Arch. Biol. Sci., Belgrade, 65 (4), 1491-1504, 2013 DOI:10.2298/ABS1304491M

EFFECT OF WATER CHEMISTRY ON THE PLANKTONIC COMMUNITIES AND RELATIONSHIPS AMONG FOOD WEB COMPONENTS ACROSS A FRESHWATER ECOTONE

T. MIECZAN*, MAŁGORZATA ADAMCZUK and DOROTA NAWROT

Department of Hydrobiology, University of Life Sciences in Lublin, 20-262 Lublin, Poland

Abstract - Most ecological research on the food web has been focused more on the pelagic zone than on the transitional zone – ecotones between lentic and lotic habitats. The specific goals of this study were to determine whether the contact zone of waters differs in hydrochemical and biological terms from the waters of the canal and the open water zone, and to evaluate the influence of particular macro-habitats on the interactions between components of the planktonic food web. The distribution of samples in ordination space led us to conclude that the studied habitats are distributed along the rising gradient of total organic carbon and nutrients. Assemblages of all investigated groups showed a strong compositional gradient correlated with conductivity and total phosphorus, while a second strong gradient in species composition was explained by nitrate nitrogen and/or phosphate concentrations. The analysis of trophic relationships in the system bacteria-ciliates-crustaceans reveals a clear differentiation and strength of mutual relations between the analyzed zones. The highest number of significant correlations was determined in the contact zone. It can also be a place of very efficient matter and energy flow in freshwater ecosystems.

Key words: Reservoir, ecotone, food web, bacteria, ciliates, crustacean

INTRODUCTION

The boundaries between water bodies (lakes, ponds, reservoirs) and adjacent terrestrial patches play an import role in coupling terrestrial with aquatic ecosystems. Ecotones are transition zones between relatively homogenous areas or patches. They are zones in which environmental gradients are steepened, where rates of change in ecological patterns and processes are increased relatively to the surrounding. The canal-reservoir system may thus be perceived a spatial system of two kinds of ecosystems or patches. Ecotonal zones of these patches would be formed by the zones of mixing the reservoir water with river/canal water (Hillbricht-Ilkowska and Węgleńska, 2003). The zone of river inflow to a reservoir functions as a barrier system and filter of incoming organic matter, nutrients and mineral suspension. The studies of the effect of ecotones on zooplankton communities have been concentrated on rotifers (Ejsmont-Karabin, 2003), bacteria and periphyton communities (Fleituch et al., 2001). However, very little is known about large-scale of bacteria, protozoan and crustacean density and their function in the ecotones of freshwater systems. Protozoa communities are integrally linked to aquatic habitats and their abundance and community structure are related to both chemical and physical conditions in lake and river, making them useful bioindicators (Xu et al., 2005). These microorganisms are important consumers of pico- and nano-sized producers; they are nutrient regenerators and an important food source for metazoans (Pierce and Turner, 1992; Biyu, 2001). Even less

knowledge exists concerning the regulating mechanisms between microorganisms with respect to species composition and abundance of crustaceans in freshwater contact zones. The classical grazer food chain and the planktonic food web are linked by several direct and indirect interactions. Metazoan grazing is important for the recycling of nutrients and production of dissolved organic substrates for bacteria, but it is also a controlling factor for the protozoan community structure. Zooplankton predation, mainly by copepods, on ciliates is well documented in pelagic zones (Jack and Gilbert, 1997). However, our knowledge of the distribution and regulating factors of microorganisms in transitional zones is still fragmentary. The first step in understanding the structural and functional significance of these microorganisms is the analysis of density and biological diversity between different zones. Because the canal/reservoir zone is recognized as an ecotone (the contact zone between two different ecosystems), it was assumed that it should show significant differences of physical and chemical water parameters from adjacent habitats, and be distinguished by high species richness and abundance of bacteria, ciliates and crustaceans, including the occurrence of species typical of the zone. According to di Castri et al. (1988), ecotones are transitional zones between relatively homogenous areas or patches and characterized by high structural and spatial diversity. They are zones in which environmental gradients are steepened, where rates of change in ecological patterns and processes are increased relatively to the surrounding. The specific goals of the study were: i) to analyze the taxonomic composition and abundance of microbial and crustacean communities, ii) to describe environmental variables responsible for the distribution of bacteria, ciliates and crustaceans in an adjacent canal, ecotone and reservoir (littoral-pelagic zone); iii) to determine whether the contact zone waters differ in hydrochemical and biological terms from the waters of the canal and the open water zone; and iv) to evaluate the influence of particular macro-habitats (canal, canal/reservoir, littoral, pelagic zone) on the interactions among components of the planktonic food web.

MATERIALS AND METHODS

Study area

Lake Dratów is a storage, eutrophic reservoir (surface area 167.9 ha, max depth 2.9 m) situated in the area of Łęczna-Włodawa Lakeland (eastern Poland) (Fig. 1). Over 80% of the lake catchment is used for agriculture purposes (mostly arable lands and meadows). In 1961, this lake was placed into the Wieprz-Krzna Canal (W-KC). Additionally, in the zone of W-KC, 13 lakes were transformed into storage reservoirs (Dawidek et al., 2004). As a result of feeding with canal waters, the rate of water exchange in the lakes increased and its quality and trophic conditions improved. The hydrotechnological facilities are efficient, carefully maintained and failure-free. The water in the reservoir is exchanged several times in a hydrological year, irrespective of the natural feeding system (Dawidek et al., 2004). Dratów reservoir is characterized by permanent and long-lasting blooms of cyanophyte. Emergent vegetation is dominated by common reed (*Phragmites australis* (Cav.) Trin. ex Steud.), bulrush (*Schoenoplectus lacustris* (L.) Palla) and broadleaf cattail (*Typha latifolia* L.). Submerged vegetation is limited to very small, single stands of rigid hornwort (*Ceratophyllum demersum* L.) and sago pondweed (*Potamogeton pectinatus* L.).

Sampling and identification

Microbial communities (bacteria and ciliates) and crustaceans (cladocerans and copepods) were examined in a transect including 1 – Wieprz-Krzna Canal (WKC), 2 – contact zone: canal/reservoir (WKC/R), 3 – littoral zone (L), 4 - pelagic zone (P) (Fig. 1). The samples were taken from April to October 2012. Collected data were presented in three seasons, spring (April), summer (July) and autumn (September). During each sampling occasion, three samples were collected from each site. At each of the sites, samples were collected with a 5-liter Bernatowicz sampler.

The bacterial abundance and biomass were determined by means of DAPI (4′6-diamidino-2-phenylindole)-staining and epifluorescence micro-

scopy, according to the method of Porter and Fleig (1980). 10 ml of water was preserved with formaldehyde to a final concentration of 2% and kept in the dark at 4°C. Four slides were made from each sample in which subsamples of 2 ml were filtered on 0.2 μm pore-size polycarbonate filters that were stained with Irgalan Black. The bacteria were counted in 250 randomly chosen fields of view.

