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Measurement of Protozoan Grazing on Bacteria by Means of [^{3}H-thymidine] - Labeled Natural Assemblages of Lake Bacteria

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Abstract

Protozoan grazing on bacteria transfers significant amounts of organic carbon to higher trophic levels in aquatic ecosystems. Therefore, the determination of protozoan grazing rates on bacteria is necessary for estimating the efficiency of microbial loops in organic matter utilization and transformation. Currently, a variety of methods are available to measure protozoan grazing on bacteria but most of them result in serious physical perturbations of the examined samples. We describe an assay for protozoan grazing on bacteria by means of [^{3}H-methyl]thymidine-labelled natural bacterial assemblages as a food tracer for protozoans. Using this method we determined the protozoan grazing rates on bacteria in ten Mazurian lakes in April and July, 2000. We compared the grazing rates with bacterial biomass production and the trophic state index of studied lakes.

Keywords: microbial loop, protozoan grazing, ^{3}H-radiolabelled bacteria

Introduction

Protozoan grazing on bacteria constitutes one of the most important microbial processes in natural aquatic ecosystems. Heterotrophic nanoflagellates (HNF), whose cell sizes range between 1 and 10 µm, are regarded to be the main bacterial consumers [1, 2, 3]. The first experiments on the role of protozoan grazing in aquatic environments were conducted in the 1920s [4]. More interest was directed to this kind of study when Azam et al. in 1983 [5] formulated the microbial loop hypothesis. The microbial loop consists of a complex of different microbial processes responsible for the utilization and transfer of organic matter from its photosynthetic production via utilization by heterotrophic bacteria resulting in bacterial biomass production. The bacterial biomass is then directly grazed by protozoans that are later consumed by metazoans within higher trophic levels in aquatic ecosystems. Studies on the microbial loop initiated an intensive search of methods to determine bacterial biomass transfer within the microbial loop and to quantify grazing rates.

There are various assays available for measuring of protozoan grazing on bacteria: fluorescent artificial particles [6], fluorescent-stained bacteria [7], dilution method [8], fractionated filtration [9], protozoan inhibitors [10], and radiolabelled-bacteria [11, 12, 13]. Most of the above-mentioned methods are very laborious and often result in serious sample perturbations during incubation. For these reasons the determined grazing rates are often underestimated. Natural bacterial assemblages labelled with radioactive isotopes can serve as food tracers for natural protozoan communities. So far different compounds (acetate, glucose, amino acids, thymidine, orthophosphate) labelled with radioisotopes (^{3}H, ^{14}C, ^{32}P) have been used [11, 13, 14, 15, 16, 17, 18].

In this paper we present a modified method that ap-

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Table 1. Basic morphological parameters and trophic conditions of the studied lakes (mean values of the trophic state indexes for the studied lakes were calculated from Secchi disc visibilities and chlorophyll, concentrations, both in April and July 2000).

Lake	Surface area (ha)	Average depth (m)	Maximum depth (m)	Chlorophyll$_a$ (µg/l)		Secchi disc (m)		Trophic state Index (mean ± SD)	Trophic condition
				April	July	April	July		
Kuc	99	8.0	28.8	4.02	2.23	4.20	4.00	40.49 ± 2.56	Oligo/mesotrophy
Przystań	115	n.d.	22.8	2.81	7.46	3.80	4.10	42.86 ± 4.97	Mesotrophy
Majcz	45	3.2	16.5	5.70	4.46	2.20	3.90	45.47 ± 3.69	Mesotrophy
Dargin	2680	10.6	37.6	14.48	8.49	3.10	3.10	48.93 ± 6.42	Meso/eutrophy
Śniardwy	11340	5.8	23.4	24.95	14.71	2.10	1.80	50.15 ± 5.28	Eutrophy
Ryńskie	671	13.5	50.8	23.41	27.69	1.60	1.15	51.28 ± 12.78	Eutrophy
Boczne	183	8.4	25.0	33.42	11.03	2.25	2.55	53.44 ± 8.38	Eutrophy
Tałtowisko	327	14.0	39.5	10.50	21.00	1.80	0.85	56.98 ± 5.22	Eutrophy
Mikołajskie	498	11.2	25.9	57.88	27.77	1.50	1.20	61.21 ± 7.18	Eutrophy
Szymoneckie	523	8.7	28.5	25.15	55.83	1.70	0.80	61.99 ± 7.30	Eutrophy

