Freshwater Biology (2001) **46,** 125–139

APPLIED ISSUES

Nitrogen uptake and the importance of internal nitrogen loading in Lake Balaton

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SUMMARY

1. The importance of various forms of nitrogen to the nitrogen supply of phytoplankton has been investigated in the mesotrophic eastern and eutrophic western basin of Lake Balaton.
2. Uptake rates of ammonium, urea, nitrate and carbon were measured simultaneously. The uptake rates were determined using ^{15}N and ^{14}C methodologies, and N_2-fixation was measured using the acetylene-reduction method. The light dependence of uptake was described with an exponential saturation equation and used to calculate surface-related (areal) daily uptake.
3. The contribution of ammonium, urea and nitrate to the daily nitrogen supply of phytoplankton varied between 11 and 80%, 17 and 73% and 1 and 15%, respectively. N_2-fixation was negligible in the eastern basin and varied between 5 and 30% in the western region of the lake. The annual external nitrogen load was only 10% of that utilized by algae.
4. The predominant process supplying nitrogen to the phytoplankton in the lake is the rapid recycling of ammonium and urea in the water column. The importance of the internal nutrient loading is emphasized.

Keywords: ammonium, nitrate uptake, N_2-fixation, remineralization, urea

Introduction

Lake Balaton is a large (surface area 596 km^2), shallow (mean depth 3.2 m) lake, that became eutrophic-hypertrophic because of increased nutrient loads in the 1970s (Herodek, 1986). The most dramatic change occurred in the western basin (surface area 38 km^2) in the shallowest (mean depth 2.5 m) and smallest basin of the lake. This basin received one-third of the increased nutrient load of the whole lake from the inflowing River Zala. During the rapid eutrophication, a proliferation of cyanobacteria species replaced the formerly dominant dinoflagellates and diatoms (Vörös & Nagy Göde, 1993). Since the 1970s, cyanobacterial blooms have appeared regularly in the western basin and gradually extended to the eastern part of the lake. To reduce the nutrient load, particularly phosphorus, several measures have been taken. In the mid-1980s reservoirs were built on the tributaries feeding Lake Balaton. The biggest reservoir (Kis-Balaton Water Protection System) was constructed on the main inflow, the River Zala, transporting close to 90% of the nutrient load of the basin. The Marcali Reservoir was built on the second largest tributary of the lake (Western Main Canal). Further-

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more, a sewage transfer system pipe around the eastern basin diverts waste water from the catchment area of the lake. As a result of these eutrophication management measures, the biologically available phosphorus load of the eastern and western basins decreased by 60% and 30%, respectively (Somlyódy & Jolánkai, 1986; Somlyódy *et al*., 1997). From the mid-1980s the phytoplankton biomass slowly decreased, but in 1992 and 1994 cyanobacterial blooms occurred. Since the latest bloom, the phytoplankton biomass has been low and, using chlorophyll *a* maxima as the criterion, the eastern basin of the lake has become mesotrophic, while the western basin remains eutrophic.

Lowering the algal biomass has improved water quality. However, the dominance of nitrogen-fixing and non-fixing cyanobacteria in the summer phytoplankton gives rise to potential concern. Although algal biomass was considered to be controlled primarily by phosphorus (Istvánovics & Herodek, 1995) it has become necessary to examine the nitrogen dynamics within the lake. Our study was designed to investigate the importance of a variety of nitrogen sources and the relative significance of the regenerated nitrogen supply to phytoplankton.

Methods

Sampling procedure

The studies were carried out in 1998 in the westernmost Keszthely basin and in the eastern Siófok basin (surface area 228 km^2, mean depth 3.5 m) of the lake (Fig. 1). Samples were taken from the middle of each basin with a 3 m long sampling tube providing a vertical sample from the whole water column, at about 9.00 and 10.00 h in the eastern and western basins, respectively. Water was immediately filtered through a plankton net (mesh size 200 μm) to remove the large zooplankton. Samples were transported to the laboratory within 30 min from the eastern and 1–2 h from the western basin, keeping them in the dark and at lake temperature. Temperature, chlorophyll *a* and nutrient concentrations were measured regularly from January to November in the eastern basin and from April to November in the western basin.

Measurements

Photosynthetically active radiation (PAR) (400–700 nm) was measured at the time of sampling with a LI-COR (LI 185B) radiometer using a flat cosine cor-

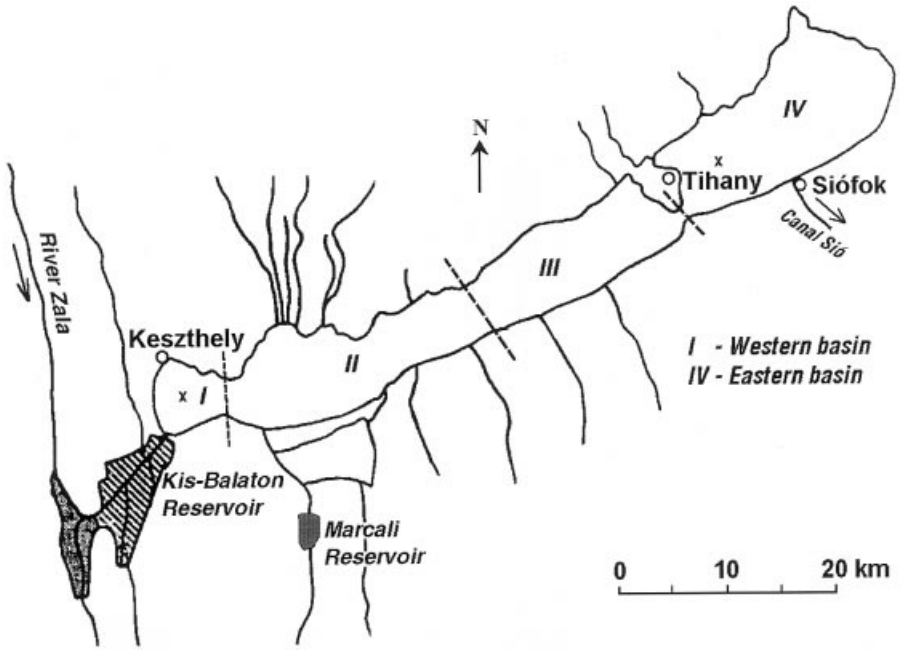

Fig. 1 Lake Balaton's basins (I–IV) with sampling stations (×) and the main water pollution control reservoirs.

rected (2π) 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. Samples for nutrient and chlorophyll *a* analysis were filtered through precombusted (450 °C) Whatman GF/C filters. Chlorophyll *a* concentrations were determined spectrophotometrically after hot methanol extraction (Iwamura, Nagai & Ishimura, 1970).

