasing of in-lake phosphorus concentration. In period from 1980s to 2001 the in-lake concentration of phosphorus has increased by about 2-fold. At the same time the average proportion of the biomass of N₂-fixing cyanobacteria during July and August increased from 13% to 29% of total phytoplankton biomass and from 27% to 56% of the biomass of cyanobacteria in L. Peipsi. The average summer biomass of N₂-fixing cyanobacteria in L. Peipsi correlated strongly positively with yearly average in-lake total phosphorus concentration ($r=0.752$, $p=0.008$). According to some published data (e. g. Smith, 1983; MacKay & Elser, 1998), cyanobacteria, both those that fix N₂ and those that do not, can be better competitors for nitrogen and poorer competitors for phosphorus than other algae. In L. Peipsi the concentration of phosphorus seems to be the most critical factor in determining cyanobacterial development, which is a widely accepted assumption (Smith et al., 1987; Willén 1992; Willén 2001). If looking at the species causing the blooms in L. Peipsi, one can notice that seasonally *G. echinulata* starts developing earlier and is followed by other cyanobacterial species (*Anabaena*, *Aphanizomenon* and *Microcystis*). *G. echinulata*, which requires lower nutrient concentrations than other indicated species is also able to migrate vertically in the water column. Under conditions of P limitation, this algae could take up phosphorus from the sediment surface and use it for growth in the euphotic zone (Pettersson et al., 1993; Jacobsen 1994). According to this it may be hypothesized that the N₂fix by *G. echinulata* as well as its ability to use sediment P could cause the enrichment of the water column with nutrients and, thus, prepare suitable conditions for more ‘eutrophic’ species like *Anabaena*, *Aphanizomenon* and *Microcystis*. In L. Peipsi the concentration of total phosphorus increased substantially after the bloom of *G. echinulata* in June 2002 (Fig. 5).

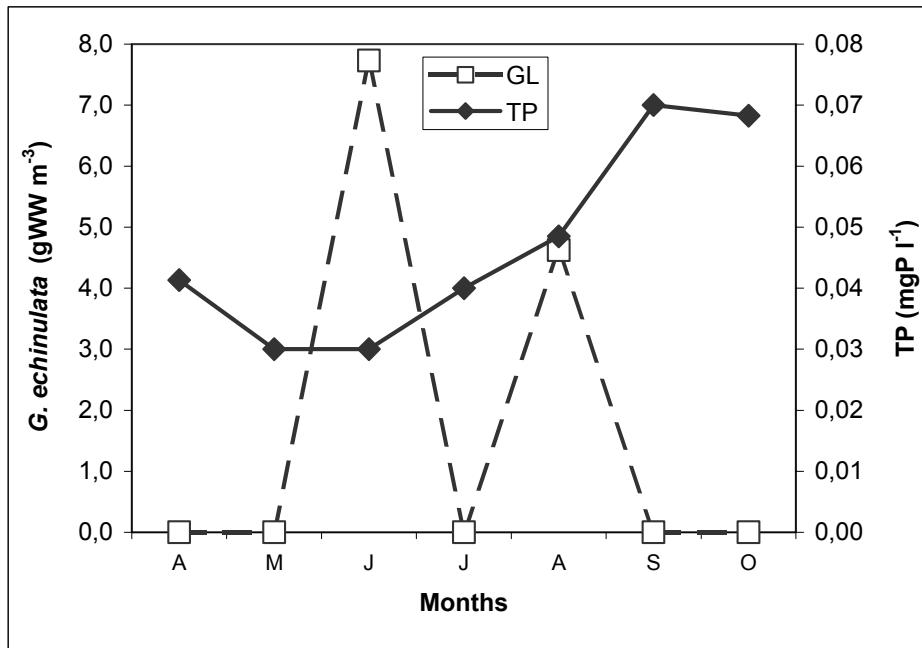


Figure 5. Seasonal course of *Gloeotrichia echinulata* (GL: gWW m⁻³) and total phosphorus concentration (TP: mgP l⁻¹) in L. Peipsi in 2002.

Another important factor affecting N₂fix and cyanobacteria is nitrogen and phosphorus ratio. Cyanobacteria usually dominate in lakes with low N/P ratio and are rare in lakes with high N/P ratio (Niemi, 1979; Smith, 1983; Helal & Culver, 1991; Bulgakov & Levich, 1999). The values of critical N/P ratios that one can find from literature are largely variable. The mass ratio of total nitrogen (TN) and total phosphorus (TP) at which cyanobacteria tend to dominate in lakes are referred to be e.g. 29 (Smith, 1983) and 5–10 (Schindler, 1977; Seip, 1994; Michard et al., 1996; Bulgakov & Levich, 1999). According to Howarth et al. (1988b), planktic N₂fix can occur if the ratio of TN/TP is equal or lower than the Redfield mass ratio 7 (Redfield, 1958). In L. Võrtsjärv N₂fix started when the mass ratio of TN/TP was about 20 and during the period when N₂fix occurred in L. Võrtsjärv, this ratio never fell below 17 (Fig. 6). These values are much higher than the Redfield ratio and also higher than the ratios given by several authors (Schindler, 1977; Seip, 1994; Michard et al., 1996; Bulgakov & Levich, 1999) as the starting point of N₂fix. As the TN/TP mass ratio reflecting the symptoms of N-limitation in L. Võrtsjärv is higher than predicted by Redfield ratio, it could be assumed that the recycling rate of P is higher than that of N and the ratio of the recycled N to recycled P is lower than TN/TP. The faster turnover of P compared with N in lakes is, in principle, a widely accepted assumption (Harris, 1986). In shallow lakes as Võrtsjärv and Peipsi the turnover

rate of P could be much higher than in deep stratified lakes. In deep lakes most of the bined phosphorus, which settles out from the euphotic zone during the vegetation period becomes available for photosynthetic organisms after the turnover in next spring. In shallow lakes where sediments are in permanent contact with the water, the same portion of phosphorus could be recycled and brought back to water column much quicker and it may support planktic photosynthesis several times during one vegetation period.

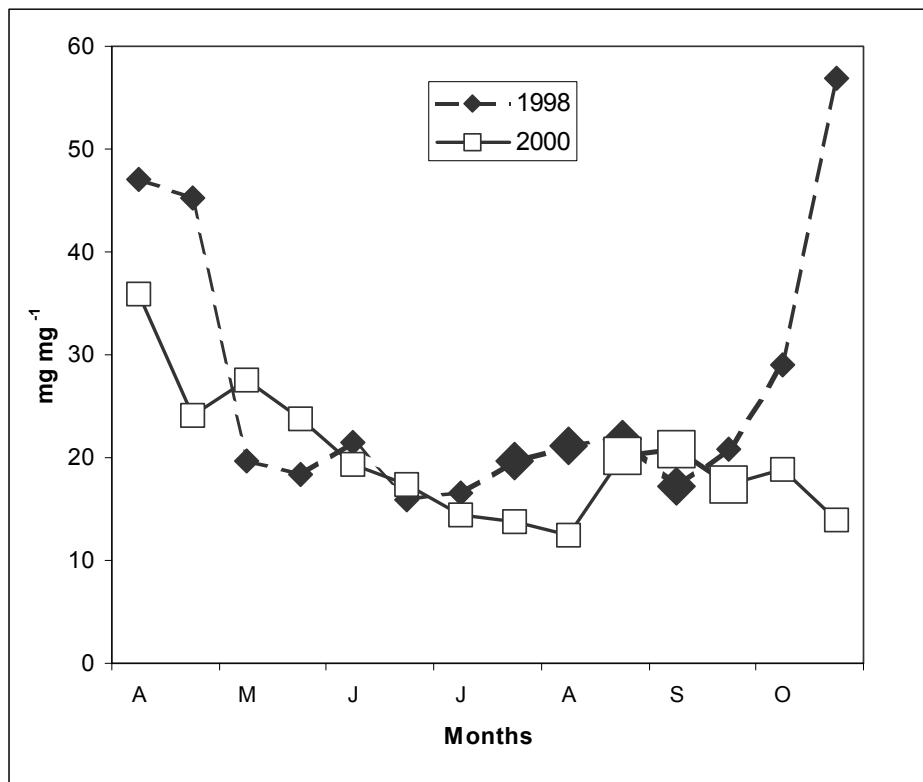
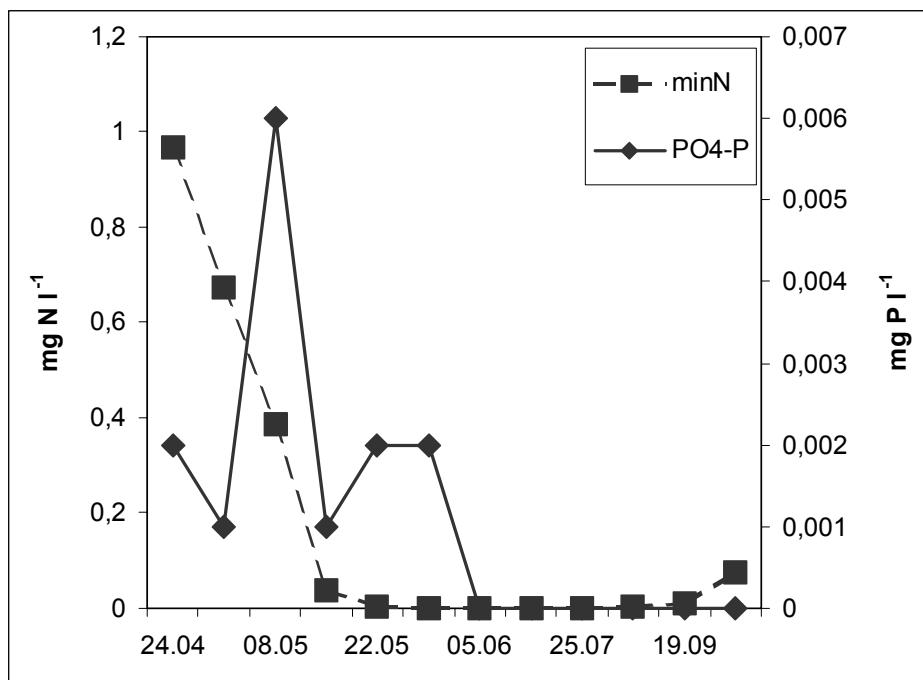


Figure 6. Dynamics of TN/TP mass ratio in L. Võrtsjärv in 1998 and 2000. The period when molecular nitrogen fixation occurred is marked as bold.

