NATURA SLOVENIAE 23(1): 5-20
SCIENTIFIC PAPER

Prejeto / Received: 20. 11. 2020
Sprejeto / Accepted: 16. 6. 2021

Harpacticoid assemblages (Copepoda: Harpacticoida) in the hyporheic zone of four streams in central Slovenia

Nataša MORI[1*], Anton BRANCELJ[1,2]

[1]National Institute of Biology, Večna pot 111, SI-1000 Ljubljana, Slovenia
[2]University of Nova Gorica, Vipavska c. 13, SI-5000 Nova Gorica, Slovenia
*corresponding author; E-mail: natasa.mori@nib.si

Abstract. Harpacticoids are an important component of meiofaunal assemblages in hyporheic zone. The goal of this study was to investigate distribution patterns of interstitial harpacticoid assemblages from four pre-Alpine streams originating in the Dinaric Karst and flowing into the Ljubljanica River. The sampling was conducted in 2002 at 12 locations distributed at a distance of approximately 1 km along each stream including tributaries, at a depth of 30–60 cm in the wetted channel (three sites per location) and depths from 65 to 160 cm on the stream banks (one site per location) using a Bou-Rouch pump. Concurrently, the interstitial water's physical and chemical parameters were measured at two sites within each location (streambed, streambank). A total of 24 harpacticoid species were found, 12 of which were stygobionts (i.e., species living exclusively in groundwaters). Among them, two previously unknown species for science were found. Harpacticoid assemblage composition, with the exception of those from the Iška stream, did not differ significantly between the streams, indicating interconnectivity of the interstitial milieu. Sediment structure, amounts of particulate organic matter, conductivity and redox conditions seemed to have certain impacts, indicating the importance of hydrological and geological settings for harpacticoid assemblages.

Key words: microcrustacea, species-environment relationship, biodiversity, distribution, groundwater

Izvleček. Združbe harpaktikoidov (Copepoda: Harpacticoida) v hiporeiku štirih rečic v osrednji Sloveniji – Cilj študije je bil raziskati vzorce porazdelitve intersticijskih združb harpaktikoidov v štirih rečicah, ki izvirajo v Dinarskem krasu in se izlivajo v reko Ljubljanico. Vzorčenje je bilo opravljeno leta 2002 na 12 lokacijah vzdolž vsake rečice s pritoki v globini 30–60 cm v omočeni rečni strugi (3 mesta) in v globinah od 65 do 160 cm na rečnih bregovih (1 mesto) s pomočjo Bou-Roucheve črpalke. Izmerili smo tudi fizikalne in kemijske lastnosti intersticialne vode v dveh vzorcih na vsaki lokaciji (omočena struga, rečni breg). Skupno smo našli 24 vrst harpaktikoidov, od tega 12 stigobiontov (tj. vrst, ki živijo izključno v podzemni vodi). Najdeni sta bili dve novi vrsti za znanost. Sestava združb harpaktikoidov, razen tistih v Iški, se med rečicami ni statistično značilno razlikovala, kar kaže na medsebojno povezanost intersticijskega življenjskega okolja. Kljub temu rezultati kažejo, da so sestava sedimentov, količine organskih delcev, prevodnost in redoks razmere tisti, ki najbolj vplivajo na sestavo združbe, kar kaže na pomembnost hidroloških in geoloških značilnosti v porečjih na sestavo združb harpaktikoidov v hiporeiku.

Ključne besede: nižji raki, odnosi med vrstami in okoljem, biodiverziteta, porazdelitev, podzemne vode

Introduction

Alluvial sediments with interstices saturated by water and located below wetted riverbed and extending laterally, the so-called »hyporheic zone«, form a transitional zone of active exchange of water, dissolved and particulate organic matter, and organisms between the river and the adjacent phreatic groundwaters (Orghidan 1955, Pennak & Ward 1986, Boulton et al. 2010, Orghidan 2010). Here, the invertebrate community is composed of surface benthic and stygobiotic species (i.e., species living exclusively in groundwaters), their ratio depending on hydrogeomorphological conditions in the hyporheic zone (Dole-Olivier & Marmonier 1992, Mori et al. 2012). In the areas where downwelling occurs, the sediments are well-oxygenated and primarily harbour organisms of benthic origin. With increasing residence time, below or lateral to the riverbed, hyporheic water becomes less oxygenated, biogeochemical processes become reductive, and the hyporheic fauna becomes dominated by stygobionts (Gibert et al. 1994, Mori & Brancelj 2011). Most common found invertebrates in the hyporheic zone are Nematoda, Oligochaeta, Gastropoda, Acarina, Crustacea (Copepoda, Ostracoda, Amphipoda, Isopoda) and insect larvae (Ephemeroptera, Plecoptera, Trichoptera, Diptera, Coleoptera) originating from benthic habitats (Mori et al. 2011, 2012, Mathers et al. 2017, Prevorčnik et al. 2019).

Biological investigation of interstitial habitats in alluvial sediments has a long tradition in Europe (e.g. Karaman 1935, Angelier 1953, Schwoerbel 1961, Danielopol 1976) and some in North America (e.g. Pennak & Ward 1986). However, there have been fewer studies on fauna in alluvial aquifers in Slovenia. Meštrov (1960) and Meštrov with his colleagues (1983) investigated interstitial communities living in different groundwater habitats of the Sava River in southern Slovenia and Croatia. This author found many species new to this region and a high number of stygobionts. In the Ljubljansko Polje alluvial plain, hydrologically connected with the Sava River, Sket & Velkovrh (1981) found highly diverse groundwater fauna compared with other zoogeographic zones of the region. During the last 20 years, several new species for science were found in Slovenia during the studies of samples from boreholes (depths from 5 to over 100 meters) (Brancelj 2000, Brancelj et al. 2011, 2016). Investigation of the Bača and Sava Rivers hyporheic zones revealed that presence of stygobiotic species depends on the intensity of hyporheic hydrological connectivity with surface water (Mori & Brancelj 2011, Mori et al. 2011, 2012). The most recent study of interstitial habitats found several geographically restricted species and newly discovered species in the Sava River hyporheic zone (Prevorčnik et al. 2019). Additionally, negative impacts of hydropower impoundments and urban land use for stygobionts inhabiting the hyporheic zone were demonstrated by Mori et al. (2020).