Ammonium analysis was carried out by the indophenol-blue method (Mackereth, Heron & Talling, 1989) and nitrate analysis was undertaken with the Cd-reduction method (Elliott & Porter, 1971). Urea concentrations were measured following Newell, Morgan & Candy (1967). Ambient nutrient and chlorophyll *a* concentrations were determined from three replicates, with a coefficient of variation of less than 10% and 5%, respectively.

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 & Vörös, 1986). At least 25 cells (or filaments) of each species were measured for biomass determination and at least 400 were counted.

N_2-fixation was measured with the acetylene reduction technique of Flett, Hamilton & Campbell (1976). Glass vessels (200 mL volume) containing 150 mL samples were suspended at the surface at 1.0 and 2.0 m depths. Measured values were integrated, over depths of 0–0.5, 0.5–1.5 and 1.5–2.0 m to obtain surface related N_2-fixation. Below 2 m, photo irradiance was considered insufficient for nitrogen fixation. Daily N_2-fixation was calculated assuming an active period of 12 h.

Primary production was measured with the ^{14}C technique (Vollenweider, 1969). Samples were kept for 2 h at lake temperature in black boxes with lids of varying transparency and illuminated by white fluorescent lamps with photo irradiance of 500 μmd photon m^{-2} s^{-1}. Photon flux densities used were: 0, 10, 20, 30, 60, 85, 120, 150, 220 and 460 μmd photon m^{-2} s^{-1}. Radioactivity was measured by a LKB RackBeta liquid scintillation counter. The exponential saturation equation of Webb, Newton and Starr (1974) was used to describe the light dependence of photosynthesis. Surface related photosynthesis was calculated using light attenuation coefficients and actual global radiation data, integrated over each hour from sunrise to sunset (from 4.00 to 20.00 h), through 0.1 m layers in the water. Forty-eight percent of the global radiation were considered to be photosynthetically active (PAR) (Wetzel & Likens, 1990).

In ammonium, urea and nitrate uptake experiments, water samples were amended with 99 atom% ^{15}N ammonium chloride, urea or potassium nitrate over the range of 10–480 μg ^{15}N L^{-1}. Samples were incubated at lake temperature and illuminated by white fluorescent lamps with photo irradiance of 110 μmd photon m^{-2} s^{-1}. In preliminary experiments this was found to be in the optimal range for nitrogen uptake. The incubation times varied between 20 and 90 min. Shorter periods were used for ammonium and urea in summer and relatively longer for nitrate.

Uptake velocity is generally not constant and decreases with increasing incubation period. Depending on the duration of the incubation, adsorption, uptake and/or growth can occur. With very short incubations, from less than 1 min to approximately 10–15 min, an elevated 'surge uptake' is observed involving mainly membrane or ion transport and even non-metabolic binding by dead cells. The extent of surge uptake varies from species to species and also depends on prehistory of the cells (Collos, 1983). It is obvious that the capacity for surge uptake has important ecological significance to take up nutrient patches. On the other hand, 'as the duration of incubation increases, so will the importance of biological processes relative to physical phenomena' (Collos, 1983). In our preliminary experiments the uptake velocity of ammonium, urea and nitrate did not decrease after 20–30, 20–40 and 30–40 min, respectively. This 'internally controlled uptake' was constant for about 1 h for ammonium and urea and for 2 h for nitrate. This duration of surge uptake and internally controlled constant uptake agrees well with the literature (Wheeler, Glibert & McCarthy, 1982; Harrison, Parslow & Conway, 1989; Lomas *et al.*, 1996). The shortest possible time was chosen for incubation, after the uptake velocity became constant and measurable ^{15}N was assimilated. We used the shortest incubation times in this constant uptake period to minimize the isotope dilution effect on uptake. This isotope dilution effect decreases with decreasing incubation time and increases with concentration of added tracer. Remineralization experiments were not undertaken to correct uptake data. It was assumed

that the uptake/regeneration ratio is close to 1, since these are usually tightly coupled to each other. This ratio was found in the lake in 1995 (Présing *et al*., 1999) and is supported by other work undertaken in similar waters (Dodds, Priscu & Ellis, 1991; Glibert *et al*., 1991). Using this ratio to evaluate the isotope dilution effect, the model of Kanda *et al*. (1987) shows that regeneration should be less than 5%, and so this has been ignored.

After incubation, samples were filtered through precombusted Whatman GF/C filters and washed with 5 mL of filtered lake water. To obtain a suitable quantity of particulate nitrogen (some 5 μg) for accurate determination of $^{15}N/^{14}N$ ratio and total nitrogen, 80–150 mL of lake water was filtered. Besides phytoplankton, the particulate organic nitrogen concentration (PON) of the water is mainly determined by the high content of non-living suspended material. However, we tried to avoid undertaking uptake experiments in windy conditions as the large quantity of non-living material causes prolonged filtration periods. This was one reason why uptake experiments with very short incubation periods were not undertaken.

The nitrogen content and $^{15}N/^{14}N$ ratio of dried samples (60 °C) were determined by an automated elemental analyser interfaced to an Isotope Ratio Mass Spectrometer (ANCA-MS, Europa Scientific Ltd., U.K.). The uptake (h^{-1}) and transport rates ($\mu g\ N\ L^{-1}\ h^{-1}$) of ammonium, urea and nitrate at ambient (*in situ*) nitrogen concentrations at this (close to optimal) photon flux density were extrapolated from measured rates with the Michaelis–Menten equation (Dugdale & Wilkerson, 1986). For these calculations we used the particulate nitrogen concentrations of samples measured at the end of incubation (Collos, 1987). In some cases, uptake did not follow Michaelis–Menten kinetics and uptake velocities were determined as in the tracer method using the lowest enrichment of the sample.

In studies of light dependence of nitrogen uptake (LDNU) by algae, samples were enriched with ^{15}N-labelled nitrate, urea and ammonium at initial substrate concentrations of 240 $\mu g\ ^{15}N\ L^{-1}$. This concentration was found to be above saturation concentration in preliminary experiments. Samples were kept in the same incubation system under the illumination used to measure photosynthesis, for the same incubation time as used for uptake experiments. Measured uptake rates at saturating substrate concentrations were used for a model describing the dependency of inorganic nitrogen transport on photon flux density (Présing *et al*., 1999). Photo irradiance was calculated from actual global radiation and light attenuation coefficients. Surface-related uptake (or areal uptake rate) was estimated hourly from sunrise to sunset by 0.1 m layers from the surface to the bottom of the water. On 5 and 19 August in the eastern basin and on 18 August in the western basin, LDNU experiments were not carried out; average values for V_D and I_k from other measurements were used to calculate daily nitrogen uptake.