The abundance of ciliates and their community composition were determined using Utermöhl's method. 5 l samples were filtered through a plankton net of 10 μm mesh size. Protozoa samples (whole sample = 500 ml) was sedimented for 24 h in a cylinder, stoppered with parafilm, then the upper was gently removed. In order to determine the density, three samples were preserved with Logol's solution and stored in the dark at a temperature of 4°C. Observation of live samples was used for the taxonomic and trophic identification. Ciliates are highly perishable, and their type of motility is a species-specific feature; for this reason, species determination and measurements were carried out on live material immediately after return to the laboratory and after silver impregnation (Augustin et al., 1984). Morphological identifications were mainly based on works by Foissner and Berger (1996), Foissner et al. (1999). Crustaceans (Cladocera and Copepoda) were collected with the use of a 5-L sampler. Double samples were collected and pooled to reduce heterogeneity in crustacean distribution and sampling variability. Samples were sieved through in a 40 μm mesh net and fixed with a formalin-glycerin solution. In the laboratory, the classification and counts of crustaceans were made with the use of the Sedgewick-Rafter cell to calculate abundance expressed as a number of individuals per 1 l^{-1}.

Biovolumes of each microbial community were estimated assuming geometric shapes and converting to carbon using the following conversion factor: heterotrophic bacteria: 1 μm^3 = 5.6 x 10^{-7} mgC; ciliates: 1 μm^3 = 1.1 x 10^{-7} mgC (Gilbert et al., 1998). Ciliate biomass was estimated by multiplying the numerical abundance by the mean cell volume (1 μm^3 = 1 pg) calculated from direct volume measurements using appropriate geometric formulas (Finlay, 1982). An obvious shrinkage of stained ciliates was observed in the silver preparation. The calculated cell volumes were multiplied with a correcting factor of 0.4 (Jerome et al., 1993). Crustacean biomass was estimated via the relations between body length and body mass of a given specimen (Dumont et al., 1975; Bottrell et al., 1976; Culver et al., 1985) by applying established mathematic formulas.

Abiotic variables

Once a month, the water samples (volume of 500 ml) for chemical analyses were taken. Temperature, conductivity, pH and dissolved oxygen (DO) were determined *in situ* with a multiparametric probe, total organic carbon (TOC) was determined using the spectrophotometer PASTEL UV and the remaining factors (TP – total phosphorus, $P\text{-}PO_4$, $N\text{-}NH_4$, $N\text{-}NO_3$, chlorophyll *a)* were analyzed in the laboratory (Golterman, 1969).

Data analyses

Diversity analysis (Shannon Wiener diversity index (log_{10}-based)) was performed using the Multivariate Statistical Package – MVSP. The differences of physical and chemical water parameters among studied habitats were analyzed by means of one-way ANOVA. Tukey's multiple range test (at $P<0.05$) was used to compare means when significant differences were found. The analysis was performed using PAST software. Spearman's rank correlation coefficients (R) were calculated for pairs of environmental variables to recognize which of the variables are inter-correlated. Principal Component Analysis (PCA) was used to measure and illustrate the variability gradients indicated by bacteria, ciliates and crustaceans. The choice of this linear ordination model was justified by the narrow range of the data (previously assessed by DCA with a length of gradient < 2 standard deviations). Redundancy analysis (RDA) was used to explore the relationships between the abundance of taxonomic groups and environmental variables. Automatic forward selection of environmental variables, the Monte Carlo permutation test (999 permu-

tations), was used to determine the most important variables (Lepš and Šmilauer, 2003). Variables whose level of significance exceeded 0.05 were plotted passively on the diagrams. On the resultant plot, the arrows representing the physicochemical variables indicate the direction of maximum change of that variable, and the length of each arrow is proportional to the rate of change. The proportion of variance explained by environmental variables was quantified using variance partitioning. The ordination analyses were performed by means of CANOCO 4.5 for Windows.

RESULTS

Abiotic variables

Physical and chemical water parameters showed visible seasonal variability (Table 1). Observed values were typical for hypertrophic lakes. Secchi disc visibility was very low and ranged from 0.40 m to 0.75 m. Statistically significant differences among the investigated sites were found in conductivity, nutrients, TOC and concentrations of chlorophyll *a* (ANOVA, F = 26.22-29.71, P = 0.001). Water pH fluctuated from 7.70 in WKC to 8.74 in L. Conductivity was significantly differentiated, attaining from >350 μS cm^{-1} in P to 542 μS cm^{-1} in WKC. The highest conductivity occurred in spring and summer, but was decidedly lower in autumn. At all sites examined, the water temperature reached the highest value in summer (>17°C), and decreased in autumn (<9°C). The highest concentrations of TOC occurred in WKC and/or WKC/R (>6 mgC L^{-1}). The concentration of total organic carbon fluctuated between 5.3-6.6 mgC L^{-1} in spring and 5.3-7 mgC L^{-1} in summer. Nutrients reached the highest values in the WKC and WKC/R, and were the highest during the spring and autumn periods. WKC had a higher concentration of chlorophyll *a* in spring and the pelagic zone in summer and autumn (Table 1).

Bacteria, ciliates and crustaceans

The highest mean bacterial numbers and biomass were noted in the littoral (L) and contact zones: canal/reservoir (WKC/R) (111 x 10^6 cells ml^{-1} and 2.49 mg C l^{-1} and 81 x 10^6 cells ml^{-1} and 1.59 mg C L^{-1}, respectively), the lower values were characteristic for the pelagic zone (P) (45 x 10^6 cells ml^{-1} and 1.09 mg C l^{-1}, respectively). The Wieprz-Krzna Canal (WKC) was characterized by the lowest numbers and biomass of bacteria (36 x 10^6 cells ml^{-1} and 0.88 mg C l^{-1}, respectively). In the pelagic and littoral zones, the numbers of bacteria were very low in summer and reached a maximum in spring and autumn; in KWC and KWC/R the highest numbers of bacteria occurred in spring and the lowest in summer.

A total of 26 ciliate taxa, 10 cladoceran taxa and 8 copepod taxa occurred in the studied area. Species richness of protozoa and crustaceans showed horizontal diversity. The highest numbers of taxa occurred in the L and/or WKC/R (21, 7 and 3 taxa, respectively) and became much lower in the P and/or WKC, where only 6 ciliate taxa and 1 crustacean taxa were identified. The highest diversity was measured in the WKC/R (Shannon-Wiener diversity index H = 2.2-2.5), and the lowest diversity was observed in the P (H = 0.75-0.83). Microorganism (bacteria and ciliates) and crustacean densities were shown to be significantly different with site and/or time of year (Table 2). The group of characteristic (exclusive) ciliate taxa that occurred only in one habitat was comparatively small. The exclusive species in KWC were *Lacrymaria olor* and *Oxytricha* sp. The most frequent taxa were *Prorodon sp.* and *Caenomorpha* spp. and four ciliate taxa had frequencies <5 %. In the littoral zone, only *Colpoda steinii* was a very constant species, and *Plagiopyla nasuta* was an accidental species. The remaining six species were accessory species. In KWC/R, *Cinetochilum margaritaceum, Colpoda cucullus, Euplotes* sp., occurred as very constant species. One taxon, *Holosticha pullaster*, was a constant species. There were also three accessory and two accidental species. Three species, *Caenomorpha* spp., and *Stylonychia mytilus-complex* were very constant in the littoral zone. The other two taxa found, *Amphileptus pleurosigma* and *Paramecium putrinum,* were constant species, one species was an accidental species, and two were accessory species. The ciliate abundances were also significantly

