
Copepod distribution as an indicator of epikarst system connectivity

Tanja Pipan · David C. Culver

Abstract The distribution of 27 copepod species was determined in 35 drips in four Slovenian caves (Dimnice, Postojna Planina Cave System, Škocjanske Jame, Županova Jama), and of ten species from 13 drips in one US cave (Organ Cave, West Virginia). The dripping water comes from epikarst, the skin of karst. A significant fraction of the copepod species found (nine species in Slovenia and three in West Virginia) occurred over a maximum linear extent of 100 m. These and other localized distributions probably resulted from colonization of epikarst by an ancestral surface population in a single location, with subsequent lateral spread in the direction of epikarst flow. This suggests that the distribution of copepods could potentially be used to trace major flow paths in epikarst without the need for the injection of dyes or other tracers.

Résumé La distribution de 27 espèces de copépodes a été déterminée dans 35 zones de percolation de quatre grottes slovènes (Dimnice, système de grottes Postojna Planina, Škocjanske Jame, Županova Jama) et grâce à dix espèces de 13 zones de percolation d'une grotte des Etats-Unis (Grotte Organ, Ouest de la Virginie). L'eau de percolation provient de l'épikarst, la partie supérieure du karst. Une fraction importante des espèces de copépodes trouvées (9 espèces en Slovénie et 3 dans l'Ouest de la Virginie) s'étendait sur une longueur maximum de 100 m. Ces dernières ainsi que d'autres distributions localisées étaient probablement issues de la colonisation par une population ancienne de surface, de zones localisées de l'épikarst, puis

par une dispersion latérale dans la direction de l'écoulement dans l'épikarst. Ceci suggère que la distribution des copépodes pourrait potentiellement être utilisée pour identifier les principales trajectoires d'écoulement dans l'épikarst, sans utiliser l'injection de colorants ou d'autres traceurs.

Resumen Se determinó la distribución de 27 especies de copépodo en 35 gotas de cuatro cavernas de Eslovenia (Dimnice, Sistema de Caverna Planina Postojna, Jame Škocjanske, y Jama Županova), y de diez especies en 13 gotas de una caverna de Estados Unidos (Caverna Órgano, Virginia Occidental). El agua que gotea se deriva del epikarst, la piel del karst. Una fracción significativa de las especies de copépodos encontradas (9 especies en Eslovenia y 3 en Virginia Occidental) se presentaron en una extensión linear máxima de 100 m. Estas y otras distribuciones localizadas resultaron probablemente de colonización del epikarst por poblaciones superficiales ancestrales en una sola localización, con una expansión lateral subsiguiente en la dirección de flujo epikárstico. Esto sugiere que la distribución de copépodos podría tener un uso potencial para trazar rutas de flujo principales en epikarst sin tener la necesidad de inyectar colorantes u otros trazadores.

Keywords Groundwater flow · Karst · Tracer tests · Copepod

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Introduction

The upper most layer of solutionally modified karst, at the rock-soil interface, is commonly termed epikarst (Jones et al. 2004). The analogy of Bakalowicz (2004), of epikarst as the skin of karst, captures the essence of this boundary zone.

There is a complex and diverse aquatic community of tiny invertebrates (size 0.05–1.5 mm) in the epikarst, including a rich array of crustaceans, most especially copepods (Pipan 2005). In open waters, organisms of this size are part of the plankton, which drifts at the mercy of currents. The possibility exists that epikarst copepods and other tiny invertebrates move at the mercy of currents in epikarst. Thus, epikarst animals have a potentially

important role in hydrogeology as tracers of water movement (Pipan and Culver 2005a).

The focus of interest in the hydrogeology of epikarst has been on the vertical movement of water, which is of considerable practical importance because of the role 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) detailed the largely vertical movement of water in the epikarst in the Hubelj aquifer, Slovenia. However, water in the epikarst also flows laterally, especially during periods of high recharge (Smart and Friederich 1987; Aley 2004).

