Entomol. Croat. 2012, Vol. 16. Num. 1-4: 7-20
ISSN 1330-6200

IZVORNI ZNANSTVENI ČLANCI **ORIGINAL SCIENTIFIC PAPERS**

ENTOMOFAUNA OF SUBMERGED MACROPHYTE STANDS IN RESERVOIRS (PAPUK NATURE PARK)

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Accepted: July, 11th 2012

In this study diversity, abundance and functional feeding guild of aquatic entomofauna were assigned within submerged macrophyte stands in Jankovac Stream reservoirs (Papuk Nature Park). The main aims of this study were to analyse: (i) physico-chemical parameters and food resources; (ii) abundance and diversity and (iii) functional feeding guilds of entomofauna. Between reservoirs non significant differences were observed in regard to abiotic or biotic parameters, except higher abundance of active filterers in vegetated littoral zone of Reservoir 1 (R1M) than in Reservoir 2 (R2M). Other parameters mostly showed significant seasonal oscillations. In total 19 taxa were identified, among them 14 in R1M, and half less in R2M. Highest abundance was reached in R1M in winter (544 ind m^{-2}), and in R2M in spring (472 ind m^{-2}). Ephemeroptera (R1M 66 %, R2M 76 %) with domination of *Cloeon* sp., and Diptera (23 % in R1M and R2M) with dominated Tanitarsini specimens contributed at most in total abundance. Among feeding guilds the greatest contribution in abundance achieved detritivores (81 %). Results of this study suggested that food resources and biotic interactions are main drivers in structuring entomofauna, i.e., macroinvertebrate community in submerged macrophyte stands.

Littoral zone, Ephemeroptera, *Cloeon*, Diptera, functional feeding guilds

M. ŠPOLJAR, D. ŠNELLER, M. MILIŠA, J. LAJTNER, M. SERTIĆ PERIĆ i I. RADANOVIĆ: Entomofauna sastojina submerznih makrofita u umjetnim ujezerenjima (Park prirode Papuk). Entomol. Croat. 2012. Vol. 16. Num. 1-4: 7-20.

U provedenom istraživanju razmatrana je raznolikost, abundancija i funkcionalne hranidbene skupine akvatičke entomofaune u submerznim sastojinama umjetnih ujezerenja potoka Jankovac (Park prirode Papuk). Glavni ciljevi ovog istraživanja bili su analizirati: (i) fizičko-kemijske čimbenike i izvore hrane; (ii) abundanciju i raznolikost te (iii) funkcionalne hranidbene skupine entomofaune. Između ujezerenja nisu utvrđene značajne razlike abiotičkih niti biotičkih čimbenika, izuzev veće abundancije aktivnih filtratora u litoralnoj zoni s makrofitima u ujezerenju 1 (R1M) u odnosu na ujezerenje 2 (R2M). Ostali istraživani čimbenici uglavnom su ukazivali na značajne sezonske razlike. Ukupno je utvrđeno 19 svojti, od kojih je 14 svojti bilo prisutno na postaji R1M, a upola manje na postaji R2M. Najveća abundancija postignuta je zimi na postaji R1M (544 jed. m^{-2}) dok je na postaji R2M maksimum abundancije postignut u proljeće (472 jed. m^{-2}). Ephemeroptera (R1M 66 %, R2M 76 %) s dominantnom svojtom *Cloeon* sp. i Diptera (23 % u R1M i R2M) s dominacijom jedinki iz skupine Tanitarsini najviše su pridonijeli ukupnoj abundanciji. Između funkcionalnih hranidbenih skupina detritovori su postigli najveći udio (81 %) u ukupnoj abundanciji. Rezultati ovog istraživanja ukazuju da su izvori hrane i interakcije unutar vodenih biocenoza bile glavne odrednice u strukturiranju entomofaune odnosno makrozoobentosa u sastojinama submerznih makrofita.