In L. Peipsi TN/TP mass ratio decreased from 38 to 14 in the period from 1980s to 2001 due to the reduced nitrogen loading and the increase of total phosphorus concentration. This favored the increase of cyanobacterial (including N₂-fixing) biomass in L. Peipsi.

In *Chara*-dominated shallow eutrophic Lake Prossa phytoplankton seasonal dynamics is different from L. Peipsi and L. Võrtsjärv. After the spring maximum, phytoplankton of L. Prossa consisting of crypto-, chryso- and

dinophytes stays on very low biomass level for the rest of the vegetation period. Negative relationship between macrophytes and phytoplankton involves several mechanisms like competition for nutrients and light (Ruggiero et al., 2003), increased grazing by zooplankton hiding themselves from predators in dense macrophyte stands, higher sinking losses of phytoplankton by mechanically suppressed water turbulence (Pluntke & Kozerski, 2003) and the allelopathic influence of macrophytes on phytoplankton (Gross et al., 2003). In summer 2001 the mineral nitrogen concentration dropped below the detection level in L. Prossia (Fig. 7). This commonly leads to the N₂-fixing cyanobacteria dominance in freshwater systems. Despite of this, in this *Chara*-dominated shallow lake cyanobacteria (both those that fix N₂ and those that do not) were missing from phytoplankton community throughout the vegetation period. In L. Prossia the concentration of PO₄-P in 2001 also decreased below the detection level by the first half of June and remained on this level during the rest of the vegetation period (Fig. 7). In such conditions the strong competition for phosphorus between *Chara* and phytoplankton could be assumed. This is possibly one reason for the missing of cyanobacteria from phytoplankton of L. Prossia as cyanobacteria are poorer competitors for phosphorus than other algae (Smith, 1983; MacKay & Elser, 1998). In L. Prossia also N/P ratio did not favor



cyanobacterial development: in 2001 TN/TP mass ratio varied during the vegetation period from 34 to 80. In *Chara*-dominated L. Prossa the influence of allelochemicals against phytoplankton (including cyanobacteria) as well as the increased net sedimentation rate in the macrophyte areas should be also considered.

Nitrogen dynamics in stratified Lake Verevi

In L. Verevi the nitrogen that accumulated in the hypolimnion was trapped in this non-mixed layer during most of the vegetation period as it is usual to stratified lakes (Scheffer, 1998). The concentration of ammonium (NH_4^+) in the hypolimnion of L. Verevi was high (average 4.8 mgN l^{-1}) compared to the other Estonian stratified eutrophic lakes (average 0.74 mgN l^{-1}), and four Canadian lakes, where the NH_4^+ content in the hypolimnion remained below 2 mgN l^{-1} (Knowles et al., 1981). The high hypolimnetic ammonium concentration implies that most of the epilimnetically derived particulate organic matter was decomposed in this region (Priscu et al., 1986). In the epilimnion the ammonium level remained low in spite of high concentrations in the hypolimnion where it was trapped and appeared useless for epilimnetic organisms. Most probably both, the metalimnetic barrier and also the nitrification detected at the oxic/anoxic interface of the upper section of the hypolimnion were responsible for preventing the penetration of ammonium into the epilimnion in the conditions of stable summer stratification. According to Hall (1982), planktonic nitrification in aerobic hypolimnetic water could be important in affecting changes in the water chemistry of this water layer. In L. Verevi, however, only some temporary coupling with NH_4^+ dynamics was documented in the hypolimnion. In the epilimnion nitrification was unable to influence nitrogen dynamics, probably because of a more open nitrogen cycle involving phytoplankton, which in the euphotic zone quickly consumes all forms of mineral nitrogen.

As a source of nitrogen, N_2 -fixation in Lake Verevi in August 2000 and 2001 was of minor importance. Abundance of N_2 -fixing cyanobacteria usually increased in August, during the period of temporal nitrogen limitation (Kangro et al., in press). Nevertheless, the daily input formed less than 1% of the total amount of TN in the water layer where N_2 fix occurred. The temporary contribution to the algal community could still be important as up to 1.4 times more nitrogen could have been fixed than available in mineral form in the euphotic water layer.

5. CONCLUSIONS

1. N₂-fixation experiments in L. Võrtsjärv showed that it is not possible to assume N₂-fixation only from the hydrochemical conditions and phytoplankton species composition. A lag period of several weeks may occur between the set-up of favorable conditions for N₂-fixation (depleted inorganic N, increased amount of N₂-fixing cyanobacteria with heterocysts) and the N₂-fixation itself. Potentially N₂-fixing cyanobacteria appear in the conditions of N deficiency but seem to be better competitors even without switching up N₂-fixation.
2. Phosphorus-enrichment experiments in L. Võrtsjärv revealed that the elevated phosphorus concentration stimulates N₂-fixation when N₂-fixing cyanobacteria are present in the ecosystem. If phosphorus is mobilized by macrophytes (in L. Prossa) cyanobacteria do not appear even in the conditions of the deficiency of bioavailable nitrogen.
3. In a large and extremely shallow polymictic lake N₂-fixation may occur at much higher TN/TP ratios (~20 by mass in L. Võrtsjärv) than referred in literature. In large shallow lakes surface sediments are continuously resuspended, the turnover rate of phosphorus between sediments and water is considerably higher and phosphorus is more readily available than in stratified lakes.
4. Reduced N/P ratio due to declined nitrogen loading and the increased in-lake phosphorus concentration are mostly responsible for the enhanced cyanobacterial blooms in Lake Peipsi in recent years.
5. In L. Peipsi a specific seasonal sequence of the development of N₂-fixing cyanobacteria occurs. *Gloeotrichia echinulata* starts developing earlier at lower nutrient concentrations. Due to its ability to fix N₂ and to use sediment phosphorus, the water column becomes enriched with nutrients, and other bloom-forming cyanobacteria (*Anabaena*, *Aphanizomenon* and *Microcystis*) can develop later in the season.
6. The role of N₂-fixation and nitrification in the total nitrogen budget of stratified eutrophic Lake Verevi is negligible. Episodically in the nitrogen-depleted epilimnion N₂-fixation could substantially contribute to the pool of mineral nitrogen; some temporary coupling with nitrification and ammonium dynamics in the hypolimnion was also documented.

REFERENCES

- Bulgakov, N. G. & A. P. Levich, A. P., (1999). The nitrogen: phosphorus ratio as a factor regulating phytoplankton community structure. Arch. Hydrobiol. 146: 3–22.
- Downing, J. A., Watson, S. B. & McCauley, E., (2001). Predicting Cyanobacteria dominance in lakes. – Can. J. Fish. Aquat. Sci. 58: 1905–1908.
- Elser, J. J., (1999). The pathway to noxious cyanobacteria blooms in lakes: the food web as a final turn. – Freshwater Biology 42: 537–543.
- Ferber, L. R., Levine, S. N., Lini, A. & Livingston, G. P., (2004). Do cyanobacteria dominate in eutrophic lakes because they fix atmospheric nitrogen? Freshwater Biology 49: 690–708.
- Grasshoff, K., Ehrhardt, M & Kremling, K., (1983). Methods of Seawater Analysis. Verlag Chemie. Weinheim. 419 pp.
- Gross, E., Erhard, D. & Iványi, (2003). Allelopathic activity of *Ceratophyllum demersum* L. and *Najas marina* spp. *intermedia* (Wolfgang) Casper. Hydrobiologia 506–509: 583–589.
- Haberman, J., Nõges, P., Pihu, E., Nõges, T., Kangur, K. & Kisand, V., (1998). Characterization of L. Võrtsjärv. Limnologica 28 (1): 3–11.
- Hall, G. H., (1982). Apparent and measured rates of nitrification in the hypolimnion of a mesotrophic lake. Apl. environm. Microbiol. 43 (3): 542–547.
- Harris, G. P., (1986). Phytoplankton Ecology. Chapman and Hall, USA: 137–165.
- Helal, H. A. & D. A. Culver, D. A., (1991). N : P ratio and plankton production in fish hatchery ponds. Verh. Int. Ver. Limnol. 24: 1508–1511.
- Howarth, R. W., Cole, J. J. & R. Marino, R., (1988b). Nitrogen fixation in freshwater, estuarine and marine ecosystems. 2. Biogeochemical controls. Limnol. Oceanogr. 33. 4: 688–701.
- Howarth, R. W., Cole, J. J., Marino, R. & J. Lane, J. (1988a). Nitrogen fixation in freshwater, estuarine and marine ecosystems. 1. Rates and importance. Limnol. Oceanogr. 33. 4: 669–687.
- Jaani, A., (2001). The location, size and general characterization of Lake Peipsi. – In: Nõges, T. (ed.). Lake Peipsi. Meteorology, hydrology, hydrochemistry. Sulemees Publishers, Tartu, pp. 10–17.
- Jacobsen, B. A., (1994). Bloom formation of *Gloeotrichia echinulata* and *Aphanizomenon flos-aquae* in a shallow, eutrophic, Danish lake. – Hydrobiologia 289: 193–197.
- Joye, S. B., Connell, T. L., Miller, L. G. & Oremland, R. S., (1999). Oxidation of ammonia and methane in an alkaline, saline lake. Limnol. Oceanogr. 44 (1): 178–188.
- Kangro, K., R. Laugaste, P. Nõges & I. Ott, I., (in press). Long-term changes and special features of seasonal development of phytoplankton in a strongly stratified hypertrophic lake. Hydrobiologia.
- Knowles, R., D. R. S. Lean & Y. K. Chan, Y. K., (1981). Nitrous oxide concentrations in lakes: Variations with depth and time. Limnol. Oceanogr. 26. 5: 855–866.
- Kostyaev, V. J., (1986). Biologiya i ekologiya azotfiksiruyushchih sinezelenyh vodoroslej presnyh vod [Biology and ecology of nitrogen fixing cyanobacteria from inland water]. Leningrad , 122 pp.