Free-living freshwater harpacticoids are an abundant component of the benthic and interstitial fauna (Galassi 2001, Galassi et al. 2009). Globally, around 640 harpacticoid species out of 1,120 freshwater species are known to be stygobionts (Boxshall & Defaye 2008, Galassi et al. 2009). A recent study of data on harpacticoids in Europe's groundwaters resulted in 408 stygobiotic species and subspecies (distributed in 7 families and 42 genera) (Iannella et al. 2020). Many such stygobionts occur in restricted geographical areas (strict endemics), while others, such as the harpacticoid *Elaphoidella elaphoides* (Chappuis, 1924) and the cyclopoid *Graeteriella unisetigera* (Graeter, 1908), seem to be more widely distributed (Galassi et al. 2009). In most cases, surface (i.e., epigean) and stygobiotic harpacticoid species occur together in the hyporheic zone, with the ratio depending on species-specific requirements and local environmental conditions (Rouch & Danielopol 1997). Due to their high abundance and species richness in hyporheic zone, their sensitivity to environmental conditions, and distinctive ratios between surface and stygobiotic species in interstitial assemblages, the harpacticoids are a good model to investigate the biodiversity patterns in the hyporheic zones.

The goal of this study was to investigate the harpacticoid biodiversity in the hyporheic zone of four small streams that are hydrologically interconnected with karst groundwaters from the Dinaric Karst biogeographical region. We explored spatial distribution patterns and analysed environmental parameters that potentially determine the assemblage composition. Moreover, this study compares the hyporheic harpacticoid fauna of streams/rivers of central Slovenia with those from different geographical areas of the country.

Methods

Study area

The streams studied are located in the central part of Slovenia, south and southwest of Ljubljana (Fig. 1). They are 4th order watercourses with relatively small catchment areas, extending between 1,100 and 300 m a. s. l., with moderate discharge, determined by the pluvio-nival regime (Tab. 1). The catchments of three streams – Želimeljščica, Iška, and Borovniščica – extend over the karst/dolomite area of the Krim and Mokrec massifs, south of Ljubljana. The catchment of the Podlipščica stream extends into the Rovte Hills, where metamorphic rocks (schists) prevail. At 310 m elevation, all of the streams enter an impermeable zone, which is the silt bottom of a lake, formed after the last glaciation; the area was a peat bog in Roman times. Between the 17th and 19th centuries, most of the peat was removed and used to heat the city of Ljubljana (Pavšič 2008).

Table 1. The main characteristics of the four streams, Želimeljščica, Iška, Borovniščica and Podlipščica, located in the central part of Slovenia. The data on discharges are provided by the Slovenian Environmental Agency.
Tabela 1. Značilnosti štirih rečic Želimeljščice, Iške, Borovniščice in Podlipščice. Podatki o pretokih so pridobljeni na Agenciji za okolje RS.

Stream	Želimeljščica	Iška	Borovniščica	Podlipščica
Catchment size (km^2)	99	151	92	74
Min. catchment altitude (m a.s.l.)	285	279	282	283
Max. catchment altitude (m a.s.l.)	1050	1100	990	885
Stream length (km)	15.4	31.3	16.7	13.3
Stream mean discharge (m^3 s^{-1})	0.9	1.4	1.4	0.7

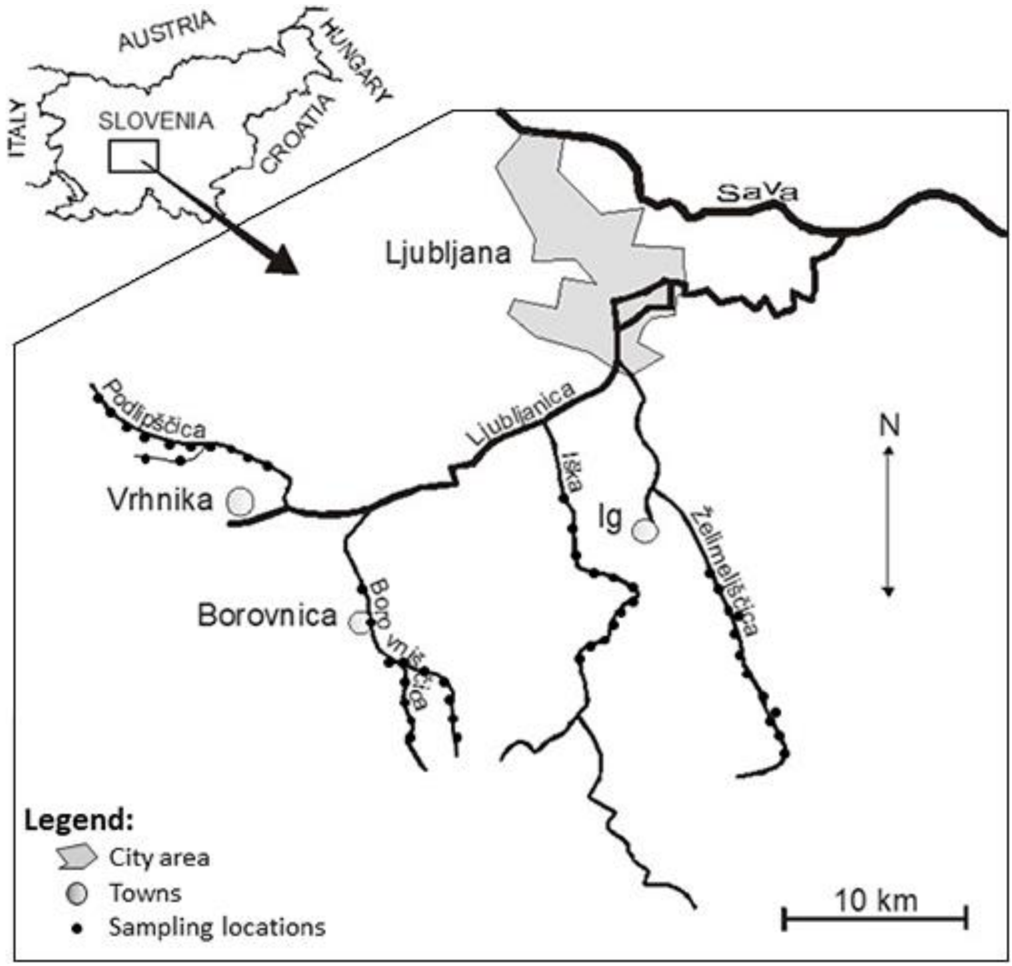

Figure 1. Map of the study area with indicated sampling locations of harpacticoids on the four streams in central Slovenia.
Slika 1. Karta območja raziskave z označenimi lokalitetami vzorčenja harpaktikoidov v štirih rečicah v osrednji Sloveniji.

The Iška and Borovniščica streams have the highest discharge, and their sediment deposits form an important porous aquifer south of Ljubljana and are far more extensive than deposits from the Podlipščica and Želimeljščica (Habič 1996). The Podlipščica valley has the highest anthropic impact due to intensive agriculture (crop production) and stream channel modifications. In the lower stretches, the Iška is subject to continuous exploitation of streambed sediments and water, the later for the drinking water supply of the city of Ljubljana. The Borovniščica is also intensively channelized and influenced by agricultural activities (cattle, crop production) in its catchment area.

