

## TUFA DEPOSITION IN A KARST STREAM AS AN INDICATOR OF WATER QUALITY (PAPUK NATURE PARK, CROATIA)

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### Summary

Tufa deposition and accompanying periphyton community were studied on artificial substrates, in karst Jankovac Stream (Papuk Nature Park, Croatia). Influence of environmental parameters on protozoans and metazoans in periphyton were assessed in two microhabitats, differed in flow velocity (fast  $1.28 \pm 0.61 \text{ m s}^{-1}$ , medium  $0.56 \pm 0.50 \text{ m s}^{-1}$ ), and across different exposure period (one and two months). Measured environmental parameters indicated oligotrophic water condition. Samples collected in fast flow velocity reached significantly higher tufa deposition ( $0.26 \pm 0.04 \text{ mg cm}^{-2} \text{ d}^{-1}$ ) contrary to those in medium flow velocity ( $0.09 \pm 0.01 \text{ mg cm}^{-2} \text{ d}^{-1}$ ). Results of our study suggested that tufa deposition increased with temperature, flow velocity, amount of organic matter and algal biomass. In total, 26 taxa were identified on artificial substrate, among them 16 ciliate and 5 rotifer taxa. Most taxa recorded low abundance in periphyton,  $< 10 \text{ ind cm}^{-2}$ . Just few taxa achieved higher maximum abundances, i.e., ciliates: *Chilodonella cucullulus* ( $28 \text{ ind cm}^{-2}$ ), *Vorticella similis* ( $68 \text{ ind cm}^{-2}$ ) and rotifers: bdelloids ( $55 \text{ ind cm}^{-2}$ ) and *Dicranophorus forcipatus* ( $64 \text{ ind cm}^{-2}$ ). Periphyton community achieved statistically significant higher abundance in fast than in medium flow velocity microhabitats, with increasing effect through the longer exposure. We presume that oligotrophic conditions in karst running water facilitate tufa deposition. In this study we revealed microscopic freshwater organisms, often neglected in investigation, but very important in food webs as link to gastropods, crustaceans, insect larvae, juvenile and adult fish.

**Key words:** oligotrophic hydrosystem, flow velocity, periphyton, artificial substrate, protozoans, rotifers

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## INTRODUCTION

All geomorphological features in karst (calcareous) area result from interaction of water and bedrock. Headwater streams on karst sediment are suitable areas for tufa deposition and barriers formation (P r i m c – H a b d i j a et al., 1997). Recently, numerous studies have shown that calcium carbonate precipitation (tufa deposition) is driven by a combination of physico-chemical and biological processes (P e d l e y, 2000; M a t o n i č k i n – K e p č i j a et al., 2005; K r a l j et al., 2006). According to the investigation of S r d o č et al. (1985) at least three conditions must be fulfilled for calcite precipitation: the saturation index in respect to calcium carbonate solution must exceed 3, the pH value of water must be over 8, and the concentration of dissolved organic carbon (DOC) must be below 10 mg L<sup>-1</sup>. Bryophytes, owing to their preference to fast running waters are very suitable substrate for tufa deposition. Their filoids and rhizoides trap detritus and periphyton and thereby mediate in calcite precipitation (S u r e n and W i n t e r b o u r n, 1991; P r i m c – H a b d i j a et al., 2000).

We undertook our study in karst headwater Jankovac Stream, on moss overgrown tufa barrier. In the former period (end of 19<sup>th</sup> century) this stream was encompassed by hidrotechnical measure which resulted in two reservoirs. They often present habitats of higher productivity, established for instance in reservoirs of River Elbe (Z i m m e r m a n – T i m m et al., 2007) and Jankovac Stream (Š p o l j a r et al., 2011 a, in press) Also, building of hiking lodge contributed to the touristic development of this area, although it could implicate destructive effect on natural process of tufa deposition. Namely, tufa deposition requires low dissolved organic carbon concentration, while anthropogenic activities often increase that value. Except physico-chemical parameters, biotic indicators also assign water quality (M i h a l j e v i ć and K e r o v e c, 2010). For instance, low trophic level of studied hydrosystem indicates the presence of brown trout (*Salmo trutta* L.) and caddisflies (M r a k o v č i ć et al., 2008; K u č i n i ć et al., 2010). Up to date, investigations in karst water were more focused on fish (M a r č i ć et al., 2011; Z a n e l l a et al., 2011) and macroinvertebrates (H a b d i j a et al., 2004; M a t o n i č k i n – K e p č i j a et al., 2006; M i l i š a et al., 2006) and less on protozoans and microscopic metazoans (P r i m c – H a b d i j a et al., 2001; Š p o l j a r et al., 2005). The main purpose of this study was to analyse tufa deposition as biogenic process in unique protected area whose aim is to conserve process of recent calcite precipitation. Main goals of our study were: (i) to establish environmental conditions of tufa deposition; (ii) to analyse periphyton (protozoa, metazoa) ecology and diversity in karst stream.

