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Urea and Ureolytic Activity in Lakes of Different Trophic Status

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Abstract

Urea and uraease (U-ase) activity were determined in water samples taken from the surface layers of 17 lakes of different trophic status. Urea concentrations were inversely correlated with the trophic status of the studied lakes and varied from below the detection limit to 25 μmol l^{-1}. Maximal potential ureolytic activity (V_{max}) ranged from 0.2 to 7.0 μmol l^{-1} h^{-1}. The highest urea concentrations and the lowest U-ase activities were recorded in the spring, whereas the lowest urea concentrations and the highest rates of urea hydrolysis were observed late in summer, during heavy phytoplankton blooms. Since in the majority of the Great Mazurian Lakes microplankton growth was limited by nitrogen supply, urea was an important N source for both auto- and heterotrophic planktonic microorganisms throughout the growth period. U-ase activity was mainly related to the seston. Only up to 25% of total activity could be attributed to free enzymes dissolved in lake water. In epilimnetic water samples the bulk of the ureolytic activity originated from seston-attached bacteria. However, a positive, statistically significant correlation between ureolytic activity and chlorophyll *a* (Chl_a) concentrations suggests that phytoplankton may also be responsible for at least a some of the observed ureolytic activity in the highly eutrophic Great Mazurian Lakes.

K e y w o r d s: algae, bacteria, lakes, urea, ureolytic activity

Introduction

Urea may be present in significant concentrations in all types of aquatic environments (Remsen, 1971; McCarthy, 1972; Kristiansen, 1983; Mitamura and Saijo, 1986; Weeb and Haas, 1986; Park *et al.*, 1997; Wilthshire and Lampert, 1999; Berman and Bronk, 2003). Numerous studies carried out in coastal and oceanic waters showed that urea plays a key role in the marine nitrogen cycle (Herbland, 1976; Savidge and Hutley, 1977). However, the function, fate, sources and turnover of urea in inland waters were only extensively studied and are still poorly known. Unlike oceanic habitats, in freshwater urea can be both of allochthonous and autochthonous origin. It is not only a major excretory product of man and terrestrial animals, but also a highly concentrated artificial N fertilizer and constituent of pesticides. Therefore, lakes receive substantial amounts of allochthonous urea as runoff from land and sewage from urban areas (Dugan, 1975). Some urea may also be introduced into natural waters due to atmospheric precipitation (Timperley *et al*., 1985).

In unpolluted freshwater ecosystems autochthonous urea predominates. The major supply routes of this compound are zooplankton excretion (Wiltshire and Lampert, 1999) and bacterial degradation of organic matter, especially purines, pyrimidines and arginine (Cho *et al*., 1996; Satoh, 1980; Pedersen *et al*., 1993; Vogels and Van der Drift, 1976; Therkildsen *et al.*, 1996). Quantitative information on urea distribution in various types of marine environments is relatively rich (Price and Harrison, 1987). However, analogous data concerning freshwater environments are scant. As reported by Berman (1974), Satoh and Hanya (1976) and Mitamura and Saijo (1986) concentrations of urea in lakes and ponds reached 9.1 μmol l^{-1} and most commonly ranged between 0.5 and 2.0 μmol l^{-1}.

Enzymatic hydrolysis of urea provides substantial amounts of NH_4^+ and CO_3^{2-} which are the basic mineral N and C forms taken up and metabolized by planktonic microorganisms. Up to now, the majority of studies

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on the assimilation and degradation of urea and on the role of this compound in growth and metabolism of aquatic microorganisms were carried out in algal and bacterial cultures. Field ecological investigations were mainly focused on the utilization of urea as a nitrogen source for the growth of marine phytoplankton (McCarthy, 1972; Ignatiades, 1986) and lately, on the role of this compound as an important N source for toxic dinoflagellates responsible for red tides (Zehr and Ward, 2002). Consequently, our knowledge concerning urea transformations and the ecological significance of this compound for aquatic bacteria and the whole microplankton community in inland aquatic habitats is limited and requires detailed investigation (Berman and Bronk, 2003).

It is generally believed that in the photic zone of marine and freshwater environments urea is utilized primarily by phytoplankton rather than by bacteria (Remsen *et al*., 1972; McCarthy, 1972; Carpenter *et al*., 1972, Satoh and Hanya, 1976; Turley, 1986). For instance, Mitamura and co-workers (Mitamura *et al*., 2000) showed that the diel changes in urea-decomposing activity exhibited a similar pattern to that of the photosynthetic assimilation number, following the diel change in photosynthetic activity. Price and Harrison (1987) found that in the ocean much of urea could be taken up by phytoplankton directly, without its hydrolysis outside the cell. They also pointed out that NH_4^+ ions liberated from this compound by intracellular enzymes was not retained by algal cells. Those observations strongly suggested that phytoplankton would not only be competitive to bacteria in terms of urea N assimilation but also could participate in NH_4^+ regeneration processes. On the other hand, little is known about the participation of bacteria in urea metabolism in aquatic habitats. Whether bacteria are a source of urea or a sink for it is still an open question (Zehr and Ward, 2002). The wide distribution of urea-decomposing bacteria (Satoh and Hanya, 1976; Satoh 1980) and cyanobacteria (Flores and Herrero, 1994) in aquatic habitats, as well as documented by Park *et al*. (1997) active urea decomposition during the night (47.1–90 % of the daily urea decomposition activity) may prove indirectly that in freshwater environments also bacteria may substantially participate in urea decomposition processes.

Some authors (McCarthy *et al.*, 1977; Probyn and Painting, 1985) considered that in marine and coastal environments urea nitrogen can be taken up by planktonic microorganisms even in the presence of nitrate and ammonium. However, as postulated by Kristiansen (1983) the uptake of urea by phytoplankton seems to be the most effective in the absence of reduced N sources. According to Kristiansen (1983) ammonium concentrations higher than 1 μmol l^{-1} effectively inhibit urea assimilation. The highest absolute as well as relative urea uptake rates were noted by Kristiansen (1983) during the summer, when concentrations of inorganic N forms in the water were low. Therefore, one can presume that urea can be one of the most important factors affecting phytoplankton growth especially significant in highly eutrophic environments often nitrogen limited. In Cheasepeake Bay urea constitutes a small percentage of total dissolved organic N pool. However, it has been shown to contribute from 60 to 80% of the nitrogen utilized throughout much of the year by the planktonic community (Glibert and Terlizzi, 1999).

The main aim of this study was to compare urea concentrations and ureolytic activity in lakes of different trophic status. Moreover we discuss the importance of this compound for N limited freshwater environments and the role of bacteria and phytoplankton in urea decomposition processes.

Experimental

Materials and Methods

Study area and sampling. The studies were carried out in the spring and the summer of two vegetation periods. During three sampling sessions (April, August and September 2002) 5 l water samples were taken under non-sterile conditions from surface layer (1 m depth) of 17 lakes of the Mazurian Lake District in northeastern Poland. From three of these lakes (Kuc, Mikołajskie and Tałtowisko) surface water samples were collected in 2001 and 2002 more intensively *i.e.* 4 times a season. Additionally, in August 2002 samples from depth profiles of Lake Tałtowisko and Lake Mikołajskie were analyzed. Lakes that were taken into consideration represented a wide range of trophic conditions – from mesotrophic to highly eutrophic (Table I).

Determination of urea concentrations. For determination of urea concentrations we modified McCarthy's "enzymatic" technique (McCarthy, 1970) by applying the highly sensitive O-phthaldialdehyde (OPA) method (Holmes *et al*., 1999) for NH_4^+ determination instead of the classical procedure of Solorzano (1969). Although according to Price and Harrison (1987) the "urease method" in comparison to chemical urea determination (Newell *et al.*, 1967) underestimates the total urea concentrations, we decided to use it as a method that provides more precise information on readily available urea for planktonic microorganisms than the "chemical" method.