Table 1. Physical and chemical characteristics of water in investigated habitats (WKC - Wieprz-Krzna Canal, WKC/R – contact zone: canal/reservoir, L – littoral zone, P – pelagic zone)

Parameters		spring				summer				autumn			
		WKC	WKC/R	L	P	WKC	WKC/R	L	P	WKC	WKC/R	L	P
SD	m	0.25	0.65	0.55	0.75	0.21	0.40	0.40	0.30	0.34	0.40	0.40	0.35
Temp.	°C	12.47	12.57	13.06	12.34	19.20	17.79	19.67	19.93	11.14	10.84	10.75	11.06
pH	pH	8.3	7.96	8.03	8.20	8.23	7.70	8.80	8.72	8.05	8.47	8.74	8.72
Conductiv.	µS cm^{-1}	531	353	352	351	542	322	250	241	526	326	322	319
Dissolv. oxygen	mg O_2 L^{-1}	109.6	87.10	93.90	98.00	102.70	58.20	139.80	162.30	8.31	8.70	8.14	8.90
N-NH_4^+	mg NH_4^+ L^{-1}	0.130	0.146	0.136	0.138	0.120	0.206	0.164	0.176	0.084	0.118	0.102	0.098
N-NO^{3-}	mg NO_3^- L^{-1}	0.801	0.189	0.184	0.238	0.370	0.156	0.168	0.070	0.701	0.051	0.047	0.025
P-PO_4^{3-}	mg PO_4^{3-} L^{-1}	0.119	0.009	0.044	0.040	0.178	0.023	0.125	0.073	0.073	0.007	0.051	0.012
Ptot	mg P L^{-1}	0.198	0.106	0.077	0.054	0.225	0.258	0.209	0.242	0.081	0.129	0.110	0.135
Chl. *a*	µg L^{-1}	64.65	34.23	24.74	17.34	70.00	51.09	46.22	95.45	37.66	91.22	99.12	109.50
TOC	mg C L^{-1}	6.6	5.5	5.3	5.3	5.7	7.0	6.8	6.6	5.6	6.9	7.0	6.9

Table 2. Results of main effects ANOVA on density of bacteria, ciliates and crustaceans testing for the effect of the time (season) and the site (horizontal distribution)

	df	SS	MS	*F*	*p*
Density of bacteria					
Intercept	2	79.01	75.01	98.28	**<0.001****
Site (Si)	2	11.00	11.03	13.97	**<0.001****
Season (Se)	6	0.46	0.54	7.43	0.055
Si x Se	2	2.18	9.12	17.35	**0.033***
Density of ciliates					
Intercept	1	21.11	21.12	58.12	**<0.001****
Site (Si)	2	5.92	2.95	50.75	**<0.001****
Season (Se)	8	0.81	0.11	8.52	0.080
Si x Se	2	0.57	3.31	8.19	**0.036***
Density of crustaceans					
Intercept	1	43.74	43.71	24.81	**<0.001****
Site (Si)	2	14.72	19.32	5.81	**<0.001****
Season (Se)	8	5.76	7.21	3.52	0.082
Si x Se	2	2.36	1.53	4.62	0.071

The significance of bold letters: * significant at the level $P < 0.05$, ** significant at the level $P < 0.01$

related to the type of macro-habitat, with the lowest numbers in the P (22 ± 2 individuals ml^{-1}) and the highest in the WKC/R (56 ± 6 individuals ml^{-1}) (ANOVA, $F = 18.5$, $p = 0.001$). Ciliate biomass, corresponding with abundances, was significantly higher in the WKC/R (137 ± 11 µg C ml^{-1}) than in L (96 ± 7 µg C ml^{-1}) (ANOVA, $F = 21.2$, $p = 0.001$). The community composition of ciliates varied greatly between habitats. *Prorodon sp.* and *Caenomorpha* spp. dominated in the KWC while *Cinetochilum margaritaceum, Colpoda cucullus, Euplotes* sp., were prevalent in the WKC/R. In L and P, the communi-

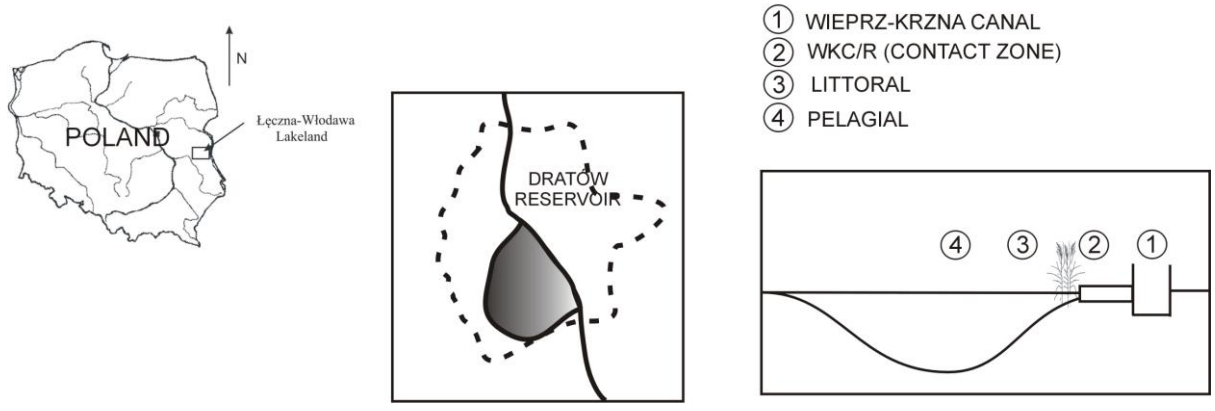

Fig. 1. Location of the study area and sampling points.

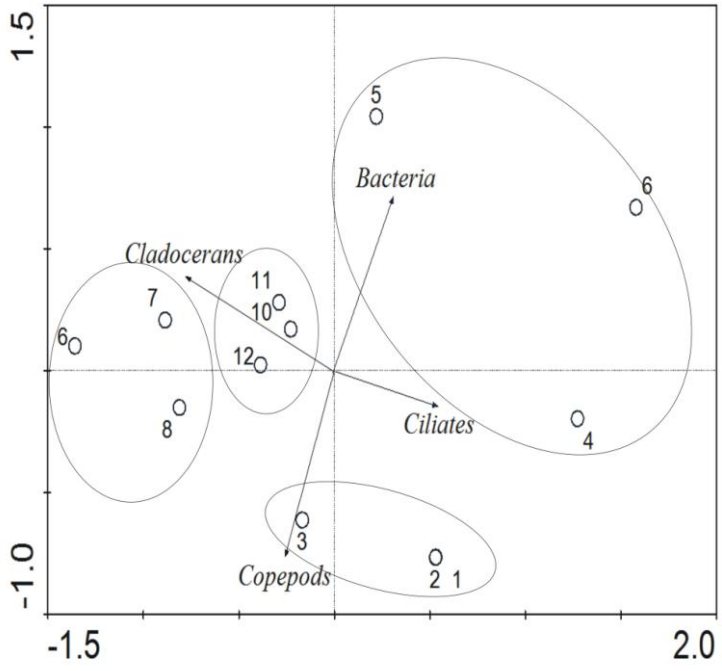

Fig. 2. Principal component analysis (PCA) biplots for axes 1 and 2 showing planktonic communities and study sites. Axes are derived from the variation in the taxonomic data-matrix. Samples collected in studied habitats are marked with an Arabic numeral: 1-3, Wieprz-Krzna Canal (WKC); 4-6, Wieprz-Krzna Canal/Reservoir (WKC/R); 7-9, littoral zone (L), 10-11, pelagic zone (P).

ties were predominantly composed of *Paramecium bursaria*, and *Halteria grandinella*.