plies [^{3}H-methyl]-thymidine for labelling natural assemblages of lake bacteria as food tracers. We have chosen [^{3}H-methyl]-thymidine because this precursor is incorporated into bacterial DNA and therefore is metabolically conservative [11, 19]. Since most aquatic bacteria are capable of assimilating exogenous thymidine in nanomolar concentrations from their surrounding environment, it is possible to label almost the whole natural bacterial assemblage. Algae and cyanobacteria cannot 'assimilate an exogenous thymidine in low, nanomolar concentrations. Thus, autotrophic microorganisms are not labelled and therefore the addition of ^{3}H-thymidine to the water sample labels bacteria only [20]. In addition, high specific activity of [^{3}H-methyl]-thymidine considerably increases the radiolabelling of bacteria [11].

Materials and Methods

Studied Lakes and Sampling

The studies were conducted in the pelagial of ten lakes (Mazurian Lakes District, Poland) during phytoplankton blooms in April and July, 2000 (Tab. 1). The studied lakes represented different eutrophication conditions (Taltowisko and Szymoneckie - hypereutrophic; Mikolajskie, Rynskie, Sniardwy, Boczne and Dargin - eutrophic; Kuc, Majcz and Przystan - oligo/mesotrophic). Water samples (1 liter) were taken from the surface (0-0.5 m) at ten sampling sites located along the length of the sampling lake and mixed vol/vol to obtain one integrated, representative sample for every studied lake.

Protozoan Grazing on Bacteria

Protozoan grazing on bacteria was estimated by means of [^{3}H-methyl]thymidine-labelled natural assemblages of lake bacteria. The general rationale for the applied method is that bacteria and protozoans differ in cell size. The cell length of aquatic bacteria usually does not exceed 1 µm and in most cases protozoans are larger than 1 µm in size. Filtration of a water sample through 1 µm pore size membrane filters roughly separates these two groups of microorganisms [21, 22]. Determination of protozoan grazing on bacteria involves the measurement of radioactivity transfer from labelled bacterial size fraction to protozoan size fraction.

The first stage involves the preparation of a suspension of radioactive bacteria (Fig. 1). Lake water sample (100 ml) was filtered through 1 µm pore size polycarbonate membrane filters (ø 47 mm, Poretics) to reduce the amount of bacterial grazers, then supplemented with [^{3}H-methyl]thymidine (TdR, spec, activity 90-97.5 Ci/nmol, NEN Du Pont; final concentration 4 nM), and incubated for 20-24 hours at 20°C in the dark. During the incubation period radioactive TdR was incorporated into bacterial DNA. ^{3}H-DNA-labelled bacteria were harvested on 0.2 µm polycarbonate membrane filters (Poretics). Labelled bacteria on filters were rinsed five times with 15 ml filter-sterilised (0.2 µm) water from the studied lake to wash out free radioactive TdR, not bound in bacterial cells. The filters were then gently sonicated for 10 min in small volume (10 ml) of the filter-sterilised (0.2 µm) water from the studied lake to release labelled bacteria from the filter surface and to resuspend them in water. The concentrated suspension of labelled bacteria

