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Original Research

Diel Horizontal Distribution of Psammonic Ciliates and Rotifers in Shallow Eutrophic Lake

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Abstract

The aim of this study was to examine the diel dynamics of the qualitative and quantitative structures of psammonic ciliates and rotifers in a shallow eutrophic lake. Furthermore, the effects of specific physical and chemical factors of water on the occurrence of microorganisms were analyzed. There were clear differences in the qualitative and quantitative structure of organisms between the examined micro-habitats in a diurnal cycle. The highest density of ciliates, irrespective of the time of day, was recorded in the higroarenal zone, while the highest density of rotifers was found in both the higro- and hydroarenal zones. The lowest diversity of ciliates was recorded in the hydroarenal zone. RDA analysis showed a clear contrast between the eupsammonic, higropsammonic, and hydropsammonic zones, regardless of the time of day. A direct impact on the occurrence of protozoa and small Metazoa was played by the content of total phosphorus (P_{tot}) and TOC in the water. In addition, the ciliates were significantly affected by $P\text{-}PO_4$ and $N\text{-}NO_3$, and also the pH affected the group of rotifers.

Keywords: lake, psammon, food relationship, rotifers, ciliates

Introduction

Organisms living in the sandy shore of seas, lakes, and rivers create a community called a psammon, which is a complex of many groups of organisms, e.g. algae, bacteria, protozoa, rotifers, gastrotrichs, and nematodes [1]. Psammonic organisms inhabit beaches of water bodies at various depths. In rivers, the psammonic zone can reach in excess of 40 cm of depth into the sand, in seas – up to a few centimeters, whereas in lakes, it rarely exceeds 2 cm [2]. Kalinowska et al. [1] distinguished three groups of organisms that inhabit different parts of the beach: hydro-, higro and eupsammon. Hydropsammon occurs in hydroarenal sand, i.e. that which is permanently submerged. Higropsammon inhabits sand wetted by lake waves (hygroarenal). However, eupsammon lives in emergent and moist sand (euarenal).

So far, studies on the occurrence of psammonic rotifers have been relatively numerous [3, 4]. However, in the available literature notably little information concerning the diel

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changes in psammonic rotifers is available [5,6]. There appear to be very few studies addressing the occurrence of other groups of organisms, particularly ciliates [2, 7, 8]. They are an important element in the trophic structure of aquatic ecosystems. In psammolittoral areas, the relationships between physical and chemical parameters and animal communities (particularly small Metazoa) are relatively well known [9]. Little or no attention is given to the abundance and biomass of protozoans and their relationships to food resources and predator pressure in these specific ecosystems [10, 11]. Rotifers also constitute one of the main components of plankton, periphyton, and psammon as consumers of bacteria, algae, and protozoa [12]. So both ciliates and rotifers are a very important element in the trophodynamics of lakes [6].

Since the psammolittoral zone is characterized by very clear dynamics of physical and chemical changes in water [13, 14], it seems that similar dynamics can be expected to exist in the case of microorganisms that inhabit it. It also appears that the correlations between the physico-chemistry of water, microorganisms (Ciliates, Rotifera) and food-consumers will be more evident in a series of daily than in a seasonal ingredients.

The specific aim of this study was to examine the community of ciliates and rotifers in daily changes, as well as to assess the influence of potential food resources (Chl-*a*) and predators (rotifers) on protozoa communities.

Study Area, Materials And Methods

The study was undertaken in a shallow, macrophyte-dominated Lake: Sumin (avg. 1.6 m, max. depth 9.5 m, area 91.5 ha) located in Łęczyńsko-Włodawskie Lake District (eastern Poland, 51ºN, 23ºE). In Lake Sumin, well developed belts of emergent (*Phragmites australis* (Car.), Trin. ex Steud and *Typha latifolia* L.) and submerged (*Myriophyllum spicatum* L.) dominate the littoral.

Fieldwork was done in spring (May, 2012). In order to determine changes in the number of microorganisms, samples were taken four times a day: at dawn (5 a.m.), at noon (11 a.m.), at dusk (4 p.m.), and at night (11 p.m.) (48 samples total). The samples of psammon were taken from three zones of arenal: euarenal – exposed sand, 1 m of shoreline; higroarenal – at the shoreline; hydroarenal – under water, 1m from the shoreline (Fig. 1). Each sample included 2 cm of sand.

