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THE FAUNA OF EPIKARST – COPEPODA (CRUSTACEA) IN PERCOLATION WATER OF KARST CAVES IN SLOVENIA

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ABSTRACT

Special attention was given to the stygobiotic species of copepods (Crustacea); their habitat is above the cave but under the surface in the so-called epikarst zone. Diversity dynamics of Copepoda were studied in six karst caves. In some caves (Postojnska jama, Pivka jama, Črna jama), samples were collected once per week. In the other three caves (Škocjanske jame, Dimnice, Županova jama), we sampled trickles once a month during 2000 and 2001. In total, 37 species were collected in the caves. From this habitat, 11 species new to science were recognized. New species living there are particularly restricted in distribution to one or few trickles of water dripping from the ceiling. The results of the faunistic research indicate that biodiversity of Copepoda in epikarst is very high on the local scale as well as over a wider area.

Key words: caves, unsaturated zone, percolation water, Copepoda, Slovenia

FAUNA DI COPEPODI (CRUSTACEA) EPICARSICI IN ACQUE DI PERCOLAZIONE DI GROTTE CARSICHE IN SLOVENIA

SINTESI

Particolare attenzione è stata rivolta alle specie stigobie di copepodi (Crustacea), che abitano gli habitat sopra le grotte ma sotto la superficie, quindi dell'area epicarsica. La dinamica di diversità dei copepodi è stata studiata in sei grotte carsiche. In alcune grotte (Grotte di Postumia, Grotta di Pivka e Grotta Nera) i campioni sono stati raccolti settimanalmente. Nelle restanti (Grotta di San Canziano, Dimnice e Grotta di Župan), i campionamenti sono stati effettuati mensilmente, negli anni 2000 e 2001. In totale sono stati raccolti 37 taxa nelle grotte. In tale habitat sono state trovate 11 nuove specie per la scienza. Tali specie hanno una distribuzione ristretta ad uno o pochi gocciolamenti d'acqua dal soffitto. I risultati della presente ricerca faunistica indicano un'alta biodiversità dei copepodi epicarsici sia su scala locale, sia considerando un'area più vasta.

Parole chiave: grotte, zona insatura, acqua di percolazione, Copepoda, Slovenia

INTRODUCTION

Karst is a special type of landscape, formed through dissolution of soluble rocks, including limestone and dolomite. The karst of Slovenia, with 43% of its territory consisting of carbonate rocks, is of great practical interest. Hypogean habitats constitute quite a significant part of nature in Slovenia, which has by far the richest aquatic hypogean fauna in the world (Sket, 1996). To understand the rarity of the organisms and the fragility of their habitats, we have to learn more about karst species, their ecosystems, and their sensitivity to environmental contamination.

The subterranean environment all over the world is inhabited by numerous taxa of Copepoda (Crustacea), and many of them are endemics. In Slovenia, 107 taxa of Copepoda have been recorded to date and about one third of these are stygobionts. At present, there are 15 endemics and all but one of them are stygobionts. All but one belong to the order of Harpacticoida (Brancelj, 1996).

The biodiversity and ecology of copepods in percolation water have rarely been systematically studied. Many species in the group of Copepoda are rarely found in streams, but are found in seeps and drip pools, although their existence in any particular set of seeps or pools is often quite ephemeral (Culver *et al.*, 1994). The primary habitat of these species is almost certainly the subcutaneous zone, *i.e.* epikarst (Holsinger, 1994). Due to the high level of endemism, the future research on the underground fauna should be carried out principally in the direction of fauna-related research. This should encompass the habitats that have remained unexplored, and these are particularly percolation waters.

In 2000 and 2001, some intensive studies of microdistribution and diversity dynamics of copepods were carried out in trickles and pools of percolation water in several cave systems in Slovenia. The working hypothesis was based upon the ecological, hydrogeological and chemical explorations of the karst unsaturated zone with an emphasis on the ecology of the copepod fauna in the percolation water. We focused on the following two main questions. First, whether there are, and what are, the causes of the biological differences in the epikarst evaluated from the differences between trickles, pools and environmental factors. Second, whether there are differences of fauna between caves in different geographical areas.