## STUDY AREA

This investigation was conducted in Jankovac Stream (Papuk Nature Park, Croatia, 45°31'07'' N, 17°41'11'' E; 475 m a.s.l.), situated on sedimentary carbonate bedrock. Jankovac is a small, approximately 700 m long, first order stream, consisting of a rheocrenous spring (JS), two man-made reservoirs (R1, R2) and the Skakavac waterfall (JW) with a recent tufa deposition (Fig. 1, Table 1). Following spring there is lotic, hypocre-

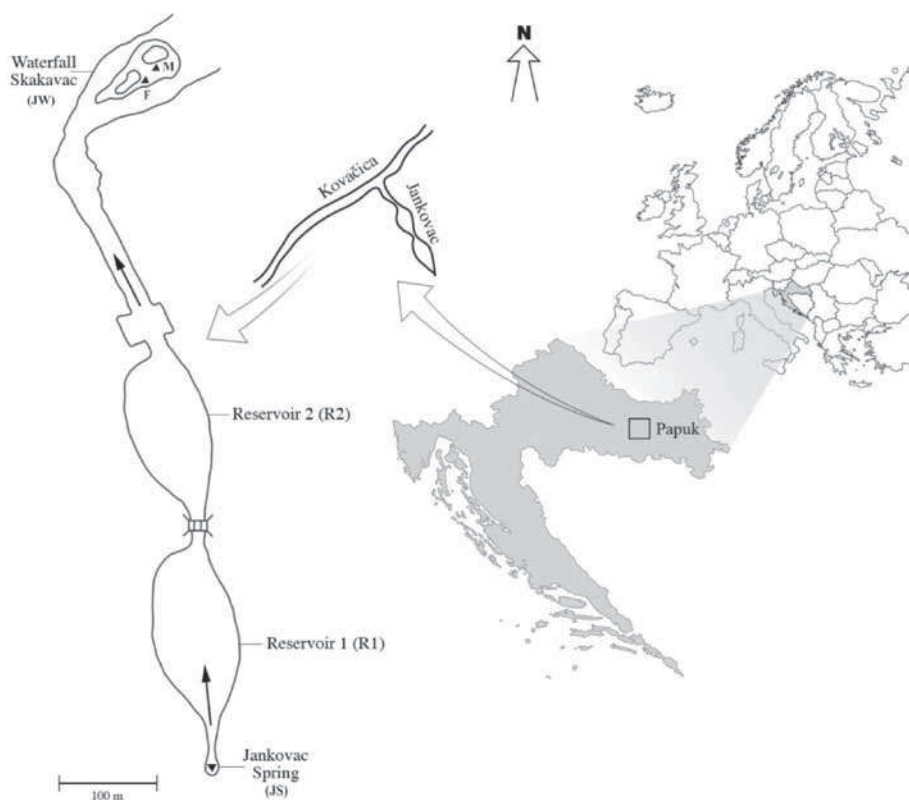


Fig. 1. Geographical position and scheme of investigated Jankovac Stream with marked position of in situ experiment, Skakavac Waterfall (JW. Abbreviations: Microhabitats - F (fast flow velocity), M (medium flow velocity))

Slika 1. Geografski smještaj i shematski prikaz istraživanog područja potoka Jankovace označena postaja slapa Skakavac (JW). Kratice: Mikrostaništa - F (brza struja vode), M (srednja struja vode)

nal stretch (length 61 m, mean width 3 m, slope 3.8°), surrounded by deciduous forest. Stream bed continues with two shallow reservoirs, with well developed submerged vegetation and a maximum depth of ~2 m (Reservoir 1, R1, length<sub>max</sub> 168 m, width<sub>max</sub> 52 m; Reservoir 2, R2, length<sub>max</sub> 130 m, width<sub>max</sub> 51 m). The outlet of the second reservoir is partially channelled and leads to Skakavac Waterfall (height 32 m, slope 63.4°). Sampling site JW is situated below waterfall. The stream bed at the Skakavac Waterfall is covered with scattered clusters of bryophytes from genera *Cratoneuron* and *Eurhynchium*. The experimental site for the tufa deposition and periphyton community was situated on the Skakavac barrier (Table 1).