Litoralna zona, Ephemeroptera, *Cloeon*, Diptera, funkcionalne hranidbene skupine

Introduction

Aquatic macrophytes play an important role for aquatic biocoenoses (Kuczyńska-Kippen & Nagengast, 2006; Cañedo-Argüelles & Rieradevall, 2009). They serve as a refuge against predators, mostly fish, and provide broad spectrum of food sources for invertebrates and vertebrates (Rooke, 1984; Scheffer, 2004; Cañedo-Argüelles & Rieradevall, 2009; Špoljar et al., 2011). Macrozoobenthos, mainly comprised of insect larvae, oligochaetes, gastropods and bivalves, are feeding on detritus, periphyton, plant tissues or are predators that pray on other invertebrates i.e., rotifers, gastropods, crustaceans (Cry & Downing, 1988; Kuczyńska-Kippen, 2003). Also, macrophytes serve as oviposition habitats for fish and water birds (Beccera-Munoz & Schramm, 2007; Klaassen & Nolet, 2007). They can reduce flow velocity, nutrient concentrations, sediment erosion and resuspension. Furthermore, macrophytes preserve physical stability of the littoral zone, and ameliorate eutrophication symptoms such as water turbidity (Horppila & Nurminen, 2005; Miliša et al., 2006; Estlander et al., 2009).

In this study we aimed to analyse diversity, abundance and feeding guilds of entomofauna within macrophyte stands in reservoirs of karst Jankovac Stream.

Reservoirs in headwater are uncommon apparition, they indicate antropogenic origin (Špoljar et al., 2008), and concurrently offer new, lentic and vegetated, habitats well known for their higher diversity and abundance in the hydrosystem (Burks et al., 2001; Zimmermann et al., 2007). According to main aim we considered following aspects of lentic biotopes of Jankovac Stream: (i) physico-chemical parameters and food resources; (ii) abundance and diversity and (iii) functional feeding guilds of entomofauna.

Materials and Methods

This investigation was carried out at the Jankovac Stream in Papuk Nature Park. Jankovac is a small, 700 m long, first order stream (45º31´07´´ N, 17º41´11´´

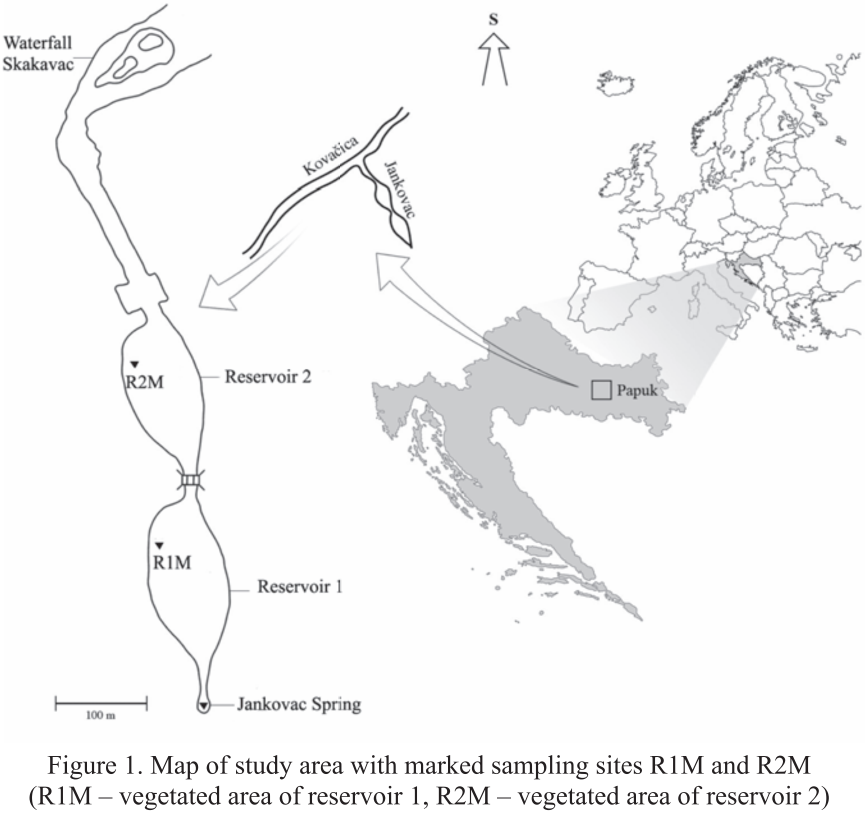


Figure 1. Map of study area with marked sampling sites R1M and R2M (R1M – vegetated area of reservoir 1, R2M – vegetated area of reservoir 2)

Table 1. Main morphometric features of study area and sampling stations in Jankovac Stream reservoirs, Papuk Nature Park