Filtered (0.2 μm Nuclepore) lake water sample was divided into six 5 ml portions and warmed up to 25°C. Three of them were supplemented with 0.25 ml (~10 U) of jack bean U-ase (Type IV, Sigma) solution whereas the next three served as controls. Commercially available U-ase was dissolved in deionized water to 40 U ml^{-1} concentrations just before using. Subsamples with U-ase

Table I
Basic morphological, physico-chemical and biological parameters of the studied lakes

Lake	Surface area (ha)	Average depth (m)	Maximum depth (m)	*Chl_a (µg l^{-1})	*Total P (µg P l^{-1})	*Water transparency (m)	Trophic condition
Kuc	99	8.0	28.8	8.3 ± 6.0	13.8 ± 8.1	4.4 ± 0.3	Mesotrophic
Mamry	2504	11.7	43.8	6.1 ± 4.5	16.3 ± 1.5	3.4 ± 0.1	Mesotrophic
Przystań	115	n.d.	22.8	6.8 ± 5.1	17.2 ± 0.7	3.6 ± 0.3	Mesotrophic
Łabap	350	8.5	13.4	10.1 ± 0.4	29.4 ± 1.3	3.1 ± 0.1	Mesotrophic
Dargin	2680	10.6	37.6	12.5 ± 2.8	31.8 ± 4.2	3.1 ± 0.0	Meso/eutrophic
Kisajno	1896	8.4	25.0	12.6 ± 3.0	33.2 ± 0.9	2.6 ± 0.1	Meso/eutrophic
Śniardwy	11340	5.8	23.4	19.9 ± 7.1	43.9 ± 4.2	2.0 ± 0.1	Eutrophic
Niegocin	2600	9.9	39.7	22.1 ± 14.5	92.1 ± 4.8	2.6 ± 0.2	Eutrophic
Boczne	183	8.4	25.0	22.3 ± 15.8	97.2 ± 9.9	2.3 ± 0.1	Eutrophic
Bełdany	941	10.0	46.0	30.7 ± 9.6	64.3 ± 5.0	1.4 ± 0.2	Eutrophic
Tałty	1160	13.5	44.7	27.4 ± 9.3	111.1 ± 11.6	1.9 ± 0.1	Eutrophic
Tałtowisko	327	14.0	39.5	14.9 ± 6.2	113.5 ± 24.1	1.9 ± 0.1	Eutrophic
Ryńskie	671	13.5	50.8	30.3 ± 9.8	100.6 ± 19.9	1.5 ± 0.1	Eutrophic
Mikołajskie	498	11.2	25.9	43.8 ± 19.9	87.3 ± 19.1	1.5 ± 0.0	Eutrophic
Jagodne	420	8.7	37.4	24.0 ± 2.2	108.3 ± 11.6	1.8 ± 0.1	Eutrophic
Szymoneckie	523	8.7	28.5	24.5 ± 0.9	107.9 ± 3.9	1.7 ± 0.17	Eutrophic
Szymon	154	1.1	2.9	24.4 ± 10.9	91.1 ± 63.9	1.0 ± 0.1	Hypereutrophic

± – standard deviation of triplicates, * – spring homothermy (April 2001)

and urease-free controls were incubated for 1.5 h at 25° C. After the incubation, 1 ml of each "sample" and "control" replicate was placed in plastic 8 ml vial and supplemented with 3 ml of combined OPA reagent (Holmes *et al*., 1999). To the controls 0.05 ml of U-ase working solution was additionally added. Combined OPA reagent was not only necessary for HN_4^+ assay but also terminated the enzymatic reaction. Addition of U-ase working solution to "controls" after their supplementation with OPA combined reagent equalized volumes of "sample" and "control" and corrected errors caused by contamination of commercially available urease by NH_4^+. The reaction with OPA reagent was carried out for 135 min at 20°C in darkness. Fluorescence of all subsamples was read in Shimadzu RF 1501 spectrofluorometer (330 nm ex., 455 nm em.). Ammonium concentrations were calculated from linear regression of the standard curve. Urea concentrations were obtained from the following equation:

$$C_{Urea} = 1.05\ (A - B) / 2$$

where: C_{Urea} – urea concentration; *1.05* – volume correction; *A* – concentration of NH_4^+ in subsamples treated with U-ase before incubation; *B* – concentration of NH_4^+ in subsamples supplemented with U-ase after incubation.

Urease activity assay. U-ase activity was determined as maximal velocity (V_{max}) of ammonium liberation in the course of hydrolysis of urea added to the tested water samples. To determine the kinetics of urea hydrolysis, a series of subsamples (10 ml) taken from each lake water sample was supplemented with 0.4 ml of working substrate solutions. Final concentrations of urea in assay were: 0.5, 1.0, 2.5, 5.0, 10.0, 20.0, 50.0 and 80.0 mmol l^{-1}. Substrate stock solution (2.5 M) and working solutions (0.0125, 0.025, 0.0625, 0.125, 0.25, 0.5, 1.25 and 2.0 M), obtained by dilution of urea stock solution in deionized water, were freshly prepared before ureolytic activity determination. Concentrations of NH_4^+ were measured twice: at time 0 and after 1.0–3.0 h of incubation at 20° by method of Holmes *et al*. (1999) described previously. Since we were interested only in an increase in fluorescence (ammonium concentration) during sample incubation, no additional controls and corrections were needed. For determination of U-ase activity in various seston size fractions differential filtration technique was used. Water samples were filtered using vacuum (up to 8000 Pa) through polycarbonate membrane filters 0.2, 1.0, 10.0 µm Nuclepore and 100.0 and 200.0 µm plankton net. To reduce possible overestimation of U-ase activity by NH_4^+ liberated by intracellular ATP-dependent urea amidolyase and released from algal cells into environment all samples were incubated in the darkness. The rate of enzymatic liberation of NH_4^+ from urea (v) in lake water samples followed first order Michaelis-Menten kinetics and could be described by the equation:

$$v = (V_{max} * [S] / (K_m + [S]).$$

U-ase kinetic parameters (V_{max} and , $K_m + S_n$) were calculated by means of nonlinear regression analysis PC software Enzpack (Biosoft, U.K.). The relative turnover time of urea (rT_t) was defined as V_{max} / K_m. In fact $rT_t = V_{max} / K_m + S_n$, where S_n is a natural (*in situ*) urea concentration. However, since natural urea concentrations were always three orders of magnitude lower than K_m, they were omitted during rT_t calculations.

Modification of U-ase activity by NH_4^+ and other environmental factors. To check whether ammonium (within range of NH_4^+ concentrations commonly observed in water of The Great Mazurian Lakes) could inhibit U-ase activity, samples taken from epilimnion (1 m depth) of L. Tałtowisko and L. Mikołajskie in August (ambient NH_4^+concentrations 0.75 and 0.5 µmol l^{-1}, respectively) were enriched with increasing amounts of NH_4Cl. U-ase activity in NH_4^+ enriched samples was measured within 0.5 h after ammonium addition. Moreover we have also tested the influence of environmental conditions on U-ase activity. Epilimnetic (1.0 m depth)

and hypolimnetic (1.0 m above of the sediment) water samples (2.0 l) were filtered through Nuclepore membrane filters (0.2 µm). Seston from each sample was suspended in 20 ml of distilled water and divided into two 10 ml portions. One portion of "epilimnetic seston" resuspension was added to 0.45 l of epilimnetic water filtrate (control) whereas another one was added to the same volume of hypolimnetic water filtrate. Similarly, hypolimnetic water filtrate was supplemented with "hypolimnetic" (control) and epilimnetic" seston resuspensions. Finally, in each combined sample U-ase activity was measured in respect to *in situ* temperature.

Other analyses. Temperature, pH, conductivity and oxygen concentrations were measured *in situ*, using a Water Analyzer H20 (Cole Palmer Instruments). Total numbers of bacterial cell (sum of seston-attached and free-living bacteria) and numbers of free-living bacteria were estimated in samples fixed with formaldehyde (2% v/v final conc.) by DAPI direct count method (Porter and Feig, 1980).

