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Ciliates versus other components of the microbial loop in the psammolittoral zone: horizontal distribution

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Abstract: The objective of this study was to determine the abundance and biomass of microbial loop components in 2 lakes of different trophic status. The lakes (mesotrophic and eutrophic ones) were located in the Łęczna-Włodawa Lakeland. The effect of selected physical and chemical water parameters on this group of organisms was also analyzed. Psammon samples were collected during 3 seasons: spring, summer, and autumn 2011. In each of the lakes, samples were collected in the euarenal, higroarenal, and hydroarenal zones. The highest abundance and biomass of the microbial loop components were recorded in spring in the eutrophic lake. The mesotrophic lake showed an increase in flagellates in autumn with a decline in the abundance of ciliates. In the mesotrophic lake, the density of individual elements of the microbial loop was correlated with temperature and total organic carbon, and in the eutrophic lake with pH, chlorophyll a, and ammonium nitrogen. In both lakes, a significant correlation occurred between bacteria and ciliate abundances. In both trophic types of lakes, the highest correlations between bacteria and heterotrophic protists were noted in hydroarenal zone. In the euarenal and higroarenal zones, the correlations were weaker.

Key words: lake, microbial loop, psammon

1. Introduction

The term "microbial loop" was originally coined by Azam et al. (1983). The microbial loop describes a trophic pathway in the freshwater food web, where dissolved organic carbon is returned to higher trophic levels via its incorporation into bacterial biomass and is then coupled with the classical food chain. The microbial loop consists of groups of microorganisms such as bacteria, nanoflagellates, and ciliates. This structure plays an important role in the trophic food web in aquatic ecosystems, affecting carbon and nutrient flows (Pomeroy, 1974; Azam et al., 1983; Kalinowska, 2004). Heterotrophic bacteria play an essential role in processes of decomposition and utilization of organic matter within the microbial loop. They also show the ability to transform the dissolved organic matter into particular organic matter and then to protistan grazing transfer (Koton-Czarnecka and Chróst, 2003; Chróst et al., 2009). In this way, bacteria transmit carbon to higher trophic levels in aquatic ecosystems (Chróst et al., 2009; Gudasz et al., 2012). Heterotrophic nanoflagellates are major consumers of bacteria and picoplanktonic algae in different freshwater habitats (Kalinowska, 2004). Apart from flagellates and bacteria, they are a source of food for other groups of protists such as ciliates. Ciliates can use multiple sources of food, e.g., pico- and nanophytoplankton (Sanders et al., 1989; Szeląg-Wasielewska and Fyda, 1999). This group of Protista represents a link to higher trophic levels (Kalinowska, 2004).

Previous studies on the structure and functioning of microbial loop communities concerned both marine and freshwater ecosystems (Hagström et al., 1988; Sanders et al., 1992). In freshwater ecosystems, the functioning of the microbial loop probably depends on the trophic status of reservoirs (Porter et al., 1988; Weisse et al., 1990). Previous studies demonstrated the important role of the microbial loop not only in hyper- and eutrophic lakes, but also in oligotrophic and humic lakes (Weisse et al., 1990; Amblard et al., 1995; Arvola et al., 1996).

The functioning of the microbial loop depends on a number of factors, including physical, chemical, and biological. The number of individual elements in the pelagic zone is also reduced by controlling "top down" or "bottom up" processes in the trophic pyramid (Chróst et al., 2009). Hardly any information is available on the functioning of the microbial loop in the psammolittoral zone in lakes of different trophic status.

The psammolittoral is a part of the lake ecosystem. Psammon is an assemblage of organisms living in the

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psammolittoral-coastal zones of rivers and both freshwater and saltwater lakes (Wiszniewski, 1934). Wiszniewski (1937) recognized 3 zones within the psammolittoral: the hydroarenal (permanently submerged), higroarenal (sand wetted by lake waves), and euarenal (including emergent sand). The zone includes assemblages of organisms with various nutritional preferences. This is probably related to its significant dynamics of transformation and the resulting necessity of organisms to adjust to the occurring environmental conditions. The composition of psammon includes bacteria, algae, protozoa, rotifers, nematodes, and also tardigrades and crustaceans (Lokko et al., 2013). According to Novitsky and MacSween (1989), bacteria constitute over 90% of the numbers of these microorganisms. Sandy beaches are habitats very rich in organic matter supplied from the pelagic zone by wave action and transported with surface flow from the surrounding land. High amounts of dissolved and particulate organic matter are absorbed on the surface of sand grains (Jędrzejczak, 1999), creating optimal conditions for bacterial growth (Mudryk et al., 2001).

The available literature includes few reports on the distribution of elements of the microbial loop in the psammolittoral zone. According to Koop et al. (1982), Jędrzejczak (1999), and Ochieng and Erftemejer (1999), bacteria play a key role in the decomposition and mineralization of beached organic matter and nutrient recycling into the near shore ecosystem. Bacteria mainly utilize the metabolic products of phytobenthos, animal feces (mainly those produced by meio- and macrofauna), and dead remains of plants and animals as their food sources (Koop and Griffiths, 1982; Mudryk et al., 2001). The high concentration of nutrients, chlorophyll *a* content and primary production (Czernaś et al., 1991), and high numbers and species richness of psammonalgae (Kalinowska et al., 2011), ciliates (Kalinowska, 2008; Mieczan and Nawrot, 2012 a, 2012b), rotifers (Ejsmont-Karabin, 2008; Nawrot and Mieczan, 2012), and crustaceans (Kalinowska et al., 2010) suggest that the structure and function of the food web components in psammolittoral habitats should be similar to those in the pelagic zone.

This research was undertaken in order to verify the following hypotheses: the fertility of habitats may significantly affect the abundance of individual elements of microbial loop components and the strength of their interrelationships; the physical and chemical parameters of waters significantly influence the abundance of bacteria, nanoflagellates, and ciliates; and the abundance of bacteria and heterotrophic Protista show considerable variation in horizontal distribution in the psammolittoral zone.