Fieldwork

Twelve sampling locations were selected in the four streams' upper and middle reaches (= 48 locations) before they enter the impermeable zone in their downstream sections (Fig. 1). The measurements and sample collection were carried out from May to August 2002. The sampling campaign was part of the European project PASCALIS (Protocols for the ASsessment and Conservation of Aquatic Life in the Subsurface), where the same methodology was implemented in six European regions (Belgium, France – two regions, Italy, Slovenia, Spain). Four replicates (=sites) were selected at each location to encompass the variability in spatial distribution of harpacticoids at a reach scale. Three replicates were obtained from the wetted stream channel, where upwelling of advected channel water or groundwater was expected to occur. This was usually at the downstream end of a riffle, gravel bar, channel step, or meander bed. We measured the vertical hydraulic gradients using T-bar to identify sampling spots with positive hydraulic gradients (i.e., upwelling areas: Malard et al. 2002). Such spots had been established in previous studies as areas with predominance of stygobiotic species (Gibert et al. 1994).

Samples were taken from a depth interval of 30–60 cm below the streambed with a Bou-Rouch pump (Bou & Rouch 1967). A steel pipe (diameter of 5 cm), with a 30 cm long perforated zone (diameter of holes = 1 cm), was hammered into gravel in the hyporheic zone to the selected depth. An additional site was selected on the streambank, at a distance of 1 to 10 m from the wetted channel. Samples were then extracted from depths between 65 and 160 cm, depending on the thickness of the gravel layer above water level in the channel and the water level of the saturated zone.

Samples extracted by a Bou-Rouch pump, 10 L in volume, were composed of a mixture of water, sediment and organisms. Afterwards, samples were filtered through a 100 µm mesh net. Invertebrates and organic matter were then transferred into plastic bottles and preserved with 4% formaldehyde solution. The remaining inorganic sediment (particles > 100 µm) was collected separately in the plastic bottle and carried to the laboratory for further analyses. At each sampling site, the time required to pump 10 L of water was measured as a proxy for measurement of hydraulic conductivity.

After fauna samples collection, an oxygen probe (WTW, Multiline P4, Oxi 320) and a conductivity probe (WTW, Tetracon 325) were inserted into the pipe at two sampling sites - one at the wetted channel and one at the stream bank. In addition, one-litre sample of water from each sampling site was collected for chemical analysis in the laboratory. Samples were stored in a cold box, in the dark, at temperature of ~ 4°C during transportation to the laboratory, where they were stored at 4°C in a refrigerator.

Laboratory work

At the laboratory, we conducted analyses within 48 hours after sampling. Chemical parameters pH, nitrate, total nitrogen and total phosphorus were measured in each water sample following standard methods (APHA 1998). The amount of particulate organic matter was determined by loss on ignition (LOI). After retrieving the fauna, the remaining particulate organic matter was put into a ceramic vessel and oven-dried (24 h, 105°C). The dried organic matter was weighed, combusted at 520°C for 2 h, put in a desiccator for 48 h, and re-weighed. The amount of particulate organic matter (POM) was expressed as mg LOI per 1 litre of water sample. The amount of mobile sediment grains (particle size 0.1–5 mm) extracted by pumping of 10 L of water was measured and expressed as ml l^{-1}. Invertebrates were sorted from the samples under a stereomicroscope at 40-x magnification and preserved in 70% ethanol. Harpacticoids were counted and identified to the species level following the key provided by Janetzky et al. (1996), the updated nomenclature follows Walter & Boxshall 2021.

Data analysis

We compiled the list of harpacticoid species, indicated either as stygobiotic or epigean species, and presented their mean abundances in different stream systems. Prior to the further analyses, we merged the data from the different spatial replicates (4 sites) of the same location. We analysed spatial variability of harpacticoid assemblages using non-metric multidimensional scaling (nMDS) on a zero-adjusted Bray-Curtis dissimilarity matrix. The correlation coefficients of nine environmental variables with nMDS axes were calculated and presented as vectors in the nMDS ordination diagram. Species were overlayed on the nMDS ordination plane using their Spearman's correlation coefficients with nMDS axes. The significant differences between streams were tested using one-way ANOSIM analysis. The analyses were carried out using computer program PAST version 3.06 (Hammer et al. 2001).

Results

Environmental characteristics

The streams included in this study differed in catchment size, length and mean annual discharge, which is reflected in the physical and chemical characteristics of hyporheic water (Tab. 2). The hyporheic zone of the Iška is well oxygenated, water conductivity is the lowest, and POM and mobile sediment amounts are substantially lower than in the other three streams, indicating high hydraulic conductivity in the hyporheic sediments. Similarly, the POM and mobile sediments are extremely low in the Borovniščica, but conductivity is higher and oxygen concentrations lower than in the Iška hyporheic water. In comparison, the oxygen saturation in the Želimeljščica hyporheic zone is close to 50% and in the Podlipščica even lower (44.7%), with POM and mobile sediment reaching three to four times higher values than in the Iška and Borovniščica. This indicates a higher input of organic matter and nutrients in the hyporheic zone, and longer retention time of water (i.e., lower hydraulic conductivity) which leads to elevated trophic conditions.

Table 2. Physical and chemical characteristics of hyporheic water of the four streams, Želimeljščica, Iška, Borovniščica and Podlipščica, located in the central part of Slovenia, measured during PASCALIS project (May to Avgust 2002). Mean values and standard deviation for 96 sites are presented (4 streams x 12 sites x 2 sites).

Tabela 2. Fizikalne in kemijske lastnosti intersticialne vode štirih rečic, Želimeljščice, Iške, Borovniščice in Podlipščice iz osrednje Slovenije, merjeno v času projekta PASCALIS (maj do avgust 2002). Predstavljene so srednje vrednosti in standardne deviacije za 96 mest (4 rečice x 12 lokacij x 2 replikata).