To detach seston-attached bacteria for counting, non-filtered water samples were supplemented with sodium pyrophosphate (100 mmol l^{-1} final conc.), cooled and sonicated with a 5 mm tip (six 5 sec. pulses, ~ 400 W) in UDM-10 Sonicator Techpan, Poland. For counting of free-living microorganisms the same water samples were filtered through a 1.0 µm Nuclepore membrane filter before fixing. The numbers of attached bacteria were calculated as the difference between numbers of total and free living bacterial cells. Chl_a was extracted from phytoplankton with 98% v/v ethanol and measured spectrophotometrically (Marker *et al*., 1980). Total phosphorus was assayed by the Koroleff (1983) method. L-leucine aminopeptidase activity (AMP) was measured according to Siuda and Chróst (2002). Concentrations of proteins and amino acids were determined fluorometrically with OPA (Roth, 1971).

Results

The results of three sampling sessions carried out in April, July and August 2002 in 17 Mazurian lakes of different trophic status and the data obtained during more intensive investigations conducted in three of these lakes during spring-autumn seasons of 2001 and 2002 are summarized in Table II and Table III. Generally, urea concentrations in surface waters of studied lakes varied from the detection limit (0.01 µmol l^{-1}) in L. Mikołajskie in September 2002 to about 25.0 µmol l^{-1} in L. Kuc in April 2001 (Table III). Typically they averaged 0.5–0.8 µmol l^{-1} and only seldom exceeded 1.0 µmol l^{-1} (Table II and Table III). Amounts of urea in lake water were inversely related to their trophic status (Fig. 1 A). Commonly, elevated urea concentrations (12.0–25.0 µmol l^{-1}) were noted in less eutrophic lakes in spring, lower – in more eutrophic environments throughout the rest of the growth period (Fig. 1 A, Table II and Table III). We found that urea was a quantitatively significant constituent of the labile dissolved organic N fraction (LDON),

Table II
Urea concentrations and urease activity in surface (1 m depth) waters of studied lakes. Lakes are ranged with respect to their trophic status

Lake	April 2002		June 2002		September 2002	
	Urea µM	Urease µmol urea l^{-1} h^{-1}	Urea µM	Urease µmol urea l^{-1} h^{-1}	Urea µM	Urease µmol urea l^{-1} h^{-1}
Kuc	0.20 ± 0.04	0.57 ± 0.15	0.60 ± 0.06	0.53 ± 0.01	0.10 ± 0.02	0.35 ± 0.02
Mamry	n.d.	n.d.	0.65 ± 0.02	1.10 ± 0.04	n.d	n.d
Dargin	n.d.	n.d.	0.68 ± 0.07	1.92 ± 0.68	n.d	n.d
Przystań	n.d.	n.d.	0.50 ± 0.02	0.97 ± 0.02	n.d	n.d
Kisajno	n.d.	n.d.	0.11 ± 0.10	3.21 ± 0.24	n.d	n.d
Łabap	n.d.	n.d.	0.55 ± 0.06	2.80 ± 0.61	n.d	n.d
Śniardwy	0.47 ± 0.07	n.d.	0.04 ± 0.03	0.23 ± 0.01	0.01 ± 0.06	1.46 ± 0.03
Bełdany	0.74 ± 0.05	n.d.	0.15 ± 0.03	4.25 ± 0.01	0.00 ± 0.06	2.32 ± 0.04
Mikołajskie	0.17 ± 0.07	2.36 ± 0.11	0.61 ± 0.03	1.16 ± 0.09	0.05 ± 0.07	1.88 ± 0.20
Niegocin	0.25 ± 0.04	n.d.	0.21 ± 0.07	2.31 ± 0.10	0.07 ± 0.08	2.37 ± 0.07
Ryńskie	0.10 ± 0.06	n.d.	0.34 ± 0.04	1.66 ± 0.03	0.01 ± 0.07	2.42 ± 0.03
Boczne	0.23 ± 0.03	n.d.	0.20 ± 0.03	2.36 ± 0.09	0.05 ± 0.09	2.98 ± 0.06
Szymoneckie	0.08 ± 0.02	n.d.	0.38 ± 0.01	6.74 ± 0.11	0.01 ± 0.06	4.70 ± 0.03
Jagodne	0.15± 0.04	n.d.	0.32± 0.04	4.87 ± 0.05	0.00 ± 0.05	4.26 ± 0.03
Tałty	0.00 ± 0.04	n.d.	0.18 ± 0.04	2.54 ± 0.05	0.03 ± 0.03	2.54 ± 0.05
Szymon	0.07 ± 0.04	n.d.	0.26 ± 0.05	7.00 ± 0.07	0.10 ± 0.08	5.93 ± 0.04
Tałtowisko	0.47 ± 0.02	1.81 ± 0.10	0.5 ± 0.04	6.80 ± 0.21	0.18 ± 0.02	4.92 ± 0.02

n.d. – not determined, ± – standard deviation of triplicate determinations

Table III
Urease activity, urea concentrations and contribution of urea-N (%) to the pool of organic NH_4^+ sources potentially easily available for planktonic microorganisms in surface (1 m depth) waters of three lakes of different trophic status

Lake	Date		Urease μmol urea $l^{-1}h^{-1}$	Amino acid N μM	Labile protein N μM	Urea N μM	%
Kuc (mesotrophic)	2001	Apr.	3.38 ± 0.43	2.03 ± 0.19	0.44 ± 0.02	25.00 ± 3.54	91
		Jun.	0.82 ± 0.01	0.81 ± 0.02	0.38 ± 0.01	0.82 ± 0.08	41
		Jul.	2.06 ± 0.13	0.54 ± 0.01	0.56 ± 0.16	0.76 ± 0.07	41
		Aug.	0.90 ± 0.08	0.86 ± 0.01	0.62 ± 0.10	1.71 ± 0.07	54
	2002	Apr.	0.57 ± 0.15	0.22 ± 0.01	0.47 ± 0.06	0.20 ± 0.04	23
		Jun.	0.54 ± 0.00	0.39 ± 0.02	0.18 ± 0.01	0.60 ± 0.03	51
		Aug.	1.75 ± 0.05	0.26 ± 0.04	0.15 ± 0.01	0.62 ± 0.04	28
		Sept.	0.35 ± 0.02	0.14 ± 0.06	0.18 ± 0.02	0.10 ± 0.02	24
	Avg.			0.66 ± 0.05	0.37 ± 0.05	3.11 ± 0.49	43
	Range		0.35–3.38	0.14–2.03	0.15–0.62	0.1–25.04	23–84
Mikołajskie (eutrophic)	2001	Apr.	4.06 ± 0.12	1.43 ± 0.25	0.49 ± 0.02	13.20 ± 0.63	87
		Jun.	5.72 ± 0.68	0.40 ± 0.01	0.70 ± 0.06	0.04 ± 0.01	4
		Jul.	2.76 ± 0.09	0.64 ± 0.01	1.77 ± 0.42	0.84 ± 0.07	26
		Aug.	6.69± 0.14	0.73 ± 0.03	2.61 ± 1.20	0.43 ± 0.01	11
	2002	Apr.	2.36 ± 0.11	0.33 ± 0.01	1.68 ± 0.22	0.17 ± 0.07	8
		Jun	1.16 ± 0.09	0.43 ± 0.01	0.30 ± 0.02	0.61 ± 0.03	45
		Aug.	2.94 ± 0.14	0.49 ± 0.01	2.42 ± 0.27	0.93 ± 0.01	24
		Sept.	1.90 ± 0.22	0.23 ± 0.02	0.02 ± 0.00	0.01 ± 0.03	5
	Avg.			0.59 ± 0.04	1.25 ± 0.28	3.55 ± 0.11	26
	Range		1.16–6.69	0.23–1.43	0.02–2.61	0.01–13.20	4–87
Tałtowisko (hypertrophic)	2001	Apr.	n.d.	1.66 ± 0.02	0.46 ± 0.03	11.37 ± 1.27	84
		Jun.	1.70 ± 0.25	0.86 ± 0.01	0.53 ± 0.05	11.75 ± 0.48	89
		Jul.	2.38 ± 0.13	0.69 ± 0.02	1.56 ± 0.11	0.39 ± 0.07	15
		Aug.	5.74 ± 0.19	1.27 ± 0.01	7.31 ± 1.25	0.25 ± 0.02	3
	2002	Apr.	1.81 ± 0.10	0.80 ± 0.01	1.09 ± 0.07	0.47 ± 0.02	20
		Jun.	6.80 ± 0.21	0.64 ± 0.01	0.47 ± 0.03	0.50 ± 0.04	31
		Aug.	7.20 ± 0.32	0.55 ± (0.01	3.06 ± 0.05	0.66 ± 0.07	15
		Sept.	7.01 ± 1.51	0.33 ± 0.01	0.67 ± 0.25	0.18 ± 0.02	15
	Avg.			0.85 ± 0.01	1.90 ± 0.23	4.45 ± 0.25	38
	Range		1.16–7.20	0.33–1.66	0.46–7.31	0.18–11.75	3–89

n.d. – not determined, ± – standard deviation of triplicate determinations

that besides urea consists of other organic N compounds relatively easily available for planktonic microorganisms *i.e.* peptides, proteins and amino acids. During the spring-autumn period urea constitutes about 26–38% (in eutrophic) and up to 44 % (in mesotrophic environments) of LDON fraction (Table III).

