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ZOOPLANKTON STRUCTURE IN TWO INTERCONNECTED PONDS: SIMILARITIES AND DIFFERENCES

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ABSTRACT

The research of zooplankton diversity, abundance and trophic structure was conducted during the summer period in pelagial zone on the longitudinal profile of the Sutla River Backwater. Investigated site consists of two interconnected basins: transparent Upper Basin with submerged macrophytes and turbid Lower Basin without macrophytes in the littoral zone. In the Upper Basin, abundance and diversity of zooplankton in the pelagial was higher in comparison to the Lower Basin, with prevailing species of genus Keratella as microfilter-feeder, and genera of Polyartha and Trihocerca as macrofilterfeeder rotifers. On the contrary, in the Lower Basin, crustaceans dominated in abundance. Microfilter-feeder cladoceran (Bosmina longirostris) and larval and adult stages of macrofilter-feeder copepod (Macrocyclops albidus) prevailed in the Lower Basin. Fish predation pressure was more pronounced in the pelagial of the Upper Basin, indicated by low cladoceran abundance in the surface layer. Although the studied basins were interconnected, results indicate significant (Mann-Whitney U test, p < 0.05) differences in the zooplankton structure as a potential result of the macrophyte impact on environmental conditions and fish predation pressure.

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INTRODUCTION

Shallow lakes have an exceptional ecological significance (biodiversity of invertebrates, fish, water birds), but they are often neglected in limnological studies (Céréghino et al., 2008). Anthropogenic impacts frequently lead to the eutrophication and acidification of these habitats, and the introduction of invasive plants and animals results in reduced biodiversity (Kruk et al., 2009). Besides ecological significance, shallow lakes have cultural and economic value, manifested through sport, recreation and tourism. Eutrophication, as

the most pronounced recent issue, is facilitated by multiple anthropogenic impacts: leaching of natural and mineral fertilizers from agricultural land, inflow of municipal and industrial waste water, fishing and recreation. In response to the increased nutrients, especially phosphorus, most of the shallow lakes have changed from transparent to the turbid state (Scheffer et al., 1993; Beklioglu et al., 2003). Turbid waters are characterized by the increasing of planktivorous and bentivorous fish, higher biomass of phytoplankton and sediment resuspension caused by wind and fish. On the other hand, transparent waters are characterized by high

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biomass of submerged macrophytes and abundant algaevorous zooplankton (large-bodied crustacean grazers). Macrophytes can significantly alter abiotic (temperature, oxygen concentration, light intensity) and biotic (food availability, competition, predation) factors in aquatic ecosystems (Cazzanelli et al., 2008; Špoljar et al., 2012). Their stands have an important role in sediment stabilization, reduction of coastal erosion and in the assimilation of nutrients into biomass, which results in decreasing of phytoplankton biomass and trophic level. Concurrently, macrophytes increased habitat diversity, provided plenty and diverse food resources, and refuge from predators (larvae and adult fish insects) for the zooplankton and benthic invertebrates (Estlander et al., 2009; Špoljar, 2013).

Fish, as visual predators, are one of the main factors modifying the abundance, diversity and horizontal migration of the zooplankton in shallow lakes. Thus, littoral zone with submerged macrophytes reduces fish predation and enables refuge for the potential prey in pelagial such as large-bodied algaevorous cladocerans (Jeppesen et al., 1999; Estlander et al., 2009). Otherwise, increased turbidity decreases fish predation in pelagial as well as the importance of macrophyte stands as a refuge for zooplankton. In these conditions, small-bodied detritivorous cladocerans and rotifers prevailed in the pelagial of turbid waters (Špoljar et al., 2011). The zooplankton structure in interconnected lakes possesses patterns that vary depending on the trophic level and biotic interactions in accordance with the theory of alternative states, i.e. transparent v. turbid state (Scheffer et al., 1993; Cotteine et al., 2001). The composition of the zooplankton in these lakes is also affected by the possibility of dispersion and colonization (Gliwicz et al., 2000; Cottenie et al., 2001; Michels et al., 2001).

