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Bacterial Secondary Production and Bacterial Biomass in Four Mazurian Lakes of Differing Trophic Status

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Abstract

This report presents results of four-year studies of bacterial production and biomass, and selected environmental variables (concentrations of total DOC, microbiologically labile DOC, chlorophyll,) in surface pelagic waters of four Mazurian lakes of differing trophic status (oligo/mesotrophic, eutrophic, hypereutrophic, polihumic) during summer stratification periods 1994-97. Bacterial production and biomass were positively proportional to the degree of lake water eutrophication. The rates of production of bacteria and their biomass turnover were primarily dependent on concentrations of microbiologically labile organic substrates in the DOC pool. In lakes with high content of suspended paniculate detritus (hypereutrophic and polihumic lakes) attached bacteria significantly predominated in total bacterial production. Importance of the "bottom-up" and "top-down" mechanisms in ecological regulation of bacterial production and biomass in the studied lakes is widely discussed.

Keywords: bacterial production, bacterial biomass, DOC, lake eutrophication

Introduction

During the past decade ecologists have become more aware of a significant role that heterotrophic bacteria play in aquatic ecosystems. In all natural waters bacteria are thought to be the major utilizers of organic matter. By virtue of their abundance, low substrate affinities, and potentially high growth rates, bacteria are capable of rapidly converting energetically low, labile dissolved organic matter (DOM) into their biomass, and that high-quality bacterial particulate organic matter can be easily utilized by bacterivorous protozoans and metazooplankton [1]. Thus production of bacterial biomass represents an important link between DOM and higher trophic levels. Moreover, bacterivores feeding on bacteria also liberate a substantial amount of macromolecular DOM that is enzymatically hydrolysable and utilizable by bacteria. Consequently, heterotrophic bacteria occupy a pivotal position in the aquatic trophic structure.

What does regulate bacterial biomass production (BP) in natural waters is a matter of considerable speculation. Literature contains the notion that BP is controlled by the availability of utilizable organic matter [2, 3, 4]. If this idea is correct, bacterial production in different systems should be correlated with the flux or standing stock of organic matter in those systems. Although it is presently almost impossible to quantify precisely a supply of labile organic matter to aquatic ecosystem, it is possible to estimate variables such as dissolved organic carbon (DOC) content, or chlorophyll concentration that are related to phytoplankton primary production [5, 6]. These variables indicate the size of organic matter production and supply.

Of the various methods available to measure bacterial

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secondary production (e.g., incorporation of ^{35}S-sulfate into bacterial proteins [7]; incorporation of ^{3}H-leucine into bacterial proteins [8, 9, 10, 11]; the dark uptake of $^{14}CO_2$ by bacteria [12,13]; incorporation of ^{32}P-phosphate into bacterial phospholipids [14, 15]; incorporation of ^{3}H-adenine into bacterial nucleic acids [16, 17]) [^{3}H-methyl]thymidine (TdR) incorporation into bacterial DNA [4] has been the most widely used method in aquatic studies. TdR estimates of bacterial production rates have been instrumental in determinations of the importance of heterotrophic bacteria in aquatic ecosystems.

The present paper reports four-year studies of bacterial secondary production and bacterial biomass in four Mazurian lakes of various degrees of water eutrophication during the summer stratification period. We discuss some environmental variables (temperature, oxygen content, pH, water transparency, chlorophyll and DOC concentrations) that affect the rates of bacterial biomass production.

Materials and Methods

Studied Lakes

The studies were conducted in the pelagial of four lakes of differing trophic status: deep oligo/mesotrophic Lake Kuc and eutrophic Lake Rynskie, shallow hypereutrophic Lake Szymon and small polihumic Lake Smolak during summer stratification periods (July-August) from 1994 to 1997.

Lake Kuc has non-significant inflow of surface waters and sewage. Lake Szymon and Lake Rynskie are supplied with a great amount of allochthonous nutrients and organic matter due to the flux of urban and agricultural sewage. Lake Smolak has a forest basin and is supplied with a high content of humic compounds. Lakes: Rynskie and Kuc are typical dimictic lakes with marked summer and winter stratification. Basic morphological and physical parameters of studied lakes are shown in Table 1.

Table 1. Surface and depth of the studied lakes (according to Cydzik [18]).

Lake	Surface (ha)	Maximum depth (m)	Average depth (m)
Kuc	98.8	28.0	8.0
Ryńskie	1831.2	50.8	13.5
Szymon	154.0	2.9	1.1
Smolak	5.5	4.0	2.6

Sampling

Water samples (0.5 1) were collected from water surface (0-0.5 m depth) at several sampling sites (5-10) located in the pelagial zone of the studied lakes. Samples taken from each sampling site were mixed together (vol/vol) to obtain one integrated representative water sample for the pelagial of the studied lake. Mixed water samples were transported to a laboratory within two hours.

Bacterial Secondary Production

Bacterial secondary production (BP) was determined by the [3H]TdR incorporation method according to Chrost [19]. Triplicate (10 ml) samples of water were dispensed to a series of tubes and supplemented with 0.1 ml [^{3}H]TdR (spec, activity 90-97.5 Ci/nmol; NEN Du Pont; final concentration [^{3}H]TdR in assays 15-20 nM). Samples were incubated for 60 min and fixed with formalin (4% final concentration). Then cold (0°C) 100% trichloroacetic acid (TCA) was added to a final concentration of 10%. After 20-30 min, the TCA-precipitate was collected on 0.2 µm membrane filters (cellulose nitrate; Sartorius), rinsed three times with 5 ml 5% TCA and once with 5 ml tap water and then placed in scintillation vials. Filters were dissolved with 5 ml of scintillation cocktail and their radioactivity was determined after 24 h in a Wallace 1400 DSA scintillation counter. Blank of abiotic adsorption of a radioisotope was prepared in duplicate (10 ml) water samples fixed with formalin for 10 min prior [^{3}H]TdR addition and then incubated and treated in the same way as studied samples.

Rates of [^{3}H]TdR incorporation into bacterial DNA were converted to bacterial cell production using the conversion factor 1.25 x 10^{6} cells/pmol [^{3}H]TdR [1]. Bacterial cell production was transformed to bacterial organic carbon production using the conversion factor 19.8 fg C/cell [20].

Bacterial Numbers, Bacterial Biomass and Turnover Rate of Bacterial Biomass

Bacterial cell numbers were determined using epifluorescence microscopy. Formalin preserved and 4,6-diamidino-2-phenyloindole (DAPI) stained samples were counted for bacterial abundance [21]. Bacterial cell numbers were transformed to bacterial biomass (BB) using the conversion factor 19.8 fg C/cell [20]. Bacterial biomass turnover rate was calculated as a ratio of bacterial biomass and bacterial secondary production.