MATERIAL AND METHODS

The six studied caves are situated in southern and southwestern Slovenia (Fig. 1). For a description of the study area, see Pipan & Brancelj (2001). We dealt with the epikarst fauna, which has been until recently an almost completely unknown segment of life within the karst underground. In hydrological division of the karst underground, the epikarst constitutes the stratum, which is the closest to the surface but remains inaccessible, if standard research methods are to be used. The epikarst fauna has thus been explored indirectly, by taking samples of the percolated water and cave pools filled with such water. The pools in the fossil parts of the caves are filled up by water, which seeps down the walls or drips directly from the ceiling. With such a selection of pools we will avoid the influence of phreatic groundwater or hypogean rivers on the composition of the fauna.

In the caves of Postojnska jama, Pivka jama and Črna jama we sampled water trickles once per week for one year. In the other three caves, samples were collected once a month during 2000 and 2001. Samples of fauna as well as samples for water quality analyses were collected from the container. During the period of a single week or month, the water from trickles was directed through a funnel into 0.25 l plastic containers. On two sides, the containers had holes covered with a net (mesh size 60 µm) to retain animals in the container. The content of these plastic containers was fixed with 4 % final solution of formaldehyde in the field and stored for further processing. In the laboratory we separated the organisms by means of stereomicroscope at 40x magnification and stored them in 70% ethanol. Further processing and identification of the organisms was performed under a compound microscope. Samples from pools were collected separately into plastic containers by means of adapted suction pump. We pumped various quantities of the pool water at different sampling points and filtered it through a 60 µm net. The samples were then processed in the same way as those from the trickles. Each water trickle or pool filled with water was treated separately.

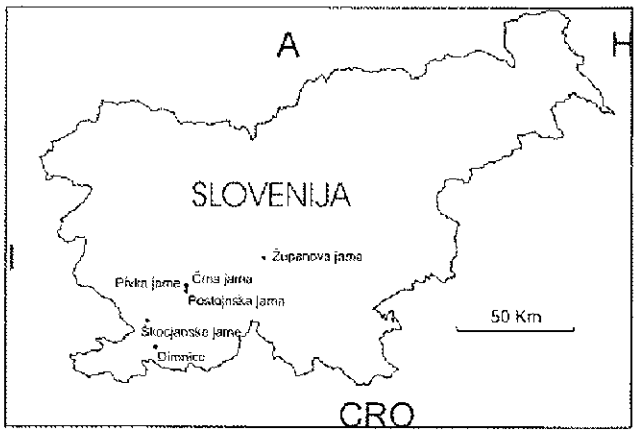

Fig. 1: Geographical location of the research caves in Slovenia.
Sl. 1: Geografska lega raziskovanih jam v Sloveniji.

RESULTS

In six karst caves, a systematic survey of two different types of habitats, trickles, and pools of percolation water was carried out. From seeps and drip pools we collected 37 species (Tab. 1). The most numerous were specimens of *Speocyclops infernus, Moraria poppei, Morariopsis scotenophila, Elaphoidella cvetkae, Bryocamptus balcanicus* and specimens of the genus *Parastenocaris*. Ten species belong to a group of troglophilous or eutroglophilous taxa, which are frequently also found in subterranean environment. The other 27 species are stygobiotic. Eleven species (from the genera *Bryocamptus, Elaphoidella, Maraenobiotus, Moraria, Nitocrella, Parastenocaris* and perhaps *Stygepactophanes)* were recognized as new to science and have to be studied in detail. It seems that they are obligate epikarst species.