- Lathrop, R. C., Carpenter, S. R., Stow, C. A., Soranno, P. A. & Panuska, J. C., (1998). Phosphorus loading reductions needed to control blue-green algal blooms in Lake Mendota. – *Can. J. Fish. Aquat. Sci.* 55: 1169–1178.
- Leppänen, J. M., A. Niemi, A. & I. Rinne, I., (1988). Nitrogen fixation of cyanobacteria (blue-green algae) and the nitrogen cycle of the Baltic sea. *Symbiosis* 6: 181–194.
- MacKay, N. A. & J. J. Elser, J. J., (1998). Nutrient recycling by *Daphnia* reduces N₂ fixation by cyanobacteria. *Limnol. Oceanogr.* 43. 2: 347–354.
- Määmets, A., (1977). *Eesti NSV järved ja nende kaitse*. Valgus, Tallinn: 202.
- Michard, M., Aleya, L. & J. Verneaux, J., (1996). Mass occurrence of the Cyanobacteria *Microcystis aeruginosa* in the hypereutrophic Villerest Reservoir (Roanne, France): Usefulness of the biyearly examination of N/P (nitrogen phosphorus) and P/C (protein/ carbohydrate) couplings. *Arch. Hydrobiol.* 135: 337–359.
- Niemi, A., (1979). Blue-green algal blooms and N : P ratio in the Baltic Sea. *Acta Bot. Fennica* 110: 57–61.
- Nixdorf, B. & Deneke, R., (1997). Why “very shallow” lakes are more successful opposing reduced nutrient loads. – *Hydrobiologia* 342/343: 269–284.
- Nõges, P. & Nõges, T., (1998). Stratification of Estonian small lakes studied during hydrooptical expeditions in 1995–97. *Proc. Estonian Acad. Sci. Biol. Ecol.* 47/4: 268–281.
- Nõges, T., Haberman, J., Jaani, A., Laugaste, R., Lokk, S., Määmets, A., Nõges, P., Pihu, E., Starast, H., Timm, T. & Virro, T., (1996). General description of Lake Peipsi-Pihkva. *Hydrobiologia* 338: 1–9.
- Pettersson, K., Herlitz, E. & Istvánovics, V., (1993). The role of *Gloeotrichia echinulata* in the transfer of phosphorus from sediments to water in Lake Erken. – *Hydrobiologia* 253: 123–129.
- Pluntke, T. & Kozersky, H.-P., (2003). Particle trapping on leaves and on the bottom in simulated submerged plant stands. *Hydrobiologia* 506–509: 575–581.
- Présing, M., Herodek, S., Vörös, L. & Kobor, I., (1996). Nitrogen fixation, ammonium and nitrate uptake during a bloom of *Cylindrospermopsis raciborskii* in Lake Balaton. *Arch. Hydrobiol.* 136. 4: 553–562.
- Priscu, J. C., R. H. Spigel, M. M. Gibbs & M. T. Downes, M. T., (1986). A numerical analysis of hypolimnetic nitrogen and phosphorus transformations in Lake Rotoiti, New Zealand: A geothermally influenced lake. *Limnol. Oceanogr.* 31. 4: 812–831.
- Redfield, A.S., (1958). The biological control of chemical factors in the environment. *Amer. Sci.* 46: 205–211.
- Ruggiero, A., Solimini, A. G. & Crachini, G., (2003). Nutrient and chlorophyll *a* in eutrophic mountain ponds with contrasting macrophyte coverage. *Hydrobiologia* 506–509: 657–663.
- Scheffer, M., (1998). Ecology of shallow lakes. Chapman & Hall, London, 49–50.
- Schindler, D. W., (1977). Evolution of phosphorus limitation in lakes. *Science* 195: 260–262.
- Seip, K. L., (1994). Phosphorus and nitrogen limitation of algal biomass across trophic gradients. *Aquatic Sciences* 56: 16–28.
- Smith, V. H., Willén, E. & Karlsson, B., (1987). Predicting the summer peak biomass of four species of blue-green algae (Cyanophyta/ cyanobacteria) in Swedish lakes. – *Water Resources Bulletin* 23 (3): 397–402.

- Smith, Val H., (1983). Low nitrogen to phosphorus ratios favor dominance by blue-green algae in lake phytoplankton. – *Science* 221: 669–670.
- Steeman-Nielsen, E., (1952). The use of radioactive carbon (^{14}C) for measuring primary production in the sea. *Journal du Conseil permanent international pour l'exploration del la mer*. 18: 117–140.
- Stewart, W. D. P., Fitzgerald, G. P. & Burris, R. M., (1967). In situ studies on N_2 fixation using the acetylene reduction technique. *Proc. natn. Acad. Sci. U.S.A.* 58: 2071–2078.
- Stolp, H., (1996). Microbial ecology: organisms, habitats, activities. Cambridge University Press, USA, 232–241.
- Tilman, D., S. S. Kilham, S. S. & P. Kilham, P., (1982). Phytoplankton community ecology: The role of limiting nutrients. *Ann. Rev. Ecol. Syst.* 13: 349–372.
- Utermöhl, H., (1958). Zur Vervollkommenung der quantitativen Phytoplankton Methodik. *Mitt. Int. Ver. Theor. Angew. Limnol.* 9: 1–38.
- Willén, E., (1992). Long-term changes in the phytoplankton of large lakes in response to changes in nutrient loading. – *Nord. J. Bot.* 12: 575–587.
- Willén, E., (2001). Phytoplankton and water quality characterization experiences from the Swedish large lakes Mälaren, Hjälmaren, Vättern and Vänern. – *Ambio*. 30 (8): 529–537.
- Williams, P. J. le B., D. N. Thomas & C. S. Reynolds, C. S., (edit.) (2002). Phytoplankton productivity. Carbon assimilation in marine and freshwater ecosystems. Blackwell Science, UK, 109–141.
- Zingel, P., & Ott, I., (2000). Vertical and seasonal dynamics of planktonic ciliates in a strongly stratified hypertrophic lake Vertical distribution of planktonic ciliates in strongly stratified temperate lakes. *Hydrobiologia*. 435: 19–26.

SUMMARY IN ESTONIA

Lämmastiku, fosfori ja N/P suhte mõju tsüanobakterite domineerimisele ning molekulaarse lämmastiku fikseerimisele mõnedes Eesti järvedes

Doktoritöö on pühendatud lämmastiku dünaamika, planktiliste tsüanobakterite (sinivetikate) arengu ning molekulaarse lämmastiku fikseerimise vaheliste seoste selgitamisele mõnedes segunevates (Võrtsjärv, Peipsi, Pross) ja kihistunud (Verevi) Eesti järvedes. Molekulaarse lämmastiku fikseerimist (N_2 fiks) uuriti madalas eutroofses Võrtsjärves aastatel 1998–2000 ning kihistunud hübertroofses Verevi järves aastatel 2000–2001. Peipsis selgitati toiteainete (P, N) kontsentratsiooni ning N/P suhte mõju tsüanobakterite biomassile ja liigilisele koosseisule aastatel 1983–2001.

Töö üheks eesmärgiks oli välja selgitada, millistel tingimustel algab suures ja väga madalas eutroofses järves N_2 fiks. Samuti püüti täpsustada fosfori, lämmastiku ja N/P suhte mõju N_2 fiks tsüanobakterite arengule madalas eutroofses järves ning selgitada N_2 fiks tsüanobakterite sesoonse arengu seoseid lämmastikuühendite kättesaadavuse muutusega. Töö teiseks eesmärgiks oli uurida N_2 fikseerimise ja nitrifikatsiooni mõju teravalt kihistunud järve lämmastikuringle.

Töö tulemusena leiti järgmist:

1. Molekulaarse lämmastiku fikseerimise katsed Võrtsjärves näitasid, et N_2 fiks pole võimalik eeldada lähtudes ainuüksi hüdrokeemilistest tingimustest ning fütoplanktoni liigilisest koosseisust. N_2 fikseerimiseks soodsate tingimuste (madal mineraalse lämmastiku kontsentratsioon, heterotsüstidega tsüanobakterite arvukuse kasv) ja tegeliku N_2 fiks vahel võib esineda mitmenädalane viivitus. Potentsiaalse N_2 fiks võimega tsüanobakterid ilmuvald küll lämmastikudefisiidi tingimustes, kuid tunduvad olevat konkurentsivõimelised isegi siis, kui nad N_2 fikseerimise võimet ei kasuta.
2. Fosfori lisamise katsed Võrtsjärves näitasid, et kõrgem fosfori kontsentratsioon stimuleerib N_2 fikseerimist siis, kui N_2 fiks sinivetikad on ökosüsteemis juba olemas. Kui fosfor on seotud suurtaimede poolt (Prossas), siis tsüanobakterid ei ilmu isegi lämmastiku puuduse korral.
3. Suure pindalaga väga madalates järvedes, mille vesi ja pindmine settekiht on lainetuse mõjul heas kontaktis, toimub N_2 fiks märksa kõrgemate N/P suhete juures kui on publitseeritud. Selle tõenäoliseks põhjuseks on suurte madalate järvede kiire fosforivahetus sette ning veemassi vahel, mis muudab fosfori paremini kättesaadavaks kui sügavates kihistunud järvedes.
4. Peipsis on viimaste aastate ulatuslike tsüanobakterite õitsengute peamiseks põhjuseks järve sissekantava lämmastiku koguse vähenemisest ning järvesisese fosfori kontsentratsiooni tõusust tingitud N/P suhte oluline langus.