Tracing the path of water movement in both the lateral and vertical direction in epikarst is notoriously difficult (Kogovšek and Šebela 2004). One of the problems is that there is rarely a water source into which dye can be injected. The injection of large amounts of water into which dye has been injected can create mounding of water (W.K. Jones, Karst Waters Institute, unpublished data, 2006), changing the hydraulic gradient, and potentially resulting in artificially induced multidirectional lateral flow. Only a very few studies have used natural precipitation to move injected dyes (Smart and Friederich 1987; Kogovšek and Šebela 2004).

Use of the epikarst fauna as natural tracers may be an attractive alternative to dye injection. Epikarst harbors a rich fauna of obligately subterranean-dwelling aquatic species, i.e., stygobionts, especially Copepoda (Pipan 2005), many populations of which have ranges whose linear extent is 100 m or less. The lateral extent of movement of dye in epikarst is of the same order of magnitude (Smart and Friederich 1987; Kogovšek and Šebela 2004), suggesting that copepods may be tracers of water movement. This work explores the possibility of using epikarst copepods as natural tracers of water movements, a technique that does not require the injection of dyes, animals, or water.

Methods and materials

The epikarst copepod fauna of four caves in Slovenia—Dimnice, Postojna Planina Cave System (PPCS),

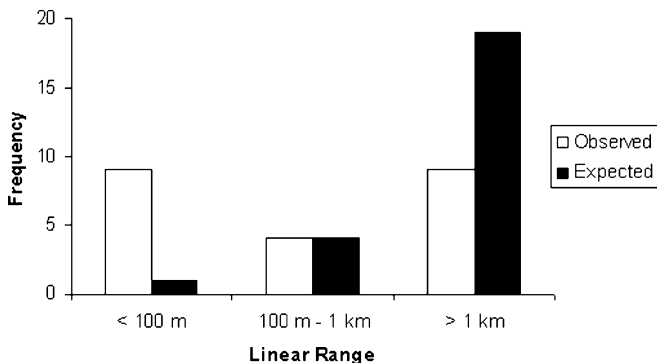


Fig. 1 Linear extent of ranges of stygobiotic copepods in four cave systems in Slovenia. Expected values were generated from the proportion of distances between drips that fall into the three categories

Škocjanske Jame, Županova Jama (see Pipan 2005)—and one cave in the US (Organ Cave, West Virginia; see Pipan and Culver 2005a,b) was sampled from a series of ceiling drips. By sampling only drip water, other sources of copepods, e.g., wet walls and streams, were eliminated. In each cave, drips were sampled by directing the water through a funnel into a plastic container. A 2×3-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

