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ORIGINAL ARTICLE

Epikarst communities: biodiversity hotspots and potential water tracers

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Abstract There is an exceptionally rich aquatic fauna in the epikarst, the skin of karst. High species richness in the epikarst, coupled with its special vulnerability as the first point of entry of nearly all toxic spills, makes its protection especially important. The epikarst fauna may also be an useful tool in tracing the potential route of pollutants. Copepods in epikarst have extremely local distributions, and their body size is such that they are largely at the mercy of directional flows. In a series of caves in southwest Slovenia and West Virginia, a significant fraction of the copepod species occur in less than 100 m of linear extent. This suggests a pattern of highly restricted lateral flow under normal conditions and the distribution of copepods could potentially be used to trace water movement. Under high flow conditions as would often be the case with toxic spill, mounding of water may increase the lateral radius of flow. Nevertheless, copepods may be useful tracers.

Keywords Epikarst · Copepods · Tracer tests

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Introduction

The upper most layer of karst, at the rock soil interface, is commonly termed epikarst. According to a recent definition, epikarst is:

> located within the vadose zone and is defined as the heterogeneous interface between unconsolidated material including soil, regolith, sediment, and vegetative debris, and solutionally altered carbonate rock that is partially saturated with water and capable of delaying or storing and locally rerouting vertical infiltration to the deeper regional phreatic zone of the underlying karst aquifer (Jones et al. 2004).

Known for decades by speleobiologists (Sket et al. 2004), water in the epikarst zone was often termed percolating water and included as part of the vadose zone, the zone of karst above the permanently saturated (phreatic) zone. The metaphor of Bakalowicz (2004) of epikarst as the skin of karst captures the essence of this boundary zone.

There is a highly complex and diverse aquatiac microfauna in the epikarst, including a rich array of crustaceans, most especially copepods (Pipan 2005). The epikarst is of interest to biologists, not only because of its species diversity, but also because it is a source of organic input as well as the location of the exchange of surface and subsurface fauna. This contribution argues that hydrogeologists should be interested in the epikarst animals for their potential role as tracers of water movement.

The focus in the hydrogeology of epikarst has been on the vertical movement of water, which is of considerable practical importance because of the importance of this pathway in contaminant flow. For example, Gabrovšek (2004) presented a model of the evolution of epikarst in the vertical direction and Trček (2003) details the largely vertical movement of water in the epikarst in the Hubelj aquifer. However, water in the epikarst can flow laterally, especially during periods of high recharge (Smart and Friederich 1987).

Purpose

In this study the potential of copepods, a group of microscopic crustaceans, as tracers of the movement and connectivity of epikarst water is examined.

Copepod biology and hydrodynamics

The subclass Copepoda belongs to the class Crustacea and comprises ten orders. About 2,600 free-living freshwater species belong to four orders: Gelyelloida, Calanoida, Cyclopoida and Harpacticoida. Approximately 650 species of these four orders occupy subterranean fresh water with a high number of stygobiotic and endemic species (Huys and Boxshall 1991). From epikarst, only Cyclopoida and Harpacticoida are known so far (Pipan 2005). The typical length of adults is 0.2–2 mm. Obligate subterranean aquatic (stygobiotic) copepods display various degrees of morphological and biological specialization. Depigmentation, eye loss, thigmotaxis and miniaturisation occur in epikarstic copepods as adaptive features. Pesce and Galassi (1986) emphasized the importance of reduction of spination on proximal segments of body of Cyclopoida in order to facilitate movement in sandy and muddy sediments likely to be found in epikarst.

Differences in swimming behaviour between stygobiotic and epigean copepods are also likely adaptations to subterranean life (Stoch 1995). Stygobiotic copepods are swimmers, crawlers or burrowers. Cyclopoids move among sediment particles by pushing their body over the grains to gain propulsion. Harpacticoids are mainly burrowers which enhances their ability to move in sediments and narrow spaces. Little is known about copepods feeding in interstitial habitats. Several large cyclopoids are predatory. The main food source for harpacticoids is organic matter either as coarse and fine particles and most probably the microbial biofilm associated with it (Pipan 2005).