5. Peipsi järves esineb eri liiki N₂fiks tsüanobakterite arengus kindel järjekord. Esmalt ilmub fütoplanktoni kootseisu madala toiteainete kontsentratsooniga kohastunud *Gloeotrichia echinulata*. Tänu selle liigi võimele fikseerida molekulaarset lämmastikku ja kasutada settes leiduvat fosforit, rikastatakse veemass toiteainetega ning arenema saavad hakata teised vee-õitsenguid põhjustavad tsüanobakterid (*Anabaena*, *Aphanizomenon* ja *Microcystis*).
6. Kihistunud Verevi järves moodustasid N₂ fikseerimine ja nitrifikatsioon üldlämmastiku kogubilansist vaid tühise osa. Siiski võib epilimnionis lühiajaliselt tekkinud lämmastikudefitsiidi korral olla N₂fiks panus mineraalse lämmastiku hulga suurendamisel oluline. Hüpolimnionis esines ammoniumi dünaamika ning nitrifikatsiooni vahel ajutisi seoseid.

ACNOWLEDGEMENTS

Funding for this study was provided by the target-financed projects 0370208s98 and 0362480s03 of Estonian Ministry of Education, by the grants 4080 and 5738 of Estonian Science Foundation, and by the European Commission's Environment and Sustainable Development Program under contracts EVK1-CT-1999-39 (ECOFRAFME), EVK1-CT-2000-00076 (MANTRA-East) and EVK1-CT-2002-00121 (CLIME). The data obtained in frames of the program of environmental monitoring were used.

First, I want to express my gratitude to my parents, who have supported me all this years. Special thanks to my supervisor Dr. Tiina Nõges for her friendship and support during my study years, especially in critical times. I want to give my thanks to Dr. Peeter Nõges who always found time to listen me and discuss with me.

Most part of the work was carried out at Võrtsjärv Limnological Station and I wish to express my gratitude to all staff for their friendly attitude.

I am grateful to prof. Uuno Mäeorg and Katrin Ott for support and advice from the very beginning of my nitrogen fixation measurements.

Many thanks to the “limnological station young gang” who showed me the pleasant side of the life.

PUBLICATIONS

Tõnno, I. & Nõges, T. 2003. Nitrogen fixation in a large shallow lake: rates and initiation conditions. *Hydrobiologia* 490: 23–30.

Nõges, T., Tõnno, I., Laugaste, R., Loigu, E. & Skakalski, B. 2004. The impact of changes in nutrient loading on cyanobacterial dominance in Lake Peipsi (Estonia/Russia). *Arch. Hydrobiol.* 160, 2: 261–279.

Tõnno, I., Ott, K. & Nõges, T. 2004. Nitrogen dynamics in steeply stratified temperate Lake Verevi, Estonia. (submitted to *Hydrobiologia*).

Tõnno, I., Künnap, H. & Nõges, T. 2003. The role of zooplankton grazing in the formation of ‘clear water phase’ in a shallow charophyte-dominated lake. *Hydrobiologia* 506–509: 353–358.

Nõges, P., Tuvikene, L., Feldmann, T., Tõnno, I., Künnap, H., Luup, H., Salujõe, J. & Nõges, T. 2003. The role of charophytes in increasing water transparency: a case study of two shallow lakes in Estonia. *Hydrobiologia* 506–509: 567–573.

Nõges, T., Tõnno, I., Blinova, I., Loigu, E., Jastremski, V., Laugaste, R. & Skakalski, B. 2003. Reduced nitrogen loading enhances cyanobacterial blooms in Lake Peipsi. In: Proceedings of the second international conference “Sustainable management of transboundary waters in Europe”. Poland, Miedzyzdroje: 501–504.

CURRICULUM VITAE

General facts

Name: Ilmar Tõnno
Date and place of birth: November 28, 1972, in Tartu (Estonia)
Nationality: Estonian
Current employment: doctoral student at the Institute of Zoology and Hydrobiology, University of Tartu; laboratory assistant at the Estonian Agricultural University, Institute of Zoology and Botany, Võrtsjärv Limnological Station
Marital status: single
Present address: Ravila 52–3, Tartu 50408, Estonia;
phone: +372 53436598
e-mail: ilmar@zbi.ee

Education

1991: graduated from Tartu Secondary School No. 5
1996: graduated from University of Tartu as hydrobiologist
1999: Msc in Hydrobiology

Professional experience

1996–1999: laboratory assistant at the Institute of Zoology and Botany, Võrtsjärv Limnological Station
2003– : laboratory assistant at the Estonian Agricultural University, Institute of Zoology and Botany, Võrtsjärv Limnological Station

Foreign languages

English, Russian

Scientific activity

Fields of research: ecology of cyanobacteria; molecular nitrogen fixation in lakes by cyanobacteria; ecology of shallow lakes

List of publications

- Tõnno, I. & Nõges, T. 2003. Nitrogen fixation in a large shallow lake: rates and initiation conditions. *Hydrobiologia* 490: 23–30
- Tõnno, I., Künnap, H. & Nõges, T. 2003. The role of zooplankton grazing in the formation of ‘clear water phase’ in a shallow charophyte-dominated lake. *Hydrobiologia* 506–509: 353–358
- Nõges, T., Tõnno, I., Blinova, I., Loigu, E., Jastremski, V., Laugaste, R. & Skakalski, B. 2003. Reduced nitrogen loading enhances cyanobacterial blooms in Lake Peipsi. In: Proceedings of the second international conference “Sustainable management of transboundary waters in Europe”. Poland, Miedzyzdroje: 501–504.
- Nõges, T., Tõnno, I., Laugaste, R., Loigu, E. & Skakalski, B. 2004. The impact of changes in nutrient loading on cyanobacterial dominance in Lake Peipsi (Estonia/Russia). *Arch. Hydrobiol.* 160, 2: 261–279
- Tuvikene, L., Feldmann, T., Tõnno, I., Künnap, H., Luup, H., Salujõe, J. & Nõges, T. 2003. The role of charophytes in increasing water transparency: a case study of two shallow lakes in Estonia. *Hydrobiologia* 506–509: 567–573

Grants

1999–2003: grant- aided at the Estonian Agricultural University, Institute of Zoology and Botany, Võrtsjärv Limnological Station

CURRICULUM VITAE

Üldandmed

Nimi:	Ilmar Tõnno
Sünnikoht ja –aeg:	Tartus, 28. november 1972
Rahvus:	eestlane
Töökoht:	Tartu Ülikooli zooloogia ja hüdrobioloogia instituudi doktorant; EPMÜ ZBI Võrtsjärve Limnoloogiaajaama van. laborant
Perekonnaseis:	vallaline
Aadress:	Ravila 52–3, Tartu 50408;
telefon:	53436598
e-post:	ilmar@zbi.ee

Haridus

1991:	lõpetanud Tartu 5. Keskkooli
1996:	lõpetanud Tartu Ülikooli hüdrobioloogina
1999:	kaitsnud Tartu Ülikoolis magistrikraadi (Msc) hüdrobioloogias

Teenistuskäik

1996–1999:	ZBI Võrtsjärve Limnoloogiaajaama laborant
2003–:	EPMÜ ZBI Võrtsjärve Limnoloogiaajaama van.-laborant

Võõrkeeled

Inglise, vene

Teadustöö

Uurimisvaldkonnad: sinivetikate e. tsüanobakterite ökoloogia; molekulaarse lämmastiku fikseerimine järvedes sinivetikate poolt; madalate järvede ökoloogia

Publikatsioonide loetelu

- Tõnno, I. & Nõges, T. 2003. Nitrogen fixation in a large shallow lake: rates and initiation conditions. *Hydrobiologia* 490: 23–30
- Tõnno, I., Künnap, H. & Nõges, T. 2003. The role of zooplankton grazing in the formation of ‘clear water phase’ in a shallow charophyte-dominated lake. *Hydrobiologia* 506–509: 353–358
- Nõges, T., Tõnno, I., Blinova, I., Loigu, E., Jastremski, V., Laugaste, R. & Skakalski, B. 2003. Reduced nitrogen loading enhances cyanobacterial blooms in Lake Peipsi. In: Proceedings of the second international conference “Sustainable management of transboundary waters in Europe”. Poland, Miedzyzdroje: 501–504.
- Nõges, T., Tõnno, I., Laugaste, R., Loigu, E. & Skakalski, B. 2004. The impact of changes in nutrient loading on cyanobacterial dominance in Lake Peipsi (Estonia/Russia). *Arch. Hydrobiol.* 160, 2: 261–279
- Tuvikene, L., Feldmann, T., Tõnno, I., Künnap, H., Luup, H., Salujõe, J. & Nõges, T. 2003. The role of charophytes in increasing water transparency: a case study of two shallow lakes in Estonia. *Hydrobiologia* 506–509: 567–573

Toetused

1999–2003: EPMÜ ZBI Võrtsjärve Limnoloogiaajaama stipendiaat

Nitrogen dynamics in steeply stratified temperate Lake Verevi, Estonia

Ilmar Tõnno^{1,2}, Katrin Ott¹ & Tiina Nõges¹

¹Estonian Agricultural University, Institute of Zoology and Botany, Võrtsjärv Limnological Station, 61101 Rannu, Tartu County, Estonia

E-mail: ilmar@zbi.ee

²University of Tartu, Institute of Zoology and Hydrobiology, Vanemuise 46, 51014 Tartu, Estonia

Key words: stratified lake, nitrogen dynamics, planktonic N₂-fixation, nitrification

This paper has not been submitted elsewhere in identical or similar form, nor will it be during the first three months after its submission to *Hydrobiologia*.