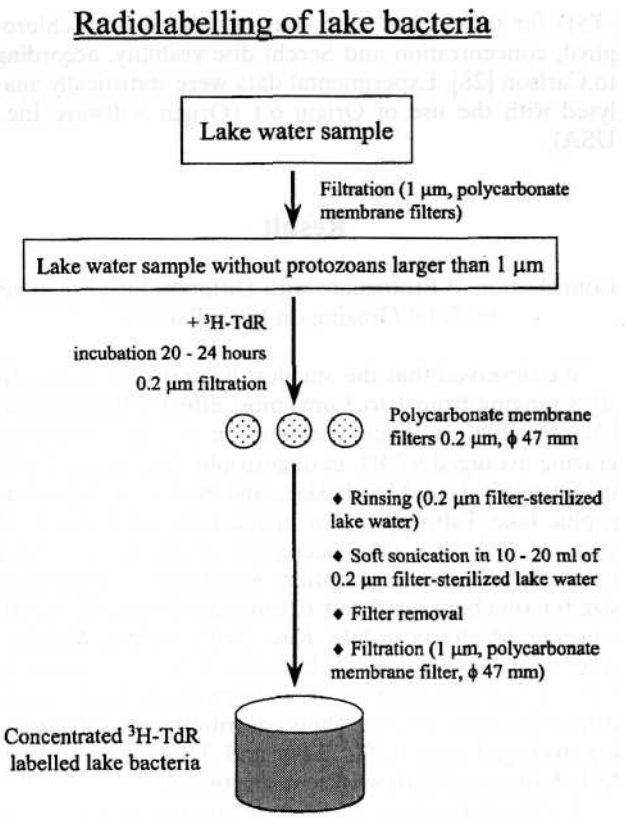

Fig. 1. Scheme of [^{3}H-methyl]thytnidine labelling of lake bacteria.

was filtered again through 1 µm pore size polycarbonate filters to remove larger bacterial cells and clumps of bacteria that may have been formed during concentration and washing procedures.

A prepared suspension of a known number of labelled bacteria was added to lake water sample (100 ml) prefiltered through 10 µm pore size membrane filters (ø 47 mm, Poretics) to remove large protozoans and metazooplankton (Fig. 2). Water samples contained a mixture of known numbers of natural bacteria and ^{3}H-TdR-labelled-bacteria and small (less than 10 µm in size) bacterivorous protozoans. Protozoan grazing on natural and labelled bacteria proceeded for 1 hour of incubation. After this time, samples were fixed with formalin (2% final concentration) and triplicate 20 ml subsamples were filtered through 1 µm pore size polycarbonate membrane filters (ø 25 mm, Poretics), i.e. protozoans were harvested on 1 µm pore size filters. Protozoans contained consumed radioactive bacteria. Filters were placed in scintillation vials, dissolved with scintillation cocktail, and their radioactivity was determined in a Wallac 1400 DSA scintillation counter. Controls of abiotic adsorption of labelled bacteria on filters and/or particulate matter were prepared in separate samples fixed with formalin before the addition of radioactive bacterial suspension. Controls were filtered through 0.2 µm and 1 µm pore size membrane filters to determine the total radioactivity of labelled bacteria added at time zero and blank radioactivity of protozoan samples, respectively.

Total numbers of bacteria (TBN) in lake water samples (i.e. sum of non-labelled + radioactive-labelled bacteria) were determined by epifluorescence microscopy (Nikon, Eclipse 400). DAPI (4,6-diamidino--2-phenyloindole) stained bacterial cells were counted on 0.2 µm pore size black polycarbonate membrane filters (ø 25 mm, Poretics) [23].

The number of bacteria consumed by protozoans (N) during incubation was calculated from the equation:

$$N = (R_1 - R_{lctrl})/R_{0.2} \bullet TBN \quad (1)$$

where:

R_1 =- radioactivity of 1 µm filters;
R_{lctrl} - radioactivity of 1 µm controls filters;
$R_{0.2}$ - radioactivity of 0.2 µm filters;
TBN - total bacterial number.

The number of bacteria consumed by protozoans during the grazing assay was transformed to the grazing rates (GR):

$$GR = N/T \quad (2)$$

where:

T - time of incubation (hours).