2. Materials and methods

Two lakes of the Łęczna-Włodawa Lakeland (eastern Poland: 51°N, 23°E; Figure 1) were selected: mesotrophic Lake Piaseczno (area: 84.7 ha, maximum depth: 38.8 m), with a well-developed sandy psammolittoral, and eutrophic Lake Sumin (area: 91.5 ha, maximum depth: 6.5 m), with a well-developed psammolittoral and a phytolittoral pond type. In the eulittoral of Lake Piaseczno, the emergent vegetation is very scarce (*Carex arenaria* L.). In Lake Sumin, well-developed belts of emergent [*Phragmites australis* (Cor.), Trin. ex Steud. and *Typha latifolia* L.] and submerged (*Elodea canadiensis* L.) vegetation dominate the littoral zone (Radwan and Kornijów, 1998).

Microbial samples were collected in spring, summer, and autumn 2011. The samples of psammon were taken from 3 zones of the arenal: the euarenal, including sand up to a distance of 1 m from the water line; the higroarenal, at the shoreline; and the hydroarenal, permanently submerged and reaching approximately 1 m into the lake (Figure 1).

The samples were collected with a plastic sharp-edged barrel, 60 mm in diameter, at 4 sites of each zone. In each term, 12 samples were taken from each lake. Each sample of bacteria was fixed in situ with formalin (final concentration of 2%). Subsamples of 1 mL of bacteria plus 9 mL of sterile water, and up to 10 mL for nanoflagellates, were stained with DAPI (final concentration: 1 µg/

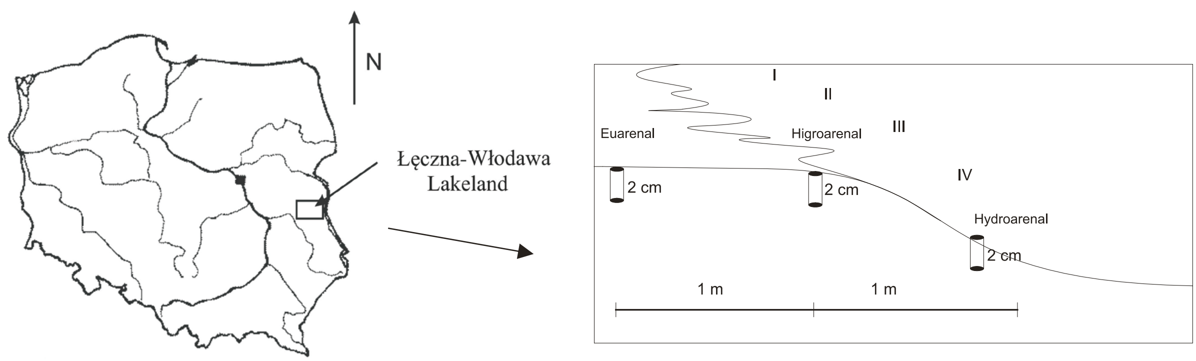

Figure 1. Location of study area.

mL) (Porter and Feig, 1980), filtered through black polycarbonate membrane filters (Millipore) with a pore size of 0.2 µm for bacteria and 0.8 µm for nanoflagellates, and enumerated by means of epifluorescence microscopy. Bacterial and nanoflagellate biovolume was calculated from measurements of cells and approximated to simple geometrical forms (Kalinowska, 2004). Ciliate samples were also shaken with filtered water and immediately fixed with Lugol's solution (0.2% final concentration). Abundance of heterotrophic protists was calculated per 1 cm^3 of sand. Observation of living samples was used for the taxonomic identification of ciliates. The species identification of ciliates was based on Foissner and Berger (1996). Ciliate biomass was estimated by multiplying the numerical abundance by the mean cell volume calculated from direct volume measurements with the application of appropriate geometric formulas. Therefore, the calculated cell volumes were multiplied by a correcting factor of 0.4 (Jerome et al., 1993).

2.1. Physical and chemical analyses

At each time point, the physical and chemical parameters [pH, conductivity, temperature, total organic carbon (TOC), total phosphorus (P_{tot}), P-PO_4, N-NO_3, N-NH_4, and chlorophyll *a*] were examined at 3 sites: euarenal (interstitial water), higroarenal, and hydroarenal. Temperature, conductivity, and pH were determined in situ using the multiparameter sensor 556 MPS (YSI). TOC was determined using the PASTEL UV. The remaining parameters were analyzed in the laboratory following the methods of Hermanowicz et al. (1976). Chlorophyll *a* was determined by means of the spectrophotometric analysis of alcohol extracts of algae retained on polycarbonate filters. In addition, granulometric analyses of the sand of the psammolittoral were performed. In order to measure the grain size structure of sand, sand samples (500 g) dried at 105 °C were divided into 8 fractions (>0.9 mm, 0.6–0.9 mm, 0.4–0.6 mm, 0.25–0.4 mm, 0.15–0.25 mm, 0.1–0.15 mm, 0.063–0.15 mm, and <0.063 mm) by means of a set of nets of different mesh sizes and were then weighed.

2.2. Statistical analyses

Ordination techniques were used to describe the relationships between the abundance of bacteria, nanoflagellates, and ciliates and the environmental variables. The same were used to determine the relationship between the occurrence of psammonic organisms and environmental parameters. The results with the largest interquartile range were logarithmized in order to normalize the distribution. The indirect multivariate method, detrended correspondence analysis, was used to measure and illustrate gradients indicated by psammonic microorganisms communities. Due to the length of the gradient with a range of 3 to 4 standard deviations, redundancy analysis (RDA) was applied in order to determine the relationships between psammonic organisms and environment parameters. The parameters of the environment with the greatest impact on the occurrence of ciliate taxa were determined by means of stepwise variable selection (Monte Carlo permutation test). Variables for which $P < 0.1$ were considered significant. The analyses were performed with the application of the ordination program CANOCO 4.5 for Windows. Pearson correlation coefficients were calculated between pairs of variables in order to determine the relationship between microbial loop components and physical and chemical parameters.