Abstract

The dynamics of different nitrogen compounds and nitrification in diverse habitats of a stratified Lake Verevi (Estonia) was investigated in 2000–2001. Also planktonic N₂-fixation (N₂fix) was measured in August of observed years. The nitrogen that accumulated in the hypolimnion was trapped in the non-mixed layer during most of the vegetation period causing an order of magnitude higher concentration than in the epilimnion. The ammonium level remained low in the epilimnion (maximum 577 mgN m⁻³, average 115 mgN m⁻³) in spite of high concentrations in the hypolimnion (maximum 12223 mgN m⁻³, average 4807 mgN m⁻³). The concentrations of NO₂⁻ and NO₃⁻ remained on a low level both in the epilimnion (average 0.94 and 9.09 mgN m⁻³, respectively) and hypolimnion (average 0.47 and 5.05 mgN m⁻³, respectively). N₂fix and nitrification ranged from 0.30–2.80 mgN m⁻³ day⁻¹ and 6.0–107 mgN m⁻³ day⁻¹, respectively; the most intensive processes occurred in 07.08.00 at depths of 2 and 5 m, accordingly. The role of N₂fix in the total nitrogen budget of Lake Verevi (in August 2000 and 2001) was negligible while episodically in the nitrogen-depleted epilimnion the N₂fix could substantially contribute to the pool of mineral nitrogen. Nitrification was unable to influence nitrogen dynamics in the epilimnion while some temporary coupling with ammonium dynamics in the hypolimnion was documented.

Introduction

Nitrogen (N) is one of the main building blocks for the production of organic matter on the planet Earth and it is required in greatest quantities (Stolp, 1996; Williams *et al.*, 2002). N, as many other elements in the world, is involved in cyclical transformations. Non-biological transformations have little importance in the nitrogen cycle by contrast with biological transformations, which are primarily controlled by microorganisms (Gorlenko *et al.*, 1977; Sprent, 1987; Stolp, 1996; Voytek *et al.*, 1999). A nitrogen cycle consists of four main components: molecular nitrogen (N_2) fixation ($N_2\text{fix}$), mineralization of organic N (ammonification), nitrification, and denitrification.

Only prokaryotes are capable of $N_2\text{fix}$. In aquatic ecosystems cyanobacteria appear responsible for most of the planktonic $N_2\text{fix}$ while heterotrophic bacteria are most important N_2 fixers in lake sediments. The fixation of N_2 by microorganisms is the only process in nature that counteracts the nitrogen losses from the environment by denitrification. The central compound of the nitrogen cycle is ammonium (NH_4^+) which is released into the water by zooplankton and represents the main decomposition product of urea of other animals like fish. In anaerobic hypolimnion where animals are scarce, ammonium is formed at amino-acid degradation of proteins carried out by ammonifying bacteria, occurring in the water column and sediments (Gorlenko *et al.*, 1977; Howarth *et al.*, 1988a; Stolp, 1996). Nitrification is a two-step oxidation of NH_4^+ through nitrite (NO_2^-) to nitrate (NO_3^-), carried out mainly by chemolithoautotrophic bacteria in aerobic conditions. The most important and intensive site in lakes for nitrification are aerobic sediments while planktonic nitrification could be also significant. Denitrification is an anaerobic heterotrophic process, which shares many of the same substrates and intermediates as nitrification. Denitrification leads to gaseous nitrogen (N_2 , N_2O) losses counteracting $N_2\text{fix}$ (Gorlenko *et al.*, 1977; Hall, 1982; Henriksen *et al.*, 1993; Stolp, 1996; Voytek *et al.*, 1999). In stratified lakes phytoplankton takes up epilimnetic mineral nitrogen and transports it to the hypolimnion via sedimentation. N may accumulate in the hypolimnion during stratification period while in the epilimnion N deficiency may occur if resupply from the inflows is limited (Scheffer, 1998).

The aim of the present study was to investigate the dynamics of different nitrogen compounds as well as the rates of N_2 -fixation and nitrification in diverse habitats of a stratified partly meromictic lake. The main processes of transformations (Figure 1) were followed on the background of the dynamics of the physico-chemical stratification regime.

Materials and Methods

Lake Verevi (0.126 km^2 , mean depth 3.6 m, maximum depth 11 m) is a small stratified hypertrophic (see Ott et al., the present issue) lake in South Estonia. The lake is characterized by strong stratification from April to September and an anoxic hypolimnion. The main N_2fix cyanobacterium in Lake Verevi during the years 2000 and 2001 was *Aphanizomenon klebahnii* (Elenkin) Pechar et Kalina (Kangro et al., the present issue).

The water samples for nitrogen determination were collected from April to December 2000 and from March to August 2001. In the year 2000 eight and in 2001 three to eight vertical samples were taken at different depths of the epi-, meta- and hypolimnion. The water from the surface layer (0.5 m) was taken directly into the bottle, for other depths a Masterflex pump was used (for details see Zingel, the present issue). Total nitrogen (TN), NH_4^+ , NO_2^- and NO_3^- was analysed at the laboratory of Võrtsjärv Limnological Station using the methods described by Grasshoff *et al.* (1983). For more detailed description of TN determination see Ott et al. (present issue). Ammonium was determined (detection error $\pm 5.5\%$) with indophenol blue method (Hansen & Koroleff, 1999). Nitrate was reduced to nitrite, and sulphanil-amide and N-(1-naphthyl)-ethylenediamine dihydrochloride was used (detection error $\pm 2\%$) for the determination of NO_2^- (Koroleff, 1982). The total amounts of measured nitrogen forms in the epilimnion, hypolimnion and in the whole water column for both years were calculated by integrating the concentrations of the compounds in different water layers. For a detailed description of the calculation method see Nõges et al. (in the present issue).

N_2 -fixation in Lake Verevi was measured on August 7, 2000 at one, on August 28, 2000 at two, and on August 2, 2001 at four different depth horizons in the epilimnion (Table 1) applying the acetylene reduction method (Stewart *et al.*, 1967; Présing *et al.*, 1996). Three 60-ml glass bottles were filled with lake water from each depth horizons and exposed for four hours in the incubator at a constant illumination of 120 W m^{-2} *in situ* temperature. For details see Tõnno & Nõges (2003).

Depths for the nitrification measurement were selected according to the supposed NH_4^+ and oxygen content (Table 1). At each depth four dark glass scintillation vials (two ‘samples’ and two ‘blanks’) with a capacity of 24 ml were filled with lake water. $\text{NaH}^{14}\text{CO}_3$ (VKI, Denmark) was added to each vial with a final activity of $0.07 \mu\text{Ci ml}^{-1}$. 100 μl of nitrification inhibitor 2-chloro-6- (trichloromethyl) pyridine (TCMP) was added (final concentration 10 mg l^{-1}) to ‘blank’ vials. Thereafter the vials were incubated 24 h in thermos flasks containing the water from the same depth where the samples had been taken from. After the incubation 100 μl of water from each vial was taken and mixed with 0.5 ml of β -phenylethylamine (PEA) for the assessment of total radioactivity by using 5 ml of Optiphase solution and LSC RackBeta 1211

(Wallac, Finland). The rest of the water sample from the vials (23.9 ml) was filtered through membranes of 0.20 µm pore size (Millipore HA). The filters were treated with concentrated HCl fumes for 5 minutes to remove the excess of inorganic ^{14}C , and air-dried for 24 h. 5 ml of toluene-PPO-POPOP cocktail was added to filters and their radioactivity was assessed with LSC RackBeta 1211.

Chemosynthetic fixation of CO_2 was calculated by the formula:

$$R = [x * C * 1.05 * 1.06 * V1 * k] / y * V2 * t$$

where R – CO_2 fixation rate ($\text{mmole m}^{-3} \text{ h}^{-1}$); x – difference of the radioactivities of the filter from the ‘sample’, and the filter from the ‘blank’; C – concentration of HCO_3^- in water (mmole l^{-1}); 1.05 – coefficient considering the difference of assimilation efficiencies of $^{12}\text{CO}_2$ and $^{14}\text{CO}_2$; 1.06 – factor considering the respiration losses of the assimilated CO_2 during the exposition; V1 – volume of the exposition vial (ml); k=1000 coefficient from litres to cubic meters; y – radioactivity of $\text{NaH}^{14}\text{CO}_3$ solution added to the vial; V2 – amount of the filtered water (ml); t – incubation time (h). To estimate nitrification, we used an average conversion factor of 8.3 moles of N oxidized per mole of carbon fixed (Owens, 1986; Joye *et al.*, 1999).