Samples were collected with a plastic sharp-edged tube, 60 mm in diameter at four points of each zone. Each sample was shaken with filtrated lake water and then filtered through a benthic mesh net and fixed immediately with Lugol's solution (0.2% final concentration). Ciliate and rotifera density were calculated per 1 cm^3 of sand. Observation of life samples was used for taxonomic identification [15, 16].

Ciliate and rotifer biomass was estimated by multiplying the numerical abundance by the mean cell volume calculated from direct volume measurements using appropriate geometric formulas.

In each plot pH, conductivity, temperature, total organic carbon (TOC), total phosphorus (P_{tot}), phosphate phosphorus ($P\text{-}PO_4$), nitrate nitrogen ($N\text{-}NO_3$), ammonium nitrogen ($N\text{-}NH_4$), and chlorophyll *a* were measured. Temperature, conductivity, and pH were determined in situ using a 556 MPS multiparameter sensor (Envag). TOC was determined using the PASTEL UV, and the other parameters were analyzed in laboratory according to Hermanowicz et al. [17]. Chlorophyll *a* was determined by the spectrophotometric analysis of alcohol extracts of the algae retained on polycarbonate filters.

Statistical analyses of results were carried out using STATISTICA 7.0 software. The differences between samplings were analyzed using t-Student test. Pearson correlation coefficients were calculated between pairs of variables in order to determine the relationship between abundance of ciliates and rotifers and physical and chemical parameters. Ordination techniques 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 distribution. The indirect multivariate method, DCA, was used to measure and illustrate gradients indicated by psammonic microorganism communities. Due to the length of the gradient with a range of 3 to 4 standard deviations (SD), the redundancy analysis (RDA) was applied in order to determine the relationships between psammonic organisms and environment parameters. The analyses were performed with the application of the ordination program CANOCO 4.5 for Windows.

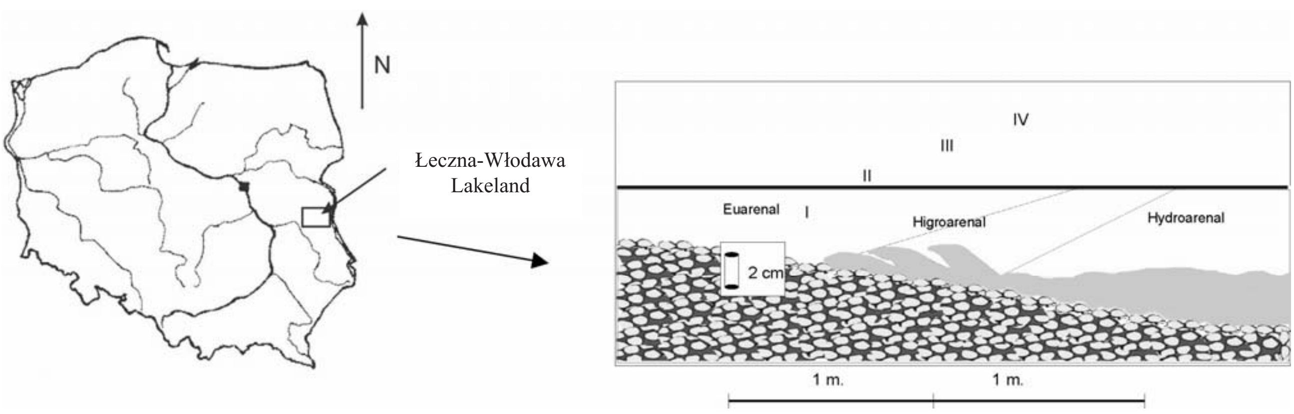

Fig. 1. Location of study area and the sampling station.

Table 1. Physical and chemical characteristics of the psammon water in the studied lake.