Results of the correlation analyses (using the Spearman correlation coefficient: Davis, 1973) and the non-parametric version of one way ANOVA (using the Kruskal-Wallis Test: Blejec, 1976) indicate that there is no correlation between thickness of the cave ceiling, temperature and discharge on the one hand and the number of specimens on the other ($p>0.05$). The precipitation shows highly positive co-variation with the discharge and with the number of specimens in two caves. Physical parameters for each cave are summarized in Table 2. The copepod abundance in different kinds of pools was not correlated with the amount of pumped water. For more precise conclusions about correlation between pool typology or amount of filtered water and the number of specimens, a higher number of samples collected in shorter intervals should be analysed. New data can be obtained from further investigations in trickles of percolation water.

From the geographical point of view, there is no correlation ($r = -0.38$, $p = 0.31$) between the distance apart of the caves and the similarity of the fauna (using the Pearson correlation coefficient (r)). The highest similarity expressed as a ratio of the species in common between two locations and the sum of taxa of both locations (using the Jaccard similarity coefficient (S_j)) was between the caves of Dimnice and Črna jama ($S_j = 0.44$), which are approximately 29 km apart (Fig. 2). In caves that belong to the same cave system (Postojnska jama, Pivka jama, Črna jama) and are 3 km apart, the similarity was lower and quite similar ($S_j = 0.33 - 0.37$). This fact is not unexpected due to the same geographical and geological situation and the same influence of external environmental factors. The lowest similarity was between the caves of Škocjanske jame and Dimnice ($S_j = 0.21$) and Škocjanske jame and Županova jama ($S_j = 0.22$). Between the most distant caves, *i.e.* Dimnice and Županova jama that are about 61 km apart, the similarity was 0.27.

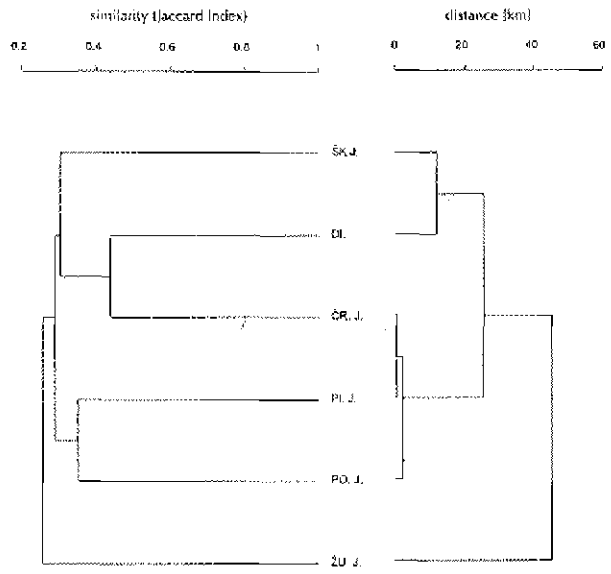

Fig. 2: Dendrogram constructed by data on similarity of copepod community and geographical distance between caves (UPGMA method, standardized on the Jaccard similarity coefficient). The abbreviations are: ČR.J. = Črna jama, DI. = Dimnice, ŠK.J. = Škocjanske jame, PO.J. = Postojnska jama, PI.J. = Pivka jama, ŽU.J. = Županova jama.

Sl. 2: Primerjava podobnosti združb ceponožcev in geografskih razdalj med jamami v dendrogramu podobnosti po metodi UPGMA (uporabljen je Jaccardov koeficient podobnosti). Okrajšave: ČR.J. = Črna jama, DI. = Dimnice, ŠK.J. = Škocjanske jame, PO.J. = Postojnska jama, PI.J. = Pivka jama, ŽU.J. = Županova jama.