Table 1. Main hydromorphometric features of sampling site JW and sampling design. Abbreviations: N (one month exposure), O (two months exposure)

Tablica 1. Osnovna hidromorfometrijska obilježja postaje istraživanja JW i plan uzorkovanja Kratice: N (ekspozicija jedan mjesec), O (ekspozicija dva mjeseca)

Postaja istraživanja/Sampling site	JW	
Obilježje staništa/Habitat specification	Lotik/Lotic	
Granulometrija podloge Bottom granulometry	Kameni blokovi, sedra Boulders, tufa	
Pokrov dna Bed coverage	Raštrkani busenovi mahovina, 50 % pokrivenosti podloge Scattered clusters of bryophytes, 50 % bottom covered	
Plan istraživanja perifitona, Chl a i stope sedrenja Sampling design of periphyton, Chl a, tufa deposition	2 mikrostaništa × 2 umjetne podloge × 2 uzorka 2 microhabitats × 2 artificial substrates × 2 samples	
Mikrostaništa/Microhabitats	F (brzo strujanje vode) F (fast flow velocity)	M (umjereno strujanje vode) M (medium flow velocity)
Brzina strujanja vode/Flow velocity (m s <sup>-1</sup> )	1.28 ± 0.61	0.56 ± 0.50
Rujan (broj uzoraka)/ September (number of samples)	8 N	0
Listopad (broj uzoraka)/ October (number of samples)	8 N, 8 O	8 N, 8 O

## MATERIALS AND METHODS

From July to October 2008 we carried out *in situ* experiment in order to measure tufa deposition, environmental variables and to analyse concomitant periphyton community on artificial substrate. We considered two diverse factors: first, flow velocity (fast flow velocity, F; medium flow velocity M) differing in microhabitats; second, exposure period (one month as new, N, two months as older, O). We assumed seasonality as summer, S (July, August) and autumn, A (September, October). In both microhabitats we submerged four bricks for periphyton community, of that two for tufa deposition and the other pair for algal biomass expressed *via* chlorophyll *a* (Chl *a*) estimation. Metal frame with 6 glass slides (dimension 26 × 76 mm, thick 1.2 mm) was fixed on each brick. Every month 2 slides from each brick were taken and replaced with new ones. We collected periphyton of one month and longer (two or three months) exposure. Although bricks were weighted with

stones to prevent downstream dislodgement, in August bricks were moved downstream either due to overturn by people or following high floods, and thus we had to replace them with new ones. We collected the most completed data for microhabitat in fast flow velocity (Table 1). Collected glass slides were transported to the laboratory in submerged plexi chamber with compartments to prevent periphyton mixing. Prior to analysis of periphyton community ( $n = 40$ ), tufa deposition ( $n = 20$ ), and chlorophyll *a* (Chl *a*,  $n = 20$ ), periphyton covered area was measured for each slide and thereafter the whole content was scraped in glass dish. First we used the samples for community analysis, and then 2 slides for tufa estimation, and two for Chl *a* estimation. Tufa deposition rate ( $\text{mg cm}^{-2} \text{d}^{-1}$ ) was calculated by dividing precipitated tufa, i.e., inorganic matter, with exposure period (Srdoč et al., 1985; Matoničkin - Kepčija, 2006).

Community was determined and counted ( $3 \times 1 \text{ mL}$ ) under inverted microscope (Opton Axiovert 35,  $100\times$ ). Protozoa (Sarcodina and Ciliata) were identified to the genus or species level according to Page and Siemensma (1991) and Foisner and Berger (1996). In this paper we use the traditional term Sarcodina, which includes naked and testate amoebae and heliozoans, although this term is no longer valid in the modern taxonomy of protozoa (Adl et al., 2005). Rotifers (except Bdelloidea) were identified to the genus or species level according to Koste (1978). Flagellated protozoa, Bdelloidea (Rotifera) Gastrotricha and Nematoda were counted, but not identified.

In the field, standard instruments were employed to determine water temperature and dissolved oxygen concentrations (OXI 96, WTW GmbH, Weilheim, Germany), pH (pH 330i WTW GmbH, Weilheim, Germany), conductivity (sensION5, HACH, Loveland, CO, USA) and flow velocity (P600, DOSTMANN electronic, GmbH, Wertheim-Reicholzheim, Germany). Other chemical parameters (alkalinity, chemical oxygen demand (COD), nitrates, orthophosphates) and algal biomass (chlorophyll *a*) were determined by analyzing 3 L of non-filtered mediated water (APHA, 1985; Hill, 1986; Nusch, 1980; review Špoljar et al., 2005, 2011). Algal biomass and particulate organic matter from water and periphyton were considered as food resources for the main groups of investigated organisms. To determine particulate organic matter (ash-free dry mass, AFDM) additional 3 L of water was first filtered (Schleicher and Schuell White Ribbon 589/2, ashless quantitative filter paper) and then dried at  $104^\circ\text{C}$  before being ashed ( $600^\circ\text{C}$  for 6 h). After periphytic community counting two replicates were dried and ashed according to described procedure. Algal biomass (Chl *a*) from periphyton was also determined according to Nusch (1980).

