

## Microbial production, utilization, and enzymatic degradation of organic matter in the upper trophogenic layer in the pelagial zone of lakes along a eutrophication gradient

Ryszard J. Chróst<sup>1</sup> and Waldemar Siuda

Department of Microbial Ecology, Institute of Microbiology, Warsaw University, Miecznikowa 1, 02-096 Warsaw, Poland

### Abstract

The major aim of this study was to evaluate the relationships between the rates of microbial activities (phytoplankton primary production, bacterial secondary production, bacterial utilization of organic matter, enzymatic activities, protozoan grazing on bacteria), bacterial numbers, and dissolved organic carbon concentrations and the trophic state index (TSI) of lakes in the upper trophogenic water layer in the pelagial zone along the trophic gradient (from oligo/mesotrophy to hypereutrophy) in 19 lakes of the Mazurian Lake District (northeastern Poland). Multiple regression analysis (analysis of variance—ANOVA) on all collected data and the TSI along eutrophication gradient showed that all studied microbial processes and parameters were very tightly coupled to the trophic conditions of the studied lakes. All studied microbial processes involved in utilization and enzymatic degradation of organic matter were strongly positively dependent on the intensity and rates of photosynthetic organic matter production and exudation that markedly increased along the eutrophication gradient of lakes.  $V_{\max}$  of alkaline phosphatase, aminopeptidase, and nonspecific esterase showed significant correlation with the TSI of the studied lakes. Protozoans removed a significant portion of bacterial production, i.e., from ~20% to 75–85% of newly produced bacterial biomass was simultaneously consumed by protozoans along the eutrophication gradient. These observations suggest that the importance of protozoan grazing on bacteria on regulation of bacterial production depends on lake productivity. The general working hypothesis that the intensity of microbial processes of organic matter can be tightly coupled to increasing eutrophication was proven in these studies.

The view of ecophysiology of aquatic microorganisms has changed dramatically in the last two decades. Several sophisticated biochemical and molecular approaches introduced to microbial ecophysiology (Overbeck and Chróst 1990; Kemp et al. 1994; Cooksey 1998) resulted in completely new ideas on the role and significance of microbial processes in the functioning of aquatic ecosystems (Azam and Worden 2004). Recent aquatic prokaryotic metagenome sequencing is providing a tremendous database for discovering metabolic capabilities and new ways to conceptualize and study microbial biodiversity (Venter et al. 2004). Environmental genomics is also revealing complex interactions of bacteria with other aquatic biota and ecosystem function. The results of these studies clearly show that bacteria are a major biological force in nutrient cycles and ecosystem structure.

Nowadays, it is commonly accepted that heterotrophic bacteria play a substantial and dominating role in the cycling of organic and inorganic matter in pelagic waters (Azam and Smith 1991). Microorganisms influence ecosystems through molecular interactions. Because of their high abundance,

biochemical and molecular diversity and dynamics, microorganisms significantly influence the chemistry of aquatic ecosystems. A large fraction of the primary production is not consumed directly by herbivorous consumers but is channeled through detrital organic matter via bacterial production to phagotrophic protozoans. With this concept of the microbial loop (Azam et al. 1983) came the recognition that planktonic food webs are very complex and include more trophic compartments than hitherto believed.

For decades, microbiologists have studied various aspects of microbial interactions with organic matter in natural waters (Fred et al. 1924; Henriki 1940). However, the recognition that detrital organic matter is a key environmental factor that affects energy flow in aquatic ecosystems and nutrient cycling (Wetzel 1995) greatly stimulated studies on organic matter production and its fate in both freshwater and marine systems. Organic matter in general and dissolved organic matter in particular constitute a basis for the metabolism of microheterotrophs, of which various representatives are present at every level of aquatic food webs. Therefore, the interactions between heterotrophic bacteria and organic matter are of utmost importance in the functioning of all aquatic ecosystems.

In the early 1940s the first attempts were made to introduce the importance of organic matter to the lake eutrophication concept. Åberg and Rodhe (1942) stated, “The trophy of a lake indicates the intensity and kind of its supply of organic matter.” Hooper (1969) proposed that a “number of aspects of production and elaboration of organic matter in natural waters may be considered as indices of eutrophication.” Birge and Juday (1927) were one of the first to incorporate bacterial processes into concepts of the features of

<sup>1</sup> Corresponding author (chrost@biol.uw.edu.pl).

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eutrophication and stated that bacteria “stand at the base of the fertility of lakes.” One of the most interesting and ecologically important issues in microbial ecophysiology and microheterotrophic organic matter transformation and utilization is the possible existence of a systematic variability in the role of microbial biomass and metabolic coupling of photoautotrophic and heterotrophic components in planktonic communities along natural gradients of productivity in freshwater and marine systems. Nowadays, it is a well-accepted and broadly known concept that microorganisms are important players in freshwater ecosystem scenarios and driving forces in nutrient cycling, biodegradation, and trophic interactions. Furthermore, eutrophication and its detrimental effects on freshwater quality is currently a very important environmental concern and many mitigation concepts and efforts have been developed and applied to protect freshwater resources and/or reverse and counteract eutrophication effects (Zalewski 2000). Environmental management, however, still needs basic knowledge about structure and function of microorganisms defined in terms of cross-ecosystem properties (Biddanda et al. 2001) and a better understanding of mechanisms and regulating factors of microbial processes along a eutrophication gradient.

In this paper, we report a study on interactions between selected microbial activities and parameters involved in organic matter production, utilization, and degradation in the upper trophogenic water layer in the pelagial zone along a trophic gradient (from oligo/mesotrophy to hypereutrophy) in 19 lakes of the Mazurian Lake District (northeastern Poland). The major aim of these studies was to evaluate the relationships between the rates of microbial activities (phytoplankton primary production, bacterial secondary production, and bacterial utilization of organic matter, enzymatic activities, and protozoan grazing on bacteria), bacterial numbers, and dissolved organic carbon (DOC) concentration and the trophic state index of lakes. We tested the working hypothesis that lake-water eutrophication and microbial production and processing of organic matter are tightly coupled and interdependent in the upper trophogenic water layer in the pelagial zone of the studied lakes.

## Methods

**Studied lakes**—The study was conducted in the pelagial zone of 19 lakes of the Mazurian Lake District (northeastern Poland; Fig. 1). The studied lakes represent the gradient of eutrophication from oligo/mesotrophy to hypereutrophy. They are typical postglacial lakes, mostly surrounded by agriculture areas that were nonintensively used in the two past decades, and forests. Seventeen studied lakes constitute the Chain of the Great Mazurian Lakes, and during the summer season (June–August), they are intensively used for recreation. The value of those lakes selected for this study is that oligo/mesotrophic and hypereutrophic lakes are located within small distances under similar geological conditions. Moreover, the studied lakes have a similarity in input of allochthonous organic matter because they share a watershed and comparable agricultural, wetland, and urban areas. Basic morphological parameters and general characteristics of the

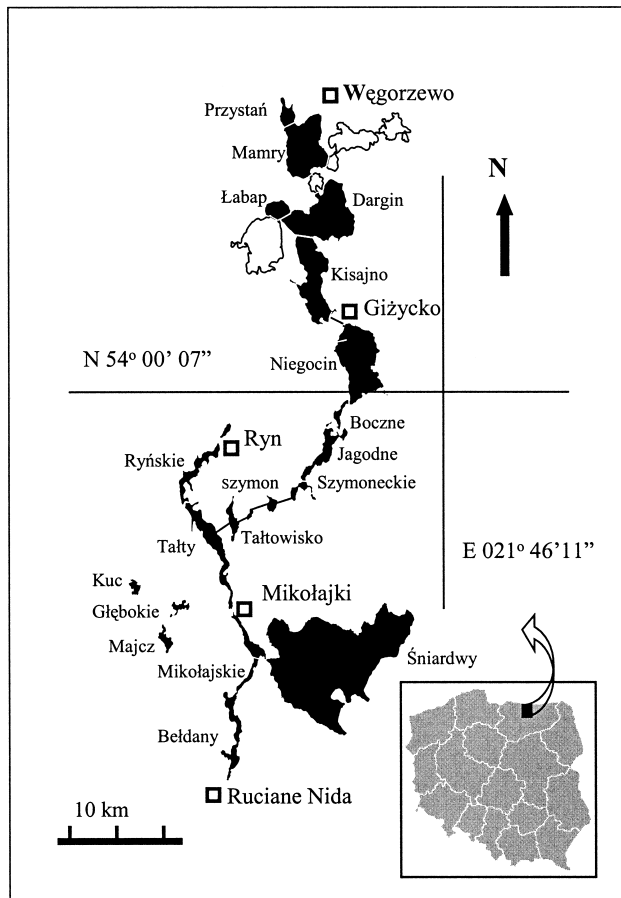


Fig. 1. The studied lakes of the Mazurian Lake District (northeastern Poland).

surrounding areas of the studied lakes are presented in Table 1.

**Sampling**—Samples were collected between April (spring homothermy) and July–August (summer thermal stratification) in 1994–1995, 1997–2000, and 2002. Integrated 1-liter samples were collected (at 0.5-m-depth interval) in the pelagial zone at the deepest site with a 4-liter (50 cm length) sampler from the upper trophogenic water layer corresponding to a maximum depth of Secchi disk at 10 sites situated in the pelagial zone along a transect in each lake. Samples taken from each sampling depth were mixed (vol/vol) together and the integrated sample was treated as representative for the studied lake. Water samples, in polyethylene 10-liter containers, were transported to the laboratory within no longer than 4 h.

**Primary production**—Phytoplankton primary production and excreted organic carbon (EOC) during photosynthesis were determined by the  $^{14}\text{C}$ -bicarbonate method (Chróst 1983) in 100-ml triplicate light- and dark-glass bottles. Samples, supplemented with  $3.7 \text{ kBq ml}^{-1} [^{14}\text{C}]\text{NaHCO}_3$  (specific activity  $1.67 \text{ GBq mmol}^{-1}$ , New England Nuclear), were incubated for 4 h in situ at  $\frac{1}{2}$  depth of Secchi disk

Table 1. Basic morphological parameters, average concentration of chlorophyll *a* and total phosphorus in the upper trophogenic water layer, and Secchi disk visibility in spring and summer during the study period, and the trophic conditions of the studied lakes.

