

Abundance and Structure of Microbial Loop Components (Bacteria and Protists) in Lakes of Different Trophic Status

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The abundance, biomass, size distribution, and taxonomic composition of bacterial and protistan (heterotrophic and autotrophic nanoflagellates and ciliates) communities were investigated in six lakes of Masurian Lake District (north-eastern Poland) differing in trophic state. Samples were taken from the trophogenic water layer during summer stratification periods. Image analysis techniques with fluorescent *in situ* hybridization (FISH) as well as [³H]-methyl-thymidine incorporation methods were applied to analyze differences in the composition and activity of bacterial communities. The greatest differences in trophic parameters were found between the humic lake and remaining non-humic ones. The same bacterial and heterotrophic nanoflagellate (HNF) cell size classes dominated in all the studied lakes. However, distinct increases in the contributions of large bacterial (>1.0 µm) and HNF (>10 µm) cells were observed in eutrophic lakes. The bacterial community was dominated by the β-Proteobacteria group, which accounted for 27% of total DAPI counts. Ciliate communities were largely composed of Oligotrichida. Positive correlations between bacteria and protists, as well as between nanoflagellates (both heterotrophic and autotrophic) and ciliates, suggest that concentrations of food sources may be important in determining the abundance of protists in the studied lakes.

Keywords: Microbial loop components, size distribution, lake trophic status

Heterotrophic bacteria play an essential role in processes of decomposition and utilization of organic matter within the microbial loop [38]. Bacteria, through the processes of

converting dissolved organic matter into particular organic matter and then protistan grazing transfer, are the source of significant amounts of organic carbon to higher trophic levels of food webs in aquatic ecosystems [10, 30]. Among protists, heterotrophic nanoflagellates (HNF) are especially recognized as major consumers of aquatic bacteria [48]. Other groups of protists, like ciliates, are only periodically significant bacterioplankton consumers, especially in lakes with high productivity [56]. However, besides protists, depending on the trophic status of a water body, rotifers, cladocerans, and autotrophic flagellates can also be important consumers of bacterioplankton [41, 51]. Thus, the trophic interactions between dissolved organic matter, bacteria, and other biotic microbial loop components are crucial for the carbon flow and nutrient cycling, functioning, and ecological stability of aquatic ecosystems [11].

Numerous studies have been undertaken to evaluate the effect of lake trophy on bacterial numbers, biomass, and microbial processes in Masurian lakes [10, 11], indicating that most of the microbial parameters are tightly coupled to the trophic condition of the studied lakes. However, the effect of lake productivity on the bacterial size–structure and taxonomic composition were rarely studied [55]. Most information about the impact of protistan grazing on bacterial community composition are derived from field and laboratory experiments with inorganic nutrients and/or organic matter enrichment [24, 32, 53]. These studies have shown that individual bacterial populations are highly dynamic and can differ strongly in their response to resources availability and to food web structure. It is evident that the top-down control by protistan grazing is an important regulator of the bacterial community, influencing their size–structure, taxonomic composition, and activity [24, 26, 32].

Little information is available about planktonic nanoflagellate and ciliate communities, or their abundance and diversity

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in Masurian lakes of different trophy [27]. Numerous investigations from other lakes have shown that the abundance of heterotrophic nanoflagellate [3, 52] as well as ciliate [4] communities increased along a trophic gradient. However, there are not much data about the influence of lake trophy on autotrophic (mixotrophic) nanoflagellates (ANF), which may also be significant grazers of bacteria [14, 21].

The aim of this study was to determine the numbers, biomass, size–structure, and taxonomic composition of bacterial and protistan communities in six lakes of Masurian Lake District of different trophic status, as well as to examine relationships between bacteria and protists in relation to increasing loading of organic matter and nutrients.

MATERIALS AND METHODS

Study Area and Sampling

The studies were carried out in the pelagial zone of six lakes (Masurian Lake District, north-eastern Poland) of different trophic status. Basic morphological, physicochemical, and biological parameters of the studied lakes are presented in Table 1. Samples were collected during summer stratification periods on three occasions: in July, August and September. On each sampling data, triplicate water samples were taken from the pelagial zone at the deepest site of the lakes, from the upper trophogenic water layer corresponding to a maximum depth of Secchi disc. Water samples were collected at 0.5 m intervals, mixed together, and treated as representative samples for the studied lakes.

Physical and Chemical Analyses

Temperature, pH, conductivity, and oxygen concentration were measured with an YSI 6600-meter (Yellow Spring Instruments, U.S.A.). Chlorophyll *a* (Chl *a*), extracted with 98% acetone, was measured using a TD-700 fluorometer [2]. Total phosphorus (TP) concentration was analyzed spectrophotometrically according to Koroleff [29]. The

concentration of dissolved organic carbon (DOC) was determined in water samples filtered through 0.2- μm pore-size polycarbonate membrane filters (Millipore, U.S.A.), using a Shimadzu TOC 5050 carbon analyzer. The trophic state index (TSI) for all the studied lakes, except L. Smolak, at each sampling time was calculated from Chl *a*, TP concentrations, and Secchi disk (SD) visibility according to equations by Carlson [7]. Lakes with a TSI below 45 were classified as mesotrophic, between 45 and 55 as meso/eutrophic, and those with TSI from 55 to 65 as eutrophic.

Bacterial Numbers and Biomass

Water samples were preserved with 37% formaldehyde (final concentration 2%). Subsamples of 1 ml were stained with DAPI (4,6-diamidino-2-phenylindole, final concentration 1 $\mu\text{g}/\text{ml}$; Invitrogen, U.S.A.) [47], filtered through 0.2- μm pore-size black polycarbonate membrane filters (Millipore, U.S.A.), and enumerated by epifluorescence microscopy Nikon Eclipse E 400 with UV-2A filter (Ex 365 nm, Em 420 nm) at 1,000 \times magnification. Bacterial biomass (BB) was calculated by converting DAPI-stained bacterial cell volume to carbon units using the biomass conversion factor of 250 $\text{fg C}/\mu\text{m}^3$ [49]. Between 500 and 1,000 DAPI-stained bacterial cells in at least 10–20 digital images of each filter were counted and measured by the automated image analysis system according to Psenner [49]. The most reliable bacterial cell parameters of pixel measurement such as volume (CV), length (CL), and width (CW) were performed according to formulas given automatically by the software LUCIA (Laboratory Imaging, Czech Republic).