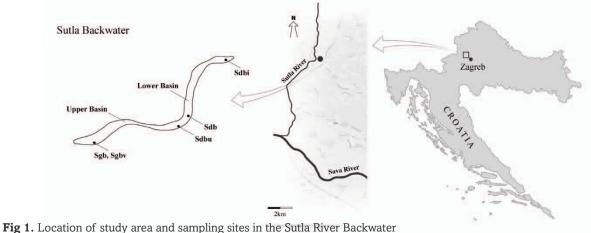
The aim of this study was to determine similarities and differences in zooplankton structure between two interconnected basins in the backwater of the Sutla River, based on the following community parameters: (i) diversity and abundance of main zooplankton groups; (ii) abundances of functional feeding guilds; and (iii) fish predation.

MATERIALS AND METHODS

This research was conducted from May to October 2013 in the backwater of the Sutla River, located in the northwest of Croatia. Sutla River Backwater consists of two interconnected basins (Fig. 1). Transparent Upper Basin (UB; width- $_{max}$ = 13 m length $_{max}$ = 124 m, depth $_{max}$ = 2.75 m) has a coverage of submerged macrophytes of 32 ± 12%, and hornwort (Ceratophyllum demersum L., 1753) as dominant species. Turbid Lower Basin (LB width_{max} = 14 m length $_{max}$ = 188 m, depth_{max} = 4.15 m) is without macrophytes in the littoral zone. Both basins are under distinct anthropogenic impacts, i.e. leaching from the surrounding meadows and fields. The Lower Basin is also exposed to the intensive sport fishing: fish stocking, the use of protein baits and food supplements. Samples from the UB were taken in the pelagial at two stations: the surface layer (Sgb) and bottom water layer (Sgbv). LB samples were collected at three stations: the water inflow from the UB to the LB (Sdbu), the surface water layer (Sdb) and the water outflow to the channel connected with the Sutla River (Sdbi).

Samples were taken on nine occasions, monthly in May, September and October, and biweekly during June, July and August. Exception was station Sdbu, where analyses started in the second half of July. From each station samples were collected in triplicate; each 30 L (3 × 10 L) sample of water was filtered through 26 µm mesh plankton net, except at the (Sgbv) station where a total of 15 L (3 × 5 L) of water was filtered by the Van Down sampler (volume 5 L).

A mean of triplicate was used as a single data point for a given occasion (n = 41). Zooplankton was fixed in 4% formalin, determined and counted. In this study, qualitative and quantitative compositions of three planktonic groups — rotifers (Rotifera), copepods (Copepoda) and cladocerans (Cladocera) — were taken into consideration, determined to the genus or species level using determination keys: Voigt and Koste (1978), Margaritora (1983) and Einsle (1993). Bdelloidea, Ostracoda, Nematoda, Gastrotricha, Oligochaeta, Tardigrada and Hydrachnidia were counted but not identi-



fied. For quantitative analysis, entire sample was counted using a Petri dish under an Opton-Axiovert 35 inverted microscope ($200\times$, $400\times$). Fish were collected at sampling stations Sgb, Sdbu and Sdb by electrofishing and their abundance was shown as catch per unit effort of 15 min during daylight hours (Hans Grassl EL 63 II, 220/440 V, 17.8/8.9 A).