Lake	Surface area (ha)	Depth (m)		Chlorophyll <i>a</i> ( $\mu\text{g L}^{-1}$ )		Total phosphorus ( $\mu\text{mol P-PO}_4^{3-} \text{L}^{-1}$ )		Secchi disk (m)		Trophic conditions
		Average	Maximum	Spring	Summer	Spring	Summer	Spring	Summer	
Kuc	99	8.0	28.8	2.6±0.6	3.3±1.4	0.4±0.1	0.3±0.1	5.5±0.5	4.5±0.2	Oligo/mesotrophy
Mamry	2,504	11.7	43.8	2.4±0.6	3.6±0.7	0.4±0.2	0.3±0.2	4.2±0.4	3.3±0.4	Oligo/mesotrophy
Przystań	115	13.4	22.8	3.1±1.1	3.3±1.7	0.5±0.1	0.4±0.1	4.1±0.2	3.8±0.3	Oligo/mesotrophy
Majcz	45	3.2	16.5	5.8±2.6	5.1±2.4	0.4±0.1	0.4±0.1	4.4±0.6	4.2±0.3	Mesotrophy
Łabap	350	8.5	13.4	10.1±1.2	9.6±1.8	0.9±0.2	0.9±0.3	3.2±0.3	2.9±0.3	Mesotrophy
Dargin	2,680	10.6	37.6	11.6±3.1	10.9±2.8	1.0±0.2	0.9±0.2	3.1±0.2	3.3±0.2	Meso/eutrophy
Śniardwy	11,340	5.8	23.4	14.4±2.8	12.3±2.7	1.3±0.3	1.1±0.2	2.1±0.3	2.2±0.2	Meso/eutrophy
Kisajno	1,896	8.4	25.0	16.2±2.4	14.1±3.3	1.0±0.3	0.9±0.2	2.6±0.5	2.7±0.3	Meso/eutrophy
Głębokie	47	15.0	34.0	15.5±3.7	14.6±5.8	2.3±0.4	1.2±0.5	2.5±0.5	2.4±0.3	Eutrophy
Niegocin	2,600	9.9	39.7	23.5±3.6	22.1±4.5	3.1±0.3	1.8±0.5	2.1±0.2	2.0±0.2	Eutrophy
Boczne	183	8.4	25.0	23.4±4.1	22.3±6.1	3.2±0.3	1.9±0.3	2.2±0.3	2.2±0.2	Eutrophy
Betdany	941	10.0	46.0	23.8±3.4	24.7±3.9	3.1±0.3	1.8±0.2	1.5±0.5	1.3±0.4	Eutrophy
Tały	1,160	13.5	44.7	23.6±3.4	25.7±2.3	3.5±0.4	1.9±0.3	1.9±0.5	1.8±0.3	Eutrophy
Ryńskie	671	13.5	50.8	34.1±5.0	31.2±4.1	3.5±0.5	2.1±0.2	1.5±0.4	1.6±0.3	Eutrophy
Mikołajskie	498	11.2	25.9	35.2±2.5	33.1±3.6	3.2±0.4	1.9±0.4	1.3±0.5	1.4±0.6	Eutrophy
Jagodne	420	8.7	37.4	51.1±6.5	47.2±5.7	3.7±0.3	2.2±0.4	1.2±0.5	1.2±0.2	Eutrophy
Szymoneckie	523	8.7	28.5	67.8±9.0	61.5±7.3	4.4±0.2	2.8±0.3	0.9±0.3	1.0±0.2	Eutrophy
Szymon	154	1.1	2.9	89.7±5.6	84.2±8.1	4.1±0.5	2.6±0.3	0.5±0.2	0.6±0.2	Hypereutrophy
Tałowisko	327	14.0	39.5	90.8±8.1	87.3±7.2	4.2±0.5	2.8±0.4	0.4±0.1	0.5±0.3	Hypereutrophy

visibility. Samples were fixed with buffered formalin (4% final concentration), and triplicate 20-ml aliquots from each bottle were filtered through 0.2- $\mu\text{m}$  pore size cellulose acetate membrane filters (25-mm diameter; Sartorius). Ten milliliters of the filtrate from each sample were acidified with 0.1 mol  $\text{L}^{-1}$  HCl to pH 2.0, bubbled with air for 10 min, and used for estimation of EOC. The radioactivity of  $^{14}\text{C}$ -EOC was determined in 5-ml aliquots. Concentration of inorganic carbon available for phytoplankton photosynthesis in lake water samples was determined by infrared spectrophotometry in a carbon analyzer (TOC 5500; Shimadzu).

**Bacterial utilization of  $^{14}\text{C}$ -EOC**—In situ measurements of bacterial utilization of EOC were performed simultaneously with primary production. Water samples (100 ml) were prepared by the same procedure as for productivity studies. Phytoplankton  $^{14}\text{C}$ -EOC release and bacterial utilization of released  $^{14}\text{C}$ -organic substrates in parallel water samples, with and without 30  $\mu\text{g L}^{-1}$  gentamicin, were determined according to Chróst (1984). Gentamicin was used to prevent bacterial utilization of released  $^{14}\text{C}$ -EOC in control samples. Samples were fixed with formalin and processed as described above for EOC determination. Bacterial utilization of EOC was estimated as the difference between EOC concentration in control samples and in antibiotic non-treated samples. Specific activity of  $^{14}\text{C}$ -EOC was determined from the ratio of concentrations of  $^{14}\text{C}$ -labeled compounds in EOC to  $^{12}\text{C}$ -DOC pools in water samples.

**Bacterial production**—Bacterial secondary production ( $^3\text{H}$ -methyl-thymidine method) was measured as described by Chróst and Rai (1994). Triplicate (10-ml) samples of water were dispensed to a series of polystyrene tubes and supplemented with [ $^3\text{H}$ -methyl]thymidine (specific activity 3.52

TBq  $\text{mmol}^{-1}$ ; New England Nuclear) to a final concentration in assays of 15–20  $\text{nmol L}^{-1}$ . Samples were incubated at in situ temperature for 1 h, fixed with formalin (4% final concentration), and cold ( $0^\circ\text{C}$ ) 100% trichloroacetic acid (TCA) was added to a final concentration of 10%. After 20–30 min of TCA precipitation at  $0$ – $1^\circ\text{C}$ , the TCA-precipitate was collected on 0.2- $\mu\text{m}$  pore-size cellulose nitrate membrane filters (25-mm diameter; Sartorius), rinsed three times with 5 ml 5% cold TCA, and finally washed with 5 ml 96% cold ethanol. Filters were placed in scintillation vials and dissolved with 5 ml scintillation cocktail, and their radioactivity was determined after 24 h. A blank of abiotic adsorption of a radioisotope was prepared in duplicate (10-ml) water samples fixed with formalin for 15 min prior to  $^3\text{H}$ -thymidine addition and then incubated and treated in the same way as studied samples. The amount of  $^3\text{H}$ -thymidine incorporated into bacterial DNA was converted to bacterial cell production using the conversion factor  $1.24 \times 10^6$  cells  $\text{pmol}^{-1}$  (Chróst and Rai 1994). Bacterial cell production was transformed to bacterial organic carbon production applying the conversion factor 19.8 fg C cell $^{-1}$  (Lee and Fuhrman 1987).

**Bacterial growth efficiency**—Bacterial growth efficiency (BGE) in April and July 1999 was determined by means of utilization of DOC naturally present in lake-water samples and in lake water supplemented with  $^{14}\text{C}$ -labeled photosynthetic dissolved organic carbon ( $^{14}\text{C}$ -PhOC) produced by phytoplankton (Siuda et al. 1991). Bacterial metabolism of organic carbon was determined from the decrease in  $^{14}\text{C}$ -PhOC and C-DOC concentrations (total utilization of organic carbon) and parallel increase in  $^{14}\text{C}$ - $\text{CO}_2$  and C- $\text{CO}_2$  content (respiration) in 250-ml water samples prefiltered through 0.8- $\mu\text{m}$  polycarbonate membrane filters (Nucleopore), and incubated at in situ temperature for 12–24 and 48–96 h, re-

Table 2. Enzymes, substrates, and their fluorescent products of hydrolysis.

Enzyme	Substrate	Product	Excitation (nm)	Emission (nm)
Leucine aminopeptidase (AMP)	L-Leucine-4-methyl-coumarinylamide hydrochloride (Leu-AMC)	7-Amino-4-methyl-coumarin	380	440
Alkaline phosphatase (APA)	4-Methylumbelliferyl phosphate (MUF-P)	4-Methylumbelliferone	365	460
Esterase (ESA)	Fluorescein diacetate (FL-DA)	Fluorescein	489	510

spectively. Bacterial utilization of  $^{14}\text{C}$ -PhOC was determined according to Siuda et al. (1991). Amount of respired  $^{14}\text{C}$ - $\text{CO}_2$  from  $^{14}\text{C}$ -PhOC metabolism, trapped in ethanolamine, was assayed by radiometry according to Sorokin and Kadota (1972). Concentrations of C-DOC (in 0.2  $\mu\text{m}$  filtrate) and C- $\text{CO}_2$  in water samples were measured using a Shimadzu TOC 5050 carbon analyzer (see below). The amount of  $\text{CO}_2$  released to water samples during respiration was corrected for simultaneous carbon dioxide uptake (chemolithotrophic and/or heterotrophic dark uptake). Chemolithotrophic  $\text{CO}_2$  fixation (mainly nitrification) in the studied water samples was inhibited by allylthiourea and/or nitrapyrin (Hall 1984). Heterotrophic dark uptake of  $\text{CO}_2$  by bacteria was determined by  $^{14}\text{C}$ - $\text{CO}_2$  method, according to Sorokin and Kadota (1972). BGE was calculated as follows:

$$\text{BGE} = \frac{[\Delta\text{OC} - (\Delta\text{CO}_2 + C_{\text{du}})]}{\Delta\text{OC}} \times 100\%$$

where  $\Delta\text{OC}$  = total utilization of C-DOC or  $^{14}\text{C}$ -PhOC, i.e., difference in organic carbon concentration between time zero and after 48–96 and 12–24 h of incubation, respectively;  $\Delta\text{CO}_2$  = respiration, i.e., increase in C- $\text{CO}_2$  or  $^{14}\text{C}$ - $\text{CO}_2$  concentration in water samples during incubation;  $C_{\text{du}}$  = C- $\text{CO}_2$  dark uptake.

**Enzyme assays**—Microbial enzyme activities in lake water samples were determined by fluorometry (Shimadzu RF 1501) with the use of artificial fluorogenic substrates as previously described (Chróst and Overbeck 1987; Hoppe 1993). Leucine aminopeptidase (AMP), alkaline phosphatase (APA) and non-specific esterase (ESA) activities were measured as an increase in fluorescence as the nonfluorescent substrates were hydrolyzed to the highly fluorescent products (Table 2). Stock solutions of the substrates 4-methylumbelliferyl phosphate (MUF-P), L-leucine-4-methyl-coumarinylamide hydrochloride (Leu-AMC), and fluorescein diacetate (FL-DA) were prepared to a concentration of 5  $\text{mmol L}^{-1}$  in deionized water, 96% ethanol and acetone, respectively, and stored at  $-25^\circ\text{C}$ . The stock substrate solutions were diluted with appropriate solvents to 0.05, 0.10, 0.25, 0.50, and 1.0  $\text{mmol L}^{-1}$  immediately before assay. For all enzyme assays, 0.5 ml of substrate solutions were added to 4.5-ml triplicate water samples, yielding final substrate concentrations in assays of 5, 10, 25, 50, and 100  $\mu\text{mol L}^{-1}$ . Fluorescence of the samples was measured in a spectrofluorometer (Shimadzu RF 1501) at time zero, immediately after addition of substrates (background fluorescence) and after incubation in the dark at  $20^\circ\text{C}$  (15–90 min, depending on substrate concentration and enzyme activity).