Percentage Contribution of Active Bacteria with Intact Membrane

The contribution of active bacteria with intact membrane (MEM+) was analyzed using LIVE/DEAD *BacLight* Bacterial Viability Kits (Invitrogen, U.S.A.) with two dyes: SYTO 9 and propidium iodide (PI), according to Schumann *et al.* [54]. Samples of water were preserved with 25% glutaraldehyde (final concentration 4%). A mixture of two stains was added (1:1 ratio, both dyes final concentration 0.15%) for a 1-ml subsample, incubated for 15 min at room temperature in the dark, filtered through a 0.2- μm pore-size black polycarbonate membrane filter (Millipore), and enumerated by epifluorescence

Table 1. Basic limnological parameters of the studied lakes.

Lake	Surface area (ha)	Average depth (m)	Maximum depth (m)	DOC (mg/l)	Chl <i>a</i> ($\mu\text{g}/\text{l}$)	TP ($\mu\text{g P}/\text{l}$)	SD (m)	Trophic conditions
Kuc	99	8.0	28.8	9.4 \pm 2.1 (5.0–12.0)	3.6 \pm 1.2 (2.3–4.7)	21.2 \pm 12.3 (11.9–39.3)	3.4 \pm 0.6 (3.0–4.1)	Mesotrophic
Przystań	115	13.4	22.8	9.5 \pm 2.5 (7.2–12.8)	7.0 \pm 1.5 (5.4–8.2)	31.7 \pm 1.9 (30.3–33.0)	3.4 \pm 0.3 (3.1–3.9)	Meso/eutrophic
Kisajno	1896	8.4	25.0	9.4 \pm 0.9 (8.3–11.0)	11.1 \pm 3.6 (8.3–15.1)	34.9 \pm 0.1 (34.9–35.0)	2.5 \pm 0.5 (2.1–3.2)	Meso/eutrophic
Mikołajskie	498	11.2	25.9	11.9 \pm 3.0 (8.3–17.2)	30.5 \pm 18 (13.9–49.6)	33.1 \pm 14.9 (19.6–58.5)	1.5 \pm 0.4 (0.9–2.0)	Eutrophic
Tałowisko	327	14.0	39.5	14.9 \pm 5.4 (10.2–25.8)	36.1 \pm 7.3 (27.7–41.0)	40.0 \pm 10.1 (26.8–50.0)	0.9 \pm 0.2 (0.7–1.1)	Eutrophic
Smolak	9	1.8	4.2	18.5 \pm 2.0 (16.2–19.9)	60.3 \pm 19.7 (39.3–81.0)	40.7 \pm 10.1 (30.0–50.0)	0.6 \pm 0.1 (0.5–0.7)	Polyhumic

Mean values \pm standard deviations and ranges in parentheses of dissolved organic carbon (DOC), chlorophyll *a* (Chl *a*), total phosphorus (TP) concentrations, and Secchi disc visibility (SD) for the trophogenic water layers during summer stratification periods. Trophic conditions of the studied lakes were based on mean TSI values.

microscopy on a Nikon Eclipse E 400 using a B-2A filter (the excitation/emission maxima for these dyes were 480/500 nm for SYTO 9 and 490/635 nm for PI). SYTO 9 labels all bacteria with intact and damaged membranes, and PI penetrates only bacteria with damaged membranes, causing a reduction in the SYTO 9 stain fluorescence when both dyes are present. Thus, with an appropriate mixture of the SYTO 9 and PI stains, bacteria with intact cell membranes stain fluorescent green, whereas bacteria with damaged membranes stain fluorescent red.

Fluorescent *in situ* Hybridization (FISH)

Water samples (5 to 10 ml) were fixed with freshly prepared buffered paraformaldehyde (PFA, pH 7.4, final concentration 2%). Subsamples were filtered through 0.2- μm pore-size white polycarbonate membrane filters (Millipore), rinsed twice with 5 ml of sterile water, dried at room temperature, and stored at -20°C . Whole-cell *in situ* hybridization of sections from the polycarbonate filters was performed with the oligonucleotide probes EUB338 [1], NON338 [59], ALF968 [39], BET42a [35], and CF319a [36], as described by Pernthaler *et al.* [44]. Oligonucleotides labeled with the cyanine dye CY3 were synthesized by Interactiva (Ulm, Germany). After FISH, the filters were air dried and mounted on glass slides in mix (Citifluor and VectaShield) amended with DAPI (final concentration 1 $\mu\text{g}/\text{ml}$) [45]. Bacterial cells on the filter sections were observed with Olympus BX 51 epifluorescence microscopy equipped with the UV-2A filter for DAPI (Ex. 330–380 nm, DM 400 nm, BA 420 nm) and G-2A filter for CY3 (Ex. 450–490 nm, DM 505 nm, BA 520 nm). The fractions of FISH-stained bacteria in at least 1,000 DAPI-stained bacterial cells per sample were quantified.

Bacterial Production and Biomass Turnover Rate

Bacterial secondary production (BP) was determined with the [^3H]-methyl-thymidine ([^3H]TdR) incorporation method [8]. The total BP and BP in two size fractions of bacterial cells, $<1.0\ \mu\text{m}$ (free-living) and $>1.0\ \mu\text{m}$ (large free-living and attached), were determined. Samples of water (5 ml) were supplemented with 0.1 ml of [^3H]TdR (spec. activity 60 Ci/nmol, final concentration [^3H]TdR in assays 16.68 nmol/l; MP BIOMEDICALS), incubated in the dark at *in situ* temperature for 60 min, and fixed with 37% formaldehyde (final concentration 4%). Then, cold (0°C) 60% trichloroacetic acid (TCA) was added to a final concentration of 6%. After 30 min of TCA precipitation at $0-1^{\circ}\text{C}$, the TCA precipitates were collected on 0.2- μm pore-size cellulose nitrates membrane filters (Sartorius) and rinsed three times with 5 ml of 5% cold TCA. Filters were placed in scintillation vials, dissolved with 5 ml of high-capacity scintillation cocktail (Rotiszint 2211), and assayed in a scintillation counter (Wallac 1400 DSA) using the external standard channel ratio model. The amount of [^3H]TdR incorporated into bacterial DNA was converted to bacterial cell production using the conversion factor of 1.25×10^6 cells/pmol TdR [8]. Bacterial cell production was transformed to bacterial organic carbon production using the conversion factor of 19.8 fg C/cell [33]. The turnover rate of bacterial biomass (BTR) was calculated as a ratio of bacterial biomass to bacterial production, according to Chróst and Faust [9].