The following parameters were measured in situ: temperature, oxygen concentration (WTW OXI 96), pH (WTW 330i) and conductivity (HACH sension 5). For analyses of chemical parameters, 3 L of non-filtered water were taken in bottles at the same study stations as the zooplankton samples. All nutrients, ortophosphates, total phosphorus, nitrates and Kjeldahl total nitrogen were determined by APHA, 1998. Nitrites and ammonium were measured using ion chromatograph (Dionex ICS-3000). Chemical oxygen demand (COD) was measured by the oxidation of dissolved organic matter, using KMnO₄ (results expressed as mg O_{2Mn} L⁻¹). Phytoplankton biomass (measured as chlorophyll α) and concentration of particulate organic matter (POM, measured as ash free dry mass, AFDM) were considered as food resources for the main zooplanktonic groups. Chlorophyll a (Chl a) concentration was analysed by ethanol extraction according to Nusch (1980). Ash free dry mass was determined from 30 L filtered water samples (mesh 26 µm); these were dried at 104°C for 4 h on ceramic dishes and ashed at 600°C for 6 h. Macrophyte coverage was calculated according to Špoljar et al. (2011). Trophic state index based on transparency (TSI_{sp}) was determined according to Carlson (1977). Rotifers were divided in functional feeding guilds (trophic groups) as microfilter-feeders (detritivorous), macrofilter-feeders (algivorous) and predators (Karabin, 1985; Špoljar et al., 2007, 2012). Generally, crustacean trophic structure was considered on the taxonomic level, because cladocerans were presented by microfilter-feeders, and larval and adult stages of copepods were presented by macrofilter-feeders. Prior to statistical analysis, all abiotic and biotic data were logarithmically transformed [log (x+1)] and their normality was checked using Shapiro-Wilk's test. As this test suggested that the data did not follow a normal distribution, even after transformation, a nonparametric Kruskal-Wallis test (comparison among multiple independent samples) or a Mann-Whitney U test (comparison between two independent samples) for environmental parameters and biotic components were used. For an explanation of relationships between the abundance of three zooplankton groups and environmental variables (conductivity, pH, Chl a, AFDW), a Spearman correlation coefficient was used. For statistical data analysis the STATISTICA software package was used (StatSoftŌ).

RESULTS

 TSI_{SD} was 60 ± 5.5 in the UB, indicating a transition of mezotrophic to the eutrophic level, while TSI_{SD} was 70 ± 2 in the

LB, indicating a transition of eutrophic to the hypertrophic level. Minimum, maximum and average values of physicochemical parameters and significance of their spatio-temporal oscillations are shown in Table 1. Transparency, POM and phytoplankton biomass were significantly higher in the UB in comparison to the LB, and the opposite was established for the conductivity.

During the study period 37 zooplankton taxa were determined. Rotifers achieved the highest diversity (27 species) and dominated in abundance (80 %) of the UB, while planktonic crustaceans (cladocerans and copepods) dominated in the LB (Fig. 2).

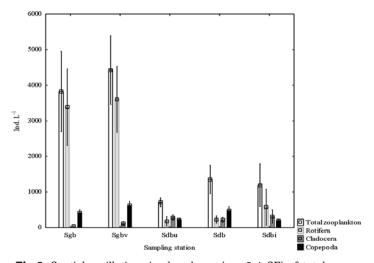


Fig 2. Spatial oscillations in abundance (n = $3 \pm SE$) of total zooplankton, rotifers, cladocerans and copepods on the longitudinal profile of the Sutla Backwater

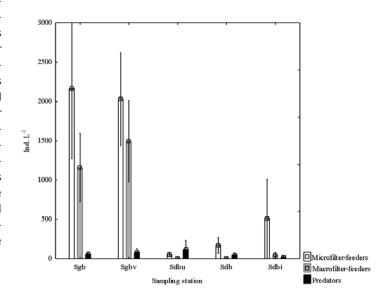


Fig 3. Spatial oscillations of rotifer trophic groups: microfilter-feeders, macrofilter-feeders and predators (n = 9 \pm SE) on the longitudinal profile of the Sutla Backwater

Table 1. Minimum (Min), maximum (Max), mean value and standard deviation (SD) of environmental parameters and the significance of spatial and temporal oscillations on the longitudinal profile of the Sutla River Backwater (Kruskal-Wallis test, p < 0.05; multiple comparison post hoc test, n = 41)