**Enzyme kinetic data analysis**—Varying amounts of substrates used in enzyme activity assays enabled the calculation of the kinetic parameters of studied enzymes. The enzymatic reactions followed Michaelis–Menten kinetics, and the plot of the initial velocity of reaction ( $v$ ) against increased concentrations of substrate ( $[S]$ ) gave a rectangular hyperbola,

$$v = \frac{(V_{\text{max}} \times [S])}{(K_m + [S])}$$

The parameters characterizing this equation were calculated from the experimental data. They were  $V_{\text{max}}$ , the maximum velocity of enzyme reaction, which is theoretically attained when the enzyme has been saturated by an infinite concentration of substrate  $[S]$ ; and  $K_m$ , the apparent Michaelis constant, which is numerically equal to the concentration of substrate for the half-maximal velocity ( $\frac{1}{2} V_{\text{max}}$ ). The kinetic parameters were calculated from the direct plot of reaction velocity ( $v$ ) versus substrate  $[S]$  concentration using the non-linear regression computer program Enzfitter (Elsevier-Bio-soft) to determine the best fit of the rectangular hyperbola.

**Protozoan grazing**—Protozoan grazing on bacteria was measured by means of  $^3\text{H}$ -thymidine-labeled, natural assemblages of lake bacteria, according to Koton-Czarnecka and Chróst (2002). Determination of protozoan grazing on bacteria involves the measurement of radioactivity transfer from  $^3\text{H}$ -labeled bacterial size fraction (0.2–1.0  $\mu\text{m}$ ) to protozoan size fraction ( $>1.0 \mu\text{m}$ ). Lake water sample (100 ml) was filtered ( $\text{Hg} < 50 \text{ mm}$ ) through 1.0- $\mu\text{m}$  pore-size polycarbonate membrane filters (47-mm diameter; Poretics) to reduce the amount of bacterial grazers, then supplemented with  $\sim 10 \text{ nmol L}^{-1}$  (final concentration) [ $^3\text{H}$ -methyl]thymidine (specific activity 3.52 TBq  $\text{mmol}^{-1}$ ; New England Nuclear), and incubated for 20–24 h at in situ temperature in the dark.  $^3\text{H}$ -DNA-labeled bacteria were harvested on 0.2- $\mu\text{m}$  pore-size polycarbonate filters (47-mm diameter; Poretics), and rinsed five times with 15-ml portions of filter-sterilized (0.2- $\mu\text{m}$ ) water from the studied lake to wash out radioactivity not bound in bacterial cells. The filters holding labeled bacteria were gently sonicated for 10 min in 10 ml of the filter-sterilized (0.2- $\mu\text{m}$ ) lake water to release bacteria from the filter surface and to resuspend them in water. The concentrated suspension of radiolabeled bacteria was filtered through 1- $\mu\text{m}$  pore size (47-mm diameter; Poretics) polycarbonate membrane filters to remove larger bacterial cells and clumps of bacteria that may have been formed during the radiolabeling procedure. A prepared suspension of a known number of labeled bacteria and their corresponding radioactivity was added to lake-water sample (100 ml) pre-

filtered through 10- $\mu\text{m}$  pore-size polycarbonate membrane filter (47-mm diameter; Poretics) to remove large protozoans, zooplankton, and other organisms and particles. Water samples contained a mixture of known numbers of natural and radiolabeled bacteria and small (less than 10  $\mu\text{m}$  in size) protozoans. Protozoan grazing on natural and labeled bacteria proceeded for 1 h of incubation, and then samples were fixed with buffered formalin (2% final concentration). Triplicate 20-ml subsamples were filtered through 1.0- $\mu\text{m}$  pore-size polycarbonate membrane filters (25-mm diameter; Poretics), i.e., the protozoan size fraction was harvested on the surface of filters. Controls were prepared in separate samples fixed with formalin before the addition of radioactive bacterial suspension. Radioactivity was determined by liquid scintillation counting.

**Radioactivity determination**—Radioactivity of samples (filters and filtrates) were assayed by liquid scintillation counting (Wallac DSA 1400) using the external standard channel ratio method. Filters and liquid samples were assayed in high water-capacity scintillation cocktail (Rotiszint 2211).

**Bacterial number**—Epifluorescence microscopy was used to determine total bacterial cell numbers and number of bacteria containing visible nucleoid (NucVB). Buffered formalin preserved (2% final concentration), and triplicate 4,6-diamidino-2-phenylindole (DAPI)-stained samples were counted for bacterial abundance (Porter and Feig 1980). Bacteria were chosen from 5–10 randomly selected fields of each microscope slide and 250–300 cells per slide were counted. The determination of the NucVB fraction was based on a staining/destaining DAPI procedure (Zweifel and Hagström 1995). DAPI-stained bacteria on filters were washed three times with 2 ml hot (70°C) 2-propanol for 3 min before they were mounted onto microscopic slides. Black, polycarbonate membrane filters, 0.2- $\mu\text{m}$  pore size (25-mm diameter; Poretics) were used for bacterial counts.

**Chemical analyses**—Chlorophyll *a* (corrected for phaeopigments), extracted with 96% ethanol, and total phosphorus concentrations were measured by spectrophotometry (Shimadzu UV/VIS 1200) according to Nusch (1980) and Koroleff (1976), respectively. Dissolved organic carbon, in lake-water samples filtered through 0.2- $\mu\text{m}$  pore-size polycarbonate membrane filters (Poretics), and  $\text{CO}_2$  concentrations were determined using a Shimadzu TOC 5050 carbon analyzer. Samples for DOC (nonpurgable organic carbon, NPOC) determination were HCl acidified ( $\text{pH} \leq 2.0$ ) and blown with oxygen for 10 min to purge  $\text{CO}_2$ . DOC and  $\text{CO}_2$  concentrations were determined with detection limit and accuracy of  $\pm 50 \mu\text{g C L}^{-1}$ , coefficient of variation  $\leq 1\%$ .

**Trophic state index**—Trophic state index (TSI) for every studied lake at each sampling time was calculated from chlorophyll *a*, total phosphorus concentrations, and Secchi depth water transparency according to equations by Carlson (1977).

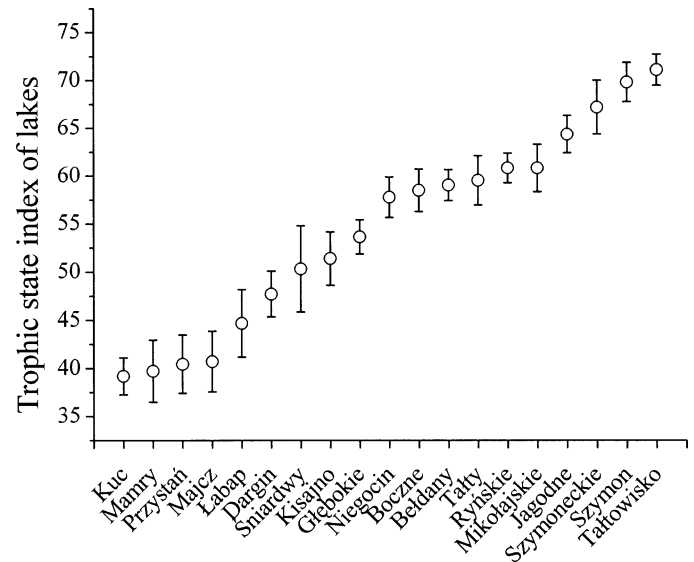


Fig. 2. Mean values of Carlson's TSI for the studied lakes determined from chlorophyll *a* and total phosphorus concentrations in the upper trophogenic water layer. Data are the mean values, and  $\pm$  standard deviations calculated from all estimates during the study period.

**Data analysis**—Data were statistically analyzed using computer software Origin v. 6.1 (Origin Lab.). One-way ANOVA and *t*-test were used to analyze similarity between the data. Linear and multiple regression models were applied to study the relationships between experimental data.

## Results

**TSI for the studied lakes**—Based on each data sets, Secchi disk, chlorophyll *a*, and total phosphorus concentration, collected during the study period, we calculated Carlson's TSI for the studied lakes:  $\text{TSI}_{\text{SD}}$ ,  $\text{TSI}_{\text{CHL}}$ ,  $\text{TSI}_{\text{PT}}$ , respectively. One-way ANOVA and *t*-test applied for statistical analyses showed that all TSI values, i.e.,  $\text{TSI}_{\text{SD}}$ ,  $\text{TSI}_{\text{CHL}}$ ,  $\text{TSI}_{\text{PT}}$ , calculated for every studied lake were not significantly different ( $p < 0.05$ ). However, the values of the TSI calculated on the basis of the Secchi depth water transparency were always lower than  $\text{TSI}_{\text{CHL}}$  and/or  $\text{TSI}_{\text{PT}}$ . Highest linear correlation among  $\text{TSI}_{\text{SD}}$ ,  $\text{TSI}_{\text{CHL}}$ , and  $\text{TSI}_{\text{PT}}$  values in all studied lakes was always found between  $\text{TSI}_{\text{CHL}}$  and  $\text{TSI}_{\text{PT}}$  ( $r^2 = 0.91$ ,  $p < 0.0001$ ). Therefore, to characterize the trophic conditions of the studied lakes, we decided to use the mean TSI value obtained from  $\text{TSI}_{\text{CHL}}$  and  $\text{TSI}_{\text{PT}}$  for a particular lake.

Figure 2 shows mean values of the TSI for the analyzed lakes during the study period. Based on TSI values, the eutrophication gradient of the studied lakes was composed of four groups of lakes with increasing trophic conditions. The four oligo/mesotrophic lakes displayed TSI values from  $39.2 \pm 1.9$  (Lake Kuc) to  $40.7 \pm 3.1$  (Lake Majcz). TSI of four transient lakes, from mesotrophy to low eutrophy, varied between  $44.7 \pm 3.5$  (Lake Łabap) and  $53.6 \pm 1.8$  (Lake Głębokie). The group of moderately eutrophicated lakes was composed of seven lakes with varying TSI between  $57.7 \pm 2.1$  (Lake Niegocin) and  $60.8 \pm 2.5$  (Lake Mikołajskie).