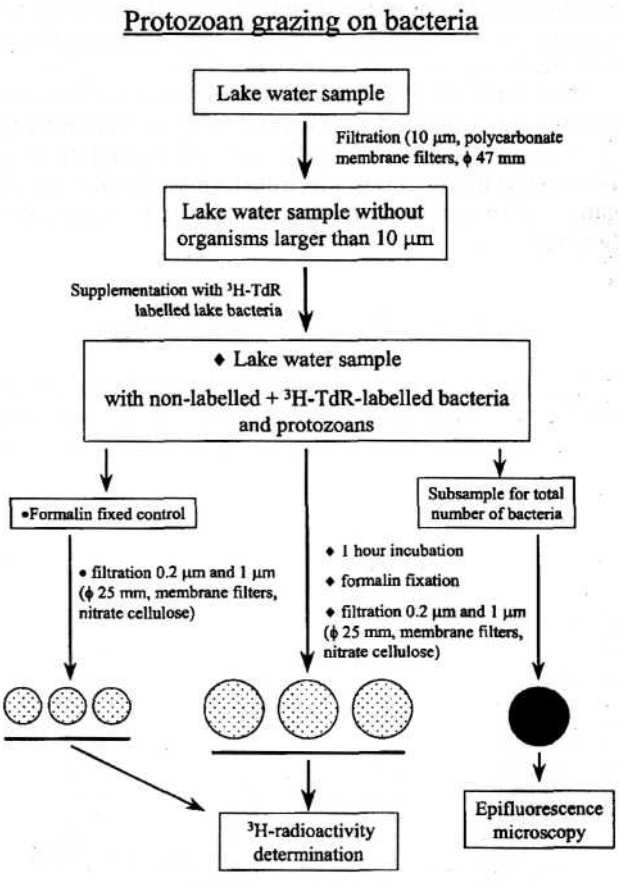

Fig. 2. Scheme of measurement of protozoan grazing on [^{3}H-thymidine]-labelled lake bacteria.

Contribution of Protozoan Differing Cell Sizes to Total Protozoan Grazing on Bacteria

Grazing on bacteria by protozoans differing in cell size was estimated by the same method as the total rates of protozoan grazing. Samples with labelled bacteria and natural grazers were filtered through 0.2 μm, 1 μm, 3 mn and 10 μ^i pores size membrane filters after incubation. Grazing rates of protozoans belonging to different size fractions were calculated using equations 1 and 2.

Bacterial Secondary Production

Bacterial secondary production (BP) was determined by the [^{3}H]TdR incorporation method according to Chrost et al. [24]. Examined samples of lake water were supplemented with 0.1 ml [^{3}H]TdR (spec, activity 90-97.5 Ci/nmol; NEN Du Pont; final [^{3}H]TdR concentration in assays 15 nM), incubated for 30 min and fixed with formalin (2% final concentration). Cold (0°C) trichloroacetic acid (TCA) was then added to a final concentration of 10%. After 20-30 min, the TCA-precipitate was collected on 0.2 μm pore size nitrate cellulose membrane filters (ø 25 mm, Sartorius), rinsed with 5% TCA and placed in scintillation vials. Radioactivity of the filters was determined in a Wallac 1400 DSA scintillation counter. A blank for abiotic adsorption of radioisotope was prepared from water samples fixed with formalin for 10 min prior to [^{3}H]TdR addition and then incubated and treated in the same way as the studied samples.

The 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 [25]. Bacterial cell production was transformed to bacterial organic carbon production using the conversion factor 19.8 fg C/cell [26].

Physico-chemical and Statistical Analyses

Chlorophyll$_a$, extracted with 96% ethanol, was measured by spectrophotometry [27]. A Secchi disc was used to determine water transparency [27]. Trophic state index (TSI) for the studied lakes was calculated from chlorophylla concentration and Secchi disc visibility, according to Carlson [28]. Experimental data were statistically analysed with the use of Origin 6.1 (Origin Software Inc., USA).

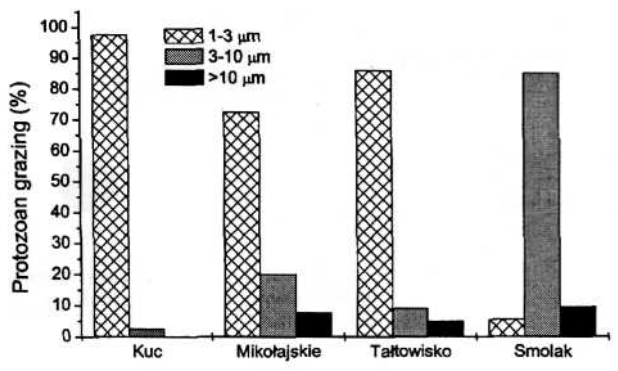

Fig. 3. Contribution of protozoan of differing cell sizes to total protozoan grazing rates on bacteria in four Mazurian lakes in April, 2000.