3. Results

3.1. Physical and chemical parameters

Irrespective of the lake's trophic status and zone analyzed, the water temperature and pH did not differ significantly (Table 1). Conductivity, N-NO_3, N- NH_4, and TOC reached their highest values in the eutrophic lake. The mesotrophic lake was distinguished by lower values of these parameters. In this lake, conductivity ranged from 83 µS cm^{-1} in the higroarenal to 92 µS cm^{-1} in the hydroarenal. The highest content of N-NH_4 was recorded in the euarenal in the mesotrophic lake at 0.73 mg NH_4 dm^{-3} and the lowest in the hydroarenal zone of the mesotrophic lake at 0.11 mg NH_4 dm^{-3}. P-PO_4 concentrations reached the highest values in the euarenal zone in both lakes (eutrophic: 0.134 mg PO_4 dm^{-3}, mesotrophic 0.029 mg PO_4 dm^{-3}). The highest total phosphorus concentrations were recorded in the euarenal of the eutrophic lake (0.134 mg P dm^{-3}), and the lowest in the hydroarenal zone (0.007 mg P dm^{-3}) of the mesotrophic lake. The average content of TOC in the eutrophic lake (18.33 mg C dm^{-3}) was higher than in the mesotrophic lake (4.12 mg C dm^{-3}). The granulometric analyses revealed the size of particles in both lakes to be between 0.15 and 0.6 mm (Table 2).

3.2. Abundance and biomass of microbial loop components in psammolittoral zone

The abundance of bacteria showed a clear differentiation between lakes and zones analyzed. The highest number of bacteria was observed in spring in the hydroarenal zone in the eutrophic lake (5.96×10^7 cm^{-3}) (Figure 2). In the mesotrophic lake, the zone with the highest density of bacteria was the euarenal in the summer season (4.1×10^7 cm^{-3}). In the mesotrophic lake, the zone with the lowest density of bacteria was the hydroarenal in autumn, and in the eutrophic lake, it was the euarenal in summer. In the eutrophic lake, regardless of the zone analyzed, the abundance of bacteria reached the highest values in spring and autumn and the lowest in summer. The mesotrophic lake showed similar trends, with the exception of the euarenal zone, where high numbers of bacteria occurred in summer (4.1×10^7 cm^{-3}) (Figure 2).

Table 1. Physical and chemical characteristic of water in investigated lakes. *: average, **: standard deviation.

Lake /	Zone	Temp.	pH	Conductivity, µS cm^{-1}	$N\text{-}NH_4$, mg N dm^{-3}	$N\text{-}NO_3$, mg N dm^{-3}	PO_4, mg PO_4^{3-} dm^{-3}	P_{tot}, mg P dm^{-3}	TOC, mg C dm^{-3}	Chlorophyll *a*, µg dm^{-3}
Mesotrophic	Euarenal	*17.76	6.66	92.00	0.731	0.084	0.029	0.224	12.12	33.92
		**9.94	0.06	13.45	0.580	0.059	0.022	0.077	9.39	43.88
	Higroarenal	*19.83	7.27	83.33	0.223	0.060	0.009	0.066	4.78	7.61
		**9.10	0.47	11.93	0.187	0.068	0.008	0.034	1.79	0.28
	Hydroarenal	*19.92	7.42	89.33	0.112	0.032	0.007	0.114	4.12	7.79
		**8.64	0.59	2.08	0.054	0.020	0.001	0.138	0.29	1.98
Eutrophic	Euarenal	*17.66	7.50	424.33	0.355	0.168	0.134	0.213	18.33	17.84
		**9.44	0.48	12.22	0.073	0.194	0.224	0.240	5.86	14.03
	Higroarenal	*17.44	7.94	364.00	0.307	0.100	0.008	0.054	14.40	8.31
		**9.65	0.60	24.33	0.040	0.028	0.009	0.015	0.76	2.84
	Hydroarenal	*17.31	8.01	380.33	0.573	0.129	0.053	0.087	14.57	9.65
		**9.69	0.50	19.73	0.204	0.070	0.055	0.055	0.29	5.03

Table 2. Grain size of sand in the psammolittoral zones examined (%).

Lake / Zone / Grain size of sand	Mesotrophic			Eutrophic		
	Euarenal	Higroarenal	Hydroarenal	Euarenal	Higroarenal	Hydroarenal
>0.9	0.16	0.90	1.27	0.04	3.89	0.38
>0.6	0.74	5.03	6.41	0.21	9.39	1.14
>0.4	6.78	16.90	23.58	1.76	20.46	6.70
>0.25	56.26	35.48	43.11	42.85	31.59	30.08
>0.15	34.51	29.48	19.98	53.32	31.68	49.63
>0.1	1.39	8.45	4.04	1.66	2.83	10.39
>0.063	0.12	2.64	1.14	0.13	0.14	1.44
Residue	0.04	1.12	0.47	0.03	0.01	0.23

The highest density of flagellates was observed in the hydroarenal zone of the eutrophic lake in the spring season (29.7×10^3 cm^{-3}) and the lowest in the hydro- and euarenal zones in the mesotrophic lake (7 and 4.8×10^3 cm^{-3}). Independently of the trophic type of lake, in the higroarenal zone, a similar density of flagellates was recorded. Irrespective of the zone, the eutrophic lake was distinguished by a higher biomass of flagellates compared to the corresponding zones of the mesotrophic lake. In the eutrophic lake, irrespective of the zone analyzed, the abundance of flagellates reached the highest values in spring and autumn and the lowest in summer. In the mesotrophic lake the highest density of these microorganisms occurred in autumn.

