

## THE IMPORTANCE OF SEVERAL NITROGEN FORMS AND SOURCES FOR PHYTOPLANKTON GROWTH IN SHALLOW FRESH WATERS

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### **Abstract**

Investigation of nitrogen metabolism of phytoplankton, especially their nitrogen uptake, started in the eighties concerning to the eutrophication of Lake Balaton. The experiments were carried out in two basins of the Lake and its two pollution control reservoirs (Marcali and Upper Kis-Balaton). Detailed description of these waters is given by Herodek and Vörös in this volume. Due to the low ambient concentrations of ammonium, nitrate and urea the <sup>15</sup>N technique was the only useful method to determine the nitrogen uptake of algae. N<sub>2</sub>-fixation was measured by acetylene-reduction and the primary production by <sup>14</sup>C method. The light dependence of nitrogen and carbon uptake was described with an exponential saturation equation and used for the calculation of surface related daily uptake. The results showed a high preference of algae for ammonium in all of the investigated waters. Its contribution to the daily nitrogen supply of phytoplankton varied between 50-90%. Despite its higher ambient concentrations nitrate uptake was generally less important with maximum of 40%. The interaction of ammonium and nitrate uptake demonstrated suppression of nitrate uptake at ammonium addition. After ammonium, urea was the other important nitrogen source of algae reaching up to 75% of daily nitrogen assimilation. N<sub>2</sub>-fixation was significant only at the time of high primary production and great nitrogen fixing cyanobacterial contribution. Nitrogen uptake experiments and loading data of the Lake suggest that annual external nitrogen load is one tenth of that utilised by algae. The predominant process supplying nitrogen to the phytoplankton is the regeneration of nitrogen in the water column and in the sediment.

**Key words:** ammonium, nitrate, urea uptake, ammonium preference-inhibition, ammonium regeneration, N<sub>2</sub>-fixation, phytoplankton

### **Introduction**

Lake Balaton is the largest shallow Lake in Central Europe on the basis of its surface area of c. 600 km<sup>2</sup>. The combination of shallow depth, intense land use within its catchment results in high productivity and vulnerability to eutrophication. The Lake is of international significance as an environmental resource and of great importance to the Hungarian economy as a recreational resort. Increasing external loading of phosphorus and nitrogen from sewage and agriculture, especially during the late 1960s and 1970s, has been detrimental to water quality and threatens species diversity and recreational use (Herodek *et al.* 1988). The western basin of the Lake was classified as hypertrophic, phytoplankton productivity and blooms of cyanobacteria have increased. There are a number of works about the relationship between the phosphorus load and algal productivity of the Lake. The algal biomass is considered to be controlled primarily by phosphorus (Istvánovics and Herodek 1995). In the eighties, an extensive programme to upgrade treatment plants, divert outflows and construct pollution control reservoirs was

initiated to reduce the external phosphorus loading. Lake Balaton is the largest Lake in Europe to have undergone such an extensive engineering programme to improve water quality. The Lake is currently meso-oligotrophic, but nitrogen fixing and non-fixing cyanobacteria in the summer phytoplankton, often with their dominance, gives rise to potential concern. As nitrogen deficiency may contribute to the development of cyanobacterium it seemed worthwhile to study the nitrogen metabolism of phytoplankton. The intense investigation of nitrogen uptake have started in 1994, when the biggest cyanobacterial bloom in the Lake has been occurred. Additional experiments were carried out in two hypertrophic pollution control reservoirs of the Lake to compare the significance of various nitrogen sources for phytoplankton and interaction of ammonium and nitrate uptake. During this research programme a number of methods were applied and newly-developed in our institute. The aim of this paper was to review the results of phytoplankton nitrogen uptake from 1994 to 1998 enlightening the need of future research in nitrogen metabolism of the Lake.

### *Materials and Methods*

#### **Study areas and sample collection**

The overwhelming majority of experiments were carried out in Lake Balaton, in the smallest, westernmost Keszthely basin and in the largest, easternmost Siófok basin. The period chosen for the research was August, the most critical time of year, when the greatest algal biomass generally occurred, often dominated by cyanobacteria. The samples were taken from the middle of the Lake basins, while in the case of reservoirs the sampling points were close to their outflows. The samples were taken with sampling tube, providing vertical sample from the whole water column. Water was filtered immediately through a plankton net to remove the large zooplankton. Samples were transported to the laboratory, keeping them in dark and at actual Lake or reservoir water temperature. At the time of sampling the photosynthetically active radiation (PAR) (400 - 700 nm) was measured with a LI-COR (LI 185B) radiometer using a flat cosine corrected ( $2\pi$ ) underwater quantum sensor at 0, 0.25, 0.5, 1.0, 1.5, 2.0, 2.5 and 3 m, and light attenuation coefficients were calculated.