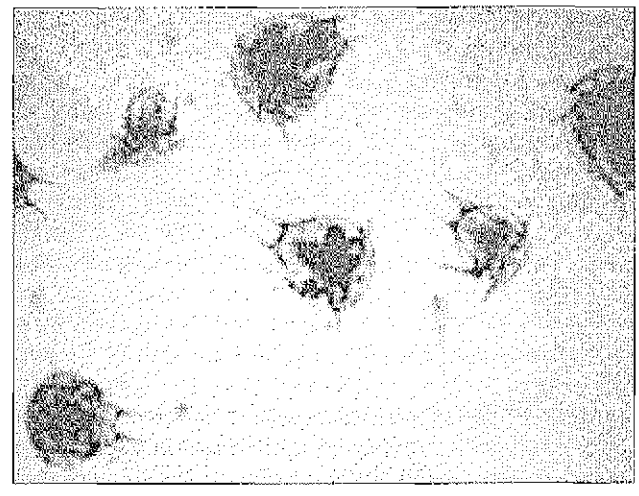

Fig. 3: The life cycle of free-living copepods includes up to six naupliar and six copepodit stages prior to the adult.

Sl. 3: Prosto živeči ceponožci imajo šest navplijskih in šest kopepoditnih stadijev.

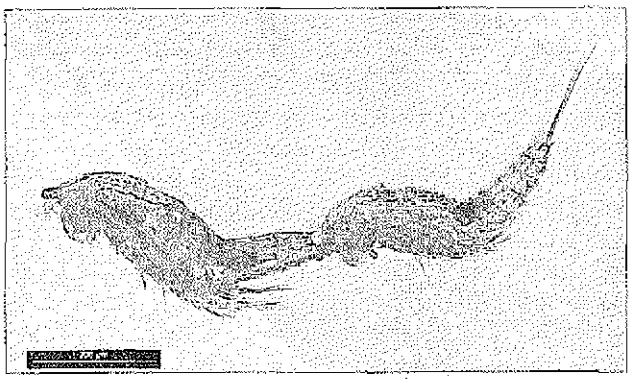

Fig. 4: Pre-copulating individuals of *Bryocamptus zschokkei.*
Sl. 4: Paritveni par vrste *Bryocamptus zschokkei.*

Nauplia (Fig. 3) were present in all samples, although they were more abundant in samples from trickles than in samples from pools. The exceptions were trickles in Postojnska jama, where they were completely absent.

Sex ratio varied, but in most samples females were predominant. Males dominated only in the samples from trickles in Škocjanske jame. Juveniles constituted from 0.5% to 20% of the population.

Precopulatory mating individuals (Fig. 4) were found in five caves (except in Postojnska jama) in six species. They were predominant in samples from pools. In trickles and in pools we found copula in *Bryocamptus balcanicus* and *Maraenobiotus* cf. *brucei*. Pre-copulating individuals of *Bryocamptus dacicus, B. typhlops, B. zschokkei* and *B. pyrenaicus* were collected only from pools. Precopulatory mating individuals were found only during the winter (from September to February). Egg sacs were observed also in August and then every month from September 2000 till March 2001. Ovigerous females were found in eight taxa. Seven of them were found only in pools (*Diacyclops* sp. *(languidoides* – group), *Paracyclops fimbriatus, Morariopsis dumonti, Bryocamptus* n. sp., *B. balcanicus, B. typhlops, B. zschokkei)* and females of *B. dacicus* also in trickles. In Dimnice cave, we found no ovigerous females. Cumulative number of copulatory individuals was 43 (the highest number in Škocjanske jame and Županova jama), and cumulative number of females with egg sacs was 34 (the highest number in Pivka jama).

Tab. 1: List of species of copepods (Crustacea: Copepoda) and total number of individuals in trickles and pools of percolation water of the six karst caves (species presumed to be new to science are marked with asterisk *).
Tab. 1: Seznam vrst ceponožnih rakov (Crustacea: Copepoda) in skupno število osebkov v prenikajoči in prenikli vodi šestih kraških jam (za znanost nove vrste so označene z zvezdico *).