Sampling stations	R1M	R2M
Coordinates	45º31´09´´ N; 17º41´11´´ E	45º31´14´´ N; 17º41´09´´ E
Area (m^2)	9240	5317
Lenght$_{max}$ (m)	168	130
Width$_{max}$ (m)	52	51
z_{max} (m)	1.9	1.95
Total volume (m^3)	8.8 x 10^4	6.8 x 10^4
WRT (day)	4.4	3.4
Habitat specification	Lentic, vegetated	
Bottom granulometry	Mud	
Bed and macrophyte coverage %	*Potamogeton natans* (25%), *Hippuris vulgaris* (50%)	*Hippuris vulgaris* (60%)

Abbreviations
R1M – reservoir 1, R2M – reservoir 2, z – depth, WRT – water residence time

E) in a submountain area at 475 m a.s. l. It is situated on sedimentary carbonate bedrock and it is comprised of a rheocrenous spring, two man-made reservoirs and the Skakavac waterfall (height 32 m, slope 63.4°) with recent tufa deposition, which represents the mouth of the Jankovac Stream (Fig. 1, Tab. 1). Across the longitudinal profile, the stream is mostly surrounded by the deciduous forest from mouth to the spring. The majority of system is lentic encompassing two shallow reservoirs, with well developed submerged vegetation and a maximum depth of approximately 2 m. Reservoirs are separated by a two-metre high bank, and water flows through a small connection between these reservoirs. Two different habitats can be identified within the reservoirs: non-vegetated and vegetated areas. Sampling sites were situated within vegetated area (R1M – vegetated area of reservoir 1, R2M – vegetated area of reservoir 2).

Macrozoobenthos samples were collected monthly from July 2008 to June 2009, thus covering all seasons of the year: spring (April, May, June), summer (July, August), autumn (September, October) and winter (December, February). Because of snow-cover and heavy torrents obstructing road to Jankovac stream, samples were not taken in November 2008 and in January and March 2009. Macrozoobenthos samples were taken in triplicate (9 months × 2 sites × 3 replicates = 54 samples) with a 25×25 cm Surber sampler (300 µm mesh), fixed in 70 % alcohol. Macrozoobenthos taxa were identified and counted using stereomicroscope Zeiss Stemi 2000-C (maximum magnification 50×) and microscope Carl Zeiss Jenaval (maximum magnification 250×). In this paper within macroinvertebrates only entomofauna would be considered. Specimens were identified to the lowermost taxonomic level according to: Nilsson (1996) for Coleoptera, Nilsson (1997) for Odonata, Megaloptera, Diptera, Heteroptera, Bauernfeind & Humpesch (2001) for Ephemeroptera. Additionally, insects were classified in respect to their feeding habits according to Moog (2002). In regard to hydromorphological features of study site, with prevailance of lentic over lotic habitats and accumulation of detritus in macrophytes' epiphyton, specimens of tribe Tanytarsini were considered as dominant detritovorous (8) with minimal share of grazers (1) and active filterers (1).

The following environmental parameters were measured *in situ*: temperature (°C, oximeter WTW OXI 96), concentration of dissolved oxygen (mg O_2 L^{-1}, oximeter WTW OXY 96), pH (pH-meter WTW 330i) and conductivity (µS cm^{-1}, conductometer Hach Sension 5). Additional 5 L of non-filtered water was taken for chemical analysis in laboratory for concentrations of: nitrates (mg $N\text{-}NO_3^-/L$), ortophosphates (mg $P\text{-}PO_4^{3-}/L$), alkalinity (mg $CaCO_3/L$), dissolved organic matter (DOM, mg $O_{2(Mn)}\ L^{-1}$) and chlorophyll *a* (Chl *a*) according to methods detailed described in publications of Špoljar et al. (2005; 2011).

Concentration of chlorophyll *a* (Chl *a*) and concentration of particulate organic matter (POM) were analyzed as indicators of food sources (phytoplankton biomass, detritus). For estimating phytoplankton biomass 3 L of non-filtered water was analysed. To determine particulate organic matter in sediment (after macrozoobenthos isolation as ash-free dry mass in benthos, mg AFDMs m^{-2}), and in water column (additional 3 L of water was first filtered through Schleicher & Schuell White Ribbon 589/2, ashless quantitative filter paper, ash-free dry mass in mg AFDMw L^{-1}) samples were dried at 104 °C and then ashed at 600 °C/6 h.