Lake	Zone	Temp	pH	Conductivity µS·cm^{-1}	N-NO$_3$ mgN·dm^{-3}	N-NH$_4$ mgN·dm^{-3}	PO$_4$ mgPO$_4$·dm^{-3}	P$_{tot}$ mgP·dm^{-3}	TOC mgC·dm^{-3}	Chlorophyll *a* µg·l^{-1}
Sumin	Hy	20,04*	8.42	373.25	0.09	0.26	0.01	0.05	13.6	19.74
		17.80-23.50**	8.32-8.52	369-379	0.06-0.12	0.24-0.27	0.011-0.012	0.04-0.06	13.5-13.7	14.8-24.69
	Hi	20.16*	8.45	371.75	0.05	0.37	0.02	0.13	14.05	14.27
		16.82-23.50**	8.31-8.57	368-376	0.054-0.056	0.33-0.40	0.009-0.028	0.12-0.13	13.9-14.1	13.98-14.55
	Eu	16.90*	7.45	382.75	0.14	0.55	0.01	0.17	16.05	30.86
		12.40-20.70**	7.32-7.75	382-387	0.12-0.15	0.54-0.55	0.002-0.025	0.14-0.20	15.8-17.2	14.77-46.96

Eu – euarenal, Hi – higroarenal, Hy – hydroarenal, *average and **range

Results

Physical and Chemical Parameters

Analysis of the water showed that the euarenal zone was characterized by the lowest pH (average 7.75) and temperature (average 16.9). The lowest level of conductivity was observed in the higroarenal zone (371.75 µS·cm^{-1}), and the highest (382.75 µS·cm^{-1}) in the euarenal zone. The zone with the highest content of mineral forms of nitrogen, phosphorus, and TOC was the euarenal zone. The lowest content of ammonium nitrogen (0.26 mgN·dm^{-3}), total phosphorus (0.05 mgP·dm^{-3}), and total organic carbon (13.6 mgC·dm^{-3}) was recorded in the hydroarenal zone. The highest content of chlorophyll *a* was observed in the euarenal zone (30.86 µg·l^{-1}). Other zones showed similar, relative to each other, contents of chlorophyll *a* in the water (Table 1). The dynamics of daily nutrient concentrations showed significant differences between the times of the day under study. The highest concentration of TOC, P-PO$_4$, N-NO$_3$, N-NH$_4$, and chlorophyll *a* was found at night, whereas the highest level of total phosphorus was observed in the day.

Statistical analysis of the significance of differences between the measurements of chemical parameters during the day showed no statistically significant differences. However, the analysis showed a number of significant differences between zones in diel cycle. Between euarenal and higroarenal in: pH ($F=3.11$, $p<0.05$), conductivity ($F= 1.37$, $p<0.05$), N-NO$_3$ ($F=123.63$, $p<0.05$), N-NH$_4$ ($F=61.81$, $p<0.05$), and TOC ($F=196.0$, $p<0.05$). Between euarenal and hydroarenal zone: pH ($F=5.95$, $p<0.05$), conductivity ($F= 2.12$, $p<0.05$), NH$_4$ ($F=10.35$, $p<0.05$), P$_{tot}$ ($F=15.63$, $p<0.05$), and TOC ($F=5.44$, $p<0.05$). The lowest numbers of significant differences was observed between the higro- and hydroarenal zones: NH$_4$ ($F=5.97$, $p<0.05$), P$_{tot}$ ($F=3.38$, $p<0.05$), and TOC ($F=36.0$, $p<0.05$).

Taxonomic Composition and Abundance of Ciliates

Species diversity of ciliates showed differences both in terms of the diurnal cycle and between the studied zones. The zone with the lowest richness of ciliates, during a prolonged period of the day was the hydroarenal zone (5 taxons), while the higroarenal zone was distinguished by the highest taxonomic richness of those microorganisms under study (23 taxons). Analysis proved that the number of identified taxa between the zones showed statistically signifi-