The highest abundance of ciliates occurred in the hydroarenal zone of the eutrophic lake in the spring season (300 ind. cm^{-3}), and the lowest occurred in the euarenal zone of the mesotrophic lake in autumn (20 ind. cm^{-3}) (Figure 2). The zone with the highest number of ciliates was the higroarenal in the spring season in the mesotrophic lake (209 ind. cm^{-3}). The lowest numbers of ciliates in the eutrophic lake were observed in the higroarenal zone in the summer season (74 ind. cm^{-3}). Microbial biomass showed a clear differentiation between the zones analyzed. The highest biomass occurred in the euarenal zone of the eutrophic lake in the spring season, and the lowest occurred in the same zone of the mesotrophic lake in autumn. In both lakes, the eu- and hydroarenal zone were

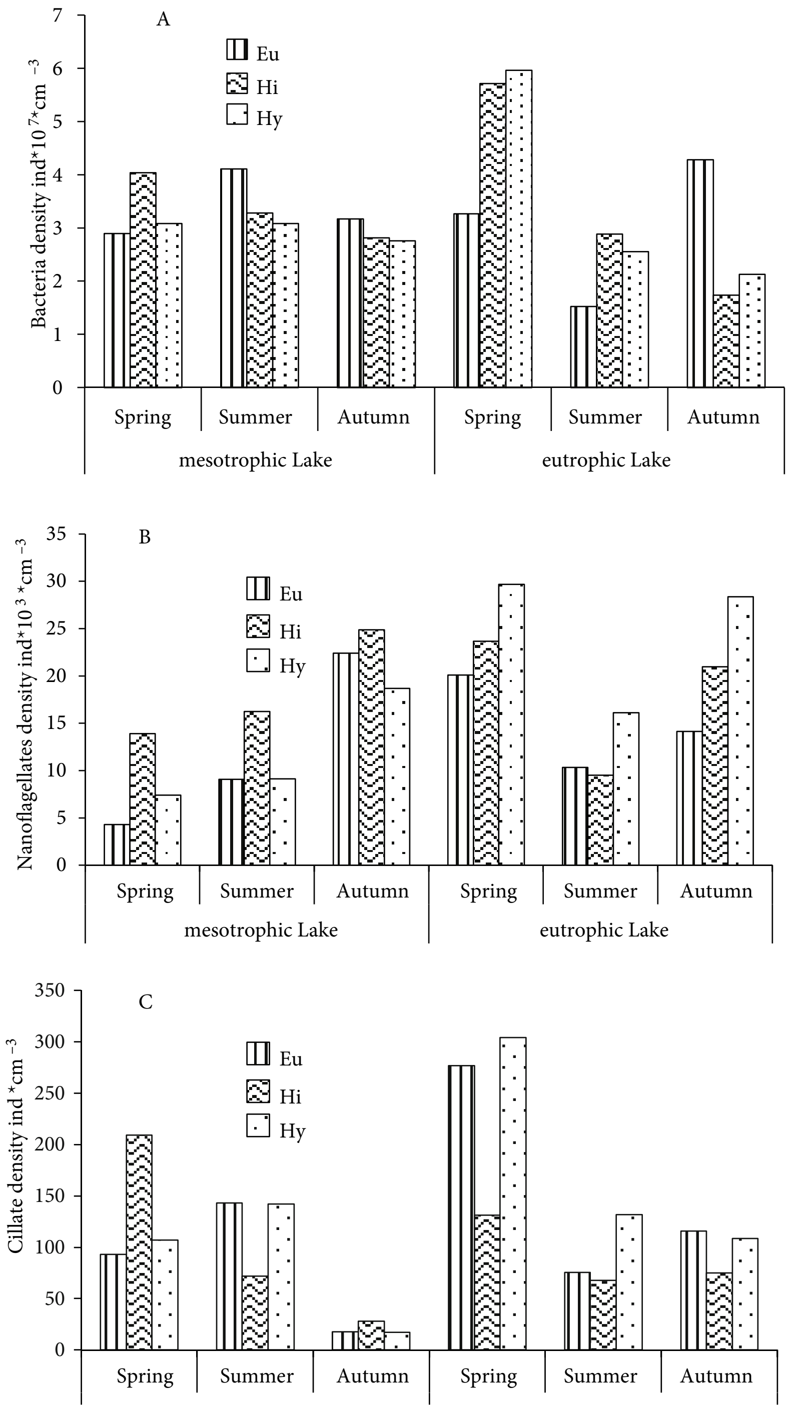

Figure 2. Density of psammon bacteria (A), nanoflagellates (B), and ciliates (C) in investigated lakes (Eu = euarenal, Hi = higroarenal, Hy = hydroarenal).

distinguished by similar biomass of individual components of the microbial loop. The aforementioned zones in the mesotrophic lake were dominated by bacterial biomass, and biomass of ciliates dominated in the eutrophic lake (Figure 3).

3.3. Diversity and trophic composition of ciliates

The taxonomic richness of ciliates remained at a similar level in both the lakes. In the mesotrophic lake, 30 ciliate taxa were recorded, and in the eutrophic lake, 31 taxa. The highest contribution in the total numbers of ciliates was