Envarimenal parametars	Min	Max	Mean	±	S SD	Spatial oscillations			Temporal oscillations		
						Н	р	Multiple comparison	Н	р	Multiple comparison
Depht (m)	0.4	4.2	1.8	±	0.9	28.8	0	Sdbi < Sdb. Sgb. Sgbv Sdb < Sdbu	5.8	ns	
Transparancy _{sp} (m)	0.3	1.5	0.8	±	0.4	22.0	0.0002	Sdb < Sgb. Sgbv	10.9	ns	
Temperature (°C)	13.1	26.6	20.1	±	3.7	1.1	ns		36.9	0 VI/2 > VI/1. IX	X < VI/2. VII/2. VIII/1
Concentration of dissolved O ₂ (mg O ₂ L ⁻¹)	2.0	9.2	5.2	±	1.8	2.6	ns		25.9	0.0011	VIII/1 < VI/1. VI/2
pН	7.4	8.4	7.7	±	0.2	7.8	ns		17.5	0.0254	X > VI/1. VI/2
								Sgb < Sdb. Sdbi			
Conductivity	324.0	681.0	526.9	±	122.7	27.0	0	Sgbv < Sdbi	2.6	ns	
								Sdbi > Sgb. Sgbv			
Orthophosphates (mg P-PO ₄ ³⁻ L ⁻¹)	0.025	1.011	0.127	±	0.176	8.0	ns		23.5	0.0028	VII/1 > VI/2
Total phosphorus (mg P L ⁻¹)	0.144	1.834	0.472	±	0.287	7.7	ns		13.8	ns	
Ammonia (mg N-NH ₃ L ⁻¹)	0.004	0.566	0.150	±	0.131	0.6	ns		28.4	0.0004 VI/1 > VIII/1. VIII/2	V > VIII/1. VIII/2
Nitrites (mg N-NO ₂ -L-1)	0.024	0.875	0.220	±	0.205	1.7	ns		31.5	0.0001	V > VIII/1. VIII/2
Nitrates (mg N-NO ₃ - L ⁻¹)	0.002	0.117	0.022	±	0.023	6.7	ns		19.0	0.015	VII/2 < V
Total nitrogen (mg N L ⁻¹)	0.248	2.518	1.189	±	0.579	1.5	ns		32.0	0.0001 X < VI/1. VIII/2	V > IX. X
DOM (mg O _{2Mn} L ⁻¹)	14.220	38.552	21.582	±	6.435	4.7	ns		33.0	0.0001 IX <vii 2<="" td=""><td>V > VII/1. IX. X</td></vii>	V > VII/1. IX. X
Phytoplankton biomass (μg Chl a L ⁻¹)	0.296	150.302	26.811	±	30.738	10.5	0.031	Sdb < Sgbv	7.1	ns	
POM (mg AFDM L ⁻¹)	0.303	9.950	1.960	±	2.025	2.1	0.46628	Sgbv > Sdb, Sdbu	24.8	ns	

^{*}ns – non significant

Within rotifers and cladocerans of both basins, microfilter-feeders prevailed (Fig. 3). Among rotifers, *Keratella cochle-aris* (Gosse, 1851) and *Trichocerca similis* (Wierzejski, 1893) dominated, within crustaceans *Bosmina longirostris* (O.F. Müller, 1776) from cladocerans and nauplii and copepodites of copepods.

In the spatial oscillations of the zooplankton structure (diversity, abundance and trophic groups), significant differences were established (Table 2). Overall, sampling stations in the UB obtained higher diversity and abundance compared to

those in the LB (Mann-Whitney U test, p < 0.05), except cladoceran abundance which achieved higher values in the LB (Mann-Whitney U test, Z = -2.90, p = 0.003).