	stygobionts / stigobionti	trickles / curki	pools / luže
CYCLOPOIDA			
1. *Acanthocyclops kieferi* (Chappuis, 1925)	x		17
2. *Diacyclops* sp. *(languidoides-group)* (Lilljeborg, 1901)	x	2	108
3. *Diacyclops languidus* (Sars, 1863)			1
4. *Megacyclops viridis* (Jurine, 1820)			11
5. *Paracyclops fimbriatus* (Fischer, 1853)			8
6. *Speocyclops infernus* (Kiefer, 1930)	x	141	794
HARPACTICOIDA			
7. *Attheyella crassa* (Sars, 1862)			1
8. *Bryocamptus balcanicus* (Kiefer 1933)	x	94	174
9. *Bryocamptus borus* Karanovic & Bobic, 1998	x		6
10. *Bryocamptus dacicus* (Chappuis 1923)		68	156
11. *Bryocamptus pygmaeus* (Sars, 1862)	x		4
12. *Bryocamptus pyrenaicus* (Chappuis, 1923)	x	8	37
13. *Bryocamptus typhlops* (Mrazek, 1893)	x		81
14. *Bryocamptus zschokkei* (Schmeil, 1893)			82
15. *Bryocamptus* sp.*	x	1	14
16. *Elaphoidella cvetkae* Petkovski, 1983	x	98	6
17. *Elaphoidella kieferi* Petkovski & Brancelj, 1985	x	5	1
18. *Elaphoidella stammeri* Chappuis, 1936	x	10	
19. *Elaphoidella* sp. 1*	x	2	8
20. *Elaphoidella* sp. 2*	x	3	
21. *Epactophanes richardi* (Mrazek, 1893)			43
22. *Maraenobiotus* cf. *brucei**	x	96	68
23. *Moraria poppei* (Mrazek, 1893)		5	754
24. *Moraria stankovitchi* Chappuis, 1924	x	1	
25. *Moraria varica* (Graeter, 1911)		10	5
26. *Moraria* sp. A*	x	4	
27. *Moraria* sp. B*	x	1	
28. *Morariopsis dumonti* Brancelj, 2000	x	3	64
29. *Morariopsis scotenophila* (Kiefer 1930)	x	2	247
30. *Nitocrella* sp.*	x	5	
31. *Parastenocaris nolli alpina* (Kiefer, 1938)	x	121	4
32. *Parastenocaris* cf. *andreji**	x	1	1
33. *Parastenocaris* sp. 1*	x	5	1
34. *Parastenocaris* sp. 2*	x	160	11
35. *Parastenocaris* sp. 3*	x	7	
36. *Phyllognathopus viguieri* (Maupas, 1892)		1	4
37. *Stygepactophanes* (?) sp.*	x	10	

Tab. 2: Minimum and maximum values for some physical parameters, measured in six caves between April 2000 and March 2001.
Tab. 2: Najnižje in najvišje vrednosti nekaterih fizikalnih podatkov v šestih jamah, merjene od aprila 2000 do marca 2001.

Parameter/Cave Podatek/Jama (min-max)	POSTOJNSKA JAMA	PIVKA JAMA	ČRNA JAMA	ŠKOCJANSKE JAME	DIMNICE	ŽUPANOVA JAMA
Thickness of the cave ceiling / debelina jamskega stropa [m]	30-110	50-70	30-65	60-110	10-70	15-50
Average temperature / povprečna temperatura [°C]	8.6-9.4	5.3-9.0	4.5-7.0	9.5-12.0	4.1-9.3	6.1-9.2
Average discharge / povprečen pretok [ml min^{-1}]	1.9-22.0	1.7-202.0	8.0-59.0	18.0-182.0	3.2-75.0	4.1-33.0
Annual precipitation / letne padavine [mm]	1827	1827	1827	1548	2019	1577

DISCUSSION

The high level of endemism, as evident from the literature published to date, and insufficient knowledge regarding the distribution of taxa inhabiting the unsaturated karst zone, were two main reasons for our decision to focus on the problems and issues related to the distribution and the ecology of the fauna in the unsaturated epikarst zone. According to rather few explorations, the major part of the fauna in percolation water consists of copepods (Galassi, 2001). During our exploration, we restricted ourselves to the community of copepods in the karst fissured system in caves. The intensive research on Copepoda in six caves in Slovenia indicated a high number of taxa, whose primary habitat are cracks and crevices filled with water in the epikarst zone. In total, 37 species were collected in six horizontal caves. Eleven species new to science were recognized. New species living there are particularly restricted in distribution to one or few trickles of dripping water. Ten species, which are frequently found in subterranean environment but transported from their epigean habitats, could be designated as ubiquitous. The rest of the species, *i.e.* 27 of them, are stygobiotic, and fifteen are endemic to Slovenia. One species, *Bryocamptus borus*, is new to the Slovenian fauna. Males of *Morariopsis scotenophila* were found for the first time.