	Želimeljščica	**Iška**	**Borovniščica**	**Podlipščica**
Temperature (°C)	11.3 ± 1.3	15.2 ± 2.6	15.3 ± 2.1	15.4 ± 1.3
Conductivity (µS cm^{-1})	522 ± 108	410 ± 23	486 ± 87	466 ± 87
pH	7.8 ± 0.3	8.2 ± 0.2	8.1 ± 0.3	8.0 ± 0.2
Oxygen (mg L^{-1})	5.6 ± 3.0	8.0 ± 1.5	6.0 ± 2.6	4.5 ± 2.5
Oxygen saturation (%)	52.8 ± 28.6	83.3 ± 15.3	61.8 ± 28.4	44.7 ± 27.4
N_{tot} (mg L^{-1})	2.6 ± 0.6	2.6 ± 0.3	2.8 ± 0.4	2.6 ± 0.7
P_{tot} (mg L^{-1})	48.2 ± 30.7	53.6 ± 13.6	61.9 ± 26.8	29.7 ± 21.9
Nitrate (mg L^{-1})	3.0 ± 2.4	3.5 ± 0.5	3.8 ± 1.6	2.7 ± 1.9
Particulate organic matter (mg DW L^{-1})	109.9 ± 112.5	27.1 ± 35.4	57.4 ± 61.1	108.6 ± 131.4
Mobile sediments (ml L^{-1})	22.3 ± 18.8	8.7 ± 11.4	6.1 ± 7.6	21.8 ± 23.0

Harpacticoid assemblages

In total, 24 harpacticoid species were collected in the hyporheic zone of the four streams (Tab. 3). Half of them, 12 species, were stygobionts. The most abundant species found in the samples were the epigean species *Bryocamptus dacicus* (Chappuis, 1923), *Pilocamptus pilosus* (Douwe, 1910) and *Bryocamptus zschokkei* (Schmeil, 1893). The rarest species, a mixture of epigean and stygobitic representatives, showed on average fewer than 10 collected specimens. In the individual samples, these species were *Attheyella wierzejskii* (Mrazék, 1893), *Elaphoidella gracilis* (Sars, G.O., 1863), *Moraria poppei* (Mrazék, 1893), *Moraria varica* (Graeter, 1911) (epigean species), and Ameridae gen. sp., *Elaphoidella jeanneli* (Chappuis, 1928), *Nitocrella* sp. and *Italicocaris* cf. *italica* (Chappuis, 1953) (stygobionts). Widespread species, defined as species occurring in the hyporheic zone of all four streams, were *Attheyella crassa* (Sars, G.O., 1853), *B. dacicus*, *B. pygmeus* (Sars, G.O., 1853), *B. typhlops* (Mrazék, 1893), *B. zschokkei*, *P. pilosus*, *Pesceus schmeili* (Mrazék, 1893), *Horstkurtcaris nolli alpina* (Kiefer, 1960) and *Parastenocaris gertrudae* Kiefer, 1968 (Tab. 3). All of these, except *B. typhlops* and representatives of family Parastenocarididae, belong to epigean/ubiquitous species.

The total number of species found in the hyporheic zone of the Želimeljščica and Iška was 16, while 15 species were found at the Borovniščica. Half of the collected species (8 species) were stygobionts. In the Podlipščica, 12 harpacticoid species were collected, but only four of them were stygobionts. Here, despite the lowest species richness, the abundances were among the highest (averaging 68 specimens in 10 L sample). Conversely, in the oligotrophic hyporheic zone of the Iška, the abundances were much lower compared to those from the other three streams (averaging 20 specimens in 10 L sample).

Table 3. List of harpacticoid species (Crustacea: Copepoda: Harpacticoida) and their mean abundances and overall species richness (number of individuals and species 10 L^{-1}) (±SD) in the hyporheic zone of the four streams in central Slovenia as found during PASCALIS project in summer 2002 (N=48, 12 locations x 4 replicates). * – stygobiont.
Tabela 3. Seznam vrst harpaktikoidov (Crustacea: Copepoda: Harpacticoida) in srednje vrednosti številčnosti in vrstne pestrosti v hiporeiku štirih rečic najdenih tekom projekta PASCALIS v poletni sezoni leta 2002 (število osebkov/vrst 10 L^{-1}) (N=48). * – stigobiont.

	Želimeljščica	**Iška**	**Borovniščica**	**Podlipščica**
	mean ± SD	mean ± SD	mean ± SD	mean ± SD
Ameridae gen. sp. *	3.0 ± 0.0			
Attheyella crassa (G.O. Sars, 1863)	6.5 ± 6.4	2.0 ± 0.0	1.0 ± 0.0	2.0 ± 1.4
Attheyella wierzejskii (Mrazek, 1893)	2.0 ± 0.0			
Bryocamptus minutus (Claus, 1863)				15.0 ± 0.0
Bryocamptus dacicus (Chappuis, 1923)	55.6 ± 85.1	15.8 ± 22.9	24.7 ± 44.3	59.3 ± 109.6
Bryocamptus pygmaeus (Sars, 1863)	1.5 ± 0.6	1.7 ± 0.6	1.0 ± 0.0	2.0 ± 0.0
Bryocamptus typhlops (Mrazek, 1893) *	3.5 ± 0.7	2.0 ± 0.0	1.0 ± 0.0	15.5 ± 20.5
Bryocamptus zschokkei (Schmeil. 1893)	16.4 ± 19.3	7.5 ± 7.8	4.1 ± 3.8	63.3 ± 78.4
Bryocamptus cf. *pyrenaicus**	3.1 ± 1.5	5.0 ± 0.0		
Pilocamptus pilosus (Van Douwe, 1910)	45.0 ± 71.0	7.2 ± 6.0	28.9 ± 41.6	29.1 ± 34.7
Elaphoidella charon Chappuis, 1936 *		24.3 ± 42.5	5.1 ± 4.5	
Elaphoidella elaphoides (Chappuis, 1924) *	28.8 ± 38.1		14.1 ± 11.5	19.0 ± 18.7
Elaphoidella gracilis (Sars, 1863)			1.0 ±	3.0 ± 1.4
Elaphoidella jeanneli Chappuis 1928 *			1.5 ± 0.7	
Elaphoidella millennii Brancelj, 2009 *		5.2 ± 4.6	8.5 ± 9.2	
Epactophanes richardi Mrazek, 1893				8.5 ± 10.6
Moraria poppei (Mrazek, 1893)	1.0 ± 0.0	1.5 ± 0.7	2.0 ± 1.4	
Moraria varica (Graeter, 1910)		1.0 ± 0.0		
Nitocrella hirta Chappuis, 1923 *	6.8 ± 9.2	5.3 ± 3.8		
Nitocrella sp. *	1.0 ± 0.0			
Pesceus schmeili (Mrazek, 1893)	8.7 ± 15.6	13.5 ± 26.0	6.1 ± 6.1	
Italicocaris cf. *italica**		1.0 ± 0.0		
Parastenocaris gertrudae Kiefer, 1968 *	2.6 ± 3.0	1.9 ± 1.1	7.0 ± 11.3	5.5 ± 6.4
Horstkurtcaris nolli alpina Kiefer, 1960 *	16.1 ± 14.5	2.7 ± 2.7	3.4 ± 2.8	8.0 ± 0.0
Overall mean abundance (specimen 10 L^{-1})	**72.6 ± 128.1**	**20.0 ± 33.7**	**46.4 ± 71.3**	**68.1 ± 124.6**
Overall mean species richness	**3.9 ± 2.4**	**2.9 ± 1.6**	**3.4 ± 1.8**	**2.1 ± 1.2**
Total number of species / stygobionts	**16 / 8**	**16 / 8**	**15 / 7**	**12 / 4**

The ANOSIM analysis indicated significant differences between the Iška and other three streams ($p<0.05$), most probably due to substantially lower harpacticoid abundances and low species richness. The nMDS ordination of sampling sites indicates a gradient in POM along first axis and a gradient in conductivity, POM, oxygen and pH along the second axis (Fig. 2). The first axis separates the Iška from the Podlipščica sites. Ordination of harpacticoid species obtained by overlaying the species on the space defined by the two nMDS axes on the basis of their correlation with the axes calculated using the Spearman's coefficient, revealed that two species showed preferences for the Želimeljščica and Podlipščica (*P. pilosus*, *B. dacicus*) and two (*Elaphoidella millenni* Brancelj, 2009, *Elaphoidella charon* Chappuis, 1936) for the Iška (Fig. 3).