Physico-Chemical Analysis

Temperature and concentration of dissolved oxygen in lake water were measured in situ with a YSI Oxygen Meter (Yellow Springs Mod. 54). A Secchi disc was used to determine water transparency. pH of lake water was measured using the pH-meter. Chlorophyll$_a$ extracted with 96% ethanol, was measured by spectrophotometry [22]. Dissolved organic carbon (DOC) in 0.2 µm filtered (polycarbonate membrane filters; Nuclepore) samples was analyzed by combustion method (680°C) and infrared CO_2 analysis with the use of organic carbon analyzer (Tocomaster model 915B, Beckman or TOC-5050, Shimadzu).

Table 2. Selected physical and chemical parameters of the studied lakes. Range values between July and August 1994 - 1997; the average values are given in parentheses.

Lake	Secchi disc visibility (m)	Temperature (°C)	Oxygen (mg O_2/l)	pH	Chlorophyll (µg/l)	DOC (mg C/l)
Kuc	4.5 – 7.5 (6.1)	19.5 – 22.5 (21.8)	9.2 – 13.0 (10.2)	8.2 – 8.7 (8.4)	2.8 – 4.5 (3.6)	5.9 – 6.8 (6.4)
Ryńskie	0.9 – 1.8 (1.3)	20.0 – 22.5 (22.2)	7.8 – 14.0 (10.6)	8.2 – 8.8 (8.6)	28.4 – 34.2 (31.3)	0.8 – 10.9 (10.8)
Szymon	0.5 – 1.3 (0.8)	19.8 – 22.0 (21.8)	9.3 – 15.6 (13.2)	8.8 – 9.5 (9.0)	102.8 – 149.4 (126.1)	13.4 – 13.6 (13.5)
Smolak	0.5 – 0.7 (0.6)	19.6 – 21.5 (21.5)	7.7 – 10.0 (9.1)	4.8 – 6.2 (5.4)	33.5 – 42.3 (37.9)	21.9 – 23.2 (22.5)

Statistics

Experimental data were statistically analyzed (multiple regression analysis and ANOVA) according to Helsel & Hirsch [23] using computer software (Origin 4.0, Microcal Software Inc., USA).

Results

Limnological Characteristics of Studied Lakes

Field studies were carried out twice during the summer stratification period (in July and August) each year between 1994 and 1997. Additionally, basic physico-chemical analyses were made in spring, summer and early fall (from April to September) 1997 to determine the trophic status of examined lakes. Basic physical and chemical parameters of studied lakes (values obtained in 1997) are shown in Table 2.

Despite different numerical values of determined parameters in sampling periods, we observed a similar pattern of data distribution among the studied lakes. There were no significant differences in temperature of water and the concentration of dissolved oxygen among water samples collected from four studied lakes. Secchi disc visibility, pH, chlorophyll$_a$ and DOC concentrations fluctuated distinctly among the studied lakes in sampling periods.

Based on examined limnological parameters we graded the studied lakes accordingly to progressing degrees of eutrophication. Lake Kuc, which had the highest water transparency and the lowest chlorophyll$_a$ and DOC concentrations, was recognized as a typical oligo/mesotrophic lake. Lower Secchi disc visibility and high chlorophyll$_a$ and DOC concentrations characterized eutrophic Lake Rynskie. Low Secchi disc visibility and the highest pH and particularly high chlorophyll$_a$ and DOC concentrations were found in water samples from Lake Szymon, which was classified as a hypereutrophic lake. There was a different situation in a polihumic Lake Smolak in comparison to Lake Szymon. Among studied lakes, in Lake Smolak we found the highest DOC concentrations, indicating a great inflow of organic matter from its forest basin. There were mostly humic substances that acidified lake water (lowest pH was noted in that lake, Table 2) and caused a dark brown colour of water (strongly affecting water transparency in this lake). The studied lakes fulfilled the criteria of their water trophic status proposed by Chapman [24].

Chlorophylla Concentration

We observed significant differences in chlorophyll$_a$ concentrations among the studied lakes (Fig. 1). The lowest chlorophyll$_a$ concentrations were found in water samples collected from Lake Kuc during summer stratification period in 1994-1997 (ranged from 2.3 to 4.5 µg/l; avg. 3.3 ± 0.8 µg C/l). Much higher chlorophyll$_a$ concentrations were noted in Lake Rynskie (ranged from 22.1 to 34.2 µg/l; avg. 28.4 ± 3.5 µg/l) and in Lake Smolak (ranged from 26.8 to 42.8 µg/l; avg. 35.65 ± 5.8 µg/l). The highest chlorophyll$_a$ concentrations were found in samples from Lake Szymon (ranged from 76.8 to 149.4 µg/l avg. 105.5 ± 23.0 µg/l).

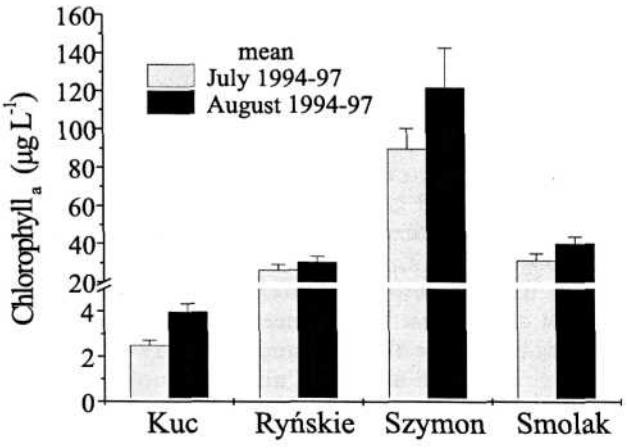

Fig. 1. Mean concentrations of chlorophyll$_a$ in July and August 1994-1997 of the studied lakes (vertical bars represent ± standard deviation).