Just as mineralized particles can be mobilized into the water column, so can copepods. It may be useful to think of copepods, not as living organisms, but as organic particles of varying sizes and shapes. A great deal is known about the mobilization of mineralized particles, e.g., Hjulstrom curves (Gordon et al. 1999). Intermediate sized particles between 0.3 and 0.6 mm are the easiest to mobilize, and this occurs at a velocity slightly greater than 0.1 m/s. This is approximately the size of the smallest copepods. Particles 3 mm in diameter can be mobilized at approximately 0.5 m/s. Smith (1975) states that the critical velocity for organic matter with a density of 1.05 g/cm^3 is about 1/6 that of an equivalent sized mineral particle, suggesting that a passive copepod in the epikarst could be mobilized at velocities of less than 0.1 m/s. In the absence of information about flow velocity in epikarst, it seems reasonable that such rates are often reached in the epikarst. Indeed, it would be hard to explain the nearly continuous washout of copepods from the epikarst via drips (Pipan and Culver 2005) if this were not the case. It is this property of copepods, i.e. their frequent occurrence in the water column, that makes them potential water tracers.

Materials and methods

Copepods and other interstitial animals were collected from ceiling drips in Organ Cave, West Virginia (Pipan and Culver 2005) and in six caves in Slovenia (Pipan 2005; Pipan and Brancelj 2004; Fig. 1):

1. Postojnska jama, Črna jama, and Pivka jama, all part of the Postojna-Planina Cave System
2. Škocjanske jame
3. Županova jama
4. Dimnice

In Organ Cave, a large multi-level cave with more than 60 km of surveyed passage, samples were taken from 13 drips in three clusters. Distances ranged from 1 m to less than 100 m and greater than 1,200 m. In Slovenia, sampled drips were generally farther apart, and ranged from 10 m to a maximum of 57 km. In all caves except Postojnska jama, five drips were sampled; in Postojnska jama, ten drips were sampled.

In each cave between five and thirteen drips were sampled by directing the water through a funnel into a plastic container. A 2 cm by 3 cm area on each of two sides of the square container was cut out and covered with a net (mesh size 60 μm) to retain animals in the container. Samples were collected for 30 days in Organ Cave and one year in the Slovenian caves.

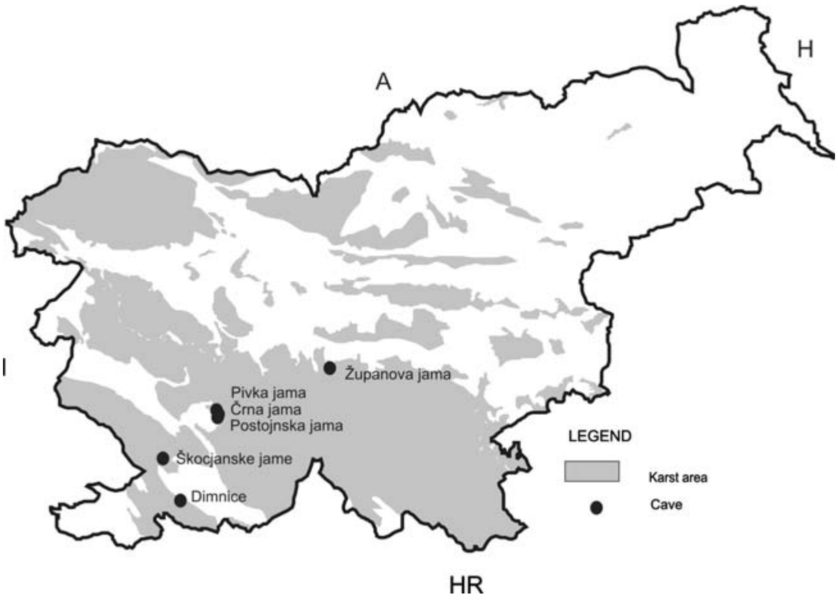

Fig. 1 Map of location of study caves in Slovenia. *Shaded areas* are karst. Bordering countries are Austria (*A*), Hungary (*H*), Croatia (*HR*), and Italy (*I*)

Results

In Organ Cave, a total of ten copepod species were found (Pipan et al. 2006). Of these, four occurred throughout the study area in Organ Cave, which had a maximum lateral extent of 1,250 m. The other six species all occurred in areas with a maximum lateral extent of less than 70 m. Two of these were known from a single specimen from a single drip. The remaining four—*Bryocamptus zchokkei alleganiensis, Bryocamptus newyorkensis* group, *Moraria cristata*, and *Rheocyclops virginianus*—by contrast were known from multiple specimens in multiple drips, and should be useful in tracing water movement in the epikarst.

The much larger sample from Slovenian caves allows for a more thorough analysis. The maximum linear extent of occurrence in the study area was divided into three categories:

1. Less than 100 m, which corresponds to drips within a single cave
2. Less than 1 km but greater than 100 m, which corresponds to drips in different caves in the Postojna Planina Cave System, and
3. Greater than 1 km, which corresponds to drips in different cave systems.