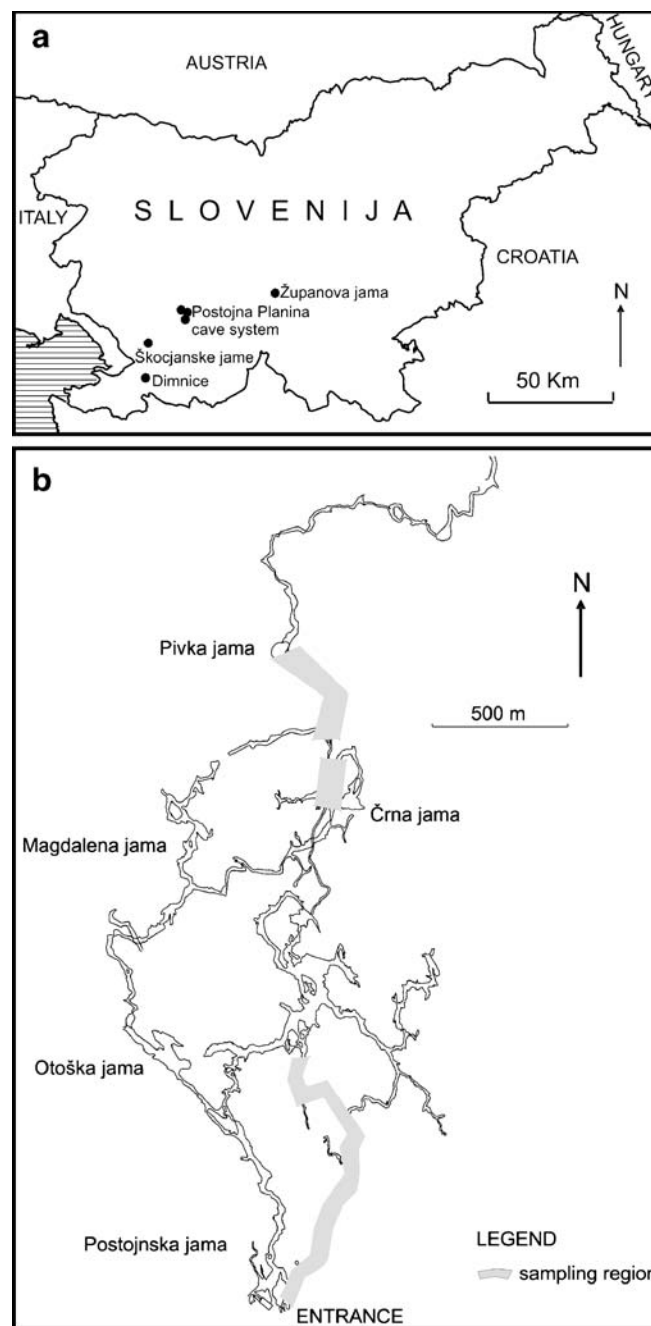


Fig. 2 a) Location map of cave systems studied in Slovenia, b) map of the Postojna Planina cave system (PPCS) with the names of the different sections of the system indicated (from Pipan 2005). The cave system is 1 km north of the town of Postojna in west central Slovenia. The shaded polygons indicate the sampling areas in caves Črna Jama, Pivka Jama, and Postojnska Jama

animals in the container. Samples were preserved in 4% formalin and identified to species.

Samples were taken over a period of 30 days in Organ Cave and one year in the Slovenian caves. Collections were taken at 10-day intervals in Organ Cave and 30-day intervals in Slovenian caves in order to minimize changes due to births and deaths (Pipan et al. 2006b). In Slovenia, sampled drips were between 10 m and 60 km apart. A total of 35 drips were sampled. In Organ Cave, 13 drips ranging in distance from 1 to 1,200 m were sampled.

The linear distance between drips was determined for all pairs of drips from cave maps (see Pipan 2005; Pipan et al. 2006b). For the more extensive Slovenian data, drip distances were categorized into three groups:

1. Drips less than 100 m apart, which correspond to drips within a single cave
2. Drips less than 1 km but greater than 100 m apart, which correspond to drips in different caves in the Postojna Planina Cave System (PPCS), the largest cave system studied in Slovenia
3. Drips greater than 1 km, which correspond to drips in different cave systems

The frequency of these three groups was used to determine the expected frequency of species ranges for these three groups. The two distributions were compared using a log-likelihood ratio for the three categories in JMP 5.1 (data analysis and experiment design software produced by SAS Institute; see Sall et al 2005).

The frequency distribution of species according to number of drips and maximum linear extent was determined for the two sites and representative range maps of copepod populations were determined by drawing the smallest ellipse that encompassed all occurrences in the study caves.

Factors other than distance, e.g., ceiling thickness and nitrate concentrations, affect species distributions (Pipan et al. 2006a), but the focus here is on distance and hence hydraulic connectivity as an explanation (see also Pipan and Culver 2005b; Pipan et al. 2006b).