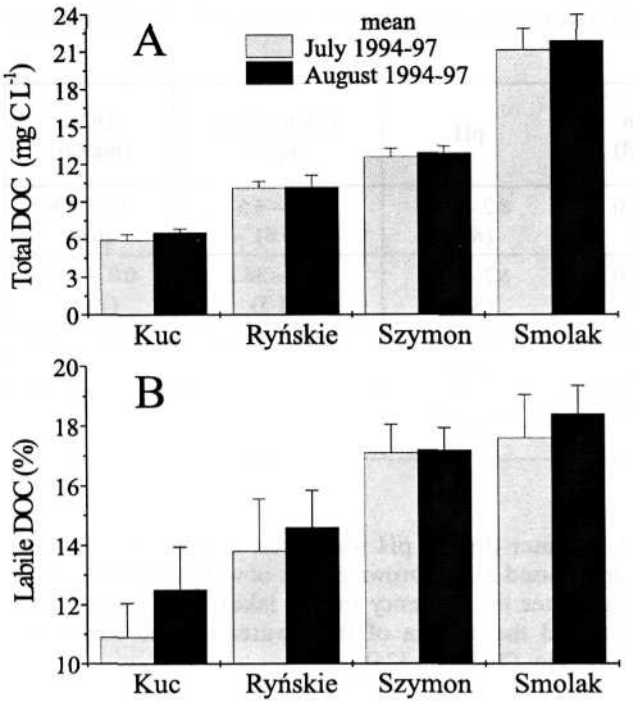

Fig. 2. (A) Mean concentrations of total dissolved organic carbon (DOC) and (B) percentage contribution of labile DOC to total amount of DOC of the studied lakes in July and August 1994-1997 (vertical bars represent ± standard deviation).

We observed significantly higher chlorophyll concentrations in August than in July of each studied year in samples collected in all studied lakes (Lake Kuc - avg. 2.5 ± 0.2 µg/l in July and 4.0 ± 0.35 µg/l in August; Lake Rynskie - avg. 26.4 ± 3.0 µg/l in July and 30.5 ± 3.0 µg/l in August; Lake Smolak - avg. 31.2 ± 3.6 µg/l and 40.1 ± 3.7 µg/l in July and in August, respectively; Lake Szymon - avg. 89.6 10.6 µg/l and 121.5 ± 21.0 µg/l and August, respectively).

DOC Concentration

There were also significant differences in the DOC concentrations among four studied lakes (Fig. 2A). The lowest DOC concentrations were found in Lake Kuc (varied from 5.4 to 6.8 mg C/l; avg. 6.2 ± 0.5 mg C/l). Lake Rynskie and Lake Szymon were characterized by greater DOC contents (ranged from 8.7 to 10.9 mg C/l; avg. 10.1 ± 0.7 mg C/l and from 11.8 to 13.3 mg C/l; avg. 12.8 ± 0.6, respectively). Differently than chlorophyll content, the highest DOC concentrations were found in samples from Lake Smolak (ranged from 19.4 to 24.1 mg C/l; avg. 21.5 ± 1.8 mg C/l). Unlike chlorophyll concentration, there were merely small differences between DOC content in lake water in July and in August (avg. 5.9 ± 0.5 mg C/l and avg. 6.6 ± 0.3 mg C/l in Lake Kuc; avg. 10.1 ± 0.5 mg C/l and 10.2 ± 1.0 mg C/l in Lake Rynskie; avg. 12.6 ± 0.7 mg C/l and avg. 12.9 ± 0.6 mg C/l in Lake Szymon; avg. 21.2 ± 1.7 mg C/l and 21.9 ± 2.1 mg C/l in Lake Smolak in July and in August, respectively).

We have also measured the contribution of microbiologically labile fraction of DOC to the total pool of dissolved organic matter in the studied lakes (Fig. 2B). The contribution of labile fraction of DOC was the greatest in lakes characterized by low Secchi disc visibility; i.e. in Lake Smolak (avg. 17.6 ± 1.4% in July and 18.4 ± 0.9% in August) and in Lake Szymon (avg. 17.1 ± 0.9% in July and 17.2 ± 0.7% in August). In lakes: Rynskie (avg. 13.8 ± 1.7% in July and 14.6 ± 1.2% in August) and Kuc (avg. 10.9 ± 1.1% in July and 12.5 + 1.4% in August) labile organic compounds constituted smaller portion of the total DOC.

Bacterial Secondary Production

The rates of bacterial secondary production (BP) were markedly lower in an oligotrophic Lake Kuc (range from 24.6 to 88.2 µg C/l/d, avg. 51.5 ± 26.2 µg C/l/d) than that determined in eutrophic Lake Rynskie (range from 177.1 to 294.5 µg C/l/d, avg. 254.6 ± 41.7 µg C/l/d) and in hypereutrophic Lake Szymon (216.7 to 795.8 µg C/l/d, avg. 435.6 ± 212.1 µg C/l/d), Fig. 3. The highest rates of BP were measured in Lake Smolak (from 528.3 to 831.8 µg C/l/d, avg. 618.6 ± 106.9 µg C/l/d). There were significant differences between BP in July and in August in the studied lakes (Lake Kuc - avg. 27.8 + 2.5 µg C/l/d and 75.2 ± 10.2 µg C/l/d, Lake Rynskie - avg. 229.4 ± 4.5 µg C/l/d and 279.7 ± 17.8 µg C/l/d, Lake Szymon - avg. 259.9 ± 36.8 µg C/l/d and 613.3 ± 142.9 µg C/l/d, Lake Smolak - avg. 555.3 ± 21.8 µg C/l/d and 681.2 ± 125.1 µg C/l/d, in samples in July and August, respectively). The greatest differences between BP in July and August were noted in lakes Kuc and Szymon.

We have measured the percent contribution of fee-living and attached bacteria to the total bacterial biomass production (Fig. 4). The greatest contribution of attached bacteria was found in polihumic Lake Smolak (66.2% in July and 77.0% in August; avg. 71.6 ± 5.4%). Attached bacteria in Lake Szymon (38.5% in July and 53.4% in August, avg. 46.0 ± 7.4%) and in Lake Kuc (30.6% in July and 43.4% in August, avg. 37.0 ± 6.4%)

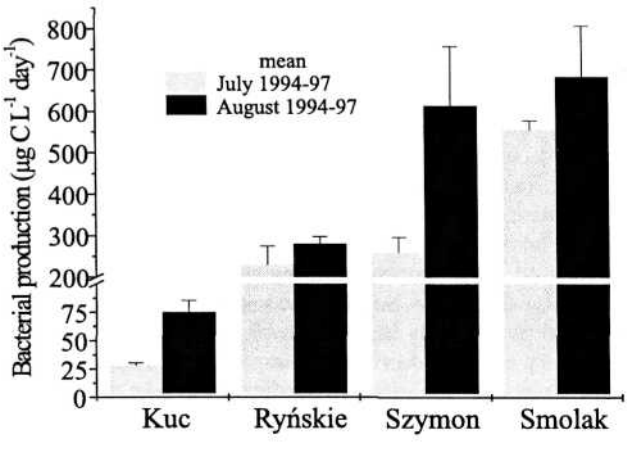

Fig. 3. Mean rates of bacterial biomass production in July and August 1994-1997 in the studied lakes (vertical bars represent + standard deviation).