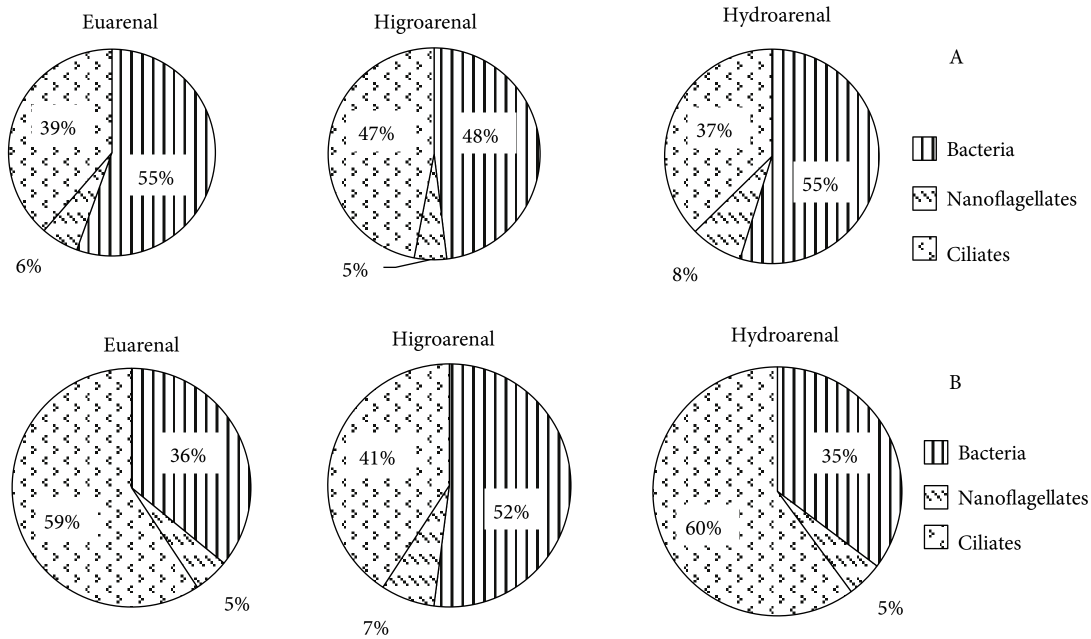

Figure 3. Biomass of components of microbial loop components in 3 zones of psammolittoral in investigated lakes: A) mesotrophic lake, B) eutrophic lake.

reached by omnivorous Hymenostomatida (from 12% to 78% of the total numbers of ciliates). In the mesotrophic lake, Scuticociliatida had a significant contribution (up to 62%), and in the eutrophic lake, Oligotrichida (up to 47%). In the hydroarenal zone of both lakes, Pleurostomatida had a significant contribution (Figure 4). The trophic structure of ciliates was clearly differentiated between the lakes. Irrespective of the zone analyzed in the mesotrophic lake, bacterivorous taxa were dominant (up 90% of the total number). In the eutrophic lake, the participation of taxa feeding on algae and flagellates increased (to 51%). In the eu- and higroarenal zones in the eutrophic lake, an increase of mixotrophic taxa was observed (Figure 5).

3.4. Ordination analyses

The Monte Carlo permutation test showed that in the mesotrophic lake, the factors with the greatest impact on the occurrence of ciliates were temperature (□ = 0.25, F = 2.38, P = 0.014), chlorophyll *a* (□ = 0.12, F = 4.06, P = 0.078), and TOC (□ = 0.20, F = 3.34, P = 0.094); in the eutrophic lake, these were pH (□ = 0.17, F = 2.17, P = 0.074), N-NO_3 (□ = 0.14, F = 2.45, P = 0.082), and N-NH_4 (□ = 0.10, F = 4.83, P = 0.088) (Figure 6). In the mesotrophic lake, the factor with the greatest impact on the occurrence of individual elements of the microbial loop were temperature (□ = 0.77, F = 23, P = 0.002), TOC (□ = 0.12, F = 7.33, P = 0.050), and P_{tot} (□ = 0.04, F = 17.81, P = 0.092). In the eutrophic lake,

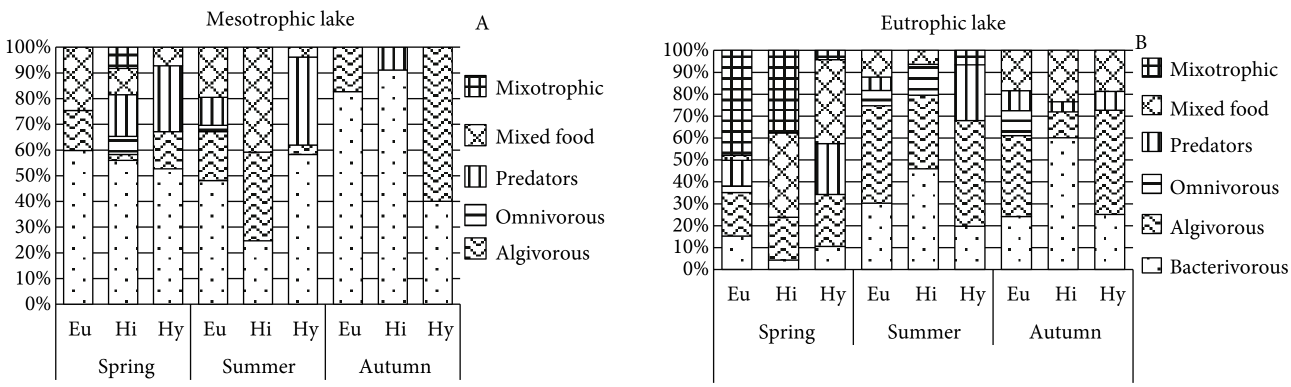

Figure 4. Trophic group of psammonic ciliates of investigated lakes.

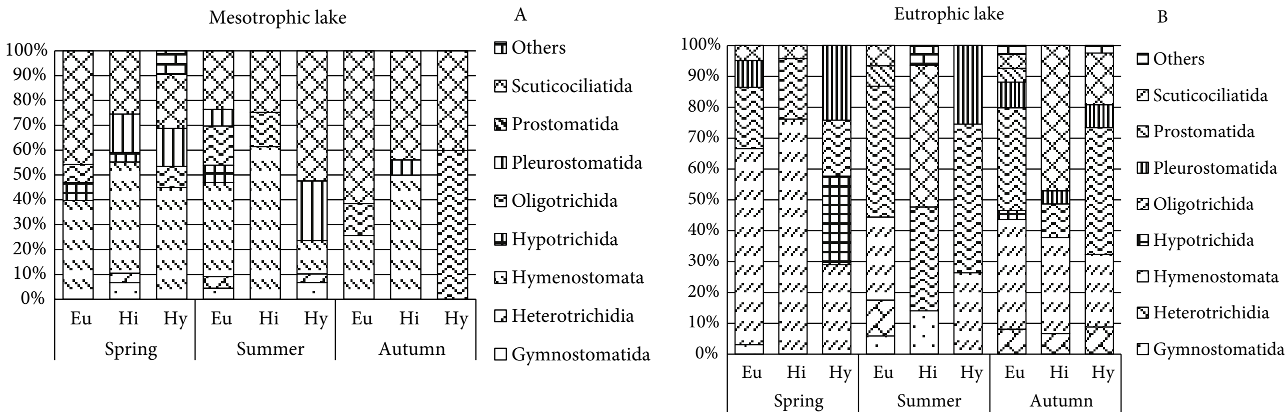

Figure 5. Domination structure of psammonic Ciliata orders in psammolittoral of investigated lakes (% of total numbers): A) mesotrophic lake, B) eutrophic lake.