Nanoflagellates Abundance, Biomass, and Size Distributions

Samples were fixed with 37% formaldehyde (2% final concentration). Subsamples of 5–20 ml, depending on the density of cells, were stained with DAPI (final concentration 1 $\mu\text{g}/\text{ml}$) [47], filtered through a

1.2- μm pore-size black polycarbonate membrane filter (Millipore), and enumerated by Nikon Optiphot 2 epifluorescence microscopy. Autotrophic (ANF) and heterotrophic nanoflagellates (HNF) were differentiated by the presence or absence of chlorophyll autofluorescence. Biovolume was calculated from measurements of cells and approximations to simple geometrical forms. The carbon content was calculated by multiplying the biovolume with a conversion factor of 200 fg C/ μm^3 [6]. Variations in DAPI-stained HNF and ANF cell length were used to subdivide their numbers and biomass into three size classes: small ($<5\ \mu\text{m}$), medium (5–10 μm), and large ($>10\ \mu\text{m}$).

Ciliate Abundance, Biomass, and Composition

Samples were fixed with Lugol's solution, and then decanted to 5–20 ml and examined with light microscopy (Nikon Optiphot 2). Species composition and measurements of ciliates were determined from living material in samples drawn together with these for quantitative enumeration. Biovolume was calculated from measurements of cell dimensions and simple geometric shapes and converted to carbon biomass using a conversion factor of 190 fg C/ μm^3 [50]. Species identifications of ciliates were based mainly on Foissner *et al.* [16].

Statistical Analyses

Statistical analyses of results were carried out using the Statistica software package. Pearson's correlation coefficients were calculated between pairs of variables in order to determine the relationships among experimental data. Correlations were performed for three combined sampling datasets ($n=13$).

RESULTS

Physical and Chemical Properties of Lake Waters

As shown in Table 1, the studied lakes differed in size, depth, water transparency, and chemistry. Most marked differences in trophic parameters among lakes were found in Secchi disc visibility (ranging from 3.4 m in lakes Kuc and Przysań to 0.6 m in humic L. Smolak) and chlorophyll *a* concentrations (from 3.6 $\mu\text{g}/\text{l}$ in L. Kuc to 60.3 $\mu\text{g}/\text{l}$ in L. Smolak). The studied lakes were characterized by the relatively low concentrations of the total phosphorus, which ranged from 21.2 $\mu\text{g P}/\text{l}$ (L. Kuc) to 40.7 $\mu\text{g P}/\text{l}$ (L. Smolak). Similar concentrations of dissolved organic carbon were found in lakes Kuc, Przysań, and Kisajno (9.5 mg/l), slightly higher in lakes Mikołajskie (11.9 mg/l) and Tańtowisko (14.9 mg/l), and the highest in L. Smolak (18.5 mg/l). No differences were recorded between lakes in water temperature ($20-23^{\circ}\text{C}$) and oxygen concentrations (9.4–11.7 mg O_2/l). Waters of lakes differed, significantly however, in pH (8.2–8.4 in non-humic lakes and 4.4–5.6 in the humic lake) and conductivity (338–390 $\mu\text{S}/\text{cm}$ in non-humic lakes and 43–60 $\mu\text{S}/\text{cm}$ in humic lake). According to the mean TSI values, the studied lakes were classified to four trophic groups: from mesotrophy (L. Kuc 44.5 ± 3.2), through meso/eutrophy (L. Przysań 48.7 ± 5.9 and L. Kisajno 52.1 ± 4.6) to eutrophy (L. Mikołajskie 57.6 ± 5.6) and high eutrophy (L. Tańtowisko 61.5 ± 4.2) (Table 1).

Bacterial Abundance and Size-Structure

The mean numbers of bacteria (BN) ranged from $8.8 \pm 2.4 \times 10^6$ cells/ml (humic L. Smolak) to $18.7 \pm 6.9 \times 10^6$ cells/ml (highly eutrophic L. Tałtowisko) (Fig. 1A). In the two meso/eutrophic lakes Przystań and Kisajno, the total BNs were similar (9.2×10^6 cells/ml), and two times lower in comparison with the eutrophic lakes. A relatively high value of total BN was recorded in mesotrophic L. Kuc ($11.5 \pm 5.7 \times 10^6$ cells/ml).

Morphological parameters of the bacterial cells (length and width) changed slightly along the trophic gradient of the studied lakes (data not shown). Similar measurements of bacterial cells were found both in meso/eutrophic and eutrophic lakes ($0.8 \mu\text{m}$ length, $0.4 \mu\text{m}$ width). The smallest bacterial cells were observed in humic L. Smolak ($0.5 \mu\text{m}$ length and $0.2 \mu\text{m}$ width). However, as shown by Pernthaler *et al.* [43], the mean cell size of bacterial assemblage may be a misleading parameter and does not correspond to the size classes of bacterioplankton. Because of that, variations in DAPI-stained bacterial cells length were used to subdivide

bacterial numbers and biomass into three size classes: small ($0.2-0.5 \mu\text{m}$), medium ($0.5-1.0 \mu\text{m}$), and large ($>1.0 \mu\text{m}$), according to Lebaron *et al.* [32]. The contribution of particular bacterial size classes to total BN did not differ markedly among the examined lakes, and small-sized bacteria ($0.2-0.5 \mu\text{m}$) dominated, constituting from 45% (humic L. Smolak) to 54% (meso/eutrophic L. Przystań) of total BN (Fig. 1A).

The mean bacterial biomass varied from $0.2 \pm 0.03 \text{ mg C/l}$ (meso/eutrophic L. Przystań) to $0.7 \pm 0.2 \text{ mg C/l}$ (eutrophic L. Tałtowisko) (Fig. 1B). Similarly to total BN, values of total BB in mesotrophic L. Kuc ($0.4 \pm 0.2 \text{ mg C/l}$) were two times higher than those in the meso/eutrophic lakes. The fraction of large-sized bacteria ($>1.0 \mu\text{m}$) was the most important constituent of total BB in all the studied lakes. Their contribution ranged from 56% (L. Przystań) to 74% (L. Smolak) (Fig. 1B).