Between 11 and 17 different species of copepods were found per cave regardless of its length. From 37 species, only two, *Speocyclops infernus* and the new species *Parastenocaris* sp., were found in all six caves. The majority of species were found in one or two caves only. Sixteen species were found only at a single locality and eleven of these were stygobiotic.

The drip pools and seeps form a distinct habitat in the cave (Culver *et al.*, 1994). The intensive survey of the two types of habitats in six karst caves showed that the ratio of copepods in the trickles was different from that in the pools of percolation water. Paradoxically, the starting point for analysis of the copepod community structure is not heterogeneity between types of habitats (trickle – pool), but self–similarity. It is important that in both types of habitats we found mostly the same groups of specimens, which differed only in the frequency of their occurrence. We established a similar species composition, which indicates that the real habitat of the stygobiotic species of copepods is above the cave but under the surface, in the epikarst zone.

The high number of specimens found in the cave and the low number of specimens involved in reproduction suggest that the water bodies sampled in the cave are probably not the breeding site for most species. This supports the idea of Brancelj (2002) who proposed that the habitat where they breed, at least for some of them, is the space beneath the soil layer. They can penetrate deeper into the rocks through small crevices, filled by water.

The results and findings of the research constitute a fundamental contribution to the understanding of distribution patterns of stygobiont copepods in the epikarst zone. Results of such studies are of general interest and value. This information helps us to better understand the interactions of organisms within the karst ecosystem. The biodiversity of epikarst fauna will be at the same time used to study and evaluate the impacts of human activities on the subterranean environment. The most urgent problem in the karst area is pollution with percolation water, which usually originates from the surface pollution. Due to the numerous newly discovered species, particularly within the subterranean habitats, the number of copepod species is rapidly increasing. New data can be obtained from further investigations of fauna in seeps and drip pools.

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Tanja PIPAN & Anton BRANCELJ: THE FAUNA OF EPIKARST – COPEPODA (CRUSTACEA) IN PERCOLATION WATER OF KARST CAVES IN SLOVENIA, 223-228