In this basin, crustaceans significantly contributed and highly positively related to the zooplankton total abundance (r = 0.72; p < 0.05). Temporal oscilations of the zooplankton structure were not statistically significant (Kruskal-Wallis test, p > 0.05). Considering fish, 15 different species were noted, 11 were cyprinids, two perciformes and one of catfish and pikes (Fig. 4). The abundance and diversity of fish did

Table 2. The significance of spatial differences in the zooplankton diversity and abundance among sampling stations on the longitudinal profile of the Sutla Backwater (Kruskal-Wallis test, p < 0.05; multiple comparison post hoc test, n = 41)

	Н	р	Multiple comparison
Zooplankton diversity	15.59	0.0036	Sgbv>Sdbu
Total zooplankton	16.35	0.0026	Sgbv>Sdbi
Rotifera	24.26	0.0001	Sgbv>Sdbu, Sdb, Sdbi
Keratatella cochlearis	23.85	0.0001	Sgb, Sgbv>Sdbu, Sdbi
Trihocerca similis	30.74	0.0001	Sgbv>Sdbu, Sdb, Sdbi Sgb>Sdb, Sdbi
Cladocera	11.28	0.0236	Sdbu>Sgb
Copepoda	11.92	0.018	Sgbv>Sdbi
Rotifera microfilter-feeders	19.52	0.0006	Sgbv>Sdbu, Sdbi Sgb>Sdbi
Rotifera macrofilter-feeders	28.11	0.0001	Sgbv>Sdbu, Sdb, Sdbi Sgb>Sdbu, Sdbi

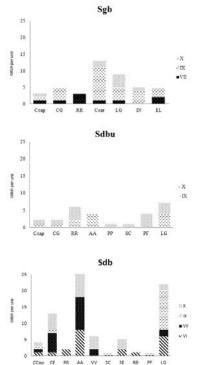


Fig 4. Oscillations in fish abundance and diversity on sampling stations Sgb, Sdbu and Sdb during the study period. Abbreviations: CCap Common carp (Cyprinus carpio); CE Loach (Cobitis elongata); CG Prussian carp (Carassius gibelio); RR Common roach (Rutilus rutilus); CCar Crucian carp (Carassius carassius); AA Bleak (Alburnus alburnus); PP Stone moroko (Pseudorasbora parva); VV Vimba bream (Vimba vimba); SC Chub (Squalius cephalus); SE Roach (Scardinius erythrophtalmus); BB White bream (Blicca bjoerkna); PF European perch (Perca fluviatilis); LG Pumpkinseed (Lepomis gibbosus); IN Brown bullhead (Ictalurus nebulosus); EL Pike (Esox luciuss)

not show any statistically significant oscillations in the spatial and even temporal scale (Kruskal-Wallis test, p > 0.05). In the environmental-biocoenotic interactions, macrophyte coverage positively correlated with transparency (r = 0.59; p < 0.05), phytoplankton biomass (r = 0.47; p < 0.05) and POM (r = 0.58; p < 0.05). Transparency and macrophyte coverage (r = 0.69; p < 0.05) positively affected zooplankton diversity and negatively impacted cladoceran abundance in pelagial (macrophyte coverage: cladocerans r = -0.52; p < 0.05). Microfilter-feeder rotifers, i.e. Keratella cochlearis, were in direct competition for food and space with microfilter-feeder cladocerans, indicated by their negative correlation. In the less transparent LB, the concentration of POM positively influenced the cladoceran abundance (r = 0.68; p < 0.05). Fish negatively impacted total zooplankton as well as abundance of a particular group (Table 3).