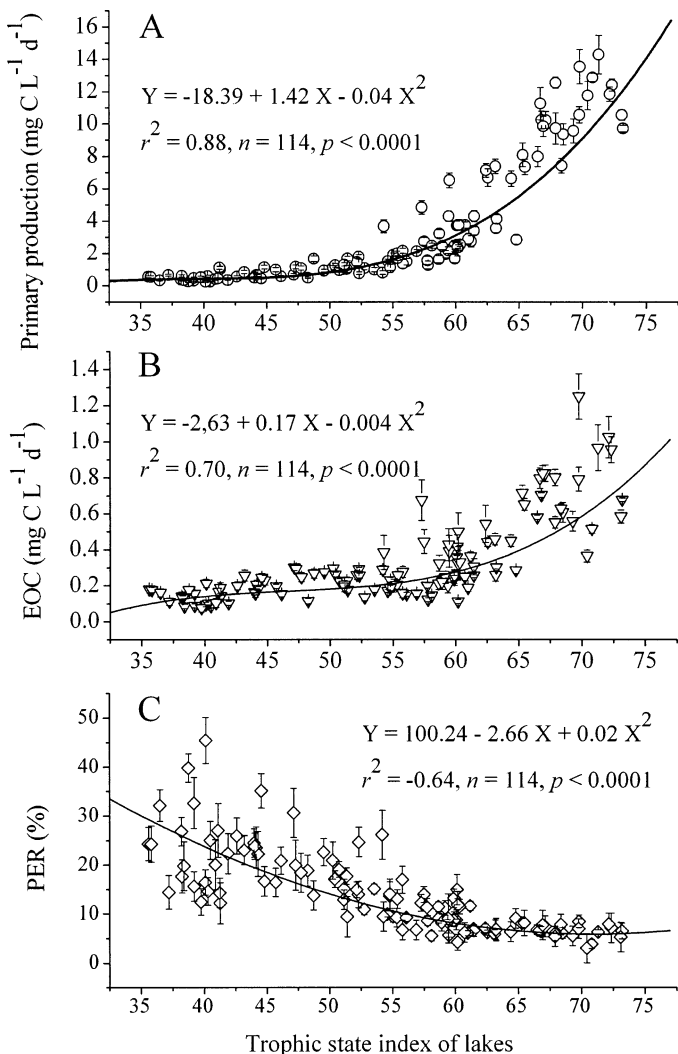


Fig. 3. (A) Rates ( $\pm$ SD) of phytoplankton primary production, (B) excreted organic carbon (EOC) by phytoplankton, and (C) the percentage of extracellular release (PER) of organic carbon in the upper trophogenic water layer in the pelagic water samples along the TSI of the studied lakes  $\pm$  standard deviation of triplicate measurements.

Four highly eutrophic lakes were characterized by TSI values from  $64.4 \pm 1.9$  (Lake Jagodne) to  $71.1 \pm 1.6$  (Lake Tałtowisko).

**Phytoplankton primary production and excreted organic carbon**—Phytoplankton primary production (PP) varied markedly along the trophic gradient of the studied lakes (Fig. 3A). Lowest rates of primary production (from  $0.255 \pm 0.014$  to  $0.650 \pm 0.078$   $\text{mg C L}^{-1} \text{d}^{-1}$ ) were determined in four lakes (Kuc, Mamry, Majecz, and Przystań) that were characterized by lowest TSI values (between  $36.3 \pm 3.5$  and  $46.1 \pm 0.4$ ). In other studied lakes, the rates of PP were exponentially proportional to their TSI and reached maximum rates  $13.755 \pm 1.872$  and  $14.285 \pm 1.056$   $\text{mg C L}^{-1} \text{d}^{-1}$  in hypereutrophic lakes Tałtowisko and Szymon (TSI between  $71.3 \pm 3.3$  and  $73.4 \pm 3.2$ ), respectively. Primary production of phytoplankton significantly correlated with

TSI in lakes (ANOVA,  $r^2 = 0.88$ ,  $n = 114$ ,  $p < 0.0001$ ). We found that, in moderately eutrophic lakes (TSI values from  $53.7 \pm 1.7$  to  $60.8 \pm 2.5$ ), the rates of primary production of phytoplankton were markedly higher in the summer than in the spring ( $t$ -test,  $n = 67$ ,  $p < 0.001$ ).

Phytoplankton communities in the studied lakes excreted into lake water various amounts of organic carbon products (EOC) that were produced in the course of photosynthesis (Fig. 3B). There was a statistically significant exponential increase in the rate of EOC with increasing eutrophication (ANOVA,  $r^2 = 0.70$ ,  $n = 114$ ,  $p < 0.0001$ ). There was also a negative relationship between percentage of primary production (PER) that was excreted as EOC and the TSI of the studied lakes (ANOVA,  $r^2 = -0.64$ ,  $n = 114$ ,  $p < 0.0001$ ), Fig. 3C. In oligo/mesotrophic lakes, EOC constituted from  $14.2\% \pm 1.5\%$  (Lake Przystań) to  $45.4\% \pm 5.5\%$  (Lake Kuc). The percentage of extracellular release of EOC decreased with increasing eutrophication conditions of the studied lakes. In highly eutrophicated lakes (TSI from  $69.7 \pm 1.9$  to  $73.2 \pm 3.2$ ), EOC constituted from  $3.5\% \pm 1.1\%$  to  $13.1\% \pm 2.0\%$  of the total primary production in lakes Szymon and Tałtowisko, respectively.

**Utilization of phytoplankton-excreted organic carbon**—Heterotrophic bacteria utilized a significant portion of phytoplankton-excreted organic carbon in the studied lakes (Table 3). Total EOC utilization rates were positively proportional to increasing eutrophication of lakes. Rates of EOC utilization varied from  $4.5 \pm 0.4$   $\mu\text{g C L}^{-1} \text{h}^{-1}$  (Lake Kuc) to  $11.1 \pm 1.4$   $\mu\text{g C L}^{-1} \text{h}^{-1}$  (Lake Szymon). Part of the utilized EOC was respired by bacteria. Respiration rates followed the utilization rates of EOC and the TSI in the studied lakes. Rates of EOC respiration fluctuated between  $2.5 \pm 0.2$  and  $7.2 \pm 0.5$   $\mu\text{g C L}^{-1} \text{h}^{-1}$  in lakes Kuc and Szymon, respectively.

**Bacterial growth efficiency**—We calculated bacterial growth efficiency (BGE) based on  $^{14}\text{C}$ -PhOC and DOC utilization and respiration by bacteria in the studied lakes (Table 4). Mean BGE values determined by two independent methods were not statistically different (one-way ANOVA,  $p < 0.05$ ) in all studied lakes in April and July 1999. BGE in all studied lakes varied from  $32.0\% \pm 9.8\%$  to  $48.0\% \pm 6.8\%$  and between  $26.0\% \pm 8.5\%$  and  $50.0\% \pm 2.2\%$  in April and July, respectively. There was no clear relationship between BGE and the TSI of the studied lakes.

**Bacterial secondary production and bacterial organic carbon demand**—Mean rates of bacterial secondary production (BP) were not significantly different (one-way ANOVA,  $p < 0.05$ ) between spring and summer in all studied lakes. Both in the spring and summer, lowest rates of BP (between  $46.9 \pm 4.6$  and  $155.2 \pm 11.5$   $\mu\text{g C L}^{-1} \text{d}^{-1}$ ) were observed in four low-TSI lakes: Kuc, Mamry, Majecz and Przystań. The rates of BP grew exponentially within increased eutrophication gradient of the studied lakes (Fig. 4A). In high-TSI lakes (TSI  $> 70$ ), BP rates varied from  $328.2 \pm 20.9$   $\mu\text{g C L}^{-1} \text{d}^{-1}$  (Lake Tałtowisko) to  $690.7 \pm 16.1$   $\mu\text{g C L}^{-1} \text{d}^{-1}$  (Lake Szymon).

Assuming that the mean BGE was, on average, 37% (Ta-

Table 3. Rates of phytoplankton excretion of organic carbon (EOC) and their bacterial utilization and respiration in the upper trophogenic water layer in the studied lakes during spring homothermy (Apr) and summer stratification (Jul) periods in 1999. Estimates are based on  $^{14}\text{C}$ -method (Chróst 1984),  $\pm$  standard deviation of triplicates.

Lake	Apr ( $\mu\text{g C L}^{-1} \text{ h}^{-1}$ )			Jul ( $\mu\text{g C L}^{-1} \text{ h}^{-1}$ )		
	EOC	Utilization	Respiration	EOC	Utilization	Respiration
Kuc	6.9 $\pm$ 0.4	4.5 $\pm$ 0.4	2.5 $\pm$ 0.2	8.1 $\pm$ 0.9	5.1 $\pm$ 0.5	3.8 $\pm$ 0.3
Mamry	8.0 $\pm$ 0.6	5.3 $\pm$ 0.8	2.8 $\pm$ 0.3	6.9 $\pm$ 0.7	5.2 $\pm$ 0.7	3.4 $\pm$ 0.3
Przystań	6.3 $\pm$ 0.4	4.3 $\pm$ 0.3	2.7 $\pm$ 0.3	6.8 $\pm$ 0.7	5.3 $\pm$ 0.5	3.8 $\pm$ 0.3
Majcz	7.7 $\pm$ 0.6	5.8 $\pm$ 0.7	3.7 $\pm$ 0.3	5.9 $\pm$ 0.6	5.4 $\pm$ 0.4	3.0 $\pm$ 0.2
Łabap	10.8 $\pm$ 0.8	7.1 $\pm$ 1.1	4.6 $\pm$ 0.2	12.1 $\pm$ 0.9	7.8 $\pm$ 1.1	4.3 $\pm$ 0.2
Dargin	8.6 $\pm$ 0.8	6.8 $\pm$ 0.6	3.8 $\pm$ 0.3	11.1 $\pm$ 1.1	8.1 $\pm$ 1.2	4.1 $\pm$ 0.3
Śniardwy	9.5 $\pm$ 0.9	7.0 $\pm$ 0.9	4.5 $\pm$ 0.2	8.9 $\pm$ 1.1	7.9 $\pm$ 0.8	4.8 $\pm$ 0.3
Kisajno	10.1 $\pm$ 1.0	8.3 $\pm$ 0.9	5.4 $\pm$ 0.5	12.3 $\pm$ 1.0	8.2 $\pm$ 0.6	5.7 $\pm$ 0.4
Głębokie	8.5 $\pm$ 0.9	6.3 $\pm$ 0.5	3.7 $\pm$ 0.2	9.8 $\pm$ 1.2	8.7 $\pm$ 0.9	5.9 $\pm$ 0.3
Niegocin	8.3 $\pm$ 0.4	6.1 $\pm$ 1.0	3.3 $\pm$ 0.4	6.9 $\pm$ 0.5	5.6 $\pm$ 0.2	2.8 $\pm$ 0.3
Boczne	8.0 $\pm$ 0.6	5.8 $\pm$ 1.0	3.8 $\pm$ 0.3	9.5 $\pm$ 0.9	8.1 $\pm$ 0.5	5.2 $\pm$ 0.4
Bełdany	11.1 $\pm$ 0.7	8.3 $\pm$ 1.2	5.3 $\pm$ 0.4	9.2 $\pm$ 0.9	8.2 $\pm$ 1.0	5.5 $\pm$ 0.4
Tały	9.8 $\pm$ 0.9	7.2 $\pm$ 0.6	4.9 $\pm$ 0.4	10.8 $\pm$ 0.9	8.3 $\pm$ 1.1	5.6 $\pm$ 0.3
Ryńskie	11.8 $\pm$ 1.2	8.7 $\pm$ 1.1	5.2 $\pm$ 0.4	7.7 $\pm$ 0.7	6.8 $\pm$ 0.5	3.8 $\pm$ 0.3
Mikołajskie	10.9 $\pm$ 1.1	7.6 $\pm$ 0.8	4.0 $\pm$ 0.3	5.4 $\pm$ 0.7	5.2 $\pm$ 0.5	3.4 $\pm$ 0.4
Jagodne	10.6 $\pm$ 1.8	7.8 $\pm$ 0.6	4.3 $\pm$ 0.4	10.5 $\pm$ 1.2	8.8 $\pm$ 0.8	5.1 $\pm$ 0.5
Szymoneckie	15.0 $\pm$ 1.9	10.2 $\pm$ 1.3	6.3 $\pm$ 1.2	10.5 $\pm$ 1.4	8.4 $\pm$ 0.9	6.8 $\pm$ 0.5
Szymon	11.0 $\pm$ 1.7	8.1 $\pm$ 0.7	4.9 $\pm$ 0.4	15.3 $\pm$ 1.2	11.1 $\pm$ 1.4	7.2 $\pm$ 0.5
Tałowisko	10.6 $\pm$ 1.1	8.1 $\pm$ 1.2	5.1 $\pm$ 0.3	10.5 $\pm$ 1.0	8.1 $\pm$ 0.7	5.8 $\pm$ 0.3