Contribution of Active Bacteria with Intact Membrane (MEM+)

The mean contribution of MEM+ bacteria to total BN was considerably higher as well as more differentiated among the studied lakes in comparison with the contribution of MEM+ to total BB (Fig. 2). The lowest share of active bacteria in total BN was recorded in L. Kisajno (16%), slightly higher in L. Przystań (18%), and the highest in lakes Tałtowisko and Smolak (24%). The share of active bacteria in total BB was very similar in all the studied lakes and ranged from 9% (eutrophic L. Tałtowisko) to 14% (eutrophic L. Mikołajskie).

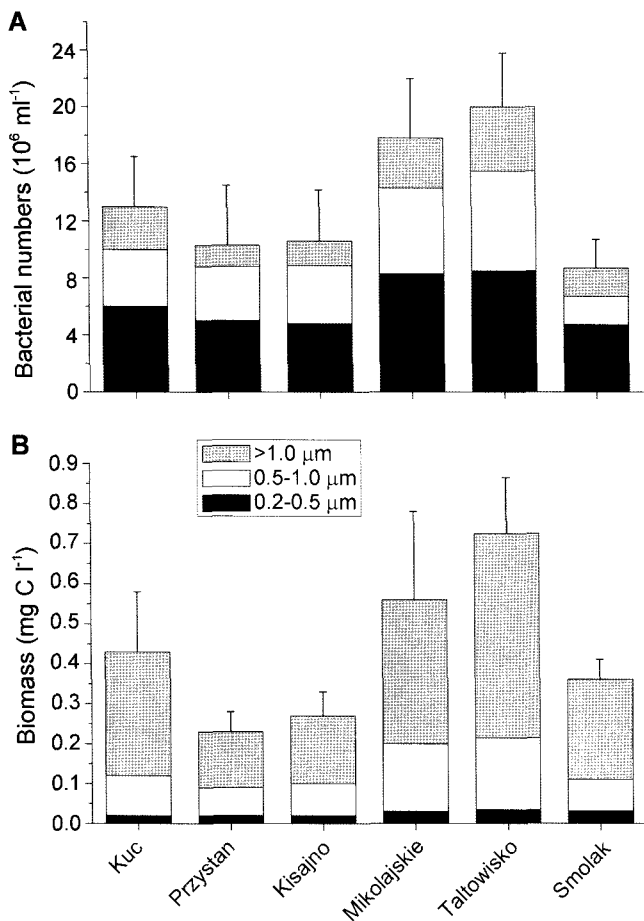


Fig. 1. Total bacterial numbers (A) and biomass (B) with three size classes, small ($0.2-0.5 \mu\text{m}$), medium ($0.5-1.0 \mu\text{m}$), and large ($>1.0 \mu\text{m}$) cells, marked in the studied lakes. Mean values from three sampling datasets \pm standard deviations for total numbers and biomass.

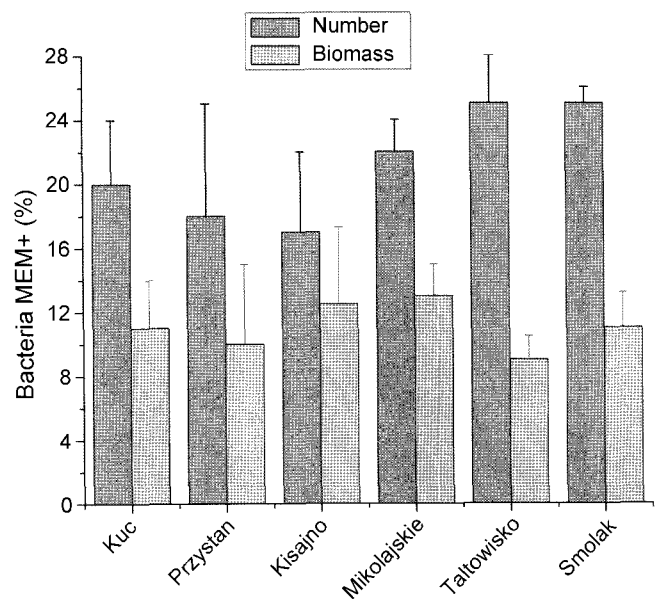


Fig. 2. Percentage contribution of active cells (MEM+) to the total numbers and biomass of bacteria, represented as a sum of MEM+ and MEM- cells in the studied lakes. Mean percentage values from three sampling datasets \pm standard deviations.

Bacterial Taxonomic Composition (FISH)

The composition and dominance structure of bacteria showed only small differences among the studied lakes. The fraction of bacterial cells hybridizing with the probe EUB338 (*Bacteria*) constituted from 44% (eutrophic L. Mikołajskie) to 62% (humic L. Smolak) of total DAPI counts (Fig. 3). β -*Proteobacteria* (probe BET42a) represented a large fraction of bacterioplankton among examined groups in most of the studied lakes, constituting from 15% (L. Tałtowisko) to 27% (L. Smolak). The mean share of *Cytophaga-Flavobacterium* cluster (probe CF319a) in total DAPI counts varied from 7% (L. Smolak) to 21% (L. Kisajno). In mesotrophic L. Kuc, the contribution of *Cytophaga-Flavobacterium* (9%) was similar to these noted in eutrophic lakes. The α -*Proteobacteria* (probe ALF968) abundances were very similar in all lakes and below 7% of total BN (Fig. 3).

Bacterial Production and Biomass Turnover Rate

The mean bacterial production changed in a wide range, from $1.7 \pm 0.4 \mu\text{g C/l/h}$ in meso/eutrophic L. Przyszań to $9.5 \pm 1.0 \mu\text{g C/l/h}$ in highly eutrophic L. Tałtowisko (Fig. 4A). The BP in mesotrophic L. Kuc ($2.7 \pm 0.6 \mu\text{g C/l/h}$) was about two times higher than in the meso/eutrophic lakes, whereas in humic L. Smolak ($3.3 \pm 1.5 \mu\text{g C/l/h}$) was three times lower than in highly eutrophic L. Tałtowisko. In all lakes, the total BP was associated with the fraction of