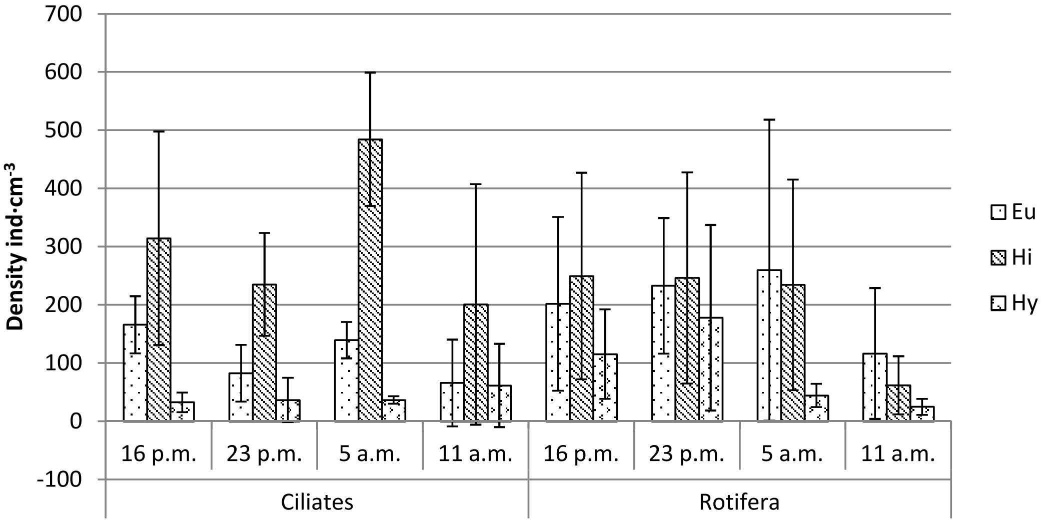

Fig. 2. Diurnal dynamics of density of psammonic ciliates and rotifers in investigated lake (Eu – euarenal, Hi – higroarenal, Hy – hydroarenal).

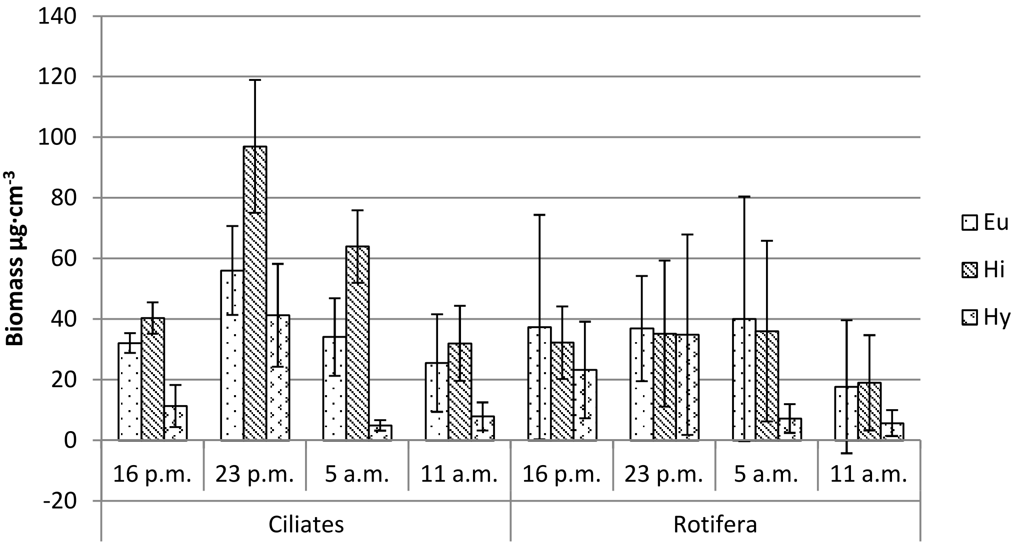

Fig. 3. Diurnal dynamics of biomass of psammonic ciliates and rotifers in investigated lake (± standard deviation) (Eu – euarenal, Hi – higroarenal, Hy – hydroarenal).