Table 4. Percentage of bacterial growth efficiency on phytoplankton photosynthetic organic carbon ( $^{14}\text{C}$ -PhOC) and dissolved organic carbon (DOC) in the upper trophogenic water layer in the studied lakes during spring homothermy (Apr) and summer stratification (Jul) periods in 1999. Mean values and  $\pm$  standard deviation of triplicate measurements, n.d., no data.

Lake	$^{14}\text{C}$ -PhOC		DOC	
	Apr	Jul	Apr	Jul
Kuc	45.0 $\pm$ 8.1	26.0 $\pm$ 8.5	39.7 $\pm$ 6.2	n.d.
Mamry	48.0 $\pm$ 6.8	35.0 $\pm$ 9.4	41.9 $\pm$ 5.2	29.8 $\pm$ 3.5
Majcz	36.9 $\pm$ 6.8	29.7 $\pm$ 7.4	34.8 $\pm$ 2.2	30.1 $\pm$ 2.3
Przystań	37.1 $\pm$ 5.9	44.0 $\pm$ 5.2	36.8 $\pm$ 2.5	41.9 $\pm$ 4.8
Łabap	35.0 $\pm$ 2.1	44.7 $\pm$ 6.2	34.2 $\pm$ 2.5	42.1 $\pm$ 1.8
Dargin	44.9 $\pm$ 6.1	49.1 $\pm$ 3.2	45.0 $\pm$ 1.1	44.2 $\pm$ 6.1
Kisajno	35.1 $\pm$ 2.3	30.3 $\pm$ 9.3	28.9 $\pm$ 6.2	n.d.
Głębokie	42.0 $\pm$ 9.8	31.9 $\pm$ 6.7	41.0 $\pm$ 1.5	32.3 $\pm$ 2.2
Śniardwy	35.1 $\pm$ 8.8	39.4 $\pm$ 5.0	37.2 $\pm$ 0.5	37.3 $\pm$ 1.8
Niegocin	46.0 $\pm$ 6.9	50.0 $\pm$ 2.2	42.4 $\pm$ 3.8	45.7 $\pm$ 3.5
Boczne	34.0 $\pm$ 9.4	36.2 $\pm$ 3.1	35.5 $\pm$ 4.1	32.3 $\pm$ 4.1
Bełdany	36.0 $\pm$ 9.2	32.3 $\pm$ 3.5	34.7 $\pm$ 2.5	33.8 $\pm$ 2.9
Tały	32.0 $\pm$ 9.8	32.9 $\pm$ 4.1	34.6 $\pm$ 1.2	37.1 $\pm$ 0.4
Tałowisko	37.0 $\pm$ 9.5	28.9 $\pm$ 7.6	35.7 $\pm$ 2.8	31.2 $\pm$ 3.8
Ryńskie	40.1 $\pm$ 7.7	45.2 $\pm$ 0.6	n.d.	44.1 $\pm$ 0.8
Mikołajskie	47.0 $\pm$ 5.4	34.9 $\pm$ 8.7	44.7 $\pm$ 1.6	31.8 $\pm$ 3.9
Jagodne	45.0 $\pm$ 9.9	41.1 $\pm$ 3.0	44.1 $\pm$ 6.2	40.2 $\pm$ 2.7
Szymoneckie	38.1 $\pm$ 9.8	18.9 $\pm$ 6.8	36.1 $\pm$ 1.1	20.2 $\pm$ 3.2
Szymon	40.0 $\pm$ 9.9	34.8 $\pm$ 6.3	38.3 $\pm$ 5.1	33.2 $\pm$ 2.2
Average	39.7 $\pm$ 5.0	36.1 $\pm$ 8.1	38.1 $\pm$ 4.4	35.7 $\pm$ 6.7

ble 4), we calculated bacterial organic carbon demand (BOCD = BP + bacterial respiration) in the studied lakes (Fig. 4B). BOCD in oligo/mesotrophic lakes of low TSI varied from  $94 \pm 16 \mu\text{g C L}^{-1} \text{ d}^{-1}$  in Lake Kuc to  $388 \pm 32 \mu\text{g C L}^{-1} \text{ d}^{-1}$  in Lake Majcz. The highest BOCD values,  $1,750 \pm 185 \mu\text{g C L}^{-1} \text{ d}^{-1}$  and  $1,681 \pm 87 \mu\text{g C L}^{-1} \text{ d}^{-1}$ , were found in hypereutrophic lakes Szymon and Tałowisko, respectively. Rates of BOCD dramatically increased nonlinearly along eutrophication gradient and they significantly correlated to the TSI (ANOVA,  $r^2 = 0.78$ ,  $n = 179$ ,  $p < 0.0001$ ).

*Phytoplankton primary production and BOCD*—We compared the rates of phytoplankton primary production of organic matter (PP) and the rates of BOCD by bacteria in the studied lakes. Figure 5A presents the percentage ratio of BOCD:PP in the studied lakes along the eutrophication gradient. Bacteria utilized a significantly higher portion of organic carbon in comparison with its production in the course of photosynthesis, in oligo/mesotrophic lakes than in highly eutrophic ecosystems. BOCD constituted from  $7.6\% \pm 5.3\%$  (eutrophic Lake Jagodne) to  $68.1\% \pm 12.5\%$  (oligo/mesotrophic Lake Kuc) of the amount of organic carbon that was autochthonously produced by phytoplankton in the studied lakes. There was an almost linear decrease of BOCD:PP ratio along the studied lake trophic gradient (ANOVA,  $r^2 = -0.77$ ,  $n = 114$ ,  $p < 0.0001$ ).

*Dissolved organic carbon*—The concentration DOC in the pelagial zone of large lakes is a result of several tightly coupled microbial processes (Münster and Chróst 1990). Thus, we decided in our studies to also measure DOC concentrations along the gradient of eutrophication of the stud-

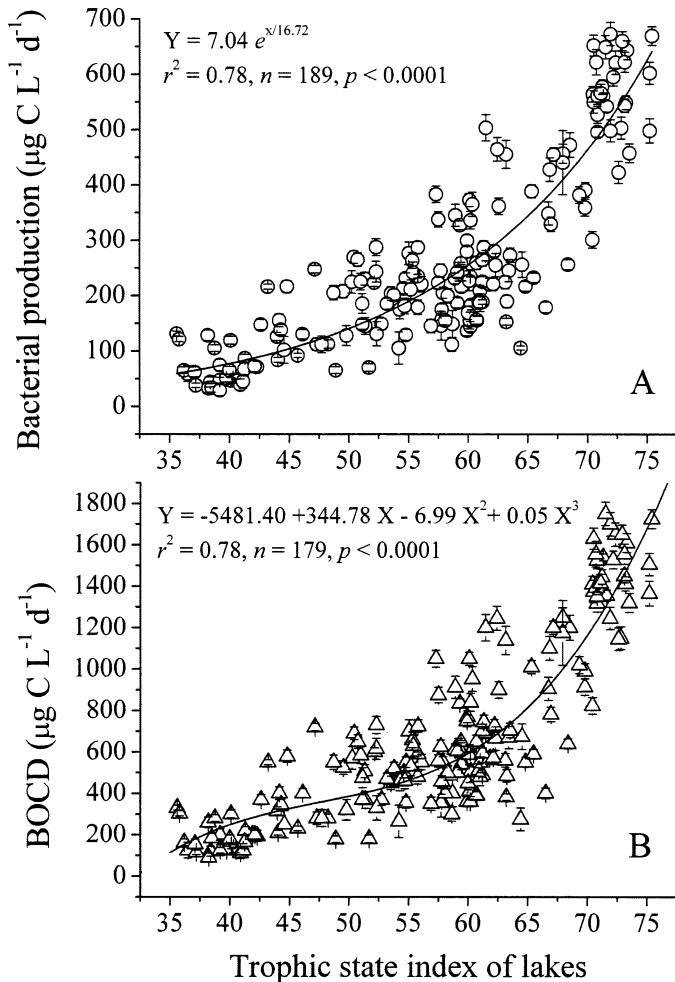


Fig. 4. (A) Rates ( $\pm$ SD) of bacterial secondary production and (B) bacterial organic carbon demand (BOCD) in the upper trophogenic water layer in pelagic water samples along the TSI of the studied lakes  $\pm$  standard deviation of triplicate measurements.

ied lakes (Fig. 5B). DOC concentrations were not significantly different (one-way ANOVA,  $p < 0.05$ ) in the spring and summer in all studied lakes (except Lake Kuc, where summer concentrations were significantly higher than spring DOC). We observed a very pronounced positive relationship between the concentration of DOC and the TSI of lakes. DOC concentration linearly increased (ANOVA,  $r^2 = 0.88$ ,  $n = 217$ ,  $p < 0.0001$ ) along eutrophication gradient and varied from  $5.15 \pm 0.06$  mg C L<sup>-1</sup> (in oligo/mesotrophic Lake Kuc) to  $14.75 \pm 0.04$  mg C L<sup>-1</sup> (in hypereutrophic Lake Szymon).

**Bacterial number and nucleoid-visible bacteria**—Total bacterial numbers (BN) were not significantly different (one-way ANOVA,  $p < 0.05$ ) between the spring and summer in all studied lakes. Total BN varied from  $2.2 \pm 0.3 \times 10^6$  cells ml<sup>-1</sup> (oligo/mesotrophic Lake Kuc) to  $15.8 \pm 3.6 \times 10^6$  cells ml<sup>-1</sup> (hypereutrophic Lake Szymon; Fig. 6A). Numbers of bacteria were linearly positively proportional to the TSI of the studied lakes (ANOVA,  $r^2 = 0.79$ ,  $n = 217$ ,  $p < 0.0001$ ).