DISCUSSION

Zooplankton structure was studied in two interconnected basins of the Sutla River Backwater: the UB, densely covered with submerged macrophytes (i.e. hornwort) and the LB, without macrophytes. The higher transparency in the UB can be explained by the presence of submerged macrophytes that prevented resuspension of sediment and reduced nutrient concentrations which commonly stimulate phytoplankton production and turbidity (Horppila and Nurminen, 2005; Špoljar, 2013). Significantly higher POM value in the UB originated from hornwort stem fragments. Instead of presence of submerged macrophytes, higher concentration of phytoplankton biomass in the UB could be induced by abundance of detritivorous species that feed on suspended organic matter and not on algae (Jeppesen et al., 1999). Higher turbidity in the LB was strongly associated

Table 3. Spearman coefficient correlations (p < 0.05, n = 41) for abiotic and biotic interactions on the longitudinal profile of the Sutla Backwater

	Transparency	Phytoplankton biomass	РОМ	Cladocera	Copepoda	Fish			
	r								
Transparency	-	ns	ns	-0.46	0.46	ns			
Phytoplankton biomass	ns	-	0.51	ns	0.48	ns			
POM	ns	0.51	-	ns	0.41	ns			
Zooplankton diversity	0.32	0.50	0.63	-0.54	0.37	ns			
Total zooplankton	0.44	0.55	0.62	ns	0.66	-0.38			
Rotifers	0.55	0.49	0.55	0.52	0.41	ns			
Keratatella cochlearis	0.60	0.40	0.47	-0.49	0.38	ns			
Trihocerca similis	0.57	0.48	0.62	-0.48	0.41	ns			
Crustracea	ns	ns	0.32	0.34	0.78	-0.50			
Cladocera	-0.46	ns	ns	-	ns	ns			
Copepoda	0.46	0.48	0.41	ns	-	-0.43			
Rotifera microfilter-feeders	ns	ns	ns	-0.44	0.44	ns			
Rotifera macrofilter-feeders	ns	ns	ns	-0.53	ns	ns			

^{*}ns - non significant

with DOM, POM and inorganic matters. It prevented light penetration and development of submerged macrophytes, thus sediment resuspension was higher (Søndergaard et al., 2007; Feldmann and Nõges, 2007). Also, higher abundance of benthic fish in the LB stimulates turbidity: loach and carp (Mičetić et al., 2008), for example, facilitated bioturbation and prevented macrophytes rooting.

Higher abundance (UB 4129 \pm 3064 ind L⁻¹, LB 1152 \pm 1341 ind L^{-1}) and diversity (UB 15 ± 4 taxa, LB 10 ± 3 taxa) of zooplankton in the UB (Mann-Whitney U test, p < 0.05), compared to the LB, can be explained by the presence of macrophyte stands in the littoral zone of UB, where many zooplankton organisms find refuge and food resources. Therefore, our results are in accordance with other studies in macrophytes-zooplankton relationships (Jeppesen et al., 1999; Estlander et al., 2009). Mainly small-bodied detritivorous rotifers (Keratella cochlearis, K. quadrata, Anuaraeopsis fissa, Filinia longiseta) were presented in the surface water layer of the UB. Due to their small body size and various protective mechanisms (sharp lorica extensions), they successfully avoided predation from adult planktivorous fish and achieved abundant and dominant populations in the UB (Stemberger and Gilbert, 1984; Spoljar et al., 2011). Zooplankton abundance in bottom water layer of the UB was particularly high (Kruskal-Wallis test, p< 0.05), caused by increased abundance of cladocerans, copepods and largebodied rotifer (e.g. Asplanchna priodonta). This indicates that even in shallow water bodies vertical migration against visual predators existed (Castro et al., 2007). The intensity of vertical migration in this study was most pronounced by cladoceras, indicated by their increased abundance

at the bottom in relation to the surface water layer of the UB. This vertical migration of microfilter-feeder cladoceras provided optimal conditions for microfilter-feeder rotifers development in the surface water layer of the UB (Špoljar et al., 2011).