Result

Contribution of Protozoans with Different Size Fractions to Total Grazing on Lake Bacteria

We observed that the smallest bacterivores (with cell sizes ranging from 1 to 3 μm) most effectively grazed on bacteria (Fig. 3). Their contribution to total protozoan grazing averaged 97.4% in oligotrophic lake Kuc, 72.9% in eutrophic lake Mikolajskie, and 87.1% in hypereutrophic lake Taltowisko. An increase in the cell size of grazers resulted in their decreased contribution to total bacterial biomass consumption. Protozoans belonging to size fraction between 3 and 10 μm grazed only 2.1% of all consumed bacteria in lake Kuc, 20.0% in lake Mikolajskie, and 9.9% in lake Taltowisko. Even less active in bacterial consumption were bacterivorous microorganisms larger than 10 μm. Their contribution to total grazing averaged only 0.5%, 7.1% and 3.0% in lakes Kuc, Mikolajskie and Taltowisko, respectively.

A different pattern of bacterial grazing was noted in polyhumic Lake Smolak. In this lake, protozoans with cell sizes ranging from 3 to 10 μm were responsible for most of the bacterial biomass consumption. They grazed 84.6% of all consumed bacteria whereas other fractions of bacterivores were much less effective. Protozoans with cell sizes between 1 and 3 μm consumed only 6.7% of bacteria and grazers larger than 10 μm consumed 8.7% of bacteria.

Protozoan Grazing on Lake Bacteria and Bacterial Biomass Production

Protozoan grazing rates on bacteria ranged from 2.2 ± 0.2 x 10^5 cells/ml/h (lake Majcz in July) to 9.9 ±1.0 x 10^5 cells/ml/h (lake Dargin in July). Rates of bacterial biomass production ranged from 3.2 + 0.2 x 10^5 cells/ml/h (lake Kuc in April) to 29.8 ± 0.6 x 10^5 cells/ml/h (lake Taltowisko in July) (Tab. 2). The ratios of bacterial cell production (BP) to protozoan grazing on bacteria (PG) in the examined lakes were usually higher than 1, which means that the rates of bacterial biomass production exceeded the rates of its consumption (Fig. 4). The highest BP/PG ratios were measured in July in lakes Kuc (5.25) and Majcz (5.45). Similar rates of bacterial biomass production and grazing on bacteria were observed in lake Dargin both in April (the ratio BP/PG = 1.55) and in July (the ratio BP/PG = 1.23). In four cases protozoan grazing on bacteria exceeded bacterial secondary production (lakes Kuc, Majcz and Przystan in April and lake Rynskie in July).

Protozoan grazing was responsible for the consumption of a significant portion of bacterial biomass (Fig. 5 A). Comparison of simultaneously determined rates of

Table 2. Comparison of the rates of bacterial production (BP) and protozoan grazing on bacteria (PG) in ten lakes of differing trophic status in April and July 2000 (± standard deviation of triplicates).

Lakes	April 2000		July 2000	
	BP 10^5 cells/ml/h	PG 10^5 cells/ml/h	BP 10^5 cells/ml/h	PG 10^5 cells/ml/h
Kuc	3.2 ± 0.2	5.3 ± 0.2	12.6 ± 0.3	2.4 ± 0.3
Przystań	5.9 ± 0.9	8.2 ± 0.2	5.9 ± 0.2	3.6 ± 0.3
Majcz	4.8 ± 0.2	7.5 ± 0.4	12.0 ± 0.5	2.2 ± 0.2
Dargin	7.6 ± 0.3	4.9 ± 0.3	12.2 ± 0.4	9.9 ± 1.0
Śniardwy	21.6 ± 1.6	6.7 ± 0.4	12.4 ± 0.9	4.6 ± 0.6
Ryńskie	13.5 ± 0.8	7.1 ± 0.3	2.8 ± 0.4	7.5 ± 0.3
Boczne	12.9 ± 0.5	5.6 ± 0.3	10.1 ± 0.1	4.6 ± 0.4
Tałtowisko	10.9 ± 0.3	5.6 ± 0.2	29.8 ± 0.6	6.0 ± 0.6
Mikołajskie	14.9 ± 0.7	4.9 ± 0.2	20.3 ± 1.9	7.9 ± 0.9
Szymoneckie	12.5 ± 0.5	5.8 ± 0.2	23.8 ± 1.3	5.3 ± 0.7