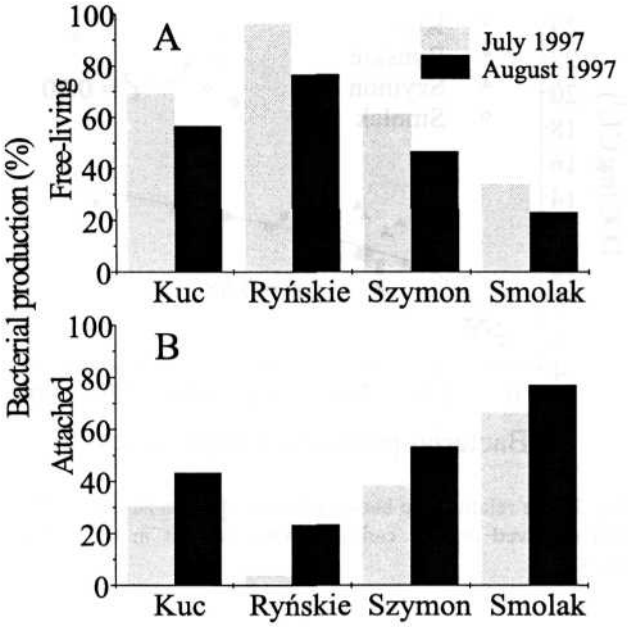

Fig. 4. Percent contribution of secondary production of free-living (A) and attached bacteria (B) to total bacterial production in July and August 1997 in the studied lakes.

produced smaller fractions of the total biomass than those bacteria in Lake Smolak. The smallest contribution of attached bacteria to the total bacterial biomass production was found in eutrophic Lake Rynskie (3.6% in July and 23.4% in August, avg. 13.5 ± 9.9%).

Bacterial Biomass

Similarly to BP, we observed changes in the amount of bacterial biomass (BB) in the lakes (Fig. 5). In Lake Kuc bacterial biomass ranged from 173.25 to 386.1 µg C/l (avg. 263.6 ± 88.8 µg C/l). BB in Lake Rynskie varied from 363.9 to 673.2 µg C/l (avg. 492.7 ± 101.4 µg C/l). Very high BB was measured in Lake Szymon (463.3 to 673.2 µg C/l, avg. 563.0 + 78.1 µg C/l). In samples from Lake Smolak, contrary to their very high BP, BB was comparable to Lake Rynskie and varied from 275.2 to 635.8 µg C/l (avg. 429.6 ± 122.5 µg C/l). In all studied lakes we found that average BB in July was smaller than in August. The most distinct differences in bacterial biomass between July and August were observed in Lake Kuc (avg. 183.6 ± 8.5 and 343.6 ± 35.4 µg C/l in July and August, respectively). In other lakes differences in BB between July and August were less pronounced (Lake Rynskie - avg. 457.4 ± 52.5 and 528.1 ± 133.8 µg C/l, Lake Szymon - avg. 529.5 ± 77.4 and 596.6 ± 72.4 µg C/l, Lake Smolak - avg. 396.3 ± 99.1 and 462.8 ± 149.1 µg C/l, in July and August, respectively).

Turnover Rate of Bacterial Biomass

The turnover rate of bacterial biomass (BTR), as a derivative of bacterial biomass and bacterial production,

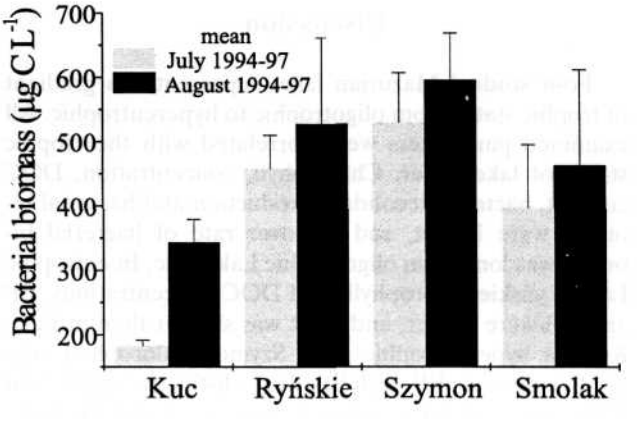

Fig. 5. Mean values of bacterial biomass in July and August 1994-1997 in the studied lakes (vertical bars represent ± standard deviation).

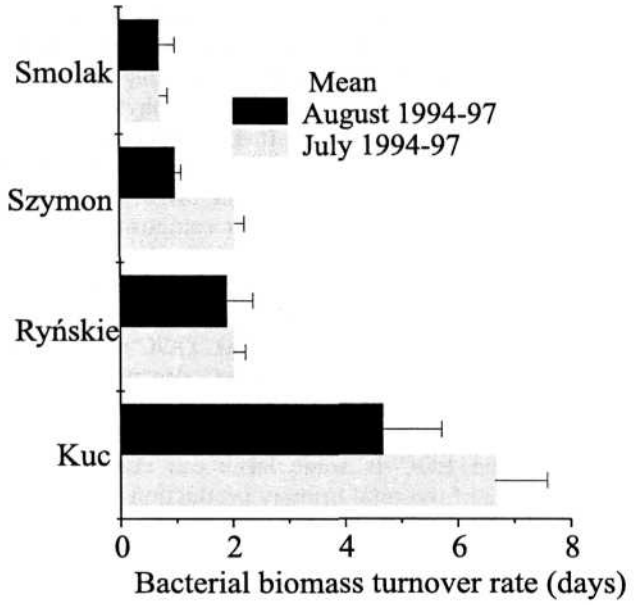

Fig. 6. Mean values of bacterial biomass turnover rate in July and August 1994-1997 in the studied lakes (vertical bars represent ± standard deviation).

was significantly longer in an oligotrophic Lake Kuc (3.8 - 7.8 days, avg. 5.7 ±1.4 days) than in eutrophic Lake Rynskie (1.2 - 2.3 days, avg. 1.9 ± 0.3 day) and in hypereutrophic Lake Szymon (0.8 - 2.2 days, avg. 1.5 ± 0.6 day). The shortest BTR, from 0.3 to 1.0 day (avg. 0.7 ± 0.2 day) was found in polihumic Lake Smolak (Fig. 6).

There were small differences between BTR in July and August in lakes Rynskie (avg. 2.0 + 0.2 and 1.9 ± 0.5 days in July and August, respectively) and in Lake Smolak (avg. 0.7 ± 0.15 and 0.7 ± 0.3 day in July and August, respectively). BTR varied significantly in lakes Kuc (avg. 6.7 ± 0.9 and 4.7 + 1.0 day in July and August, respectively) and Szymon (avg. 2.0 ± 0.2 and 1.0 + 0.1 days in July and August, respectively).