To test whether relatively high concentrations of urea noted in the early spring could be an effect of slow urea decomposition in this period of the year the relationship between temperature and U-ase activity was examined. We found that ureolytic activity (urea decomposition rate) increased exponentially with the temperature and at 5°C was about 2.25 times lower than at 20°C (Fig. 2).

Analysis of the depth profile of eutrophic L. Tałtowisko in August exhibited the lowest amounts of urea (0.8–1.1 μM) in the upper epilimnion (Fig. 3A). In spite of some irregularities (distinct peaks in the central metalimnion and at 16 m depth) urea concentrations generally increased with depth and reached 6 μmols l^{-1} above the bottom sediments. In contrast to L. Tałtowisko, in waters of L. Mikołajskie in August urea content decreased with the depth (Fig. 3B). Higher urea concentrations were found in epilimnetic waters (0.40–0.75 μmol l^{-1}), while the lowest (<0.2 μmol l^{-1}) were observed at the bottom of the hypolimnetic zone. Similarly to L. Tałtowisko, a distinct peak of urea (to 2.7 μmol l^{-1}) was observed in the middle of the metalimnetic zone.

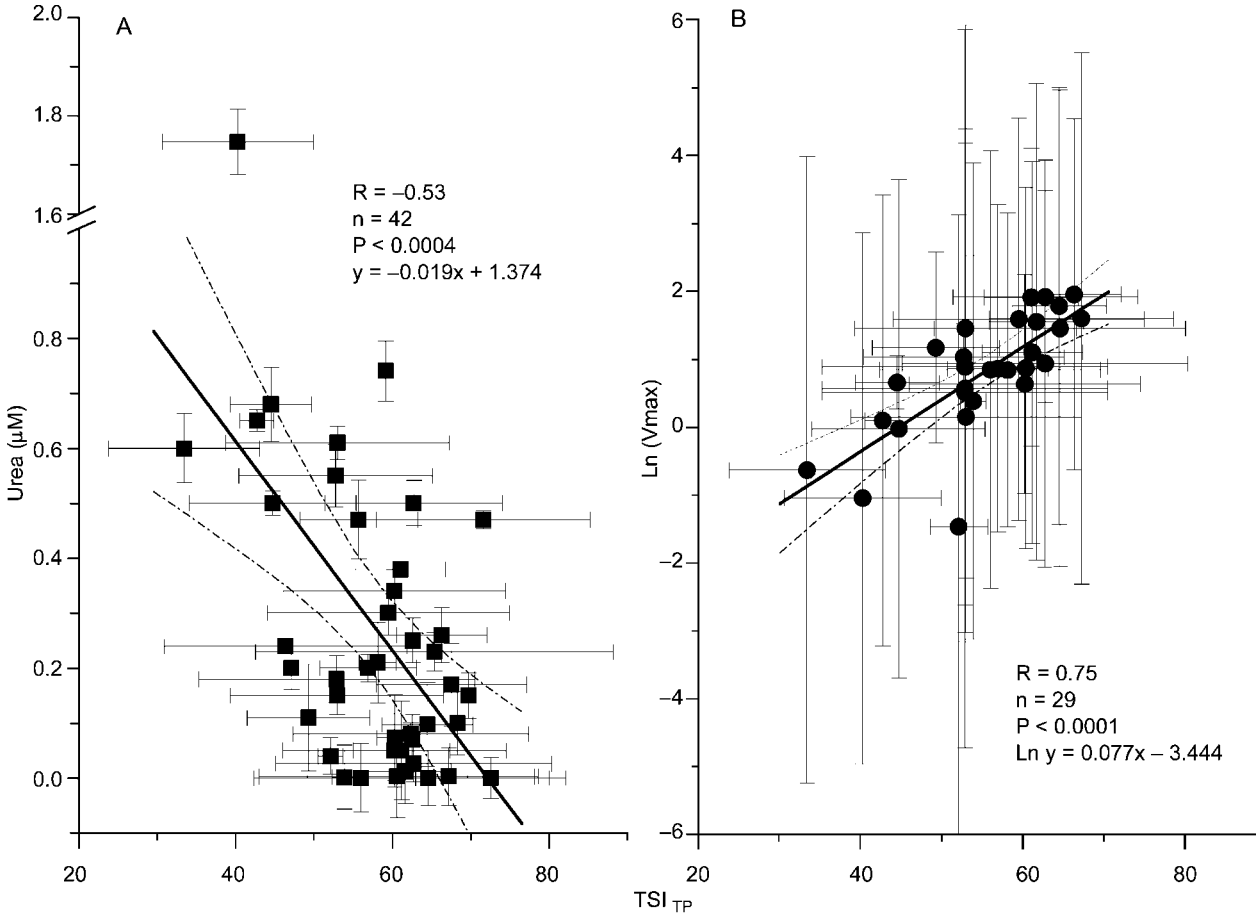

Fig.1. Urea concentrations (A) and urease activity (B) as a function of the trophic state index of the studied lakes. Trophic state index was calculated from total phosphorus according to Carlson (1977).

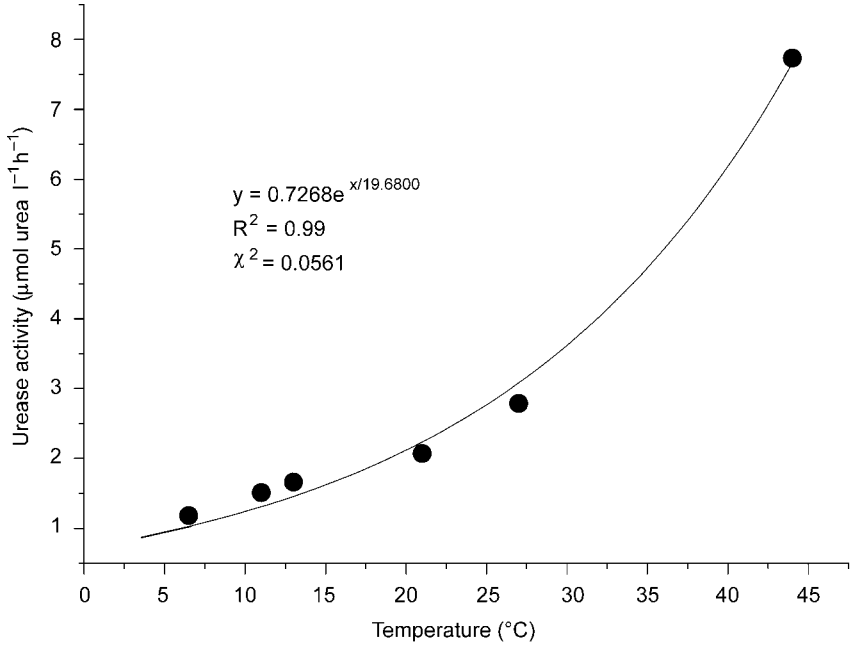

Fig. 2. The influence of temperature on activity of urease in lake water sample taken from surface layer of L. Mikołajskie in August 2002.