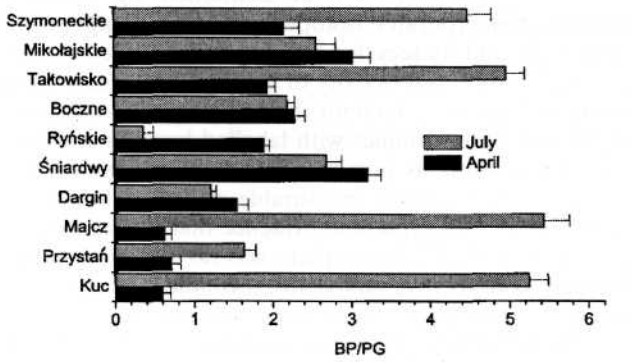

Fig. 4. Ratio of bacterial cell production (BP) to protozoan grazing on bacteria (PG) in ten Mazurian lakes in April and July, 2000.

PG and BP showed that grazing on bacteria was more efficient in April than in July. Protozoans grazed from 31.0% to 165.6% (mean 78.3 ± 53.2%) and from 22.3% to 61.0% (mean 39.9 ± 11.7%) of simultaneously produced bacterial biomass in the studied lakes in April and July, respectively.

In July 2000, we found a statistically significant and strong positive correlation between the rates of protozoan grazing on bacteria and the trophic state index of the studied lakes ($r = 0.71$, $P < 0.02$, ANOVA), (Fig. 5 B). Such a statistically significant correlation was not found, however, in April 2000 for all studied lakes.

Discussion

The idea of the microbial loop explains how dissolved organic matter (DOM) that is not available as a carbon source for eucaryotic organisms becomes available for

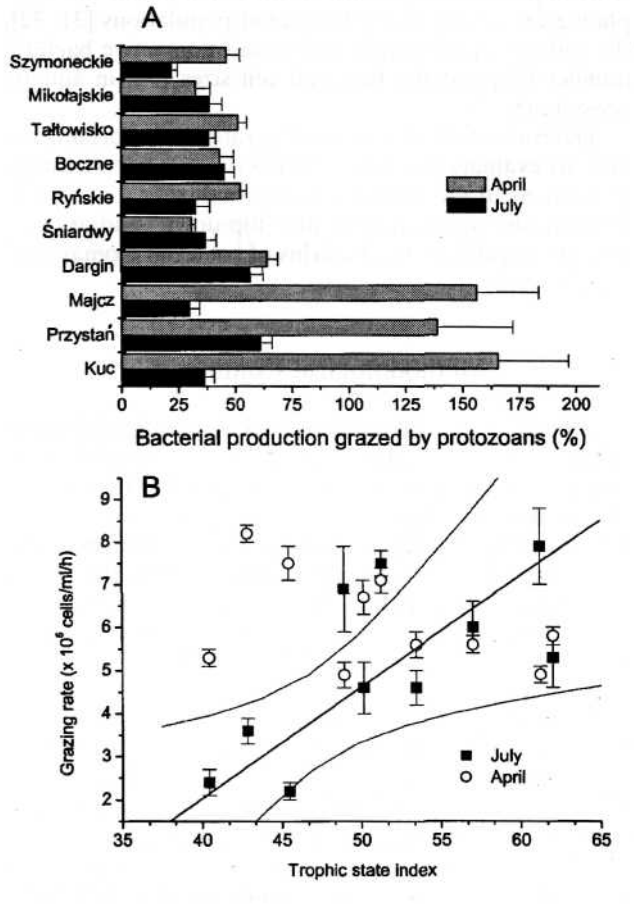

Fig. 5. (A) Percentage of bacterial production grazed by protozoans, and (B) relationship between protozoan grazing rates on bacteria and the trophic state index in ten Mazurian lakes in April and July 2000. Linear regression and confidence bands (95% confidence level) are shown for the July data.

organisms of higher trophic levels. The key process that enables the transfer of organic matter to higher trophic levels is conversion of DOM into bacterial particulate organic matter. Heterotrophic bacteria that are capable of assimilating dissolved organic compounds and incorporating them into their biomass are responsible for this bioconversion. Bacterial cells constitute food sources for bacterivorous protozoans and later protozoans are consumed by larger planktonic metazoans. How much DOM assimilated by bacteria is transferred to the higher trophic levels (in other words the efficiency of the microbial loop) depends on the activity of bacterivorous organisms because they are the direct consumers of heterotrophic bacteria.