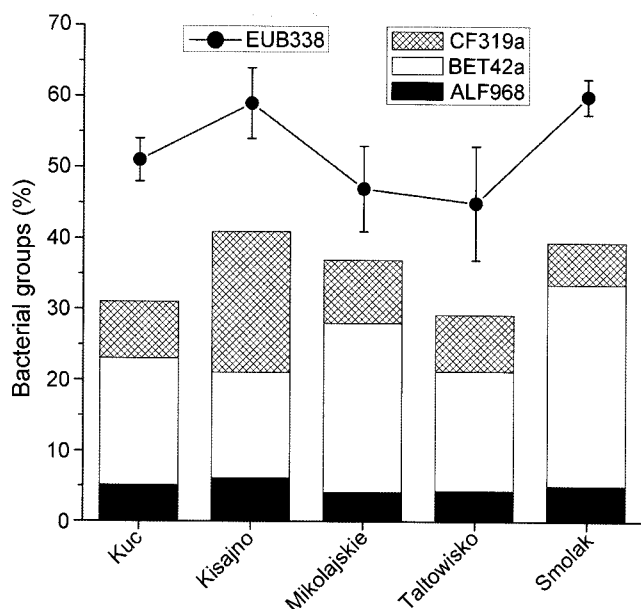


Fig. 3. Percentage contribution of bacterial taxonomic groups determined by fluorescent *in situ* hybridization (FISH) with rRNA-targeted oligonucleotide probes: EUB338 for *Bacteria*, ALF968 for α -*Proteobacteria*, BET42a for β -*Proteobacteria*, and CF319a for *Cytophaga-Flavobacterium* cluster, related to the total DAPI-stained bacteria in the studied lakes. Mean percentage values from three sampling datasets \pm standard deviations for *Bacteria*.

bacteria $<1.0 \mu\text{m}$, which constituted 85% of total BP in mesotrophic lake, but only 55% in meso/eutrophic L. Kisajno and eutrophic L. Mikołajskie.

The longest turnover rate of bacterial biomass (BTR) was found in meso/eutrophic L. Kisajno (19.8 h), slightly shorter in mesotrophic L. Kuc (16.2 h), and the shortest in eutrophic L. Tałtowisko (7.4 h). Similar BTR values were found in lakes Smolak and Mikołajskie (9.4 h) (Fig. 4B).

Nanoflagellate Abundance, Biomass, and Size Distribution

Similarly as in the case of BN, the lowest mean number of heterotrophic nanoflagellates was recorded in humic L. Smolak ($0.7 \pm 0.7 \times 10^3/\text{ml}$), and the highest in eutrophic lakes Mikołajskie ($5.2 \pm 0.7 \times 10^3/\text{ml}$) and Tałtowisko ($5.8 \pm 1.0 \times 10^3/\text{ml}$) (Fig. 5A). In all the studied lakes, the small size class of HNF ($<5 \mu\text{m}$) dominated in total HNF numbers (69%–83%). There was observed large ($>10 \mu\text{m}$) HNF cells in the mesotrophic and both eutrophic lakes; however, their proportion was only of marginal importance ($<1\%$) (Fig. 5A).

The mean HNF biomass increased along a trophic gradient of the lakes (from $10.2 \pm 4.7 \mu\text{g C/l}$ in L. Kuc to $27.4 \pm 8.5 \mu\text{g C/l}$ in L. Tałtowisko). Relatively low HNF biomass was recorded in humic L. Smolak ($2.4 \pm 1.6 \mu\text{g C/l}$) (Fig. 5C). In all the studied lakes, medium-sized cells dominated in the HNF biomass, constituting from 64% to 77%.

In the majority of studied lakes, the numbers of autotrophic nanoflagellates were lower in comparison with the HNF number, whereas values of their biomass were about 2 times higher than biomass of HNF. Only in L. Smolak were both numbers and biomass of ANF higher than HNF

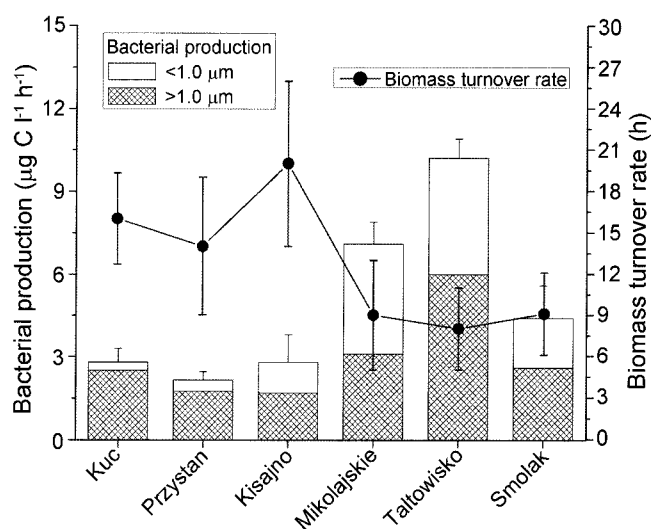


Fig. 4. Total bacterial production of two fractions of bacterial cell sizes, free-living ($<1.0 \mu\text{m}$) and large free-living and attached ($>1.0 \mu\text{m}$), and bacterial biomass turnover rates in the studied lakes.

Mean values from three sampling datasets \pm standard deviations.