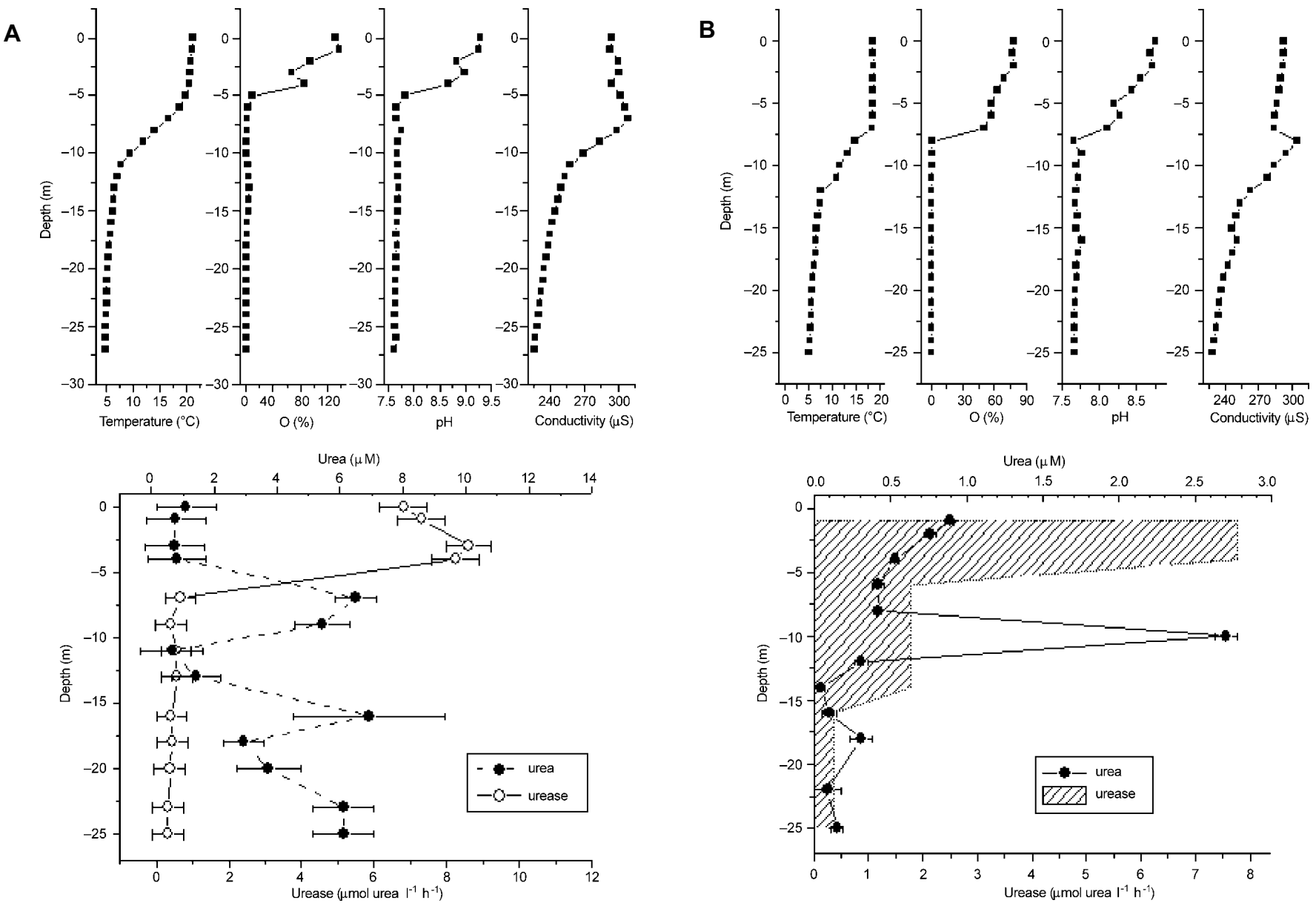

Fig. 3. Urea concentrations and urease activity in depth profiles of L. Tałtowisko in August 2002 (A) and L. Mikołajskie in September 2002 (B).
For determination of urease activity in L. Mikołajskie 2 l samples taken from 1, 2, 4, 6 m (epilimnetic layer); 8, 10, 12, 14 m (metalimnetic layer) and from 18, 22, 25 m (hypolimnetic layer) were mixed and further analyzed as one integrated sample.

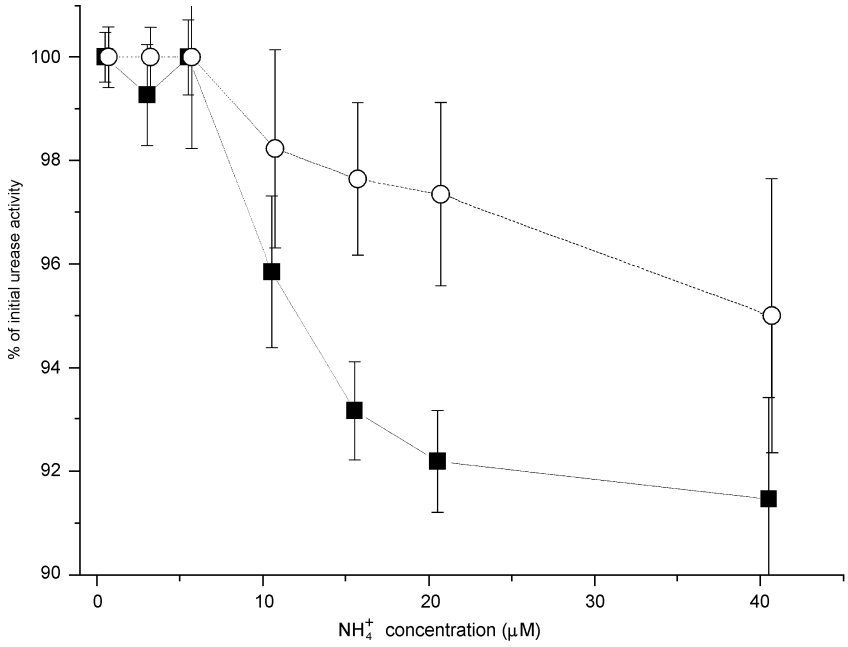

Fig. 4. Inhibition of urease activity by ammonium.
Samples were taken in August 2002 from epilimnion (1.0 m depth) of L. Mikołajskie (circles) and L. Tałtowisko (squares).

The maximal potential U-ase activity (V_{max}) was tightly correlated with the trophic status of the studied lakes (Fig 1B) and increased in the summer months from 0.18 µmol urea l^{-1} h^{-1} (Table II) to 7.25 µmol urea l^{-1} h^{-1} (Table III). The highest U-ase activity (4.5–7.2 µmol urea l^{-1} h^{-1}) was observed in July and September in four of the most eutrophic lakes connected to each other and placed along the water flow (L. Jagodne, L. Szymoneckie, L. Szymon and L. Tałtowisko) see Table II. In the rest of the studied lakes maximal potential activity of this enzyme was relatively stable and varied between 2.5 and 3.5 µmol urea l^{-1} h^{-1}. Analysis of U-ase in depth profile of L. Tałtowisko in August (Fig. 3A) showed that quantitatively important activity of this enzyme (8.1–10.1 µmol urea l^{-1} h^{-1}) was only found in the epilimnion. In the other parts of the depth profile U-ase was almost undetectable (0.5–0.9 µmol urea l^{-1} h^{-1}). Additional evidence suggesting minimal U-ase activity in the deeper parts of the eutrophic lake was also provided by analysis of integrated epi- meta- and hypolimnetic samples taken from L. Mikołajskie in August (Fig. 3B), when V_{max} of U-ase in the meta- and hypolimnion was respectively about 3.5 and 21.5 times lower than in the epilimnion.