Information on protozoan activity is necessary for estimation of microbial loop efficiency in organic carbon utilization in lake waters. It extends our knowledge of energy flow and organic matter cycling in aquatic ecosystems. This is also very important and useful insight for lake management to prevent eutrophication and to reduce organic pollution.

Another important role of bacterivorous protozoans is control of bacterial biomass (i.e. top-down control) [29] and remineralization of organic matter [30]. Trophic activity of protozoans also affects the taxonomical and morphological composition of bacterial populations [31, 32], the ratio of metabolically active and non-active bacteria number [33] and the bacterial cell sizes [34] in aquatic ecosystems.

Determination of real grazing rates is important, not only to evaluate the role of bacteria and bacterivorous protozoans in the overall aquatic food-webs, but also to estimate the significance of the "top-down" and/or "bottom-up" regulatory mechanisms of bacterial biomass and their diversity.

Methodological Comments

Protozoan grazing on bacteria was investigated intensively but the methodological difficulties caused that the results obtained through various methods to be not fully comparable [35]. Most of the presently applied methods for measuring grazing on bacteria strongly alter the natural conditions of water samples and therefore the results are often non-realistic. The described method of [^{3}H-methyl]-thymidine for labelling natural bacterial assemblages, as a food source for protozoans in grazing assays, seems to be the best.

The first experiments using radioisotope-labelled bacterial cells were conducted in the early 1970s [36]. At that time bacteria from laboratory cultures were labelled. Cultured bacteria are much larger than aquatic bacteria and therefore in later studies natural aquatic bacteria were used for metazoan [37] and protozoan grazing measurements [11, 38]. In these studies individual grazers (ciliates or dinoflagellates) were micropipetted to water samples with labelled bacteria. The grazers were then retained and their radioactivities were determined. Ducklow et al. [39] labelled bacteria in a natural lake water sample and then measured the appearance of radioactive label in the plankton fraction larger than 1 µm.

Radiolabelling lake water bacteria for determining protozoan grazing rates has several advantages. It is suitable for measurement of total grazing rates of whole natural protozooplankton community or specific grazing rates of individual protozoan species (in combination with some isolation techniques). Combined with size fractionation method this assay gives information on specific grazing rates for particular size fractions of protozoa [40]. The addition of radiolabelled bacteria to natural water samples does not influence grazer activity because it causes only minimal physical perturbations and changes in microbial communities in comparison to methods that require preliminary filtration or dilution. Estimated grazing rates are more realistic than those obtained by methods using artificial preys (FLB), which are selectively avoided by grazers because of their large size or their being "untasty". For example, Nygaard and Hessen [12] reported that grazing rates of *Bodo* sp. and *Paraphysomonas* sp. obtained by the FLB method constituted only 10% of rates determined by means of radioactive-labelled bacteria. Moreover, the proposed method is simple and rapid and allows processing of a large number of samples.

Grazing rates by means of radiolabelled bacteria, however, are also criticised. When applying this method, several precautions have to be mentioned, e.g. potential release of incorporated isotope from bacterial and protozoan cells and its recycled uptake, and insufficient concentration and radioactivity of labelled bacteria used in assay with grazers. To limit the recycling of isotopes we incubated water samples with labelled bacteria and protozoans as short as possible. One hour incubation was sufficient to result in measurable grazer radioactivity. Caron et al. [40] presented evidence that during incubation shorter than 2 hours there was no release of metabolically conservative radioactive thymidine into the environment. The use of thymidine concentration of about 1.0 µCi/ml for labelling bacteria and long incubation with radioisotope (up to 24 hours) are good ways for sufficient increase of labelled bacterial radioactivity [13]. In order to increase the concentration of labelled bacteria we propose concentrating bacteria after labelling using filtration [13], and bacteria retained on 0.2 µm pore size filters should then be resuspended in a small volume of sterilised lake water [11]. Some bacteria, however, are strongly bound to the surface of the filter and it is difficult to resuspend them. Jarvis and Hart [13] found only from 4 to 21% of the theoretically expected bacterial number in their inoculum obtained in this way. Concentrations of labelled bacteria on 0.2 µ^i pore-size filters also helps in removal of non-incorporated isotope from bacterial suspension [13]. For this purpose, after retaining labelled bacteria on membrane filters, we rinsed them several times with sterilised lake water. It is important that membrane filters be wet before filtration because bacteria strongly adhere to dry filters, resulting in poor recovery of radiolabelled cells [11]. We recommend the use of polycarbonate membrane filters due to their precise nominal pore-size, high filtration rates, and low adsorption properties.