#### **Measurements**

The samples for nutrient and chlorophyll-*a* analysis were filtered through precombusted (450 °C) Whatman GF/C filters in the laboratory. Chlorophyll-*a* concentrations were determined spectrophotometrically after Iwamura *et al.* (1970). Ammonium analysis was carried out by the indophenol-blue assay (Mackereth *et al.* 1989) and nitrate analysis was undertaken with the Cd-reduction method (Elliott and Porter 1971). Urea concentrations were measured from 1996 with modified direct oxidation method of Newell *et al.* (1967) and with urease method of McCarthy (1970). Phytoplankton samples were preserved by Lugol's solution. Algal species were enumerated with an inverted plankton microscope. The wet weight of each species was calculated from cell volumes (Németh and Vörös 1986). At least 25 cells (or filaments) of each species were measured for biomass determination and at least 400 were counted. N-fixation was measured with the acetylene reduction technique of Flett *et al.* (1976). Data

N<sub>2</sub>-fixation was calculated assuming an active period of 12 hours and below 2 m depth the photosynthetically active radiation was considered insufficient for nitrogen fixation. Primary production was measured with the <sup>14</sup>C technique (Vollenweider 1969). In ammonium, urea and nitrate uptake experiments, water samples were enriched with 99 atom% <sup>15</sup>N ammonium chloride, urea or potassium nitrate over the range of 10 - 480 µg <sup>15</sup>N/l. Samples were incubated at Lake temperature and illuminated by white fluorescent lamps with photo irradiance of 110 µE m<sup>-2</sup> s<sup>-1</sup>. The shortest possible time was chosen for incubation, after the uptake velocity became constant and measurable <sup>15</sup>N was assimilated (20 - 90 minutes). Shorter periods were used for ammonium and urea and relatively longer for nitrate. The interaction of ammonium and nitrate uptake was determined by the method of Harrison *et al.* (1996). The measurements were carried out in pollution control reservoirs, where the ambient concentration of nitrate was high enough for enrichment of tracer experiment. The light dependence of photosynthesis and nitrogen uptake were measured in separate experiments worked out during our investigation (Présing *et al.* 1999). The results were used for calculation of surface related daily carbon and nitrogen uptake.

### Results

The chlorophyll-*a* concentrations were extremely high in the first year of the study (Fig.1). Both in the eastern and western basins they were the highest values have ever been measured (180 and 88 µg/l, respectively) in the Lake (Présing *et al.* 1996). Over the following years the algal biomass declined to approximately one third of its maximum (Présing *et al.* 1998; 1999; 2000). In 1994 August almost exclusively a single species the *Cylindrospermopsis raciborskii* (Wolosz.) Seenayya et Subba Raju, a N<sub>2</sub>-fixing cyanobacteria, was found in the phytoplankton of the entire Lake. After this bloom the cyanobacterial dominance decreased, but their proportion in phytoplankton biomass remained high, especially in the western basin. Ammonium and nitrate concentrations were generally very low (1-20 µg/l) in summer (Table 1.), often close to their detection limit. The urea concentrations (measured with both methods) were often higher than that of ammonium, but fell in the same range of low ambient concentrations. Uptake of measured nitrogen sources, with two exceptions, followed the Michaelis-Menten kinetics (Table 1.).

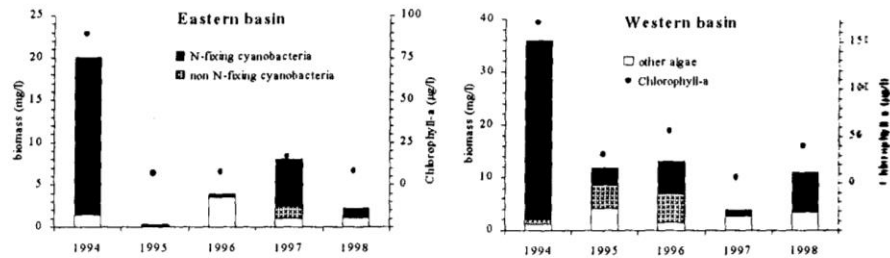


Fig.1. Chlorophyll-a, phytoplankton composition and biomass in eastern and western basins of Lake Balaton in August.