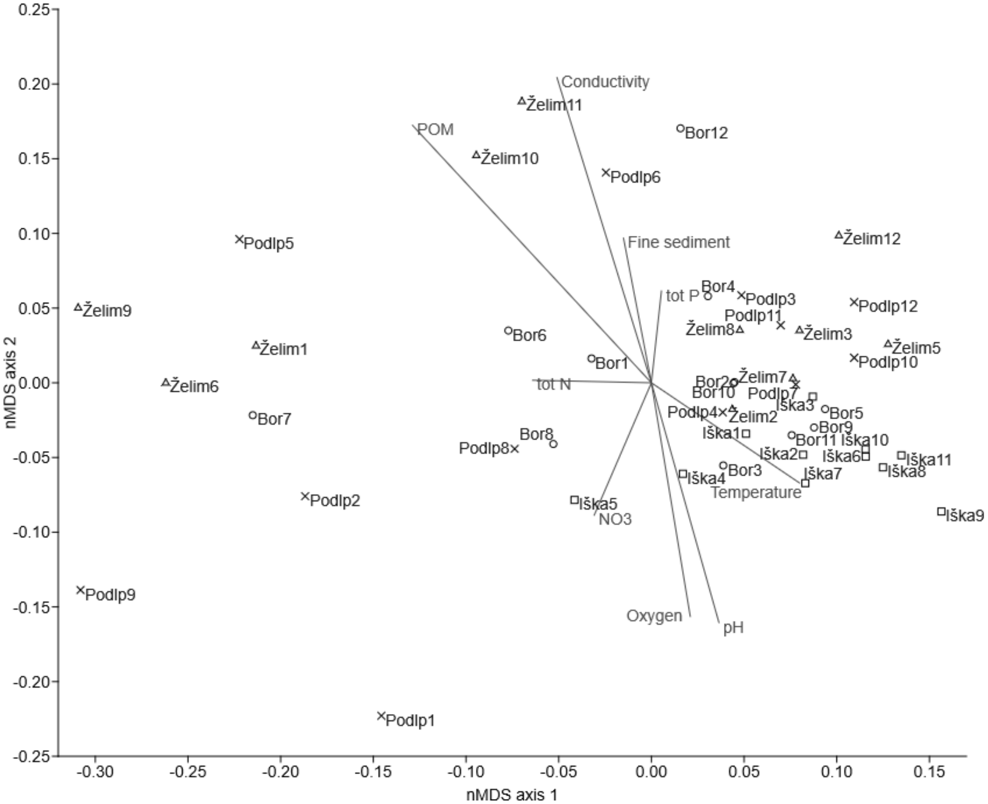

Figure 2. nMDS ordination diagram presenting ordination of sampling locations based on zero-adjusted Bray-Curtis dissimilarity matrix of harpacticoid assemblages and correlations of environmental variables with nMDS axes, presented as vectors (stress = 0.1403).

Slika 2. nMDS ordinacijski diagram, ki prikazuje razporeditev vzorčnih lokalitet na osnovi prilagojenega Bray-Curtisovega indeksa različnosti združb harpaktikoidov in korelacije okoljskih spremenljivk z nMDS osmi, prikazanih kot vektorji (stress = 0.1403).

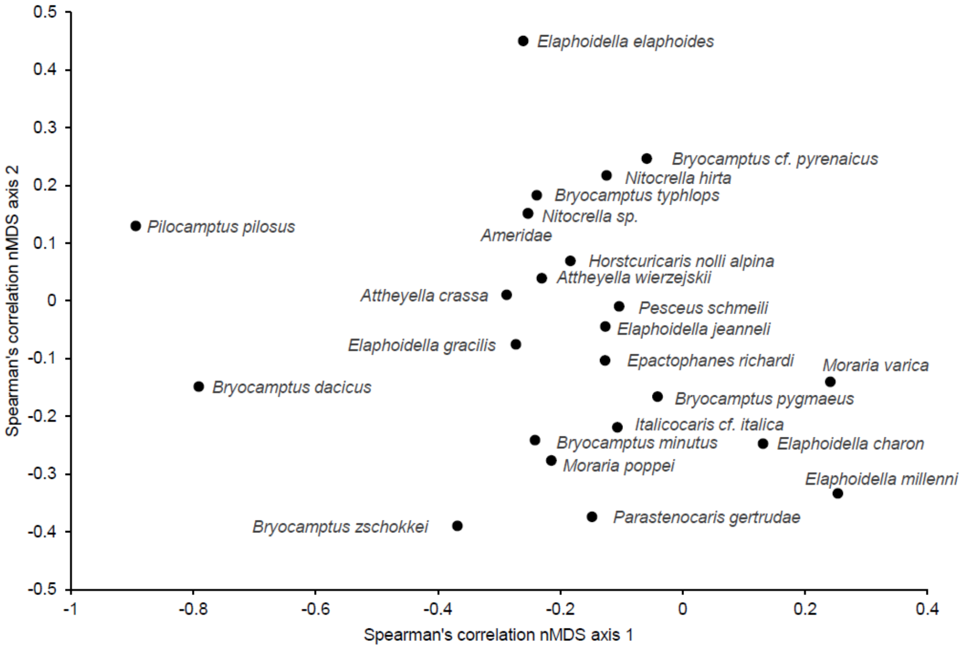

Figure 3. Ordination of harpacticoid species obtained by overlaying the species on the plane defined by the two nMDS axes on the basis of their correlation with the axes calculated using the Spearman's coefficient.
Slika 3. Razporeditev vrst harpaktikoidov v prostoru, definiranem z dvema nMDS osema na osnovi izračuna Spearmanovega koeficienta korelacije med abundancami vrst in vrednostmi osi.