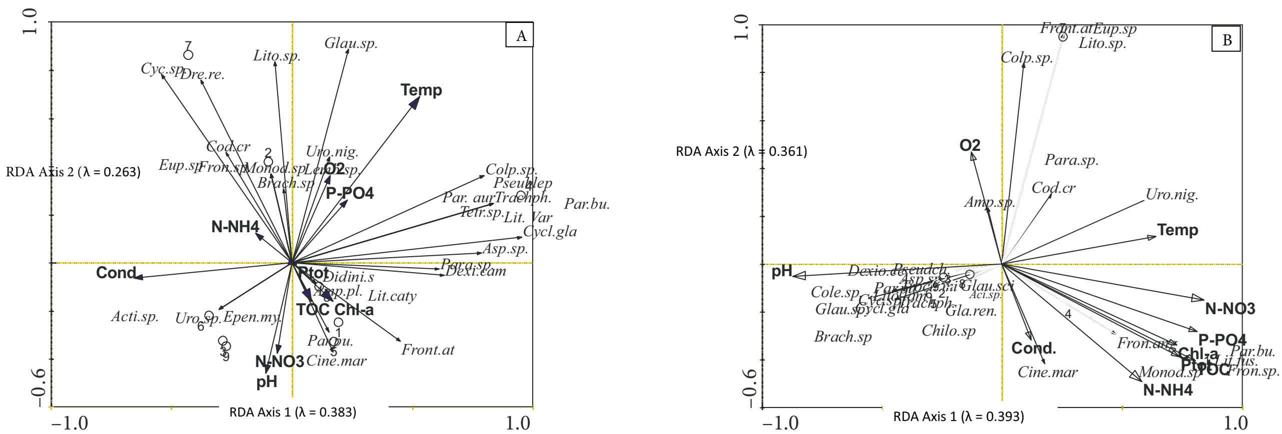

Figure 6. Redundancy analysis (RDA): A) mesotrophic lake, B) eutrophic lake triplots for samples collected, ciliate species, and environmental variables. Temp = water temperature, Cond. = conductivity, O2 = dissolved oxygen, Chl-a = chlorophyll-a, N-NH4 = ammonium nitrogen, N-NO3 = nitrate nitrogen, Ptot = total phosphorous, P-PO4 = dissolved orthophosphates, TOC = total organic carbon. *Trochilia minuta*: *Troch.min.*, *Pseudochilodonopsis* sp.: *Pseud.sp.*, *Didinium* sp.: *Didini.sp.*, *Monodinium* sp.: *Monod.sp.*, *Tracheophyllum* sp.: *Trachph.sp.*, *Brachonella spiralis*: *Brach.spir.*, *Pseudoblepharisma tenue*: *Pseudobl.t.*, *Colpidium* sp.: *Colp.sp.*, *Dexiostoma campylum*: *Dexi.cam.*, *Dexiotrichides centralis*: *Dexio.ce.*, *Epenardia myopyri*: *Epen.my.*, *Frontonia* sp.: *Fron.sp.*, *Frontonia angusta*: *Fron. ang.*, *Frontonia atra*: *Front.at.*, *Frontonia angusta*: *Front.an.*, *Glaucoma* sp.: *Glau.sp.*, *Glaucoma reniforme*: *Gla.ren.*, *Glaucoma scinitalis*: *Glau.sci.*, *Lembadion* sp.: *Lemb.sp.*, *Paramecium* sp.: *Para.sp.*, *Paramecium aurelia*: *Par. aur.*, *Paramecium bursaria*: *Par.bu.*, *Paramecium putrinum*: *Par.pu.*, *Tetrahymena* sp.: *Tetr.sp.*, *Uronema* sp.: *Uro.sp.*, *Uronema nigricans*: *Uro.nig.*, *Aspidisca* sp.: *Asp.sp.*, *Euplotes* sp.: *Eup.sp.*, *Chilodontopsis* sp.: *Chilo.sp.*, *Drepanomonas revolute*: *Dre.re.*, *Codonella cratera*: *Cod.cr.*, *Acineria* sp.: *Aci.sp.*, *Amphileptus* sp.: *Amp.sp.*, *Amphileptus pleurosigma*: *Amp.pl.*, *Litonotus* sp.: *Lito.sp.*, *Litontus catynematum*: *Lit.caty.*, *Litonotus fusidens*: *Lit.fus.*, *Litonotus lamella*: *Lito.lam.*, *Litonotus varsaviensis*: *Lit.var.*, *Coleps* sp.: *Cole.sp.*, *Cinetochilum margaritaceum*: *Cine.marg.*, *Cyclidium* sp.: *Cyc.sp.*, *Cyclidium glaucoma*: *Cycl.gla.*, *Actineta* sp.: *Acti.sp.*

the abundance of individual elements of the microbial loop depended on pH (λ = 0.34, F = 3.59, P = 0.058), dissolved oxygen (λ = 0.27, F =4.13, P = 0.034), and conductivity (λ = 0.14, F = 2.79, P = 0.094) (Figure 7).