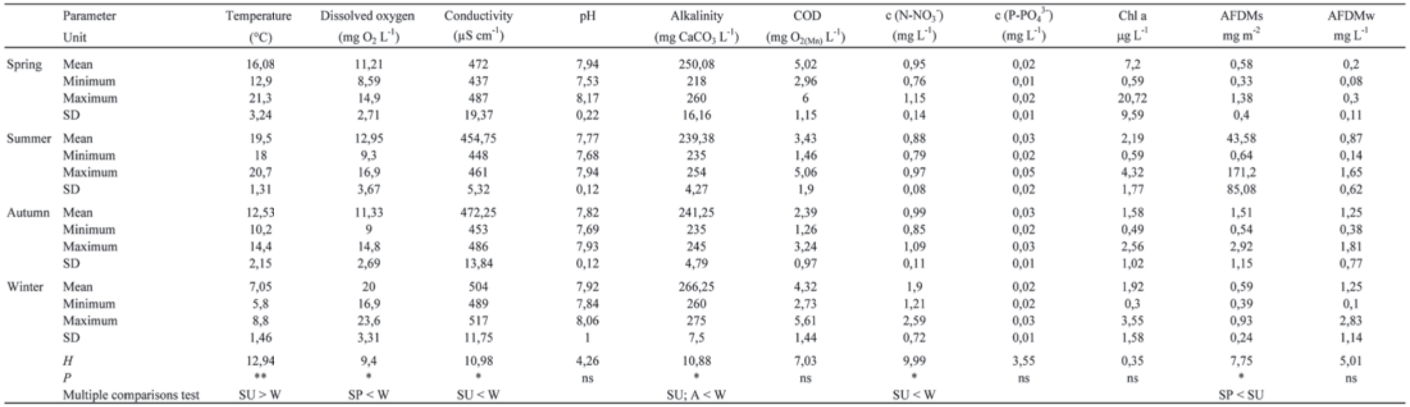
Macrophyte coverage (%) was estimated from the ratio of transect length occupied by macrophytes to total transect length at five locations (Lau & Lane, 2002). Species similarity between samples from both sampling sites was calculated using the Sørensen index. In further analyses the mean of triplicate samples was used as a single data point for a given date and site ($N_{R1M} = 9$, $N_{R2M} = 8$, R2M samples for September were destroyed during the transport). Prior to statistical analysis, all abiotic and biotic data were transformed [*log* (x+1)]; because they did not follow a normal distribution (Shapiro-Wilk's test) nonparametric analyses were used. These were as follows: Spearman correlations, Mann-Whitney U test (comparison between two independent samples for spatial distribution of environmental parameters and biotic components) and Kruskal-Wallis test (comparison among multiple independent samples for seasonal distribution of environmental parameters and biotic components), as well as accompanying *post-hoc* multiple comparisons were carried out using Statistica 9.1 (Statsoft, Inc. 2010).

Results and Discussion

Environmental conditions

Results of nonparametric Mann-Whitney U test suggested no differences between two study sites according to physico-chemical parameters. We anticipated environmental conditions as uniform at both study sites and analysed them through the seasonal oscillations using Kruskal-Wallis test. Oscillations of temperature, dissolved oxygen, conductivity, alkalinity, nitrates and POM in the

Table 2. Seasonal oscillations of environmental conditions and food resources analyzed by Kruskal-Wallis test
Abbreviations: SP – spring, SU – summer, A – autumn, W – winter