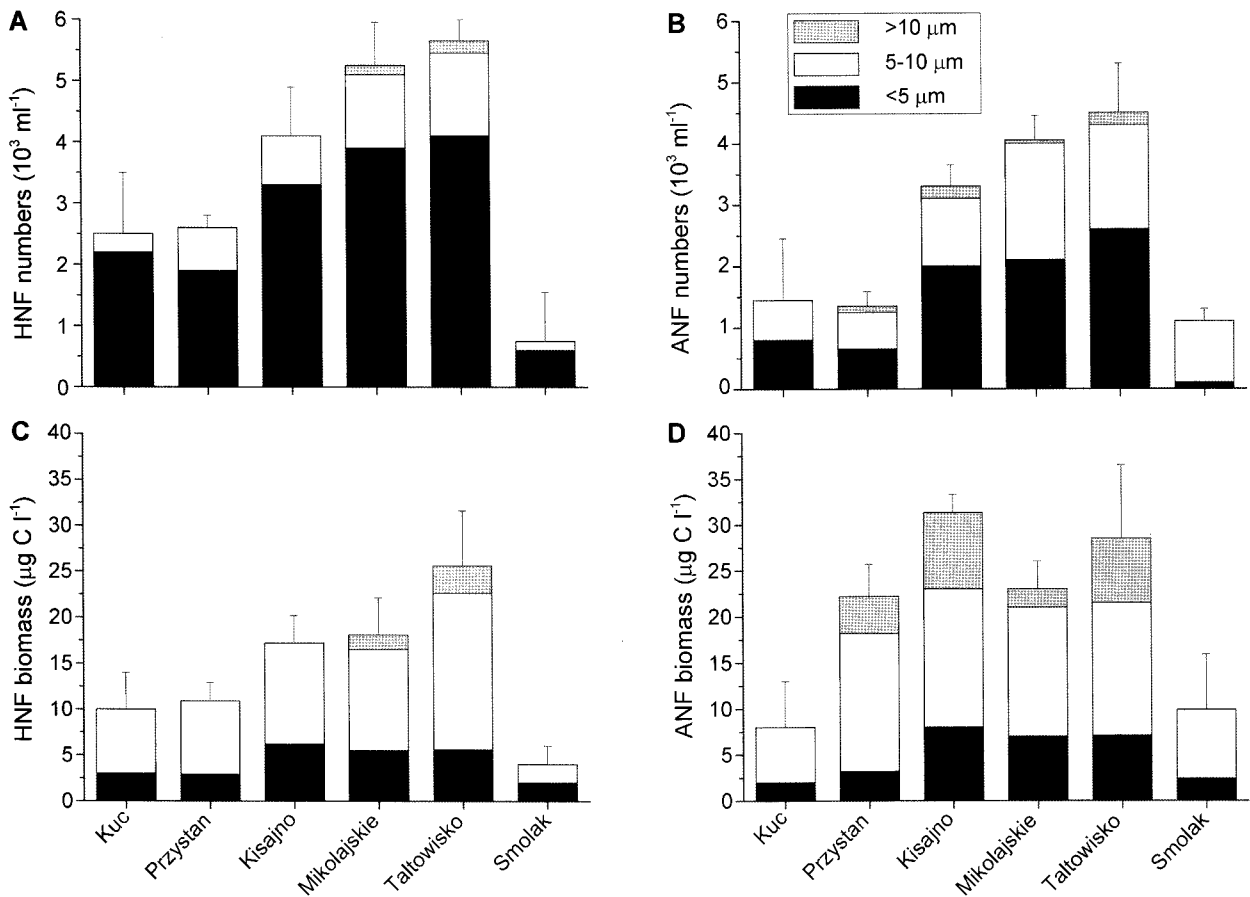


Fig. 5. Total numbers and biomass of heterotrophic (HNF) and autotrophic nanoflagellates (ANF) with three size classes: small (<5 µm), medium (5–10 µm), and large (>10 µm) cells marked in the studied lakes. A, HNF numbers; B, ANF numbers; C, HNF biomass; D, ANF biomass. Mean values from three sampling datasets±standard deviations for total numbers and biomass.

values (Fig. 5B). The mean ANF numbers ranged from $1.4 \pm 0.3 \times 10^3/\text{ml}$ (L. Przystań) to $3.8 \pm 0.8 \times 10^3/\text{ml}$ (L. Tałowisko). Similarly as HNF, relatively low ANF numbers were noted in L. Smolak ($0.7 \pm 0.01 \times 10^3/\text{ml}$). The small ANF cells dominated in lakes Kuc, Kisajno, and Tałowisko (53%–61%), whereas medium-sized ANF prevailed in the remaining lakes (50%–94%). The lowest mean ANF biomass of $7.5 \pm 5.4 \mu\text{g C/l}$ was noted in L. Smolak and the highest in L. Kisajno ($36.5 \pm 4.3 \mu\text{g C/l}$) (Fig. 5D). The significant part of ANF biomass was composed of medium-sized forms, which constituted from 39% (eutrophic L. Tałowisko) to 99% (humic L. Smolak). The large cells (>10 µm) of ANF were observed in most of the studied lakes; their proportion in L. Mikołajskie was negligible (2%), whereas in the remaining lakes was distinctly higher (20%–35%).

Ciliate Abundance, Biomass, and Composition

The mean numbers of ciliates ranged from $5.2 \pm 3.0 \text{ ind/ml}$ (L. Kuc) to $27.8 \pm 19.0 \text{ ind/ml}$ (L. Tałowisko) (Fig. 6A). Species belonging to the order of Haptorida (mainly small omnivorous *Mesodinium* sp.) dominated in lakes Kisajno and Mikołajskie (40% and 36% of total numbers, respectively).

In the remaining lakes, Oligotrichida, mainly composed of *Strombidium* and *Halteria*, was the most common ciliate group (37%–46% in non-humic lakes and 82% in humic lake).

The lowest mean value of ciliate biomass was noted in meso/eutrophic L. Przystań ($14.9 \pm 3.1 \mu\text{g C/l}$) and the highest in humic L. Smolak ($90.0 \pm 102.5 \mu\text{g C/l}$) (Fig. 6B). Oligotrichida was the dominant component of ciliate biomass in meso/eutrophic (54% and 45% of total biomass) and eutrophic lakes (43% and 55%), whereas Heterotrichida, was represented by large omnivorous *Stentor* in the mesotrophic L. Kuc (55%). Mass occurrence and intensive growth of mixotrophic species *Stokesia vernalis* (Hymenostomatida) contributed to the high value of ciliate biomass in L. Smolak (70%).

DISCUSSION

Differentiation of Microbial Loop Components Among Lakes

In our study, a majority of the studied microbial parameters differed among the studied lakes. However, only the bacterial

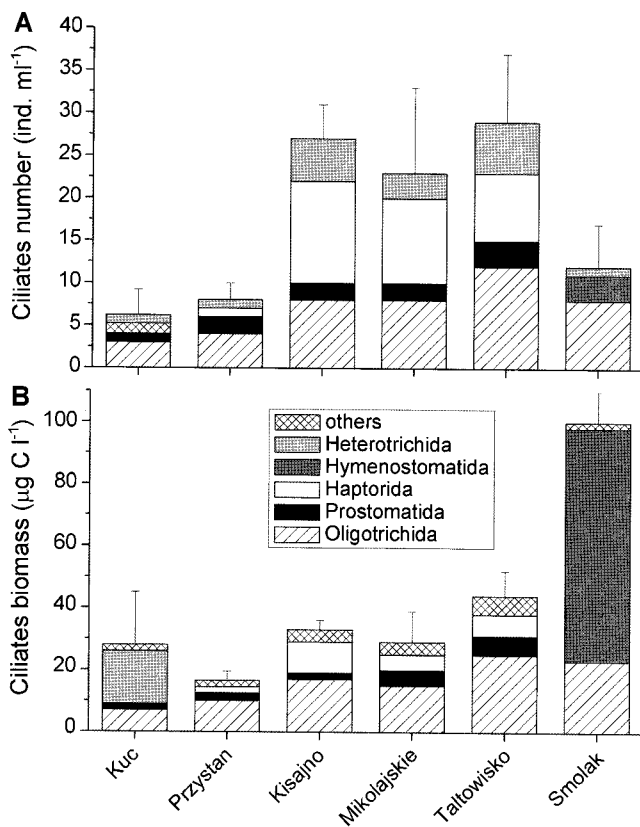


Fig. 6. Total ciliate numbers (A) and biomass (B) of ciliate taxonomic groups in the studied lakes. Mean values from three sampling datasets \pm standard deviations for total numbers and biomass.