Table 1. Characteristics of ammonium, urea and nitrate uptake in eastern and western basins of Lake Balaton in August

		Eastern basin				Western basin			
		1994	1995	1996	1998	1994	1995	1996	1998
$S_n$ ( $\mu\text{g N/l}$ )	$\text{NH}_4$	1.00	0.70	3.50	0.70	2.60	0.70	5.10	5.80
	Urea	-	-	21.00	35.10	-	-	21.20	7.00
	$\text{NO}_3$	1.10	4.00	3.40	6.30	0.20	0.70	40.00	9.40
$V_{max}$ ( $\mu\text{g N l}^{-1}\text{h}^{-1}$ )	$\text{NH}_4$	20.00	5.20	3.60	5.30	80.00	14.28	39.60	19.60
	Urea	-	-	0.90	-	-	-	18.20	4.30
	$\text{NO}_3$	4.80	0.73	0.12	-	17.50	1.30	3.90	0.90
K ( $\mu\text{g N/l}$ )	$\text{NH}_4$	6.50	5.30	22.00	24.70	27.10	10.30	4.00	5.30
	Urea	-	-	85.00	-	-	-	1.70	2.60
	$\text{NO}_3$	7.20	1.10	2.70	-	2.40	1.58	2.40	10.60
$\alpha$	$\text{NH}_4$	3.10	1.00	0.20	0.20	2.90	1.40	9.90	3.70
	Urea	-	-	0.01	-	-	-	10.70	1.70
	$\text{NO}_3$	0.70	0.70	0.04	-	7.30	0.80	1.60	0.10
v ( $\mu\text{g N l}^{-1}\text{h}^{-1}$ )	$\text{NH}_4$	7.70	0.61	0.50	0.14	7.00	0.91	10.50	16.30
	Urea	-	-	0.20	1.38	-	-	11.20	3.20
	$\text{NO}_3$	1.10	0.57	0.10	0.31	1.40	0.40	3.60	0.40

In 1998 urea and nitrate uptake did not fit a saturation curve in the eastern basin. Typical uptake velocity patterns of ammonium, urea and nitrate are shown in Fig.2. The behaviour of different nitrogen sources was similar in the two basins. The same added

nitrogen concentration of various nitrogen source resulted always the biggest increase of uptake velocity in the case of ammonium and the smallest change with nitrate. Usually, ammonium had the highest and nitrate had the smallest maximal uptake velocity (Table 1.). Half saturation constant of nitrate was generally smaller than in the case of ammonium. In majority, the initial slope of uptake curves ( $V_{max}/K$ ) was the biggest in ammonium uptake experiments. The kinetic parameters of urea were between the values of ammonium and nitrate, close to that of ammonium. The calculated actual uptake velocity of ammonium at ambient nitrogen concentration exceeded those of urea and nitrate. The light dependence of nitrogen uptake followed the modified equation of Webb *et al.* (1974). The weakest light dependence and the highest dark uptake were observed in ammonium experiments (Fig. 2.). Dark uptake velocities of ammonium were 30-80 % of the values measured at optimal photo irradiance. The same ratios of urea were slightly smaller, ranging between 18-74 %. Dark uptake of nitrate had less importance and was only 1-67 % of the optimal illuminated uptake velocities. The proportion of dark uptake to the daily uptake in the case of ammonium, urea and nitrate varied between 65-80%, 48-71% and 0-76% respectively. The light adaptation parameters ( $I_k$ , photon flux density at which the initial slope of light saturation curve equals the maximal uptake velocity) were always the smallest for ammonium and the highest for nitrate. In the case of urea they were very close to those of ammonium. The calculated daily  $N_2$ -fixation, carbon uptake and uptake of various forms of nitrogen per unit surface area ( $m^2$ ) are shown in Fig. 3. In the inhibition experiments ammonium addition reduced the uptake of nitrate (Fig. 4.). The resulting curves fitted the Michaelis-Menten equation in which the nitrate uptake was normalised to the maximum observed uptake rate (at zero  $^{14}NH_4$  addition). The nitrate uptake decreased with increasing ammonium concentration. The depression was stronger in the reservoir in which the nitrate concentration and the cyanobacteria abundance were lower. Generally in both basins the ammonium was the main nitrogen source for algae.