Results

In Lake Verevi the concentration of total nitrogen in the hypolimnion (annual average 6646 mgN m^{-3}) was by an order of magnitude higher than in the epilimnion (annual average 948 mgN m^{-3}). TN concentration in the epilimnion and in the hypolimnion from April to September was 781 and 4007 mgN m^{-3} , respectively, increasing by the end of the vegetation period up to 1284 and 11922 mgN m^{-3} , respectively (Figure 2a). Mean epilimnetic concentration of ammonium from May to August was 6.4 mgN m^{-3} , followed by a sharp increase. In the hypolimnion the concentration of NH_4^+ was about 35 times higher (average from May to August 2118 mgN m^{-3}) than in the epilimnion but followed the same dynamics (Figure 2b). The NO_2^- content in the epi- and hypolimnion stayed on a low level from April to October (average 0.21 and 0.16 mgN m^{-3} , respectively), increasing up to 6.8 and 2.9 mgN m^{-3} , respectively by November/December (Figure 2c). The mean epilimnetic concentration of NO_3^- from April to October was 1.5 mgN m^{-3} increasing abruptly up to 130 mgN m^{-3} by November/December (Figure 2d). Mean hypolimnetic nitrate concentration in spring was 3.4 mgN m^{-3} , by the end of June it decreased to undetectable values, and two peaks occurred in autumn: in September (4.1 mgN m^{-3}) and in December (58 mgN m^{-3}).

N_2 -fixation was measured in 2000 on August 7th at a depth of 2 m ($2.80 \text{ mgN m}^{-3} \text{ day}^{-1}$), and on August 28th at a depth of 0.5 m (0.30 mgN m^{-3}

day⁻¹) forming, respectively, 0.31% and 0.043% of TN, and 140% and 15% of the amount of mineral nitrogen in the investigated depth horizons. On August 2, 2001 the N₂fix occurred only at a depth of 3 m (0.38 mgN m⁻³ day⁻¹), taking up 0.05% of TN and 5.37% of mineral nitrogen in the investigated depth horizon.

Nitrification occurred in 2000 on August 7th at a depth of 5 m (107 mgN m⁻³ day⁻¹), and on August 28th at a depth of 5.5 m (54.4 mgN m⁻³ day⁻¹). In 2001 we were unable to detect any nitrification on April 23rd and on May 7th (Table 1) while on April 30th and June 5th nitrification occurred at a depth of 0.5 m (6.0 and 7.5 mgN m⁻³ day⁻¹, respectively) and 5.5 m (13.3 and 17.7 mgN m⁻³ day⁻¹, respectively). On August 2nd we detected nitrification at four depths: 0.5, 3, 6, and 7m (26.1, 50.7, 79.3 and 308 mgN m⁻³ day⁻¹, respectively).

Discussion

As it is common to stratified lakes (Scheffer, 1998), in Lake Verevi the nitrogen that accumulated in the hypolimnion was trapped in the non-mixed layer during most of the vegetation period remaining inaccessible to the epilimnetic community.

The concentration of ammonium in the hypolimnion of Lake Verevi was high (maximum 12223 mgN m⁻³, average 4807 mgN m⁻³) compared to the other Estonian stratified eutrophic lakes, where according to the database of Võrtsjärv Limnological Station (108 lakes), the average is 742 mgN m⁻³, and four Canadian lakes, where the NH₄⁺ content in the hypolimnion remained below 2000 mgN m⁻³ (Knowles *et al.*, 1981). The high hypolimnetic ammonium concentration implies that most of the epilimnetically derived particulate organic matter was decomposed in this region (Priscu *et al.*, 1986). According to Tammert *et al.* (present issue) bacteria are one of the most important pools of nutrients (nitrogen, phosphorus) in the hypolimnion of Lake Verevi. In the epilimnion the ammonium level remained low in spite of high concentrations in the hypolimnion (Figure 2b). Most probably the metalimnetic barrier but also nitrification detected at the oxic/anoxic interface of the upper section of the hypolimnion were responsible for preventing the penetration of ammonium into the epilimnion in conditions of stable summer stratification. As many microorganisms prefer NH₄⁺ as a nitrogen source (Wetzel, 1983; Ahlgren *et al.*, 1994), the ammonium leaking from the hypolimnion could be trapped also by phytoplankton. In Lake Verevi euglenophytes were numerous important in the upper part of hypolimnion, where the concentration of ammonium was high (Kangro *et al.*, in the present issue). Although there was high NH₄⁺ content in the hypolimnion, it was trapped and useless for epilimnetic organisms. Such a sharp gradient of nutrients composed a number of niches in the water column for the phyto- and bacterioplankton (see Kangro *et al.*; Tammert *et al.*, in the present issue). An increase in the epilimnetic ammonium concentration in

autumn could be caused by the disturbance of stratification and mixing up of some hypolimnetic water of high ammonium concentration. This assumption is, however, not supported by the results of our measurements showing increasing concentrations both in the epi- and hypolimnion. As the whole year 2000 and the September of 2001 were poor rather than rich in precipitation, a high external loading was not likely either. The only explanation for this pronounced increase could be the mixing up of the nutrient-rich water from the thin near-bottom layer which was not detected by our sampling strategy. It is possible that we could not collect the nearest to the bottom water layer by applied water sampler. As shown by Kõiv & Kangro (the present issue), the concentration of total phosphorus (TP) and SRP in the whole water column also increased in September, which supports the hypothesis that the near-bottom water layer had been mixed up. The hypothesis, however, remains speculative as we have no evidence of such near bottom nutrient rich layer. The concentrations of NO_2^- and NO_3^- remained on a low level both in the epi- and hypolimnion (Figures 2c, d). The nitrogen mineralized in the epilimnion was probably quickly assimilated by the phytoplankton, causing temporal nitrogen limitation and increase of N₂-fixing cyanobacteria (see Kangro et al., in the present issue). In Lake Verevi denitrification probably could occur not only in the sediments but also in the water column close to the anoxic hypolimnion, and, thus, use nitrite and nitrate, as it was found also in other stratified lakes (Golterman, 1975; Lampert & Sommer, 1997).

It must be emphasized that there is no information about the seasonality of N₂-fixation in Lake Verevi. As a source of nitrogen, N₂-fixation in Lake Verevi in August 2000 and 2001 was of minor importance. Abundance of N₂-fixing cyanobacteria (mainly *Aphanizomenon klebahnii*) usually increased in August, during the period of temporal nitrogen limitation (Kangro et al., the present issue). Nevertheless, the daily input formed less than 1% of the total amount of TN in the water layer where occurred. The temporary contribution to the algal community could still be important as up to 1.4 times more nitrogen could have been fixed than available in mineral form in the euphotic water layer. According to Kostjaev (1986), in eutrophic lakes N₂fix could form up to 50% of the yearly nitrogen budget.

As an oxygen (O₂) demanding process, nitrification occurred in the epilimnion and the upper part of the hypolimnion (5–5.5 m), where some O₂ was present (Figure 3). By Tammert et al. (present issue) the total number of bacteria was highest in the hypolimnion. According to Knowles et al. (1981), nitrification has been reported to occur more rapidly at low O₂ concentrations. Accordingly, in Lake Verevi also more intensive nitrification occurred in the upper hypolimnion (except on August 2, 2001). High rates of dark assimilation of ¹⁴CO₂ measured on August 2nd, 2001 in anoxic H₂S- rich water at depths of 6 and 7 m could probably indicate not the nitrification but rather the oxidation of H₂S by sulphur chemoautotrophes, which can also assimilate inorganic carbon in darkness (Gorlenko et al., 1977). Nitrification intensity in Lake

Verevi (up to $308 \text{ mgN m}^{-3} \text{ day}^{-1}$, Figure 3) was much higher than recorded by Hall (1982) in the hypolimnion of mesotrophic L. Grasmere (in all cases less than $8 \text{ mgN m}^{-3} \text{ day}^{-1}$). According to Hall (1982), planktonic nitrification in aerobic hypolimnetic water could be important in affecting changes in the water chemistry of this water layer. In Lake Verevi, however, a remarkable influence on nitrogen dynamics could be quantified in some cases. For example, a decrease in ammonium during the period after a rather high nitrification rate had been detected in late August/early September 2000, and at the beginning of May 2001 in the hypolimnion (Figure 4). In the epilimnion the coupling was absent probably because a more open nitrogen cycle involving phytoplankton, which in the euphotic zone quickly consumes all forms of mineral nitrogen.

Conclusions

- In Lake Verevi steep stratification trap ammonium in the hypolimnion during most of the vegetation period causing a concentration that is by an order of magnitude higher than in the epilimnion.
- The role of N₂-fixation in the total nitrogen budget of Lake Verevi in our investigation period was negligible while episodically in the nitrogen-depleted epilimnion N₂fix could substantially contribute to the pool of mineral nitrogen.
- Nitrification was unable to influence nitrogen dynamics in the epilimnion while some temporary coupling with ammonium dynamics in the hypolimnion was documented.

Acknowledgements

This work was supported by the core grants No 0370208s98 and 0362480s03 of Estonian Ministry of Education, and by grants No 3579, 4835 and 5738 of the Estonian Science Foundation. We would like thank I. Ott, A. Rakko, D. Sarik, T. Kõiv, P. Nõges, K. Kübar, E. Lill, H. Tammert, H. Künnap, H. Starast, A. Lindpere, K. Kangro, R. Laugaste for taking part in the project and making their data available for analysis. We would also like to thank Dr E. Veldi for revising the language and the anonymous reviewer for revising the manuscript.