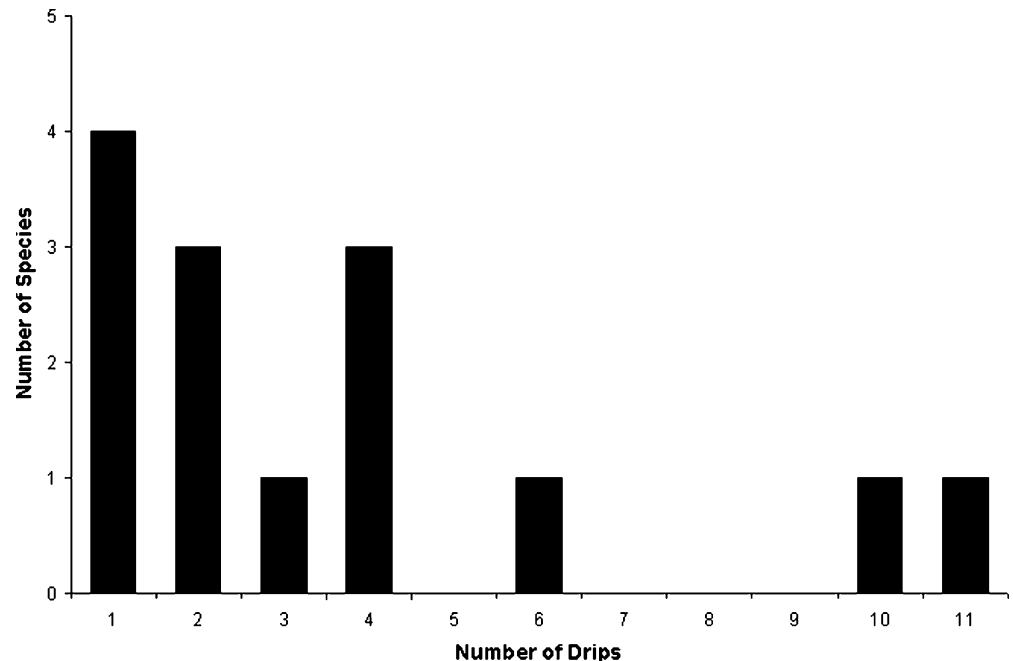
Results

A total of 27 copepod species were found in the four Slovenian caves, 22 of which were stygobionts, i.e., strictly limited to subterranean habitats (Pipan 2005). Most stygobiotic species either have very small ranges or occur throughout the study area (Fig. 1). When the potential bias is due to differing numbers of drip pairs in the different categories, species with very small ranges (<100 m) are highly over-represented. There is a statistically significant difference between the observed number of maximum linear extents in the different categories and the expected number based on the number of drip pairs in that category (likelihood χ^2 test=16.11, degrees of freedom=2, probability of type II errors <0.024).

Since the data for PPCS are more extensive, they are worth examining in more detail (Fig. 2). Of the 12 stygobiotic species, no species was found in more than 11 of 20 drips and 7 were found in less than 3 drips (Fig. 3). The median number of drips in which a species occurred was two.

Actual distribution of species in PPCS was also informative. Two species—*Parastenocaris* n.sp. and cf. *Stygopactophanes* n.sp.—were found only at drip sites in the Črna Jama section of PPCS (Fig. 2b). The maximum linear distance of sampled drips in Erna Jama was 141 m. Five species—*Bryocamptus dacicus*, *Bryocamptus* n.sp., *Elaphoidella* n.sp., *Maraenobiotus brucei*, and *Moraria varica*—were found only at drip sites in Pivka Jama,

Fig. 3 Frequency distribution of stygobiotic epikarst copepods according to the number of drips in PPCS in which they occurred. A total of 20 drips were sampled



another section of PPCS (Fig. 2b). The maximum linear distance of sampled drips in Pivka Jama was 206 m. These species may occur in unsampled drips but it seems unlikely that their ranges are significantly greater, especially given the close proximity of the sampling sites in Črna Jama and Pivka Jama (Fig. 2b).

The final analysis for PPCS shows the distributional data for Pivka Jama in more detail (Fig. 4). All stygobiotic copepods found in Pivka Jama, not just those exclusively found in Pivka Jama are shown. Only two of nine species were found in all five drips. The others formed a partial nesting pattern. All species were found in one or both of the sampling points in the northwest. From these drips, there is a spreading of distributions in a regular way such that no drips within an ellipse lack the species.

In Organ Cave, a total of ten species were found, seven of these were stygobionts. Of these seven, four occurred throughout the study area in Organ Cave, which had a maximum lateral extent of 1250 m. The other three species had maximum lateral extents of considerably less

than 1250 m. Two species were limited to a section of the cave named Sively No. 2 and one was limited to Sively No. 3. The maximum lateral extent in these three areas ranged from 25 m (Sively No. 3) to 65 m (Sively No. 2). The pattern in Sively No. 3 (Fig. 5) is similar to that of Pivka Jama in Slovenia. The highest concentration of species was in drip number 3, with a consistent and regular spreading of distributions from that point.