As shown in Fig. 2, the preponderance of species either have very small ranges or occur throughout the study area. Even when there is potential for bias due to differing numbers of drip pairs in the different categories (Fig. 2), there is still a strong, statistically significant difference between observed number maximum linear extents in the different categories and expected number based on the number of drip pairs in that category (likelihood $\chi^2 = 20.99$, df = 2, $P < 0.0001$). In particular, there is an excess of species with large linear extents and a deficiency of species with moderately sized linear extents. If the analysis is limited to stygobionts, very similar results are obtained. Out of 27 stygobiontic species, 11 had maximum linear extents of less than 100 m, i.e. were limited to a single cave, while the other 16 had ranges greater than 1 km, i.e. were found in multiple caves. Among the most abundant species found in multiple drips in a single cave are the stygobiotic *Acanthocyclops kieferi* Chappuis and *Morariopsis dumonti* Brancelj.

Discussion

Some of the species with very small ranges are also extremely rare, such as *Epactophanes* and *Eucyclops*, known from single specimens in Organ Cave. It may be that these species are accidentals in the epikarst, but in any case are not numerous enough to be of any use in tracing water movement. However, most of the species with limited liner extent of occurrence do not fall into this category. In Postojna Planina cave system, there are 11 stygobionts that are known from a single cave in the study area, four are only known from a single cave anywhere, but the other seven are known from other caves. These 11 single cave stygobionts were not found in all the drips in the caves where they were found. Similarly, *Paracyclops* and *Moraria* in Organ Cave were not found in all the drips within their areas of occurrence. While some species may be absent from a

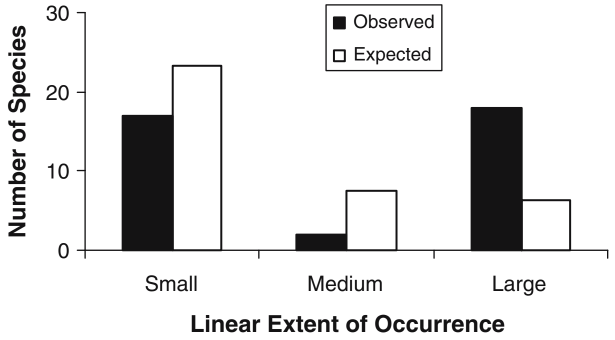

Fig. 2 Linear extent of occurrence of 37 species of copepods in six Slovenian caves

drip because environmental conditions are not suitable, this seems to be generally unlikely (Pipan and Culver 2005). Likewise, a species may be absent because of inadequate sampling, but this also seems unlikely in the caves studied, based on plateauing of species accumulation curves and estimates of total species richness that are in close agreement with observed species richness (Pipan 2005; Pipan and Culver 2005).

Those species that were found over the entire study area in Slovenia or over the maximum linear extent of drip sites in Organ Cave were in general not ubiquitous. The authors only found two cases where a copepod species occurred in all drips sampled in a cave—*Speocyclops infernus* (Kiefer) and *Parastenocaris* n.sp., both stygobionts from Pivka jama. In Organ Cave, the maximum coverage of drips was the genus *Microcyclops*, which was found in 9 of 13 drips sampled. This suggests that the distribution of copepods may have value in determining water movement in small areas, such as that above a single cave. While those species that were widespread have limited value in tracing the movement of water, they are likely to represent multiple populations, or perhaps even cryptic species. Pipan et al. (2005), using the Organ Cave data, showed that community similarity, as measured by the Jaccard index, declined with geographic distance, but after a few hundred meters, the average similarity and range of values increased, as ‘‘new’’ communities appeared. The overall pattern, even among widespread species, appears to be a relatively fine-scale patchwork of communities of populations and communities at a scale of a hundred or so meters.

How does this pattern compare with what is known about the lateral movement of water in epikarst? Two known studies address this problem directly. The first is that of Smart and Friederich (1987) in GB Cave in England. They found that injected fluorescent dyes moved a lateral distance of 80 m in a maximum of 6 days. They claim to have located all trickles and seeps in the cave but it is not clear from their discussion whether the injected dye appeared in all of them. They also showed that some of the injected dye was retained in the epikarst for at least 13 months.