References

- Ahlgren, I., F. Sörensson, T. Waara & K. Vrede, 1994. Nitrogen budgets in relation to microbial transformations in lakes. *Ambio* 23: 367–377.
- Golterman, H. L., 1975. Physiological limnology. An approach to the physiology of lake ecosystems. Elsevier, Amsterdam – Oxford – New York.
- Gorlenko, V. M., G. A. Dubinina & C. I. Kuznetsov, 1977. Ekologija vodnyh mikroorganizmov [Ecology of water micro-organisms]. Moscoy.
- Grasshoff, K., M. Ehrhardt & K. Kremling, 1983. Methods of Seawater Analysis. Verlag Chemie. Weinheim.
- Hall, G. H., 1982. Apparent and measured rates of nitrification in the hypolimnion of a mesotrophic lake. *Applied and Environmental Microbiology*. 43: 542–547.
- Hansen, H. P. & F. Koroleff, 1999. Determination of nutrients. In Grasshoff, K., K. Kremling & M. Ehrhardt (eds), Methods of Seawater Analysis. WILEY-VCH, Weinheim. New York. Chichester. Brisbane. Singapore. Toronto: 600 pp.
- Henriksen K., T. H. Blackburn, B. A. Lomstein & C. P. McRoy, 1993. Rates of nitrification, distribution of nitrifying bacteria and inorganic N fluxes in northern Bering- Chukchi shelf sediments. *Continental Shelf Research* 13: 629–651.
- Howarth, R. W., J. J. Cole, R. Marino & J. Lane, 1988. Nitrogen fixation in freshwater, estuarine and marine ecosystems. 1. Rates and importance. *Limnology and Oceanography* 33: 669–687.
- Joye, S. B., T. L. Connell, L. G. Miller & R. S. Oremland, 1999. Oxidation of ammonia and methane in an alkaline, saline lake. *Limnology and Oceanography* 44: 178–188.
- Kangro, K., R. Laugaste, P. Nõges & I. Ott, Long-term changes and special features of seasonal development of phytoplankton in a strongly stratified hypertrophic lake (Present issue).
- Knowles, R., D. R. S. Lean & Y. K. Chan, 1981. Nitrous oxide concentrations in lakes: Variations with depth and time. *Limnology and Oceanography* 26: 855–866.
- Kõiv, T. & K. Kangro, Resource ratios and phytoplankton species composition in a strongly stratified lake (Present issue).
- Koroleff, F., 1982. Total and organic nitrogen. In K. Grasshoff (ed), Methods of seawater analysis. Verlag chemie: 162–168.
- Kostyaev, V. J., 1986. Biologiya i èkologiya azotfiksiruyushchih sinezelenyh vodoroslej presnyh vod. Leningrad. [Biology and ecology of nitrogen fixing cyanobacteria from inland water].
- Lampert, W. & U. Sommer, 1997. Limnoecology: The Ecology of Lakes and Streams. Oxford University Press.
- Nõges, P. Water and nutrient mass balance of the partly meromictic temperate Lake Verevi (Present issue).
- Ott, I., T. Kõiv, P. Nõges, A. Kisand, A. Järvalt & E. Kirt. General description of partly meromictic hypertrophic Lake Verevi, its ecological status, changes during the past eight decades and restoration problems (Present issue).
- Owens, N. J. P., 1986. Estuarine nitrification: A naturally occurring fluidized bed reaction? *Estuarine, Coastal and Shelf Science* 22: 31–44.
- Présing, M., S. Herodek, L. Vörös & I. Kobor, 1996. Nitrogen fixation, ammonium and nitrate uptake during a bloom of *Cylindrospermopsis raciborskii* in Lake Balaton. *Archiv für Hydrobiologie* 136: 553– 562.

- Priscu, J. C., R. H. Spigel, M. M. Gibbs & M. T. Downes, 1986. A numerical analysis of hypolimnetic nitrogen and phosphorus transformations in Lake Rotoiti, New Zealand: A geothermally influenced lake. *Limnology and Oceanography* 31: 812–831.
- Scheffer, M., 1998. Ecology of shallow lakes. Chapman & Hall, London.
- Sprent, J. I., 1987. The ecology of the nitrogen cycle. Cambridge University Press, USA.
- Stewart, W. D. P., G. P. Fitzgerald & R. M. Burris, 1967. In situ studies on N₂ fixation using the acetylene reduction technique. *Proceedings of the National Academy of Sciences of the U.S.A.* 58: 2071–2078.
- Stolp, H., 1996. Microbial ecology: organisms, habitats, activities. Cambridge University Press, USA.
- Tammert, H., V. Kisand & T. Nõges, Bacterioplankton abundance and activity in a small hypertrophic stratified lake (Present issue).
- Tõnno, I. & T. Nõges, 2003. Nitrogen fixation in a large shallow lake: rates and initiation conditions. *Hydrobiologia* 490: 23–30.
- Voytek, M. A., J. C. Priscu & B. B. Ward, 1999. The distribution and relative abundance of ammonia- oxidizing bacteria in lakes of the McMurdo Dry Valley, Antarctica. *Hydrobiologia* 401: 113–130.
- Wetzel, R., 1983. Limnology. Saunders College Publishing.
- Williams, P. J. le B., D. N. Thomas & C. S. Reynolds (eds), 2002. Phytoplankton productivity. Carbon assimilation in marine and freshwater ecosystems. Blackwell Science, UK, 109–141.
- Zingel, P., Vertical and seasonal dynamics of planktonic ciliates in a strongly stratified hypertrophic lake (Present issue).

Table 1. Dates, sampling depths and measured parameters in Lake Verevi in 2000–2001.

Date	Depth m	Measured parameters					
		Nitrification rate mgN m^{-3} day $^{-1}$	TN mgN m^{-3}	NH $_4^+$ mgN m^{-3}	NO $_2^-$ mgN m^{-3}	NO $_3^-$ mgN m^{-3}	N $_2$ -fixation mgN m $^{-3}$ day $^{-1}$
07.08.2000	2.0	–	+	+	+	+	+
07.08.2000	4.0	+	+	+	+	+	–
07.08.2000	5.0	+	+	+	+	+	–
28.08.2000	0.5	–	+	+	+	+	+
28.08.2000	1.0	–	–	–	–	–	+
28.08.2000	5.0	+	+	+	+	+	–
28.08.2000	5.5	+	+	+	+	+	–
23.04.2001	0.5	+	+	+	+	+	–
23.04.2001	5.5	+	+	+	+	+	–
23.04.2001	8.5	+	+	+	+	+	–
30.04.2001	0.5	+	+	+	+	+	–
30.04.2001	5.0	+	+	+	+	+	–
30.04.2001	5.25	+	+	+	+	+	–
30.04.2001	5.5	+	+	+	+	+	–
07.05.2001	0.5	+	+	+	+	+	–
07.05.2001	5.0	+	+	+	+	+	–
07.05.2001	5.25	+	+	+	+	+	–
07.05.2001	5.5	+	+	+	+	+	–
05.06.2001	0.5	+	+	+	+	+	–
05.06.2001	5.0	+	+	+	+	+	–
05.06.2001	5.25	+	+	+	+	+	–
05.06.2001	5.5	+	+	+	+	+	–
02.08.2001	0.5	+	+	+	+	+	+
02.08.2001	1.0	+	+	+	+	+	+
02.08.2001	2.0	+	+	+	+	+	+
02.08.2001	3.0	+	+	+	+	+	+
02.08.2001	4.0	+	+	+	+	+	–
02.08.2001	5.0	+	+	+	+	+	–
02.08.2001	6.0	+	+	+	+	+	–
02.08.2001	7.0	+	+	+	+	+	–

Figures

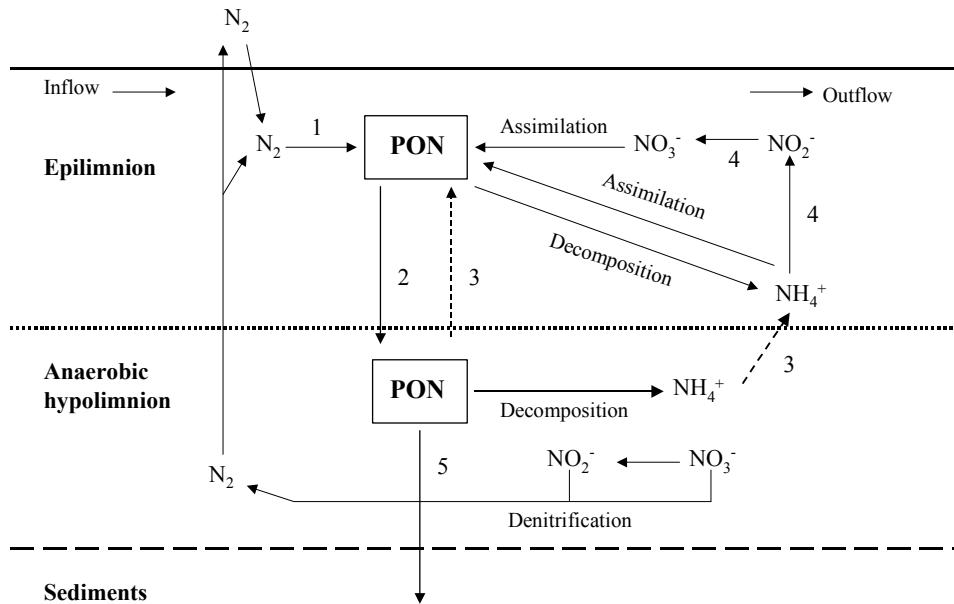


Figure 1. Conceptual scheme of the main processes of the nitrogen cycle in a stratified lake according to Lampert and Sommer (1997): 1 – planktic molecular nitrogen fixation (N_2 fix); 2 – a part of the PON (particular organic nitrogen) sinks to the hypolimnion; 3 – due to water mixing some PON is carried from hypolimnion to epilimnion; 4 – in epilimnion ammonium (NH_4^+) is subject to nitrification; 5 – a part of PON is switched off from nitrogen cycle due to sedimentation.