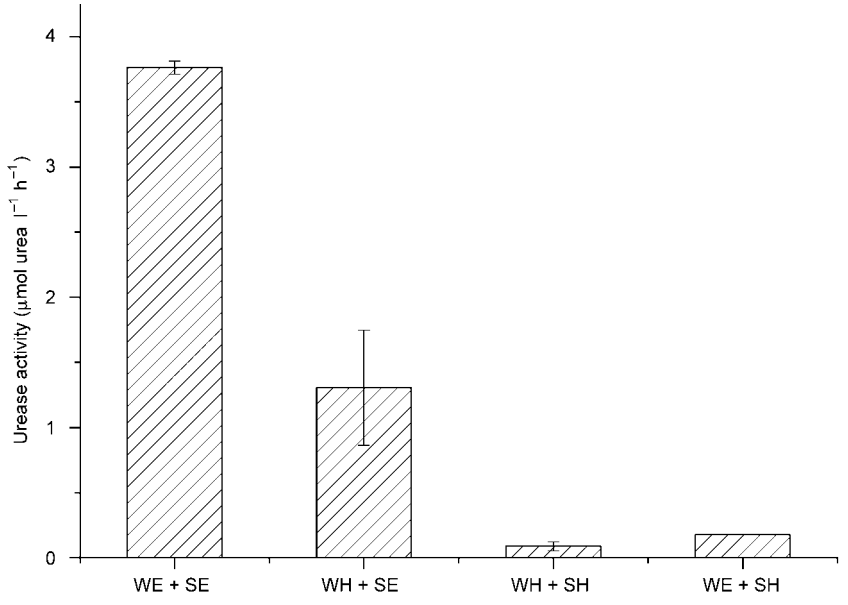

Fig. 5. Modification of urease activity by environmental conditions.
WE – epilimnetic water filtrate, SE – epilimnetic seston, WH – hypolimnetic water filtrate, SH – hypolimnetic seston.

Looking for factors that, like the low temperature, could cause depletion of U-ase activity in the hypolimnion, possible inhibition of this enzyme by high NH_4^+ concentrations or other inhibitors was also examined. As arises from Figure 4, ammonium concentrations up to 40.0 μmol l^{-1} inhibited "epilimnetic U-ase" in only about 5%. However, in the hypolimnion U-ase could be evidently inhibited by unknown factors other than NH_4^+ (Fig. 5). "Epilimnetic enzyme" placed in hypolimnetic water exhibited only about 35% of its normal activity whereas "hypolimnetic U-ase" carried to epilimnetic water increased its activity by about 100%.

U-ase activity similarly as AMP activity was mainly associated with the seston. In surface waters of mesotrophic L. Kuc, eutrophic L. Mikołajskie and hypereutrophic L. Tałtowisko only about 20 % of total U-ase activity was found in the liquid phase (fraction <0.2 μm) whereas 80 % was related to particulate material (Table IV). Simultaneously, the relative turnover time (rT_t) of urea calculated for free urease activity was 3–11 times longer than calculated for the enzyme bound to the particles. The relative turnover time of urea calculated for the total ureolytic activity varied from 10 days to 2 months in highly eutrophic and from 3 to 4 months in less eutrophic environments.

A more detailed pattern of urease activity in various fractions of the seston demonstrated in Fig. 6A shows, that microorganisms (and perhaps free extracellular U-ase) attached to particulate material in fractions 10.0–100.0 μm and 1.0–10.0 μm were mainly responsible for urea hydrolysis in epilimnetic waters of L. Mikołajskie (31 % and 29 % of the total activity, respectively). Relatively low U-ase activities (up to 5% of the total activity) were found in fractions of larger particles (100.0–200.0 μm and >200.0 μm).

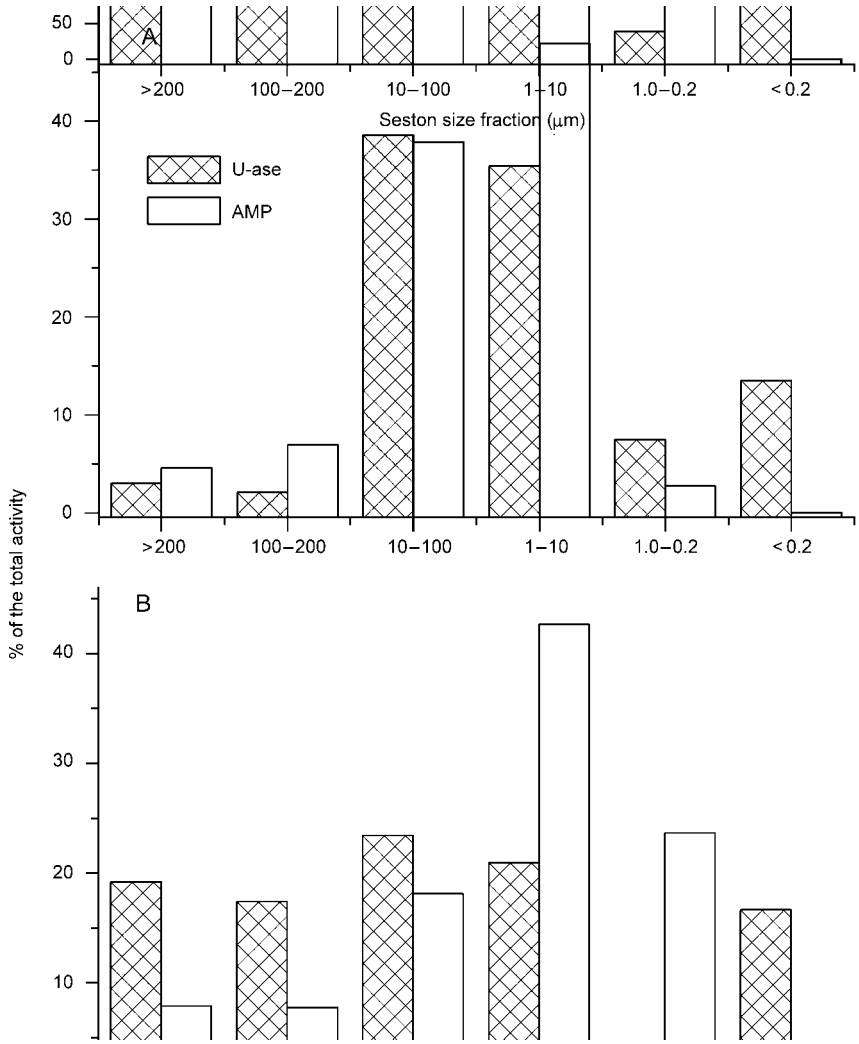

Fig. 6. Contribution of various seston size fractions to total urease and aminopeptidase activities in epilimnetic (A) and hypolimnetic (B) water of Lake Mikołajskie.

Table IV
Partition of total urease activity between particulate (>0.2 μm) and cell-free (<0.2 μm) fractions of integrated, epilimnetic water samples taken in August from the epilimnion of three lakes of different trophic status in August 2002

	V_{max} (μm urea l^{-1} h^{-1})		K_m (mM)		rT_t (day^{-1})		% of total activity	
	>0.2 μm	<0.2 μm	>0.2 μm	<0.2 μm	>0.2 μm	<0.2 μm	>0.2 μm	<0.2 μm
Kuc (mesotrophic)	1.27 ± 0.05	0.39 ± 0.04	3.06 ± 0.47	10.84 ± 2.44	100	1158	77	23
Mikołajskie (eutrophic)	2.34 ± 0.02	0.60 ± 0.02	1.54 ± 0.07	1.18 ± 0.21	28	82	80	20
Tałtowisko (hypertrophic)	7.19 ± 0.30	1.57 ± 0.03	1.60 ± 0.40	1.04 ± 0.14	9	28	82	18

± – standard errors

Table V
Relationships between urease activity and bacterial numbers, aminopeptidase activity of (AMP-ase) and chlorophyll a (Chl_a) concentrations in surface waters of The Great Mazurian Lakes

		Bacteria			AMP	Chl_a
		Total	Attached	Free living		
U-ase	June	R = 0.54 n = 17 P < 0.0250	R = 0.33 n = 17 n. s.	R = 0.81 n = 17 P < 0.0001	R = 0.84 n = 17 P < 0.0001	R = 0.93 n = 17 P < 0.0001
	September	R = 0.68 n = 12 P < 0.0220	R = 0.51 n = 11 n. s.	R = 0.86 n = 12 P < 0.0004	R = 0.62 n = 11 P < 0.0414	R = 0.90 n = 12 P < 0.0001
	All results	R = 0.44 n = 36 P < 0.0080	R = 0.24 n = 36 n. s.	R = 0.65 n = 36 P < 0.0001	R = 0.78 n = 28 P < 0.0001	R = 0.75 n = 37 P < 0.0001
Chl_a	June	R = 0.61 n = 17 P < 0.0089	R = 0.42 n = 17 n. s.	R = 0.83 n = 17 P < 0.0001	R = 0.92 n = 16 P < 0.0001	–
	September	R = 0.67 n = 11 P < 0.0249	R = 0.57 n = 11 n. s.	R = 0.67 n = 12 P < 0.0172	R = 0.62 n = 11 P < 0.043	–
	All results	R = 0.57 n = 131 P < 0.0001	R = –0.02 n = 41 n. s.	R = 0.43 n = 42 P < 0.0100	R = 0.78 n = 38 P < 0.0001	–

n.s. – correlation non-significant

It was notable that in epilimnetic waters, excluding fraction <0.2 μm where L-leucine-aminopeptidase activity was almost undetectable, the distribution of U-ase and AMP activities among the tested seston size fractions was almost identical ($R = 0.96$, $n = 5$, $P<0.01$). In the hypolimnion, participation of U-ase activities in each fraction compared to overall activity of this enzyme was similar and did not exceed 23%. Moreover, in hypolimnetic waters, the correlation between U-ase and AMP activities in various fractions of the seston was not found.