The second study that considered the lateral movement of dye injected water is that of Kogovšek and Šebela (2004) on epikarst water tracing in Postojnska jama, one of the caves reported on in this study (see also Pipan and Brancelj 2004). They injected uranin dye at the bottom of a septic tank four metre below the surface. They injected 60 g of dye and flushed it with 5.5 m^3 of water, during a relatively dry period of high evapotranspiration. Dye was recovered at one trickle 60 m away in a horizontal direction from the injection point. Dye was not present after 17 days but was present after 90 days. Their studies of the appearance of dye in different drips suggested considerable heterogeneity of flow rates. During a rainy period in 1996, dye injected at the same point was recovered in 30 h. Taking vertical distance into account as well, water moved a minimum distance of 85 m in 30 h, approximately 0.0007 m/s. This is well below the threshold velocity transport of copepods in the water column, but this calculation assumes both minimum distance and uniform flow rates. They also showed that the temporal movement of the injected dye did proceed in a linear fashion, i.e. injected dye did not appear in some trickles closer to the injection than some trickles that did contain the injected dye. The major point of interest is that water moves to a distance approximately the same as the distribution limits of many copepod species (this study) and the extent of overall copepod community similarity (Pipan et al. 2005).

The results presented here show that the mapping of copepod microdistribution on the basis of their occurrence in drips is a potentially useful technique to determine the path of water in the epikarst. Dye injection studies are difficult both because the time course of dye movement is generally unknown and injection often requires the addition of a considerable volume of water (Kogovšek and Šebela 2004), which may produce spurious results due to mounding of the water. An interesting study would be the simultaneous sampling of epikarst copepods and the injection of dye.

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References

Bakalowicz M (2004) The epikarst, the skin of karst. In: Epikarst. In: Jones WK, Culver, DC, Herman JS (eds) Proceedings of the symposium held October 1 through 4, 2003, Shepherdstown, West Virginia, USA, Karst Waters Institute, Charles Town, pp 16–22

Gabrovšek F (2004) Attempts to model the early evolution of epikarst. In: Jones WK, Culver DC, Herman JS (eds) Epikarst. Proceedings of the symposium held October 1 through 4, 2003, Shepherdstown, West Virginia, USA, Karst Waters Institute, Charles Town, pp 50–55

Gordon ND, McMahon TA, Finlayson BL (1999) Stream hydrology: an introduction for ecologists. Wiley, Chichester, 511 pp

Huys R, Boxshall GA (1991) Copepod evolution. The Ray Society, London, 468 pp

Jones WK, Culver DC, Herman JS (eds) (2004) Epikarst. In: Proceedings of the symposium held October 1 through 4, 2003, Shepherdstown, West Virginia, USA, Karst Waters Institute, Charles Town, 160 pp

Kogovšek J, Šebela S (2004) Water tracing through the vadose zone above Postojnska jama, Slovenia. Env Geol 45:992–1001

Pesce GL, Galassi DP (1986) Taxonomic and phylogenetic value of the armature of coax and antenna in stygobiont cyclopoid copepods. Bull Zool Suppl 53:58–72

Pipan T (2005) Epikarst—a promising habitat. Copepod fauna, its diversity and ecology: a case study from Slovenia (Europe). Založba ZRC, Inštitut za raziskovanje krasa ZRC SAZU, Postojna, 101 pp

Pipan T, Brancelj A (2004) Distribution patterns of copepods (Crustacea: Copepoda) in percolation water of the Postojnska Jama cave system, Slovenia. Zool Stud 43:206–210

Pipan T, Christman MC, Culver DC (2006) Dynamics of epikarstic communities: microgeographic pattern and environmental determinants of epikarstic copepods in Organ Cave, West Virginia. Am Midland Nat 156(1):75–87

Pipan T, Culver DC (2005) Estimating biodiversity in the epikarstic zone of a West Virginia cave. J Cave and Karst Stud 67:103–109

Sket B, Trontelj P, Žagar C (2004) Speleobiological characterization of the epikarst and its hydrological neighborhood: its role in dispersion of biota, its ecology and vulnerability. In: Jones WK, Culver DC and Herman JS (eds) Epikarst. Proceedings of the symposium held October 1 through 4, 2003, Shepherdstown, West Virginia, USA, Karst Waters Institute, Charles Town, pp 104–113

Smart PL, Friederich H (1987) Water movement and storage in the unsaturated zone of maturely karstified carbonate aquifer, Mendip Hills, England. In: Proceedings of conference on environmental problems in Karst Terranes and their solutions, National Water Well Association, Dublin, USA, pp 59–87

Smith IR (1975) Turbulence in lakes and rivers. Special Publication 29, Fresh Water Biological Association, UK

Stoch F (1995) The ecological and historical determinants of crustacean diversity in groundwaters, or why are there so many species? Mém Biospéol 22:139–160

Trček B (2003) Epikarst zone and the karst aquifer behavior. A case study of the Hubelj Catchment, Slovenia. Geološki zavod Slovenije Ljubljana, 100 pp