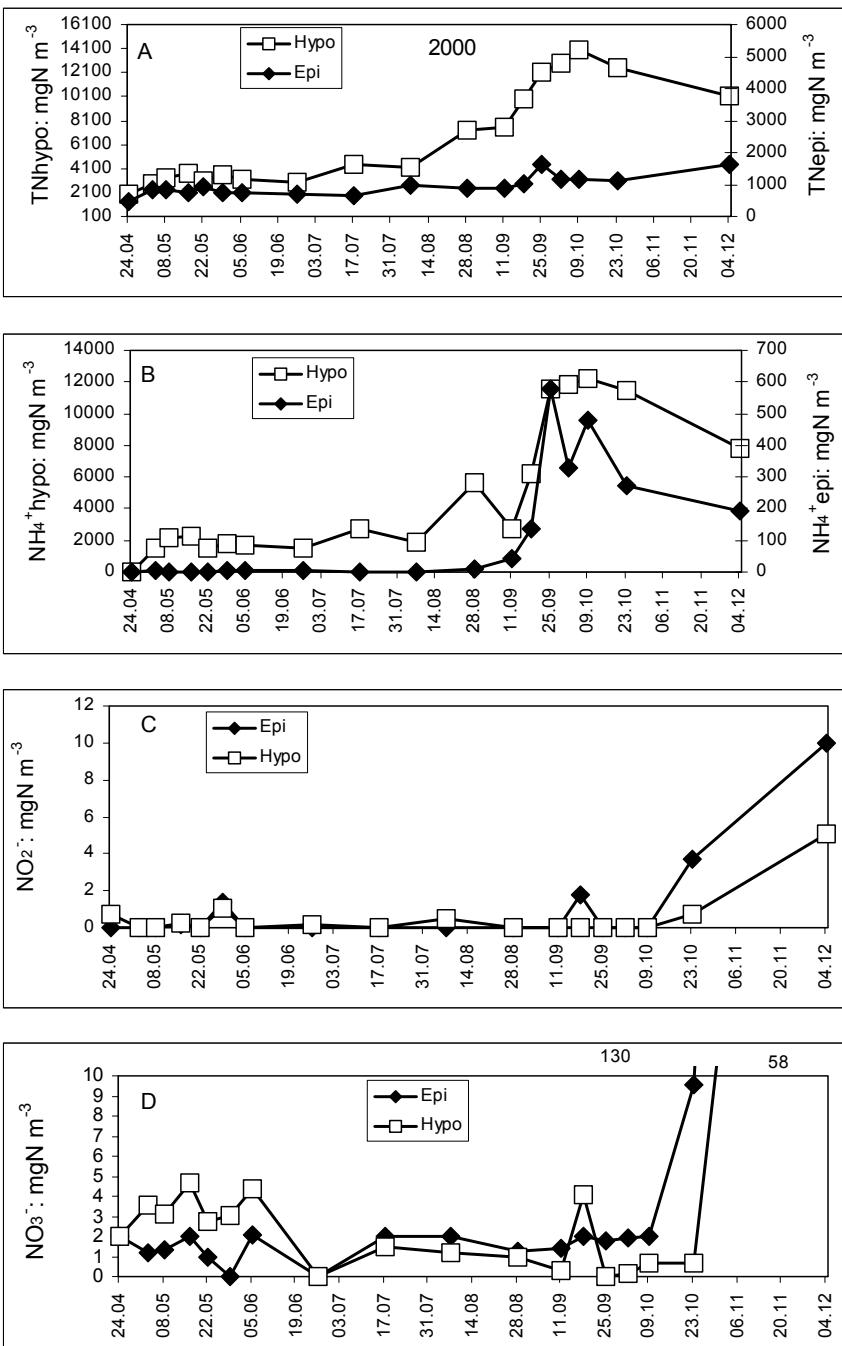


Figure 2. Seasonal course of the epilimnetic (Epi) and hypolimnetic (Hypo) A: total nitrogen (TN) B: ammonium (NH_4^+) C: nitrite (NO_2^-), and D: nitrate (NO_3^-) in Lake Verevi in 2000.

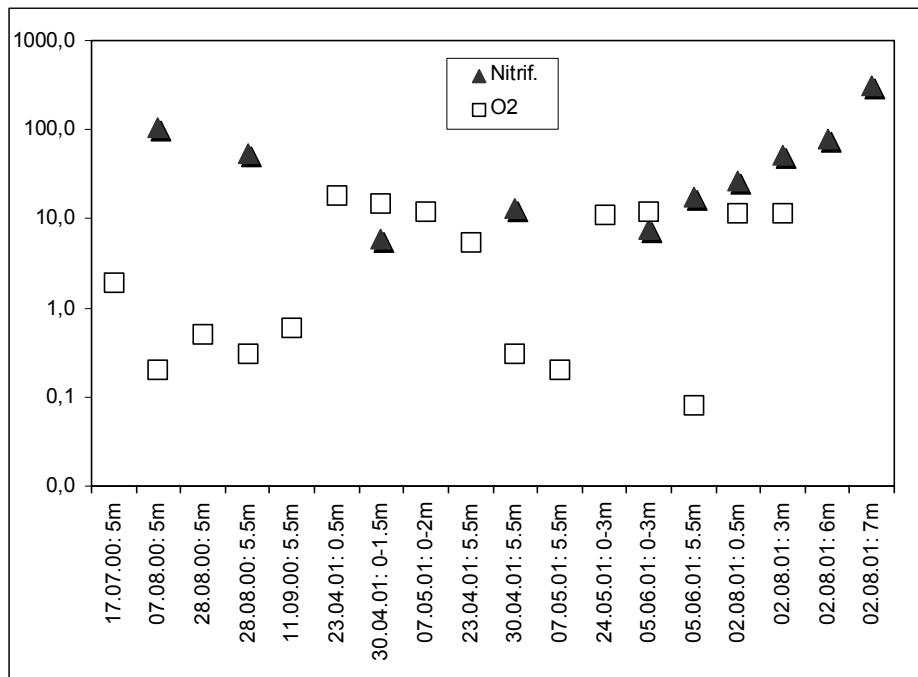


Figure 3. Dynamics of nitrification (Nitrif: $\text{mgN m}^{-3} \text{ day}^{-1}$) and dissolved oxygen (O_2 : mg l^{-1}) in Lake Verevi in 2000–2001 (depth-integrated water samples were collected from water layers 0–1.5m, 0–2m and 0–3m).

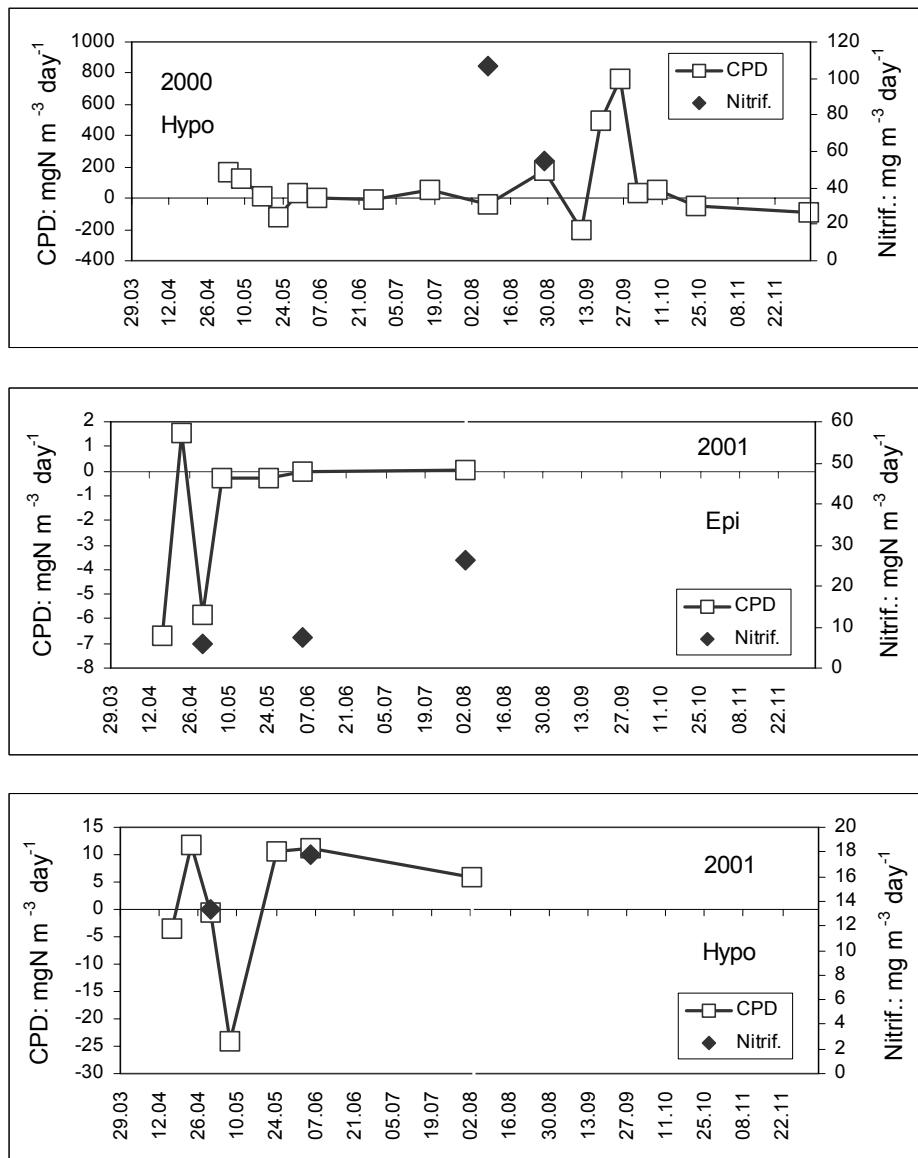


Figure 4. Daily changes in the amount of ammonium (CPD) and nitrification (Nitrif.) in the epi- and hypolimnion of Lake Verevi in 2000–2001.