3.5. Relations between microbial loop components

The correlation analysis (Pearson correlation coefficients) shows that, irrespective of the lake's trophic status, ciliate biomass was correlated with concentrations of chlorophyll *a* and abundance of bacteria (mesotrophic lake: r = 0.31 and r = 0.58, P = 0.05; eutrophic lake: r = 0.55 and r = 0.61, P = 0.05). In the mesotrophic lake, a strong negative correlation occurred between the density of bacteria and flagellates (r = –0.69, P = 0.05), and in the eutrophic lake a significant relationship was observed between nanoflagellates and ciliates (r = –0.31, P = 0.05). The strength of the interactions among bacteria, flagellates, and

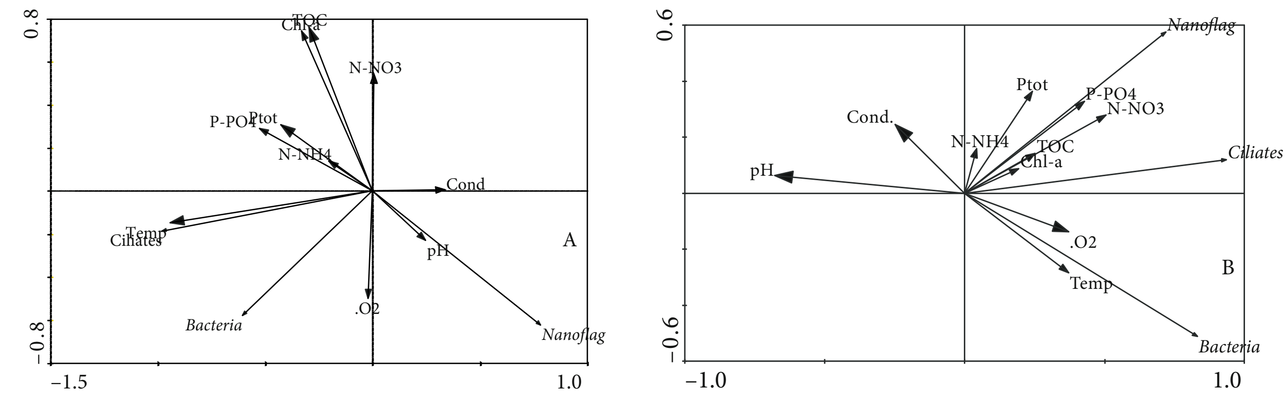

Figure 7. Redundancy analysis (RDA): A) mesotrophic lake, B) eutrophic lake triplots for samples collected, groups of organisms, and environmental variables. Temp = water temperature, Cond. = conductivity, O2 = dissolved oxygen, Chl-a = chlorophyll-a, N-NH4 = ammonium nitrogen, N-NO3 = nitrate nitrogen, Ptot = total phosphorous, P-PO4 = dissolved orthophosphates, TOC = total organic carbon.

different trophic groups of ciliates differed significantly. In both lakes, significant correlations between ciliate and bacteria abundance were recorded ($r = 0.31$ and $r = 0.55$, $P = 0.05$). In the mesotrophic lake, a significant correlation also occurred between bacteria and flagellates (Table 3). In both type of lakes, the highest significant values of correlation coefficients were calculated from bacteria and ciliates in the higroarenal zone ($r = 0.97$, $P = 0.05$).

4. Discussion

4.1. Microbial loop components: general results

In the available literature, information on the functioning of the microbial loop and concentration of particular groups of organisms in the psammolittoral zone is fragmentary (Mudryk et al., 2001; Kalinowska et al., 2012; Mieczan and Nawrot 2012a, 2012b; Nawrot and Mieczan, 2012). The psammolittoral is a changeable environment, and as stated by Schmid-Araya (1998), it is convenient for organisms with the ability of fast population growth and a wide scale of ecological tolerance. Therefore, it seems that the psammon zone is a good environment for organisms adapting well to variable environmental conditions, such as bacteria, flagellates, and ciliates. Due to the variable environmental conditions of the psammolittoral, as well as the fast population growth of the aforementioned groups of organisms, they show high differentiation in terms of abundance, both between seasons and among various trophic types of lakes. Numbers of bacteria in the psammolittoral zones analyzed ($2.9–3.55 \times 10^7$ cm^{-3}) are similar in their density to the hydroarenal in a eutrophic lake (the Mazurian Lakeland, northern Poland: from 10^8 to 10^9 cm^{-3}) (Kalinowska et al., 2012). The available literature contains hardly any information on the numbers, biomass, and roles of flagellates in the psammolittoral zone. In the water lakes studied, the numbers of flagellates amounted to $4.3–29 \times 10^3$ cm^{-3}. In the hydroarenal of the eutrophic lake, the numbers of flagellates ranged between 4.3 and 78.2×10^3 cm^{-3} (Kalinowska et al., 2012). In the littoral zone of the eutrophic Lake Gooimeer, the abundance of these microorganisms reached from 0.7 to 167.9×10^3 cm^{-3} (Starink et al., 1996). The numbers of ciliates in the lakes analyzed are comparable with the data from the psammolittoral of meso- and eutrophic lakes (Kalinowska 2004, 2008; Mieczan and Nawrot, 2012a, 2012b).

4.2. Microbial loop components vs. physical and chemical parameters

The abundance of particular components of the microbial loop showed clear correlation with physical and chemical parameters. In the eutrophic lake, significant correlations were determined between the abundance of particular components of the microbial loop and the content of dissolved oxygen in water, pH, and conductivity. Moreover, the content of nutrients and chlorophyll *a* in the lake correlated with the numbers of flagellates and ciliates. According to Ejsmont-Karabin et al. (2004), ciliates, especially small-bodied forms, can play an important role in phosphorus regeneration, supplying nutrients for algae and bacteria growth. That is why the positive relationship between ciliates and trophic parameters was found. In the mesotrophic lake, significant correlations were determined between the abundance of components of the microbial loop and temperature, TOC, and P_{tot}. Studies on the development and growth of bacteria reveal that they are determined by, among others, the temperature of the environment and its pH (Chróst et al., 2009). These factors, and the content of phosphorus and chlorophyll *a* in water, also strongly affect the occurrence of protists in limnic ecosystems (Mieczan, 2008). It seems that these parameters largely affect the abundance of bacteria constituting the main source of food for heterotrophic

Table 3. Pearson correlations between microbial loop components in investigated lakes (P ≤ 0.05). n.s. = not significant.