To analyse community composition we used following metrics: Shannon-Wiener biodiversity index,  $H'$  (calculated by PRIMER v6 package, Clarke and Gorley, 2006); Sørensen similarity index (SI) between microhabitats and exposure periods. Prior to statistical analysis, all abiotic and biotic data were transformed [ $\log(x+1)$ ] and their normality was checked using Shapiro-Wilk's test. Pearson product moment correlation and analysis of variance (ANOVA) were calculated using Statistica 9.1 (Statsoft, Inc. 2010). Two-way analysis of variance (ANOVA) was used to compare tufa deposition and periphyton assemblages across microhabitats (F, M) and exposure periods (N, O).

## RESULTS

Environmental conditions in JW during the experiment of tufa deposition and associated community development are shown in Table 2a. Positive correlation was established between COD and particulate organic matter, AFDM, in the water, ( $r = 0.85$ ,  $P < 0.05$ ). Flow velocity, higher in F ( $1.28 \pm 0.61 \text{ m s}^{-1}$ ) and lower in M ( $0.56 \pm 0.50 \text{ m s}^{-1}$ ), was determinant variable in distinguishing of two microhabitats (ANOVA,  $F = 5.75$ ,  $df = 1$ ,  $n = 18$ ,  $P < 0.05$ ).

Table 2. a) Temporal oscillations of environmental parameters at sampling site JW; b) parameters connected to tufa deposition on artificial substrates, means  $\pm$  SD. Abbreviations: Microhabitats - F (fast flow velocity), M (medium flow velocity), Exposure - N (one month), O (two months); Season - S (summer), A (autumn)

Tablica 2. a) Vremenske promjene okolišnih čimbenika na postaji JW; b) čimbenici vezani uz taloženje sedre na umjetnim podlogama, srednje vrijednosti  $\pm$  SD. Kratice: Mikrostaništa - F (brza struja vode), M (srednja struja vode), Ekspozicija - N (jedan mjesec), O (two months); Sezona - S (ljetno), A (jesen)

a)						
Month	Temperature (°C)	Dissolved oxygen (mg O <sub>2</sub> L <sup>-1</sup> )	Conductivity (μS cm <sup>-1</sup> )	pH	Alkalinity (mg CaCO <sub>3</sub> L <sup>-1</sup> )	Nitrates (mg NO <sub>3</sub> <sup>-</sup> -N L <sup>-1</sup> )
Mjesec	Temperatura (°C)	Otopljeni kisik (mg O <sub>2</sub> L <sup>-1</sup> )	Konduktivitet (μS cm <sup>-1</sup> )	pH	Alkalinitet (mg CaCO <sub>3</sub> L <sup>-1</sup> )	Nitrati (mg NO <sub>3</sub> <sup>-</sup> -N L <sup>-1</sup> )
VIII	21.3	9.9	388	8.0	205	0.793
IX	10.4	13.8	424	8.4	225	0.793
X	15.5	10.5	429	8.4	225	0.910

b)						
Month	Orthophosphates (mg PO <sub>4</sub> <sup>3-</sup> -P L <sup>-1</sup> )	COD <sub>KMnO<sub>4</sub></sub> (mg O <sub>2(Mn)</sub> L <sup>-1</sup> )	Chl a (μg L <sup>-1</sup> )	Dry mass (mg L <sup>-1</sup> )	Ashed mass (mg L <sup>-1</sup> )	AFDM (mg L <sup>-1</sup> )
Mjesec	Ortofosfati (mg PO <sub>4</sub> <sup>3-</sup> -P L <sup>-1</sup> )	COD <sub>KMnO<sub>4</sub></sub> (mg O <sub>2(Mn)</sub> L <sup>-1</sup> )	Chl a	Suha masa (mg L <sup>-1</sup> )	Žarena masa (mg L <sup>-1</sup> )	AFDM (mg L <sup>-1</sup> )
VIII	0.024	4.3	0.296	4.870	3.280	1.590
IX	0.034	3.5	1.282	3.980	3.040	0.940
X	0.023	1.7	0.233	2.120	1.653	0.467