Its contribution to the daily nitrogen supply of phytoplankton always exceeded 50% even when  $N_2$ -fixing cyanobacteria were the predominant species. The nitrogen fixation was only in a single case (in 1995, in western basin) more important than ammonium uptake. After ammonium, urea was the most significant nitrogen source for algae. Nitrate uptake did not play dominant role in the nitrogen supply of phytoplankton. Nitrogen fixation was negligible in the eastern basin, but significant in the western basin in 1994-1995. The surface related daily C:N uptake ratios varied from 5.2 to 12.5 (weight:weight). In 1995 from July to August the phytoplankton assimilated 140 t of ammonium, 17.5 t of nitrate and fixed 143 t of  $N_2$  in western, 631.6 t of ammonium, 163.9 t nitrate and fixed 45.5 t of  $N_2$  in eastern basin. During the same time the external ammonium and nitrate loading of the basins was 1.3 and 1.9 t, respectively (Somlyódy and Jolánkai 1986). In 1998 from July to September in western basin the phytoplankton took up 470 t of ammonium, 256 t of urea, 25 t of nitrate and fixed 120 t of  $N_2$ . In this period the external loading of the basin by Zala River was 8.6 tons ammonium and 6 t of nitrate.

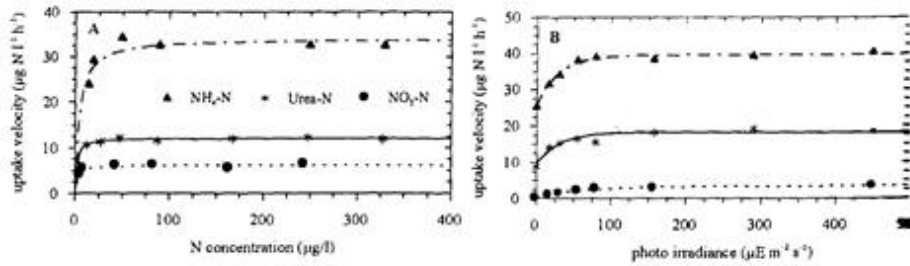


Fig. 2. Uptake velocity of ammonium, urea and nitrate as a function of nitrogen concentrations at optimal photo irradiance (A) and as a function of photo irradiance at saturation nitrogen concentrations (B)

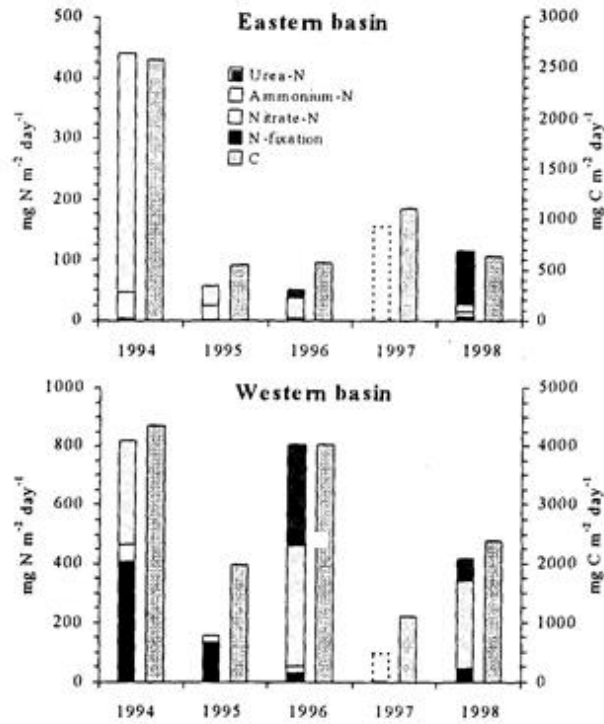


Fig. 3. Daily carbon and nitrogen uptake from different sources in eastern and western basins of Lake Balaton in August. (In 1997 the uptaken N was calculated by the C uptake data and by C:N ratios measured in previous years.)