Discussion

Four studied Mazurian lakes represented a gradient of trophic status from oligotrophic to hypereutrophic. All examined parameters were correlated with the trophic status of lake water. Chlorophyll$_a$ concentration, DOC content, bacterial secondary production and bacterial biomass were lowest, and turnover rate of bacterial biomass was longest in oligotrophic Lake Kuc. In eutrophic Lake Rynskie chlorophyll$_a$ and DOC concentrations, BP and BB were higher, and BTR was shorter than in Lake Kuc. In hypereutrophic Lake Szymon chlorophyll$_a$ and DOC contents, BP and BB were distinctly higher and BTR was markedly shorter than in Lake Rynskie. Chlorophyll$_a$ concentration and bacterial biomass in Lake Szymon were the highest among the studied lakes. Lakes Kuc, Rynskie and Szymon were graded accordingly to progressing degrees of eutrophication. Lake Smolak differs significantly from these lakes. It contained the greatest DOC concentration (almost twice higher than in lakes Rynskie and Szymon) but a relatively low chlorophylla amount (comparable to Lake Rynskie). We suggest that organic matter in Lake Smolak originates from other sources than that in three other lakes. In lakes Kuc, Rynskie and Szymon organic carbon is mainly generated in the process of primary production of phytoplankton (autochthonic organic matter). In Lake Smolak a great part of organic carbon is allochthonous and originates from the forest watershed. Different origin of organic matter may also indicate a different composition of organic compounds in lake waters.

Products of photosynthesis of phytoplankton of lakes Kuc, Rynskie and Szymon were much more microbiologically labile than those in Lake Smolak. DOC released by phytoplankton, called extracellular organic carbon (EOC), may be released by healthy algal cells [5, 6, 25], or can originate from dead or dying algal cells [26, 27]. Phytoplankton EOC in some lakes can constitute as much as 70% of the total primary production [28] but in most natural aquatic systems it was found to be in the range of 10-20% [6, 29]. Amino acids, simple sugars, organic acids and polymers like proteins and polysaccharides are among the most common exudates [5].

The organic material liberated from dead or dying algal cells is composed mainly of the constituents that build living cells. Polymers, such as proteins, polysaccharides, nucleic acids [30], and lipids [31] are predominant. These polymers have high molecular weight and therefore they cannot be directly transported through the bacterial membranes. Polymeric substrates, before their incorporation into bacterial cell polymers must be degraded by hydrolytic enzymes into monomers that are transportable [32]. These degradative processes in the water column are mainly mediated by bacterial ectoenzymes [33]. Heterotrophic bacteria synthesize a wide range of ectoenzymes acting outside the cells [34, 35], and they are capable of utilizing a variety of polymeric organic compounds that are not otherwise utilizable. These enzymes belong to ectoenzymes that are associated with external bacterial cell structures, and/or they may occur as free, dissolved in water extracellular enzymes [36]. Microbial enzymatic activity protects aquatic environments against accumulation of detrital organic matter and supplies photosynthetic organisms with nutrients [37]. It was found that enzymatic decomposition of polymeric organic matter increases with the degree of lake eutrophication [32, 38].

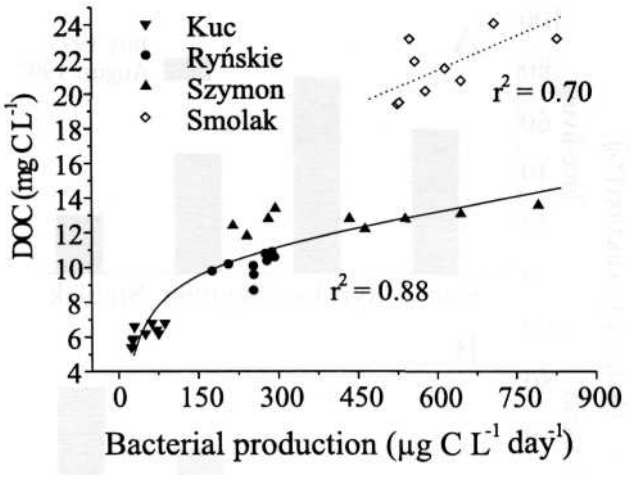

Fig. 7. The relationship between bacterial secondary production and dissolved organic carbon (DOC) content in the studied lakes.

In polihumic Lake Smolak organic matter is principally composed of refractory high molecular weight humic compounds originating from partially degraded material of vascular plants grown in the surrounding area of the lake (structural polymers: lignin, cellulose and hemicellulose which constitute the bulk of vascular plants biomass), and also from microbial products and animal remains. A chemical definition of humic matter is very difficult. Humic substances are generally considered to be of heterogeneous origin and may be formed by several mechanisms, acting independently or in combination. These polihumic substances are submitted to processes of biological transformation and act as secondary metabolites of an inhibitory nature (for example phenols of lignin) and those jointly with very low pH of lake water (caused by great amounts of organic acids) can inactivate bacterial ectoenzymes [39, 40]. Bacterial assemblages in Lake Smolak seem to be very well adapted to specific chemical properties of humic environment. This can be a result of long-drawn processes of evolution and natural selection among microorganisms in these waters. It was found that bacteria isolated from polihumic waters contain a great amount of plasmid-DNA that code many enzymes responsible for degradation of polihumic substances requiring synergistic activity of many hydrolytic enzymes [41].

Results of many studies indicated that the rates of dissolved organic matter supply are a major regulator of bacterial activity, production and growth rate in both freshwater and marine ecosystems [3, 42]. Dissolved organic matter predominates (>90%) in the pool of the total organic matter in all aquatic ecosystems and heterotrophic bacteria are the major utilizers of DOC [41]. The hypothesis that bacterial growth and activity are regu-

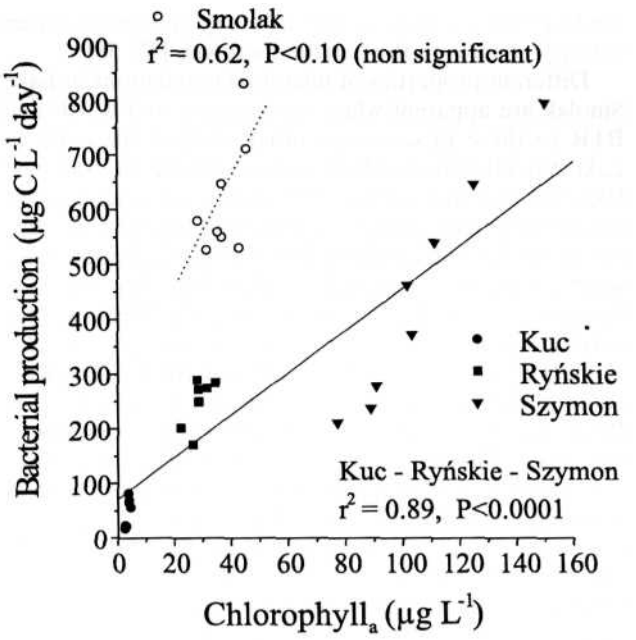

Fig. 8. The relationship between the rates of bacterial secondary production and chlorophylla concentrations in the studied lakes.