cant differences: euarenal vs. higroarenal ($F=5.07$, $p<0.05$), euarenal vs. hydroarenal ($F=3.64$, $p<0.05$), and higroarenal vs. hydroarenal ($F=18.45$, $p<0.05$). The highest average density of ciliates were found in the higroarenal zone (484.83 ind·cm^3). Significantly lower values were reordered in the hydroarenal zone (33 ind·cm^3). Statistical analysis showed significant differences between zones in the diurnal cycle: euarenal vs. higroarenal (at night: $F=3.29$ and at dawn: $F=13.5$, $p<0.05$), euarenal vs. hydroarenal (at dusk: $F=8.46$ and at dawn $F=23.02$, $p<0.05$), higroarenal vs. hydroarenal (at dusk: $F=118.33$, at night: $F=5.41$ and at dawn: $F= 310.93$, $p<0.05$). In the higroarenal zone the density of ciliates ($F=1.43$, $p<0.05$) and biomass ($F=2.02$, $p<0.05$) shoved statistically significant differences between dusk and night. The diurnal cycle with the greatest changes, both in the numbers and biomass of ciliates, occurred in the higroarenal zone. An almost double increase in the density of protozoa in the morning was observed here. The lowest values of abundance and biomass were observed in the afternoon hours (Figs. 2 and 3). Only between the higroarenal and hydroarenal zones did we observe statistically significant differences in biomass of ciliates (at dusk: $F= 1.76$ and at dawn: $F= 47.61$ $p<0.05$).

Regardless of the time of the day, the euarenal zone was dominated by taxa belonging to Hymenostomatidia (*Paramecium* sp., *Colpidium* sp.) (from 41% to 70% of the total numbers of ciliates). A large percentage of species belonging to Prostomatidia (from 25% in the early morning to 60% at noon) was observed in the higroarenal zone. Taxa belonging to Oligotrichida (*Codonella cratera*) occurred mainly in the hydroarenal zone (from about 60% in the day to about 23% at night). The morning saw a significant increase in the percentage of Prostomatida (*Coleps* sp., *Holophyra* sp.) and Hypotrichia (*Euplotes* sp., *Oxytrichia* sp.), while in the afternoon and evening there was an increased percentage of Pleurostomatida (*Litonotus* sp.) and Oligotrichida.

Taxonomic Composition and Abundance of Rotifers

The zone with the lowest richness of rotifers, at any time of the day, was the hydroarenal zone, while the euarenal zone was distinguished by the highest richness of those microorganisms under study. The highest average density of rotifers was found in the euarenal zone. Significantly lower values were recorded in the hydroarenal zone. In the diurnal cycle, the highest changes both in the density and biomass of rotifers occurred in the hydroarenal zone. The lowest values of abundance and biomass were observed in the afternoon hours (Figs. 2 and 3). Statistical analysis of the abundance of rotifers has not demonstrated any significant differences.

The rotifer community was dominated by taxa belonging to the genera Lecanidae, Cephalodella, and Keratella. Taxa of the genus Lecane achieved the highest percentage in the higroarenal zone (from 53% at noon to 84% in the evening). Taxa of the genus Keratella dominated in the hydroarenal zone, with the highest percentage at night (28% in total numbers). However, in the morning and at noon a marked clear increase was recorded in the percentage of taxa belonging to the genus Cephalodella in this zone.

Relationship between Chemical Parameters and Abundance of Ciliates and Rotifers

Pearson correlation analysis between the physical and chemical properties of the water, and the abundance of groups of microorganisms, showed significant differences between the zones. In the hydroarenal zone the abundance of ciliates and rotifers correlated with nutrients and chlorophyll *a* (respectively: $r=0.39$, $p=0.03$; $r=0.56$, $p=0.023$). In the higroarenal zone, a significant correlation was recorded between the abundance of ciliates and water temperature ($r=0.56$, $p=0.24$) and pH ($r=0.58$, $p=0.13$). In the euarenal

Table 2. Pearson correlation between ciliate and rotifer density and physical and chemical factors of investigated lake (p≤0.05).