Nucleoid-visible bacteria (NucVB) constituted from

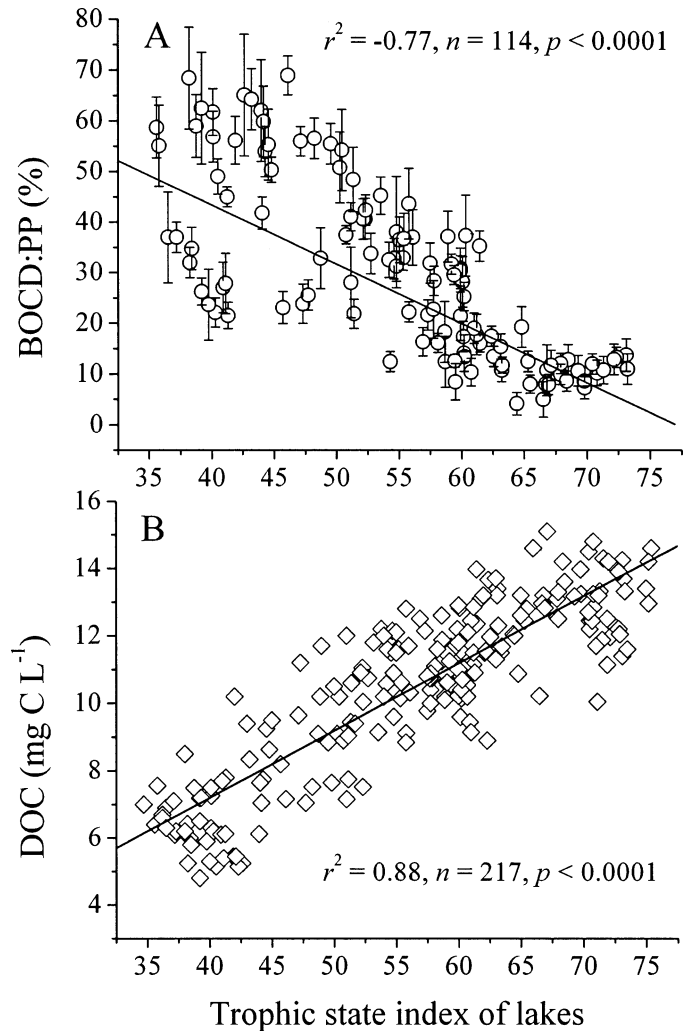


Fig. 5. (A) Percentage ratio BOCDD:PP and (B) dissolved organic carbon (DOC) concentrations in the upper trophogenic water layer in pelagic water samples along the TSI of the studied lakes  $\pm$  standard deviation of triplicate measurements, DOC determined with coefficient of variation  $\leq 1\%$ .

$46.3\% \pm 6.5\%$  (Lake Szymon) to  $97.3\% \pm 7.3\%$  (oligo/mesotrophic Lake Przysań) of the total number of bacteria (Fig. 6B). The percentage of NucVB was not significantly different (one-way ANOVA,  $p < 0.05$ ) in spring and summer periods. In both study periods, we found distinct negative relationships between percentage of NucVB and the TSIs of lakes (ANOVA,  $r^2 = -0.82$ ,  $n = 186$ ,  $p < 0.0001$ ). The highest numbers of NucVB were usually found in moderately eutrophicated lakes, e.g.,  $8.6 \pm 0.9 \times 10^6$  cells ml<sup>-1</sup> and  $7.2 \pm 0.7 \times 10^6$  cells ml<sup>-1</sup> in lakes Beldany and Śniardwy, respectively. NucVB contributed on average 75–95% to the total number of bacteria in oligo/mesotrophic lakes, 40–70% in moderately eutrophic lakes, and 30–50% in hypereutrophic environments. Generally, bacterial communities of hypereutrophic lakes had a lower percentage of NucVB.

**Protozoan grazing on bacteria**—Along the studied eutrophication gradient of lakes, we determined the rates of pro-

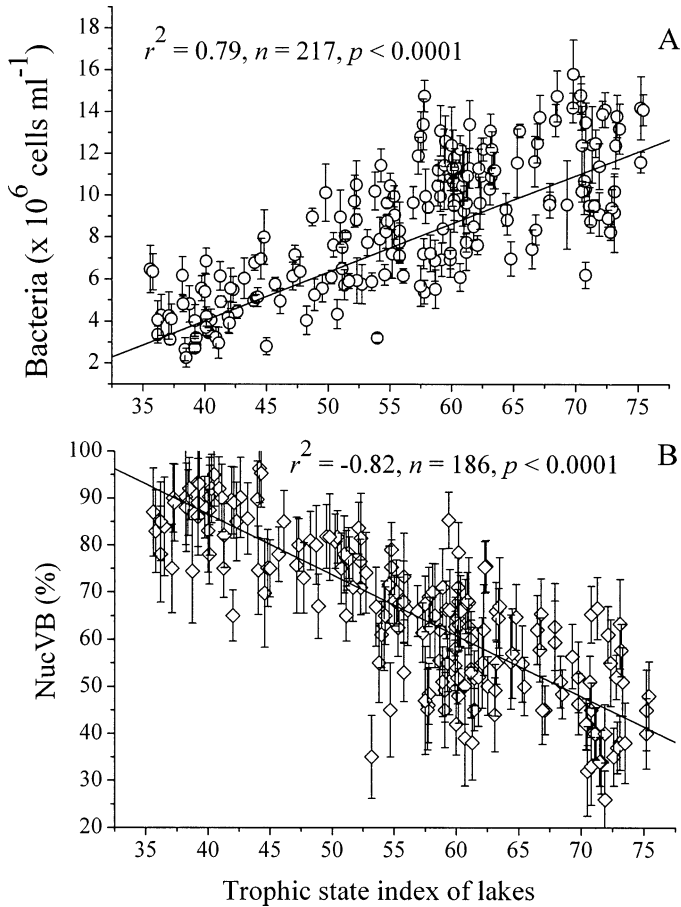


Fig. 6. (A) Total number of bacteria and (B) the percentage of nucleoid visible bacteria (NucVB) in the upper trophogenic water layer in pelagic water samples along the TSI of the studied lakes  $\pm$  standard deviation of triplicate measurements.

tozoan grazing on bacteria (PGB), Fig. 7A. We found that the rates of PGB correlated positively to the rates of BP (ANOVA,  $r^2 = 0.76, n = 50, p < 0.0001$ ) and to the TSI of examined lakes (ANOVA,  $r^2 = 0.86, n = 50, p < 0.0001$ ). Low rates of PGB were noted in oligo/mesotrophic lakes Kuc ( $1.4 \pm 0.3 \times 10^5$  bacteria ml<sup>-1</sup> h<sup>-1</sup>) and Majcz ( $2.2 \pm 0.2 \times 10^5$  bacteria ml<sup>-1</sup> h<sup>-1</sup>). PGB rates exponentially increased along the eutrophication gradient of the studied lakes, and in hypereutrophic environments, PGBs were approximately 5–7 times higher, e.g., Lake Szymon ( $15.5 \pm 3.3 \times 10^5$  bacteria ml<sup>-1</sup> h<sup>-1</sup>), Lake Tałtowisko ( $16.4 \pm 3.9 \times 10^5$  bacteria ml<sup>-1</sup> h<sup>-1</sup>). Protozoan grazers consumed from 20.7% to 81.0% of bacterial cell production in the studied lakes (Fig. 7B). There was observed a significant exponential relationship between the percentage ratio of PGB:BP and the TSI of the studied lakes (ANOVA,  $r^2 = 0.71, n = 50, p < 0.0001$ ).

**Microbial enzymatic activities**—We determined the activities of three microbial ectoenzymes, aminopeptidase (AMP), nonspecific esterase (ESA), and alkaline phosphatase (APA), that are responsible for degradation of the high-molecular-weight components of organic matter. Natural

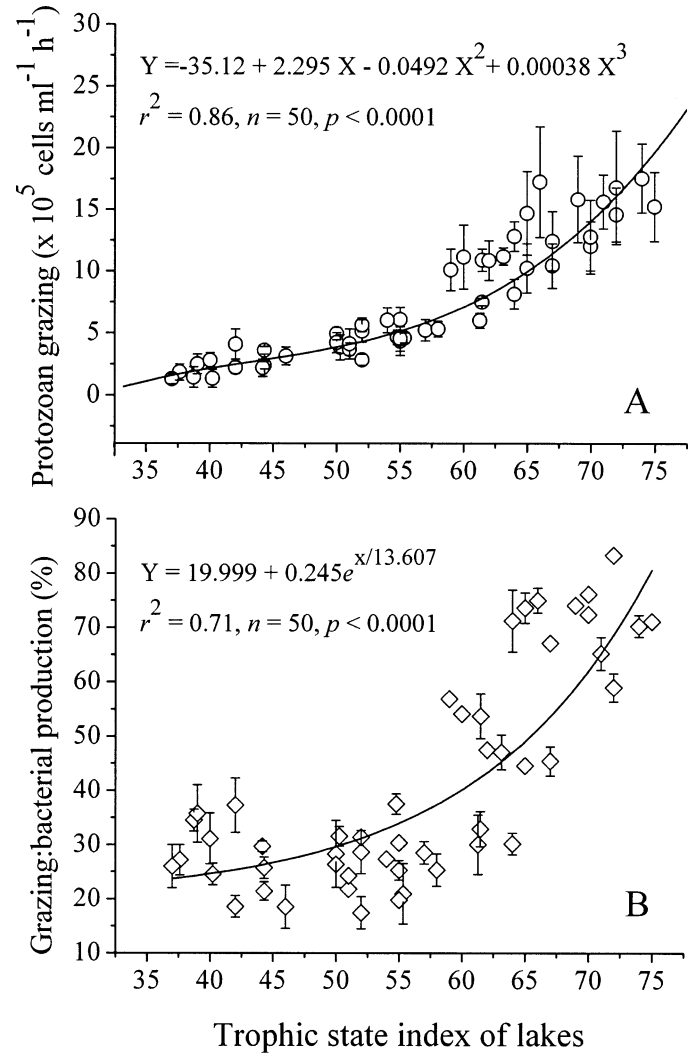


Fig. 7. (A) Rates ( $\pm$ SD) of protozoan grazing on bacteria and (B) percentage ratio of grazing:bacterial production in the upper trophogenic water layer in pelagic water samples along the TSI of the studied lakes  $\pm$  standard deviation of triplicate measurements.

substrates for these enzyme reactions are predominating components of aquatic organic matter and include polysaccharides, proteins, polypeptides and peptides, lipids, nucleic acids, and phosphoesters (Chróst 1994). All enzyme activities reported here are  $V_{\max}$  values of velocity of enzymatic reactions and they were determined using Michaelis–Menten's enzyme kinetic approach.