Discussion

In the hyporheic zone of the four streams studied, a total of 24 harpacticoid species were found during a single sampling period. Six species of them belong to the genus *Bryocamptus*, five to *Elaphoidella*, and three to the family Parastenocarididae. Twelve species are stygobionts, and two are new species for science. In this study, the species *Nitocrella hirta* Chappuis, 1924, was found in Slovenia for the second time. Before this study, it had been reported only once, at the phreatic zone of the Sava River (Sket & Velkovrh 1981). A 2-year study of the hyporheic zone of the Sava River near Ljubljana in 2012, reported 12 harpacticoid species. Out of them, five of the species were stygobionts, with *Bryocamptus* as the most frequent genus; one representative of *Elaphoidella* and Parastenocarididae each, were also found (Mori et al. 2012). In both study areas, the commonest taxa found were six epigean species (i.e., *Bryocamptus dacicus*, *B. zschokkei*, *P. pilosus*, *P. schmeili*, *Attheyella crassa*, *Epactophanes richardi* Mrazék, 1893), and one stygobiont (*Elaphoidella elaphoides*). Two thirds of harpacticoid species present in the Sava River hyporheic zone were found also in this study.

In 1996, a total number of 44 harpacticoid species were known in Slovenia (Brancelj 1996). In 2001, 49 species and subspecies of harpacticoids were reported for Slovenia, 23 of them being stygobionts (Pipan & Brancelj 2001). From 2006 to present, additional four new species, previously unknown to the scientific world, have been found in Slovenian caves and interstitial habitats, as described by Brancelj (2006, 2009, 2011). Currently, there is a total of 53 harpacticoid species known to exist in Slovenia; among them, nearly half were found in the hyporheic zone of the four streams analysed in this study. Species composition of the studied stream assemblages was quite similar to that found in the Dinaric Karst region in southern Slovenia, with one third of species present in the Dinaric Karst region also present at the hyporheic zone of the studied streams. Additionally, almost half of the species from the studied hyporheic zone are also known from caves in the Dinaric Karst region (Brancelj 1986, Pipan & Brancelj 2003). Species found in common within different regions were primarily the epigean species that typically have wide distribution patterns, such as *B. dacicus*, while the common stygobionts present were rarer and found less frequently. Among the latter, *B. typhlops*, *Bryocamptus pyrenaicus* (Chappuis, 1923), and *H. nolli alpina* were found in both Dinaric Karst caves and hyporheic zone of the studied streams. A possible reason is the hydrological connectivity of the Dinaric Karst with the study area. This is possibly due to the complexity of the Ljubljanica River surface and subsurface flow. This flow starts in the south of Slovenia and flows over the Dinaric Karst region, passes few karst caves, and flows as a surface river into the Sava River near Ljubljana (Bonacci 2015). This hydrological connectivity, combined with a broad ecological tolerance of these species and their high ability to disperse, could explain the observed distributional patterns (Galassi et al. 2009). Another possible explanation, especially for stygobionts could be that current species encompasses complex of cryptic species (Lefébure et al. 2007).

The most frequently occurring and also the most abundant species found were the stygophiles *B. dacicus*, *B. zschokkei*, and *P. pilosus*. *Bryocamptus dacicus,* which have the Balkan distribution, is widespread in Slovenia. Its preferred habitat is groundwater (caves and interstitial groundwater), but it was found also in springs and in the benthos of alpine lakes (Jersabek et al. 2001). *Bryocamptus zschokkei* and *P. pilosus* are widespread and found in a variety of habitats (Gaviria 1998, Rundle et al. 2002), while the *Elaphoidella charon – jeanelli – millennii* complex is endemic to the Slovenian part of the Dinaric Karst region (Mori & Brancelj 2008, Brancelj 2009). *Attheyella wierzeyskii and Bryocamptus minutus* are widely distributed epigean species in Europe (Janetzky et al. 1996, Gaviria 1998). In Slovenia, *M. varica* has been, until now only found in the percolating water of karst caves, as it is a rare species that is limited in its distribution (Brancelj 1986, Pipan & Brancelj 2003). Nevertheless, it is well represented in groundwater of central Europe, as reported from seven localities of south-eastern Germany (Gaviria & Defaye 2017) and springs in Austria (Löffler & Neuhuber 1970, Gaviria 1998).

Species richness of harpacticoids was similar in the hyporheic zones of the Želimeljščica, Iška and Borovniščica, where oxygen concentrations were above 5 mg l^{-1}, but lower in the Podlipščica, where average oxygen concentrations were 4.5 mg l^{-1}. In opposite, abundances were low at the Iška and Borovniščica and high in the Podlipščica and Želimeljščica hyporheic zones. In the latter two streams, the sediments were finer and POM content higher. The causal factors for the composition and density of interstitial fauna are not always clear, in part because of the interplay between sediment pore size and interstitial water velocity. Another factor is the distribution of key environmental variables, such as temperature, oxygen and particulate organic matter (POM) (Strayer et al. 1997). Rouch (1988) demonstrated a functional relationship between permeability, concentration of oxygen, and the distribution of interstitial fauna. Harpacticoids were abundant and dominated over surface species in areas of high permeability and high oxygen saturation (>75%). In contrast, in areas of lower permeability and lower oxygen saturation, harpacticoids were low in abundance and stygobionts were the dominating species. Strayer et al. (1997) found that interstitial harpacticoids were found most frequently with the combination of high levels of dissolved oxygen and coarse-grained sediments, which have high hydraulic conductivity. Thus, according to both latter authors, interstitial harpacticoids are most abundant at sites with high levels of both oxygen and POM. In this study, our results indicated that with decreased permeability, the increased mobile sediment amounts and accumulation of POM in the interstitial spaces resulted in higher harpacticoid abundances, while higher oxygen concentrations lead to higher species richness.

The nMDS analysis indicated small differences in species composition among sites and streams due to strong dominance of the few abundant species *B. dacicus* and *P. pilosus*. Only the sites from the Iška significantly differed from others, primarily due to the low harpacticoid abundances. Qualitatively, the harpacticoid assemblages of the Iška and Borovniška were the most similar. The alluvial fans of both streams are extensive and interconnected (Mencej 1981). Their hyporheic zones have similar characteristics in regard to geomorphology, hydrology, and water chemistry. However, it seems that anthropic pressures (gravel extraction, channelization, water abstraction for drinking water and agriculture) and coarse-grained sediments with low POM amounts are not favourable for the abundance of harpacticoids. This conclusion is in accordance with Strayer et al. (1997), who stressed that the combined parameters that drive the hyporheic communities are hydrology, geomorphology, disturbance history of specific sites and biological interactions combined with temperature, seasonal changes and interannual variation.