	Zone	Temp	pH	Cond	$N\text{-}NO_3$	$N\text{-}NH_4$	$P\text{-}PO_4$	P_{tot}	TOC	Chl-*a*
Ciliates	Euarenal	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s
	Higroarenal	-0.56	-0.58	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	Hydroarenal	n.s.	n.s.	n.s.	0.39	-0.39	0.39	-0.39	0.39	0.39
Rotifera	Euarenal	n.s.	-0.31	-0.31	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	Higroarenal	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	Hydroarenal	n.s.	0.38	0.38	0.56	-0.56	0.56	-0.56	0.56	n.s.

zone, the density of rotifers was correlated with pH and conductivity (r=-0.31), but this correlation was not statistical significant (p<0.05). In addition, there was a significant and positive correlation between the abundance of protozoa and rotifers (r=0.69, p=0.01) (Table 2).

RDA analysis demonstrated a clear differentiation between the eu-, hygro- and hydroarenal zones. A Monte Carlo permutation test analysis showed that the ciliates were significantly affected by: $N\text{-}NO_3$ (λ=0.15; F=1.75; p=0.034), P_{tot} (λ=0.16; F=2.13; p=0.014), $P\text{-}PO_4$ (λ=0.11; F=1.44; p=0.092), and TOC (λ=0.11; F=1.6; p=0.09) (Fig. 4). Rotifers were decisively affected by: $P\text{-}PO_4$ (λ=0.38; F=6.15; p=0.002), TOC (λ=0.18; F=5.22; p=0.012), and pH (λ=0.08; F=3.62; p=0.038) (Fig. 5).

Discussion

Ciliates and rotifers inhabiting the psammolittoral zone live in an environment where they are exposed to continuous changes in physical and chemical properties. Thus, it is possible that such stress is causing the observed changes in

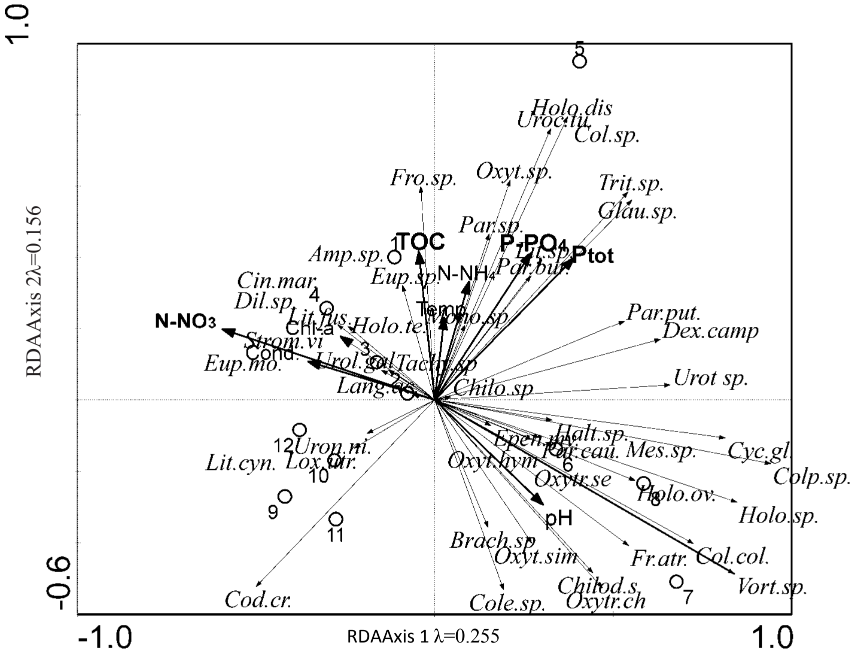

Fig. 4. Triplots of RDA of ciliates. Arrows marked as bold indicate significant parameters in Monte Carlo permutation test at P<0.1. Samples collected in studied habitats are marked with an Arabic numeral: 1-4, euarenal; 5-8, higroarenal; 9-12, hydroarenal.