	Parameter	Temperature	Dissolved oxygen	Conductivity	pH	Alkalinity	COD	c (N-NO_3^-)	c (P-PO_4^{3-})	Chl a	AFDMs	AFDMw
	Unit	(°C)	(mg O_2 L^{-1})	(µS cm^{-1})		(mg $CaCO_3$ L^{-1})	(mg $O_{2(Mn)}$ L^{-1})	(mg L^{-1})	(mg L^{-1})	µg L^{-1}	mg m^{-2}	mg L^{-1}
Spring	Mean	16,08	11,21	472	7,94	250,08	5,02	0,95	0,02	7,2	0,58	0,2
	Minimum	12,9	8,59	437	7,53	218	2,96	0,76	0,01	0,59	0,33	0,08
	Maximum	21,3	14,9	487	8,17	260	6	1,15	0,02	20,72	1,38	0,3
	SD	3,24	2,71	19,37	0,22	16,16	1,15	0,14	0,01	9,59	0,4	0,11
Summer	Mean	19,5	12,95	454,75	7,77	239,38	3,43	0,88	0,03	2,19	43,58	0,87
	Minimum	18	9,3	448	7,68	235	1,46	0,79	0,02	0,59	0,64	0,14
	Maximum	20,7	16,9	461	7,94	254	5,06	0,97	0,05	4,32	171,2	1,65
	SD	1,31	3,67	5,32	0,12	4,27	1,9	0,08	0,02	1,77	85,08	0,62
Autumn	Mean	12,53	11,33	472,25	7,82	241,25	2,39	0,99	0,03	1,58	1,51	1,25
	Minimum	10,2	9	453	7,69	235	1,26	0,85	0,02	0,49	0,54	0,38
	Maximum	14,4	14,8	486	7,93	245	3,24	1,09	0,03	2,56	2,92	1,81
	SD	2,15	2,69	13,84	0,12	4,79	0,97	0,11	0,01	1,02	1,15	0,77
Winter	Mean	7,05	20	504	7,92	266,25	4,32	1,9	0,02	1,92	0,59	1,25
	Minimum	5,8	16,9	489	7,84	260	2,73	1,21	0,02	0,3	0,39	0,1
	Maximum	8,8	23,6	517	8,06	275	5,61	2,59	0,03	3,55	0,93	2,83
	SD	1,46	3,31	11,75	1	7,5	1,44	0,72	0,01	1,58	0,24	1,14
	H	12,94	9,4	10,98	4,26	10,88	7,03	9,99	3,55	0,35	7,75	5,01
	P	**	*	*	ns	*	ns	*	ns	ns	*	ns
	Multiple comparisons test	SU > W	SP < W	SU < W		SU; A < W		SU < W			SP < SU	

* $P < 0.05$; ** $P < 0.001$

sediment significantly differed among the seasons, and detail measurements are shown in Table 2. We argue that vicinity of the two reservoirs is the reason for the lack of physico-chemical differences. Interconnected reservoirs are thus characterised by similar hydromorphological features and macrophyte coverage. Similar physico-chemical conditions were also established along the longitudinal profile of similar hydrosystem, in Plitvice Lakes (Špoljar et al., 2007). Littoral zone is characterised by higher diurnal as well as seasonal oscillations of environmental conditions also established in this study (Scheffer, 2004).

Biocenotical analyses

Among 19 identified taxa, 14 were found in R1M, while half less in R2M, 7 taxa (Fig. 2). Thus, Sørensen similarity index between two sampling sites was very low, only 19 %. Most diverse were orders Coleoptera and Diptera, 5 taxa, each. We found positive correlation between diversity and abundance ($r = 0.83$, $P < 0.05$) at both sampling sites (Fig. 2).

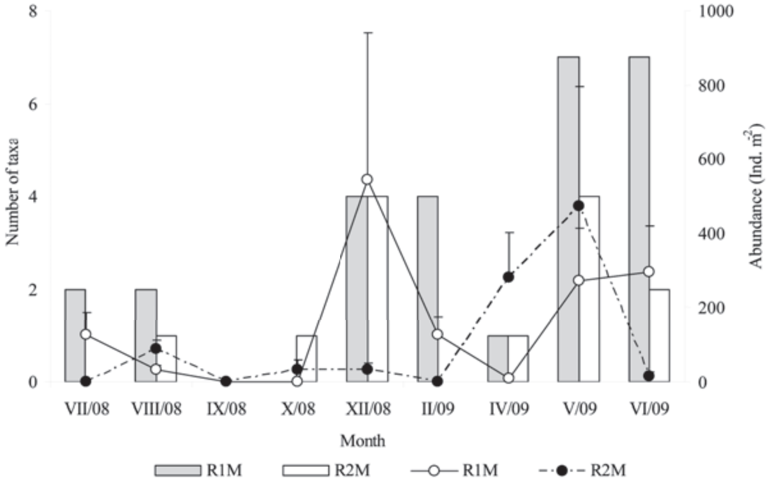


Figure 2. Seasonal and spatial oscillations of entomofauna diversity and abundance. Whisker mark standard deviations (SD). R1M – vegetated area of reservoir 1, R2M – vegetated area of reservoir 2