Povzetek

Biološke in ekološke raziskave podzemnih voda rečnih (aluvialnih) vodonosnikov so v Sloveniji, v primerjavi z Evropo in Severno Ameriko, precej manj intenzivne, saj je večji del bioloških raziskav v Sloveniji že od začetka usmerjen predvsem na kraške podzemne vode. Cilj te raziskave je bil raziskati pojavljanje harpaktikoidov (Crustacea: Copepoda: Harpacticoida = raki: ceponožci: harpaktikoidi) v hiporeku štirih manjših rečic, ki izvirajo na območju Dinarske biogeografske regije in se stekajo v Ljubljanico. Harpaktikoidi so, poleg ciklopidov, med najbolj številčnimi in vrstno pestrimi predstavniki vodnih nevretenčarjev, ki naseljujejo hiporeik in globje plasti podtalnice. Raziskali smo tudi možne okoljske parametre, ki vplivajo na sestavo združb harpaktikoidov. Harpaktikoide smo vzorčili na 12 mestih vzdolž toka štirih rečic (Želimeljščica, Iška, Borovniščica, Podlipščica), in sicer v sezoni najnižjih letnih pretokov (maj-avgust) v letu 2002. Vzorčili smo v omočeni strugi, 30–60 cm globoko pod rečnim dnom in na bregu rek, v globinah od 65 do 160 cm. Na vsakem vzorčevalnem mestu smo, s pomočjo Bou-Roucheve črpalke, odvzeli vzorce vode, ki smo jih prefiltrirali prek 0.1 mm mrežice in tako prestregli harpaktikoide v načrpani vodi. Merili smo tudi fizikalno-kemijske lastnosti vode v hiporeiku in prisotnost anorganskih hranil, organskega drobirja v načrpani vodi ter ocenili stopnjo zamuljevanja medzrnskih prostorčkov. Določili smo 24 vrst harpaktikoidov, od tega 12 vrst, ki živijo izključno v podzemni vodi (tj., stigobionti). Najdeni sta bili dve novi vrsti za znanost. Združbo večinoma sestavljajo splošno razširjene vrste, pogoste v Sloveniji in tudi v Evropi. Razlike med štirimi rečicami so bile predvsem v številčnosti osebkov in v manjši meri v bogastvu vrst in njihovi sestavi.

Acknowledgements

The research was supported by the Slovenian Research Agency (P1-0255 & Young Researcher Programme). The study was partly funded by the EU project PASCALIS (EVK2-CT-2001-00121, 2002-2004). The authors would like to thank Gregor Bračko, Milijan Šiško and Andreja Jerebic for their assistance in the field and technical support in the laboratory.

References

Angelier E. (1953): Recherches écologique et biogéographiques sur la faune des sables submergés. Arch. Zool. Exp. Gen. 90: 37-161.

APHA, AWWA, WEF. (1998): Standard methods for the examination of water and wastewater, 20th edition. Clesceri L.S., Greenberg A.E., Eaton A.D. (Eds.), United Book Press, Baltimore, 1162 pp.

Bonacci O. (2015): Karst hydrogeology/hydrology of dinaric chain and isles. Environ. Earth. Sci. 74: 37-55.

Bou C., Rouch R. (1967): Un nouveau champ de recherches sur la faune aquatique souterraine. C.R. Hebd. Séances Acad. Sci. Ser. III. Sci. Vie 265: 369-370.

Boulton A.J., Datry T., Kasahara T., Mutz M., Stanford J.A. (2010): Ecology and management of the hyporheic zone: stream-groundwater interactions of running waters and their floodplains. J. N. Am. Benthol. Soc. 29: 26-40.

Boxshall G.A., Defaye D. (2008): Global diversity of copepods (Crustacea: Copepoda) in freshwater. Hydrobiologia 595: 195-207.

Brancelj A. (1986): Rare and lesser known harpacticoids (Harpacticoida, Copepoda) from the Postojna-Planina Cave System (Slovenia). Biol. Vestn. 34: 13-36.

Brancelj A. (1996): Favna rakov ceponožcev (Crustacea: Copepoda) v celinskih vodah. In: Gregori J., Martinčič A., Tarman K., Urbanc-Berčič O., Tome D., Zupančič M. (Eds.), Narava Slovenije, stanje in perspektive: zbornik prispevkov o naravni dediščini Slovenije. Društvo ekologov Slovenije, Ljubljana, pp. 242-246.

Brancelj A. (2000): *Parastenocaris andreji* n. sp. (Crustacea; Copepoda) - the first record of the genus in Slovenia (SE Europe). Hydrobiologia 437: 235-239.

Brancelj A. (2006): The epikarst habitat in Slovenia and the description of a new species. J. Nat. Hist. 40: 403-413.

Brancelj A. (2009): Fauna of an unsaturated karstic zone in Central Slovenia: two new species of Harpacticoida (Crustacea: Copepoda), *Elaphoidella millennii* n. sp. and *E. tarmani* n. sp., their ecology and morphological adaptations. Hydrobiologia 621: 85-104.

Brancelj A. (2011): Copepoda from a deep-groundwater porous aquifer in contact with karst: description of a new species, *Paramoraropsis brigitae* n. sp. (Copepoda, Harpacticoida). In: Defaye D., Suárez-Morales E., Vaupel Klein J. Carel (Eds.), Studies on freshwater Copepoda: a volume in honour of Bernard Dussart. Brill. Crustaceana Monographs 16: 85-104.

Brancelj A., Žibrat U., Jamnik B. (2016): Differences between groundwater fauna in shallow and in deep intergranular aquifers as an indication of different characteristics of habitats and hydraulic connections. J. Limnol. 75: 248-261.

Danielopol D.L. (1976): The distribution of the fauna in the interstitial habitats of riverine sediments of the Danube and the Piesting (Austria). Int. J. Spel. 8: 23-51.

Dole-Olivier M.-J., Marmonier P. (1992): Patch distribution of interstitial communities: prevailing factors. Freshw. Biol. 27: 177-191.

Galassi D.M.P. (2001): Groundwater copepods: diversity patterns over ecological and evolutionary scales. Hydrobiologia 453: 227-253.

Galassi D.M.P., Huys R., Reid J.W. (2009): Diversity, ecology and evolution of groundwater copepods. Freshw. Biol. 54: 691-708.

Gaviria S. (1998): Checklist and distribution of the free-living copepods (Arthropoda, Crustacea) of Austria. Ann. Nat. Hist. Mus. Wien 100 B: 539-594.

Gaviria S., Defaye D. (2017): A new species of *Moraria* (Copepoda, Harpacticoida, Canthocamptidae) from groundwaters of Germany, including a key for the identification of the species of the western Palearctic region. Crustaceana 90: 1537-1561.

Gibert J.D., Danielopol L., Stanford J.A. (1994): Groundwater Ecology. Academic Press, New York, 570 pp.

Habič P. (1996): Ogroženost in varstvo voda v občini Vrhnika. Vrhniški razgl. 1: 78-83.

Hammer Ø., Harper D.A.T., Ryan P.D. (2001): PAST: Paleontological Statistics Software Package for Education and Data Analysis. Palaentol. Electron. 4: 9 pp.