Although our data suggest a strong relation of U-ase activity with the seston, we did not observe a direct relationship between U-ase activity and the number of seston-attached bacteria (Table V). In samples taken during June and September, no correlation between these parameters was observed. This was confirmed by analysis of all data collected during our investigations. On the other hand, we found an unexpected, strong correlation between U-ase activity and number of free-living bacteria ($R = 0.81$, $n = 17$, $P<0.0001$; $R = 0.86$, $n = 12$, $P<0.0004$ and $R = 0.65$, $n = 36$, $P<0.0001$ – for June, September and all collected results, respectively) and not so strong, but still statistically significant correlation U-ase activity with the total number of the bacteria ($R = 0.54$, $n = 17$, $P<0.03$; $R = 0.68$, $n = 12$, $P<0.02$ and $R = 0.44$, $n = 36$, $P<0.008$, respectively). The bacterial origin of U-ase can be confirmed additionally by comparing its activity with the activity of other, almost exclusively bacterial, enzyme – AMP. As can be seen in Figure 7, the activities of both of these enzymes were proportional to each other (U-ase activity $=0.0047 \times$ AMP activity $+ 1.0215$) and tightly correlated ($R = 0.78$, $n = 27$, $P<0.0001$) in the surface waters of all studied lakes.

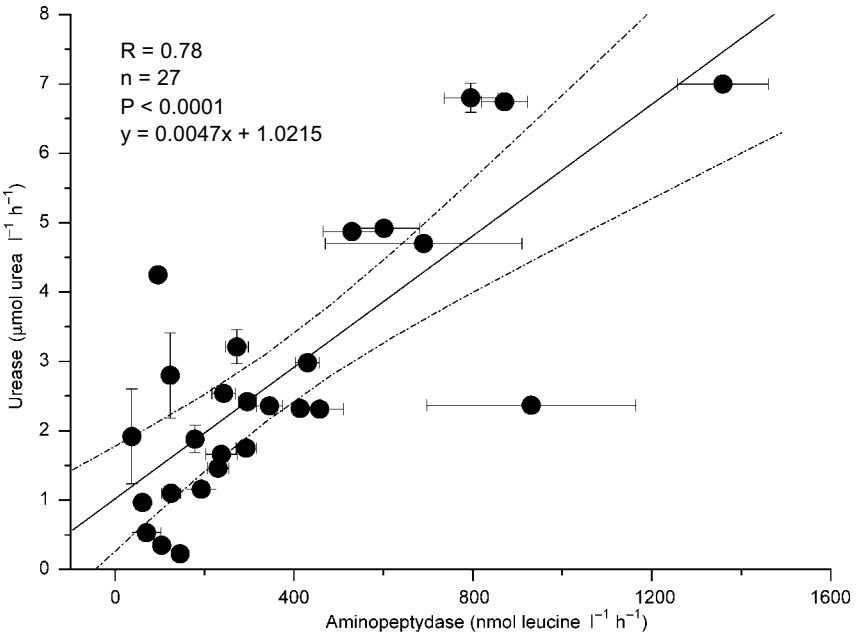

Fig. 7. Correlation between urease and aminopeptidase activity in surface water of the studied lakes

Ureolytic activity was correlated not only with bacterial number but also with Chl_a concentrations ($R = 0.93$, $n = 17$, $P < 0.0001$; $R = 0.90$, $n = 12$, $P < 0.0001$ and $R = 0.75$, $n = 37$, $P < 0.0001$ – calculated for June, September and from all collected results, respectively) (Table V). However, it should be pointed out that these correlations might only be apparent, because U-ase activity is a function of bacterial number, which depends directly on organic matter originating from algal production. To test this hypothesis, we examined relationships between Chl_a and bacterial numbers (total, seston-attached and free-living) for results obtained in July, August and during the whole investigation period (Table V). Analysis of the results collected in Table V showed that, like ureolytic activity, also Chl_a concentrations were well correlated with total bacterial number ($R = 0.61$, $n = 7$, $P < 0.001$; $R = 0{,}67$, $n = 11$, $P < 0.001$; $R = 0.57$, $n = 131$, $P < 0.001$; June, September and all results, respectively) and with the number of free-living bacteria ($R = 0.83$, $n = 17$, $P < 0.001$; $R = 0.67$, $n = 11$, $P < 0.0172$; $R = 0.43$, $n = 42$, $P < 0.0100$ June, September and all results, respectively). Simultaneously, they were not correlated with the number of seston-attached bacteria.

Discussion

Urea is the one of the most significant components of the DON pool and easiest available N sources for aquatic microorganisms. Although this simplest organic N compound is ubiquitous in natural waters its content, biological transformations and importance to planktonic microorganisms and dissolved organic N pool in freshwater environments are still insufficiently investigated. Concentrations of urea in lake water noted during our investigations commonly changed from 0.2 to 7.0 µmol l^{-1} h^{-1}. However, they were unusually as high as 11.37, 13.2 or even 25.0 µmol l^{-1}. In surface waters of the studied lakes concentrations of urea were inversely and closely correlated with their trophic status. Some indirect evidence suggested that in water of Mazurian Lakes urea was mostly of autochthonous origin. This is not fully consistent with the observations of other authors. For instance, the data obtained by Lomas *et al*. (2002) during long-term studies in waters of eutrophic Chesapeake Bay suggested that the external input of urea from the watershed was extremely significant in this estuarine system. Berman and Bronk (2003) also stated that human-derived pollution and runoff from agricultural areas are important allochthonous sources of urea in many lakes reservoirs and coastal marine waters. On the other hand, we did not observe higher urea concentrations in lakes Niegocin, Ryńskie and Mikołajskie receiving more sewage from surrounding urbanized areas than others. Indeed, relatively low and unpredictable urea concentrations could be there a consequence of high and dynamically changing rate of enzymatic decomposition of this compound by planktonic microorganisms. However, in these lakes systematically enriched with allochthonous urea U-ase activities did not differ distinctly from that noted in other lakes, less exposed to urea supply. Moreover in

waters of the studied lakes no statistically significant negative correlation between urea concentrations and U-ase activity was found. Such a correlation should be observed if urea concentrations would be depended directly on U-ase activity.

Increase in urea concentrations in the lower part of epilimnion and in metalimnion of L. Mikołajskie at the beginning of August (Fig. 3) followed not only massive phytoplankton blooms in surface waters but also the peak of the sailing season. This, by contrast, may suggest that, at least periodically, urea of allochthonous origin creates a substantial part of the total urea pool. The highest urea concentrations (up to 25 μmol l^{-1}) noted during the early spring in the surface waters of all studied lakes and in the deepest part of L. Tałtowisko in July, if not overestimated, could be or an effect of urea accumulation in the environment resulted from low temperature of water (4.5–5.0°C) and/or from inhibition of U-ase activity at this time of the year and part of the lake depth profile. We found that the rate of urea decomposition in natural lake water sample increased exponentially with temperature and at 5°C was about 2.25 times lower than at 20°C (Fig. 2). Mitamura (1986) suggested that the optimal temperature for urea degradation in lake water was about 30°C. Q10 coefficient calculated for this reaction varied from 1.7 to 2.0.