The limitation of the proposed method is that it is not suitable for estimation of grazing rates on large bacterial cells, bacterial aggregates and bacteria attached to particulate matter. Because this assay is based on size differ-

ence between bacteria and their consumers, it is necessary to remove from the studied samples all bacteria with sizes similar to protozoan sizes (i.e., larger than 1 μm). Moreover, there are some bacterial strains that do not incorporate exogenous thymidine [41]. Also, we cannot exclude the existence of positive or negative protozoan chemotaxis to radioactive-labelled bacteria; however, there still is no information available on this topic.

Protozoan Grazing on Bacteria in Aquatic Environments

The most popular morphotypes of aquatic bacteria present in aquatic environments are short rods and cocci with cell size less then 1 μm. Hansen et al. [42] proved that most bacterivorous protozoans efficiently graze on particles which are three times smaller than themselves. Following these two facts the most efficient bacterial grazers should be protozoans smaller than 3 μm. Our results obtained by means of radiolabeled bacterial preys confirmed this hypothesis (Fig. 3). The fraction of the smallest flagellates with cell sizes ranging from 1 μm to 3 μm displayed the highest contribution to total grazing on bacterial biomass in three of the examined lakes - Kuc, Mikolajskie and Taltowisko. A different situation was observed in polihumic lake Smolak where protozoans with cell sizes ranging from 3 μm to 10 μm were the most effective bacterial grazers. Lake Smolak is characterised by a large amount of allochthonous particulate matter organic attached by bacteria. Probably attached bacteria predominate in the total number of bacteria, and free-living small bacteria are less abundant. Only larger protozoans can efficiently graze large organic particles with attached bacteria. That is why larger protozoans acquired dominance in the protozooplankton community of this lake. Moreover, it is well known that protozooplankton communities are composed of larger organisms in acidified environments with high amounts of humic substances, such as Lake Smolak [43, 44].

To keep the bacterial abundance in the ecosystem constant, the number of newly produced bacterial cells must be balanced by their mortality. Protozoan grazing is the main reason of bacterial mortality in environments where similar rates of bacterial biomass production and grazing on bacteria are observed. In our studies we found this situation only in Lake Dargin. In the other studied lakes bacterial biomass production significantly exceeded its consumption by protozoans, which suggests the existence of other regulatory mechanisms of bacterial growth (Tab. 2). Fukami et al. [45] also found a high ratio (8.4) of bacterial biomass production in comparison to its consumption (in hypolimnion). The uncoupling between bacterial production rates and protozoan grazing rates was repeatedly observed in different environments [2, 46, 47, 48, 49]. This is an often observed phenomenon, especially in eutrophic ecosystems, where bacterial productivity is very high and protozoan grazing is much lower. In these environments larger planktonic animals (e.g. crustaceans and rotifers) graze on bacterivorous protozoans, reducing their abundance and thus limiting efficient protozoan grazing on bacteria [50, 51]. Another explanation for the observed uncoupling between bacterial and protozoan activity may be the predominance of "grazing-resistant" bacteria in microbial communities [52] or the preference by protozoans for other food sources such as: autotrophic picoplankton [46, 47], viruses [53], detritus particles [54], and colloidal organic macromolecules [49, 55].

A review of literature data [35] and the results of our studies in July 2000 suggest that protozoan grazing rates on bacteria correlate significantly to the trophic state index of the studied lakes (Fig. 5). This means that the "top-down" regulatory mechanism of bacterial biomass (grazers pressure) is more important in eutrophic and hypereutrophic ecosystems than in clear waters. In oligotrophic and mesotrophic environments the availability of nutrients ("bottom-up" control) is probably the main factor regulating bacterial biomass. Similar conclusions have been drawn earlier by others [56]. Further studies on the correlation between protozoan grazing on bacteria and the trophic state index are needed for use of protozoan grazing rates as a helpful microbial indicator of the state of lake eutrophication.

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