lated by supply of available organic matter is supported by two types of evidence. Firstly, bacterial production often correlates with DOC concentration. Cole et al. [43] has cited several reports that bacterial production was controlled and/or was directly related to the standing stock or supply of decomposable organic matter. In our studies we also noted a significant and strong positive correlation between bacterial secondary production and DOC concentrations in all studied lakes (r^2 = 0.88, P < 0.0001 in lakes: Szymon, Rynskie and Kuc, and r^2 = 0.70, P = 0.0001 in Lake Smolak), Fig. 7. BP linearly increased with DOC contents in lake water samples, which means that bacterial production was strongly dependent on DOC content.

The second piece of evidence supporting the hypothesis that bacterial production is regulated, at least partially, by readily available dissolved organic matter is the fact that additions of low molecular weight DOC (e.g., glucose, free amino acids, algal hydrolysate) frequently stimulate bacterial production, whereas changes in bacterial abundance are negligible or much less than the changes in bacterial production. The average bacterial growth rate also greatly increases after organic matter additions [3, 4, 44, 45, 46, 47]. The stimulation of bacterial production suggests that DOC limits, or at least partially determines, growth rates and metabolic activities of bacterioplankton. Furthermore, the lack of large changes in bacterial biomass, even when bacterial production increases greatly after DOC addition, and lack of correlation between DOC and bacterial biomass in Mazurian lakes observed in our studies, point to the importance of grazing and/or viral activity in controlling bacterial density.

The suggestion that bacterial populations are capable of increasing their heterotrophic activity in response to an increase in organic carbon load is supported by the results of our studies. In lakes characterized by low Secchi disk visibility (Smolak and Szymon) with high content of organic suspended matter we determined the greatest contribution of attached bacteria to the total bacterial secondary production (Fig. 4). It is well known that attached bacteria display much higher enzymatic and metabolic activity than free-living bacteria.

Robarts et al. [2] noted that the molecular composition, quality of DOC, and the rates of DOC supply are important factors regulating bacterial activities in pelagic ecosystems because several compounds of the DOC pool may be rapidly assimilated by these microorganisms. We noted that lakes in which the labile fraction of DOC constituted a greater part of total DOC (Smolak and Szymon) were characterized by much higher bacterial secondary production and shorter bacterial biomass turnover rates.

It is widely accepted that EOC is rapidly taken up by bacterioplankton. This indicates that photosynthetic organic carbon excreted by phytoplankton constitutes the major source of organic substrates for aquatic bacteria [5, 6]. In large lakes, marine systems and other habitats where the loading with allochthonous materials occurs at low rates and water residence time is long, bacteria must be dependent on phytoplankton photosynthetic activity. Literature reviews show that phytoplankton EOC supports 50% or more of bacterial organic carbon demand in most pelagic systems [28, 48, 49, 50, 51]. That is why bacterial production and algal primary production are often closely and positively correlated and this relation is essentially linear across differing levels of primary production [32, 43].

Results of many studies indicated the tight relationship between bacterial production and phytoplankton biomass (standing stock), measured by chlorophyll concentration [29, 43]. It was found that bacterial production often correlates more significantly with chlorophyll than with phytoplankton primary production [52] because DOC stimulating bacterial activity originates not only from the current primary production of living algal cells but also from other mechanisms of carbon supply (such as viral lysis and/or decomposition of algal cells) [52, 53]. We found a significant positive correlation between bacterial secondary production and chlorophylla concentration (r^2 = 0.89; P < 0.0001) in three studied lakes (Kuc, Rynskie and Szymon) where primary production of phytoplankton is assumed to be the main source of DOC for heterotrophic bacteria (Fig. 8). The distinct response of bacterioplankton to variations in chlorophyll$_a$ concentration, which we observed in those three Mazurian lakes, indicated that phytoplankton indeed is the dominant source of substrates for bacteria in these lakes. The differences in primary production can therefore explain the variations in bacterial production among lakes Kuc, Rynskie and Szymon.

We did not find a statistically significant correlation between BP and chlorophyll in Lake Smolak. This means that BP was not strongly coupled to phytoplankton production in this lake. This weak correlation between BP and primary production in Lake Smolak, and

the fact that BP was too high to be fully supported by input of autochthonous organic matter suggested that other factors, such as allochthonous loading of organic carbon were more important than phytoplankton photosynthesis in regulation of BP. It was found that humic lakes have an exceedingly high ratio of respiration to primary production [54, 55, 56]. Major sources of energy in such ecosystems are terrestrial inputs of humic carbon. Moreover, the bacterioplankton biomass and activity have been positively correlated to humic content in a number of lakes [57, 58]. Pure cultures of bacteria isolated from humic lake water were able to mineralize or cometabolize humic matter [59]. Tranvik and Hofle [60] found a great potential of natural bacterial assemblages from humic lake water for the degradation of phenolic compounds suggesting that bacteria in humic lakes utilize the humic material. It was also observed increases in bacterial biomass and activity following the addition of humic substances at concentrations above those naturally present in lake waters [61]. Based on these observations, it is reasonable to consider humic matter as a potentially significant source of organic carbon for bacterioplankton in Lake Smolak. On the other hand, mineralization of humic matter is known to be an extremely slow process and humic substances have been suggested to be highly refractory to bacterial degradation due to their recalcitrant nature [62]. However, these two circumstances not necessary are contradictory. A large pool of dissolved humic substances could be of great importance as a bacterial substrate, even if they are utilized with low efficiency [60] and it can be accounted for a fraction of DOC that is biologically available, particularly in highly humic environments such as Lake Smolak. Hessen [57] found that allochthonous organic matter accounted for almost 90% of the carbon required for bacterial growth in small humic lakes while the influence of photosynthetic DOC on bacterial production was negligible. Our results from Lake Smolak indicated a very similar situation to those described for other polihumic lakes.