## The Importance of Several Nitrogen Forms & Sources for Phytoplankton Growth

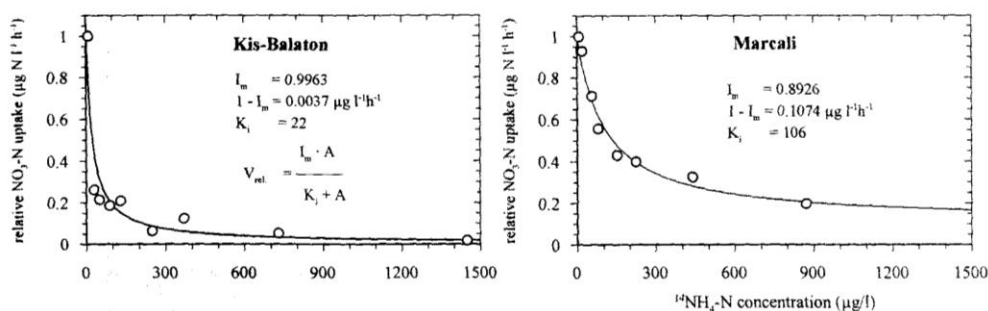


Fig. 4. Interaction of ammonium and nitrate uptake in Kis-Balaton and Marcali reservoirs

### Discussion

After five years research on the Lake we have the opportunity to compare the significance of several nitrogen forms for different phytoplankton assemble. The parameters measured and calculated in this study are appropriate to characterise the substrate affinity and the nitrogen supply of phytoplankton. The similarly low ambient concentrations of ammonium, urea and nitrate in the summer can not give enough information about the preference of phytoplankton. But their low level gave the evidence at least that nitrogen loading of the water did not exceed the nitrogen consumption of algae. From the kinetic parameters  $V_{\text{max}}$  and  $\alpha$  were found right to describe the substrate affinity of phytoplankton (Healey 1980; Takamura *et al.* 1987; Lomas *et al.* 1996; Présing *et al.* 1999). The maximal uptake velocity and the initial slope of uptake were the highest in the case of ammonium, independent of basin and species composition. While the same values of urea were close to those of ammonium, the nitrate ones was significantly lower. In agreement with increasing energy cost of uptake the order of preference was ammonium, urea and nitrate, like in many other waters (Mitamura *et al.* 1995). This was confirmed with the results of dark uptake, where the smallest response to irradiance of ammonium relative to urea and especially to nitrate was observed. Consequently, the biggest contribution to the daily integrated N demand of phytoplankton had the two reduced form (ammonium and urea) of nitrogen. When the ambient concentration of urea was high, its contribution was close or even higher than that of ammonium. Uptake of ammonium for a phytoplankton cell needs less energy than uptake of urea or especially uptake of nitrate (Syrett 1981). On the other hand ammonium uptake can inhibit nitrate uptake (Harrison *et al.* 1996). We found significant preference of ammonium in our experiments, without a distinct threshold concentration of inhibition. The nitrate uptake decreased with increasing ammonium concentration but did not stop even at saturation ammonium level. In agreement with Dortch's (1990) definition, this was more likely preference of ammonium uptake, than inhibition of nitrate uptake by ammonium. The general rule that ammonium is the preferred source by phytoplankton holds true especially for cyanobacteria. They take advantage of their ability to assimilate ammonium at low ambient concentrations (Blomqvist *et al.* 1994), furthermore, when the support of reduced nitrogen forms is not sufficient they can fix nitrogen from the unlimited atmospheric source. The interaction of different nitrogen forms' uptake and the mechanism of change for uptake and fixation will aim future laboratory experiments. The daily C:N uptake

ratios integrate a number of several measurements and calculations; the uptake of dissolved nitrogen and carbon, N<sub>2</sub>-fixation, their extrapolation for 24 hours and 3 m depth. Therefore the deviations from the classical Redfield ratio (5.6 weight:weight) are not necessarily inform about the N deficiency of phytoplankton, but can control the rightness of these measurements and calculations. Our results are in the range of the Redfield ratio, confirming the correct estimation of nitrogen and carbon uptake. Comparing the uptake data to the external load the importance of internal nitrogen loading is obvious. The nitrogen recycling, especially ammonium, has a very high rate within the Lake. But not only the majority of uptaken ammonium and urea, the nitrate also has an internal origin. The rapid nitrogen regeneration in the water column and in the sediment can support the summer standing crop of phytoplankton. The nitrogen demand of net increase in the phytoplankton biomass could be supported only from remineralization in the sediment and from fixation of atmospheric nitrogen. The sediment N pool is potentially high. In the interstitial water in the upper 5 cm of a square metre there is on average approximately 7 mg of ammonium, 2 mg of urea and 1.5 mg nitrate nitrogen. Future study needs to make clear the conditions of processes, especially the role of temperature, controlling the internal nitrogen loading of the Lake.

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