Sample	Exposure period days (d)	Chl a (μg cm <sup>-2</sup> )	Dry mass (mg cm <sup>-2</sup> )	Ashed mass (mg cm <sup>-2</sup> )	AFDM (mg cm <sup>-2</sup> )	Tufa rate (mg cm <sup>-2</sup> d <sup>-1</sup> )
Uzorak	Vrijeme izlaganja dani (d)	Chl a (μg cm <sup>-2</sup> )	Suha masa (mg cm <sup>-2</sup> )	Žarena masa (mg cm <sup>-2</sup> )	AFDM (mg cm <sup>-2</sup> )	Stopa sedrenja (mg cm <sup>-2</sup> d <sup>-1</sup> )
FNS	28	3.08 $\pm$ 2.15	7.96 $\pm$ 3.64	7.37 $\pm$ 2.75	0.59 $\pm$ 0.32	0.27 $\pm$ 0.09
FNA	28	1.19 $\pm$ 0.83	6.55 $\pm$ 2.67	6.20 $\pm$ 2.59	0.35 $\pm$ 0.14	0.21 $\pm$ 0.07
FOA	56	4.77 $\pm$ 3.74	17.45 $\pm$ 5.72	15.84 $\pm$ 4.48	1.61 $\pm$ 0.67	0.28 $\pm$ 0.05
MNA	28	1.77 $\pm$ 1.12	2.80 $\pm$ 0.86	2.23 $\pm$ 1.19	0.57 $\pm$ 0.71	0.08 $\pm$ 0.13
MOA	56	0.74 $\pm$ 0.87	5.84 $\pm$ 2.13	5.50 $\pm$ 1.37	0.34 $\pm$ 0.18	0.10 $\pm$ 0.58



After one month exposure higher tufa deposition was measured in F than in M microhabitats (ANOVA,  $F = 34$ ,  $df = 1$ ,  $P < 0.01$ ). Hereafter, also O exposures were comprised almost twofold higher amount of inorganic matter (considered as tufa) than in N exposures, but it was not significant (ANOVA,  $P > 0.05$ ) (Table 2b). Results of our study suggested that tufa deposition increased with temperature ( $r = 0.52$ ), flow velocity ( $r = 0.43$ ) and organic matter ( $r = 0.61$ ) but insignificantly ( $P > 0.05$ ), while significantly increased with amounts of chl *a* in periphyton ( $r = 0.82$ ,  $P < 0.05$ ). In total 26 taxa were identified on artificial substrate, comprising of protozoans and metazoans. Among them ciliates (16 taxa) and rotifers (5 taxa) were most diverse groups (Table 3a). Diversity index ( $H'$ ) varied non significantly though the microhabitats and exposure periods (two way ANOVA,  $P > 0.05$ ). The Sørensen similarity index was 59%, with 10 and 12 common taxa between different microhabitats (F, M) and exposure periods (N, O), respectively.

In general, low abundance was observed on slides,  $< 10$  ind  $\text{cm}^{-2}$  per taxon (Table 3a). Few taxa recorded population abundances  $\geq 20$  ind  $\text{cm}^{-2}$ : i.e., ciliates *Chilodonella cucullulus* (max 28 ind  $\text{cm}^{-2}$ ) and *Vorticella similis* (max 68 ind  $\text{cm}^{-2}$ ), and from rotifers bdelloids (max 55 ind  $\text{cm}^{-2}$ ) and *Dicranophorus forcipatus* (max 64 ind  $\text{cm}^{-2}$ ). In microhabitat F ciliates contributed with 50% in total periphyton abundance, while in microhabitat M they were dominated presenters of periphytic community (Fig. 2). In M they shared only few percentages with flagellates and rotifers. Results of two way ANOVA suggested that

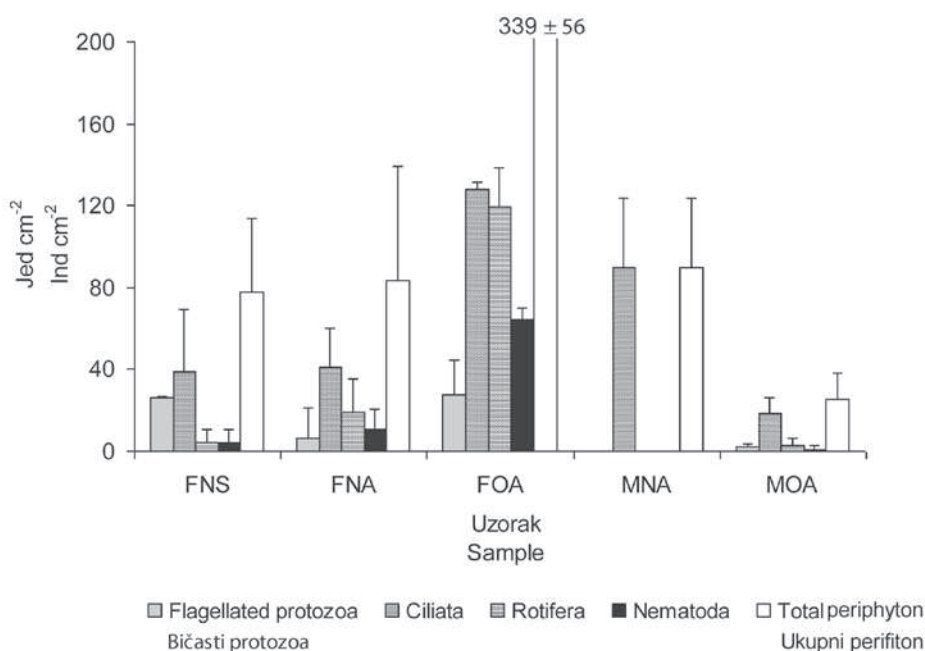


Fig. 2. Abundance of analyzed groups in periphyton. Bars mark SD.