production and HNF abundance increased clearly along the trophic gradient. The size distribution both of bacteria and HNF did not differ markedly among the studied lakes and the same size classes dominated. However, a distinct increase of large-sized bacteria and HNF contribution to total numbers and biomass with increasing lakes trophicity (from 10% to 23%) was observed. It is in line with results of studies carried out by Auer and Arndt [3] in 55 lakes of different trophic status in north-eastern Germany. The authors showed that the size distribution of HNF changed with lake productivity, with the higher proportion of large forms in hypertrophic lakes. There are not much data on bacterial size-structure in lakes. Results of studies conducted by Jürgens and Stolpe [25] in shallow eutrophic lake indicated that large bacteria (11%–33% of total biomass), mainly filamentous forms resistant to protistan grazing, appeared parallel to the HNF maxima. In our investigations, large-sized bacteria constituted 60%–77% of total BB, but we did not observe such long bacterial cells as for example in laboratory experiments, where bacterial cells of 10–50 μm length were observed as a consequence of strong grazing pressure by protists (mainly HNF) [20, 32].

Our results showed high variability of both bacterial and protistan numbers and biomass over short time scales (July,

August), as well as between studied years in the same lakes, reaching 2–3 orders of magnitude. The highest variability of microbial parameters was observed in eutrophic Lake Mikołajskie, in which ciliate biomass was more variable (coefficient of variation [CV]=121%) than the biomass both of HNF (CV=67%) and bacteria (CV=65%). These facts may indicate that microbial communities were strongly regulated by bottom-up and top-down controls. As shown by Weisse [60], the significance of bottom-up versus top-down controls may alter with lake productivity and season. Sanders *et al.* [52] found that in oligo- and/or mesotrophic environments, the bottom-up control is mostly important in regulating bacterial abundances; whereas in eutrophic systems, the top-down control may be more significant.

Considering mean TSI values as well as microbial parameters in the studied lakes, the most unexpected results were found in mesotrophic Lake Kuc. Despite the mean TSI value, indicating until now an oligo/mesotrophic [11] or mesotrophic (this study) character of this lake, it seems that Lake Kuc is undergoing the process of water eutrophication. This lake was characterized by the highest mean value of SD visibility (3.4 m) and the lowest concentrations of chlorophyll *a* (3.6 $\mu\text{g/l}$) and TP (21.2 $\mu\text{g P/l}$). The DOC concentration (9.4 mg/l) was very similar to values measured in both meso/eutrophic lakes. Kufel [31] demonstrated that mesotrophic lakes differ from meso/eutrophic ones not merely in nutrient and chlorophyll concentrations, but more in the way of nutrient utilization. As shown by Chróst and Siuda [11], organic matter in general and dissolved organic matter in particular constitute a basis for the metabolism of microheterotrophs. Because of their high abundance, and biochemical and molecular diversity and dynamics, microorganisms significantly influence the chemistry of aquatic ecosystems [11]. Therefore, the interactions between bacteria and organic matter are very important in the functioning of all aquatic ecosystems. In this study, values of bacterial numbers and biomass, as well as rates of bacterial production, were several times higher than those noted by Chróst *et al.* [10] and Chróst and Siuda [11] in the years 1994–1997. That is why Lake Kuc was similar to the remaining two meso/eutrophic studied lakes. Additionally, morphological parameters of bacteria such as cell length and width were similar to values noted in eutrophic lakes. We observed a high contribution of large-sized bacteria (>1.0 μm) to total BN (21%) and BB (77%), which was similar and even higher than in eutrophic lakes, probably indicating high grazing pressure by protists. A high proportion of bacterivorous ciliates in the mesotrophic Lake Kuc (24% of the total numbers; two times higher than in eutrophic lakes) seemed to be also surprising. Although ciliate numbers were the lowest among all the studied lakes, their biomass was higher than in meso/eutrophic Lake Przyszań. Moreover, the percentage share of ciliates in the total biomass of

protists was higher than nanoflagellates. Thus, all mentioned above findings seem to confirm Muylaert *et al.* [37] conclusions, who showed that ciliates were probably more important with respect to bacterivory in the four eutrophic shallow lakes owing to very low HNF biomass compared with ciliate biomass. Taking into account that, in the studied mesotrophic lake, values of microbial parameters were much higher than we expected basing on TSI value, we suggest that changes in microbiological parameters, particularly in bacterial and protistan biomass, may be useful indicators of lake trophic in addition to chemical parameters.

From among all the studied lakes, the greatest differences in examined microbial parameters were found in humic Lake Smolak. Many studies pointed out that humic lakes have specific chemical and biological properties, and thus bacterial response to protistan grazing may be different from that in non-humic lakes. It is mainly because of a high concentration of DOC of allochthonous origin, which is not readily assimilated by bacteria [10], and low pH values of water, which restrict growth of protists [27]. In our study, the humic lake was characterized by the lowest SD visibility (0.6 m), but the highest concentrations of chlorophyll *a* (60.3 µg/l), TP (40.7 µg P/l), and DOC (18.5 mg/l). However, the mean concentration of TP did not differ markedly from those in eutrophic lakes. Bacterial numbers were the lowest, and the biomass was higher in comparison with meso/eutrophic ones. Both HNF and ANF numbers and biomass were the lowest among the six studied lakes. Among nanoflagellates, mixotrophic forms composed of medium-sized cells were more abundant than heterotrophs. An analysis of ciliate species composition showed that this lake was especially poor in species, among which large-bodied mixotrophic taxa (oligotrichs *Strombidium* and heterotrichs *Stokesia vernalis*) dominated and determined the much higher biomass as compared with the remaining lakes. These mixotrophs are able to ingest nanoplanktonic algae in addition to bacteria and may be important components in plankton, especially during periods of nutrient limitation [4, 5]. The mixotrophic mode of nutrition allows them to compete for some food sources with other microorganisms. On the other hand, according to data found by Domaizon *et al.* [14], autotrophic flagellates may feed on bacteria more effectively than heterotrophic forms. Chróst *et al.* [10] showed that the highest grazing rate among all studied Masurian lakes existed in polyhumic Lake Smolak, because of the decidedly highest BP and relatively low BB. Results of our studies confirm this assumption, because values of morphological parameters (length and width of bacterial cells) were the lowest among the studied lakes, bacterial production and biomass were higher than in meso/eutrophic lakes, and BTR was similar or slightly longer than in eutrophic ones. However, taking into account that the biomass of autotrophic nanoflagellates was 3 times

higher than HNF, it seems that these forms might be responsible for predation pressure on bacteria in the studied humic lake.