Interaction of abiotic and biotic factors in the UB enabled optimal conditions for development of numerous r-strategist rotifer populations in the food-rich environment in the pelagial surface water layer of the UB (Lapesa et al., 2002; Kuczyńska-Kippen, 2007). Although in this study horizontal migrations of zooplankton were not considered, it was presumed that low abundance of cladocerans and absence of large-bodied ones in the surface water layer of the UB was a consequence of their horizontal migration in macrophyte covered littoral zone (Kuczyńska-Kippen and Nagengast, 2006; Estlander et al., 2009). Thus, negative correlation between transparency and cladocerans abundance (r = -0.46, p < 0.05) can be explained by their escape in the littoral zone or bottom water layer. Absence of large-bodied cladocerans in the UB is in concordance with results reported by other authors and size efficiency theory where large-bodied individuals were first eliminated, due to the fish prey selectivity (Estlander et al., 2009).

In the LB, turbidity was the most significant factor in the zooplankton structuring. Fish predation pressure was reduced and large-bodied cladocerans appeared, although small planktonic cladocerans prevailed in abundance. Considering copepods, their fast movement is an efficient tool against fish predation (Špoljar et al., 2012). Appearance and abundance of juvenile perch negatively affected copepods, while adult perch is a much more efficient predator than

other planktivorous fish (Horpilla et al., 2000; Piria et al., 2005; Nurminen et al., 2007). High turbidity, reduced fish predation and plenty of food resources in the pelagial of the LB allowed abundant growth of detritivorous cladocerans (*Bosmina longirostris*) and resulted in rotifers suppressing (Špoljar et al., 2011). Also, *B. longirostris* consumess a wider range of food and is competitively dominant in presented conditions in relation to detritivorous rotifers.

Results of Castro et al. (2005) and Miracle et al. (2007) suggest that higher TSI, turbidity and the absence of submerged macrophytes may result in reduced abundance and diversity of zooplankton, which is in accordance with our results. Therefore, lower abundance and diversity of zooplankton in the LB was found as the result of macrophyte absence in the littoral zone and depletion of food resources. Simultaneously, by increasing trophic level, diversity is reduced and abundance of detritivorous species increased (May and O'Hare, 2005). Results of our study indicated different zooplankton structures in view of diversity, abundance and functional feeding guilds in two interconnected basins, caused by the multiple interaction of biotic (predation, macrophytes) and abiotic (turbidity, nutrient concentrations) factors. The importance of macrophytes as the main factor governing ecosystem functioning is emphasized.

Sažetak

STRUKTURA ZOOPLANKTONA U MEĐUSOBNO POVEZANIM PLITKIM JEZERIMA

Istraživanje raznolikosti, abundancije i trofičke strukture zooplanktona provedeno je tijekom vegetacijskog razdoblja na pelagičkim postajama longitudinalnog profila rukavca rijeke Sutle koji se sastoji od dva međusobno povezana bazena. Gornji bazen veće je prozirnosti i sa submerznim sastojinama makrofita, Donji bazen veće je mutnoće i bez makrofita. U Gornjem bazenu, brojnost i raznolikost zooplanktona u pelagijalu je bila veća u odnosu na Donji bazen, a prevladavali su kolnjaci, detritivorni-mikrofiltratori roda Keratella i algivorni-makrofiltratori rodova Polyartha i Trihocerca. U Donjem bazenu brojnost planktonskih rakova bila je veća u odnosu na Gornji bazen, a prevladavali su mikrofiltratorski rašljoticalci (Bosmina longirostris) te makrofiltratorski ličinački i adultni stadiji veslonožaca (Macrocyclops albidus). Predacijski pritisak riba bio je izraženiji u pelagijalu Gornjeg bazena, na što ukazuje manja brojnost Cladocera u površinskom sloju. Iako su istraživani bazeni međusobno povezani, rezultati rada ukazuju na značajne razlike (Mann-Whitney U test, p < 0.05) u strukturi zooplanktona kao rezultat utjecaja makrofita i predacije riba na promjenu uvjeta okoliša.

Ključne riječi: submerzni makrofiti, prozirnost, mutnoća, predacija, ribe

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