Slika 2. Abundancija analiziranih skupina u perifitonu. Stupiči označavaju SD

higher flow velocity and longer period of exposure are suitable for development of higher abundance in periphyton (Table 3b). According to exposure period, it was significant that rotifers, especially bdelloids, achieved one third of total abundance in microhabitat F (Fig. 2, Table 3a). All observed groups in periphyton positively related with tufa ratio ( $r = 0.36$  to  $0.65$ ,  $P > 0.05$ ), whereas flagellates showed highest correlation ( $r = 0.93$ ,  $P < 0.05$ ).

Table 3. a) Taxa in periphyton community on the artificial substrate at different microhabitats and exposure. Abundance is expressed as follows: 1: 1-5 ind cm<sup>-2</sup>; 3: 6-19 ind cm<sup>-2</sup>; 5: 20-70 ind cm<sup>-2</sup>.

Tablica 3. a) Svoje perifitonske zajednice u različitim staništima i ekspozicijama. Abundancija je izražena prema slijedu: 1: 1-5 ind cm<sup>-2</sup>; 3: 6-19 ind cm<sup>-2</sup>; 5: 20-70 ind cm<sup>-2</sup>.

a)

Skupina/Group	Uzorak/Sample Mjesec/Month Svojtā/Taxon	FNS IX	FNA X	FOA X	MNA X	MOA X
Flagellated protozoa	Bičasti protozoa/Flagellated protozoa	3	1	3		1
Sarcodina	<i>Vahlkampfia limax</i>	1				1
	<i>Actinophrys sol</i>		1			
Ciliata	<i>Aspidisca lynceus</i>	3	1		3	3
	<i>Chilodonella cucullulus</i>	1	1	3		
	<i>Ciliata non det.</i>	1	1	5		1
	<i>Epistyllis hentschelli</i>				1	
	<i>Euplotes muscicola</i>		1			
	<i>Frontonia acuminata</i>					1
	<i>Glaucoma scintillans</i>			3		
	<i>Holosticha sp.</i>			3		
	<i>Lionotus lamella</i>		1			
	<i>Loxophyllum meleagris</i>				1	
	<i>Nassula ornata</i>					1
	Oxytrichidae	3	1	3		1
	<i>Pleuronema crassum</i>			3		
	<i>Pseudochilodonopsis piscatoris</i>	3				
	<i>Trochilia minuta</i>			3	1	
	<i>Vorticella similis</i>		3		5	1
Gastrotricha	<i>Chaetonotus sp.</i>		1			
Nematoda	Nematoda	1	3	5		1
Rotifera	Bdelloidea		1	5		
	<i>Cephalodella sp.</i>	1	1			
	<i>Colurella uncinata</i>		3	3		
	<i>Dicranophorus forcipatus</i>		1	5		1
	<i>Lepadella patella</i>					1
	H'	2.8	3.5	3.4	1.3	3.1



Table 3. b) Results of two way ANOVA for periphyton group abundances between microhabitats (M) and exposure (E). Abbreviations: Microhabitats - F (fast flow velocity), M (medium flow velocity), Exposure - N (one month), O (two months); Season - S (summer), A (autumn)

Tablica 3. b) Rezultati analize varijance s dva promijenjiva čimbenika (mikrostaništa, ekspozicija) na temelju abundancija pojedinih skupina u perifitonu. Kratice: Mikrostaništa (M) - F (brza struja vode), M (srednja struja vode), Ekspozicija (E) - N (jedan mjesec), O (two months); Sezona - S (ljetno), A (jesen)

b)

Skupina/Group		M	E	M*E
Bičasti protozoa/Flagellated protozoa	F	6	1	0.3
	P	0.133	0.509	0.634
Ciliata	F	938	76	6573
	P	0.001	0.013	0.000
Rotifera	F	101	76	68
	P	0.010	0.013	0.014
Nematoda	F	171	115	107
	P	0.006	0.009	0.009
Ukupni perifiton/Total periphyton	F	3879	1585	4364
	P	0.000	0.001	0.000