Discussion

Some of the species with very small ranges are also extremely rare such as *Epactophanes richardi*, known from a single specimen in Organ Cave. It may be that these species are accidentals in the epikarst, but in any case are not numerous enough and of limited use in tracing water movement. However, most of the species with limited linear extent of occurrence do not fall into this category. While some species may be absent from a drip because of unsuitable environmental conditions, this seems to be generally unlikely (Pipan and Culver 2005b). 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 2005b).

Even those species that were found over the entire study area in Slovenia or over the maximum linear extent

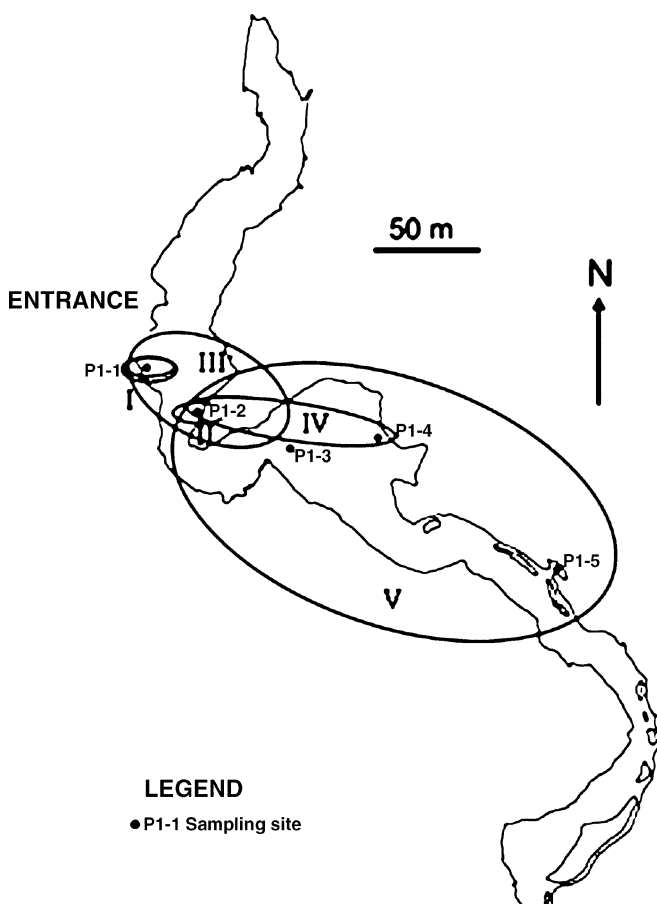


Fig. 4 Map of distribution of stygobiotic epikarst copepods in the Pivka Jama section of PPCS. Ranges are indicated by ellipses. Points numbered from P1-1 to P1-5 are the locations of the epikarst drips sampled. Roman numerals refer to different distributions: Ellipse I—P1-1 only (*Bryocamptus* n.sp.); II—P1-2 only (*Bryocamptus balcanicus* and *Elaphoidella* n.sp.); III—P1-1 and P1-2 (*Mar-aenobiotus* cf. *brucei*); IV—P1-2 and P1-4 (*Parastenocaris nolli alpina*); and V—P1-2, P1-3, P1-4 and P1-5 (*Elaphoidella cvetkai*). Not shown in the figure are two species (*Parastenocaris* n.sp. and *Speocyclops infernos*) found in all five drips