Nitrogen uptake experiments were undertaken in the eastern basin from February to November. In the western basin they were started in July, when phytoplankton biomass significantly exceeded that of the eastern basin. Carbon uptake was measured from the beginning of April to the end of October in both basins. Carbon and nitrogen uptake experiments were undertaken on the same day on most occasions. When this was not possible, incubations were typically less than 1 week apart. In February and November, carbon uptake was estimated using a model developed for the lake (Vörös & V.-Balogh, 1998) based on water temperature and chlorophyll *a* concentration. Total nitrogen uptake in the eastern basin from February to November and in the western basin from July to November were calculated from the daily ammonium, urea, nitrate uptake and nitrogen fixation measurements by interpolating the approximately monthly periods between experiments.

In July 1997, sediment samples were taken along the longitudinal axis of the lake. Ammonium, urea, nitrate and orthophosphate concentrations of sediment interstitial water and the total carbon and nitrogen content of the sediment in the 5 cm upper layer were determined.

Results

The lake was not ice-covered in winter 1997 and the water temperature rose to 10 °C in March (Fig. 2). After a cold March the water temperature started to rise in April and it reached 20 °C in late May/early June and remained above this until September. Although the water temperature was above 20 °C from June, two relatively cold fortnights at the beginning of June and July prevented the lake from warming further until July, unlike previous years. The typically

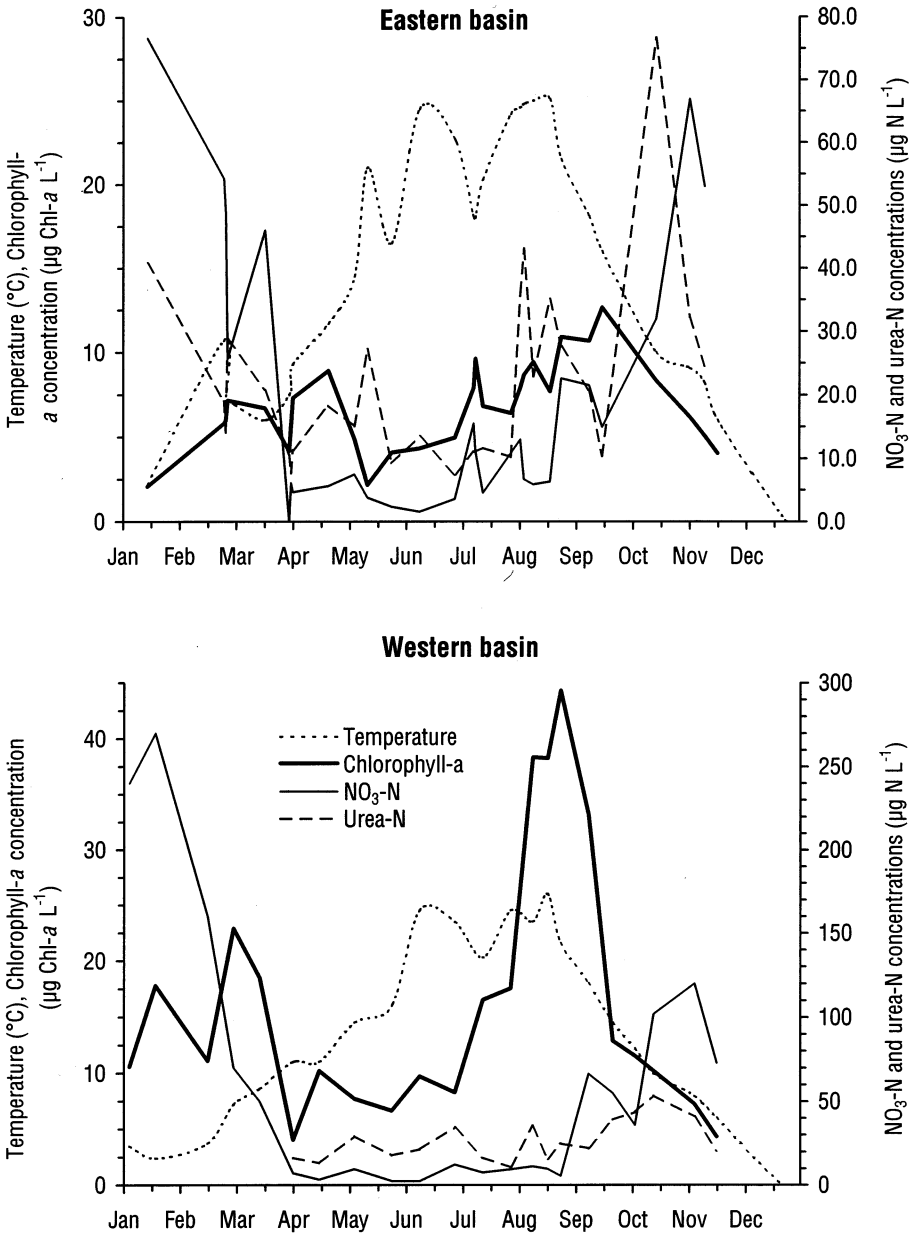

Fig. 2 Water temperature, chlorophyll *a* and nitrogen concentration in eastern and western basins of Lake Balaton in 1998.

warm summer started some 2 weeks later than usual. Consequently the water did not reach its usual maximum of 27–28 °C. In the warming up periods the deeper eastern basin was generally delayed by 2 weeks compared to the shallower western basin and its water temperature was 2–4 °C lower. From September to December the lake cooled down rapidly.

In the eastern basin, chlorophyll *a* concentrations were low, as in each year since the bloom in 1994, ranging between 2 and 14 µg L^{-1}. There were two small maxima, one in spring from the end of February to middle of May and the other in late summer–autumn from July to October. In the western basin, chlorophyll *a* concentrations were higher and peaked at 20 µg L^{-1} in February–March and at 45 µg L^{-1} in August–September. The summer 'peaks' occurred a few weeks later than in previous years, most probably due to the 'delayed summer' (Fig. 2).

Ammonium concentration varied between 3 and 20 μg L^{-1} during the year. Only once in July, in the western basin, was the measured ammonium concentration close to its detection limit (0.7 μg L^{-1}). Nitrate concentration was highest in winter (above 75 and 250 μg L^{-1} in the eastern and western basins, respectively), dropped rapidly during the spring and increased from autumn to winter as usual. The summer nitrate concentration was close to that of ammonium and slightly higher than in previous years (Fig. 2). The urea concentration was surprisingly high in winter (40 μg L^{-1}), decreased towards spring–summer, but was still higher than that of ammonium. From late summer urea concentration increased again and in some cases even exceeded the concentrations of nitrate (Fig. 2).