The numbers of cladocerans ranged from 465 ± 16 individuals l^{-1} in L to 6490 ± 11 individuals l^{-1} in WKC/R. In all the examined habitats the highest abundance of these organisms occurred in summer (from 155 ± 8 individuals l^{-1} in the WKC to 10490 ± 13 individuals l^{-1} in the LG), while the lowest values were recorded in spring. The cladoceran biomass was significantly higher in the WKC (151 ± 11 mg l^{-1}) than in the other zones (<39 ± 7 mg l^{-1}) (ANOVA,

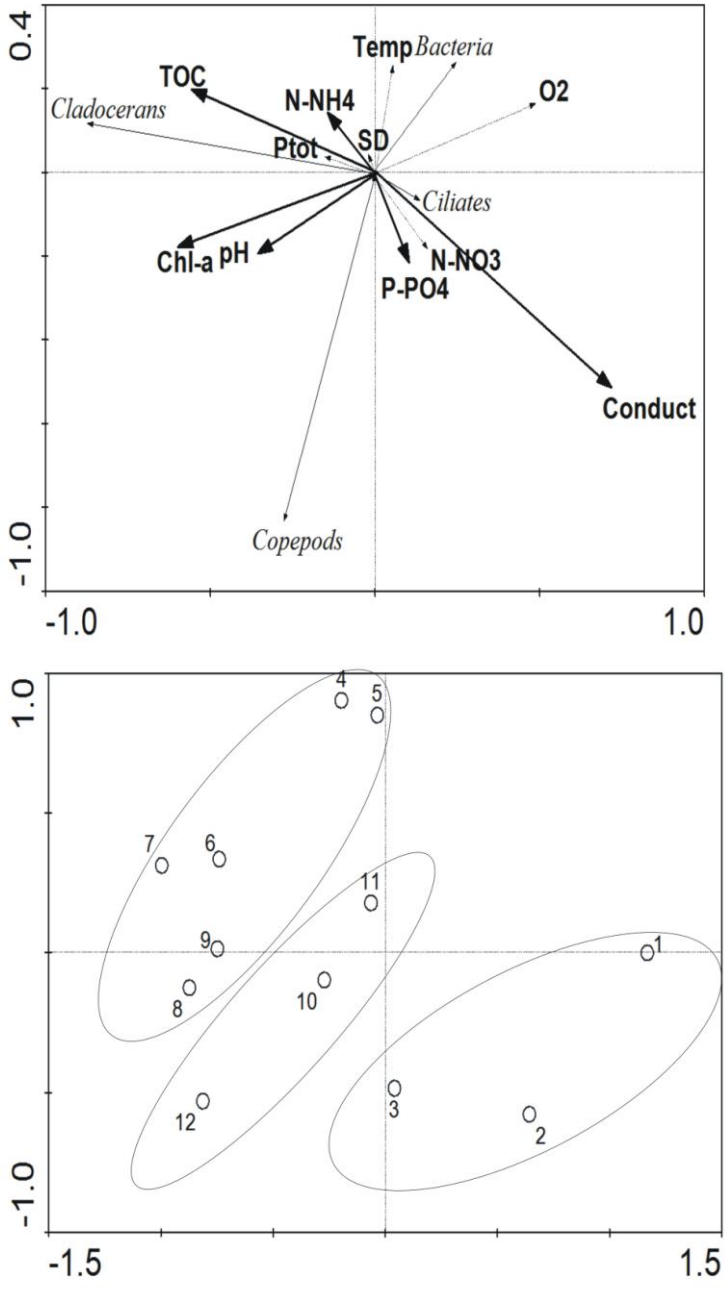

Fig. 3. Redundancy analysis (RDA) biplots showing: A) planktonic communities and environmental variables, B) studied habitats. Solid arrows indicate significant variables based on Monte Carlo permutation test ($P < 0.05$). Samples collected in studied habitats are marked with an Arabic numeral: 1-3, Wieprz-Krzna Canal (WKC); 4-6, Wieprz-Krzna Canal/Reservoir (WKC/R); 7-9, littoral zone (L), 10-11, pelagic zone (P). Environmental variable codes: SD, Secchi disc depth; Temp, water temperature; Conduct, conductivity; 02, dissolved oxygen; Chl-a, chlorophyll-a; N-NH4, ammonium nitrogen; N-NO3, nitrate nitrogen; Ptot, total phosphorus; P-PO4, dissolved orthophosphates; TOC, total organic carbon.