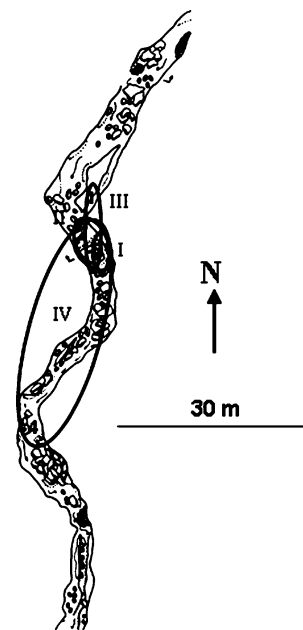


Fig. 5 Map of distribution of stygobiotic epikarst copepods in the Sively No. 3 section of Organ Cave in southcentral West Virginia. Ranges are indicated by ellipses. Points numbered from 1 to 4 are the locations of the epikarst drips sampled. Roman numerals refer to different distributions: Ellipse I—3 only (*Epactophanes richardi* and *Parastenocaris* n.sp. 1); II—2 and 3 (*Parastenocaris* n.sp. 2); III—1 and 3 (*Rheocyclops virginianus*); and IV—2, 3, and 4 (*Rheocyclops* cf. *virginianus*). See Pipan et al. (2006b)

of drip sites in Organ Cave were, in general, not ubiquitous. No species occurred in all drips in either PPCS or Organ Cave, the two most thoroughly sampled caves. In Organ Cave, the maximum coverage of drips was the *Rheocyclops* cf. *virginianus*, which was found in 9 of 13 drips sampled. In PPCS, *Speocyclops infernus* was found in 11 of 20 drips.

These results suggest that copepod distribution patterns may have value in determining water movement within small karst system 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. If molecular genetic data were available, these widespread species might be of use as well.

Just as mineralized particles can be mobilized into the water column, so can copepods. It is instructive 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 mobilization occurs at a velocity slightly greater than 0.1 m/s. This is approximately the size of the smallest copepods; body size of adult copepods ranges from 0.2 to 2 mm. Particles 0.3 mm in diameter can be mobilized at approximately 0.5 m/s. Smith (1975), who states that the critical velocity for organic matter with a density of 1.05 g/cm³ is about 1/6 that of an equivalent-sized mineral particle, suggested that a passive copepod in the epikarst could be mobilized at velocities of less than 0.1 m/s. Smart and Friederich (1987) report that epikarst flow rates reach 4.5×10^3 m/day (0.52 m/s), well above the critical mobilization velocity. 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 2005b) if this were not the case. It is this property of copepods, i.e., their frequent occurrence in the water column as well as their small size that makes them potential water tracers.

Little is known about the specifics of colonization of epikarst habitats by surface-dwelling ancestors, but it is likely that invasion occurred from above, especially the soil, as well as superficial aquatic habitats such as wet leaves (Reid 2001). In any case, the colonization of epikarst habitats likely occurred at a single site. It is the analogy between a point-source contaminant spill and the invasion of epikarst by copepods that lies at the core of this work.

How does this pattern compare with what is known about the lateral movement of water in epikarst? The authors know of only three studies that address this problem directly. The first is that of Smart and Friederich (1987) in GB Cave in England, UK. They found that injected fluorescent dyes moved a lateral distance of 80 m in 6 days. 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 in this study. They

injected uranin dye at the bottom of a septic tank 4 m below the surface. Dye was recovered at one trickle 60 m away in a horizontal direction from the injection point. Aley (2004) reported travel distances of up to 200 m in low flow and 1,100 m in high flow in Tumbling Creek Cave, Missouri, USA. The longer distances reported by Aley may result from epikarst sites with more integrated lateral flow than is typical.

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, one that would not require the injection of either dye or water. Validation of the utility of copepods as tracers could come from the simultaneous sampling of epikarst copepods and the injection of dye. If copepods are useful tracers, then their distribution should correspond to that of injected dye.

This is not the first suggestion that living organisms can be useful groundwater tracers. Of particular interest has been the identification of surface water contamination of groundwater (reviewed by Hancock et al. 2005). Danielopol (1981) used ostracods to identify wells contaminated with surface water and Malard et al. (1994) used cyclopoid copepod and oligochaetes to follow contaminant transport in a fractured limestone aquifer 10–40 m deep. Bacteria and other microbes have been useful tracers, especially in porous aquifers (see Griebler 2001 for a review and Bricelj and Čenčur Curk 2005 for a recent example). What is suggested here is that the use of biological tracers need not be confined to surface-water contamination, and that epikarst is an especially appropriate place for such studies.

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