Figure 8 presents  $V_{\max}$  of the studied enzyme activities along eutrophication gradients of the studied lakes. Aminopeptidase activities were exponentially proportional to the TSI of the studied lakes, (ANOVA,  $r^2 = 0.74, n = 181, p < 0.0001$ ), Fig. 8A. A pronounced increase in AMP activities during the summer in highly eutrophicated lakes was observed. AMP activities did not vary greatly in the spring and summer in lakes of low and moderate trophic conditions (one-way ANOVA,  $p < 0.05$ ). Along the trophic gradient of the studied lakes, activity of AMP varied between  $44 \pm 24$

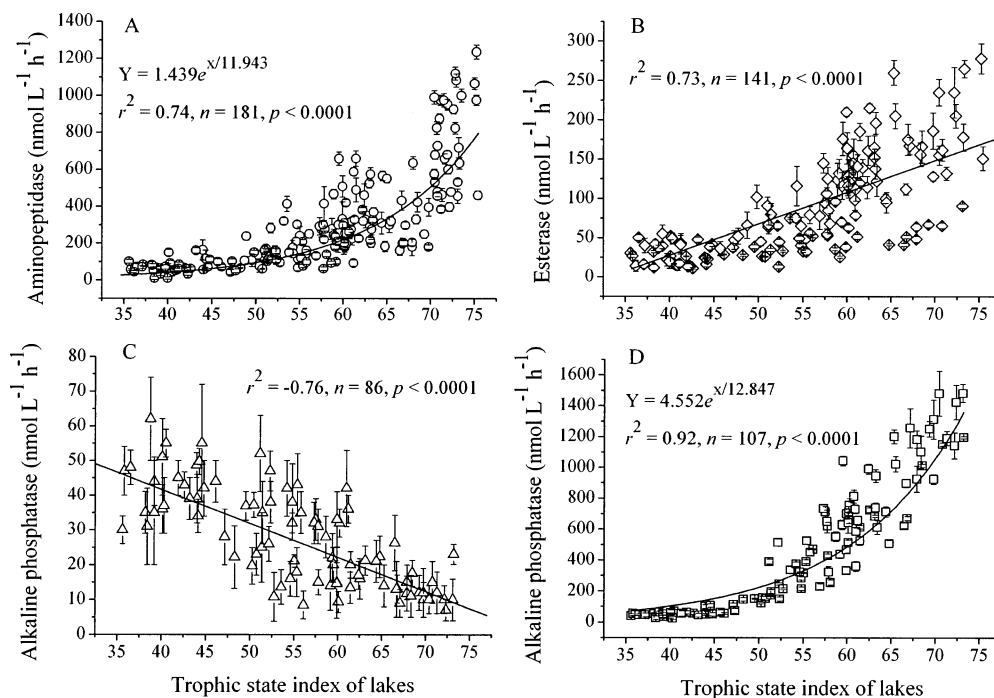


Fig. 8.  $V_{\max}$  activity of (A) aminopeptidase, (B) esterase, and (C) spring and (D) summer alkaline phosphatase in the upper trophogenic water layer in pelagic water samples along the TSI of the studied lakes  $\pm$  standard deviation of triplicate measurements.

$\text{nmol L}^{-1} \text{h}^{-1}$  (Lake Kuc) and  $1,180 \pm 155 \text{ nmol L}^{-1} \text{h}^{-1}$  (Lake Szymon).

Similar to aminopeptidase, we observed high ESA activities ( $215 \pm 5$ – $276 \pm 21 \text{ nmol L}^{-1} \text{h}^{-1}$ ) in highly eutrophicated and hypereutrophic lakes during the summer periods (Fig. 8B). These were several times higher than in the spring. ESA activities in oligo/mesotrophic lakes were much lower ( $11 \pm 1$ – $52 \pm 5 \text{ nmol L}^{-1} \text{h}^{-1}$ ), and there were no significant differences between activities in the spring and summer (Fig. 8B). ESA  $V_{\max}$  linearly positively correlated with the TSI of the studied lakes (ANOVA,  $r^2 = 0.73$ ,  $n = 141$ ,  $p < 0.0001$ ).

The rates of APA  $V_{\max}$  in the spring (Fig. 8C) were notably lower than in the summer study periods (Fig. 8D). There were evident, but different in the spring and summer periods, proportional relationships between APA and the TSIs of the studied lakes.  $V_{\max}$  of APA during spring homothermy of the studied lakes varied from  $8.4 \pm 2.2 \text{ nmol L}^{-1} \text{h}^{-1}$  in hypereutrophic Lake Szymon (TSI = 72.1) and  $62.5 \pm 8.9 \text{ nmol L}^{-1} \text{h}^{-1}$  in oligo/mesotrophic Lake Kuc (TSI = 38.6), Fig. 8C. Spring APA  $V_{\max}$  displayed lower activities in highly eutrophicated lakes and higher activities in oligo/mesotrophic lakes, i.e.,  $V_{\max}$  of APA was inversely proportional to the increase of TSI values of the studied lakes (ANOVA,  $r^2 = -0.76$ ,  $n = 86$ ,  $p < 0.0001$ ). In summer study periods (Fig. 8D), however, APA  $V_{\max}$  displayed exponentially increased rates (from  $48.5 \pm 14$  to  $1,496 \pm 105 \text{ nmol L}^{-1} \text{h}^{-1}$ ) along the eutrophication gradient of the analyzed lakes. Extremely high  $V_{\max}$  of APA was measured in hypereutrophic lakes Szymon ( $1,150 \pm 70$ – $1,290 \pm 125 \text{ nmol L}^{-1} \text{h}^{-1}$ ) and Taltowisko ( $1,250 \pm 138$ – $1,496 \pm 105 \text{ nmol L}^{-1} \text{h}^{-1}$ ).

Aminopeptidase apparent Michaelis constants ( $K_m$ ) esti-

mated from enzyme kinetics varied between  $5.3 \pm 3.5$ – $5.9 \pm 2.8 \mu\text{mol L}^{-1}$  in oligo/mesotrophic lakes and  $15.2 \pm 9.7$ – $15.8 \pm 9.1 \mu\text{mol L}^{-1}$  in hypereutrophic environments (Table 5). Esterase was characterized by lower values of  $K_m$  than AMP. Similar to AMP,  $K_m$  of ESA increased along eutrophication gradient from  $0.6 \pm 0.4$ – $0.9 \pm 1.2 \mu\text{mol L}^{-1}$  in oligo/mesotrophic samples to  $3.3 \pm 2.1$ – $3.5 \pm 2.7 \mu\text{mol L}^{-1}$  in hypereutrophic lakes (Table 5).

## Discussion

The Mazurian study lakes represent a gradient of trophic status from meso/oligotrophic to hypereutrophic. Multiple regression analysis (ANOVA) on all collected data and the TSI along eutrophication gradient showed that all studied microbial processes and parameters were very tightly coupled ( $r^2 = 0.95$ ,  $F = 98.3$ ,  $p < 0.0001$ ) to the trophic conditions of the studied lakes, although there were some differences between spring and summer. A very tight correlation was found between the TSI of the studied lakes and concentration of DOC (Fig. 5B), and organic matter production, i.e., phytoplankton primary production, EOC, and PER (Fig. 3). Moreover, all studied microbial processes involved in utilization (BP, BOCD, BN, NucVB, and PGB) and enzymatic (AMP, APA, ESA) degradation of organic matter were also strongly positively dependent on the intensity and rates of photosynthetic organic matter production and exudation that markedly increased along the eutrophication gradient of lakes. Among activities of the studied microbial enzymes, summer APA displayed the highest posi-

Table 5. Range and mean values  $\pm$  SD of apparent Michaelis constants ( $\mu\text{mol L}^{-1}$ ) of aminopeptidase and esterase in the upper trophogenic water layer in the studied lakes during spring homothermy (Apr) and summer stratification (Jul) periods in 1999.  $n$  = number of assays.

Lake	Aminopeptidase			Esterase		
	Range	Mean $\pm$ SD	$n$	Range	Mean $\pm$ SD	$n$
Kuc	1.6–16.4	5.3 $\pm$ 3.5	14	0.6–1.2	0.7 $\pm$ 0.3	10
Mamry	5.6–7.5	5.5 $\pm$ 2.5	5	0.5–1.1	0.6 $\pm$ 0.4	5
Przystań	5.4–7.4	5.5 $\pm$ 1.6	5	0.7–1.2	0.7 $\pm$ 0.3	5
Majcz	4.2–6.9	5.9 $\pm$ 2.8	5	0.5–3.1	0.9 $\pm$ 1.2	7
Łabap	5.2–7.6	6.3 $\pm$ 2.5	5	1.1–3.8	2.7 $\pm$ 0.8	5
Dargin	6.7–7.8	7.1 $\pm$ 1.4	5	1.3–3.3	2.4 $\pm$ 1.1	5
Śniardwy	3.4–15.1	11.2 $\pm$ 4.8	8	1.2–3.3	1.8 $\pm$ 1.1	9
Kisajno	5.8–18.3	13.1 $\pm$ 5.8	5	1.7–2.9	2.1 $\pm$ 0.6	7
Głębokie	4.7–5.5	5.3 $\pm$ 2.7	4	1.2–3.4	1.8 $\pm$ 1.3	6
Niegocin	5.6–11.4	10.2 $\pm$ 3.5	6	1.8–3.6	2.7 $\pm$ 1.6	8
Boczne	4.9–13.7	11.8 $\pm$ 5.1	6	1.7–3.5	2.7 $\pm$ 1.4	8
Bełdany	3.3–14.3	11.5 $\pm$ 6.1	8	2.2–2.8	2.5 $\pm$ 0.6	8
Tały	2.8–14.1	10.8 $\pm$ 6.1	8	1.3–5.2	2.8 $\pm$ 2.2	12
Ryńskie	3.6–22.6	13.5 $\pm$ 7.4	12	1.7–3.4	2.3 $\pm$ 1.3	12
Mikołajskie	3.2–15.6	12.8 $\pm$ 4.7	14	1.1–3.5	2.3 $\pm$ 1.4	15
Jagodne	4.8–18.5	12.7 $\pm$ 7.7	6	2.2–5.6	2.7 $\pm$ 1.8	8
Szymoneckie	4.8–14.7	12.7 $\pm$ 6.6	8	1.9–6.1	3.1 $\pm$ 2.6	8
Szymon	5.8–38.2	15.2 $\pm$ 9.7	12	2.6–6.6	3.5 $\pm$ 2.7	10
Tałtowisko	5.4–26.1	15.8 $\pm$ 9.1	12	1.9–6.4	3.3 $\pm$ 2.1	14

tive correlation with the TSI (Fig. 8D),  $V_{\text{max}}$  of AMP and ESA showed also high positive correlation with the TSI of the studied lakes. An interesting relationship with the TSI was found in the case of APA activities in the analyzed lakes. There was no correlation between  $V_{\text{max}}$  of APA and the TSI, when both the spring and summer activities of an enzyme were fitted together. Activities of APA determined in the spring periods of study displayed a high negative correlation while summer APA  $V_{\text{max}}$  correlated positively with the TSI of lakes (Fig. 8C,D). The general working hypothesis that the intensity of microbial processes of organic matter can be tightly coupled to increasing eutrophication was, however, proven in these studies.