## DISCUSSION AND CONCLUSIONS

Microscopic organisms are often neglected in ecosystem, i.e., hydrosystem investigations (S u r e n, 1992; R e i s s and S c h m i d – A r a y a, 2008). More studies investigate macroinvertebrates (M a t o n i č k i n et al., 2001; H a b d i j a et al., 2004; S e r t i ć P e r i ć et al., 2011) and fish (Ć a l e t a et al., 2009; M a r č i ć et al., 2011) compared to microscopic protozoans and metazoans. However, they are very important in matter cycling and energy transfer, both in plankton (Š p o l j a r et al., 2005, 2011) and benthos (S t e a d et al., 2005) as well as first colonisers of new substrates (W ö r n e r et al., 2000).

We provided experiment of tufa deposition during the summer, because many authors established positive correlation of deposition process with temperature (S r d o č et al., 1985; M a t o n i č k i n – K e p č i j a, 2006). Later authors established that higher temperatures facilitate carbon dioxide outgassing, and thus increasing calcite precipitation. Unfortunately, bricks with artificial substrates were often removed from water, presumably by uninformed visitors or were dislocated downstream by flooding. Because of that we collected relatively lower quantity of samples for estimating tufa deposition. Therefore, we modified our experiment to collect adequate data corroborated with abiotic and biotic interpretation on tufa deposition. According to quoted arguments, we sampled short time series which are also well documented in the literature (P r i m c - H a b d i j a et al., 2000, 2001).

According to the Croatian Directive of Water Quality (NN 77, 1998) measured parameters (dissolved oxygen, pH, alkalinity, conductivity, orthophosphates) indicated oli-

gotrophic level on Jankovac Stream outflow, i.e., site JW. According to same Directive we observed COD limiting values for oligotrophic water, which could negatively affect tufa deposition (S r d o č et al., 1985). Higher COD values could be explained with two arguments. First, the hicking lodge is situated upstream with effluents entering and more visitors through the summer period. Also, higher production through the summer in upstream reservoirs could result in increasing organic matter and due to degradation processes higher COD values (Š p o l j a r et al., 2008). Therefore positive correlation between COD and suspended organic matter is in agreement with our presumptions. Higher nitrate concentrations are common in first order stream, related to adjacency of source and groundwater (M a l a r d et al., 1997).

Amounts of tufa deposition are comparable with those on Plitvice Lakes (mostly 0.2 to 0.4 mg cm<sup>-2</sup> d<sup>-1</sup>), considering same type of artificial substrate and environmental conditions, i.e., temperature, flow velocity, alkalinity, nutrients (M a t o n i č k i n – K e p č i j a, 2006). Higher temperature and flow velocity facilitate outgassing and calcite precipitation, and it is in concordance with higher tufa deposition through the summer period and in microhabitat F. Tufa deposition increased positively with Chl *a* and organic matter. According to personal observation on artificial substrate and examination of epiphyton on barrier mosses we concluded that diatoms and cyanobacteria were pioneers on new substrate (Š p o l j a r et al., 2008; Š p o l j a r et al., 2011 a, in press). This is confirmed by other studies in karst hydrosystem, for instance on Plitvice Lakes (P l e n k o v i ć – M o r a j et al., 2002) and Krka River (K r a l j et al., 2006). Namely, diatoms and cyanobacteria produce mucopolysaccharides which trapped calcite in primary tufa deposition (C h a f e t z and F o l k, 1984). Positive correlation between tufa deposition and organic matter in Jankovac Stream are similar to results established by P r i m c – H a b d i j a et al. (2001), M a t o n i č k i n – K e p č i j a (2006) and M i l i š a et al. (2006). In general tufa deposition and tufa-forming periphyton community was guided by similar principles as other colonisation processes. W ö r n e r et al. (2000) concluded that colonization starts with sedimentation of organic matter, and it serves as media facilitating bacterial attachment and food quickly after few hours. Bacterial activity performs the suitable surface for a life of autotrophic and heterotrophic organisms. First heterotrophic organisms appearing in periphyton are flagellates, then sarcodins and ciliates as link between bacteria and metazoa.

According to provided investigation in Jankovac Stream that recorded low abundance of protozoans and metazoans in seston, epiphyton (Š p o l j a r et al., 2008; Š p o l j a r et al., 2011 a, in press) we found also low abundance on artificial substrate. Low abundance is a probable consequence of low trophy and productivity in headwater stream. Flagellates could be pioneer colonisers of tufa substrates, suggested by their positive correlation with tufa deposition. Presumably, ciliate species *Chilodonella cucullulus* is among first colonisers of tufa substrate, fed by detritus and diatoms. It is in agreement with findings of M a t o n i č k i n – K e p č i j a (2006), where she considered *C. uncinata* as primary coloniser of new substrates. Results of analyses indicated that all investigated groups and total periphyton ( $r = 0.16$  to  $0.44$ ,  $P > 0.05$ ) except ciliates ( $r = -0.05$ ,  $P > 0.05$ ) preferred higher flow velocity. These could be explained by higher availability of food resources represented as suspension of bacteria and detritus for ciliates and rotifers, and more input of new coloniser species from upstream. P r i m c – H a b d i j a et al.