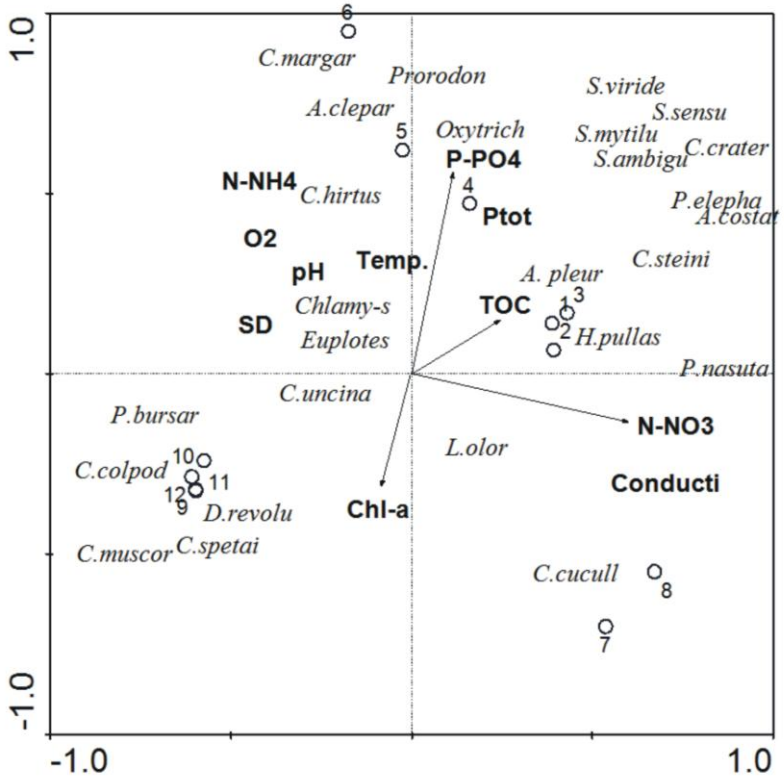

Fig. 4. Biplot of RDA of ciliates species, and samples. Arrows indicate significant parameters in Monte Carlo permutation test at P<0.05. Samples collected in studied habitats are marked with an Arabic numeral: 1-3, Wieprz-Krzna Canal (WKC); 4-6, Wieprz-Krzna Canal/ Reservoir (WKC/R); 7-9, littoral zone (L), 10-11, pelagic zone (P). Environmental variable codes: SD, Secchi disc depth; Temp, water temperature; Conduct, conductivity; 02, dissolved oxygen; Chl-a, chlorophyll-a; N-NH4, ammonium nitrogen; N-NO3, nitrate nitrogen; Ptot, total phosphorus; P-PO4, dissolved orthophosphates; TOC, total organic carbon. Species codes: A.clapar, *Amphileptus cleparedei;* A.pleur, *Amphileptus pleurosigma*; A.costat, *Aspidisca costata*; C.cucull, *Colpoda cucullus*; C.colpod, *Colpidium colpoda*; C.hirtus, *Coleps hirtus*; C.crater, *Codonella cratera*; C.steini, *Colpoda steinii*; C.margar, *Cinetochilum margaritaceum*; C.muscor, *Cyrtohymena muscorum*; C.spetai, *Coleps spetai*; Chlamy-s, *Chlamydonella*-spr; C.uncina, *Chilodonella uncinata*; D.revolu, *Drepanomonas revoluta*; Euplotes, *Euplotes* sp.; H.pullas, *Holosticha pullaster*; L.olor, *Lacrymaria olor*; Oxytrich, *Oxytrichia* sp.; P.elepha, *Paradileptus elephantinus*; P.bursar, *Paramecium bursaria*; P.nasuta, *Plagiopyla nasuta*; Prorodon, *Prorodon* sp.; S.ambigu, *Spirostomum ambiguum*; S.mytilu, *Stylonychia mytilus*-komplex; S.sensu, *Spathidium sensu lato*; S.viride, *Strombidium viride*.

F = 27.2, p = 0.001). Distinct horizontal differences were noted in the domination structure of cladocerans. *Bosmina longirostris* dominated in the KWC/R and L, while *Chydorus sphaericus* and *Bosmina coregoni* were prevalent in the pelagic zone. In WKC, the communities were predominantly composed of *B. coregoni*, and nauplii. In the littoral zone, only one species of Cladocera typical of the zone occurred, namely *Alona quadrangularis.* In the KWC and P, no typical species were recorded. The numbers and biomass of copepods varied between 1 and 555 ± 8 individuals l^{-1} and 0.001 and 2.7 ± 0.4 mg l^{-1} , respectively, with the highest mean numbers and biomass in the L and the lowest in the WKC (ANOVA, F = 23.6, p = 0.05). The highest abundances and biomass of copepods communities were noted in spring, whereas in summer we observed a remarkable decrease in abundance. In addition, the community composition of copepods varied greatly from WKC to P. The most abundant in WKC/R and L were *Cyclopoidae*, while *Eudiaptomus graciloides* was dominant in P. Exclusive species for the P were *Cyclops strenuus* and *Cyclops*

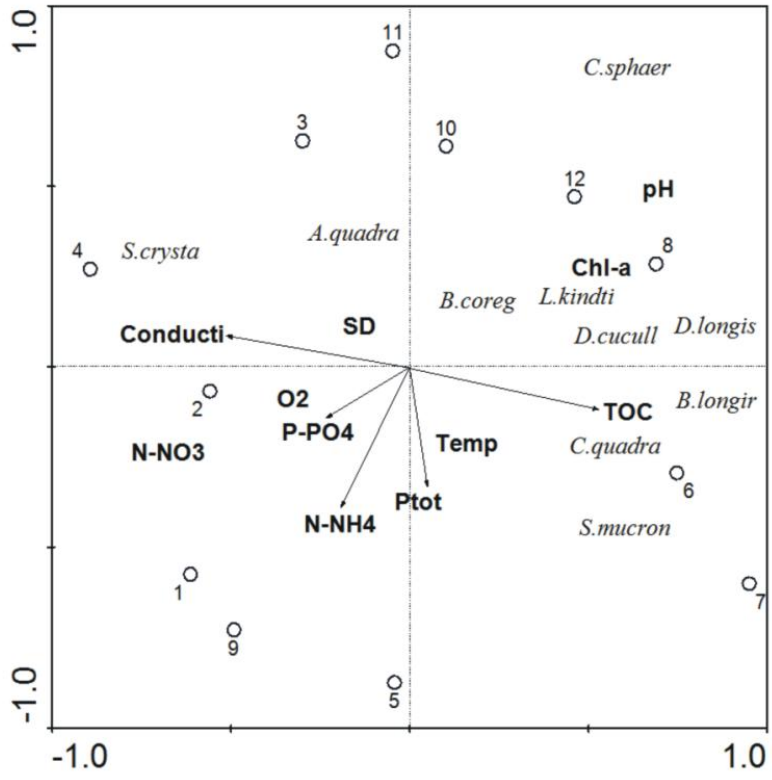

Fig. 5. Biplot of RDA of cladoceran species, and samples. Arrows indicate significant parameters in Monte Carlo permutation test at P<0.05. Samples collected in studied habitats are marked with an Arabic numeral: 1-3, Wieprz-Krzna Canal (WKC); 4-6, Wieprz-Krzna Canal/Reservoir (WKC/R); 7-9, littoral zone (L), 10-11, pelagic zone (P). Environmental variable codes: SD, Secchi disc depth; Temp, water temperature; Conduct, conductivity; 02, dissolved oxygen; Chl-a, chlorophyll-a; N-NH4, ammonium nitrogen; N-NO3, nitrate nitrogen; Ptot, total phosphorus; P-PO4, dissolved orthophosphates; TOC, total organic carbon. Species codes: A.quadra, *Alona quadrangularis*; B.coreg *Bosmina coregoni*; B.longir, *Bosmina longirostris*; C.sphaer, *Chydorus sphaericus*; C.quadra, *Ceriodaphnia quadrangula*; D.cucull, *Daphnia cucullata*; D.longis, *Daphnia longispina*; L.kindti, *Leptodora kindtii*; S.mucron, *Scapholeberis mucronata*; S.crysta, *Sida crystallina*.

vicinus. In the case of the KWK, only one exclusive species, *Eucyclops macrurus*, occurred.

Ordination analyses

PCA axis 1 (λ = 0.394) and axis 2 (λ =0.182) explained 57.4% of the total variance in planktonic composition. The first two axes showed a clear separation of the studied planktonic communities and habitats. The abundances of cladocerans and copepods were most strongly correlated with the main direction of variation (axis 1). Axis 2 was the most strongly correlated with abundance of bacteria and ciliata as well as with samples collected in WKC/R (Fig. 2). RDA performed in order to specify the direct relationships between abundances of taxonomic groups of planktonic communities and environmental variables, showed evident differences between the studied habitats. All variables together explained 45.7% of total variance. However, variables that significantly ($P < 0.05$, Monte Carlo permutation test) explained the variance in planktonic communities abundance were TOC ($\lambda = 0.17$; $F = 4.66$; $P = 0.006$), N-NH_4 ($\lambda = 0.09$; $F = 3.21$; $P = 0.014$), conductivity ($\lambda = 0.11$; $F = 3.16$; $P = 0.02$), pH ($\lambda = 0.09$; $F = 3.06$; $P = 0.02$), P-PO_4 ($\lambda = 0.06$; $F = 3.47$; $P = 0.026$) and chlorophyll-a ($\lambda = 0.16$; $F = 2.66$; $P = 0.044$). In the RDA biplot for plankton taxonomic groups, axis 1 appeared to separate the different