Species codes: *Amphileptus* sp. – *Amp.sp.*; *Brachonella sprialis* – *Brach.sp.*; *Chilodonella* sp. – *Chilo.sp.*; *Chilodontopsis* sp. – *Chilod.sp.*; *Cinetochilum margaritaceum* – *Cin.mar.*; *Codonella cratera* – *Cod.cr.*; *Coleps* sp. – *Cole.sp.*; *Colpidium coploda* – *Col.col.*; *Colpoda* sp. – *Col.sp.*; *Coplidium* sp. – *Colp.sp.*; *Cyclidium glaucoma* – *Cyc.gl.*; *Dexiostoma campylum* – *Dex.camp.*; *Dileptus* sp. – *Dil.sp.*; *Epenardia myopyri* – *Epen.my.*; *Euplotes moebusi* – *Eup.mo.*; *Euplotes* sp. – *Eup.sp.*; *Frontonia atra* – *Fr.atr.*; *Frontonia* sp. – *Fro.sp.*; *Glaucoma* sp. – *Glau.sp.*; *Halteria* sp. – *Halt.sp.*; *Holophyra discolor* – *Holo.dis.*; *Holophyra* sp. – *Holo.sp.*; *Holophyra teres* – *Holo.te.*; *Holpohyra ovum* – *Holo.ov.*; *Lagynophyra acuminate* – *Lang.ac.*; *Litonotus cyngus* – *Lit.cyn.*; *Litonotus fusidens* – *Lit.fus.*; *Litonotus* sp. – *Lit.sp.*; *Loxophyllum utriculaire* – *Lox.utr.*; *Mesodinium* sp. – *Mes.sp.*; *Monodinium* sp. – *Mono.sp.*; *Oxytrichia chlorelligera* – *Oxytr.chl.*; *Oxytrichia hymenostoma* – *Oxyt.hym.*; *Oxytrichia setigera* – *Oxytr.set.*; *Oxytrichia similis* – *Oxyt.sim.*; *Oxytrichia* sp. – *Oxyt.sp.*; *Paramecium bursaria* – *Par.bur.*; *Paramecium caudatum* – *Par.cau.*; *Paramecium putrinum Par.put.*; *Paramecium* sp. – *Par.sp.*; *Strombilidium viride* – *Strom.vi.*; *Tachysoma* sp. – *Tachy.sp.*; *Trithigmostoma* sp. – *Trit.sp.*; *Urocentrum turbo* – *Uroc.tu.*; *Urolpetus gallina* – *Urol.gal*; *Uronema nigricans* – *Uron.ni.*; *Urotrichia* sp. – *Urot sp.*; *Vorticella* sp. – *Vort.sp.*

the density and richness of the species of microorganisms in both treatment groups, both in seasonal and diel cycles [6, 7, 18]. Mieczan and Nawrot [7] consider that protozooans that inhabit continuously changing habitats are more qualitively diversified than those living in more stable and homogenous habitats. The present study observed significant differences in the qualitative and quantitative structure of ciliates in both the diurnal cycle and between the two zones. The average number of taxa classified as psammonic ciliates in the lake were similar to those recorded in eutrophic lakes [1, 2, 7]. In the diurnal cycle, the lowest taxonomic richness and abundance of ciliates were recorded in the morning in the hydroarenal zone. The higroarenal saw the biggest drop in taxonomic diversity. This zone was characterized by high dynamics of change that are likely to have an impact on the density and taxonomic richness of the microorganisms that inhabit it [19]. The maximum abundance of ciliates in this zone coincided with the maximum concentration of chlorophyll *a*, mineral forms of nitrogen, and TOC. It seems that nutrients and total organic carbon have an indirect influence on the prevalence of ciliates through the control food abundance (mainly bacteria) [20]. Also, in the eutrophic lakes of northern Poland, it was observed that pH and content of nutrients and chlorophyll *a* in the water have a significant effect on the occurrence of these microorganisms [19].

Also, the abundance of rotifers varied depending on the area and time of day, which is consistent with studies conducted in hydroarenal in eutrophic lake Mikołajskie [5]. The study also showed that the area with the lowest density of rotifers at any time of the day was the hydroarenal zone. The density of rotifers in the hydroarenal zone was correlated with the physical and chemical properties of the water (pH, conductivity, and nutrients). It seems therefore that in the littoral zone density of psammonic rotifers is most dependent on the amount of nutrients in the water. The lowest abundance of these organisms was observed in the morning and at noon, and the samples collected at night were more abundant, which is consistent with studies carried out in the eutrophic lakes [6, 9]. High numbers of these organisms during the night may have resulted from a bottom-up control mechanism. As evidenced by the analysis of correlation, at the time the rotifer abundance correlated with the abundance of ciliates. Similar relationships were observed in the eutrophic lake [6, 9].