In the period under investigation the phytoplankton biomass varied between 1.0 and 3.5 mg L^{-1} in the eastern basin (Fig. 3). There was a well-defined spring diatom maximum in April with a predominance of centric diatoms (*Cyclotella* spp.). In early summer, the phytoplankton community became more diverse with increasing significance of dinoflagellates (*Ceratium hirundinella* (O.F.M.) Schrank) and non N_2-fixing cyanobacteria (*Snowella lacustris* (Chodat) Komárek et Hindák). In August and September, N_2-fixing

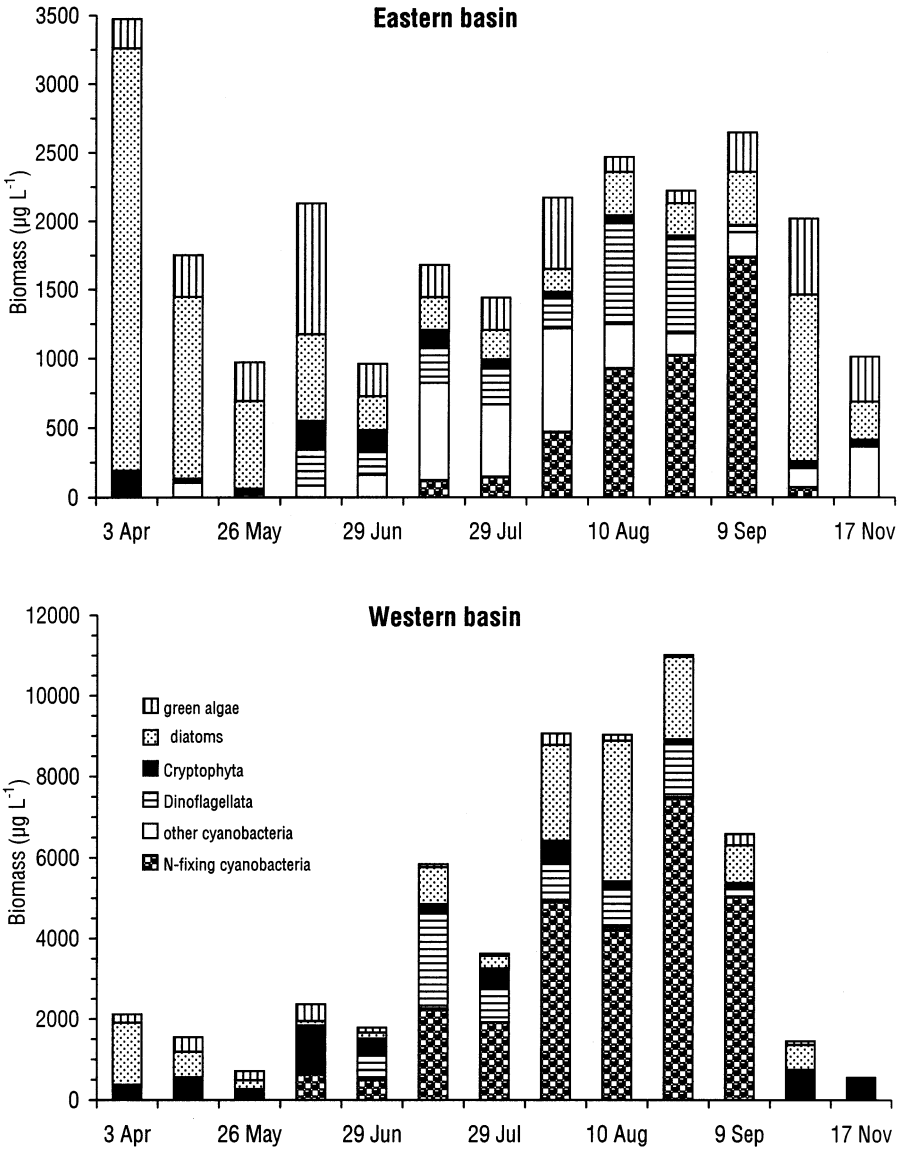

Fig. 3 Seasonal changes in phytoplankton composition and biomass in eastern and western basins of Lake Balaton in 1998.

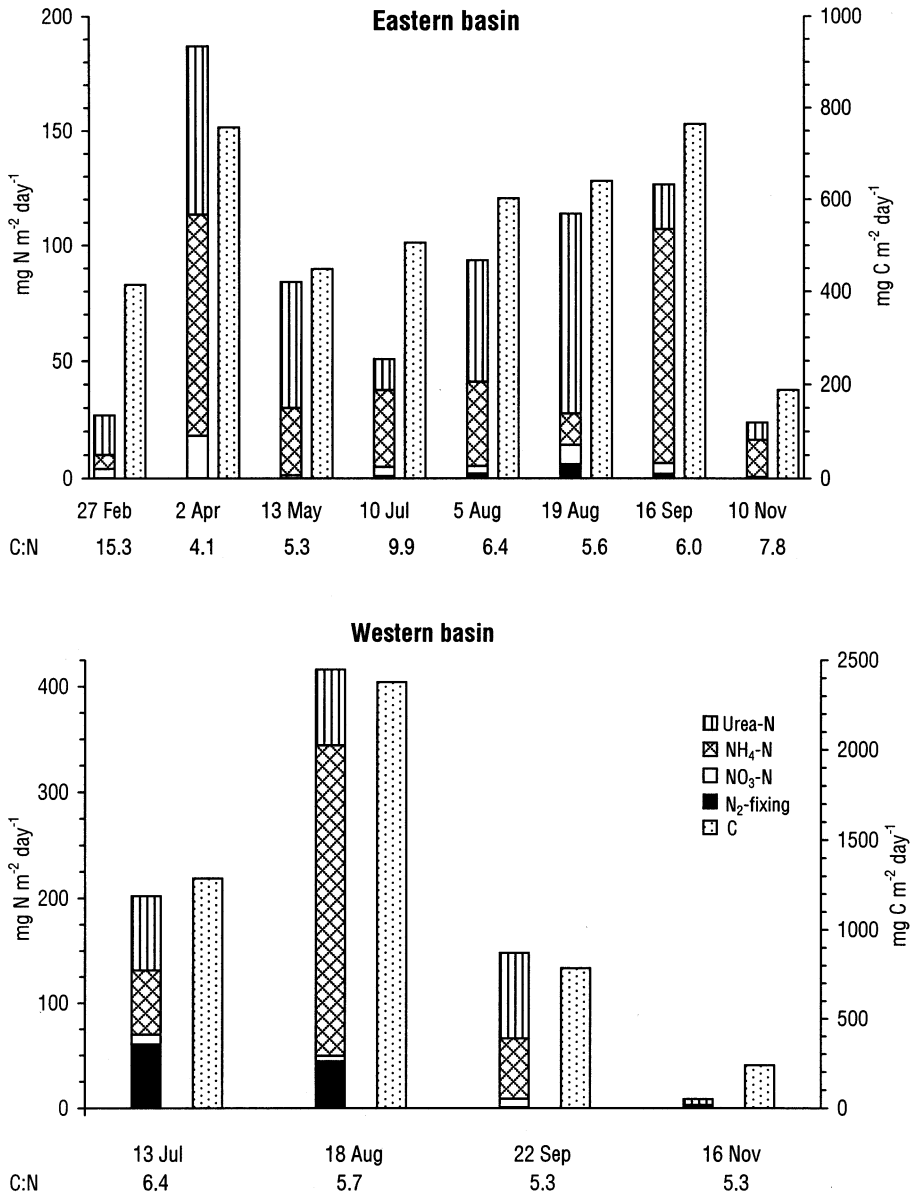

Fig. 4 Daily carbon and nitrogen uptake from different sources and their C:N ratios in the eastern and western basins of Lake Balaton in 1998.