Phytoplankton production and bacterial utilization of organic matter occur simultaneously and both processes are tightly coupled in the euphotic zone of lakes. A close relationship between phytoplankton primary production and bacterial secondary production in natural waters was often described in numerous studies (Ducklow and Carlson 1992; Chróst and Rai 1993). The results of our studies agreed well with the published data. We found a very significant correlation between primary production and bacterial secondary production (ANOVA,  $r^2 = 0.82$ ,  $p < 0.0001$ ) in the studied lakes along the eutrophication gradient. A tight relationship between phytoplankton and bacterial production in the upper trophogenic water layer in the pelagial zone of the studied lakes indicates that a significant part of phytoplankton-EOC was transferred to bacterial biomass production and metabolism (Table 3). EOC is a very important source of organic substrates for aquatic bacteria (Cole et al. 1982). EOC creates a direct link that tightly couples microbial autotrophic and heterotrophic production in natural waters. The tight coupling between EOC and bacterial production ( $r^2 = 0.78$ ,  $n = 78$ ,  $p < 0.0001$ ), bacterial numbers ( $r^2 = 0.70$ ,  $n =$

114,  $p < 0.0001$ ), and number of NucVB ( $r^2 = 0.53$ ,  $n = 114$ ,  $p < 0.0001$ ) was also observed during our studies. High standing stock of bacterial numbers and rates of biomass production in eutrophic lakes resulted in a very high bacterial demand for organic substrates (Fig. 4A) and leads to efficient bacterial utilization of EOC in those lakes (Table 3). This resulted in PER being negatively correlated with increased rates of bacterial production and bacterial numbers along the eutrophication gradient (Fig. 3C).

An interesting finding was the dramatic decrease of the percentage of NucVB along the eutrophication gradient (Fig. 6B), which corresponded well with increased rates of PGB (Fig. 7A). This observation agrees well with studies on selective and preferential grazing of protozoans on metabolically active bacteria (Del Giorgio et al. 1996). Several studies had shown that bacterial cells with intact visible nucleoids were metabolically active and numbers of NucVB were tightly correlated with other parameters of bacterial metabolic activity (Williams et al. 1998; Berman et al. 2001). Results of experiments in Mazurian lakes previously described by Koton-Czarnecka and Chróst (2003) clearly demonstrated that PGB rates on live bacteria were almost two times higher than on dead bacteria. Protozoans, and among them predominately heterotrophic nanoflagellates (HNF), grazed reluctantly on the whole fraction of metabolically inactive bacteria, which did not contain visible nucleoid (dead, dormant, or very slowly growing bacteria), clearly preferring NucVB. This preferential feeding was observed as a great decrease of the percent contribution of NucVB to the total BN in lake-water samples containing bacterivorous HNF. Thus, PGB probably eliminates mainly newly produced bacterial cells (BP) rather than cell standing stock (BN) in lakes. Protozoans show chemotaxis toward chemical compounds released by actively metabolizing bacteria (Ny-

gaard and Hessen 1994) or they distinguish between active and inactive bacteria because of their different cell surface properties (Sanders 1988). They probably select growing and dividing bacteria because these cells are much richer in nutrients (Goldman et al. 1985). Our studies demonstrated that PGB removed a significant portion of BP and that BP consumption exponentially increased from approximately 20% to 75–85% along the eutrophication gradient from oligo/mesotrophy to hypereutrophy (Fig. 7B). High BP consumption by PGB was proportional to both BN and NucVB (Fig. 6), i.e., increased numbers of bacterial cell (higher food concentration) stimulated grazing rates of protozoans. These observations suggest that the importance of PGB on regulation of BP depends on lake productivity. These observations of protozoan bacterivorous activity are consistent with the prevailing hypotheses that bacterial biomass and production are largely controlled by bottom-up control (substrate and nutrient availability) in low-productivity waters and grazing activity (top-down control) in high-productivity waters (Sanders et al. 1992).

Our results also showed that a larger fraction of the primary production (~45–65%) was utilized by bacteria in oligo/mesotrophic lakes than in highly eutrophicated lakes (Fig. 5A). Increased eutrophication, i.e., increased primary production, caused excessive production of organic carbon that was nonbalanced by simultaneous rates of its utilization and degradation by microheterotrophic communities. Unbalanced production and utilization of organic carbon resulted in significantly lower percent ratio BOC<sub>D</sub>:PP (10–15%) and higher DOC concentrations in highly eutrophicated lakes (Fig. 5B). DOC concentration is another parameter that indicates an overall efficiency of micropkton community production and metabolism of organic carbon in lake water. Accumulation of the DOC in lake water, as displayed by higher DOC concentration, is a distinct result of an excessive allochthonous input, photosynthetic and chemolithotrophic production and processing (viral lysis, zooplankton floppy feeding, excretion, etc.) of organic carbon in the ecosystem (Münster and Chróst 1990; Williamson et al. 1999). Nonextensive farming, low human density, lack of industry in the watershed area, and relatively well-developed littoral zone protect the pelagial zone in the studied lakes from high inputs of allochthonous organic matter (Hilbricht-Ilkowska and Pieczyńska 1993). We assumed that most of the DOC originated from primary production and internal processing of organic matter in the studied lake ecosystems. Unbalanced rates of organic matter high production in eutrophic waters in comparison with its slower microbial utilization resulted in accumulation of DOC in lake water. Therefore, not surprisingly, a strong correlation was found between percent ratio BOC<sub>D</sub>:PP and DOC concentrations (ANOVA,  $r^2 = -0.84$ ,  $n = 114$ ,  $p < 0.0001$ ) and between the DOC and TSI along the trophic gradients of the studied lakes (ANOVA,  $r^2 = 0.88$ ,  $n = 217$ ,  $p < 0.0001$ ). DOC increased linearly from low concentrations, determined in oligo/mesotrophic lakes, to the highest concentrations measured in highly eutrophicated lakes (Fig. 5B).

The stepwise degradation of the high-molecular-weight constituents of dissolved and particulate organic matter, prior to their utilization by bacteria, requires enzymatic hydrolysis

by ectoenzymes to yield monomeric products and/or nutrients that are readily assimilable by microorganisms (Chróst and Siuda 2002). Among aquatic microorganisms, heterotrophic bacteria, cyanobacteria, eukaryotic algae, and protozoans (especially heterotrophic nanoflagellates) are excellent producers of ectoenzymes (Chróst 1994; Karner et al. 1994). Most of the microbial ectoenzymes are inducible catabolic enzymes. It is well known and widely accepted that the nutritional status of the environment and the physiological state of the microbial cell regulate the rates of ectoenzyme synthesis and activity in lake water (Chróst 1991).  $V_{\max}$  and apparent  $K_m$  of ectoenzyme activity in lake water are the resultants of several variables, such as enzyme-specific activity, amount of enzyme molecules, physicochemical properties of lake water, quantity and quality of microbial enzyme producers, concentration of enzyme substrates, etc. Therefore, the  $V_{\max}$  activity of ectoenzymes may vary markedly with space and time in aquatic environments and usually display environmental response of various members of micropkton communities to nutrient status, amount of organic matter, and interspecific relations among microbial assemblages (Chróst and Siuda 2002; Hoppe et al. 2002). Moreover, increasing values of an enzyme apparent  $K_m$  along eutrophication gradient indicate increasing concentration of natural enzyme substrates present in the pool of organic matter in lake water (Chróst 1991).

There are not many published attempts to correlate enzyme activities with the trophic conditions of lakes (Chróst et al. 1999; Chróst and Siuda 2002). Most of the published freshwater data describe enzyme activities related to the physiological state of microbial cells, e.g., alkaline phosphatase activity was used as an indicator of phosphorus deficiency in phytoplankton and bacteria (Rose and Axler 1998). Others reported temporal and spatial variations in enzyme activities related to decomposition of organic matter in lakes (Münster et al. 1999). Because microbial ectoenzyme activity is specifically, functionally, and biochemically tightly coupled with the presence of high-molecular-weight substrates that are predominating constituents of organic matter, the activity of some enzymes may respond to their concentrations and thus they may depend on the gradient of trophic conditions of lakes. In our studies, AMP and ESA activities confirmed well this assumption. We observed a proportional increase in both  $V_{\max}$  and apparent  $K_m$  of these enzymes along the eutrophication gradient of the studied lakes. Similar results were earlier observed in studies by Chróst et al. (1999) in the case of esterase activity of several Mazurian lakes, and aminopeptidase and  $\beta$ -glucosidase activities in freshwater mesocosm experiments by Chróst and Rai (1993). Alkaline phosphatase is an enzyme known to be significantly inhibited by inorganic orthophosphate present in water samples (Chróst and Overbeck 1987). Therefore, activities of alkaline phosphatase determined during spring homothermy and mixing of lake water displayed significant negative correlation to the TSI (Fig. 8C) due to increasing concentrations of inorganic orthophosphate in lake water along eutrophication gradient of the studied lakes. In the upper trophogenic water layer in eutrophicated lakes, we determined high  $V_{\max}$  of APA (Fig. 8D) and very low inorganic phosphate concentration during the summer stratification period and the

enzyme activities and the TSI were also positively correlated. This means that synthesis of alkaline phosphatase was probably derepressed by low inorganic orthophosphate concentrations in lake water and, thus, high enzyme activity was produced due to phosphorus deficiency by actively photosynthesizing phytoplankton (Nedoma et al. 2003).

Our studies have demonstrated that microbial production, utilization, and enzymatic degradation of organic matter in the upper trophogenic water layer in the pelagial zone varied systematically along the eutrophication gradient in lakes. Similar results were reported on DOC and chlorophyll *a* concentrations, and bacterial biomass, production, and respiration across a trophic gradient in 10 Minnesota kettle lakes and Lake Superior (Biddanda et al. 2001). There is some evidence in the recent literature for the existence of a systematic variability in the role of biomass and metabolic coupling of photoautotrophic and heterotrophic components in planktonic communities along the eutrophication gradients in freshwater and marine systems (Cole et al. 1988). The ecophysiological context underlying the predominance of biomass and activity of heterotrophic prokaryotes in oligotrophic but phototrophic eukaryotes in eutrophic environments needs to be clearly defined in terms of cross-ecosystem properties (Biddanda et al. 2001). Recognizing the supremacy of such a trend in biomass distribution and activity among autotrophs and microheterotrophs across trophic gradients is likely to have very important consequences for our understanding of food-web interactions and carbon and nutrient cycling in aquatic ecosystems. Therefore, understanding the nature of microbial autotrophic and heterotrophic processes and interrelations is central to the study of biogeochemical cycles of nutrients controlling eutrophication of freshwater resources.

These studies have also shown that microbial processes and activities may be useful indices of the actual trophic conditions of lakes. Moreover, the quantification of DOC and measurements of BP and enzyme activities in water sampled from lakes of differing trophic status are very important because they provide functional ecological information that is very useful for management and protection of lakes against eutrophication.

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