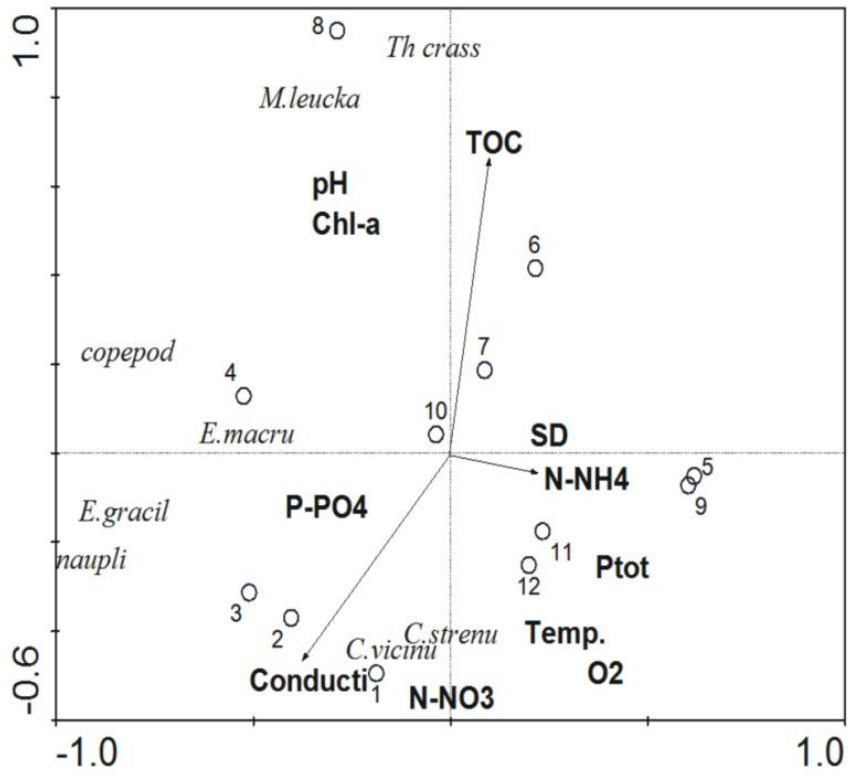

Fig. 6 Biplot of RDA of copepod species, and samples. Arrows indicate significant parameters in Monte Carlo permutation test at P<0.05. Samples collected in studied habitats are marked with an Arabic numeral: 1-3, Wieprz-Krzna Canal (WKC); 4-6, Wieprz-Krzna Canal/Reservoir (WKC/R); 7-9, littoral zone (L), 10-11, pelagic zone (P). Environmental variable codes: SD, Secchi disc depth; Temp, water temperature; Conduct, conductivity; 02, dissolved oxygen; Chl-a, chlorophyll-a; N-NH4, ammonium nitrogen; N-NO3, nitrate nitrogen; Ptot, total phosphorus; P-PO4, dissolved orthophosphates; TOC, total organic carbon. Species codes: C.vicinu, *Cyclops vicinus*; C.strenu, *Cyclops strenuus*; E.gracil, *Eudiaptomus graciloides*; copepod, copepodites; M.leucka, *Mesocyclops leuckarti*; Th.crass, *Thermocyclops crassus*.

components of planktonic food web; bacteria and ciliates are separated from cladocerans and cyclopoids (Fig. 3A). The RDA biplot for sites shows the direct effect of habitat on planktonic community. On the biplot it can be observed that samples collected in WKC (I group) are separated from those collected in P (II group) and WKC/R – L (III group) (Fig. 3B), and this may correspond with abundances of ciliata (Fig. 3A). However, RDAs performed on each planktonic community separately (ciliates, cladocerans, copepods) showed differences in relations between environmental variables and their density. The RDA for spatial distribution of ciliates showed that all environmental variables together explained 77% of the total variance. Monte Carlo permutation test showed the significance of P-PO_4 (λ = 0.34, F = 11.28, P = 0.002), N-NO_3 (λ = 0.06, F = 2.78, P = 0.04), TOC (λ = 0.08, F = 3.40, P = 0.048) and chlorophyll-a (λ = 0.06, F = 2.73, P = 0.049) in explaining the variability of ciliates in all habitats. On the RDA biplot, samples are divided into three groups (habitats): WKC, WKC/R and L-P. The species commonly observed in WKC (*Amphileptus pleurosigma*, *Holosticha pullaster*, *Plagiopyla nasuta*, *Colpoda steinii*) correspond with the gradient of N-NO_3. The species observed in the WKC/R habitat, *Cinetochilum margaritaceum*, *Prorodon* sp., *Amphileptus cleparedei* and *Oxytricha* sp., correspond with P-PO_4. The ciliates, *Colpoda cucul-*

lus, *Cyrtohymena muscorum*, *Coleps spetai*, *Drepanomonas revoluta*, *Colpidium colpoda* and *Parameciumbursaria* mostly correspond with L and P habitats and may be influenced by chlorophyll-a (Fig. 4). For cladocerans, all environmental variables explained 54.5% of the total variability. Monte Carlo permutation test showed the significance of five variables: TOC ($\lambda = 0.22$, $F = 6.16$, $P = 0.0024$), P-PO_4 ($\lambda = 0.1$, $F = 4.90$, $P = 0.014$), N-NH_4 ($\lambda = 0.07$, $F = 4.22$, $P = 0.02$), P-tot ($\lambda = 0.09$, $F = 3.45$, $P = 0.024$) and conductivity ($\lambda = 0.11$, $F = 3.59$, $P = 0.026$) in explaining the variability of cladocerans in the habitats. Axis 1 correlates mostly with TOC affecting the abundance of *Scapholeberis mucronata*, *Ceriodaphnia quadrangula* and *Bosmina longirostris*, and corresponding with WKC/R and L habitats as well as with conductivity affecting the abundance of *S. crystallina* and *A. quadrangularis* that may correspond with WKC habitat (Fig. 5). For copepods, the environmental variables explained 52% in their total variance in the studied habitats. Three variables, TOC ($\lambda = 0.11$, $F = 4.68$, $P = 0.024$), conductivity ($\lambda = 0.042$, $F = 3.55$, $P = 0.042$) and N-NH_4 ($\lambda = 0.07$, $F = 9.94$, $P = 0.046$), showed significant importance in the Monte Carlo permutation test. Axis 1 correlates weakly with N-NH_4. Axis 2 is mostly correlated with TOC and conductivity. The former variable may influence the presence of *Thermocyclops crassus* and *Mesocyclops leuckarti* and correspond with the WKC/R and L habitats, whereas the latter variable influences the density of *Cyclops strenuus* and *Cyclops vicinus* and correspond with the WKC habitat (Fig. 6)

Relationships among planktonic food-web components

Generally, the abundance of bacteria were correlated with the abundance of ciliates and concentrations of chlorophyll *a* (from r=0.27, $P \leq 0.05$ to r=0.58, $P \leq 0.01$). However, the number of significant correlations between the main groups of microorganisms forming the planktonic food web was different among the sites. In WKC/R, the relations between food-web components were stronger. Bacterial density and biomass correlated positively with the density and biomass of ciliates (r=0.58, r=0.63, $P \leq 0.01$) and negatively with density of cladocerans (r=-0.53, $P \leq 0.01$). The biomass of bacteria correlated positively with the chlorophyll *a* (r=0.42, $P \leq 0.05$). In the littoral zone, there was a significant and positive correlation between bacterial density and ciliates (r=0.35, $P \leq 0.05$). Ciliate density and biomass correlated with the abundance of bacteria (r=0.51, r=0.53, $P \leq 0.01$) and copepods (r=-0.27, r=0.32, $P \leq 0.05$) in the pelagic zone. In WKC, the total numbers and biomass of bacteria correlated with the density of ciliates (r=0.33, r=0.38, $P \leq 0.05$).