cyanobacteria species (*Aphanizomenon flos-aquae*, (L.) Ralfs, *Cylindrospermopsis raciborskii* (Wolosz.) Seenayya et Subba Raju, and *Raphidiopsis mediterranea* Skuja) dominated. In October, cyanobacteria disappeared and centric diatoms increased again.

Total phytoplankton biomass was much higher in the western basin of the lake, varying between 0.5 and 12 mg L^{-1} (Fig. 3). In April, centric diatoms prevailed but towards summer they were slowly replaced by cryptophytes (*Cryptomonas* sp., *Rhodomonas minuta* Skuja), dinoflagellates and N_2-fixing blue-greens. Phytoplankton biomass was the highest (7–12 mg L^{-1}) in August and September, being dominated by N_2-fixing cyanobacteria, *A. flos-aquae*, *C. raciborskii* and *R. mediterranea*. In October and November, total biomass decreased significantly and cryptophytes were the most abundant organisms.

The measured and calculated carbon uptake results are plotted in Fig. 4. In the eastern basin, carbon uptake showed a spring peak of 757 mg C m^{-2} day^{-1}. After the collapse of the diatom population in May, the carbon uptake increased

linearly until the middle of September, reaching the same value as in April. In the western basin the greatest carbon uptake (2377 mg C m^{-2} day^{-1}) occurred a few weeks earlier than in the eastern basin.

The results of nitrogen uptake experiments are shown in Tables 1 and 2. The uptake rates for all the calculated parameters in the eastern basin were highest for ammonium in half of the measurements and for urea in the other. The absolute rates were not high, varying around 1 μg N L^{-1} h^{-1} with peaks of 1.56 μg L^{-1} h^{-1} of ammonium in the middle of September. The highest urea transport (1.38 μg L^{-1} h^{-1}) was observed a month earlier. Their chlorophyll *a* related transports (μg N μg Chl a^{-1} h^{-1}) were the highest at the same time (216.7 and 191.7 for ammonium and urea, respectively), and were also close to this in April. The maximum uptake velocities for ammonium, urea and nitrate could only be calculated together in a few cases. Usually the values for urea were highest. Half saturation constants were relatively high (from 7.37 to 215 μg N L^{-1}) but always less for ammonium than for urea. Despite the greater uptake maximum for urea than ammonium, lower half saturation constants resulted in higher initial slopes for uptake rate versus nutrient concentration curves (α) for ammonium than for urea. The uptake of nitrate was much lower than these in the eastern basin. The absolute uptake rates were only 10–20% of those for ammonium and urea. However, nitrate uptake exceeded that of ammonium at the end of February and in the middle of August, when its ambient concentration was four and nine times higher than ammonium, respectively. In the western basin the uptake velocities of urea and nitrate were some three

Table 1 Characteristics of ammonium, urea and nitrate uptake in eastern basin*

Date 1998		S_n	p	p/Chl *a*	v	P_{max}	P_{max}/Chl *a*	V_{max}	K_s	α	PN
27 February	NH_4-N	6.9	0.11	15.3	0.57	2.04	28.3	10.6	120	0.09	193
	Urea-N	28.8	0.40	55.1	1.79	3.36	46.7	15.2	215	0.07	222
	NO_3-N	26.7	0.16	22.3	0.75	0.31	4.3	1.4	24.9	0.06	214
2 April	NH_4-N	4.0	1.33	234.1	7.93	3.95	69.3	23.5	7.8	3.02	168
	Urea-N	7.7	1.11	195.0	6.47	3.97	69.6	23.1	19.7	1.17	172
	NO_3-N	6.1	0.39	68.4	2.45						159
13 May	NH_4-N	5.8	0.42	190.0	5.48	1.61	73.2	21.1	16.4	1.29	76
	Urea-N	30.3	0.80	363.7	10.27	3.27	148.6	42.0	93.4	0.45	78
	NO_3-N	3.8	0.03	12.6	0.41	0.06	2.5	0.8	3.9	0.20	74
10 July	NH_4-N	3.4	0.54	55.7	3.14	2.75	28.6	16.1	13.9	1.16	171
	Urea-N	11.1	0.36	37.4	2.15						168
	NO_3-N	11.1	0.15	15.6	0.91						165
5 August	NH_4-N	3.0	0.54	67.5	3.08	3.57	44.6	20.4	16.8	1.22	175
	Urea-N	43.2	1.05	131.3	5.73						183
	NO_3-N	6.7	0.10	13.1	0.60	0.22	2.8	1.3	7.4	0.17	176
19 August	NH_4-N	0.7	0.14	18.8	1.09	5.25	68.2	39.5	24.7	1.60	133
	Urea-N	35.1	1.38	179.2	9.99						138
	NO_3-N	6.3	0.31	40.3	2.25						138
16 September	NH_4-N	6.8	1.56	123.3	7.58						206
	Urea-N	10.4	0.34	26.9	1.63						208
	NO_3-N	14.9	0.14	11.1	0.67						208
10 November	NH_4-N	16.0	0.23	18.2	3.72						62
	Urea-N	24.5	0.12	9.5	2.04						59
	NO_3-N	53.0	0.02	1.3	0.29						59

* S_n, ambient concentrations (μg N L^{-1}); p, transport rate (μg N L^{-1} h^{-1}); p/Chl *a*, chlorophyll *a* related transport rate (μg N μg Chl a^{-1} $h^{-1} \times 10^{-3}$); v, uptake velocity ($h^{-1} \times 10^{-3}$); P_{max}, transport rate at the saturation concentration (μg N L^{-1} h^{-1}); P_{max}/Chl *a*, chlorophyll *a* related transport rate at saturation concentration (μg N μg Chl a^{-1} $h^{-1} \times 10^{-2}$); V_{max}, uptake rate at the saturation concentration ($h^{-1} \times 10^{-3}$); K_s, half saturation constant, where $v = V_{max}/2$ (μg N L^{-1}); α, initial slope of uptake rate versus nutrient concentration curve (V_{max}/K_s), PN, total particulate nitrogen (μg N L^{-1}).