Relations Among Various Components of the Microbial Loop

Numerous studies demonstrate that both bacterial and protistan parameters (*e.g.*, abundance, biomass) as well as intensity of microbial processes (*e.g.*, utilization of organic matter, enzymatic activity, biomass production) are tightly coupled to the trophic conditions of lakes [3, 4, 10, 11, 40]. However, there are not much data about the relations between bacteria and their potential consumers in lakes along the eutrophication gradient [11]. In this study, changes in BN and BB along the trophic gradient of the studied lakes were not related to DOC concentrations. It suggests that grazing might be a crucial factor to control bacterial density. Statistical analysis showed significant positive correlation between bacterial and HNF numbers ($r=0.59$, $p<0.05$). Total ciliate numbers did not show significant correlation with bacteria. However, when the taxonomic groups of ciliates were included in the analysis, the numbers of bacteria were positively related to the numbers of bacterivorous Peritrichida ($r=0.60$, $p<0.05$). Šimek *et al.* [58] showed that the role of HNF grazing decreased along the trophic gradient from meso- to eutrophy, in contrast with the increasing role of ciliates as bacterivores. In our study, peritrichs ciliates were mainly attached to the filamentous cyanobacteria *Anabaena*, and thus they were protected against metazoan predation. These sessile bacterivorous ciliates, even at low densities, may strongly affect bacteria because of the high grazing rate [27, 57].

The selective HNF grazing pressure on bacterial structure has been well demonstrated mainly in laboratory experiments [*e.g.*, 18, 26, 32]. Thus, in our study, we additionally calculated correlations for size classes of bacteria and nanoflagellates that revealed significant relations among them. Small bacteria (0.2–0.5 µm) had significant relationship with the small HNF ($r=0.65$, $p<0.05$), whereas medium-sized bacteria (0.5–1.0 µm) were related to the large HNF ($r=0.58$, $p<0.05$) as well as to the medium-sized ANF ($r=0.81$, $p<0.001$). These correlations may suggest that nanoflagellate species of various sizes differed in their relation to size of bacteria, and that medium-sized bacteria were preferentially consumed by nanoflagellates. Only large-sized (>1.0 µm) bacteria were positively related to the numbers of peritrichs ciliates ($r=0.57$, $p<0.05$). As given in Kivi and Setälä [28], each ciliate species shows a specific particle size preference (the optimal size from 1.4 to 9.8 µm), suggesting a possible ability to utilize nanoflagellate-size food as well as bacteria-size prey.

Heterotrophic nanoflagellates are recognized as effective predators of metabolically active bacteria because they are

rich in nutrients [17]. As given in Koton-Czarnecka and Chróst [30] and Chróst and Siuda [11], protistan grazing rates on live bacteria were almost two times higher than on inactive bacteria. There is also evidence that the proportion of active cells tends to increase with increasing trophic state; however, factors regulating their numbers and proportion (temperature, resources limitation, grazing, and viral lysis) are still unclear [12, 13]. In our study, the contribution of MEM+ bacteria to total BN and BB did not follow increasing trend with trophy. Grazing pressure of HNF appeared to be one of the most important factors responsible for keeping bacterial numbers constant in the studied lakes. The numbers of active bacteria (MEM+) were positively related to the total HNF numbers ($r=0.64$, $p<0.05$).

Depending on the species diversity of protistan assemblages in lakes, grazing may affect the taxonomic composition of bacterial communities by preferential elimination of bacteria belonging to various groups. Selective predation by HNF has already been demonstrated in many studies [20, 22, 34]. Lebaron *et al.* [32] in mesocosm studies found that γ -*Proteobacteria* were preferentially consumed, whereas α -*Proteobacteria* were protected from grazing pressure. On the contrary, Jezbera *et al.* [23] demonstrated that two nanoflagellates species (*Bodo saltans* and *Goniomonas* sp.) preferably ingested β -*Proteobacteria* over γ -*Proteobacteria*, whereas ciliate *Cyclidium glaucoma* showed no clear preferences. In our study, β -*Proteobacteria* was clearly the most abundant group in all lakes, except Lake Kisajno. It is in agreement with others studies in freshwater habitats [42]. The contribution of bacteria belonging to the α -*Proteobacteria* was very small and similar in all lakes (3%–6%). This group of bacteria was significantly and negatively correlated with the total numbers of HNF ($r=-0.63$, $p<0.05$). Contrary to the above statement by Lebaron *et al.* [32], this finding implies that increased HNF density may lead to considerable reduction of this group of bacteria in the Mazurian lakes. In our study, bacteria belonging to the *Cytophaga-Flavobacterium* cluster were present in high density only in meso/eutrophic Lake Kisajno (2–3 times higher than in the remaining lakes). Several authors suggest that some members of the *Cytophaga-Flavobacterium* cluster possess a defense strategy against grazing and may play a key ecological role along the eutrophication gradient [19, 32, 46].

As shown by Weisse [60], ciliated protists may be significant predators of HNF in lakes. In our study, both ANF and HNF numbers showed positive correlations with dominating taxonomic groups of ciliate communities, with the exception of Peritrichida mentioned above. The abundance of Haptorida, mainly composed of omnivorous *Mesodinium*, was positively correlated with the ANF abundance ($r=0.60$, $p<0.05$). Among heterotrophic nanoflagellates, medium-sized HNF exhibited strong relationships with the abundance of ciliate orders such as Oligotrichida ($r=0.80$, $p<0.01$) and

Prostomatida ($r=0.71$, $p<0.01$). Thus, it seems that ciliate groups, growing with increasing flagellate abundance, preferentially grazed on particles of nanoflagellate size between 2 and 20 μm [15].

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