DISCUSSION

Microbial communities

So far, comparative data concerning the distribution of bacteria, protozoa and crustaceans in the horizontal transect (canal – contact zone (canal/reservoir) – littoral - open water zone) are very scarce. This study suggests significant relationships between the species richness of protozoa and metazoa and the type of habitat. A visible increase of the number of taxa usually occurred in contact zone Wieprz-Krzna Canal/reservoir (WKC/R); the lowest diversity was observed in pelagic zone. The most species found in all stations were eurytopic species, which have a rapid ecological adaptability and whose tolerance limits to environmental changes are wide. The small Scuticociliatida are frequently observed in productive systems. This group of ciliates presents an opportunistic bacterivorous behavior (Foissner and Berger, 1996). In the canal, the highest proportions were reached by prostomatids and pleurostomatids. Prostomatids are often reported as a relatively common component of protozooplankton, especially in high productive systems (Foissner and Berger, 1996). In the KWC, we observed low species diversity, probably as a consequence of current velocity, which confirms the studies of Primc-Habdija et al. (1998). In the ecotone we noted a very high number of ciliate, cladoceran and copepod taxa. Due to the presence of a "contact zone", the ecotone zone is usually inhabited by a large number of species. At particular sites, some physical or chemical water properties influenced the quality and quantity structure of planktonic ciliates. Along

with the increase of organic matter and nutrient concentrations, we observed a higher number of taxa and abundance of microorganisms. Similar relationships were noted in eutrophic lakes (Takamura et al., 2000, Mieczan, 2008). Ordination analyses performed on planktonic communities confirmed a clear separation of the studied habitats in their distribution, and RDA computed for each planktonic group separately showed that the spatial separation was strongest for ciliates and shaped along with the environmental gradient. The higher abundances of ciliates at the flow of Wieprz-Krzna Canal into the Dratów Reservoir were probably caused by a high nutrient concentration. Besides, in this particular zone, the total organic carbon (TOC) concentration in the water positively correlated with the density of bacteria, ciliates and crustaceans. This is in agreement with the results obtained during studies on bacterio- and zooplankton in lakes (Beaver and Crisman, 1990). It seems that the increase of ciliate density (especially Scuticociliatida) can be a reflection of good food conditions for that group, which is then foraged by crustaceans. In addition, the presence of emergent vegetation in the ecotone zone may influence the abundance of zooplankton through a slowing of the current and the development of microhabitats. Similar tendencies were observed in phyto- and zooplankton in the river-lake system of Krutynia River in Poland (Hillbricht-Ilkowska and Węgleńska, 2003). We observed a ninefold higher abundance and biomass of zooplankton in the contact and littoral zones than in the open water or sparsely vegetated areas. Several mechanisms may account for the contact zone and macrophyte beds being a more favorable habitat for zooplankton to develop. As reviewed by Jeppesen et al. (1998) and Mieczan (2007), in eutrophic lakes macrophyte beds provide zooplankton with a refuge against predatory effects of fish. On the other hand, that is also a zone of forage for obligatory planktivorous fish fry, which plays an important role in eliminating zooplankton (Whiteside, 1988; Zalewski et al., 1990). Our results showed that cladocerans and copepods, although occurring abundantly within the littoral zone, were mainly small-bodied species, whereas bigger crustaceans were mostly restricted to the open water zone. Hence, it cannot be excluded that planktivorous fish, being visual predators on larger crustaceans, have modified the crustacean community size structure by eradicating bigger species.

Relationships between food-web components

The analysis of trophic relationships in the system bacteria-ciliates-crustaceans reveals a clear differentiation and strength of mutual relations between the zones analyzed. The highest number of significant correlations was determined in the contact zone (KWC/R), and the lowest number in the water of the canal (KWC) and in the pelagic zone (P). Irrespective of the zone analyzed, bacteria and ciliates correlated with cladocerans and copepods. This suggests that the microorganisms could constitute a potential source of food for a number of groups within the planktonic food web. In the pelagic zone (P) and the canal (WKC), significant correlations were also determined between chlorophyll *a* concentrations and bacteria. This suggests that organic substances excreted by planktonic algae constitute one of the main sources of carbon for bacteria. Similar patterns were also observed in the littoral and pelagic zones of eutrophic lakes (Mieczan and Nawrot, 2012; Kalinowska et al., 2012). In the ecotone zone (WKC/R) and in the littoral (L), a substantially higher density of all the groups of organisms studied was determined in comparison to the remaining zones analyzed. Jeppesen et al. (1998) suggest that at an increased volume of zooplankton, both algal biomass (chlorophyll *a* concentration) and abundance of microorganisms should be effectively reduced by zooplankton inhabiting the macrophyte zone. Our study, however, reveals high numbers of bacteria in that zone. According to McQueen et al. (1986), "top-down" control of bacteria communities by zooplankton is much more effective in the presence of large filtrators, such as *Daphnia*, that occurred only sparsely in the ecotone zone (WKC/R) and in the littoral (L). It seems that in the remaining zones studied, zooplankton could effectively control the abundance of ciliates. According to a study conducted by Wickham (1995), ciliates are frequently consumed by crustaceans from the genus *Cyclops*, e.g. small Scuticociliatida or *Halteria*, to the amount of 20-30 ciliates copepod^{-1} h^{-1}. The strong

predation pressure of crustaceans on ciliates in Lake Dratów was particularly confirmed by observations in the summer season, when the numbers of protozoa clearly decreased along with an increase in the numbers of cladocerans and copepods. Crustaceans probably selectively consumed protozoan communities, which was also reflected in the predominance of small taxa of ciliates, particularly in the summer season. Similar patterns were also observed in eutrophic estuaries (Urrutxurtu et al., 2003).

CONCLUSION

The present study showed clear horizontal distribution patterns of bacteria, ciliates and crustaceans. The highest abundance and biomass of microorganisms and crustaceans were observed in canal-reservoir contact zone (WKC/R), while the lowest values were noted in the pelagic zone. Hence, the contact zone in Lake Dratów could fulfill the function of an ecotone, distinguished by a significant increase in biodiversity, abundance, and species specificity of micro- and macroorganisms. Irrespective of the zone analyzed, correlative gradients between bacteria-ciliates-crustaceans were found, suggesting strong trophic relations between planktonic communities. Nevertheless, the number of significant correlations between the main groups of microorganisms forming the planktonic food web was different among sites, and in the canal/reservoir contact zone the relations between food-web components were the strongest. These correlations were strongly supported by environmental variables. Taking into account that the canal-reservoir contact zone (WKC/R) could also be a place of very efficient matter and energy flow into the lake ecosystem, it could indirectly strengthen the relations between successive components of the food web in lakes. Therefore, knowledge of the relationships between planktonic communities and their environment is of crucial importance in understanding the functioning of freshwater ecosystem.