Table 2 Characteristics of ammonium, urea and nitrate uptake in western basin*

Date 1998		S_n	p	p/Chl a	v	P_{max}	P_{max}/Chl a	V_{max}	K_s	α	PN
13 July	NH_4-N	4.5	1.93	57.2	5.38	10.51	31.2	29.3	19.9	1.48	358
	Urea-N	26.8	2.78	82.5	7.80						356
	NO_3-N	5.6	0.52	15.4	1.46						355
18 August	NH_4-N	5.8	10.27	268.5	21.08	19.62	51.3	40.3	5.3	7.63	487
	Urea-N	7.0	3.17	83.0	6.50	4.33	11.3	8.9	2.6	3.48	488
	NO_3-N	9.4	0.42	11.0	0.86	0.90	2.3	1.8	10.6	0.17	489
22 September	NH_4-N	7.8	1.74	135.1	8.36	3.24	25.1	15.5	6.7	2.31	209
	Urea-N	38.9	2.63	203.9	11.78						223
	NO_3-N	55.0	0.34	26.4	1.53						222
16 November	NH_4-N	8.6	0.07	16.5	0.96	1.07	24.9	14.5	120.8	0.12	74
	Urea-N	20.5	0.15	34.3	1.94	1.22	28.4	16.1	149.1	0.11	76
	NO_3-N	72.9	0.03	7.0	0.35						78

* S_n, ambient concentrations (μg N L^{-1}); p, transport rate (μg N L^{-1} h^{-1}); p/Chl a, chlorophyll a related transport rate (μg N μg Chl a^{-1} $h^{-1} \times 10^{-3}$); v, uptake velocity ($h^{-1} \times 10^{-3}$); P_{max}, transport rate at the saturation concentration (μg N L^{-1} h^{-1}); P_{max}/Chl a, chlorophyll a related transport rate at saturation concentration (μg N μg Chl a^{-1} $h^{-1} \times 10^{-2}$); V_{max}, uptake rate at the saturation concentration ($h^{-1} \times 10^{-3}$); K_s, half saturation constant, where $v = V_{max}/2$ (μg N L^{-1}); α, initial slope of uptake rate versus nutrient concentration curve (V_{max}/K_s), PN, total particulate nitrogen (μg N L^{-1}).

to four times higher than in the eastern one. In the case of ammonium this difference was even more pronounced. During the present study the highest uptake rates were found for ammonium in mid-August. The behaviour of different nitrogen sources was similar to that found in the eastern basin.

Dark uptake velocities of ammonium were 51–80% of the values measured at optimal photo irradiance in the eastern basin and 30–36% in the western basin. In the case of urea they were between 39 and 74% and 7 and 43% in the eastern and western basins, respectively. Dark uptake of nitrate was only 9–39% of the optimal illuminated uptake velocities in the eastern and 0–1% in the western basin (Table 3). The I_k values (light adaptation parameter) were generally slightly lower for ammonium than for urea. The proportion of dark ammonium uptake to the total daily uptake of ammonium in both basins was much higher than that in the light, ranging between 62 and 93% and 56 and 67% in the eastern and western basins, respectively. The contribution of dark nitrate uptake to the integrated daily nitrate uptake ranged from 13 to 64% in the eastern and from 0 to 25% in the western basin; these ratios were always less than for ammonium or urea (Table 3).

Calculated daily N_2-fixation, ammonium, urea and nitrate uptake per unit surface area in the two basins are summarized in Fig. 4. In the eastern basin from February to November the contribution of ammonium to daily nitrogen supply of phytoplankton varied from 11 to 81%. On four of the eight sampling days, ammonium was the most important of the measured nitrogen sources. The contribution of urea to the daily nitrogen uptake was between 15 and 73% and on the four other occasions it made the largest contribution to daily nitrogen demand. Nitrate uptake did not play a dominant role in the nitrogen supply of phytoplankton. In the eastern basin, the highest nitrate values (4 and 18 mg N m^{-2} day^{-1}) in February and April only provided 15% and 9% of daily uptakes. From May to November these contributions varied from only 1.5 to 5%. N_2-fixation was negligible, ranging from only 2 to 5%, even when 70% of the phytoplankton biomass was comprised of N_2-fixing cyanobacteria. In the western basin from July to November, the ratios of daily ammonium, urea and nitrate uptake per unit surface area to the total nitrogen utilization by phytoplankton were in the same range as in the eastern basin. Nitrogen fixation played an important role in the middle of July (when half of the phytoplankton biomass was N_2-fixing cyanobacteria) and provided some 30% of the daily nitrogen supply. In August, when N_2-fixing cyanobacteria contributed 70% of biomass, nitrogen fixation only contributed 10% of the daily nitrogen supply. From July to September 470 t ammonium,

256 t urea and 25 t nitrate-nitrogen were taken up and 120 t N_2 was fixed by phytoplankton in the basin.

The sum of ammonium, urea and nitrate uptake together with nitrogen fixation, compared with carbon uptake measurements, indicated C:N uptake ratios (weight:weight) of 4.1–9.9 in the eastern and of 5.3–6.4 in the western basin from April to November and from July to September, respectively (Fig. 4). At the end of February in the eastern and in the middle of November in the western basin these ratios were extremely high with values of 15.3 and 28, respectively.

The transport rate of carbon at optimal photo irradiance ($P_{opt\,C}$) was compared to the sum of ammonium, urea and nitrate transport rates measured at saturation concentration (240 μg L^{-1}) and optimal photo irradiance $P_{max\,N}$) (Table 3). The ratios varied in a relatively narrow range from 2.5 to 9.8 in the eastern basin. They were higher but still remained in this range (7.2–10.2) from July to September in the western basin, with the exception of 23.1 in November.

The external load of the western basin was assumed to be determined by the loading of the single significant inflow, the River Zala. It was calculated using daily water discharge and nutrient concentrations. The external load was estimated monthly and is shown in Table 4. The ammonium load was only about 1 t $month^{-1}$ in the first half of the year. It increased from September and peaked in October with more than 12 t $month^{-1}$. Nitrate showed a clear annual trend with high quantities in autumn and winter of up to 42 and 34 t $month^{-1}$, respectively. In spring and summer the monthly load was low, close to that of ammonium. The total nitrogen load was also highest in the autumn–winter period (up to 132 t $month^{-1}$ in November) but did not decrease in the summer months like nitrate. The orthophosphate load from the river varied between 0.4 and 3.4 t $month^{-1}$, with smaller amounts from January to March.