FAVNA EPIKRASA – COPEPODA (CRUSTACEA) V PRENIKAJOČI VODI KRAŠKIH JAM V SLOVENIJI

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POVZETEK

V članku so podani kvalitativni in kvantitativni podatki o favni ceponožnih rakov v curkih prenikajoče vode in v lužah, ki jih ta polni. Ceponožni raki so najbogateje zastopana živalska skupina v epikrasu. Nezasičena kraška cona je s standardnimi raziskovalnimi metodami nedostopna, zato je bilo vzorčevanje epikraške vodne favne posredno, z vzorčenjem prenikajoče vode. V šestih kraških jamah je bilo opravljeno sistematično vzorčenje v curkih prenikajoče vode in lužah, ki jih ti curki napajajo. Curki prenikajoče vode so se stekali v lijak, od koder je stekla voda v plastenko z dvema odprtinama, zamreženima z gosto mlinarsko svilo z odprtinami 60x60 µm. Plastenka je bila v večji posodi z vgrajeno odtočno cevjo. Voda iz plastenke je odtekala skozi mrežico v posodo z odtokom, živali pa so ostale ujete v plastenki. Vzorci so bili fiksirani z dodatkom 37% formalina do končne koncentracije okrog 4% in shranjeni za nadaljnjo obdelavo v laboratoriju. Voda in usedlina v lužah je bila posesana s pomočjo prirejene sesalne črpalke ter precejena skozi mrežico z odprtinami 60x60 µm. Najdenih je bilo 37 vrst ceponožnih rakov, med njimi 27 stigobiontskih. Vrsta Bryocamptus borus *je bila prvič ugotovljena v Sloveniji. Prvič so bili najdeni samci vrste* Morariopsis scotenophila. *Ugotovljenih je bilo še 11 vrst iz 7 rodov, novih za znanost. Za 10 vrst je znano, da v jame zaidejo naključno s curki prenikajoče vode in da so v površinskih in podzemeljskih vodah pogoste. Osebki drugih vrst, ki so bili najdeni v posameznih jamah ali na posameznih vzorčevalnih mestih v jami, so stigobionti. Petnajst med njimi je endemitov, omejenih le na ozemlje Slovenije. V posamezni jami je bilo med 11 in 17 vrst ceponožcev. Pojavljanje in številčnost vrst ter vrstna sestava v prenikajoči vodi niso odvisni od velikosti jame. Ugotovljena je bila velika vrstna raznolikost med jamami. Samo* Speocyclops infernus *in nova vrsta* Parastenocaris *sp. 2 sta bili v vseh šestih jamah. Šestnajst vrst je bilo najdenih le v eni jami, od tega 11 stigobiontov. V curkih in lužah s prenikajočo vodo je maloštevilna, a vrstno bogata favna ceponožcev. Podobnost kopepodnih združb v jamah ni v soodvisnem razmerju z geografsko oddaljenostjo med jamami, z debelino stropa, s temperaturo in pretokom, ampak morda z obstojem podobnih mikrohabitatov v epikraški coni. Za večino curkov je soodvisnost med padavinami in pretokom statistično značilna. Številčnost kopepodov v različnih tipih luž ni sorazmerna s količino prefiltrirane vode. Zaradi visoke stopnje ekološke specializacije kopepodov iz epikraške cone bi lahko bile mnoge vrste uporabne kot bioindikatorji pri ocenjevanju vplivov človekove dejavnosti na podzemeljske habitate.*

Ključne besede: jame, nezasičena cona, prenikajoča voda, Copepoda, Slovenija

REFERENCES

Blejec, M. (1976): Statistične metode za ekonomiste. Univerza v Ljubljani, Ekonomska fakulteta, Ljubljana, 497-498 str.

Brancelj, A. (1996): Favna rakov ceponožcev (Crustacea: Copepoda) v celinskih vodah. The Fauna of Copepods (Crustacea: Copepoda) in Inland Waters. Narava Slovenije, stanje in perspektive. Zbornik prispevkov o naravni dediščini Slovenije. Društvo ekologov Slovenije, Ljubljana, 242-246 str.

Brancelj, A. (2002): Microdistribution and high diversity of copepoda (Crustacea) in a small cave in central Slovenia. Hydrobiologia, 477, 59-72.

Culver, D. C., W. K. Jones, D. W. Fong & T. C. Kane (1994): Organ Cave Karst Basin. In: Gibert, J., D. L. Danielopol & J. A. Stanford (eds.): Groundwater Ecology. Academic press, New York, p. 451-473.

Davis, J. C. (1973): Statistics and Data Analysis in Geology. John Wiley & Sons, Inc., New York, p. 59-106, p. 456-473.

Galassi, D. M. P. (2001): Groundwater copepods: diversity patterns over ecological and evolutionary scales. Hydrobiologia, 453-454, 227-253.

Holsinger, J. R. (1994): Pattern and process in the biogeography of subterranean amphipods. Hydrobiologia, 287, 131-145.

Pipan, T. & A. Brancelj (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(2), 257-265.

Sket, B. (1996): Biotic Diversity of Hypogean Habitats in Slovenia and its Cultural Importance. In: Cimerman, A. & N. Gunde-Cimerman (eds.): Biodiversity, International Biodiversity Seminar ECCO XIV. Gozd Martuljek, Slovenia, 30 June – 4 July, 1995. p. 59-74.