In conclusion, we can say that the apparent correlation between DOC and bacterial production in all examined lakes (Fig. 7) constitutes the evidence that ambient DOC pools of different lakes along the gradient of increasing DOC content are approximately equally available for bacterioplankton. Moreover, as polyhumic lakes generally have higher DOC concentrations than nonhumic lakes (Fig. 2), this would mean that there must be greater biodegradation of DOC by planktonic bacteria in humic lakes. The organic matter from terrestrial sources can therefore constitute important input to the metabolism of heterotrophic bacteria counteracting its negative effect on phytoplankton primary production due to light attenuation.

A main question of recent works is whether bacterioplankton acts rather as a DOC sink (through the processes of mineralization of organic matter) or rather is a source of organic carbon in aquatic food webs (through the processes of converting DOC into particular organic matter, i.e. bacterial cells) [63]. In environments with a significant allochthonous input of bacterial substrates (e.g. humic lakes), humus DOC converted into bacterial biomass certainly do not constitute a sink for carbon, but its real source that is transferred to higher trophic levels. Bacteria in those systems import the organic matter from external sources to the aquatic food web [57, 58].

Different properties of microbial populations in Lake Smolak are apparent when we compare its PB, BB and BTR to these processes in other studied lakes. BP in Lake Smolak was decidedly highest among all examined lakes but bacterial biomass in that lake was relatively low (even lower than in lakes: Rynskie and Szymon). Results of our studies indicated that BP and bacterial abundance were positively correlated in lakes Kuc, Rynskie and Szymon ($r = 0.84$, $P < 0.0001$) (Fig. 9). Much weaker correlation was found in Lake Smolak ($r^2 = 0.63$, $P < 0.001$). Relation between PB and BB in lakes Kuc, Rynskie and Szymon is bilateral because an increase in PB results in an increase in BB, and greater amounts of bacterial cells result in higher rates of BP. In polihumic Lake Smolak very high BP does not arise from a great abundance of bacteria but rather is due to decidedly greater metabolic activity of bacterial cells, which is probably caused by a greater amount of active cells in relation to metabolically inactive cells. It was found that the range of variation across systems in the number of metabolically active cells is at least 10-fold greater than that of the total number of cells, and that the proportion of metabolically active cells tends to increase with increasing system productivity [64]. Microbial communities thus seem to be more dynamic and less invariant across systems than it is evidenced from patterns in the total abundance or biomass alone. Factors regulating the number and proportion of active and inactive cells in bacterioplankton (like temperature, viral infection, resource limitation and grazing) are still unclear and intensively investigated [65].

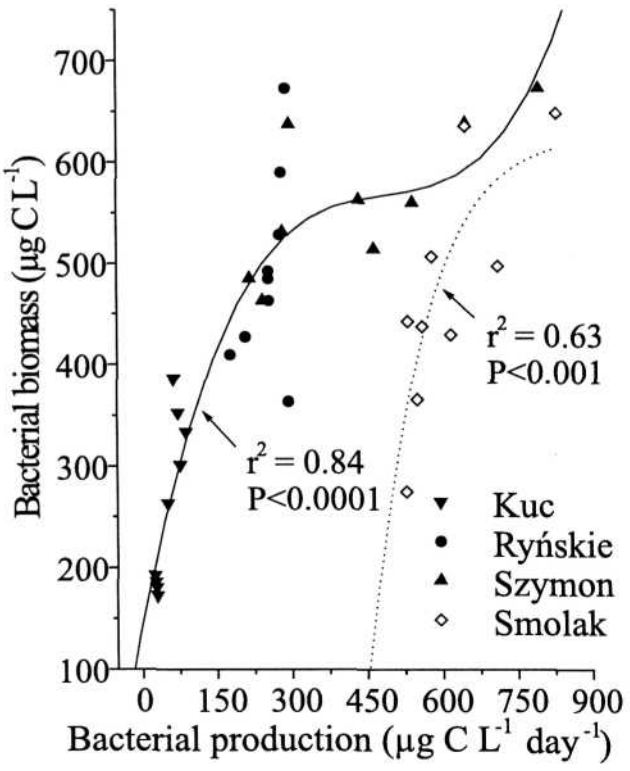

Fig. 9. The relationship between bacterial secondary production and bacterial biomass in the studied lakes.

The correlation between PB and BB in all four examined lakes (Fig. 9) displayed a polynomial function. When PB was relatively low, bacterial biomass was increasing together with BP. However, when PB was high - bacterial biomass began to be rather constant. That suggests efficient and rapid removal of bacterial biomass originating from BP by bacterivorous heterotrophic nannoflagellates (HNF), other protozoans and/or metazooplankton in environments with high bacterial abundance. Wikner et al. [66] found that on average, the daily graz ing rate equalled 60% of the bacterial standing stock, sometimes exceeding bacterial growth. We observed very high activity of bacterivores in lakes Szymon, Rynskie and Smolak that were characterized by high BB. In Lake Kuc (characterized by low BB), however, bacterial graz ing pressure was much lower. The reason for this is the fact that grazing on bacterial populations may continue until the concentration of bacteria decrease below threshold value (we estimated that in our studies this was - 450 µg C/l), where grazing becomes energetically un favourable.

The grazing rate is consequently supposed to be one of the regulatory mechanisms keeping the concentration of bacteria rather constant in planktonic communities despite differential productivity of lake waters [66]. Therefore, DOC concentration in lake water is a factor regulating bacterial biomass in aquatic systems in relatively narrow range. Most of the researchers suggest the importance of combination of resource limitation, predation, viruses, pH and temperature in the regulation of bacterial abundance and productivity [e.g. 67, 68]. Grazing on bacteria, one of the most important regulatory factors, was recently intensively studied. Previous studies have considered the relationship between bacterial growth and losses due to grazing and have inferred that growth and grazing are in close balance in many aquatic ecosystems [69, 70, 71]. Bacteria are grazed by a wide variety of protozoans and metazoans, including mixotrophic algae, heterotrophic flagellates, ciliates, microzooplankton and macrozooplankton. The relative abundance of these consumers varies dramatically across aquatic systems and seasonally within a system [72]. Many researchers suggested that bacteria are ingested mainly by HNF, and subsequently HNF are consumed by larger organisms such as ciliates or micro- and macrozooplankton (copepods and cladocerans) [73]. Abundance of small (2 to 20 µm) heterotrophic flagellates is at least 1000 individuals per ml [67] and because the diet of these organisms tends to be restricted to bacterial size particles [73] their grazing could be a very important sink for bacterial production. It was found that HNF community grazing rates could be very high (~ 100% of newly produced cells) and could reflect in a rapid drop of bacterial biomass [74].