Assimilation of urea by various groups of microorganisms in natural environments is still poorly understood. It is commonly believed that bacteria and cyanobacteria can utilize urea nitrogen as NH_4^+, after enzymatic hydrolysis of urea by intracellular U-ase. The mechanism of urea assimilation by algae seems to be more complicated and is still not fully known. Some algal species (particularly green algae) and yeasts produce ATP-dependent urea amidolyase instead of U-ase. The activity of this enzyme seems to be induced by urea and repressed by ammonium. Other planktonic algae (some diatoms and dinoflagellates), similarly to bacteria produce intracellular U-ase, or assimilate urea using metabolic pathways that are not yet known (Syrett, 1962; Morris, 1974; Park *et al*., 1993). The literature concerning utilization of urea by algae in pure cultures is relatively rich (Syrett, 1962). Moreover, field data (Remsen *et al*., 1972; Cho and Azam, 1995) also strongly suggest that urea is decomposed/assimilated primarily by phytoplankton rather than by bacteria in most freshwater and marine habitats.

Park *et al*. (1997) pointed out that in hypertrophic freshwater environments not only algae but also bacteria participate substantially in urea decomposition processes. Our results suggest that the role of bacterial U-ase was also significant in the surface waters of moderately eutrophic Mazurian Lakes. In meta- and hypolimnetic zones, bacterial U-ase produced *in situ* as well as exported from the epilimnion with bacteria attached to sinking seston was probably strongly inhibited/repressed by high HN_4^+ concentrations or other not yet defined factors. Although we did not observe substantial U-ase inhibition by 40 μmol l^{-1} of HN_4^+ in laboratory conditions, but the concentrations of ammonium noted in the deepest layers of The Great Mazurian Lakes in late summer were occasionally as high as 140 μmol l^{-1}. Thus, one can expect that facultative and obligate anaerobic bacteria living in the profundal zone and in the bottom sediments may have produced urea rather than hydrolyzed it. Nucleic acid and protein decomposition supporting bacteria with urea precursors and inhibition or/and reduction of U-ase activity at low temperature caused accumulation of urea in deep waters during the summer and its preservation in cold environment during the winter. Water mixing processes substantially increased urea concentrations in the photic zone of the studied lakes during the spring, when urea might serve, instead of NO_3^-, as a "trigger" for early spring diatoms blooms.

It should be pointed out that the relatively low urea concentrations found in deep waters of L. Mikołajskie in August (Fig. 3B) contradict the hypothesis of urea conservation in the hypolimnion. However, they were probably only an exception to the rule. Later investigations showed that urea concentrations varied from 1 to 6 μM in profundal zone of the studied lakes and were generally much higher than in surface waters (Siuda, unpublished). The consistently elevated urea concentrations were also observed by Lomas *et al*. (2002) in the deep waters of Cheaseapeake Bay. According to these authors relative high concentrations of urea in deep waters could be an effect of decomposition of recently settled particulate organic matter by benthic microorganisms and/or could be due to decreases in authotrophic utilization of urea, relative to surface waters.

U-ase (E.C.3.5.1.5) activity is a widely distributed property of aquatic bacteria including aerobes, facultative anaerobes and obligate anaerobes (Satoh and Hanya, 1976; Satoh, 1980). This mainly intracellular enzyme is specific for urea and hydroxyurea (Fishbein and Carbone, 1965) has a pH optimum between 6.7 and 8.0 and K_m of milimolar range (Palińska *et al*., 2000, Mobley and Hausinger, 1989). We found more than 75% of total U-ase activity in the particulate fraction, whereas the rest represented free dissolved enzyme activity. The relative turnover time (rT_t) of urea *via* U-ase varied from 10 days to 2 months in highly eutrophic and from 3 to 4 months in meso- and moderately eutrophic lakes. In water of the same lakes T_t calculated for L-leucine-4-methyl-coumarinylamide (AMP substrate) commonly does not exceed 15 days and the half-life time of labile dissolved proteins reaches 8 days (Siuda *et al*., in preparation). Therefore,

one can speculate that urea may be a poorer N source for aquatic bacteria compared to peptides or proteins. Especially, as more complicated organic N compounds (like peptides or proteins) additionally support bacteria with organic C radicals.

Though the total U-ase activity was tightly correlated with the number of free-living bacteria (Table II), other collected evidence strongly suggests that it was produced mainly by bacteria attached to particles having dimensions from 1.0 to 100.0 μm. This fraction of the seston commonly exhibits also the highest overall microbial activity (Kiersztyn, unpublished, Long and Azam, 1996; Grossard and Simon, 1998). The crucial role of attached bacteria in urea decomposition processes was confirmed definitely by some other observations. First, by strong, statistically significant ($P<0.0001$) correlation between U-ase and AMP (Table V), an enzyme that is commonly produced by more efficiently attached bacteria (Siuda and Chróst, 2002). Second, by the fact, that U-ase activity in the studied lakes was proportional to their trophic state indexes, which were rather a function of quantity of the seston, than the number of free living bacteria in lake water, and finally by the partition of U-ase activity between various seston size fractions observed by Siuda in eutrophic Zegrzyński Reservoir (Siuda, unpublished). Contribution of free-living bacteria to the total U-ase activity was there smaller than 4%. Decisive role of attached bacteria in processes of urea hydrolysis was also postulated by Satoch and Hanya (1976). They found that in freshwater pond free-living bacteria did not decompose urea efficiently.

The data collected in Table V lead to more general conclusion that may define the function of attached and free-living bacteria in the environment. If we presume that, similarly as U-ase and aminopeptidase activity, also activities of other bacterial enzymes are primarily due to "colonists" – attached bacteria, we have to also accept that the number of free-living bacteria ("explorers") in lake water must be dependent on "colonists" activity. Thanks to overproduction of variety of hydrolytic enzymes, attached bacteria support free-living heterotrophic microorganisms with readily utilizable monomeric substrates (Siuda and Chróst, 2002) and thus facilitate them to colonize other particles. Considering this, one could rather not expect the correlation of U-ase and AMP activities with the number of attached bacteria, especially that both of those enzymes might be also preserved in the matrix of biofilms, even after death of their producers. Inversely, the tight correlation between both of enzyme activities and the number of free-living bacteria observed during our investigations was obvious and well understood.

To explain the high correlation between ureolytic activity and Chl_a concentrations (Table V) we propose four hypotheses: (1) as opposed to many individual algal species tested in pure cultures, natural phytoplankton assemblages (in the case of tested lakes dominated by cyanobacteria, diatoms and dinoflagellates), synthesized urease and utilized urea nitrogen after its hydrolysis to NH_4^+ similarly as bacteria. Assimilation of urea could be especially favorable for algal species without anhydrase activity. During periods of intensive photosynthesis they could not take up inorganic C from insoluble carbonates but could, at least theoretically, obtain it from urea hydrolysed inside their cells; (2) urease was mainly produced by bacteria and correlation of its activity with Chl_a was only apparent and misleading. It arose from the fact that Chl_a concentrations, similarly as U-ase activity were well correlated with the total number of bacteria as well as with the number of free-living microorganisms; (3) considering that nitrogen limited primary production in majority of Great Mazurian Lakes and that urea composed the bulk of easy utilizable DON fraction, one could presume that NH_4^+ liberated in excess from urea by bacterial U-ase not only covered bacterial N demand but also stimulated phytoplankton growth, (4) tight correlation of U-ase and AMP activities with Chl_a concentrations was a consequence of peculiar algal-bacterial metabolic coupling (Francoeur and Wetzel, 2003). Bacteria attached to the living algal cells cooperated with algae supporting them, thanks to U-ase and AMP activities, with surplus NH_4^+ and amino acids and utilizing easy available organic C compounds released extracellularly by algal cells. This mode of algal NH_4^+ nutrition seems to be less energy-consuming than liberation of NH_4^+ from urea *via* ATP-dependent urea amidolyase system.

Although none of the above hypotheses can be definitely proven on the basis of our experimental data, the last presumption seems to be the most promising and elegant. For better understanding the role of algae and bacteria in urea metabolism, importance of this compound in freshwater environments and mechanisms responsible for its production, assimilation and decomposition further extensive investigations are needed.

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