Iannella M., Fiasca B., Di Lorenzo T., Biondi M., Di Cicco M., Galassi D.M.P. (2020): Jumping into the grids: mapping biodiversity hotspots in groundwater habitat types across Europe. Ecography 43: 1-17.

Janetzky W., Enderle R., Noodt W. (1996): Crustacea: Copepoda: Gelyelloida und Harpacticoida. Süsswasserfauna von Mitteleuropa 8/4-2. Schwoerbel J., Zwick P. (Eds.), Gustav Fisher Verlag, Stuttgart, 208 pp.

Jersabek C.D., Brancelj A., Stoch F., Schabetsberger R. (2001): Distribution and ecology of copepods in mountainous regions of the Eastern Alps. Hydrobiologia 453/454: 309-324.

Karaman S.T. (1935): Die fauna der unterirdischen Dewässer Jugoslawiens. Verh. Int. Ver. Limnol. 7: 46-73.

Lefébure T., Douady C.J., Malard F., Gibert J. (2007): Testing dispersal and cryptic diversity in a widely distributed groundwater Amphipod (*Niphargus rhenorhodanensis*). Mol. Phylogenet. Evol. 42: 676-686.

Löffler H., Neuhuber F. (1970): Catalogous Fuana Austria. Ein systematisches Verzeichnis aller auf österreichischem Gebiet festgestellten Tierarten. Teil VIII: Crustacea, VIIIc Harpacticoida. Österreichische Akademie der Wissenschaften, Wien, 10 pp.

Malard F., Dole-Olivier M.J., Mathieu J., Stoch F. (2002): Sampling manual for the assessment of regional groundwater biodiversity. European Project PASCALIS: 1-74.

Mathers K.L., Hill M.J., Wood P.J. (2017): Benthic and hyporheic macroinvertebrate distribution within the heads and tails of riffles during baseflow conditions. Hydrobiologia 794: 17-30.

Mencej Z. (1981): Aluvialni vršaj Želimeljščice. Prvo obvestilo. Geologija 24: 169-171.

Meštrov M. (1960): Faunističko-ekološka i biocenološka istraživanja podzemnih voda savske nizine. Period. Biol. 13: 73-108.

Meštrov M., Stilinovic B., Habdija I., Lattinger R., Maloseja Z., Kerovec M., Čičin-Šain L. (1983): The ecological characteristics of intertstitial underground waters in relation to the water of the river Sava. JAZU, Prirodoslovna istraživanja 48. Acta Biol. 9: 5-33.

Mori N., Brancelj A. (2008): Distribution and habitat preferences of species within the genus *Elaphoidella* Chappuis, 1929 (Crustacea: Copepoda: Harpacticoida) in Slovenia. Zool. Anz. 247: 85-94.

Mori N., Brancelj A. (2011): Spatial and temporal variability of hyporheic invertebrate community within a stream reach of the River Bača. Nat. Slov. 13: 2-38.

Mori N., Simčič T., Lukančič S., Brancelj A. (2011): The effect of in-stream gravel extraction in a pre-alpine gravel-bed river on hyporheic invertebrate community. Hydrobiologia 667: 15-30.

Mori N., Simčič T., Žibrat U., Brancelj A. (2012): The role of river flow dynamics and food availability in structuring hyporheic microcrustacean assemblages: a reach scale study. Fund. Appl. Limnol. 180: 335-349.

Mori N., Debeljak B., Zagmajster M., Fišer C., Brancelj A. (2020): Pogled pod površje - živi svet v podzemnih vodah rečnih nanosov. In: Cerkvenik S. (Ed.), Vodni dnevi 2020 : simpozij z mednarodno udeležbo: zbornik referatov. 17.-18. september 2020, Rimske Toplice, Kongresni center Rimske terme. Slovensko društvo za zaščito voda, Ljubljana, pp. 103-115.

Orghidan T. (1955): Un nouveau domaine de vie souterraine aquatique: le biotope hyporhéique. Bull. Biol. Acad. R. P. Romania 7: 657-676.

Orghidan T. (2010): A new habitat of subsurface waters: the hyporheic biotope. Fund. Appl. Limnol. 176: 291-302.

Pavšič J. (2008): Ljubljansko barje. Neživi svet, rastlinstvo, živalstvo, zgodovina in naravovarstvo. Slovenska matica, Ljubljana, 214 pp.

Pennak R.W., Ward J.V. (1986): Interstitial faunal communities of the hyporheic and adjacent groundwater biotops of a Colorado mountain stream. Arch. Hydrobiol. Suppl: 74: 356-396.

Pipan T., Brancelj A. (2001): Ratio of copepods (Crustacea: Copepoda) in fauna of percolation water in six karst caves in Slovenia = Delež ceponožcev (Crustacea: Copepoda) v favni preniklih voda v šestih kraških jamah v Sloveniji. Acta carsologica 30: 257-265.

Pipan T., Brancelj A. (2003): The fauna of epikarst: Copepoda (Crustacea) in percolation water of Karst caves in Slovenia. Annales: anali za istrske in mediteranske študije, Series historia naturalis 13: 223-228.

Prevorčnik S., Remškar A., Fišer C., Sket B., Bračko G., Delić T., Mori N., Brancelj A., Zagmajster M. (2019): Interstitial fauna of the Sava River in Eastern Slovenia. Nat. Slov. 21: 13-23.

Rouch R. (1988): Sur la répartition spatiale des Crustacés dans le sous-écoulement d'un ruisseau des Pyrénées. Ann. Limnol. 24: 213-234.

Rouch R., Danielopol D.L. (1997): Species richness of microcrustacea in subterranean freshwater habitats. Comparative analysis and aproximate evaluation. Int. Rev. ges. Hydrobiol. 82: 121-145.

Rundle S.D., Bilton D.T., Galassi D., Shiozawa D.K. (2002): The geographical ecology of freshwater fauna. In: Rundle S.D., Robertson A.L., Schmid-Araya J.M. (Eds.), Freshwater meiofauna: biology and ecology. Backhuys Publishers, Leiden, the Netherlands, pp. 279-293.

Schwoerbel J. (1961): Über die Lebensbedingungen und die Besiedlung des hyporheischen Lebensraumes. Arch. Hydrobiol. Suppl. 25: 182-214.

Sket B., Velkovrh F. (1981): Phreatische Fauna in Ljubljansko Polje (Ljubljana - Ebene, Jugoslawien) - ihre ökologische Verteilung und zoogeograpische Beziehungen. Int. J. Speleol. 11: 105-121.

Strayer D.L., May S.E., Nielsen P., Wolheim W., Hausam S. (1997): Oxygen, organic matter and sediment granulometry as controls on hyporeic animal communities. Arch. Hydrobiol. 140: 131-144.

Walter T.C., Boxshall G. (2021): World of Copepods database. http://www.marinespecies.org/copepoda/ [accessed on 8.5.2021]