Table 3. Diversity, abundance and functional feeding guilds of investigated aquatic entomofauna at sampling sites R1M and R2M. Abbreviations: AFIL (active filterers), SHR (shredders), GRA (grazers), DET (detritivores) and PRE (predators)

	Sampling site	R1M					R2M					
Order	Taxon	Min	Max	Mean		SD	Min	Max	Mean		SD	FFG
Coleoptera	*Elmis aenea* P. Muell.						0	8	3	±	4	GRA
	Hydrophilidae	0	24	5	±	10						SHR, GRA, DET
	Limnius volckmari Panzer	0	8	1	±	3						GRA
	Haliplus sp.	0	8	2	±	4						GRA, PRE
	Helophorus sp.	0	8	1	±	3						GRA, DET
Coleoptera Total		0	24	9	±	2	0	8	3	±	4	
Diptera	Chironomidae (Chironominae/Tanytarsini)	0	192	45	±	69	0	168	31	±	67	AFIL, GRA, DET
	Oplodontha lindneri James	0	8	1	±	3						GRA, DET
	Oxycera sp.						0	8	1	±	3	GRA,DET
	Psychodidae						0	8	1	±	3	GRA,DET
	Tipulidae						0	8	1	±	3	SHR, DET
Diptera Total		0	200	46	±	68	0	176	35	±	70	
Ephemeroptera	*Cloeon* sp.	0	520	118	±	182	0	288	115	±	135	DET
	Centroptilum sp.	0	112	16	±	42						GRA, DET
	Ephemera sp.						0	8	1	±	3	AFIL
Ephemeroptera Total		0	520	134	±	186	0	296	116	±	137	
Heteroptera	*Nepa cinerea* Linnaeus	0	16	2	±	6						PRE
	Micronecta sp.	0	8	1	±	3						DET
Heteroptera Total		0	16	3	±	6						
Megaloptera	Megaloptera	0	8	1	±	3						PRE
	Sialis lutaria Linnaeus	0	8	2	±	4						PRE
	Sialis morio Klingstedt	0	16	5	±	6						PRE
Megaloptera Total		1	9	8	±	8						
Odonata	Zygoptera-Coenagrionidae	0	8	1	±	3						PRE
Odonata Total		0	8	1	± ±	3						
Total		32	544	201	±	186	32	472	153	±	184	

Aquatic entomofauna reached highest abundance at R1M in winter (544 ind m^{-2}), and at R2M in spring (472 ind m^{-2}) (Fig. 2, Tab. 3). In total benthic macroinvertebrate abundance it constituted up to 25 % and 20%, in R1M and R2M, respectively. Ephemeroptera (R1M 66 %, R2M 76 %) and Diptera (23 % in R1M and R2M) contributed the most to total abundance. *Cloeon* sp. was dominant among Ephemeroptera and Tanytarsini among Diptera (Tab. 3). High positive relation between Ephemeroptera and total entomofauna abundance (R1M $r = 0.83$, R2M $r = 0.92$; $P < 0.05$) was established at both sampling sites. We explain higher diversity and abundance in littoral zone of reservoir 1 (R1M) contrary to reservoir 2 (R2M) by organisms drifting from upstream lotic stretch (Miliša et al., 2006), avoidance of flow velocity (Zimmermann-Timm et al., 2007, Sertić Perić et al., 2011) and simultaneously development of limnophylic taxa, i.e., mayfly *Cloeon* sp. Contrary, in lotic stretch prevailed congenial mayfly taxa *Baetis* sp. (Špoljar et al., 2009). Miliša et al., (2006) were also established higher macroinvertebrates and entomofauna diversity and abundance in vegetated area with slower flow velocity on Plitvice Lakes (Croatia). Also study by González-Sagrario et al., (2009) confirmed higher macroinvertebrates abundance in submerged stands in Los Padres Lake (Argentina). In underwent investigation in Jankovac Stream we focused on comprehensive analysis of food web in vegetated area, therefore we did not collect benthic macroinvertebrate in nonvegetated area. Namely, vegetated area occupied majority reservoir’s bottom (cca. 70 %), and non-vegetated area occupied main stream. Thus we presumed that vagile aquatic insect larvae or adults quickly avoid flow velocity and find refuge within macrophytes. For less vagile zooplankton we collected samples in vegetated and non-vegetated area and established higher zooplankton diversity and abundance in vegetated area (Špoljar et al., 2012). Vertebrate predation over entomofauna was not quantitatively investigated in this study, because it was carried out in protected area, and second all amphibians in Croatia, presented in reservoirs by newts, tadpoles and salamanders, are protected. However, we presume, that vertebrate predation, especially by fish, plays important role in entomofauna assemblage (Mičetić et al., 2008). According to Mrakovčić et al. (2008) main visual predator in Jankovac reservoirs are trout (*Salmo trutta* L.). We suppose, that trout as opportunistic feeders relay on benthic prey and affected at most macroinvetebrate community (Winder et al., 2003), while predation by newts, due to their slow ingestion, has weaker impact (Schabetsberger et al., 2006).