Ammonium, urea, nitrate and orthophosphate concentrations of sediment interstitial water were 1000–2400, 20–60, 10–60 and 70–140 μg L^{-1}, respectively. No clear trend could be discerned along the longitudinal axis of the lake. In the upper 5 cm of a square metre of sediment, there was on average approximately 7 mg of ammonium-, 2 mg of urea-, 1.5 mg of nitrate-nitrogen and 4 mg of phosphate-phosphorus.

Table 3 Relationship between light and dark N-transport rates and maximum transport rates of C and N at optimal photon flux density*

	Ammonium				Urea				Nitrate					
Date 1998	P_{max}	P_d%	I_k	Dark%	P_{max}	P_d%	I_k	Dark%	P_{max}	P_d%	I_k	Dark%	$P_{opt\,C}$	$P_{opt\,C}/P_{max\ (total\ N)}$
Eastern basin														
27 February	1.69	51	32	78	1.40	61	34	78	0.29	9	148	33	21.4	6.3
2 April	3.97	77	25	90	4.71	66	51	85	0.30	35	46	63	57.7	6.4
13 May	2.41	63	35	78	2.61	66	34	82	0.11	24	99	45	24.3	4.7
10 July	3.01	66	102	92	0.27	39	38	89	0.23	7	60	21	20.5	5.8
5 August	3.80	65	36	85	0.50	42	45	71	0.31	9	58	32	17.5	3.8
19 August	5.50	63	34	62	1.38	52	39	69	0.28	6	51	13	17.6	2.5
16 September	3.24	65	37	84	1.09	54	52	79	0.23	12	58	31	44.7	9.8
10 November	0.93	80	34	93	0.38	74	44	90	0.03	32	47	64	9.6	7.2
Western basin														
13 July	9.66	30	50	56	1.77	7	53	16	0.43	0	73	0	85.8	7.2
18 August	19.60	32	49	65	4.80	18	50	48	0.80	0	83	0	244.2	9.7
22 September	3.97	34	48	62	1.75	30	48	57	0.27	0	92	0	61.3	10.2
16 November	0.55	36	37	67	0.13	43	33	68	0.05	1	37	25	17.0	23.1

* P_{max}, transport rate at the saturation concentration at optimal photon flux density (μg N L^{-1} h^{-1}); P_d%, dark transport rate in % of maximum transport rate measured at optimal photon flux density; I_k, photon flux density at which the initial slope equals P_{max} (μmd photon m^{-2} s^{-1}); Dark%, contribution of dark transport in % of the total daily transport; $P_{opt\,C}$, optimum photosynthetic rate (μg C L^{-1} h^{-1}).

Table 4 External load from the River Zala

Date 1998	NH_4-N (t)	NO_3-N (t)	Total N (t)	PO_4-P (t)	Water inflow ($m^3 \times 10^6$)
January	1.57	18.81	47.13	0.83	26.1
February	0.7	6.93	26.89	0.43	17.5
March	0.39	2.84	23.61	0.82	21.2
	2.66	28.58	97.63	2.08	64.8
April	0.59	1.89	26.08	1.4	21.3
May	0.59	1.29	21.71	2.29	15.6
June	1.05	1.07	17.69	2.43	10.7
	2.23	4.25	65.48	6.12	47.6
July	2.08	1.34	34.93	3.48	20.3
August	0.74	0.89	14.87	1.37	7.3
September	5.8	3.83	51.71	3.28	32.7
	8.62	6.06	101.51	8.13	60.3
October	12.21	4.55	69.9	3.91	43.6
November	5.8	42.38	132.24	2.7	80.7
December	2.08	33.75	82.3	1.92	47.9
	20.09	80.68	284.44	8.53	172.2
Total	33.6	119.57	549.06	24.86	344.9

Discussion

Our previous work has been concerned with the various parameters characterizing the degree of nutrient limitation or substrate affinity of algae in relation to summer nitrogen uptake in Lake Balaton (Présing *et al*., 1999). The parameters measured and calculated in the present work are appropriate to characterize the nitrogen supply of phytoplankton.

Ambient concentrations

Ammonium concentrations were low, but values were higher than in previous years and ammonium was not depleted to its detection limit, which often occurred in both basins during cyanobacterial blooms (Présing *et al*., 1996, 1999). This perhaps indicates a reduced demand for ammonium—assumed to be the preferred nitrogen source. The total dissolved organic nitrogen (DON) concentration in the lake water exceeded 1 mg L^{-1} throughout the year (Transdanubian Water Authority, unpublished data). Of several possible organic nitrogen compounds only urea was measured and found relatively high. Potentially, other organic nitrogen compounds, such as amino acids, can also support phytoplankton production (Preston *et al*., 1996). During the growing period the concentration of reduced nitrogen was far above the detectable limit in the water. From late autumn to spring, due to the high nitrate and urea concentration, there were luxurious amounts, of nitrogen in the water. There was less available nitrogen during spring in the eastern basin and during the middle of August in the western basin.

Michaelis–Menten kinetics

One underlying assumption in the use of the Michaelis–Menten equation to describe the uptake is a certain limitation of nutrient. However, several factors are involved in dictating the N uptake pattern; generally non-linear uptake is associated with N limited algal cells and linear uptake with N sufficient algal cells (Collos, 1983). In the eight experiments carried out in the eastern basin, ammonium, urea and nitrate uptake showed Michaelis–Menten saturation from spring to summer. From July to August, ammonium uptake fitted a saturation curve. In the western basin, August was the most 'critical period', when uptake of every measured nitrogen source followed Michaelis–Menten kinetics. In the other experiments, ammonium uptake could always be described by saturation kinetics and urea uptake in one case.

Kinetic parameters

In the eastern basin the highest V_{max} (both absolute and chlorophyll *a* related) and the highest initial slopes of uptake for ammonium were measured in April, May and after mid-August, at the time of spring and late summer algal peaks, respectively. But these parameters were significantly lower, and the half saturation constant higher, in the present study than in 1995 (Présing *et al.*, 1999). The K_s values for urea were higher than those for ammonium. The V_{max} values for urea exceeded those of ammonium in spring, but in summer, most probably due to the high urea ambient concentration; we could not calculate them. The lowest α values with the lowest maximum uptakes (despite the smallest values of K_s) in the case of nitrate indicated the weakest preference among the three nitrogen substrates. In the western basin, as in the eastern basin, the highest maximum uptakes and α values for ammonium were observed in the middle of August, at the time of highest phytoplankton biomass. The order of preference in both basins was ammonium > urea > nitrate, as in previous years and in many other waters (Mitamura *et al.*, 1995). This order of preference was expressed also according to I_k and P_{max} values for dark uptake. The I_k values did not show a significant change with seasons as found in previous years (Présing *et al.*, 1999) and were lowest for ammonium and highest for nitrate. The values for urea were very close to those of ammonium.