In both studied groups of organisms, significant correlations between the physical and chemical factors of water and the density of psammonic organisms are shown only in the hydroarenal zone. A significant correlation has also been observed in the eutrophic lake [2, 19]. Irrespective of the zone analyzed, the abundance of ciliates correlated with rotifers. This suggests that the microorganisms could constitute a potential food source for a small Metazoa. According to a study conducted by Arndt [21], ciliates are frequently consumed by rotifers, eg. small Scuticociliatides and Oligotrichs to the amount of 0.6-4.8 ciliates·rotifers^{-1}·h^{-1}. The strong predation pressure of rotifers on ciliates in the investigated lake were particularly confirmed by observations in the night, when the numbers of Protozoa clearly decreased along with an increase in the numbers of rotifers. Rotifers probably selectively consumed protozoan communities, which was also reflected in the predominance of small taxa of ciliates.

The results confirm the assumption that the environment was characterized by a high variability of physical and chemical conditions ideal for organisms that have a short

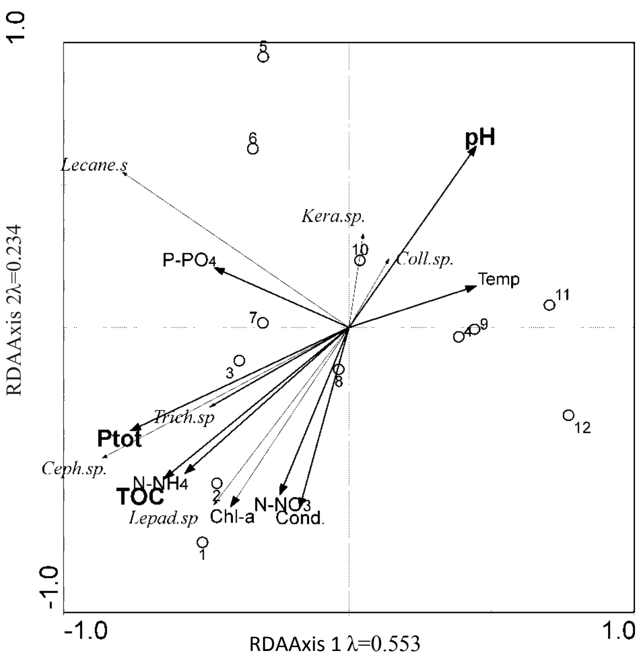

Fig. 5. Triplots of RDA of Rotifera. Arrows marked as bold indicate significant parameters in Monte Carlo permutation test at P<0.1. Samples collected in studied habitats are marked with an Arabic numeral: 1-4, euarenal; 5-8, higroarenal; 9-12, hydroarenal.
Species codes: *Cephalodella* sp. – *Ceph.sp.*; *Colurella* sp. – *Coll.sp.*; *Keratella* sp. – *Kera.sp.*; *Lecane* sp. – *Lecane.sp.*; *Lepadella* sp. – *Lepad.sp.*; *Trichocerca* sp. – *Trich.sp.*

generation time and can colonize habitats quickly and have broad ecological niches, i.e. eurybionts [13]. Fluctuations in environmental conditions cause changes in dynamic psammonic assemblages that are observable even within a few hours.

Conclusion

In summary, studies have shown significant differences in the horizontal distribution of ciliates and rotifers in the diurnal cycle in the psammolittoral zone. The highest abundance of the investigated organisms was observed at night. In the hydroarenal zone, the physical and chemical properties of the water have the greatest impact on the organisms, while that zone was characterized by the lowest abundance of test organisms at any time of the day. The largest differences in taxonomic composition were reported in the higroarenal zone. The results presented show ciliate and rotifer abundances are impacted by contents of total organic carbon and total phosphorus. However, with the aim of clarifying the understanding of the role of factors conditioning the presence of these microorganisms, it is necessary in future research to explain the biotic factors such as, among others, the abundance of bacteria and heterotrophic flagellates.

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