Functional feeding guilds (FFG) analyses

In respect to the functional feeding guilds (FFG, Moog, 2002) observed specimens were categorised in five groups, according to food type and collection: AFIL (active filterers), SHR (shredders), GRA (grazers), DET (detritivores) and PRE (predators). DET constituted 81 % of feeding aspects among presented aquatic entomofauna, owing mostly to *Cloeon* sp. abundance. Significantly higher abundance of AFIL was found in R1M than in R2M ($Z = 1.99$, $P < 0.05$), while for other FFG significant spatial differences were not recorded ($P > 0.05$). Significant seasonal differences within each functional feeding guild were found (Kruskal-Wallis test, $H = 8.8$ to 14.6, $P < 0.05$). All FFG achieved maximum abundances in spring, except shredders that peaked in winter (Fig. 3). Negative correlations between detritus in sediment and DET ($r = -0.65$, $P < 0.05$) indicate strong interrelation between the dominant FFG and food sources even outside the macrophyte stands indicating foraging feeding behaviour of insects outside their native habitat. For better insight in food webs of reservoir's submerged stands we included data for possible food/prey from previous studies (Špoljar et al., 2008;

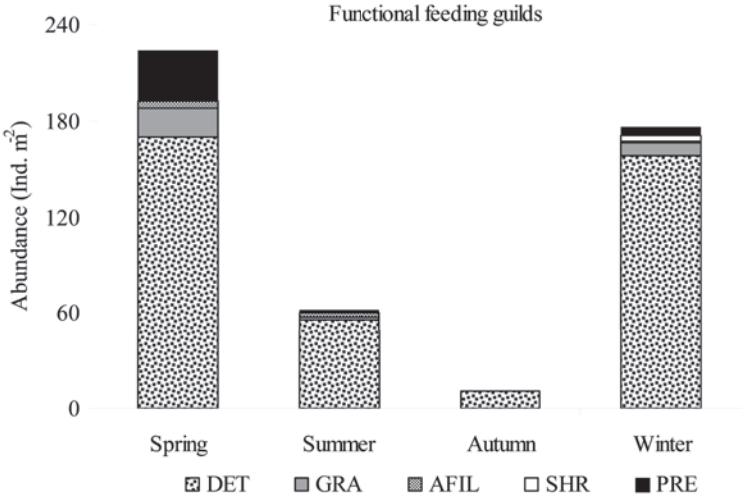


Figure 3. Seasonal assemblage of functional feeding groups. Abbreviations: AFIL (active filterers), SHR (shredders), GRA (grazers), DET (detritivores) and PRE (predators)

2009), i.e. rotifers, cladocerans, copepods. We obtained negative correlations between insect predators and copepods ($r = -0.75$, $P < 0.05$) and rotifers ($r = -0.05$, $P > 0.05$), indicated on predation of tactile predators *i.e.* insect larvae (Meerhoff et al., 2007; González-Sagrario et al., 2009).

We suppose that more frequent sampling regime and sampling points would reveal better data (diversity, abundance) for better understanding of plankton-benthos coupling and freshwater food webs. Results of our study showed importance of aquatic entomofauna in freshwater food webs, especially in macrophyte stands where they can find various food resources such as detritus, epiphyton, sediment, plant periderm and over predation influence on plankton assemblage. We predict that food resources and biotic interactions are main drivers in structuring macroinvertebrate community in submerged macrophyte stands.

Acknowledgments

This study was funded by the Papuk Nature Park and the project 11900000001205 of the Ministry of Education, Science and Sport, Republic of Croatia. We would like to thank the personnel of the Papuk Nature Park for their help in administrative and field support. We are thankful to technician Svjetlana Vidović for macroinvertebrate isolation.

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