REFERENCES

Augustin, H., Foissner, W. and *H. Adam* (1984). An improved pyridinated silver carbonate method which need few specimens and yields permanent slides of impregnation ciliates (Protozoa, Ciliophora). *Mikroskopie* **41**, 134-137.

Beaver, J.R. and *T.L Crisman* (1990). Seasonality of planktonic ciliated protozoa in 20 subtropical Florida lakes of varying trophic state. *Hydrobiologia* **190**, 127-135.

Biyu, S. (2000). Planktonic protozooplankton (ciliates, heliozoans and testaceans) in two shallow mesotrophic lakes in China – a comparative study between a macrophyte-dominated lake (Biandantang) and algal lake (Houhu). *Hydrobiologia* **434**, 151-163.

Bottrell, H.H., Duncan, A., Gliwicz, Z.M., Grygierek, E., Herzig, A., Hillbricht – Ilkowska, A., Kurasawa, H., Larsson, P. and *T. Węgleńska* (1976). Review of some problems in zooplankton production studies. *Norw. J. Zool*. **24**, 419-456.

Culver, D.A., Boucherle, M.M., Bean, D.J. and *W.J. Fletcher* (1985). Biomass of freshwater crustacean zooplankton from length-weight regression. *Can. J. Fish. Aquat. Sci*. **42**, 1380-1390.

Dawidek, J., Sobolewski, S. and *M. Turczyński* (2004). Transformations of catchment areas of lakes converted into storage reservoirs in the Wieprz-Krzna Canal system. *Limnological Review* **4**, 67-74.

Di Castri, F., Hansen, A.J. and *M.M. Holland* (1988). A new look at ecotones: emerging international projects on landscape boundaries. *Biol. Int*. **17**, 1-163.

Dumont, H.J., Van de Velde, I. and *S. Dumont* (1975). The dry weight estimate of biomass in selection of Cladocera, Copepoda and Rotifera from the plankton, periphyton and benthos of continental waters. *Oecologia* **19**, 75-97.

Ejsmont-Karabin, J. (2003). Is sandy beach of the lake an ecotone? Psammon rotifera in a mesotrophic Lake Kuc (Masurian Lakeland, Northern Poland). *Pol. J. Ecol.* **51**, 219-224.

Fleituch, T., Starzecka, A. and *T. Bednarz* (2001). Spatial trends in sediment structure, bacteria and periphyton communities across a freshwater ecotone. *Hydrobiologia* **464**, 165-174.

Finlay, B.J. (1982). Procedures for the isolation, cultivation and identification of protozoa. *Exp. Microb. Ecol*. **1**, 44-65

Foissner, W. and *H. Berger* (1996). A user-friendly guide to the ciliates (Protozoa, Ciliophora) commonly used by hydrobiologists as bioindicators in rivers, lakes and wastewaters, with notes on their ecology. *Freshwat. Biol.* **35**, 375-470.

Foissner, W., Berger, H. and *J. Schaumburg* (1999). *Identification and Ecology of Limnetic Plankton Ciliates*. Informationsberichte des Bayer. Landesamtes für Wasserwirtschaft, München, 1-800.

Gilbert, D., Amblard, C., Bourdier, G. and *A.J. Francez* (1998). The microbial loop at the surface of a peatland: structure,

functioning and impact of nutrients inputs. *Microb. Ecol*. **35**, 89-93.

Golterman, H.L. (1969). *Methods for chemical analysis of freshwaters*. Blackwell Scientific Publications, Oxford, Edinburgh, 1-172.

Hillbricht-Ilkowska, A. and *T. Węgleńska* (2003). River-lake system as mosaic pattern of landscape patches and their transition zones (ecotones). *Pol. J. Ecol.* **2**, 163-174.

Jack, J.D. and *J.J. Gilbert* (1997). Effects of metazoan predators on ciliates in freshwater plankton communities. *J. Euk. Microbiol*. **44**, 194-199.

Jeppesen, E., Lauridsen, T.L., Kairesalo, T. and *M.R. Perrow* (1998). Impact of submerged macrophytes on fish-zooplankton interactions in lakes, In: *The structuring role of submerged macrophytes in lakes* (Eds. E. Jeppesen, M. Sondergaard and K. Christofersen), 91-114, Springer, New York.

Jerome, C.A., Montagnes, D.J.S. and *F.J.R. Taylor* (1993). The effect of the quantitative protargol stain and Lugols and Buenos fixatives on cell size: A more accurate estimate ciliate species biomass. *J. Euk. Microbiol*. **40**, 254-259.

Kalinowska, K., Ejsmont-Karabin, J., Gorelysheva, Z. and *I. Rybak* (2012). Biomass distribution and dynamics and food web relations in psammon community of an eutrophic lake. *Pol. J. Ecol.* **3**, 443-453.

Lepš, J. and *P. Šmilauer* (2003). Multivariate Analysis of Ecological Data using CANOCO. University Press, Cambridge, 1-251.

McQueen, D.J., Post, J.R. and *E.L. Mills* (1986). Trophic relationships in freshwater pelagic ecosystems. *Can. J. Fish. Aquat. Sci.* **43**, 1571-1581.

Mieczan, T. (2007). Size spectra and abundance of planktonic ciliates within various habitats in a macrophyte - dominated lake (Eastern Poland). *Biologia* **62**, 189-194.

Mieczan, T. (2008). Influence of emergent and submerged macrophytes on the structure of planktonic ciliate communities in shallow freshwater lakes (Eastern Poland). *Int. Rev. Hydrobiol*. **93**, 269-283.

Mieczan, T. and *D. Nawrot* (2012). Vertical micro-distribution of psammonic ciliates and their relationship to physical and chemical parameters in two lakes of different trophic status. *Oceanol. Hydrobiol. St.* **2**, 48-56.

Pierce, R.W. and *J.T. Turner* (1992). Ecology of plankton ciliates in marine food webs. *Rev. Aquat. Sci.* **6**, 139-181.

Porter, K.G. and *Y.S. Fleig* (1980). The use of DAPI for identifying and counting aquatic microflora. *Limnol. Oceanogr*. **25**, 943-948

Takamura, N., Shen, Y. and *P. Xie* (2000). Species richness of protozoa in Japanese lakes. *Limnology* 1, 91-106.

Urrutxurtu, I., Orive, E. and *A. Sota* (2003). Seasonal dynamics of ciliated protozoa and their potential food in an eutrophic estuary (Bay of Bascay). *Estuar. Coast. Shelf Sci.* **57**, 1169-1182.

Whiteside, M.C. (1988). 0+fish as major factors affecting abundance patterns of littoral zooplankton. *Verh. Int. Verein. Limnol.* **23**, 1710-1714.

Wickham, S.A. (1995). Cyclops predation on ciliates: species-specific differences and functional responses. *J. Plankton Res*. **17**, 1633-1646.

Xu, M., Cao, H., Xi,e P., Deng, D., Feng, W. and *J. Xu* (2005). The temporal and spatial distribution, composition and abundance of Protozoa in Chaohu Lake, China: relationship with eutrophication, *Eur. J. Protistol*. **41**, 183-192.

Zalewski, M., Brewińska-Zaraz, B. and *P. Frankiewicz* (1990). Fry communities as a biomanipulation tool in a temperate lowland reservoir. *Arch. Hydrobiol. Beih. Ergebn. Limnol.* **33**, 763-774.