(2001) also recorded higher abundance of ciliates at flow velocity  $50 \text{ cm s}^{-1}$  than in  $5 \text{ cm s}^{-1}$  on artificial substrate at Skradinski buk (Krka National Park, Croatia). Results indicate that among rotifers, bdelloids and monogonont benthic species *Dicranophorus forcipatus* prefer fast flow velocity (Schmid-Araya, 1998; Dražina et al., pers. comm. data) and it correspond with our findings as they were most abundant in microhabitat F. Moreover, their abundance increased with exposure period, accordingly to observed order of substrate colonisation (Wörner et al., 2000). High flow velocity ensure plenty bacteria-detritus suspension as main food resources for many bdelloids, while *Dicranophorus* as predator species presumably preys on ciliates. Both of later taxa have morphological and physiological features which allow them to live in high flow velocity, i.e., foot glands to adhere on surface. We could suspect that organic matter secreted from this gland could contribute in calcite trapping and tufa formation for instance like simuliids and trichopteran silk pads established by Matoničkin-Kepčija et al. (2006).

We can presume that oligotrophic water condition arise tufa deposition where algae, presumably with protozoans and metazoans, play important role in calcite precipitation. Human impact should be controlled through monitoring of physico-chemical and biological parameters to protect and conserve habitats and biocoenoses of this karst hydrosystem.

#### Sažetak

### TALOŽENJE SEDRE U KRŠKOM POTOKU KAO INDIKATOR KVALITETE VODE (PARK PRIRODE PAPUK, HRVATSKA)

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Taloženje sedre i naseljavanje perifitonske zajednice istraživani su na umjetnim podlogama, u krškom potoku Jankovac (Park prirode Papuk, Hrvatska). Razmatran je utjecaj okolišnih čimbenika na zajednicu protozoa i metazoa u perifitonu, u dva mikrostaništa, različita s obzirom na brzinu strujanja vode (brza struja vode  $1,28 \pm 0,61 \text{ m s}^{-1}$ , srednja struja vode  $0,56 \pm 0,50 \text{ m s}^{-1}$ ) i vremensku ekspoziciju (jedan ili dva mjeseca). Mjereni okolišni čimbenici ukazuju na oligotrofiju sustava. Uzorci u brznoj struji vode postigli su veće vrijednosti taloženja sedre ( $0,26 \pm 0,04 \text{ mg cm}^{-2} \text{ d}^{-1}$ ) za razliku od uzoraka u srednjoj struji vode ( $0,09 \pm 0,01 \text{ mg cm}^{-2} \text{ d}^{-1}$ ). Rezultati ovog istraživanja ukazuju da su na taloženje sedre pozitivno utjecali temperatura, brzina strujanja vode, količina organske tvari i biomasa algi. Ukupno je determinirano 26 svojiti u perifitonu na umjetnim podlogama, 16

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svoji pripadalo je trepetljikašima, a 5 kolnjacima. Većina svoji bila je prisutna s malom abundancijom, < 10 jed. cm<sup>-2</sup>. Samo je nekoliko svoji postiglo veću abundanciju, na primjer, trepetljikaši: *Chilodonella cucullulus* (28 jed. cm<sup>-2</sup>), *Vorticella similis* (68 jed. cm<sup>-2</sup>) i kolnjaci: bdeloidni (55 jed. cm<sup>-2</sup>) i *Dicranophorus forcipatus* (64 jed. cm<sup>-2</sup>). Perifitonska zajednica postigla je statistički značajno veću abundanciju u mikrostaništu s brzom u odnosu na mikrostanište sa srednjom brzinom strujanja vode, a abundancija se također povećavala s vremenom ekspozicije. Pretpostavljamo da oligotrofni uvjeti u krškim vodama pogoduju taloženju sedre. U ovom istraživanju prikazani su mikroskopski slatkovodni organizmi, koji su često zanemareni u istraživanjima, ali su vrlo važni u hranidbenim lancima, kao poveznica prema puževima, rakovima, ličinkama kukaca, ličinačkim i adultnim stadijima riba.

**Ključne riječi:** oligotrofni hidrosustav, brzina strujanja vode, perifiton, umjetne podloge, protozoa, kolnjaci (Rotifera)

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