Results of recent studies have shown that pelagic ciliates can significantly graze on bacterioplankton [70, 75, 76, 77]. They can consume both heterotrophic flagellates and bacteria [70, 75, 76]. On average 19% (range from 0.8 to 62%) of the bacterial production was consumed by ciliates during studies conducted in 17 lakes in eastern Norway [77]. This means that ciliates are serious competitors for flagellates. Despite this, ciliates are only occasionally important grazers for bacteria, while bacterial mortality due to grazing by heterotrophic flagellates always seems to be very significant. This can probably be explained by the low number of bacterivorous ciliates. Information on seasonal abundance or trophic preferences of ciliates is very rare, but they are probably more common in eutrophic than in oligotrophic lakes [70, 76, 78, 79, 80, 81]. This can explain higher bacterial grazing rates in lakes Rynskie, Szymon and Smolak than in Lake Kuc observed in our studies (Fig. 9). Other studies suggested that also *Daphnia sp.* is a significant grazer of bacterioplankton. Pace et al. [72] found that in surface waters of Upton Lake flagellates were the primary consumers of bacteria only in winter and fall. In other periods *Daphnia galeata* consumed most of the bacterial production.

Besides the composition of grazer populations, the selective activity of bacterivores is very important. It was found that HNF and other protists selectively eliminate larger cells of bacterioplankton [76, 82, 83]. It means that biomass of smallest bacterial cells should be only weakly related to the changes in protists predation, whereas slightly larger bacteria should reflect protists bacterivory much more clearly. Bacterial cells above a certain size range are subjected to size-selective feeding. Protists size selecting grazing probably defines a threshold size for bacterial cells above which loss rates increase substantially [74]. The relation between size of bacterial cells and losses of BB due to grazing is a very good explanation of our results in the studied Mazurian lakes. We know that in systems characterized by high productivity large bacteria are very common, but in low productive systems small bacteria dominate. Most of the bacteria in oligotrophic Lake Kuc are probably very small cells (below the threshold size for larger animals) and they are exposed to grazing only to a little extent. That is why the relation between

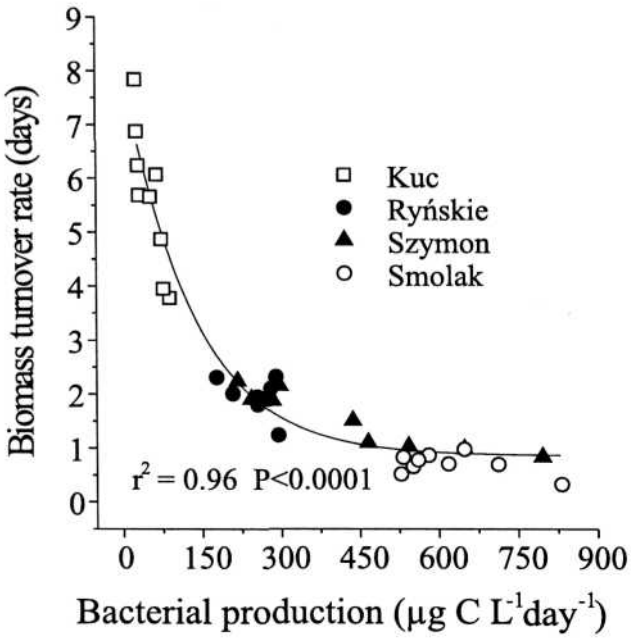

Fig. 10. The relationship between the rates of bacterial secondary production and bacterial biomass turnover in the studied lakes.

BB and BP is linear in Lake Kuc. Large bacteria are predominant in lakes Rynskie, Szymon and Smolak; therefore we observed permanently very strong predation pressure on bacteria in these lakes. Because bacterial cell size increases during growth of cell, size-selected predators are expected to graze more intensively on bacteria shortly before or during cell division [84] and the most actively growing cells probably suffer highest mortality rates. Giorgio et al. [65] found that grazing rates on metabolically active bacteria were four or more times higher than those on inactive cells. Heterotrophic nannoflagellates thus seem to control bacterial density by skimming newly growing cells rather than cropping the standing stock of bacteria. All that entail in lack of linear correlation between bacterial biomass and bacterial secondary production in highly productive lakes where dominate large and metabolically active bacteria. Even if bacterial production is very high most of the newly produced large cells are immediately ingested by HNF.

These results indicate that bacterioplankton in lakes Rynskie, Szymon and Smolak were strongly negatively influenced by top-down regulatory mechanisms and the grazing pressure of HNF appears to be an important factor responsible for keeping bacterial numbers rather constant in epilimnion and metalimnion. Probably the highest grazing rate among all studied lakes existed in polihumic Lake Smolak, because we observed decidedly highest BP and low BB (lower than in lakes Szymon and Rynskie). This fact was probably caused by greater metabolic activity of bacterioplankton in Lake Smolak and very fast rate of production of new bacterial cells. Possibly, the higher prey density in a humic lake allowed bacterivores to operate at higher efficiencies [58].

Very high BP and relatively low BB in Lake Smolak caused that bacterial biomass turnover rate to be very short (it was over twice shorter than in Lake Szymon), Fig. 6. However, BTR in Lake Szymon was still much shorter than in lakes Rynskie and Kuc. A strong correlation between PB and BTR was found in all studied lakes ($r = 0.96$, $P < 0.0001$) (Fig. 10). Low bacterial activity and low bacterial production entail long BTR in Lake Kuc. High BP in lakes Rynskie, Szymon and Smolak resulted in markedly shorter BTR in these lakes. The values of BTR indicate that bacteria in Lake Kuc were metabolically less active than in the other three lakes. This situation is probably a result of very low DOC concentration and small amounts of labile DOC fraction found in samples from this lake.

Our results have shown that the concentration of labile organic substrates determines bacterial growth rates (bottom-up effect) and grazing probably limits increases in bacterial abundance (top-down effect). Thus, resource limitation of growth and predatory influence on abundance interacted simultaneously and determined final bacterial biomass and productivity in the studied lake ecosystems.

We found significant differences in most examined parameters between values obtain in July and August. Because chlorophyll concentrations were always much higher in August than in July, we suppose that summer phytoplankton blooms in Mazurian lakes occur in August. The period of the greatest bacterial activity follows phytoplankton bloom and therefore we measured higher BP and BB in August than in July in all studied lakes. Observed increased values of examined parameters ($chlorophyll_a$ and DOC) from year to year in all studied Mazurian lakes may also indicate their progressive eutrophication.

Concluding our studies, we have found that heterotrophic bacteria responding to eutrophication of lake water displayed their higher growth rates, and therefore we determined increasing rates of bacterial production and bacterial biomass along a trophic gradient of the studied lakes. The quantification of DOC, and measurements of BP and BB in water sampled from lakes of differing trophic status are very important because they provide ecological information that is very useful for management of lakes against eutrophication and pollution.

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