Mesotrophic lake	Trophic group of ciliates									
	Chlorophyll a	Bacteria	Flagellates	Ciliates	Bacterivore	Algivore	Omnivore	Predator	Mixed food	Mixotrophic
Chlorophyll *a*	-	n.s.	n.s.	0.31	n.s.	0.32	n.s.	n.s.	0.36	0.41
Bacteria	n.s.	-	–0.69	0.58	0.51	0.62	0.75	0.30	n.s.	0.38
Flagellates	n.s.	–0.69	-	n.s.	n.s.	–0.74	–0.60	n.s.	–0.44	n.s.
Ciliates	0.31	0.58	n.s.	-	-	-	-	0.37	-	-
Eutrophic lake	Trophic group of ciliates									
	Chlorophyll a	Bacteria	Flagellates	Ciliates	Bacterivore	Algivore	Omnivore	Predator	Mixed food	Mixotrophic
Chlorophyll *a*	-	n.s.	–0.53	0.55	n.s.	n.s.	0.42	n.s.	n.s.	0.82
Bacteria	n.s.	-	n.s.	0.61	0.26	0.47	n.s.	n.s.	0.75	n.s.
Flagellates	–0.53	n.s.	-	–0.31	n.s.	n.s.	0.26	n.s.	n.s.	–0.35
Ciliates	0.55	0.61	–0.31	-	-	-	-	0.34	-	-

protists. Moreover, studies conducted so far also reveal a strong correlation between the content of organic matter in water (mainly in the form of dissolved organic matter) and bacterial numbers (Chróst et al., 2009; Kalinowska, 2012). Because algae are the main source of organic substance for bacteria, it seems that they can also indirectly determine the occurrence of ciliates. According to Kalinowska (2004) and Chróst et al. (2009), bacteria constitute their main source of food, particularly in water bodies with high trophic status. The highest density of bacteria is observed in places of accumulation of decomposing organic matter (the wave zone) (Podgórska et al., 2008). Another factor directly affecting the quantitative and qualitative structure of the psammon assemblage is the granulometric composition of sand (Ejsmont-Karabin, 2008). It determined the structure of size of protozoan cells and the surface inhabited by bacteria. In the lakes studied, a much higher abundance of heterotrophic protozoa was recorded in the hydro- and euarenal zones in the eutrophic lake, and in the higroarenal zone in the mesotrophic lake. These zones were dominated by sand fractions with sizes of 0.15–0.4 mm. The zones were strongly dominated by ciliate cells with sizes of approximately 50 µm.

4.3. Relationships among microbial loop components

The structure of the microbial loop in lakes was already quite thoroughly studied in the pelagic zone (Mayer et al., 1997; Kalinowska, 2004; Chróst et al., 2009). Like in the psammolittoral zone thus far, studies on the microbial loop have only been conducted in the hydroarenal zone (Kalinowska, 2012). In the lakes studied, a number of correlations were determined (correlation r) between particular elements of the microbial loop. The correlations were stronger in the eutrophic lake, which is in accordance with the study on the eutrophic Lake Mikołajskie (Kalinowska, 2012). Studies conducted on the bottom sediments in shallow lakes evidenced that both flagellates and ciliates can be important bacteriovores (Fenchel, 1987; Epstein, 1997). Some studies show that benthic Protista only use a slight portion of bacterial production, and therefore their role in transferring carbon to higher trophic levels is insignificant (Alongi, 1986; Kemp, 1988; Starink et al., 1996; Glucker and Fischer, 2003). In oligo- and mesotrophic lakes, e.g., in the pelagic zone, rotifers are the most important link in the transfer of carbon from bacterial biomass to macrozooplankton (Stockner and Shortreed, 1989). In the scope of this study, the microscopic analyses reveal that ciliates reach numbers of up to 300 ind cm^{-3}. This suggests their important role in the processes of matter and energy circulation to higher trophic levels (Nawrot and Mieczan, 2012). In the lakes studied, in all the psammolittoral zones, high bacterial production was recorded. In the mesotrophic lake, it was probably determined by high amounts of plant organic matter subject to decomposition in the coastal zone. Moreover, a strong negative correlation between the abundance of bacteria and flagellates occurred in the lake. Therefore, it seems that in the lake, it was mainly ciliates that could control the numbers of bacteria. This is confirmed by the fact that the total numbers of ciliates were dominated by small bacterivorous taxa. In the mesotrophic lake, no significant correlations were recorded between the numbers of heterotrophic flagellates and ciliates. In the eutrophic lake, significant correlations between the abundance of bacteria and ciliates were determined. In the lake, due to the relatively high density of ciliates belonging to Scuticociliatida and Hymenostomatida, they can be ascribed with the crucial effect on the abundance of bacteria. According to Šimek et al. (1995), the rate of feeding of the groups of ciliate on bacteria is very high, varying from 380 to as much as 2130 bacteria h^{-1}. Lack of significant correlations between flagellates and bacteria in the eutrophic lake could result from the fact that the population of flagellates was dominated by large forms, often exceeding 10 µm. According to Auer and Arndt (2001), large forms of flagellates do not only feed on bacteria but also on algae and other flagellates. In the conditions of the psammolittoral, flagellates could therefore favor a more autotrophic or mixotrophic manner of feeding. In the mesotrophic lake, the abundance of heterotrophic flagellates significantly correlated with the numbers of bacteria. The lake was dominated by small forms of flagellates, which probably effectively control the abundance of bacteria. Similar patterns were observed in the pelagic zone in the lakes of northeastern Germany (Auer and Arndt, 2001).

4.4. Conclusions

Depending on the trophic status of a lake, the physical and chemical water parameters (pH, conductivity, temperature, TOC, P_{tot}, $P\text{-}PO_4$, $N\text{-}NO_3$, $N\text{-}NH_4$, and chlorophyll *a*) affected the abundance of the analyzed groups of microorganisms to a various degree. The relation between bacteria and heterotrophic protists suggests a significant process of transferring carbon to the higher trophic levels in the psammolittoral zone, whereas significant components of the microbial loop in the mesotrophic lake are more numerous and statistically more significant. In the eutrophic lake, mainly omnivore and mixed-food Hymenostomatida correlated with total bacteria numbers, while in the mesotrophic lake, the influence of bacterivorous Scuticociliatida increased. In both trophic types of lakes, the highest correlation between bacteria and heterotrophic protists were noted in the hydroarenal zone. In the euarenal and higroarenal the correlation were weaker.

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