Lean & Pick (1981) introduced an index of PO_4-P limitation comparing the P_{max} of carbon (or $P_{opt\,C}$) to the equivalent value for phosphorus uptake at optimal photo irradiance ($P_{opt\,C}/P_{max\,P}$). Their intention was to normalize luxury P uptake according to another metabolic rate, since it is impossible to measure the nutrient content of cells in natural phytoplankton populations. Similarly to the P limitation index, Lean, Murphy & Pick (1982) tried to apply an index of N limitation ($P_{opt\,C}/P_{max\,N}$). Index values from 6 to 16 were accepted to be close to the cellular ratio and indicate a non-N-depleted population. In our experiments the $P_{opt\,C}/P_{max\,N}$ values in both basins were close to the ratio to maintain the phytoplankton and did not relate to N limitation. However, we note that the lowest ratios were calculated in July and August.

The daily C:N uptake ratios (weight:weight) were very close to the Redfield ratio except for two very high values, measured in February and November in the eastern and western basins, respectively. Because of high ambient concentrations, low algal biomass and low temperature, nitrogen limitation was unlikely. Carbon uptake was probably overestimated in these circumstances. In this good fit to the Redfield number a high contribution of urea to the total nitrogen uptake was observed. Generally, the contribution of urea to the total N utilized by phytoplankton is intermediate between that of ammonium and nitrate, somewhat closer to that of ammonium (reviewed by Takamura, Iwakuma & Yasuno, 1987; Mitamura *et al.*, 1995). Urea contribution can be as high as 70–80% of the daily nitrogen demand (Glibert *et al.*, 1991). Measurements during 1996 in Lake Balaton showed the contribution of urea to phytoplankton nitrogen supply reached 50–70% of ammonium uptake (Présing *et al.*, 1998). While ammonium was the preferred nitrogen source of phytoplankton, urea contribution to daily total nitrogen uptake sometimes exceeded that of ammonium in 1998, due to much higher urea ambient concentrations. The origins of urea in natural waters include bacterial degradation of particulate and dissolved organic nitrogen, zooplankton excretion, allochthonous input, and sediment (including bioturbation) (Mitamura & Saijo, 1986). Vörös, V.-Balogh & Herodek (1996) showed that approximately 50% of phytoplankton primary production (130000 t C $year^{-1}$ in the whole lake, in 1998) can be utilized by bacteria (65000 t C $year^{-1}$ in the whole lake). Assuming a carbon/nitrogen ratio of 5.6 in the phytoplankton and bacterioplankton, this is equivalent to 23000 and 11600 t N, respectively. We have no data on the rates of regenerated ammonium and urea from mineralization. Mitamura & Saijo (1986) calculated for Lake Biwa that regenerated urea contributed 35–115% of urea nitrogen assimilated by phytoplankton and 16–49% of that originated from bacterial mineralization. Ammonium formation from urea was not detectable in their study. Assuming a similar ratio of urea produced from the mineralization of phytoplankton in Lake Balaton, this would equate to 2000–6000 t urea nitrogen originating from phytoplankton degradation. Zooplankton grazing is estimated to consume 22% (Vörös *et al.*, 1996) of phytoplankton primary production, utilizing 5000 t N $year^{-1}$ in the whole lake. The percentage that urea excretion contributes to the total excretion rate of zooplankton (as the sum of urea and ammonia excretion) varies from 3 to 30% (reviewed by Mitamura & Saijo, 1986).

Assuming an average 16% excretion rate of urea by zooplankton in Lake Balaton, the urea-N originating from zooplankton excretion was some 800 t. The fresh weight of fish in Lake Balaton varies from 100 to 300 kg ha^{-1} (Bíró, 1997) with an average of 150 kg ha^{-1}, equivalent to 10 000 t fresh weight in the whole lake. This biomass of fish should consume some 100 000 t fresh food, containing approximately 7000 t carbon and roughly 1300 t nitrogen. The average percentage of urea excretion of the total nitrogen excretion by fish is about 30% (Brett & Groves, 1979; Lovell, 1989). The same proportion was found (19–40%) in the excretion of bream (*Abramis brama* L.) (Tátrai, 1981) in Lake Balaton. Because cyprinids, mainly bream, provide the bulk of fish biomass in the lake (Bíró, 1997), we assumed a value of 30% for urea excretion. On this assumption, the fish populations produce close to 400 t urea-N in the lake per year. The external total annual nitrogen load of the whole lake is approximately 3000 t (Jolánkai, 1995). Unfortunately the proportion of urea in this loading is unknown, but the value will certainly be smaller than those of the above listed sources. It seems to be clear that the overwhelming majority of the 23 000 t N taken up by phytoplankton during 1998 in the whole lake had an internal origin and only about one tenth came from external sources. The difference between N uptake and the sources listed and calculated above is only about 2000 t. The difference could be due to invertebrates other than zooplankton, sediment sources (including bioturbation), N fixation.

The significance of the internal load is obvious when the external nutrient load of the western basin from July to September (8.6 t ammonium and 6 t nitrate nitrogen) is compared to ammonium, urea and nitrate assimilated by phytoplankton in the whole basin during the same period (470, 256 and 25 t, respectively; calculated from the transport rates in Table 2 and the volume of the western basin, 95×10^6 m^3). Similarly, if the change in the nitrogen standing crop between July and August (an increase of 12.5 t N) is compared to the external ammonium plus nitrate loading (2.7 t N), the significance of sediment supply to the internal loading is clear. Indeed, analysis of the sediment interstitial water reveals a N:P ratio of 2.63:1. This ratio is likely to reflect the total P:total N ratio of the sediment (Andersen, 1974). If significant quantities of sediment nutrient reserves are supporting the phytoplankton standing crop in summer, this is likely to be more limited by nitrogen availability than phosphorus.

Very fast recycling of ammonium and urea is the predominant process supporting the nitrogen demand of the summer standing crop of phytoplankton in the lake. There is no doubt that nitrogen recycling is occurring at a very high rate within the water column. However, any net increase in phytoplankton nitrogen above that supplied from the external loading and water column, such as observed when the maximum standing crop was established, must have originated from the sediment nitrogen reserve. Future study should be directed at the processes controlling the internal nutrient supply of the lake and thereby potentially providing novel approaches for managing and evaluating the progress of oligotrophication in the lake.

Acknowledgments

We wish to thank Western-Transdanubian District Water Authority (NYU-VIZIG) for nutrient loading data. This work was supported by the Hungarian Research Found (OTKA) grant no. T 016303 and the Research Found of the Hungarian Academy of Sciences (AKP) grant no. 97-111 3,3/47. Support from the Royal Society is gratefully acknowledged in the form of a Travelling Fellowship to Tom Preston.

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(*Manuscript accepted 1 March 2000*)